Phylogenetics of Cystopteridaceae:  
Reticulation and Divergence in a Cosmopolitan Fern Family

by

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Michael Windham

Dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Biology in the Graduate School of Duke University

2012
ABSTRACT
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Abstract

The fern family Cystopteridaceae has been a thorn in the side of fern phylogeneticists, on many levels. Until this thesis, its basic existence (as a deeply isolated clade) and composition were unrecognized, hypotheses as to the relationships of its constituents within the broader fern tree-of-life were wildly inconsistent, the relationships of its genera to each other were contested, the species limits within those genera weakly understood, and the relationships among those species unknown. This thesis first establishes the broad evolutionary context for the family, which is that it is the first-diverging branch in Eupolypods II (it is sister to the rest of the eupolypod II clade). Eupolypods II is a large clade, containing nearly a third of extant fern species; this is a position pivotal to a full understanding of fern evolution.

The evolution of the Eupolypods II is marked by an “ancient, rapid radiation” at the base of the clade, which helps to explain the difficulty that this broad group has historically posed to evolutionary biologists. Molecular data from five plastid loci show that Eupolypods II consists of 10 deeply divergent lineages, each worthy of recognition at the rank of family: Cystopteridaceae, Rhachidosoraceae, Diplaziopsidaceae, Hemidictyaceae, Aspleniaceae, Thelypteridaceae, Woodsiaceae, Onocleaceae, Blechnaceae, and Athyriaceae. The ancestors of Cystopteridaceae diverged from those of the rest of the clade approximately 100 million years ago, and the family is now composed of five extant genera: Acystopteris, Cystoathyrium (the only genus for which we lack molecular data—it may be extinct), Cystopteris, Gymnocarpium, and ×Cystocarpium.

Within the family, the relationships of Cystoathyrium are unknown. Acystopteris is sister to Cystopteris, and those two genera, together, are sister to Gymnocarpium. Gymnocarpium is the maternal parent of ×Cystocarpium, so that genus falls within Gymnocarpium in phylogenetic trees based on maternally transmitted loci (i.e., plastid or
mitochondrial loci). Plastid data resolve a basal trichotomy in Gymnocarpium, among the G. disjunctum clade, the G. robertianum clade, and core Gymnocarpium. The earliest diverging branch of core Gymnocarpium is the morphologically anomalous G. oyamense, followed by a split that separates G. appalachianum and G. jessoense parvulum (on one side) from G. remotepinnatum and G. jessoense jessoense, on the other. In Acystopteris, the first division surprisingly separates A. taiwaniana (which is frequently treated as a variety of A. japonica) from A. japonica + A. tenuisecta (which are morphologically very distinct from each other).

The evolution of Cystopteris is, as expected, more complex. The first lineage to diverge from the rest of the genus is the one that gave rise to C. montana. The next division, however, is unclear; molecular data infer a trichotomy among the sudetica clade (containing C. sudetica, C. moupinensis, and C. pellucida), the bulbifera clade (containing C. bulbifera and its related allopolyploids C. tennesseensis and C. utahensis), and the C. fragilis complex. Within the C. fragilis complex relationships (and species limits) get particularly messy. The diploid species of eastern North America—C. protrusa—is sister to the rest of the complex, but after that point the major named species (including C. fragilis and C. tenuis) cease to be monophyletic, being found on both sides of a major split, alongside such taxa as the Australian/New Zealand C. tasmanica, the Hawaiian C. douglasii, and the Mexican C. membranifolia and C. millefolia.

In the context of the deep divergence of Gymnocarpium from Cystopteris, and the complicated species-level patterns of relationship within each genus, it is particularly surprising that molecular data confirm that ×Cystocarpium is a hybrid between Gymnocarpium dryopteris and a European tetraploid member of the Cystopteris fragilis complex. The ancestors of Cystopteris diverged from those of Gymnocarpium approximately 58 million years ago, meaning that the ×Cystocarpium hybridization event
(which happened very recently) united genomes that contain, between them, over 100 million years of independent evolution. This breadth of divergence makes ×Cystocarpium the most extreme example of wide hybridization currently documented, with important implications for the pace of evolution of reproductive isolation, and thus for species formation.

This thesis ends with a tentative synopsis of the Cystopteridaceae (Appendix E). The family, as construed here, contains five genera and approximately 36 species (three in Acystopteris, one in Cystoathyrium, ~25 in Cystopteris, seven in Gymnocarpium, and one in ×Cystocarpium), plus two named subspecies (one each in Cystopteris and Gymnocarpium), and eight named sterile hybrids (three in Cystopteris and five in Gymnocarpium). Each of these tallies is highly subjective—much further research, with an emphasis on cytological and low-copy nuclear data, is necessary before we can hope to have any confidence in the species limits and finer-scale evolutionary patterns in this family.
Dedication

To my parents, Paul Rothfels and Margaret Almack, the best field and non-field support team, respectively.
Cystopteris is not the sort of problem I'd wish on anyone …
Alan R. Smith, 2008

Cystopteris is dominated by the widespread and polymorphic C. fragilis complex, which constitutes perhaps the most formidable biosystematic problem in the ferns.
John Lovis, 1978: 356
# Table of Contents

Abstract .......................................................................................................................... iv

List of Tables .................................................................................................................. xiii

List of Figures ................................................................................................................. xiv

Acknowledgements ......................................................................................................... xvi

Introduction .................................................................................................................... 1

1. Overcoming deep roots, fast rates, and short internodes to resolve the ancient rapid radiation of eupolypod II ferns ................................................................. 6
  1.1 Introduction ............................................................................................................. 6
  1.2 Materials and Methods ......................................................................................... 10
    1.2.1 Taxon Sampling ............................................................................................. 10
    1.2.2 Amplification and Sequencing ...................................................................... 11
    1.2.3 Alignment and Tree Search .......................................................................... 14
    1.2.4 Phylogeny Evaluation .................................................................................... 16
  1.3 Results .................................................................................................................. 18
    1.3.1 Data and Topology Point Estimate .............................................................. 18
    1.3.2 Bayesian Star-tree Paradox Artifact ............................................................. 20
    1.3.3 Lineage-specific Rate Heterogeneity ............................................................ 23
    1.3.4 Rooting Uncertainty ...................................................................................... 24
    1.3.5 Eupolypod II Phylogeny ................................................................................. 27
  1.4 Discussion ............................................................................................................ 29
    1.4.1 Bayesian Star-tree Paradox Artifact ............................................................. 29
    1.4.2 Lineage-specific Rate Heterogeneity ............................................................ 30
    1.4.3 Rooting Uncertainty ...................................................................................... 31
    1.4.4 Eupolypod II Phylogeny: Major Clades ....................................................... 32
<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.2.3 Sequence Alignment and Phylogenetic Analysis</td>
<td>89</td>
</tr>
<tr>
<td>3.3 Results</td>
<td>90</td>
</tr>
<tr>
<td>3.3.1 Phylogenetic Analyses</td>
<td>90</td>
</tr>
<tr>
<td>3.3.2 Cystopteridaceae Phylogeny</td>
<td>91</td>
</tr>
<tr>
<td>3.4 Discussion</td>
<td>93</td>
</tr>
<tr>
<td>3.4.1 Phylogenetic Analyses and Intralinkage Incongruence</td>
<td>93</td>
</tr>
<tr>
<td>3.4.2 Cystopteridaceae Phylogeny</td>
<td>95</td>
</tr>
<tr>
<td>3.4.3 Gymnocarpium Phylogeny</td>
<td>95</td>
</tr>
<tr>
<td>3.4.4 Acystopteris Phylogeny</td>
<td>98</td>
</tr>
<tr>
<td>3.4.5 Cystopteris Phylogeny</td>
<td>99</td>
</tr>
<tr>
<td>3.4.5.1 Cystopteris montana and the sudetica and bulbifera Clades</td>
<td>100</td>
</tr>
<tr>
<td>3.4.5.2 Cystopteris fragilis Complex</td>
<td>102</td>
</tr>
<tr>
<td>3.5 Acknowledgments</td>
<td>103</td>
</tr>
<tr>
<td>4. ( \times ) Cystocarpium is a natural intergeneric hybrid between parents that diverged over 50 million years ago</td>
<td>105</td>
</tr>
<tr>
<td>4.1 Introduction</td>
<td>105</td>
</tr>
<tr>
<td>4.2 Methods</td>
<td>107</td>
</tr>
<tr>
<td>4.2.1 Determining Parentage</td>
<td>107</td>
</tr>
<tr>
<td>4.2.2 Divergence Dating</td>
<td>113</td>
</tr>
<tr>
<td>4.3 Results and Discussion</td>
<td>118</td>
</tr>
<tr>
<td>4.3.1 Origin of ( \times ) Cystocarpium</td>
<td>118</td>
</tr>
<tr>
<td>4.3.2 Depth of Divergence</td>
<td>120</td>
</tr>
<tr>
<td>4.4 Acknowledgments</td>
<td>122</td>
</tr>
<tr>
<td>Conclusions</td>
<td>123</td>
</tr>
<tr>
<td>Appendix A: Voucher table for Chapter One</td>
<td>128</td>
</tr>
<tr>
<td>Appendix B: Supplementary figures for Chapter One</td>
<td>135</td>
</tr>
</tbody>
</table>
List of Tables

Table 1: Primers used for amplification and sequencing in Chapter One......................... 13
Table 2: Dataset statistics for Chapter One........................................................................ 15
Table 3: Primers used for amplification and sequencing in Chapter Three....................... 88
Table 4: Statistics for the datasets used in Chapter Three............................................... 89
Table 5: Best-fitting models for the $gapCp$ partitions for Chapter Four......................... 113
List of Figures

Figure 1: Challenges inherent in resolving the eupolypod II phylogeny ......................... 8

Figure 2: Broad phylogeny of ferns.................................................................................. 9

Figure 3: Phylogeny evaluation: Rate heterogeneity, and the Bayesian star-tree paradox artifact ......................................................................................................................... 19

Figure 4: Discrepancies between maximum likelihood and Bayesian support values........ 20

Figure 5: Effects of outgroup composition on ingroup backbone support values .......... 25

Figure 6: Maximum likelihood (ML) phylogram of the concatenated data .................. 28

Figure 7: Representative eupolypod II ferns..................................................................... 42

Figure 8: Morphological characteristics of eupolypod II taxa........................................ 43

Figure 9: Divergence and diversification in the Eupolypods II ...................................... 44

Figure 10: Silhouettes of representative Cystopteridaceae species ................................. 83

Figure 11: Geographic ranges of the sampled Cystopteridaceae taxa ............................. 87

Figure 12: Maximum likelihood phylogram of the concatenated data ............................ 93

Figure 13: Intralinkage incongruence .............................................................................. 94

Figure 14: A most-parsimonious tree from the “all-unique” dataset ............................... 109

Figure 15: A most-parsimonious tree from the “trimmed-1” dataset ............................. 110

Figure 16: Maximum likelihood phylogeny of gapCp alleles from the “trimmed-2” dataset................................................................................................................................. 112

Figure 17: The hybrid origin of ×Cystocarpium, and nested empirical Bayesian analysis of divergence time .................................................................................................................. 117

Figure 18: Representative chromosome squashes from ×Cystocarpium roskamianum .... 119

Figure 19: Majority-rule consensus tree from Phycas with C=1 .................................. 135

Figure 20: Majority-rule consensus tree from Phycas with C=e ................................. 136

Figure 21: Majority-rule consensus tree from Phycas with C=10. ................................. 137

Figure 22: Majority-rule consensus tree from MrBayes with the branch-length prior (mu0/mu1) = 0.01. .................................................................................................................. 138
Figure 23: Majority-rule consensus tree from MrBayes with the branch-length prior (μ0/μ1) = 0.001 ................................................................. 139

Figure 24: Majority-rule consensus tree from MrBayes with the branch-length prior (μ0/μ1) = 0.0001 ................................................................. 140

Figure 25: Majority-rule consensus tree from BEAST ......................................................... 141
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Introduction

The fern family Cystopteridaceae presents a unique combination of challenges—and unique opportunities—for systematic biologists. Until very recently, virtually all elements of the family’s phylogeny were unknown, including its existence as a discrete and long-isolated lineage. This fact—the close relationship of the genera *Acystopteris* Nakai (1933), *Cystopteris* (L.) Bernh. (1805), and *Gymnocarpium* Newman (1851), and their deep divergence from their closest relatives—became apparent only with the advent of fern molecular phylogenetics (Hasebe et al., 1995; Sano et al., 2000a; Wolf et al., 1994). Prior to these data, *Cystopteris* and *Gymnocarpium* were typically not considered to be closely related (e.g., Kramer et al., 1990b; Sledge, 1973; Tryon & Tryon, 1982), and were individually placed within a heterogeneous assemblage of “dryopteroid” and/or “athyrioid” taxa.

The systematics community did not immediately accept the early molecular phylogenetic results. While classifications in the first decade of the 2000s recognized the alliance of *Cystopteris* (and *Acystopteris*) with *Gymnocarpium*, they continued to group these taxa together with distantly related lineages, most particularly members of the Athyriaceae (Schmakov, 2001; Smith et al., 2006; Wang et al., 2004; Wang, 2008). This hesitancy to recognize Cystopteridaceae was almost certainly due to the obscure patterns of morphological evolution within Eupolypods II (of which Cystopteridaceae is a part), and the long-standing instability of fern familial concepts in general (Rothfels et al., 2012a; Smith et al., 2006; Sundue & Rothfels, 2012 (in prep)).

The delayed acceptance of Cystopteridaceae as a distinct family, however, also reflects past uncertainty about the family’s phylogenetic position. The backbone branches within the Eupolypods II are short, following the pattern of an ancient rapid radiation (Rothfels et al., 2012a), and it was not until 2007 that the critical position of
Cystopteridaceae as sister to the rest of the eupolypod II clade was first well supported (Schuettpelz & Pryer, 2007). This phylogenetic position places the divergence of Cystopteridaceae very deep in the eupolypod phylogeny; the family shared its last common ancestor with other fern groups approximately 100 million years ago (Rothfels et al., 2012b; Schuettpelz & Pryer, 2009).

In contrast to the historically contentious familial and ordinal relationships of the Cystopteridaceae, genus-level relationships have been relatively straightforward, at least by fern standards. Two segregate genera (*Currania* Copel. (1909) and *Rhizomatopteris* Khokhr. (1985)) were each infrequently recognized, and are nested in *Gymnocarpium* and *Cystopteris*, respectively (Rothfels et al., 2012b; Rothfels et al., 2012c). The status of *Acystopteris* has been somewhat more contentious, with some authors recognizing it as a separate genus (e.g., Nakai, 1933; Wang et al., 2012b) and others subsuming it within a broad circumscription of *Cystopteris* (e.g., Blasdell, 1963; Tagawa, 1935). Molecular data (Rothfels et al., 2012a; Rothfels et al., 2012c; Sano et al., 2000a) show *Acystopteris* to be sister to *Cystopteris* s.s., so either option is defensible from a cladistic perspective. Given their relatively deep divergence from each other, and their ease of diagnosis, I favor their recognition as distinct genera (Rothfels et al., 2012b; Rothfels et al., 2012c). Based on morphological characters, the affinities of the enigmatic *Cystoathyrium* Ching (1966) are presumed to be with Cystopteridaceae, but it may well represent an isolated lineage (Rothfels et al., 2012b). It is known from only the type collection, and has not been included in any phylogenetic analyses. The only other genus recognized in the family is the nothogenus ×*Cystocarpium* Fraser-Jenk. (2008), a sterile hybrid between *Gymnocarpium dryopteris* and a member of the *Cystopteris fragilis* complex (see Chapter Four).
Within the Cystopteridaceae genera, however, species-level relationships have been virtually unknown, and species boundaries remain extremely tentative (see Appendix E). This situation is particularly surprising given that the two main genera—Cystopteris and Gymnocarpium—are common, conspicuous, and widespread in the Northern Hemisphere and thus might be expected to have been the focus of much early taxonomic attention. However, the complexity and geographic breadth of these two genera has discouraged global systematic investigations. The only such investigation of Cystopteris is Blasdell’s monograph from 1963 (he included Acystopteris as a subgenus of Cystopteris), which recognized far fewer species than do current treatments (e.g., Haufler et al., 1993; Paler & Barrington, 1995; Japanese Society for Plant Systematics, 2012; Wang, 2008), and it had a lukewarm reception even when initially published (Crabbe, 1965; Lovis, 1978). For Gymnocarpium, the only global treatment is a short (five page) synopsis by Sarvela (1978), which is likewise out of step with the current consensus (e.g., Pryer, 1993; Pryer & Haufler, 1993).

As currently treated, both Cystopteris and Gymnocarpium are dominated by widely distributed taxa, each of which likely includes multiple independent lineages. The most egregious example is Cystopteris fragilis, which, as broadly defined, ranges nearly pole to pole, occurs on every continent except for Antarctica, and includes ploidy levels ranging from diploid to octaploid (Blasdell, 1963). It is possibly the most widely distributed vascular plant in the world (excluding introduced species). Both Cystopteris and Gymnocarpium have been the subject of focused regional studies and, in all cases, those studies have concluded with the recognition of multiple entities within the broad cosmopolitan taxa (Haufler & Windham, 1991; Haufler et al., 1990; Pryer & Haufler, 1993; Vida, 1974; Vida & Mohay, 1980).
Much of the taxonomic difficulty posed by *Cystopteris* (and, to a lesser extent, *Gymnocarpium*) is due to extensive hybridization and polyploidy (allopolyploidy) between morphologically similar lineages (Lovis, 1978; Pryer & Haufler, 1993). Unlike in many polyploid fern groups, however, polyploidy in Cystopteridaceae is not associated with apogamy or apomixis—the higher polyploids in this family remain sexual. The family, with its broad geographic range and diverse sexual polyploids, thus presents a rare research opportunity for investigating the processes of polyploid evolution, free of the complicating considerations of asexuality. These processes include both reticulate evolution and primary speciation at the polyploid level, with a myriad of associated factors and effects spanning biogeography, morphological evolution, population genetics, and macroevolution. Although many of the genetic and molecular resources available for some angiosperm groups are not yet developed for the Cystopteridaceae (or ferns in general), research in the family has several advantages: relatively high levels of sequence divergence (even for coding regions of plastid genes); the occurrence of common, widely distributed, and easily collected taxa; the presence of an independent gametophytic generation (as in all ferns), which permits segregating allelic variation to be distinguished from non-segregating gene duplication; ease of cultivation; and ploidy level that can be inferred from spore size measurements.

In this thesis, I provide the beginnings of a modern phylogenetic understanding of the Cystopteridaceae, with the aim of facilitating its development as a system for investigating polyploid evolution. Chapter One provides the first well-supported phylogeny of the Eupolypods II, and thus establishes the broader evolutionary context for the family. This phylogeny forms the basis for Chapter Two, a family-level taxonomic treatment of the eupolypod II clade. This treatment summarizes the taxonomic, phylogenetic, ecological, and morphological data available for
Cystopteridaceae and its relatives. In Chapter Three, I provide the first phylogeny focused on the family, a three-gene plastid phylogeny encompassing an ingroup sample of 75 accessions: 23 from Gymnocarpium, six from Acystopteris, and 46 from Cystopteris. Finally, Chapter Four begins to explore the extent of reticulation in the family by using nuclear and plastid sequence datasets to demonstrate that ×Cystocarpium is a recently formed hybrid between Gymnocarpium (G. dryopteris) and Cystopteris (one of the members of the C. fragilis clade). Nested empirical Bayesian analyses of these data provide a mean estimate for the divergence of the ancestor of Gymnocarpium from that of Cystopteris + Acystopteris at 57.9 million years ago. The ×Cystocarpium hybridization event, then, united lineages that have a mean estimate of nearly 116 million years of independent evolution between them, the widest such natural hybridization event yet documented with modern dating methods.

Chapters 1 and 2 have been published in peer-reviewed journals, in both cases with equally-contributing coauthors. For Chapter 1 Anders Larsson performed much the analyses and a good proportion of the labwork, while Li-Yaung Kuo supplied essential then-unpublished data (matK sequences), did nearly all of the matK sequencing, and provided phylogenetically critical Asian taxa (most notably, the Rhachidosorus species). For Chapter 2, Michael Sundue did the majority of the morphological research, including constructing the final key, and writing the character synopses for each family. For both chapters I was responsible for study design and manuscript writing, and all aspects of project management, among other contributions.
1. Overcoming deep roots, fast rates, and short internodes to resolve the ancient rapid radiation of eupolypod II ferns


1.1 Introduction

A classic problem in phylogenetics is the reconstruction of “ancient rapid radiations,” broadly defined as evolutionary histories where long branches are intercalated among a series of short backbone internodes (see Fig. 1; Jian et al., 2008; Whitfield & Lockhart, 2007). Accurately resolving such topologies is a well-documented challenge for phylogenetic inference (Anderson & Swofford, 2004; Gaut & Lewis, 1995; Huelsenbeck, 1995; Jackman et al., 1999; Wang et al., 2009), and is also of considerable practical importance—this ancient rapid radiation model is a prominent feature of many phylogenetic problems (Whitfield & Lockhart, 2007). Furthermore, the ancient rapid radiation pattern rarely exists unaccompanied; rather, it tends to coincide with other well-recognized analytical challenges. First, the phylogenetic root is often long with respect to ingroup branches (Fig. 1; Bergsten, 2005; Schuettpelez & Hoot, 2006). Because signal deteriorates along phylogenetic branches (in a likelihood framework), long branches are less likely than short ones to strongly affix to any single point in the topology (Huelsenbeck et al., 2002; Swofford et al., 1996; Wheeler, 1990). Furthermore, while the monophyly of the ingroup and of all ingroup relationships may be fully
supported, uncertainty in the placement of the root may nonetheless reduce apparent support for relationships among ingroup clades when one uses consensus-based measures to assess support (Roberts et al., 2009; Wilkinson, 1996). Second, lineage-specific heterogeneity in rates of substitution is common, making “fast” taxa particularly difficult to place (Fig 1.; Drummond et al., 2006; Felsenstein, 1978; Hillis & Bull, 1993; but see Ho & Jermiin, 2004; Nickrent et al., 2004; Soltis et al., 1999; Takezaki & Gojobori, 1999). Finally, the presence of both very short and very long branches—regardless of their topological arrangement—poses additional challenges. While long-branch attraction has been well characterized (Anderson & Swofford, 2004; Bergsten, 2005; Felsenstein, 1978), other branch-length related inconsistencies are just beginning to attract attention (Brown et al., 2010; Lewis et al., 2005; Marshall, 2009; Roberts et al., 2009; Yang, 2008; Yang & Rannala, 2005).

One option for tackling problems associated with reconstructing ancient rapid radiations is to amass character-rich (often genome-scale) datasets (e.g., Hallstrom et al., 2007; Jian et al., 2008; Pereira & Baker, 2006; Regier et al., 2010; Wang et al., 2009). However, the specific challenges inherent to this sort of phylogenetic problem are not necessarily amenable to resolution by greatly expanded character data (Philippe et al., 2011). Rather, increasing character data can yield increasingly strong support for erroneous relationships, especially in cases of branch-length variation such as is inherent in the long-root, short internode, and rate heterogeneity features common under the ancient rapid radiation model (Bergsten, 2005; Gaut & Lewis, 1995; Philippe et al., 2005; Rannala & Yang, 2008; Soltis et al., 2004; Steel & Matsen, 2007; Susko, 2008; Whitfield & Lockhart, 2007; Yang, 2008). Here, we focus on resolving an ancient rapid radiation, that of the eupolypods II clade, using moderate amounts of character data but a strongly expanded taxon sample (for a recent application of this “moderate data” approach, see
This fern clade has resisted elucidation by both morphological and molecular data (Ching, 1964a; Kuo et al., 2011; Sano et al., 2000a; Schuettpelz & Pryer, 2007; Sledge, 1973; Smith, 1995; Smith et al., 2006), and previous molecular studies indicate that it exhibits all of the analytical challenges outlined above (see Fig. 1).

![Figure 1: Challenges inherent in resolving the eupolypod II phylogeny.](image)

**Figure 1: Challenges inherent in resolving the eupolypod II phylogeny.** Eupolypods II phylogram modified from Schuettpelz & Pryer (2007), in a) unrooted and b) rooted form. i) Outgroup taxa are on long branches. ii) Backbone internodes are very short, suggesting an “ancient rapid radiation.” iii) The ingroup is marked by significant heterogeneity in rates of evolution, with the members of Aspleniaceae on much longer branches than other eupolypod II taxa.

The Eupolypods II, together with its sister group, Eupolypods I, constitute the large eupolypod clade, which encompasses two-thirds of living fern species (Fig. 2; Pryer et al., 2004; Schneider et al., 2004b; Smith et al., 2006). The ancestors of Eupolypods I and II diverged from each other in the Early Cretaceous (Pryer et al., 2004; Schneider et al., 2004b; Schuettpelz & Pryer, 2009). The eupolypod II clade started to diversify shortly thereafter (its crown-group is approximately 100 million years old; Schuettpelz & Pryer, 2009) and has subsequently grown into a lineage-rich clade comprising nearly 30% of extant fern diversity. Eupolypods II includes some of the most familiar groups of ferns...
(the ladyferns, ostrich fern, sensitive fern, marshferns, and spleenworts), as well as some of the most species-rich genera: *Thelypteris* s.l. (~950 species); *Asplenium* (~700 species); *Diplazium* (~350 species); *Athyrium* (~220 species); and *Blechnum* s.l. (~150 species).

![Figure 2: Broad phylogeny of ferns. Approximate number of species per clade is given in parentheses. Modified from Smith et al. (2006).](image)

The eupolypod II clade is cosmopolitan in distribution, with the subgroups primarily temperate to tropical, and the larger subclades each well represented in both areas. However, many of the phylogenetically enigmatic taxa in this clade are limited to the Himalayas or Southeast Asia, and critical members of several genera are rare and/or infrequently collected. This pattern of rarity and endemism, in conjunction with the richness and geographical breadth of the clade as a whole, is undoubtedly a contributing factor to the incomplete sampling of these ferns in previous phylogenetic studies.

Not surprisingly, given the clade’s size and age, eupolypod II taxa are morphologically disparate and seemingly incohesive. However, early workers did tend to recognize the close affinities among many of the taxa in this clade, although
frequently with members of Eupolypods I interdigitated among them (Holttum, 1947; Mickel, 1974; Sledge, 1973; Tryon & Tryon, 1982). The cohesiveness of the Eupolypods II started to become apparent with the earliest applications of molecular phylogenetic techniques to ferns (Hasebe et al., 1995; Sano et al., 2000a; Wolf et al., 1994), and has been strongly supported in recent broad studies (Kuo et al., 2011; Schneider et al., 2004b; Schuettpelz & Pryer, 2007). None of these studies, however, found support for the backbone relationships within Eupolypods II, and only Kuo et al. (2011) attempted to sample its major lineages. It remains one of the few areas of the fern tree-of-life where the backbone relationships remain elusive (Schuettpelz & Pryer, 2007; Smith et al., 2006).

Our approach to resolving the eupolypod II phylogeny couples a considerably expanded taxon sample with moderate character sampling. Our objectives include identifying well-supported major (approximately “family-level”) clades and determining the backbone relationships among these clades. Given the anticipated phylogenetic challenges and potential for artifacts in our data, we explicitly evaluate our phylogenetic hypothesis against these analytical pitfalls, placing strong emphasis on the use of the reduced consensus technique (Wilkinson, 1996) to isolate the effects of signal weakness from those of signal conflict (e.g., see: Cobbett et al., 2007; Wiens, 2003). Our study aims for a comprehensive and well-supported phylogeny of this important group of ferns, and for novel inferences about the behavior of our choice of methods, gleaned from their performance on this dataset.

1.2 Materials and Methods

1.2.1 Taxon Sampling

We selected an ingroup of 67 species, intended to maximize our capture of the deep divergences (Zwickl & Hillis, 2002) within Eupolypods II. Decisions for inclusion were based on data from previous molecular (Cranfill & Kato, 2003; Gastony & Ungerer,
1997; Kuo et al., 2011; Murakami et al., 1999; Sano et al., 2000a; Sano et al., 2000b; Schneider et al., 2004a; Schuettpelz & Pryer, 2007; Smith & Cranfill, 2002; Tzeng, 2002; Wang et al., 2003) and morphological studies (Brown, 1964; Kato, 1975a, 1975b, 1977, 1979, 1984; Kato & Darnaedi, 1988; Sano et al., 2000b; Wang et al., 2004; Wang, 2008).

While 67 species is sparse coverage of the approximately 2600 species in the clade, our utilization of past results (both molecular and morphological/taxonomic) in selecting our taxon sample allows us a high degree of confidence that we have captured a great majority of the deepest branches, if not all of them. Most unsampled taxa are known to be deeply nested in crown clades, especially in the large genera Asplenium, Athyrium, Blechnum, Diplazium, and Thelypteris (s.l.).

Wherever possible, we included generic and familial types, to facilitate future taxonomic revisions. Based on data from Schuettpelez & Pryer (2007) and Liu et al. (2007), our broad outgroup sample included 10 representatives from the sister group to the Eupolypods II (Eupolypods I, see Fig. 2). To better evaluate the effect of uncertainty in outgroup placement on the ingroup topology, and to better understand the divergence between Eupolypods I and II, we also included two representatives from each of the two potentially successive sister groups to the Eupolypods (Notholaena and Cryptogramma from Pteridaceae; Dennstaedtia and Pteridium from Dennstaedtiaceae; see Fig. 2 and Schuettpelez & Pryer 2007). Our total sample has 81 terminal taxa (Appendix A).

### 1.2.2 Amplification and Sequencing

DNA was extracted from silica-dried or herbarium material, using either 1) a modified Carlson-Yoon protocol (<0.01g dried plant material, silica beads, 750 ul Carlson buffer, and 20 ul mercaptoethanol added to a 2 ml tube and ground for 45 s using a Mini-Beadbeater (BioSpec Products), followed by incubation at 65 °C for 45 min; Yoon et al., 1991), or 2) the protocol of Pryer et al. (2004), or 3) the protocol of Kuo et al.
For material extracted under the Carlson-Yoon protocol, the extracted DNA was purified by Illustra GFX PCR DNA and Gel Band Purification Kit (GE Healthcare).

Five plastid loci were selected for analysis: \textit{atpA}, \textit{atpB}, \textit{matK}, \textit{rbcL}, and the \textit{trnG-trnR} intergenic spacer (henceforth “\textit{trnG-R}”). All loci, except for \textit{matK}, were amplified according to either the “standard” or “difficult” reaction protocols (below) depending on the source of the material (standard for most extractions; difficult for those from herbarium specimens greater than 10 years old), using the primers listed in Table 1. The “standard” amplification reaction used standard taq polymerase with the following cycle: a 3 min initial denaturation at 95 °C, followed by 35 cycles of 30 s denaturation at 95 °C, 1 min annealing at 54 °C, and 2 min elongation at 72 °C, followed by a final elongation of 10 min at 72 °C. The “difficult” amplification reaction, using Phusion High Fidelity DNA Polymerase (Finnzymes), was: 1 min initial denaturation at 98 °C, followed by 35 cycles of 10 s denaturation at 98 °C, 30 s annealing at 58 °C, and 1 min elongation at 72 °C, followed by a final elongation of 8 min at 72 °C. Amplification of all \textit{matK} sequences followed the protocol of Kuo et al. (2011).
<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer</th>
<th>Usag</th>
<th>Sequence (5’– 3’)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>atpA</td>
<td>ESATP553F</td>
<td>F, S</td>
<td>ACAGCAGTAGCTACAGATAC</td>
<td>Schuettpelz et al. (2006)</td>
</tr>
<tr>
<td>atpA</td>
<td>ESATP557R</td>
<td>R, S</td>
<td>ATTTATGTTAGCTACTGC</td>
<td>Schuettpelz et al. (2006)</td>
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<tr>
<td>atpA</td>
<td>ESATP856F</td>
<td>R, S</td>
<td>CGAGAAGCATATCCCAGATG</td>
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<td>atpA</td>
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<td>R, S</td>
<td>CATCCTCCGGATGCTTCTCG</td>
<td>Schuettpelz et al. (2006)</td>
</tr>
<tr>
<td>atpA</td>
<td>ESATPF412F</td>
<td>F, A, S</td>
<td>GARCARGTTGAGCAAGATG</td>
<td>Schuettpelz et al. (2006)</td>
</tr>
<tr>
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<td>ESTRN46F</td>
<td>R, A, S</td>
<td>GATATCTCATTAGGCTTGGAG</td>
<td>Schuettpelz et al. (2006)</td>
</tr>
<tr>
<td>matK</td>
<td>ASPmatKrKVh</td>
<td>R, A, S</td>
<td>CTACATTGCACTATTGCAAAACAA</td>
<td>Rothfels et al. (2012a)</td>
</tr>
<tr>
<td>matK</td>
<td>AthmatKrHTY</td>
<td>A, S</td>
<td>CACATGTTAGCTACAGCATG</td>
<td>Rothfels et al. (2012a)</td>
</tr>
<tr>
<td>matK</td>
<td>BLEmatKrDCF</td>
<td>C, R, A, S</td>
<td>AATGATGRTTACTATGCAGTGAAC</td>
<td>Rothfels et al. (2012a)</td>
</tr>
<tr>
<td>matK</td>
<td>CYSmatKrCG</td>
<td>R, A, S</td>
<td>AACATGAGTRTACTATGCAGTGAAC</td>
<td>Rothfels et al. (2012a)</td>
</tr>
<tr>
<td>matK</td>
<td>DematKrHTY</td>
<td>A, S</td>
<td>ACAGAAGTTTGTACGTGGAA</td>
<td>Rothfels et al. (2012a)</td>
</tr>
<tr>
<td>matK</td>
<td>DImatKrTY</td>
<td>A, S</td>
<td>CCACACRAGTTTTTGTATGG</td>
<td>Rothfels et al. (2012a)</td>
</tr>
<tr>
<td>matK</td>
<td>DIPZmatKrDS</td>
<td>R, A, S</td>
<td>GTCCATAAAACTACATATGGAAT</td>
<td>Rothfels et al. (2012a)</td>
</tr>
<tr>
<td>matK</td>
<td>EuUmatKrHLL</td>
<td>n, R, A, S</td>
<td>GTGARAAACATCTTTGATAGT</td>
<td>Kuo et al. (2011)</td>
</tr>
<tr>
<td>matK</td>
<td>EuUmatKSIH</td>
<td>A, F, S</td>
<td>TCRRAAAATBGCAGTCTACCTC</td>
<td>Rothfels et al. (2012a)</td>
</tr>
<tr>
<td>matK</td>
<td>FERMatKrEDR</td>
<td>F, A, S</td>
<td>ATTCTCRATRTTTTTATHTGGA</td>
<td>Kuo et al. (2011)</td>
</tr>
<tr>
<td>matK</td>
<td>FERMatKrAGK</td>
<td>R, A, S</td>
<td>CGTRTTGTACTYYRTTGTTRCAGC</td>
<td>Kuo et al. (2011)</td>
</tr>
<tr>
<td>matK</td>
<td>FERNchiByFAA</td>
<td>F, A, S</td>
<td>GATGTRAYGTATGRCYCAAGA</td>
<td>Kuo et al. (2011)</td>
</tr>
<tr>
<td>matK</td>
<td>FERNJps16fQCGR</td>
<td>F, A</td>
<td>CRMTGTGGAGAACAAGC</td>
<td>Kuo et al. (2011)</td>
</tr>
<tr>
<td>matK</td>
<td>FERNJps16fSRQF</td>
<td>F, A</td>
<td>CCCGRMRAGAGGGGAR</td>
<td>Kuo et al. (2011)</td>
</tr>
<tr>
<td>matK</td>
<td>ONOmatKrIRD</td>
<td>I, R, A, S</td>
<td>GTRGAAATGCGCACAATTCCTAAT</td>
<td>Rothfels et al. (2012a)</td>
</tr>
<tr>
<td>matK</td>
<td>PtmatKrHy</td>
<td>I, R, A, S</td>
<td>TTTCTMYATCTTSCRTARTGAAT</td>
<td>Kuo et al. (2011)</td>
</tr>
<tr>
<td>matK</td>
<td>THEmatKrVRL</td>
<td>L, R, A, S</td>
<td>TCGACCAGAACAGGCAAC</td>
<td>Rothfels et al. (2012a)</td>
</tr>
<tr>
<td>matK</td>
<td>WOmatKrVRL</td>
<td>I, R, A, S</td>
<td>TCKACGAAACAGGGGCAC</td>
<td>Rothfels et al. (2012a)</td>
</tr>
<tr>
<td>rbcL</td>
<td>PKRBCl556F</td>
<td>F, S</td>
<td>GGTAGRGGCGTCTGAYGATAGC</td>
<td>Rothfels et al. (2012a)</td>
</tr>
</tbody>
</table>

Notes: F = forward; R = reverse; A = used in amplifications; S = used in sequencing reactions.

While most primers were applied across the phylogeny, superscripts following primer names indicate lineage-specificity: *Aspleniacae; *athyriids; *Blechnaceae; *Cystopteris/Gymnocarpium; *depariiids; *diplaziids; *Diplaziopsis/Homalosorus; *Rhachidosorus; Onocleaceae; *Pteridaceae; *Thelypteridaceae; Woodsia & allies.
PCR products from Carlson-Yoon extractions were purified using MultiScreen Plates in a vacuum manifold (Millipore) and sequenced by Macrogen Inc. (South Korea). For material extracted under the protocol of Pryer et al. (2004), each PCR product was cleaned using 0.5 μl of exonuclease I and 1 μl of Shrimp Alkaline Phosphatase (USB, Cleveland, Ohio); reaction tubes were incubated at 37 °C for 15 min and then heated to 80 °C for 15 min to inactivate the enzymes, prior to sequencing on a Applied Biosystems 3730 xl at the Duke IGSP Sequencing Facility (Duke University, USA). Material extracted under the protocol of Kuo et al. (2011) was sequenced at Genomics (Taipei, Taiwan). We completed our sampling with an additional 100 previously published sequences from GenBank (Appendix A).

1.2.3 Alignment and Tree Search

Sequences were manually aligned in Mesquite 2.72 (Maddison & Maddison, 2009). Indels (limited to matK, trnG-R, and the ends of the atpA alignment) were assessed by eye, and ambiguously aligned areas were excluded prior to phylogenetic analysis. Any gaps associated with unambiguous indel regions were treated as missing data. In one rapidly evolving region of the trnG-R alignment we were unable to confidently align the Pteridaceae sequences to those of the other taxa. In order to retain this otherwise unambiguous region, we excised those portions of the Pteridaceae sequences, replacing them with question marks.

To evaluate congruence among our loci, we performed maximum likelihood (ML) tree searches on 1000 bootstrap datasets for each locus individually, under a GTR+I+G model using the default settings in Garli v1.0.695 (Zwickl, 2006). The majority rule bootstrap consensus trees from each locus were manually compared and examined for strongly supported conflicts (Mason-Gamer & Kellogg, 1996), after which we concatenated the full data with abioscript (Larsson, 2010), producing a single, annotated,
five-locus dataset, with excluded regions removed. This alignment is largely complete (361 of the possible 405 sequences are present, for an average of 4.5 loci per terminal taxon), and contains 13.3% missing data and 6595 characters, of which 3641 are variable (Table 2). Our alignment is available on TreeBase (accession number S11464); the full length of all newly generated sequences (including any portions excluded prior to analysis) are deposited in GenBank (see Appendix A).

Table 2: Dataset statistics for Chapter One

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Taxa</th>
<th>Sites</th>
<th>Variable sites</th>
<th>Missing data (%)</th>
<th>Mean MLBS</th>
<th>Bipartitions with:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>≥70% MLBS</td>
</tr>
<tr>
<td><em>atpA</em></td>
<td>74</td>
<td>1706</td>
<td>809</td>
<td>3.1</td>
<td>79%</td>
<td>69%</td>
</tr>
<tr>
<td><em>atpB</em></td>
<td>69</td>
<td>1278</td>
<td>507</td>
<td>0.8</td>
<td>74%</td>
<td>58%</td>
</tr>
<tr>
<td><em>matK</em></td>
<td>75</td>
<td>1377</td>
<td>1142</td>
<td>9.0</td>
<td>84%</td>
<td>75%</td>
</tr>
<tr>
<td><em>rbcL</em></td>
<td>78</td>
<td>1308</td>
<td>417</td>
<td>1.0</td>
<td>76%</td>
<td>68%</td>
</tr>
<tr>
<td><em>trnG-R</em></td>
<td>65</td>
<td>926</td>
<td>706</td>
<td>3.4</td>
<td>81%</td>
<td>71%</td>
</tr>
<tr>
<td>combined</td>
<td>81</td>
<td>6595</td>
<td>3641</td>
<td>13.3</td>
<td>92%</td>
<td>91%</td>
</tr>
</tbody>
</table>

Notes: Missing data include both uncertain bases (e.g., ?, R, Y) and gaps (–). Support values are listed as maximum likelihood bootstrap support (MLBS) or Bayesian posterior probabilities (PP).

To obtain a point estimate of the phylogeny, we performed 10 tree bisection-and-reconnection heuristic searches of the concatenated (unpartitioned) data, each from a different random-addition-sequence starting tree, under maximum likelihood using a GTR+I+G model as implemented in PAUP* 4.0b10 for Unix (Swofford, 2002). The values for the exchangeability parameters, base frequencies, gamma shape parameter, and proportion of invariant sites were fixed at their maximum likelihood values as optimized under a Garli 0.951 (Zwickl, 2006) tree search, using default genetic algorithm and termination settings.

We assessed support using ML bootstrapping and Bayesian inference. For the ML bootstrapping, we performed 5000 replicates in each of PAUP* 4.0b10 for Unix.
(Swofford, 2002), Garli 1.0.695 (MPI parallel version; Zwickl, 2006), and RAxML v7.2.6 (Stamatakis, 2006). The PAUP* runs were performed with the parameters optimized as above, reconnection limit set to eight (“reconlim=8”), and with only a single random-addition-sequence per bootstrap replicate. In Garli we ran 5000 bootstrap replicates on the concatenated data, under a GTR+I+G model using the default genetic algorithm and termination settings. In RAxML we ran 5000 bootstrap replicates on the data partitioned by locus, with each locus assigned a GTR+G model. For Bayesian inference, we ran four runs of four chains each (one cold; three heated), for 15 million generations, under a partitioned GTR+I+G model in the parallel version of MrBayes v3.1.1 (Altekar et al., 2004; Ronquist & Huelsenbeck, 2003). Each of the five loci was assigned its own partition, with substitution parameters unlinked among partitions, and branch-lengths linked (with a proportionality parameter to account for rate heterogeneity among partitions); the posterior was sampled every 1000 generations. Visual inspection in AWTY (Nylander et al., 2008; Wilgenbusch et al., 2004) revealed that the runs converged within the first 500,000 generations. To be conservative, we excluded the first 2 million generations of each run as burnin prior to summarizing the posterior. The posterior thus comprised 52,000 samples (13,000 post-burnin samples from each of the four runs).

1.2.4 Phylogeny Evaluation

As stated above, earlier studies (e.g., Schuettpelz & Pryer, 2007, see Fig. 1), indicate that the eupolypod II phylogeny is likely to include several key challenges for phylogenetic inference, specifically a series of long branches among very short backbone internodes (an “ancient rapid radiation”), lineage-specific rate heterogeneity, and a distantly related outgroup. Given these concerns, we sought to explicitly evaluate our topology and support values against these potential artifacts, with particular emphasis on the support values along the backbone of the tree.
Our approach to phylogeny evaluation involved permutations of both the models and the implementation of those models (i.e., programs). The models were deliberately selected according to their varying degrees of susceptibility for each of the risk factors in question, in an attempt to isolate potential model-based biases. The choice to additionally vary the programs used was in part due to constraints of implementation—no single program offered all the models we wished to compare. This approach has the added benefit of demonstrating a further level of robustness: if our phylogenetic results are insensitive to both the differing models and to the myriad of incompletely quantified differences among programs, we can be all the more confident in our conclusions. Additionally, varying both the models and their implementation more closely matches the options available to empirical phylogeneticists seeking to resolve ancient rapid radiations. This approach suffers a clear liability, however, in that the effects of model differences and implementation differences are conflated. In the event of differing results, we may not be able to isolate the effects of one from the other; therefore, the added value to empirical phylogeneticists comes at the cost of reduced utility of our results to program developers and theorists.

The specific evaluations performed are described more thoroughly in the Results. Computation-intensive analyses were run either on the Duke Shared Cluster Resource (https://wiki.duke.edu/display/SCSC/DSCR) or the Uppsala Multidisciplinary Center for Advanced Computational Science (UPPMAX). When appropriate, multiple tree files were summarized onto a target phylogram with SumTrees 2.0.2 (Sukumaran & Holder, 2010) for subsequent inspection or manipulation in FigTree 1.3.1 (Rambaut, 2006).
1.3 Results

1.3.1 Data and Topology Point Estimate

Tree-wide mean maximum likelihood bootstrap support (MLBS) values (summed bootstrap values on the maximum likelihood tree divided by the number of internodes in that tree) for the individual loci ranged from 74% \((\text{atpB})\) to 84% \((\text{matK})\). The concatenated data have a mean MLBS of 92% and strongly support (i.e., have \(\geq 70\%\) MLBS and \(\geq 0.95\) posterior probability [PP]) 90% of the partitions (Table 2). Across datasets, ML and Bayesian inference consistently inferred strong support for a comparable number of bipartitions (Table 2), a result that offers further empirical corroboration for the approximate equivalence of 70% MLBS and 0.95 PP (Alfaro et al., 2003; Hillis & Bull, 1993).

There are two well-supported conflicts among the individual-locus ML trees. The first involves a tip relationship \((\text{matK} \text{ unites Deparia pterorachis with } \text{D. unifurcata, with } 72\% \text{ MLBS, whereas } \text{rbcL places } \text{D. pterorachis as sister to the rest of the genus, with } 75\% \text{ support})\) that is peripheral to the focus of this study. The second is deeper in the tree: \(\text{matK} \text{ has } 80\% \text{ MLBS for a clade uniting Thelypteridaceae with the athyrioids, Blechnaceae, and Onocleaceae, to the exclusion of Woodsia and allies, whereas both } \text{atpA} \text{ and } \text{atpB place Woodsia and its allies within that clade (92\% in } \text{atpA}; 71\% \text{ in } \text{atpB}).\) Given that we confirmed this conflict to not be attributable to laboratory or identification errors, and because the loci involved are linked and the taxa are long-diverged, we do not ascribe this conflict to differences in evolutionary history, and proceeded to concatenate all the data for subsequent analyses.

Each of our ten ML best-tree searches of the concatenated data in \(\text{PAUP}^*\) (from different random-addition-sequence starting trees) inferred the same tree (Fig. 3a), suggesting that tree space is unimodal for our dataset. Most partitions in this topology
point estimate were strongly supported by both maximum likelihood bootstrapping and Bayesian posterior probabilities (Table 2); the different ML programs (*PAUP*, *Garli*, *RAxML*) inferred very similar MLBS levels (data not shown).

**Figure 3: Phylogeny evaluation: Rate heterogeneity, and the Bayesian star-tree paradox artifact.**

(a) Unrooted maximum likelihood (ML) phylogram of the concatenated data, with the backbone internodes highlighted and labeled. (b) Accounting for the impact of rate heterogeneity on backbone support values. The four values listed for each backbone internode are: ML bootstrap support (MLBS) on full data; MLBS with Aspleniaceae (*Asplenium* and *Hymenasplenium*) pruned from trees; MLBS with Aspleniaceae removed from analysis; and posterior support from *BEAST*. (c) Controlling for the Bayesian star-tree paradox artifact using the polytomy prior in *Phycas*. The four posterior probabilities listed for each internode are from: MrBayes 3.1.1 (susceptible to the star-tree artifact); *Phycas* with C=1; *Phycas* with C=e; and *Phycas* with C=10. (d) Controlling for the Bayesian star-tree paradox artifact using the Yang branch-length prior. The four posterior probabilities listed for each internode are from: MrBayes 3.1.2 with branch-length prior mu1/mu0=0.01; MrBayes 3.1.2 with branch-length prior mu1/mu0=0.001; and MrBayes 3.1.2 with branch-length prior mu1/mu0=0.0001.
1.3.2 Bayesian Star-tree Paradox Artifact

For certain branches, we observed very high Bayesian posterior probabilities from the MrBayes analysis, but much lower levels of support from the ML bootstrapping (Fig. 4a); these support discrepancies are disproportionately represented among short branches (Fig. 4b). This pattern is consistent with artificially high Bayesian support due to the star-tree paradox artifact—most implementations of Bayesian phylogenetic inference do not consider polytomies among the option set for the MCMC sampler, and thus can return high posterior probabilities for branches that are unsupported by the data (Lewis et al., 2005; Yang, 2008; Yang & Rannala, 2005; see early hints in Cummings et al., 2003).

Figure 4: Discrepancies between maximum likelihood and Bayesian support values. a) Maximum likelihood phylogram of the concatenated data, internal branches only (all tip branches have been deleted). Branches are colored according to the magnitude of the difference between their posterior probability (PP; from MrBayes 3.1.1) and their percent maximum likelihood bootstrap support (MLBS; from 5000 pseudoreplicates in PAUP*). b) Internal branches rotated to be vertical and sorted by length. Colors follow Figure 4a.

To ensure that this “star-tree paradox” artifact was not influencing our assessment of support, we compared the results from our original MrBayes 3.1.1 analysis (Ronquist & Huelsenbeck, 2003; MrBayes 3.1.1 is potentially vulnerable to the star-tree paradox artifact) with those of a non-Bayesian analysis (ML bootstrapping in PAUP*...
from our initial assessment of support), as well as with two implementations of Bayesian inference that use different approaches to reduce their vulnerability to the star-tree paradox artifact.

First, we analyzed our data with Phycas 1.1.2-r (Lewis et al., 2010). Phycas uses reversible-jump MCMC to allow the sampling of incompletely resolved topologies, controlled via the incorporation of a polytomy prior, “C.” A value of C=1 means that unresolved and fully resolved topologies are weighted equally; under a value of C=10 a trichotomy is 10 times more likely, a priori, than either of its fully resolved resolutions (Lewis et al., 2005). We performed three runs of our full concatenated data, under a GTR+I+G model, using a branch-length hyperprior (default values), for 200,000 cycles (sampling from the posterior every 10 cycles; note: Phycas makes proposals to each free parameter in each cycle, and thus Phycas cycles are not comparable to MrBayes generations, which include a proposal to only a single parameter), with C=1, C=e (2.718), and C=10, respectively (Appendix B). Inspection of the AWTY-type plots (see Nylander et al., 2008) and sojourn plots (see Lewis & Lewis, 2005) revealed that the runs converged before 40,000 cycles; to be conservative, we excluded the first 50,000 cycles (5000 samples) as burnin.

We then reanalyzed our data with a modified version of MrBayes 3.1.2 that incorporates exponential priors on internal and external branch lengths (Yang, 2007; Yang, 2008). These “Yang branch-length priors” allow the concentration of the prior mass on topologies with very short internal branches, for an intended effect similar to that of the polytomy prior (above); only if the data strongly support a branch can the short internal branch prior be overcome. We performed three runs of four chains each under the settings used in the initial MrBayes analysis, but with the addition of the branch-length priors. The mean of the external branch-length prior (mu1) was set to 0.1,
and the mean of the internal branch-length prior (mu0) to 0.00001, 0.0001, and 0.001, successively (Appendix B). As in the initial analyses, 2 million generations from each run were discarded as burnin.

The results of these analyses show that four of the backbone internodes (A, B, C, D; Fig. 3a) were largely insensitive to either the polytomy prior (Fig. 3b) or the Yang branch-length prior (Fig. 3c); their PPs stayed at or within three percentage points of 1.0 for all seven analyses (the original MrBayes 3.1.1 analysis, three Phycas runs with increasingly strong polytomy prior values, and three MrBayes 3.1.2 runs with increasingly strong Yang branch-length prior values). Interestingly, the two approaches (polytomy prior vs. branch-length prior) had very different effects on the other backbone internodes, despite the approaches being designed to overcome the same shortcoming in Bayesian phylogenetic inference. For example, the only backbone internode that was not well supported by the original ML and Bayesian analyses (internode F; Fig. 3a) exhibited increased support under weak versions of either the polytomy prior or the branch-length prior. However, the norms of reaction for the two priors were opposed: as the polytomy prior increased in strength (C=1, e, and 10), the posterior support for internode F decreased (PP=.93, .89, .83; Fig. 3b), whereas as the branch-length prior strength increased (mu0/mu1=0.01, 0.001, 0.0001), the PP on internode F also increased (PP=.97, 1.0, 1.0; Fig. 3c).

The remaining three internodes (E, G, H; Fig. 3a) were well supported by the original ML and Bayesian analyses, but showed some sensitivity to either the polytomy or branch-length prior, again in opposing ways. Internode E was largely insensitive to the branch-length prior (Fig. 3c), but was strongly weakened by the polytomy prior (Fig. 3b), whereas internodes G and H were largely unaffected by the polytomy prior but were unsupported under strong values of the branch-length prior (Fig. 3b, c).
1.3.3 Lineage-specific Rate Heterogeneity

To investigate whether the rapid rate of evolution for the Aspleniaceae (Figs. 1 and 3) was biasing tree reconstruction, we attempted to isolate the effects of this rate heterogeneity in three ways. First, we pruned the Aspleniaceae from 1000 full-data Garli ML bootstrap trees prior to building the consensus tree and evaluating support. This “reduced consensus” approach (Burleigh et al., 2009; Wilkinson, 1996) removes any effects due solely to uncertainty in the placement of these long-branch taxa. If the remaining relationships are well supported, then overall support values will appear low in the standard consensus, but will be restored under the reduced consensus. Second, we reran the Garli ML bootstrap analysis on a dataset where the Aspleniaceae had been removed prior to analysis, to eliminate any effect that these taxa might have on the optimization of model parameters, and to allow the model to better fit the remaining data (the “reduced data” approach). Third, we ran the full dataset in BEAST 1.5.4 (Drummond & Rambaut, 2007), incorporating a relaxed-clock model that explicitly models lineage-specific rate variation (Drummond et al., 2006) and thus should be less sensitive to any artifacts induced by the strongly heterogeneous rates in our data. We ran three independent runs on the full, concatenated dataset, each for 20 million generations (sampling the posterior every 1000 generations), with the following settings: birth-death tree prior; lognormal uncorrelated relaxed clock; and GTR+I+G substitution model. Priors were left at their default values, with the exception of those for six time-to-most-recent-common-ancestor (TMRCA) age parameters, which were each given normal distributions with a mean equal to the inferred age estimated for that clade by Schuettpelz & Pryer (2009) and a standard deviation equal to 10 percent of that mean. The relevant taxon sets, and their TMRCA prior means, are: tree root (165.6 MA); Dennstaedtiaceae (119.3 MA); Eupolypods (116.7 MA); Pteridaceae (110.8 MA);
Eupolypods II (103.1 MA); and Eupolypods I (98.9 MA). None of the taxon sets was constrained to be monophyletic. The use of secondary constraints such as these is clearly inferior to the use of fossil data for divergence time dating (Magallón, 2004), but as no such data are available, and our interest is more in the relative than absolute divergence times, this approach seemed best. Visual inspection in Tracer (Rambaut & Drummond, 2007) demonstrated that the runs converged before 1 million generations; to be conservative, we excluded the first 3 million generations of each run prior to analyzing the pooled posterior of 51,000 samples (17,000 from each run; Appendix B). For this sample, the effective sample size for each parameter was above 300.

None of these attempts to mitigate potential effects of the increased rates of molecular evolution associated with Aspleniaceae strongly affected support values along the backbone. Support values from the full taxon-sample consensus data (Fig. 3d, first values) differed from those from the reduced consensus (Fig. 3d, second values) by at most one percentage point. Removing Aspleniaceae from the dataset prior to the bootstrap tree searches had a larger effect (up to a five percentage point change in support; Fig. 3d, third values), but in no case resulted in an internode moving from well supported (≥70% MLBS) to poorly supported, or vice-versa. The support values from the BEAST analysis (Fig. 3d, fourth values) were concordant with those of the maximum likelihood runs, especially in that the internode uniting Rhachidosorus with the diplazioids, Hemidictyum, and Aspleniaceae (internode F; Fig. 3d) was the only one without strong support (it had a PP of .90).

1.3.4 Rooting Uncertainty

To evaluate any effects that uncertainty in root-branch placement might have on apparent levels of support within the ingroup, we compared ingroup backbone MLBS values from the analysis of our complete data (full outgroup) with those from each of six
different variations in outgroup composition: i) ingroup only; ii) ingroup + _Dryopteris_; iii) ingroup + _Dryopteris_ and _Didymochlaena_; iv) ingroup + _Dryopteris_ and _Notholaena_; v) ingroup + _Dryopteris_ and _Notholaena_ and _Pteridium_; and vi) ingroup + our full eupolypod I sample (Fig. 5).

Figure 5: Effects of outgroup composition on ingroup backbone support values. Values on each internode indicate the percentage point difference in maximum likelihood bootstrap support between the focal analysis and the analysis with the full taxon sample. The first values are those from the reduced consensus approach; second values are from the reduced data approach. NA indicates an internode not present with that outgroup sample. Excluded taxon branches are in grey; included outgroup branches are black. This figure shows only outgroup and ingroup backbone branches; most ingroup branches have been deleted (but were included in the analyses). Labeling of the backbone internodes follows Figure 3a.
This outgroup sampling regime was selected to successively bisect the longest outgroup branches, with a particular emphasis on breaking the proximate root branch (the branch connecting the ingroup to the first outgroup node).

We evaluated support for each of the six outgroup sampling regimes using both the reduced consensus approach (full data included in the analysis, but with the outgroup pruned down to the desired sample prior to forming the consensus tree; Burleigh et al., 2009; Wilkinson, 1996) and a reduced data approach (outgroup reduced to the desired sample prior to the analyses; Fig. 5, second values). The former approach controls for uncertainty in outgroup placement alone (i.e., it offers a metric of the signal strength), while the latter approach additionally accounts for model fit. All analyses were based on 1000 ML bootstrap replicates of the concatenated data in Garli 1.0.695 (MPI parallel version; Zwickl, 2006), under a GTR+I+G substitution model, using the default genetic algorithm and termination settings.

The results of these rooting comparisons demonstrate that our initial concerns—that the outgroup would wander and thus reduce support measures within the ingroup—were largely unfounded. The reduced consensus support values were minimally different from those with the full outgroup (Fig. 5, first values). When a “consensus interference” effect did appear (first values > 0 in Fig. 5b, c, f), it was correlated with the maximum root length rather than with proximate root length, i.e., it is the long Notholaena branch that wanders, rather than the outgroup as a whole.

In stark contrast, outgroup composition had a strong effect on backbone support if the outgroup was changed prior to the tree searching steps. When we reduced our outgroup sample and reran the ML bootstrapping (the reduced data approach), backbone internode support values changed from their full-outgroup values by up to 32 percentage points (Fig. 5, second values). The largest of these changes are reductions in
support for branches proximate to the root (internodes E, F, and G; Fig. 3a), and are due to uncertainty in the ancestral state of the smaller outgroup sample (as demonstrated by the reduced consensus values from each of the smaller datasets; data not shown).

1.3.5 Eupolypod II Phylogeny

Our results demonstrate that the vast majority of internodes in the ML tree are strongly supported by both ML bootstrapping and Bayesian posterior probabilities (Fig. 6; Table 2), and these support values proved robust to our phylogeny evaluations. In particular, the ML tree has 10 highly supported major (approximately “family unit”) ingroup clades: Cystopteris/Gymnocarpium; Rhachidosorus; Diplaziopsis/Homalosorus; Hemidictyum; Aspleniaceae; Thelypteridaceae; Woodsia and allies; Onocleaceae; Blechnaceae; and the athyrioids (Fig. 6b). Of these, Cystopteris/Gymnocarpium is sister to the remaining eupolypod II taxa, followed by the loosely supported assemblage of Rhachidosorus with Diplaziopsis/Homalosorus + Hemidictyum + Aspleniaceae. Blechnaceae is sister to Onocleaceae, and they together are successively sister, in a pectinate pattern, to the athyrioids, then to Woodsia and allies, and finally to the Thelypteridaceae (Fig. 6). This broad phylogeny is in general agreement with earlier molecular phylogenetic studies that included members of the Eupolypods II (Cranfill & Kato, 2003; Gastony & Ungerer, 1997; Kuo et al., 2011; Murakami et al., 1999; Sano et al., 2000a; Sano et al., 2000b; Schneider et al., 2004a; see particularly Schuettpelz & Pryer, 2007; Smith & Cranfill, 2002; Tzeng, 2002; Wang et al., 2003). However, the backbone of the phylogeny is strongly supported for the first time; the only backbone internode lacking such support is the one attaching the Rhachidosorus branch to the rest of the tree (Fig. 6, internode F). Additionally, we are finally able to confidently place the enigmatic genera Cheilanthopsis, Diplaziopsis, Homalosorus, Protowoodsia, and Woodsia.
Figure 6: Maximum likelihood (ML) phylogram of the concatenated data. Bayesian posterior probabilities follow ML bootstrap support values. Bold branches have ≥70% ML bootstrap support and ≥0.95 posterior probability. Support values of 100% or 1.0 are indicated with an asterisk (*).

a) Family designations of Smith et al. (2006), with the paraphyletic Woodsiaceae highlighted.

b) Major clade names used in this study: Cyst/Gymno = Cystopteris s.l. & Gymnocarpium; Rha = Rhachidosorus; Dipls = Diplazizyopsis & Homalosorus; H = Hemidictyum; Aspleniaceae; Thelypteridaceae; Woods & allies = Woodsia & allies; Onoclea = Onocleaceae; Blechnaceae; athyrioids. Backbone internodes labeled A through H following Figure 3a.
1.4 Discussion

1.4.1 Bayesian Star-tree Paradox Artifact

While one would anticipate that internodes across a topology would differ in their sensitivity to the star-tree paradox approaches (not all short branches are inferred equal), it is unclear what is driving the different responses in our data—neither the original Bayesian posteriors or the MLBS levels correlate with the behavior of a given internode under the additional priors (Fig. 3). This study is the first to examine the performance of these star-tree paradox methods on empirical data; their non-parallel effects were perhaps the most surprising result of this portion of the analyses. However, while they were developed for the same function, the methods differ strongly in their approach, and should not be expected to result in similar behavior. The branch-length prior, in effect, flattens the posterior for topologies. As the mu0/mu1 ratio decreases, the relative influence of any data supporting an internal branch is reduced, and the external branches come closer to being randomly arranged. However, each topology sampled from the posterior must still be fully resolved and thus any reduction of support for a particular topology must be accompanied by increased support for some other one. In this sense, the branch-length prior is less a measure of intrinsic support for a given internode than it is a measure of whether that node is better supported than all alternative resolutions. Under circumstances of low support for an entire set of relationships, the branch-length prior favors the best of a bad lot. The polytomy prior, on the other hand, allows the direct comparison between a given resolution and a polytomy. Strong values of the branch-length prior lead to many trees, each with a low posterior, whereas strong values of the polytomy prior lead to a star tree, with a high posterior.
In interpreting the performance of these two methods on our data, it is important to stress that we did not attempt to tightly isolate the effects of the Yang branch-length prior and those of the polytomy prior. Rather, each was bundled with other elements of its host program (MrBayes 3.1.2 and Phycas 1.1.2-r, respectively), which differ from each other in both their models, and their implementation of those models. In particular, important model differences include data partitioning in MrBayes (the Phycas runs were on unpartitioned data) and the incorporation of a branch-length hyperprior in Phycas (there is no such hyperprior in MrBayes); important implementation differences include the limitation of Phycas to Larget-Simon moves (Larget & Simon, 1999), whereas MrBayes utilizes a broader suite of topology proposals.

Regardless of the different performance of the two methods, the backbone support levels in our data were generally robust to the star-tree paradox artifact approaches (Fig. 3b, c), suggesting that the high Bayesian support values for these internodes are valid. Even under extreme values of the polytomy prior, for example (C=10, or trichotomies 10 times more likely, a priori, than their fully resolved alternatives), the posterior consensus tree still resolved each of the eight critical backbone internodes, and only one fell below 0.95 PP (internode E; Fig. 3b). The differences between Bayesian PP and MLBS values in our data (Fig. 4), therefore, reflect something other than the failure of the original Bayesian analyses to include polytomies in the option set; these differences may simply be due to Bayesian inference being more sensitive to small amounts of data than is bootstrapping, and thus more likely to support short internodes (Alfaro et al., 2003).

1.4.2 Lineage-specific Rate Heterogeneity

The absence of any effect of lineage-specific rate heterogeneity on our topology estimation or support levels is particularly interesting in light of recent questions
(Drummond et al., 2006) about the general applicability of the unrooted model (aka “no clock” model; Wertheim et al., 2010; Yang & Rannala, 2006) in phylogenetic inference. Given the dramatic lineage-specific rate heterogeneity that is present in our dataset, one might expect the unrooted and relaxed-clock models to fit very differently, and given that the fast lineages in our data are intercalated among short internodes, our topology would be expected to be sensitive to such model differences. However, no effects are seen; our data, at least, do not support concerns about the application of the unrooted model in phylogenetic inference, a result that provides empirical support to the simulation results of Wertheim et al. (2010).

1.4.3 Rooting Uncertainty

The effects of differing outgroup compositions on support levels for branches phylogenetically distant from the root were unexpected, and may reflect a combination of both stochastic variation in maximum likelihood bootstrapping and of factors of model optimization on the different datasets. Neither of these explanations is heartening. The latter—the “model mediated” effect—requires that changes in outgroup composition have strong and somewhat idiosyncratic effects on support levels on parts of the tree phylogenetically distant from the root itself (for a similar case, see Roberts et al., 2009). These effects are not due to the outgroup itself changing position (that possibility is eliminated by comparison with the reduced consensus values) and must instead be mediated through non-topological factors. The former explanation—stochastic variation in bootstrap support values—would suggest that 1000 pseudoreplicates are insufficient to get accurate support estimates for these data. Regardless of the precise mechanism by which the outgroup affects support values, these results emphasize the wisdom of including a broad outgroup sample, particularly
when the outgroup is distantly related to the taxa of interest (Graham & Iles, 2009; Swoford et al., 1996).

### 1.4.4 Eupolypod II Phylogeny: Major Clades

The affinities of *Cystopteris* s.l. (including *Acystopteris*, e.g., Blasdell, 1963) and *Gymnocarpium* have been the object of considerable taxonomic disagreement. Both genera, individually or in tandem, have been thought to be allied with the Dryopteridaceae (in Eupolypods I) or the Athyriaceae; in either position they were inevitably highlighted as being anomalous (see Sledge, 1973). Early molecular studies supported *Cystopteris* and *Gymnocarpium* as sister species and demonstrated their lack of close affinity to either Dryopteridaceae or *Athyrium*, but were unable to pinpoint their phylogenetic position (Hasebe et al., 1995; Wolf et al., 1994). Recent studies (Kuo et al., 2011; Schuettpelz & Pryer, 2007) were the first to support a sister group relationship between a *Cystopteris/Gymnocarpium* clade and the rest of Eupolypods II, a result corroborated and strengthened by our data (Fig. 6, internode E), the first to include multiple accessions of *Acystopteris* and *Cystopteris* s.s.

Historically, arguments about *Rhachidosorus* focused on its validity as a genus, distinct from either *Athyrium* or *Diplazium* (Ching, 1964a; Kato, 1975a). Early molecular phylogenies (Sano et al., 2000a; Tzeng, 2002; Wang et al., 2003) provided the first evidence that *Rhachidosorus* might not be closely related to either, a result further emphasized by the three-gene results of Kuo et al. (2011). In our study, the two included *Rhachidosorus* species form a tight clade phylogenetically distant from any other taxon; their closest relatives appear to be *Diplaziopsis, Homalosorus, Hemidictyum*, and Aspleniaceae. While our data do not strongly support a precise position for *Rhachidosorus* (Fig. 6, internode F), of note is the 100% MLBS and 1.0 PP for internode D (Fig. 6), which separates *Rhachidosorus* from the athyrioids. Thus, our data very strongly
reject a close relationship between *Rhachidosorus* and its presumed allies, the athyrioids, an unanticipated conclusion based on morphological data (Kato, 1975a). Indeed, our data suggest that the two groups last shared a common ancestor nearly 100 million years ago (Appendix B).

As with *Rhachidosorus*, *Homalosorus* and *Diplaziopsis* were long thought to be allied with the athyrioids, where they are typically treated as members of *Diplazium* (Ching, 1964b; Kato, 1975b, 1977; Kato & Darnaedi, 1988; Wang et al., 2004). The first indication that this placement might be inaccurate came from the study of Sano et al. (2000a), which strongly supported *Homalosorus* (a monotypic genus) as sister to their lone *Diplaziopsis* accession and placed the two genera distant from *Diplazium*, a result corroborated by Wei et al. (2010) and Kuo et al. (2011). Our study includes two *Diplaziopsis* species, which are strongly supported as sister to each other, and together are sister to *Homalosorus*. These two genera form a clade that is strongly supported, for the first time, as sister to *Hemidictyum* + Aspleniaceae (Fig. 6, internode G).

*Woodsia* has been under-represented in molecular phylogenetic studies to date; no study has included more than one species, and none has been able to strongly infer the position of that species, either. Here we establish that *Woodsia s.l.* is likely to be monophyletic (Fig. 6, seven species included in our analysis) and we demonstrate that two of the three segregate genera (*Cheilanthopsis* and *Protowoodsia*) recognized by Shmakov (2003) are nested within *Woodsia s.s.*; only *Hymenocystis* is as-yet unsampled. Additionally, our study finds strong support for the position of *Woodsia s.l.* to be far from the other Woodsiaaceae genera (*Athyrium*, *Acystopteris*, *Cornopteris*, *Cystopteris*, *Deparia*, *Diplazium*, *Gymnocarpium*, *Rhachidosorus*, *Diplaziopsis*, *Homalosorus*, *Hemidictyum*), as circumscribed in the most recent family level fern classification (Smith et al., 2006); cf. Figure 6a with 6b.
The “athyrioids” have been a source of great disagreement in fern systematics (e.g., Alston, 1956; Ching, 1940a, 1964a; Sledge, 1973; Tryon & Tryon, 1982). Molecular data confirmed their distant relationship to the dryopteroid ferns (Dryopteridaceae, in Eupolypods I), but uncertainty regarding their delimitation and affinities has persisted until very recently. Sano et al. (2000a) were the first to extensively sample the athyrioids, and they provided the initial evidence that the group, as then understood, was strongly heterogeneous. Our data corroborate the results of earlier studies (Sano et al., 2000a; Schuettpelez & Pryer, 2007; Wang et al., 2003) in revealing three major clades within the athyrioids s.s.: one containing *Athyrium* and close allies (“athyriids”); one containing *Diplazium* s.l. (“diplaziids”); and one containing *Deparia* s.l. (“depariids”). Our novel finding is the well-supported, early diverging position of *Athyrium skinneri* with respect to the other athyriids included in our sample. This species belongs to a small group of predominantly Mexican taxa, none of which had been included in previous phylogenetic studies. Its position as sister to the rest of the included athyriids (including *Cornopteris* and *Pseudocystopteris*) emphasizes the paraphyly of *Athyrium* as currently circumscribed, and has important implications for our understanding of the evolution of both the athyriids and the diplaziids. Our study provides additional novel support for the placement of the athyrioids as phylogenetically distant from *Rhachidosorus, Cystopteris, Gymnocarpium, Woodsia, Diplaziopsis, Hemidictyum* and *Homalosorus*, a topology that is in conflict with the recent classifications of the group (Smith et al., 2006; Wang et al., 2004); both Athyriaceae *sensu* Wang et al. (2004) and Woodsiaceae *sensu* Smith et al. (2006) are shown here to be strongly paraphyletic (Fig. 6).

Our results for the remaining five major clades—Aspleniaceae, Thelypteridaceae, *Hemidictyum*, Blechnaceae, and Onocleaceae—agree in all important respects with earlier studies of these groups (Cranfill, 2001; Gastony & Ungerer, 1997; Kuo et al., 2011;
1.4.5 Eupolypod II Phylogeny: Morphological Stasis and Disparity

A striking pattern in our phylogeny is its incongruence with previous morphology-based hypotheses of relationship, particularly with respect to the position of the genera of Woodsiaceae *sensu* Smith et al. (2006): *Acystopteris*, *Cystopteris*, *Diplaziopsis*, *Gymnocarpium*, *Hemidictyum*, *Homalosorus*, *Rhachidosorus*, *Woodsia* and allies, as well as the athyrioids (Fig. 6). Some of these groups have been historically difficult to place, and thus their isolation from *Woodsia* or the athyrioids (the bulk of Woodsiaceae *sensu* Smith et al. (2006) is in the athyrioids) is not particularly surprising. Smith et al. (2006) themselves noted that their Woodsiaceae might prove to be not monophyletic. The placement of three genera, however, was utterly unanticipated by morphological data: *Diplaziopsis*, *Homalosorus*, and *Rhachidosorus*. These taxa have not only been considered closely related to the athyrioids, they have been nearly universally considered to be members of the large genera *Diplazium* (first two) or *Athyrium* (*Rhachidosorus*). Their phylogenetic position, deeply isolated from their presumed relatives, underscores the complex patterns of morphological evolution in Eupolypods II; further morphological investigations are necessary to determine whether the apparent similarities between these three genera and the athyrioids are due to convergence or symplesiomorphy.

This trend of shared morphological syndromes across very deep splits in the tree by some members of the “Woodsiaceae” is in contrast to the interdigitation, among those same taxa, of a series of distinct, morphologically unique groups, including the Aspleniaceae, Blechnaceae, Onocleaceae, and Thelypteridaceae. The coarse picture of eupolypod II morphological evolution, then, is marked by two seemingly opposing
patterns. On the one hand are the autapomorphy-rich clades, whose individual phylogenetic coherence is strong, but whose deep relationships were obscure based on morphological data. And, on the other, the morphologically consistent yet phylogenetically incoherent members of the “Woodsiaceae.”

While not the focus of this study, our phylogeny contains rich information on relationships closer to the tips of the tree, within the approximately “family unit” clades. For example, within the athyrioids and Blechnaceae, morphological evolution is complex, and non-monophyletic generic concepts are common. Generic delimitation within these families is in need of much further study. In addition, a cursory comparison between the Onocleaceae and their sister group, the Blechnaceae, is revealing. Both clades have approximately the same crown ages (Appendix B), yet exhibit strikingly different patterns of diversification. The Onocleaceae branch is marked by few, well-spaced, divergences leading to the five extant species. Conversely, the Blechnaceae branch features multiple, very short internodes; this family includes approximately 200 extant species.

1.4.6 Phylogeny Evaluation

Despite the presence in our dataset of each of the anticipated challenges to robust phylogenetic inference (long outgroup branch; strong lineage-specific rate heterogeneity; “ancient rapid radiation” model; Figs. 1, 3a), we were able to infer a phylogeny with strong backbone support (Fig. 6), and our various evaluations gave no indication that the support for the internodes in our maximum likelihood tree is due to artifacts. However, different approaches to controlling for the Bayesian star-tree paradox artifact, and different outgroup sampling regimes all influenced support levels; only lineage-specific rate heterogeneity had a negligible effect.
These effects give further weight to arguments for rigorously evaluating phylogenies against potential artifacts. While specific vulnerabilities may be dataset-dependent, the core elements of our analysis regime are broadly applicable, including the inspection of preliminary phylogenetic hypotheses for potential confounding factors, the investigation of those factors through scrutinizing the performance of multiple models and multiple implementations of those models, and the utilization of the reduced consensus approach to isolate topological effects of signal weakness from those of signal conflict. Although this study is focused on the post-dataset steps, pre-analysis components (taxon sampling, character sampling, character evaluation) are also vital. In particular, in our case, the use of a broad taxon sample with moderate character data proved effective.

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2. A revised family-level classification for eupolypod II ferns (Polypodiidae: Polypodiales)


2.1 Introduction

Despite intensive studies spanning the late 1930s to 1980s (Alston, 1956; Ching, 1940a, 1978a, 1978b; Christensen, 1938; Copeland, 1947; Holttum, 1947; Mickel, 1974; Nayar, 1970; Pichi Sermolli, 1973; Sledge, 1973; see Smith, 1995; Tryon & Tryon, 1982), evolutionary relationships within ferns remained obscure, and suprageneric treatments varied wildly. Holttum lamented in 1971 that “most family names of ferns have had such different meanings, as used by different authors, that such names are only intelligible if we associate them with the names of particular authors.” He suggested “in the meantime it would best serve the ultimate stability of nomenclature if we regard all family names of ferns as informal and tentative (which in fact they have always been)” (Holttum, 1971a). Thirty-five years later, Hennipman (1996) voiced a similar sentiment, that "modern higher classifications of ferns are a jungle for the user." As recently as 1990, for example, the schizaeoid ferns (Schizaeales sensu Smith et al. 2006) and pteroid ferns (Pteridaceae sensu Smith et al. 2006) were hypothesized to be each other’s closest living allies (Tryon et al., 1990); current evidence, however, suggests these lineages shared a most recent common ancestor over 260 million years ago (Schuettpelz & Pryer, 2009; their Table S3), and that pteroids are more closely related to other Polypodiales, the
Cyatheales, and the Salviniales (in total, the vast majority of ferns) than they are to the schizaeoids. Suprageneric fern classifications had fallen into such disrepute that some recent floras avoided them altogether, opting instead to present genera in alphabetical order (e.g., Mickel & Smith, 2004; Palmer, 2002; Smith, 1981; Zuquim et al., 2008).

For nearly two decades, renewed investigations using molecular (Hasebe et al., 1994; Hasebe et al., 1995; Korall et al., 2006a; Korall et al., 2006b; Kranz & Huss, 1996; Manhart, 1994, 1995; Pahnke et al., 1996; Pryer et al., 2004; Sano et al., 2000a; Schneider et al., 2004b; Schuettpelz et al., 2006; Schuettpelz & Pryer, 2007; Vangerow et al., 1999; Wang et al., 2003; Wikström & Pryer, 2005; Wolf, 1995; Wolf, 1997; Wolf et al., 1998; Wolf et al., 1999; Wolf et al., 1994), morphological (Schneider, 1996; Stevenson & Loconte, 1996), and combined molecular and morphological data (Pryer et al., 2001; Pryer et al., 1995) have yielded increased support for the relationships that shape the major branches of the fern tree of life. In 2006, these phylogenetic hypotheses were consolidated and presented in a revised classification for ferns (Smith et al., 2006).

Smith et al. (2006) recognized a monophyletic Polypodiales (“Polypods”) within which the majority of species fall into two large “eupolypod” clades, sister to each other and christened Eupolypods I and Eupolypods II, respectively (Fig. 2; Schneider et al., 2004b). Together, the eupolypod lineages include over 6000 species—more than half of extant fern diversity. The large eupolypod clades had been hinted at, rather presciently, by earlier workers, including Sledge (1973, his Aspidiaceae and Athyriaceae approximate the Eupolypods I and II, respectively) and Mickel (1974, who grouped members of what are now called Eupolypods together in a “derived” position on his tree, Polypodiaceae being the chief exception). The existence of the eupolypod clade was further suggested by early molecular (Hasebe et al., 1994; Hasebe et al., 1995), morphological (Stevenson & Loconte, 1996), and combined analyses (Pryer et al., 1995).
Schneider et al. (2004b) were the first to adopt the names Eupolypods I and II for these two clades, and it was not until the Smith et al. (2006) compilation that their composition was broadly understood.

As currently circumscribed, Eupolypods II is a large clade, comprising over 2500 species, including those associated with the large genera *Asplenium* (~700 spp.), *Cyclosorus* (~650 spp.), *Diplazium* (~400 spp.), *Athyrium* (~180 spp.), and *Blechnum* (~150 spp.; estimates from Kramer et al., 1990a; Kramer et al., 1990b; Kramer & Viane, 1990; Smith, 1990). It encompasses great morphological and ecological variation, including taxa as disparate as the diminutive dry-rock dwelling *Asplenium tenerrimum* Mett. ex Kuhn, large arborescent tropical *Blechnum auratum* (Fée) R.M. Tryon & Stolze, high-arctic plants of *Woodsia glabella* R.Br. ex Richardson, and the temperate floodplain understory (and frequently sautéed) *Matteuccia struthiopteris* (L.) Tod. (Fig. 7). Given its species richness, morphological disparity, and lack of historical recognition, it is not surprising that unequivocal morphological synapomorphies for Eupolypods II are lacking. However, some clear trends exist that are particularly useful for distinguishing Eupolypods II from Eupolypods I. Most eupolypod II taxa have two vascular bundles in the stipe (vs. many bundles in Eupolypods I), and many eupolypod II species have linear, indusiate sori (in the rare cases where members of Eupolypods I have linear sori, they are not indusiate; Fig. 8).

In their treatment of Eupolypods II, Smith et al. (2006) recognized not only that the backbone relationships within the clade were unresolved, but that Woodsiaceae as it was then circumscribed was possibly not monophyletic; the data then available did not support a monophyletic Woodsiaceae, but they also did not support any alternative set of relationships (Hasebe et al., 1995; Pryer et al., 2004; Sano et al., 2000a; Schneider et al., 2004b). In recognizing a potentially non-monophyletic Woodsiaceae, Smith et al.
Figure 7: Representative eupolypod II ferns. Photographers are credited after the species names.

**ASPLENIACEAE**—A: Asplenium nidus L. s.l. [M. Sundue]. B: Asplenium montanum Willd. [S. Zylinski].

**ATHYRIACEAE**—C: Athyrium asplenoides (Michx.) A.A. Eaton [S. Zylinski].

**BLECHNACEAE**—D: Woodwardia areolata (L.) T. Moore [C. Rothfels]. E: Blechnum schomburgkii (Klotzsch) C. Chr. [M. Sundue].


**DIPLAZIOPSIDACEAE**—I: Diplaziopsis javanica (Blume) C. Chr. [L.-Y. Kuo].

**HEMIDICTYACEAE**—J: Hemidictyum marginatum (L.) C. Presl [M. Sundue].

**ONOCLEACEAE**—K: Onocleopsis hintonii F. Ballard [C. Rothfels]. L: Matteuccia struthiopteris (Hook.) Hayata [M. Sundue].

**RHACHIDOSORACEAE**—M: Rhachidosorus mesosorus (Makino) Ching. [L.-Y. Kuo].

**THELYPTERIDACEAE**—N: Thelypteris noveboracensis (L.) Nieuwl. [C.W. Cook].

**WOODSIACEAE**—O: Woodsia alpina (Bolton) Gray [A. Larsson].
(2006) issued the caveat that, while “it is premature to adopt the alternative of erecting (or resurrecting) numerous small families to house its constituent genera … further sampling will likely shed additional light on this subject, and the recognition of several additional families may be warranted” (Smith et al., 2006).

Figure 8: Morphological characteristics of eupolypod II taxa. Photographers are credited within square brackets. A: Cross-section of Diplaziopsis javanica (Blume) C. Chr. (Diplaziopsidaceae) showing two vascular bundles at the base of the petiole [L-Y. Kuo]. B: Close-up of abaxial leaf surface of Asplenium platyneuron (L.) Britton, Sterns & Poggenb. (Aspleniaceae), showing sporangia arranged in linear, indusiate sori. The sporangia are visible under the flap-like erose indusium, which opens away from the vein [C.J. Rothfels]. C: Abaxial leaf surface of Blechnum occidentale L. (Blechnaceae), again showing sporangia arranged in linear, indusiate sori. In this species the sori are contiguous along the main vein of each pinna, and the indusium opens towards the vein [R.C. Moran; modified with permission from www.plantsystematics.org].

Further studies were rapidly forthcoming. In their 400-taxon, three-gene study, Schuettpelz & Pryer (2007) showed that three genera—Cystopteris, Gymnocarpium, Hemidictyum—tentatively placed in Woodsiaceae (Smith et al., 2006) were only distantly related to other members of Woodsiaceae sensu Smith et al. (2006). This general pattern—Woodsiaceae sensu Smith et al. (2006) not monophyletic and the backbone relationships within Eupolypods II only weakly supported—was also uncovered by the two-gene analyses of Wei et al. (2010), the three-gene analyses by Kuo et al. (2011), and the four-gene study of Li et al. (2011a).

To directly address the composition of the major clades within Eupolypods II and the relationships among them, Rothfels et al. (2012a) assembled an expanded molecular dataset (five plastid loci) for 67 eupolypod II species and 14 outgroup taxa. Their taxon sampling was designed to capture the deepest divergences across Eupolypods II and those within each major clade, as well as any potentially isolated
lineages, as suggested by previous molecular (particularly Kuo et al., 2011; Sano et al., 2000a; Schuettgelz & Pryer, 2007; Tzeng, 2002) or morphological studies (chiefly Kato & Darnaedi, 1988; Wang et al., 2004). Although the results of Rothfels et al. (2012a) were consistent with those of earlier studies, the more comprehensive taxon and data sampling provided higher levels of support for relationships and helped to resolve most of the taxonomic challenges in Eupolypods II. We base our classification on their inferred phylogeny (see Figs. 6, 9), with the caveat that, like all phylogenetic studies of the Eupolypods II to date, their phylogeny is based solely on plastid data; no loci from the nucleus or mitochondrion were included. This phylogeny is similar in outline to the linear sequence recently proposed by Christenhusz et al. (2011), but is further informed by the critical data of Kuo et al. (2011), Li et al. (2011a), and Rothfels et al. (2012a).

Figure 9: Divergence and diversification in the Eupolypods II. i) Eupolypod phylogeny, with branch lengths approximately proportional to time (from the relaxed clock analyses of Rothfels et al. 2012, Appendix B). The tip of the grey triangles along each branch marks the first sampled divergence within each family (Rothfels et al. 2012). All branches in this phylogeny are well supported (maximum likelihood bootstrap support ≥70% and Bayesian posterior probability ≥0.95) with the exception of the grey branch, marked with an asterisk (*), which had 63% maximum likelihood bootstrap support and 0.89 posterior probability. ii) Family-level nomenclatural status: U: Unchanged from Smith et al. (2006); R: Recircumscribed (family name existed, but was not adopted by Smith et al. (2006)); N: Newly described since Smith et al. (2006). Letters that are encircled indicate those families that have been segregated from Woodsiaceae sensu Smith et al. (2006). iii) Family names, and approximate species richness, for the classification adopted here.
2.2 Classification of the Eupolypods II

The aim of our classification is to recognize families within the eupolypod II phylogeny that balance the somewhat conflicting criteria of maximizing evolutionary informativeness (we thus adhere to the principle of monophyly) and minimizing nomenclatural instability (we retain long-established circumscriptions as much as possible). This conflict is most difficult to reconcile for *Asplenium* and its allies. Both choices (to recognize an expanded Aspleniaceae that includes *Hemidictyum*, *Diplaziopsis*, and *Homalosorus*, or to create new families to accommodate the latter three genera) yield justifiable, monophyletic families. We take the latter approach—to recognize Aspleniaceae, Hemidictyaceae, and Diplaziopsidaceae—despite the addition of two small families, in order to preserve the long-standing use of Aspleniaceae in the more restricted sense, to highlight the deep divergence of each of the respective groups (Fig. 9), and because there are no clear morphological synapomorphies for the expanded family concept.

Many generic concepts in Eupolypods II are in flux and although not a focus of our classification, we attempt to account for all generic names in current general usage, and provide a familial placement. For each family, we provide: a list of defining morphological characters (from the references cited in the family header, from our direct observations, and from the following general references: Gifford & Foster, 1989; Ogura, 1972; Tryon & Tryon, 1982; Wilson, 1959), nomenclatural data, and a list of included genera and the estimated number of species. In addition, we recommend possible English family names, and summarize information on ecology, geographic range, and phylogenetic relationships. Each family is accompanied by a concept map (see Franz et al., 2008), mapping our treatment onto previous classifications. For example, the
following entry under Rhachidosoraceae: “=Athyriaceae: Rhachidosoroideae sensu Wang et al. (2004); <Woodsiaceae sensu Smith et al. (2006)” indicates that our treatment of that family is equivalent in composition to Wang et al.’s concept of subfamily Rhachidosoroideae of Athyriaceae, and is a subset of Smith et al.’s concept of Woodsiaceae. Family names are based on those in Hoogland & Reveal (2005), except for Diplaziopsidaceae and Rhachidosoraceae, which are from Christenhusz et al. (2011), and Hemidictyaceae, from Christenhusz & Schneider (2011).

2.2.1 Cystopteridaceae (Payer) Schmakov, Turczaninowia 4 (2001).

Cystopteridoids: Bladderferns, Brittleferns, Oakferns, and allies. Approximately 30 species in the genera Acystopteris (3 species), Cystoathyrium (1 species), Cystopteris (~20 species; incl. Rhizomatopteris), and Gymnocarpium (~7 species; incl. Currania); (Blasdell, 1963; Haufler & Windham, 1991; Pryer & Haufler, 1993; Sarvela, 1978; Tagawa, 1935; Vida, 1974).

<Polypodiaceae: Aspleniodeae + Polypodiaceae: Dryopteridoideae sensu Christensen (1938); <Dennstaedtiaceae: Dryopteridoideae + Athyriodeae sensu Holttum (1947); <Dryopteridoideae: Dryopteridoideae + Athyriodeae sensu Nayar (1970); <Athyriaceae sensu Pichi Sermolli (1977); <Athyriaceae sensu Ching (1978a); <Dryopteridoideae: Athyriodeae sensu Lovis (1978); <Dryopteridoideae: Phymatidaceae sensu Tryon & Tryon (1982); <Dryopteridoideae: Athyriodeae: Phymatidaceae sensu Kramer et al. (1990b); <Cystopteridaceae sensu Schmakov (2001); <Athyriaceae: Cystopteridoideae sensu Wang et al. (2004); <Woodsiaceae sensu Smith et al. (2006); =Cystopteridaceae sensu Christenhusz et al. (2011).

Characters.—Plants terrestrial; roots blackish, wiry, inserted radially, non-proliferous; rhizomes epigean or more often subterranean, short- to more often long-creeping, occasionally sub-erect (Cystopteris), commonly branched, bearing scales and sometimes golden hairs similar to the root-hairs (e.g., C. protrusa (Weath.) Blasdell); rhizome scales lanceolate, clathrate or non-clathrate, the margins glandular or not, without distinct pubescence, entire to ciliate, the teeth when present not formed by two adjacent cells; leaves green and not covered in mucilage during any stage of development, spirally arranged, monomorphic, bulbiferous in a few Cystopteris, closely spaced to distant, bearing scales and sometimes gland-tipped hairs, the scales sometimes
reduced to filiform proscales (*Cystopteris*) or catenate hairs (*Acystopteris*); petioles stramineous throughout or proximally darkened, the base narrow, or conspicuously thickened and then starch-filled and persistent (trophopods), without conspicuous aerophores, without a proximal articulation, sometimes with golden hairs similar to the root hairs (e.g., *C. moupinensis* Franch.); petiolar vascular bundles two, the bundles with hippocampiform-shaped xylem, distally uniting to form a single V-shaped bundle; laminae thin-herbaceous, 2–3-pinnate-pinnatifid (pinnate-pinnatifid in *Cystoathyrium*), broadest at the base or lanceolate, the apex non-conform, the leaf marginal cells differentiated into nodulose hyaline cells (*Acystopteris, Cystopteris*) or not (*Gymnocarpium*); pinna axes distinctly articulate in *Gymnocarpium*, otherwise non-articulate, sulcate adaxially, lacking a free central ridge; the rachis grooves continuous or not, the sulcus wall of the rachis continuing as a prominent ridge onto the sulcus wall of the costa or not; veins free, terminating at the leaf margin, the vein endings not differentiated; sori dorsal along veins, not terminal, round or slightly elongate (*Gymnocarpium*), indusiate (*Acystopteris, Cystoathyrium, Cystopteris*) or exindusiate (*Gymnocarpium*); soral receptacle distinctly raised and hardened (*Acystopteris, Cystopteris*) or flat (*Gymnocarpium*); indusia basal (*Acystopteris, Cystoathyrium, Cystopteris*); sporangia with stalks two or three cells wide in the middle; spores monolete, non-chlorophyllous, tan (*Acystopteris*) or brown, the perispore echinate, tuberculate, or with broad folds, the folds sometimes perforate; chromosome base number \(x=40\) (*Gymnocarpium*; Kato et al., 1992; Pryer & Hafler, 1993) or 42 (*Acystopteris, Cystopteris*; Blasdell, 1963; Mitui, 1975; Vida, 1974). Reports of \(x=41\) (e.g., Christenhuz et al., 2011) are not substantiated.

Although the genera are distinctive, Cystopteridaceae as a whole are not easily characterized. Among families with petioles that contain two vascular bundles, they can
be distinguished by an absent or hood-like indusium, usually long-creeping and subterranean rhizome, and veins that terminate at the leaf margin.

The indusia of *Acystopteris, Cystoathyrium*, and *Cystopteris* are unique in being attached at the base of the sporangia and curving, hood-like, around them. The sorus itself is situated upon a raised and hardened receptacle; we know of no other taxa within Eupolypods II with a similar receptacle. *Woodsia* also has a basally attached indusium, however, it can be distinguished by having a flat receptacle, the indusium encircling the sorus, usually dissected into multiple lobes, and veins that do not reach the leaf margin.

*Gymnocarpium* can be diagnosed by its articulate pinnae (that do not disarticulate) with a swollen protuberance at the base of each pinna. Among Eupolypods II, articulate pinnae also occur in *Stenochlaena* and *Woodwardia virginica*. Those articulations differ, however, by lacking the basal protuberance present in *Gymnocarpium*. *Gymnocarpium* can also be distinguished from other Eupolypods II by having slightly elongate sori that lack an indusium. These sori are about twice as long as wide, and appear round until the sporangia are removed to reveal an elongate patch of sporangial stalks spreading along the vein.

*Biology and phylogeny.*—Cystopteridaceae are unusual in their primarily temperate distribution and tendency to occupy montane habitats. Both *Cystopteris* and *Gymnocarpium* are common ferns of the north temperate zone, with *Cystopteris* also ranging south in montane habitats through the Andes and Himalaya, and to Australia, New Zealand, Hawaii, and southern Africa. Within the family, *Acystopteris* is the only genus found commonly in tropical areas; it is most rich in East Asia (Blasdell, 1963; Pryer, 1993; Sarvela, 1978).

The relationships of genera within the Cystopteridaceae have been the subject of unusually strong disagreement; their affinities have been extremely difficult to infer
from morphology, even more so than is typical for most eupolypod II taxa. Individually, both *Cystopteris* s.l. (i.e., including *Acystopteris*; Blasdell, 1963; Tagawa, 1935) and *Gymnocarpium* have been thought to be allied with the Dryopteridaceae (in Eupolypods I) or the Athyriaceae; in either position they were inevitably highlighted as being anomalous (see Sledge, 1973). Ching (1940a) was an early exception, however, in placing both *Cystopteris* and *Gymnocarpium* together, but among the athyrioids.

Early molecular data supported *Cystopteris* and *Gymnocarpium* as sister genera (one accession each; *Acystopteris* was not sampled), and demonstrated their lack of close affinity to either Dryopteridaceae or *Athyrium*, but were unable to resolve their position within a broad assemblage of eupolypod II taxa (Hasebe et al., 1995; Wolf et al., 1994). In their landmark study, Sano et al. (2000a) included four representatives from this clade: one *Acystopteris*, one *Cystopteris*, and two *Gymnocarpium* species. Their within-clade relationships were consistent with earlier studies (*Acystopteris* + *Cystopteris* sister to *Gymnocarpium*), but they did not find support for the clade’s placement within Eupolypods II. Conversely, Schuettpelz & Pryer (2007) in their broad study across ferns, included fewer taxa from this clade (a single *Gymnocarpium* and a single *Cystopteris*) but more character data; they were the first to find support for a sister group relationship between this clade and the rest of Eupolypods II. Similarly, Kuo et al. (2011), using three plastid loci and single accessions of *Acystopteris* and *Gymnocarpium*, also recovered the sister group relationship of this clade to the rest of Eupolypods II. The results of Rothfels et al. (2012a) corroborate and strengthen that finding (Fig. 9).

*Cystopteris* and *Acystopteris* are strongly supported as sister, and are in turn sister to *Gymnocarpium*. The type species of all three genera have been included in molecular phylogenetic studies, as have those of the segregates *Rhizomatopteris* and *Currania*: *Rhizomatopteris* is sister to the remaining species of *Cystopteris* s.s.; *Currania* is embedded
within *Gymnocarpium* (Rothfels et al., 2012a; Sano et al., 2000a). Cystopteridaceae was first circumscribed by Schmakov (2001), who included *Pseudocystopteris* (which belongs in the Athyriaceae) and omitted *Cystopteris* and *Cystoathyrium* (which were not in his geographic range).

The position of the monotypic genus *Cystoathyrium* is uncertain. In describing the genus, Ching (1966) emphasized its morphological intermediacy between *Cystopteris* and *Athyrium*, and little progress has since been made towards resolving its affinities. Wang et al. (2004; 2008) treated it as allied to *Cystopteris*, whereas Kramer et al. (1990b) place it in *Athyrium*, a position also advocated by Pichi Sermolli (1977). *Cystoathyrium* has yet to be included in any phylogenetic study, and we know it only from photographs and the illustration provided in the protologue; it is possibly extinct (X.-C. Zhang, pers. comm.). Other genera historically thought to be allied with *Cystopteris* (most notably *Pseudocystopteris*; Ching, 1964a) have been shown to be nested within the Athyriaceae (Liu, 2008; Rothfels et al., 2012a; Sano et al., 2000a), however, we tentatively include *Cystoathyrium* here in the Cystopteridaceae based on four characters: round sori, hood-like indusium, strongly echinate spores, and veins that terminate at the leaf margin. Although homoplastic within Eupolypods II, these character states occur most frequently in Cystopteridaceae. More research is needed; *Cystoathyrium* may be an isolated lineage within the Eupolypods II.

### 2.2.2 Rhachidosoraceae X.C. Zhang, Phytotaxa 19 (2011)

Lacquer Ferns. Four to seven species of the genus *Rhachidosorus*; (Ching, 1964a; Kato, 1975a; Li et al., 2011a).

<Polypodiaceae: Aspleniodeae sensu Christensen (1938); <Dennstaedtiaceae: Athyriodeae sensu Holttum (1947); <Dryopteridaceae: Athyriodeae sensu Nayar (1970); <Athyriaceae sensu Tagawa & Iwatsuki (1972); =*Diplazium mesosorum group* sensu Kato (1977); <Athyriaceae sensu Pichi Sermolli (1977); <Athyriaceae sensu Ching (1978a); <Dryopteridaceae: Athyriodeae sensu Lovis (1978); <Dryopteridaceae: Physematieae sensu Tryon & Tryon (1982); <Dryopteridaceae: Athyriodeae: Physematieae sensu Kramer et al. (1990b); =Athyriaceae: Rhachidosoroideae sensu Wang et al. (2004); <Woodsiaceae sensu Smith et al. (2006); =Rhachidosoraceae sensu Christenhusz et al. (2011).
**Characters.**—Plants terrestrial; roots inserted radially, non-proliferous; rhizomes creeping or short-creeping, not commonly branched, bearing scales; rhizome scales lanceolate, clathrate, the margins entire, without distinct pubescence; leaves green and not covered in mucilage during any stage of development, spirally arranged, monomorphic, not articulate to the rhizome, closely spaced, sparsely scaly; petioles reddish to stramineous throughout, narrow at the base, not forming trophopods, without conspicuous aerophores, without a petiolar articulation; petiolar vascular bundles two, each with hippocampiform xylem, the bundles distally uniting to form a single U-shaped bundle; laminae herbaceous, 2–3-pinnate-pinnatifid, broadest at the base, the apex non-conform, the leaf marginal cells differentiated into nodulose hyaline cells; pinna axes not articulate, sulcate adaxially, lacking a free central ridge; the rachis grooves U-shaped, continuous, the sulcus wall of the rachis continuing as a prominent ridge onto the sulcus wall of the costa, and then departing on the costule of the first basiscopic segment; veins free, terminating before the leaf margin, the vein endings not differentiated; sori dorsal along veins, not terminal, elongate, indusiate; soral receptacle flat; indusia lateral, non-glandular; sporangia with stalks two or three cells wide in the middle; spores monolete, non-chlorophyllous, brown, the perispore echinate, tuberculate, or with broad folds, the folds sometimes perforate; chromosome base number x=41 (Kato, 1975a; Kato et al., 1992; Takamiya et al., 2000). The count of x=40 (Kurita, 1960) is unsubstantiated.

Rhachidosoraceae can be distinguished by the combination of subterranean creeping rhizomes, leaves without abundant anthocyanins or mucilage at any stage, petioles with two vascular bundles, elongate sori restricted to one side of the vein, with indusia, and laminae provided with narrow filiform scales, and lacking hairs. This suite of characters, however, renders it difficult to distinguish from either Aspleniaceae or
Athyriaceae. With Aspleniaceae it shares clathrate scales and elongate sori that are largely confined to one side of the vein. It does not, however, have the pinna-costa architecture characteristic of Aspleniaceae—a non-sulcate petiole where wings are formed by a decurrent lamina margin. (See the key provided below for additional technical characters distinguishing Rhachidosoriaceae from Aspleniaceae). More difficult is distinguishing Rhachidosoraceae from Athyriaceae; both families have similar pinna-costa architecture. This architecture is characterized by a sulcate rachis that is not alate, and that has a prominent flange on the basiscopic side of the pinna costa formed by the sulcus wall as it continues from the rachis onto the pinna costa itself. In addition, *Rhachidosorus* has minute corniculae and scales adaxially at the junction of the pinna and rachis, which are similar to those of *Athyrium* and *Cornopteris*. The most useful characters for distinguishing between these two families are the clathrate scales and linear sori confined to one side of the vein in Rhachidosoriaceae; most Athyriaceae have sori on two sides of a single vein, either back-to-back, or in a hooked arrangement.

**Biology and phylogeny.**—Endemic to Asia, *Rhachidosorus* is a genus of approximately eight species of understory terrestrial ferns, which are often found in limestone habitats, and are very similar in gross morphology to species of *Athyrium*. Based on morphology, *Rhachidosorus* was previously included in either *Athyrium* (Makino, 1899) or *Diplazium* (Kato, 1975a), or considered a closely allied segregate (Ching, 1964a; reviewed in Sano et al., 2000a). Early molecular phylogenies (Sano et al., 2000a; Tzeng, 2002; Wang et al., 2003), however, unexpectedly suggested that *Rhachidosorus* was not closely related to either *Athyrium* or *Diplazium*. These results were later corroborated by the three-gene study of Kuo et al. (2011) who resolved *Rhachidosorus* as sister to the large clade of Thelypteridaceae + *Woodsia* + Athyriaceae + Blechnaceae + Onocleaceae, but with only weak support, and by Li et al. (2011a) who
placed the genus as sister to the clade recognized here as Diplaziopsidaceae. These studies each included a single *Rhachidosorus* accession (*R. mesosorus* in Sano et al. (2000a); *R. consimilis* in Wang et al. (2003); *R. pulcher* in Tzeng (2002) and Kuo et al. (2011)), except for Li et al. (2011a) who included both *R. consimilis* and *R. blotianus*. The five-locus dataset of Rothfels et al. (2012a) also included two species (*R. mesosorus* and *R. pulcher*), but as with the other studies was unable to strongly support the phylogenetic position of the genus; it was weakly placed as sister to Diplaziopsidaceae + Hemidictyaceae + Aspleniaceae.

Molecular data from four species (including *R. mesosorus*, the type of the genus) and six independent studies consistently support the surprising finding that *Rhachidosorus* is not phylogenetically close to *Athyrium* or *Diplazium*, but instead is an isolated lineage within Eupolypods II. The phylogeny of Rothfels et al. (2012a) suggests that *Rhachidosorus* diverged from its nearest relatives approximately 90 million years ago, long before, for example, the ancestors of *Blechnum* diverged from those of *Athyrium* (Fig. 9; Rothfels et al., 2012a, their Supplementary Fig. 1).

### 2.2.3 Diplaziopsidaceae X.C. Zhang & Christenh. Phytotaxa 19 (2011)

Glade Ferns. Approximately four to six species of the genera *Diplaziopsis* (2 to 4 species), *Homalosorus* (1 species), plus *Diplazium flavoviride* Alston; (Ching, 1964b; Kato, 1975b; Kato & Darnaedi, 1988; Li et al., 2011a; Wei et al., 2010).

*<Dennstaedtiacea: Athyriodeae sensu* Holttum (1947); <Dryopteridaceae: Athyriodeae sensu Nayar (1970); <Athyriaceae sensu Tagawa & Iwatsuki (1972); "Diapazium javanicum group" sensu Kato (1977); <Athyriaceae sensu Pichi Sermolli (1977); <Athyriaceae sensu Ching (1978a); <Dryopteridaceae: Athyriodeae sensu Lovis (1978); <Dryopteridaceae: Physematieae sensu Tryon & Tryon (1982); <Dryopteridaceae: Athyriodeae: Physematieae sensu Kramer et al. (1990b); <Athyriaceae: Diplazioideae sensu Wang et al. (2004); <Woodsiaceae sensu Smith et al. (2006); <Diplaziopsidaceae + Athyriaceae sensu Christenhusz et al. (2011).

**Characters.**—Plants terrestrial or epipetric; roots fleshy, pale, inserted radially, non-proliferous; rhizomes erect to sub-erect (*Diplaziopsis, Diplazium flavoviride*) or short-creeping (*Homalosorus*), commonly unbranched, bearing scales, and sometimes golden
hairs similar to the root hairs (*Homalosorus*); rhizome scales lanceolate, non-clathrate, the margins entire, non-glandular, without distinct pubescence; leaves green and not covered in mucilage during any stage of development, spirally arranged, monomorphic, non-bulbiferous, closely spaced, glabrous (*Diplaziopsis*) or with filiform proscales (*Homalosorus*); petioles stramineous throughout or proximally darkened, thin, without a proximal thickening, conspicuous aerophores, or proximal articulation, sometimes with golden hairs similar to the root hairs (*Homalosorus*); petiolar vascular bundles two, each with hippocampiform xylem, the bundles distally uniting to form a single V-shaped bundle; laminae soft-herbaceous, 1-pinnate, the apex conform (*Diplaziopsis*) or non-conform (*Homalosorus*), the leaf marginal cells differentiated into nodulose hyaline cells; pinna axes not articulate, sulcate adaxially, lacking a free central ridge; the rachis grooves V-shaped, not continuous, the sulcus wall of the rachis continuing as a prominent ridge onto the sulcus wall of the costa; veins free (*D. flavoviride, Homalosorus*) or anastomosing toward the pinna margins (*Diplaziopsis*), the areoles without free included veinlets, usually terminating before the leaf margin, however some veins reaching the leaf margin in *D. flavoviride* and *Homalosorus*, the vein endings differentiated, slightly raised and expanded; sori singular along one side of the vein, rarely paired back to back along the same vein, elongate, indusiate, not terminal; soral receptacle flat; indusia lateral, vaulted or essentially flat, glabrous or glandular (*Diplaziopsis*), opening along the lateral margin or sometimes rupturing irregularly (*Diplaziopsis*); sporangia with stalks two or three cells wide in the middle; spores monolete, non-chlorophyllous, brown, the perispore folded with thin crests, the crests erose; chromosome base numbers $x=40$ (*Diplazium flavoviride, Homalosorus*; Kato, 1993; Kato & Darnaedi, 1988; Löve et al., 1977) or 41 (*Diplaziopsis*; Mitui, 1975; Takamiya & Ohta, 2001).
Diplaziopsidaceae can be recognized by the combination of petioles with two vascular bundles, 1-pinnate laminae, elongate sori that are usually along one side of the vein only, vein endings that are thickened and raised adaxially, and by the sulcus wall of the rachis forming a ridge that connects to the pinna sulcus wall of the pinna costa. The thickened and raised vein endings are a particularly useful diagnostic character among Eupolypods II, because they are otherwise only found in *Hemidictyum* (Hemidictyaceae). *Hemidictyum* also has a 1-pinnate lamina, a conform terminal segment, and veins that anastomose towards the pinna margins, similar to *Diplaziopsis*; however, it differs in several other respects, most conspicuously by having a sub-marginal collecting vein, and pinna margins that have a broad, pale membranaceous edge.

As Price (1990) noted, leaves of Diplaziopsidaceae are conspicuously soft, green, and fleshy. The pale fleshy roots appear to be unique among Eupolypods II, and anatomical study may provide synapomorphies for the family. However, the claim by Price (1990) that the plants entirely lack sclerenchyma is overstated; sclerenchyma occurs in the cortex and xylem, as evidenced by staining with toluidine blue (Sundue & Rothfels, unpublished data). Diplaziopsidaceae are most likely to be confused with Athyriaceae, particularly *Diplazium*, which is morphologically similar. Diplaziopsidaceae differs from most *Diplazium* species, however, by the usually singular linear sori, non-continuous groove of the adaxial pinnae-costae junctions, the near absence of indument on the lamina, the narrow petiole bases that do not form trophopods, thickened vein endings, and vaulted indusium, when it is present.

**Biology and phylogeny.**—Diplaziopsidaceae are medium-sized ferns of mesic understory habitats. They show an interesting pattern of disjunction, with the monotypic *Homalosorus* being a common member of rich temperate forests of eastern
North America, while the *Diplaziopsis* species and *Diplazium flavoviride* are found in Asia, extending east to the Pacific islands (Kato & Darnaedi, 1988).

The history of typification of *Diplaziopsis* is convoluted. Christensen (1906; p. XXXII) published it as a replacement name for *Allantodia* Wall., 1830 (a later homonym of *Allantodia* R. Br., 1810). As a replacement name, therefore, *Diplaziopsis* takes the type of *Allantodia* Wall., which is *A. brunoniana* Wall. However, Christensen did not publish a combination for *A. brunoniana* under *Diplaziopsis* (he considered *A. brunoniana* to be a synonym of *Asplenium javanicum* Blume); the combination *Diplaziopsis brunoniana* (Wall.) W.M. Chu was made only recently (Chu & Zhou, 1994). Christensen (1906) listed *Asplenium javanicum* (= *Diplaziopsis javanica*) as the type of *Diplaziopsis*, but this is prohibited under Art. 7.3 of the Vienna Code. However, if *Diplaziopsis brunoniana* is regarded as a heterotypic synonym of *D. javanica*, as Christensen (1906: p. CCXXVII) indicated, then *D. javanica* has priority and must be used as the name of the species, and the type of the genus. Alternatively, if *D. brunoniana* and *D. javanica* are recognized as distinct (as by Chu & He, 1999), then the type of the genus remains *D. brunoniana*.

Prior to the availability of molecular data, members of this clade were consistently thought to belong with the athyrioids, and both *Diplaziopsis* and *Homalosorus* were typically treated as members of *Diplazium* (Ching, 1964b; Kato, 1975b, 1977, 1993; Kato & Darnaedi, 1988; Wang et al., 2004). The first indication that this placement might be inaccurate came from the study of Sano et al. (2000a), in which the monotypic *Homalosorus* was strongly supported as sister to *Diplaziopsis cavaleriana*, with these two taxa forming an isolated lineage distant from *Diplazium*. The next molecular phylogenetic study to include members of this clade was by Wei et al. (2010), and their results placed the two genera together in an unresolved position within the Eupolypods. Kuo et al. (2011), with more character data and denser taxon sampling, again resolved
Diplaziopsis as sister to Homalosorus. Their results showed that this combined lineage—Diplaziopsis + Homalosorus—diverged from the rest of the Eupolypods II at an unsupported position deep along the eupolypod II backbone. The results of Li et al. (2011a) were similar (Diplaziopsis sister to Homalosorus, and that clade distant from Diplazium), with the exception that their study placed the Diplaziopsis + Homalosorus clade as sister to Rhachidosorus.

In the analyses of Rothfels et al. (2012a), Diplaziopsis cavaleriana and D. javanica are strongly supported as sister, and together they are sister to Homalosorus. These data allow either for the recognition of a monotypic Homalosorus, or its treatment within Diplaziopsis, as D. pycnocarpa (Spreng.) M.G. Price (Price, 1990). Diplaziopsidaceae diverged from Hemidictyaceae + Aspleniaceae early in the diversification of Eupolypods II—these two lineages shared a most recent common ancestor some 90 million years ago (Fig. 9; Rothfels et al., 2012a, their Supplementary Fig. 1)—further supporting the recognition of Diplaziopsidaceae rather than merging it into an expanded Aspleniaceae. Diplazium flavoviride has not been included in any phylogenetic analyses, but is included here based on the arguments of Kato and Darnaedi (1988). Hemidictyum, however, does not fall in the Diplaziopsidaceae; its inclusion in that family by Christenhusz et al. (2011) rendered their concept of Diplaziopsidaceae paraphyletic, an error they subsequently corrected (Christenhusz & Schneider, 2011).

2.2.4 Hemidictyaceae Christenh. & H. Schneid. Phytotaxa 28 (2011)

Hemidictyum. One species of the genus Hemidictyum; (Kato, 1975b).

<Dennstaedtiaceae: Athyriodeae sensu Holttum (1947); <Dryopteridaceae: Athyriodeae sensu Nayar (1970); <“Diplazium javanicum group” sensu Kato (1977); <Athyriaceae sensu Pichi Sermolli (1977); <Thelypteridaceae sensu Lois (1978); <Dryopteridaceae: Phylematieae sensu Tryon & Tryon (1982); <Dryopteridaceae: Athyriodeae: Phylematieae sensu Kramer et al. (1990b); <Woodsieae sensu Smith et al. (2006); <Diplaziopsidaceae sensu Christenhusz et al. (2011); =Hemidictyaceae sensu Christenhusz and Schneider (2011).
Characters.—Plants terrestrial; roots inserted radially, proliferous; rhizomes erect or sub-erect, commonly unbranched, bearing scales; rhizome scales lanceolate, weakly-clathrate, the margins entire, non-glandular, without distinct pubescence; leaves green and not covered in mucilage during any stage of development, spirally arranged, monomorphic, non-bulbiferous, closely spaced, glabrous; petioles stramineous throughout or proximally darkened, thin, not forming trophopods, lacking conspicuous aerophores, without a petiolar articulation; petiolar vascular bundles two, each with hippocampiform xylem, the bundles distally uniting to form a single U-shaped bundle; laminae herbaceous, 1-pinnate, the apex conform, the lateral pinnae sub-opposite, the pinna bases cordate, the leaf margin differentiated into a broad membranaceous edge; pinna axes not articulate, sulcate adaxially, lacking a free central ridge; the rachis grooves not continuous, the sulcus wall of the rachis not continuing as a ridge along the costa; veins anastomosing toward the pinna margins, the areoles without free included veinlets, terminating before the leaf margin and forming a sub-marginal collecting vein, the vein endings differentiated, slightly raised and expanded; sori usually singular along one side of the vein, occasionally back-to-back along both sides of the vein, elongate, indusiate, not terminal; soral receptacle flat; indusia lateral, essentially flat, glabrous; sporangia with stalks two or three cells wide in the middle; spores monolette, non-chlorophyllous, brown, the perispore with broad folds and tubercles, the folds sometimes perforate; chromosome base number x=31 (Wagner, 1980a; Walker, 1973a).

Although its conform apical pinnae, pattern of anastomosing veins, and thickened and raised vein endings are shared with Diplaziopsis, Hemidictyaceae can be distinguished from all other Eupolypods II by the combination of its sub-marginal collecting vein and pinna margin that is differentiated into a broad membranaceous border. Hemidictyaceae is sister to Aspleniaceae, but the two families together share no
known synapomorphies. One character that should be investigated further is whether roots are proliferous, yielding new plants asexually. Walker (1985, p. 217) reported such roots in *H. marginatum*; they also occur in some species of *Asplenium* (Mickel & Smith, 2004), although sporadically enough that a synapomorphy for the two families is unlikely.

**Biology and phylogeny.**—*Hemidictyum* is a monotypic genus of the New World tropics—from southern Mexico to southeastern Brazil—where it grows at low to mid elevations in wet forests. The genus has always been an awkward fit in fern classifications, with opinions alternating for an alliance with thelypteroid ferns (based on spore morphology, e.g., Lovis, 1978), *Diplaziopsis* (based on its sagenoid venation, e.g., Kato, 1975b), or with Dryopteridaceae (in Eupolypods I; e.g., Tryon & Tryon, 1982).

Kato’s (1975b) study was the first to emphasize commonalities between *Hemidictyum* and *Diplaziopsis*, and he argued that they might be isolated from much of *Diplazium* (Kato, 1975b). Molecular data (Kuo et al., 2011; Rothfels et al., 2012a; Schuettpelz & Pryer, 2007) corroborated this morphology-based hypothesis, in part; *Hemidictyum* (like *Diplaziopsis*) is not closely related to *Diplazium s.s.*—*Hemidictyum* and *Diplaziopsis* + *Homalosorus* are more closely related to each other than to any eupolypod II lineage outside of the Aspleniaceae.

Given its sister relationship with *Asplenium* + *Hymenasplenium*, *Hemidictyum* could be subsumed within an expanded concept of Aspleniaceae while retaining the monophyly of the latter family (Fig. 9). However, we favor recognizing Hemidictyaceae, even though it is monotypic, because the most recent common ancestor of *Hemidictyum* and the Aspleniaceae dates to the Late Cretaceous (approximately 85 million years ago; Fig. 9; Rothfels et al., 2012a, their Supplementary Fig. 1), *Hemidictyum* would be
morphologically anomalous within Aspleniaceae, and Aspleniaceae has a long history of taxonomic treatment excluding *Hemidictyum*.

### 2.2.5 Aspleniaceae Newman, Hist. Brit. Ferns (1840)

Spleenworts. Approximately 700 species of one to ten genera, dominated by the large genus *Asplenium* (incl. *Antigrama*, *Asplenidictyum*, *Biropteris*, *Camptosorus*, *Ceterach*, *Diellia*, *Diplora*, *Holodictyum*, *Loxoscaphe*, *Neottopteris*, *Phyllitis*, *Pleurosorus*, *Schaffneria*, *Scolopendrium*, and *Sinephropteris*) and with a small genus *Hymenasplenium* (incl. *Boniniella*); (Gastony & Johnson, 2001; Murakami, 1995; Murakami et al., 1999; Schneider et al., 2004a).

<Polypodiaceae: Aspleniodeae sensu Christensen (1938); =Dennstaedtiaceae: Aspleniodeae sensu Holtum (1947); =Aspleniaceae sensu Nayar (1970); =Aspleniaceae sensu Tagawa & Iwatsuki (1972); =Aspleniaceae sensu Pichi Sermolli (1977); =Aspleniaceae sensu Lovis (1978); =Aspleniaceae sensu Tryon & Tryon (1982); =Aspleniaceae sensu Kramer & Viane (1990); =Aspleniaceae sensu Smith et al. (2006); =Aspleniaceae sensu Christenhusz et al. (2011).

**Characters.**—Plants terrestrial, epipetric, or epiphytic, sometimes rheophytic; roots blackish, wiry, inserted radially or ventrally (*Hymenasplenium*), proliferous or non-proliferous; rhizomes usually odorless, rarely with the odor of wintergreen (e.g., *A. longissimum* Blume), short- to long-creeping, or suberec, branched or more commonly unbranched, sometimes massive and forming a detritus-collecting basket (e.g., *A. nidus* L.), bearing scales; rhizome scales lanceolate, clathrate, usually with blackish cell walls and hyaline lumens, sometimes brown or golden-brown, the margins glandular or not, entire to dentate or ciliate, without distinct pubescence; leaves green and not covered in mucilage during any stage of development, usually monomorphic, sometimes hemic-dimorphic, spirally arranged or distichous and dorsal (*Hymenasplenium*), occasionally bulbiferous, the bulbils frequently at the leaf apex, in a distal pinna axil, or at the base of the lamina, leaves usually closely spaced, sparsely to densely scaly, occasionally pubescent, rarely glandular (e.g., *A. platyneuron* (L.) Britton, Sterns & Poggenb.), also
frequently with minute filiform proscales; petioles dull and greenish, gray, or brownish, or lustrous and castaneous, atropurpureous, or ebenous, the bases expanded in *Hymenasplenium*, otherwise not usually expanded, persistent or not, articulate to the rhizome in *Hymenasplenium*, otherwise not; petiolar vascular bundles two, each with “C”-shaped xylem, the bundles distally uniting to form a single “X”-shaped bundle; laminae soft-herbaceous to coriaceous, simple to 4-pinnate, the apex usually pinnatifid or non-conform, occasionally conform (e.g., *Asplenium davisii* Stolze), the leaf marginal cells usually not differentiated; pinna axes not articulate, among species with divided leaves the axes usually alate, with wings derived from a decurrent and thickened leaf margin, or the wings thin, fragile, and apparently derived from the rachis; the rachis axes usually not sulcate adaxially, without a free central ridge; veins free or anastomosing, the areoles without free included veinlets, reaching the leaf margin or terminating before it, some species with a sub-marginal collecting vein, the vein endings forming hydathodes, or not differentiated; sori elongate, along one side of the vein, rarely paired back to back, and then usually not along the same vein, and if so then usually where groups of veins converge, indusiate; soral receptacle flat; indusia lateral, essentially flat, glabrous or sometimes glandular, opening along the lateral margin; sporangia with stalks one cell wide in the middle; spores monolete, non-chlorophyllous, brown, the perispore with sharp ridges or broad folds, sometimes echinulate, fenestrate, or reticulate; chromosome base numbers x=36 (most species, e.g., Bir & Shukla, 1967; Smith & Mickel, 1977; Walker, 1973a), 38 (*Hymenasplenium*; Murakami, 1995), and 39 (*Hymenasplenium*; Kato et al., 1990; Murakami, 1995).

Aspleniaceae exhibit a broad spectrum of morphological diversity, yet identification of the family is usually not difficult. Diagnostic for the Aspleniaceae are the linear sori with lateral indusia restricted to one side of the vein. The so-called “back-
to-back” or “diploazioid” sori occur in some Aspleniaceae, however, they tend to be restricted to small portions of the lamina. As Holttum (1954) pointed out, patterns of major vein groups suggest that these instances are likely the result of lamina fusion or reduction. Some species of *Hymenasplenium* have been confused with *Diplazium* (Smith, 1976). However, numerous technical apomorphies of the Aspleniaceae serve to distinguish these two genera. See the first lead in the key to families provided below for a list of characters serving to separate Aspleniaceae from other eupolypod II families.

**Biology and phylogeny.** — Aspleniaceae are a distinctive element within Eupolypods II; the family has usually been regarded as readily definable, in its current circumscription, even before Eupolypods II or the Polypodiales (*sensu* Pryer et al., 2008; Smith et al., 2006) were recognized as cohesive entities (e.g., Nayar, 1970). Aspleniaceae are somewhat unusual considering their species-richness, in that they show strong patterns of diversification in both temperate and tropical areas (rather than being predominantly tropical), and have approximately equal numbers of epiphytic and terrestrial species (Schneider et al., 2004a). These two major habit types—epiphytic versus terrestrial—are both scattered across the Aspleniaceae phylogeny, although there is some evidence that the most recent common ancestor of the Aspleniaceae crown clade was epiphytic (Schneider et al., 2004a). Our circumscription is identical to that of Smith et al. (2006), who include further information on this family.

**2.2.6 Thelypteridaceae Ching ex Pic. Serm., Webbia 24 (1970)**

Thelypteroids; marsh ferns, beech ferns, and allies. Approximately 950 species, divided among *Cyclosorus* (incl. *Ampeleopteris, Amphineuron, Chingia, Christella, Cyclogramma, Cyclosorus s.s., Glaphyropteridopsis, Goniopteris, Meniscium, Menisorus, Mesophlebion, Pelazoneuron, Plesioneuron, Pneumatopteris, Pronephrium, Pseudocyclosorus, Sphaerostephanos, Stegnogramma, Steiropteris, Trigonospora*), *Macrothelypteris, Phegopteris,*

<Polypodiaceae: Dryopteridoideae *sensu* Christensen (1938); =Thelypteridaceae *sensu* Holttum (1947); =Thelypteridaceae *sensu* Nayar (1970); =Thelypteridaceae *sensu* Tagawa & lwatsuki (1972); =Thelypteridaceae *sensu* Pichi Sermolli (1977); =Thelypteridaceae *sensu* Ching (1978a); <Thelypteridaceae *sensu* Lovis (1978); =Thelypteridaceae *sensu* Tryon & Tryon (1982); =Thelypteridaceae *sensu* Smith (1990); =Thelypteridaceae *sensu* Smith et al. (2006); =Thelypteridaceae *sensu* Christenhuzs et al. (2011).

**Characters.**—Plants terrestrial, sometimes epipetric or rheophytic, rarely scandent (*Thelypteris* subg. *Amauropelta* sect. *Lepidoneuron*); roots blackish, wiry, inserted radially, non-proliferous; rhizomes not usually branched, short- to long-creeping, sub-erect, or erect, rarely sub-arborescent, bearing scales; rhizome scales lanceolate, non-clathrate, grayish to tan or brown, entire or dentate, the margins and often the surfaces usually bearing distinct pubescence similar to that of the leaves; leaves usually greenish in all stages, occasionally reddish when young (e.g., some *Cyclosorus* species treated in *Mesophlebion* and *Pronephrium,* possibly others), sometimes covered in mucilage when young, usually monomorphic, sometimes sub-dimorphic, spirally arranged, closely to distantly spaced, occasionally bulbiferous, the bulbils usually distal or apical on the leaf, scaly or not, almost always pubescent, the hairs whitish or hyaline, acicular, or sometimes forked, stellate, stalked-stellate, or hamate, also often provided with sessile or stalked glands; petioles greenish to stramineous, sometimes darker, the bases not articulate to the rhizome, not expanded at the base, and generally not persistent on the rhizome; petiole with two vascular bundles (rarely more), the bundles with hippocampiform-shaped xylem, distally uniting to form a single “U”-shaped bundle; laminae thin-herbaceous to coriaceous, simple and entire to 3-pinnate-pinnatifid, in divided leaves, the base with or without a series of reduced pinnae, the apex conform or non-conform, the leaf marginal cells not clearly differentiated; pinna axes not articulate, the pinna base often with a conspicuous aerophore, these usually appearing as a low
elongate or orbicular protuberance, or erect and vermiform, up to ca. 1 cm long; the rachis axes sulcate adaxially or not, when present the sulcae not continuous onto the next order, lacking a free central ridge; veins reaching the leaf margin or terminating before it, free, connivent at or below the sinus in lobed pinnae, or anastomosing in various patterns, the areoles without free included veinlets, the vein endings expanded or not differentiated; sori circular or elongate, on top of veins, not terminal, indusiate or exindusiate; soral receptacle flat; indusia lateral, reniform, sometimes pubescent and or glandular; sporangia with stalks more than one cell wide in the middle, often bearing hairs or glands (paraphysate); spores usually monolette, sometimes trilete (Cyclosorus treated as Trigonospora), non-chlorophyllous, the perispore brown, often with sharp crests, or reticulate or echinulate; chromosome base numbers x=27 (e.g., Parathelypteris; Weng & Qiu, 1988), 29 (e.g., Amauropelta; Walker, 1985), 30 (e.g., Phegopteris; Mitui, 1975), 31 (e.g., Lastrea, Macrothelypteris, Pseudophegopteris, Wagneriopteris; Loyal, 1991; Mitui, 1975; Tindale & Roy, 2002), 34 (e.g., Oreopteris; Holttum, 1981; Manton, 1950), 35 (e.g., Metathelypteris, Pseudocyclosorus, Thelypteris; Loyal, 1991; Mitui, 1975; Walker, 1985), or 36 (e.g., Abacopteris, Ampelopteris, Amphineuron, Christella, Cyclogramma, Cyclosorus, Dictyocline, Goniopteris, Lastrea, Leptogramma, Meniscium, Pronephrium, Stenogramma; Loyal, 1991; Mitui, 1975; Tindale & Roy, 2002; Walker, 1985).

Thelypteridaceae are a large and diverse family, however, they can usually be recognized by the presence of distinctive acicular hairs. These hairs are whitish or hyaline, and usually 1-celled. In addition to being on the leaves, these hairs also regularly occur upon the margins and faces of the rhizome scales. While dentate or ciliate scales are common, as far as we know, no other family in Eupolypods II has rhizome scales that bear hairs similar to those found upon the leaves. Hamate, forked, and stellate hairs also occur in Thelypteridaceae, which being uncommon in the
Eupolypods, are also useful diagnostic characters. Thelypteridaceae also frequently have conspicuous aerophores at the bases of their pinnae. These often differ in color and texture from the surrounding tissue, and are frequently raised. In some cases, elongate vermiform aerophores are present; these frequently occur in species in which the crosiers and young leaves are surrounded by thick mucilage.

**Biology and phylogeny.**—This large family is morphologically cohesive and has been long recognized as such, in its current circumscription (but cf. Hennipman, 1996). Within the Thelypteridaceae, however, generic classifications vary widely, and only two molecular phylogenetic studies have included a substantial representation of the family (approximately 30 species each; Schuettpelez & Pryer, 2007; Smith & Cranfill, 2002). Our circumscription is identical to that of Smith et al. (2006), who discuss this family in further detail.

### 2.2.7 Woodsiaceae Herter, Revista. Sudamer. Bot. 9 (1949)


<Polypodiaceae: Woodsieae: Woodsiinae *sensu* Diels in Engler & Prantl (1897); <Polypodiaceae: Gymnogrammeoideae + Polypodiaceae: Woodsioideae *sensu* Christensen (1938); <Sinopteridaceae + Woodsiaceae *sensu* Ching (1940a); <Dennstaedtiaceae: Dryopteridoideae *sensu* Holttum (1947); =Woodsiaceae *sensu* Herter (1949); <Dryopteridaceae: Dryopteridoideae *sensu* Nayar (1970); <Athyriaceae *sensu* Tagawa & Iwatsuki (1972); =Woodsiaceae *sensu* Pichi Sermolli (1977); =Woodsiaceae *sensu* Ching (1978a); <Dryopteridaceae: Athyrioidae *sensu* Lovis (1978); <Dryopteridaceae: Physematiae *sensu* Tryon & Tryon (1982); <Dryopteridaceae: Athyrioidae: Physematiae *sensu* Kramer et al. (1990b); =Woodsiaceae *sensu* Wu and Ching (1991); <Woodsiaceae *sensu* Smith et al. (2006); =Woodsiaceae *sensu* Christenhusz et al. (2011).

**Characters.**—Plants epipetric, or occasionally terrestrial; roots blackish, wiry, inserted radially, non-proliferous; rhizomes short-creeping, horizontal to sub-erect, commonly unbranched, bearing scales; rhizome scales lanceolate, non-clathrate, the margins glandular or eglandular, without distinct pubescence, entire to denticulate or ciliate, the teeth when present formed by two adjacent cells, or not; leaves green and not
covered in mucilage during any stage of development, usually spirally arranged, monomorphic, closely spaced, bearing scales and hairs, the hairs catenate or terete, sometimes gland-tipped (e.g., *W. mollis* (Kaulf.) J. Sm.), sometimes the scales forming a reduction series that terminates in broad-based, catenate, hair-like scales (e.g., *W. mollis*); petioles stramineous, castaneous or dark purple throughout, or proximally darkened, the base thin, not forming trophopods, persistent, usually forming a thick mantle of old petiole bases, without conspicuous aerophores, in some species with a petiolar articulation, the articulation usually proximal (e.g., *W. ilvensis* (L.) R. Br.) or just below the lamina; petiolar vascular bundles two, the bundles with hippocampiform-shaped xylem, distally uniting to form a single U-shaped bundle; laminae herbaceous, 1-pinnate–2-pinnate-pinnatifid, usually broadest in the middle, the base with a series of reduced pinnae or not, the apex non-conform, the leaf marginal cells differentiated into nodulose hyaline cells or not; pinna axes not articulate, sessile or slightly petiolate; the rachis axes sulcate adaxially, lacking a free central ridge, the grooves not continuous; veins free, terminating before the leaf margin, the vein endings usually expanded and forming hydathodes; sori dorsal along veins, sub-terminal, or terminal (e.g., *W. elongata* Hook.), round, indusiate; soral receptacle distinctly flat; indusia basal, composed of a series of scale-like or filamentous segments or sometimes sac-like globose, glandular, pubescent, or not; sporangia with stalks two or three cells wide in the middle; spores monolete, non-chlorophyllous, tan or brown, the perispore echinate, tuberculate, or with broad folds or narrow crests, these sometimes forming a reticulum; chromosome base numbers \( x = 33 \) (*W. manchuriensis*; Kurita, 1965), 38, 39, or 41 (Brown, 1964; Manton, 1950).

When fertile, Woodsiaceae are easily diagnosed by the unique basal indusium composed of multiple scale-like or filamentous segments (occasionally as a single
globose structure enclosing the sorus), which is unique among ferns. Many Cyatheaceae (in the Cyatheales; see Fig. 2) have basal scale-like indusia, but these are generally more robust, are often spherical or cup-shaped, and do not consist of multiple segments. Some other taxa in the Cyatheaceae have sori protected by scaly indument (e.g., Sphaeropteris subsect. Fourniera (J. Bommer ex Fourn.) P.G. Windisch); in these cases the scales resemble those of the lamina whereas in Woodsia they do not. Sterile leaves of Woodsiaceae, however, are not as easily characterized. When present, the petiolar articulation is a powerful diagnostic character, because it is unique in Eupolypods II, and rare outside of this clade. However, its utility is hindered by its absence from most species. Nonetheless, all species tend to accumulate large mats of persistent petiole bases, which are characteristic. In addition, the combination of 1-pinnate–2-pinnate-pinnatifid leaves with sessile or short-petioled pinnae, laminae usually bearing scales and hairs (that are not acicular), and veins that terminate before the leaf margin in hydathodes serve to diagnose Woodsiaceae. Sterile plants of Cystopteris can appear surprisingly similar to those of Woodsia, however, they can be distinguished by having veins that reach the leaf margin.

**Biology and phylogeny.**—As circumscribed here, species of Woodsiaceae typically occur in rocky, montane areas, predominantly in the northern hemisphere. Areas of particular species-richness include the mountains of Eurasia, and arid areas of Mexico and southwestern USA; one polymorphic species (W. montevidensis (Spreng.) Hieron.) extends south through the Andes and also occurs in South Africa. They have remarkable ecological and morphological resemblance to members of Cystopteris (in the Cystopteridaceae), to which they are only distantly related (Figs. 7, 9).

This family—comprising Woodsia and its segregates—is an isolated lineage, not closely related to the other taxa frequently included in broad concepts of Woodsiaceae.
(e.g., Smith et al., 2006). Protowoodsia and Cheilanthopsis are nested within Woodsia s.l. (Rothfels et al., 2012a), as is Hymenocystis, the other segregate genus recognized by Shmakov (2003; A. Larsson, unpublished). The molecular phylogeny of Woodsia is marked by a remarkably deep split between a clade of Old World or holarctic species, and a clade of predominantly New World species. This deep dichotomy essentially mirrors the results of Brown’s (1964) groundplan divergence scheme (Wagner, 1980b) based upon morphological characters.

### 2.2.8 Athyriaceae Alston, Taxon 5 (1956)

Athyrioids; ladyferns, and allies. Approximately 600 species, in Anisocampium (4 species; incl. Kuniwatsukia), Athyrium (~220 species; incl. Pseudocystopteris), Cornopteris (9 species; incl. Neoathyrium), Deparia (~70 species; incl. Athyriopsis, Lunathyrium, Dictyodroma, Dryoathyrium, and Triblenma), and Diplazium (~300 – 400 species; incl. Allantodia, Anisogonium, Callipteris, Monomelangium; excl. Diplazium flavoviride Alston); (Adje et al., 2008; Kato, 1979, 1984; Liu, 2008; Liu et al., 2011; Pacheco & Moran, 1999; Sano et al., 2000b; Tryon & Tryon, 1982).

<Polypodiaceae: Aspleniodeae sensu Christensen (1938); <Dennstaedtiaceae: Athyriodeae sensu Holttum (1947); <Athyriaceae sensu Alston (1956); <Dryopteridaceae: Athyriodeae sensu Nayar (1970); <Athyriaceae sensu Tagawa & Iwatsuki (1972); <Athyriaceae sensu Ching (1978a); <Dryopteridaceae: Athyriodeae sensu Lovis (1978); <Dryopteridaceae: Physematieae sensu Tryon & Tryon (1982); <Dryopteridaceae: Athyriodeae: Physematieae sensu Kramer et al. (1990b); <Athyriodeae: Athyriodeae + Depariodeae + Diplazioideae sensu Wang et al. (2004); <WOODSIAESE SENSU Smith et al. (2006); <Athyriaceae sensu Christenhusz et al. (2011).

**Characters.**—Plants terrestrial or epipetric, sometimes rheophytic; roots blackish, wiry, inserted radially, non-proliferous; rhizomes short- to long-creeping, or suberect to erect, branched or more commonly unbranched, bearing scales, and sometimes golden hairs similar to the root hairs (e.g., Athyrium skinneri (Baker) Diels); rhizome scales lanceolate, not or only weakly clathrate, the margins usually non-glandular, sometimes glandular (some Deparia), without distinct pubescence, entire or dentate, when present the teeth commonly formed by two adjacent cells (Diplazium); leaves sometimes
internally mucilaginous (some Deparia and Diplazium spp., particularly those treated as Callipteris), not externally covered in mucilage during any stage of development, green in Diplazium and Deparia, the petiole and rachis frequently with a pink hue in Athyrium presumably due to anthocyanins, monomorphic, spirally arranged or sometimes distichous and dorsal (e.g., Athyrium skinneri), occasionally bulbiferous, closely to distantly spaced, sparsely to moderately scaly and occasionally pubescent (Athyrium and Diplazium) or with a reduction series beginning with scales at the base of the leaf that gradually reduce to catenate hairs distally (Deparia, and some Diplazium); petioles castaneous, stramineous, or proximally darkened, often with a proximal thickening forming trophopods that may be starch-filled (Athyrium, Cornopteris, and Diplazium), often with conspicuously elaborated aerophores (elsewhere termed pneumatophores; Cornopteris, some Athyrium, some Deparia), the bases usually persistent, rarely articulate to the rhizome (e.g., Anisocampium and Athyrium skinneri), and sometimes with golden hairs similar to the root hairs (e.g., A. skinneri); petiolar vascular bundles two (rarely more; Kato, 1972), each with hippocampiform xylem, the bundles distally uniting to form a single U- or V-shaped bundle; laminae soft-herbaceous to coriaceous, simple to 3-pinnate-pinnatifid, the apex usually pinnatifid or non-conform, sometimes conform in Diplazium, the leaf marginal cells differentiated into nodulose hyaline cells or not; pinna axes not articulate, sometimes muricate (Diplazium), sulcate adaxially, lacking a free central ridge; the rachis grooves V-shaped (Anisocarpium, Athyrium, and Cornopteris) or U-shaped (Deparia and Diplazium), continuous (Anisocampium, Athyrium, Cornopteris, most Diplazium) or not continuous (Deparia, some Diplazium), the sulcus wall of the rachis usually continuing as a prominent ridge onto the sulcus wall of the costa (but not in Deparia); veins free or sometimes anastomosing (Deparia species treated as Dictyodroma, and some Diplazium), the areoles without free included veinlets, usually
terminating before the leaf margin, the vein endings slightly raised and expanded, or forming hydathodes, or not differentiated; sori usually elongate, sometimes round, not terminal, on top of the vein, or along one side, singular or paired back-to-back along the same vein, or hooked in most Athyrium (i.e., paired back-to-back and crossing over the vein at one end in J- or U-shapes), or rarely sori marginal (e.g., Deparia prolifera (Kaulf.) Hook. & Grev.), at the tips of vein endings, usually indusiate; soral receptacle flat; indusia lateral, vaulted or essentially flat, glabrous or glandular, opening along the lateral margin; sporangia with stalks two or three cells wide in the middle; spores monolete, non-chlorophyllous, brown, the perispore nearly plain to coarsely tuberculate, echinate, or folded, the folds short and low, forming a rugate surface, or broad and wing-like; chromosome base numbers x=40 (Athyrium, Deparia and some Cornopteris; Kato, 1979; Liu et al., 2011; Manton & Sledge, 1954; Sano et al., 2000a; Sano et al., 2000b), or 41 (Diplazium, and some Cornopteris; Dawson et al., 2000; Kato, 1979; Manton & Sledge, 1954). Reports of x=41 for Deparia require confirmation (reviewed in Sano et al., 2000a), and reports for individual Cornopteris species are occasionally inconsistent (alternating between x=40 and x=41), indicating that further cytological study is needed.

Several genera of Athyriaceae can be diagnosed by unique or rare character states. However, character state reversals and homoplasy render these characters imperfect diagnostics for the family. Deparia typically has broad scales present at the base of the leaf that transition along a homologous series to septate hairs. Similar hairs occur in Diplazium (species treated in Callipteris by Pacheco & Moran, 1999), Acystopteris, and some species of Woodsia (e.g., W. mollis)—other species of Woodsia have septate hairs, but these are not reduced from broad scales. Many eupolypod II ferns have reduced filiform scales, but in most cases these never approach the septate hairs found in Deparia (see Figure 3 in Sano et al., 2000b). Deparia also differs from most Eupolypods...
II in having sulcate rachises that are not continuous with the sulcae of the pinna costae. Many *Athyrium* and *Cornopteris* species have red-tinged leaves. This color is visible in live plants as well as on herbarium specimens. Blechnaceae also have reddish leaves and this has been attributed to the presence of anthocyanins (Crowden & Jarman, 1974). That family, and the other eupolypod II families with reddish leaves, differ in that the red coloration is only present in developing leaves and is not visible by maturity.

Another useful character of limited distribution is the corniculae/scales that are present adaxially at the junction of the pinna costa and the rachis in many *Athyrium* and *Cornopteris*. In addition, many *Athyrium* and some *Diplazium* species have small epidermal spinules along the adaxial pinna costae. Outside of the Athyriaceae, adaxial corniculae occur only in Rhachidosoraceae and *Onocleopsis* (Onocleaceae). In the Eupolypods I, similar structures also occur in *Didymochlaena*, and outside of the Eupolypods similar structures occur in *Pteris* (Pteridaceae). Athyriaceae also frequently have well-developed trophopods, which consist of a thickened petiole base that is often starch-filled, persistent upon the rhizome, and in some cases highly sclerified. The trophopods of some *Athyrium* and *Deparia* are additionally adorned with toothed or wing-like protuberances, referred to as pneumatophores by Iwatsuki (1970) and Kato (1984).

**Biology and phylogeny.**—Athyriaceae are mostly medium-sized understory terrestrial ferns, comprising three major clades that correspond to the subfamilies Athyrioideae, Diplazioideae, and Deparioideae of Wang et al. (2004); with the exception of *Diplaziosis* and *Homalosorus,* which Wang et al. (2004) include in Diplazioideae, and which we place in Diplaziopsidaceae). This alliance of “athyriid,” “diplaziid,” and “depariid” ferns (Rothfels et al., 2012a) has a long history; at some point they have all been treated in a broad concept of *Athyrium* (e.g., Copeland, 1947). The sister-group
relationship of the athyriids and diplaziids, and they together as sister to the depariids, was anticipated first by Hiraoka (1978), suggested by the single-locus data of Sano et al. (2000a), and strongly supported by multi-locus molecular data (Rothfels et al., 2012a; Schuettpelz & Pryer, 2007). Character evolution in the Athyriaceae is complex, and the generic-level relationships within the two large clades (athyriids and diplaziids) need further investigation (e.g., Liu et al., 2011).

2.2.9 Blechnaceae Newman, Hist. Brit. Ferns (1844)

Blechnoids; deer ferns, chain ferns, and allies. Approximately 200 species in Blechnum s.l. (~150 species; incl. many potential segregates), Brainea (1 species), Diploblechnum (2 species), Doodia (~15 species), Pteridoblechnum (2 species), Sadleria (6 species), Salpichlaena (3 species), Steenisoblechnum (1 species), Stenochlaena (8 species), and Woodwardia (14 species; incl. Anchistea, Lorinseria); (Cranfill & Kato, 2003; Holttum, 1971b; Moran, 1990).

<Polypodiaceae: Pteridoideae + Polypodiaceae: Blechnoideae sensu Christensen (1938);
=Dennstaedtiaceae: Blechnoideae sensu Holttum (1947); =Blechnaceae sensu Nayar (1970);
<Blechnaceae + Pteridaceae sensu Tagawa & Iwatsuki (1972); =Blechnaceae sensu Pichi Sermolli (1977);
=Blechnaceae + Stenochlaenaceae sensu Ching (1978a); =Blechnaceae sensu Lovis (1978); =Blechnaceae sensu Tryon & Tryon (1982); =Blechnaceae sensu Kramer et al. (1990a); =Blechnaceae sensu Smith et al. (2006); =Blechnaceae sensu Christenhuz et al. (2011).

Characters.—Plants terrestrial or climbing (by means of rhizomes in Stenochlaena and Blechnum sect. Lomaria, or by leaves in Salpichlaena), rarely epiphytic or rheophytic; roots blackish, wiry, inserted radially, non-proliferous; rhizomes short- to long-creeping, suberect, or erect, sometimes massive and arborescent (Blechnum sect. Lomarioycas (J. Sm.) C. V. Morton, and Sadleria), branched, or more commonly unbranched, sometimes stoloniferous (Blechnum sect. Eublechnum Hooker & Baker), bearing scales; rhizome scales lanceolate to linear-lanceolate, non-clathrate, light-brown to blackish, the margins glandular or not, entire or dentate, without distinct pubescence; leaves reddish when young, green at maturity, sometimes covered in mucilage when young (some Blechnum),
monomorphic or dimorphic, spirally arranged, occasionally bulbiferous, the bulbils frequently in a distal pinna axil, leaves usually closely spaced, sparsely to densely scaly, sometimes pubescent, sometimes with glandular nectaries (e.g., *Stenochlaena palustris* (Burm. f.) Bedd., *Blechnum orientale* L.); petioles greenish to dark brown or atropurpureous, the bases not expanded, not articulate to the rhizome, usually not persistent; petiolar vasculature with two large bundles on the adaxial side of the petiole and an arc of smaller bundles on the abaxial side of the bundle, rarely petioles with only two bundles (e.g., *Woodwardia areolata* (L.) T. Moore), the larger bundles with hippocampiform-shaped xylem, distally uniting to form a single “U”-shaped bundle; laminae soft-herbaceous to more often coriaceous, pinnatifid to 2-pinnate-pinnatifid, the base with or without a series of reduced pinnae, the apex conform or not, the leaf marginal cells differentiated and scarious or membranaceous, or non-differentiated; pinna axes articulate (*Stenochlaena*), or usually non-articulate, pinna bases sometimes with conspicuous aerophores in *Blechnum*, the aerophores appearing as a low protuberance or elongate and vermiform (e.g., *Blechnum violaceum* (Fée) C. Chr.); rachis axes sulcate adaxially, rarely not (e.g., some *Woodwardia*), the sulcae not continuous onto the next order, lacking a free central ridge; veins anastomosing, or more commonly with costular areoles and otherwise free, the areoles without free included veinlets, reaching the leaf margin or terminating before it, the vein endings forming hydathodes, or not differentiated; sori elongate, along one side of the costular commissural vein, indusiate, or sori acrostichoid and exindusiate (*Stenochlaena*); soral receptacle flat; indusia lateral, essentially flat, glabrous or sometimes glandular, opening along the lateral margin with the opening facing the costa; sporangia with stalks more than one cell wide in the middle; spores monolete, occasionally chlorophyllous (e.g., *Blechnum nudum* (Labill.) Leurss.; Sundue et al., 2011), usually non-chlorophyllous, usually pale brown or tan, the
perispore with sharp ridges, broad folds, echinulate, tuberculate, foliose, or nearly plain;
chromosome base numbers $x=27$ (Pteridoblechnum; Tindale & Roy, 2002), 29 (Blechnum;
Walker, 1973a), 31 (Blechnum; Walker, 1973a), 32 (Doodia; Tindale & Roy, 2002), 33
(Blechnum, Sadleria; Smith & Mickel, 1977; Wagner, 1995; Walker, 1973a), 34 (Blechnum,
Woodwardia; Manton & Sledge, 1954; Tryon & Tryon, 1982), 35 (Brainea, Woodwardia; Aziz
Bidin, 1995; Britton, 1964; Wagner, 1955), 36 (Blechnum; Walker, 1973a), 37 (Stenochlaena;
Manickam & Irudayaraj, 1988), or 40 (Salpichlaena; Walker, 1973b).

Blechnaceae are unique among ferns in having elongate sori along a sub-costular
commissural vein that is parallel to the pinna costa, with an indusiate sorus that opens
to face the costa. Other ferns with elongate sori lack a commissural vein, and have
indusia that face the costa at a low angle (not parallel) or are exindusiate. Blechnaceae
also differ from nearly all other Eupolypods II by having petioles with a vascular
anatomy that resembles those of Eupolypods I. That is, in addition to the two large
bundles on the adaxial side of the petiole, there is an arc of smaller bundles along the
abaxial side of the petiole. However, Woodwardia areolata is aberrant among Blechnaceae
in having only two.

Some genera of the Onocleaceae, such as Matteuccia and Pentarhizidium, have a
strong superficial resemblance to Blechnaceae. Those genera can be differentiated by
fertile leaves with a modified leaf margin that opens to face the costa and the indusium
itself, which is inconspicuous and faces away from the costa. Plagiogyria (Cyatheales; see
Fig. 2) also appears similar; the shape of the lamina, dimorphic leaves, and young leaves
covered in mucilage are all reminiscent of Blechnum sect. Parablechnum. Other characters
of Plagiogyria, however, support its position among the Cyatheales, namely the
sporangial capsule with an oblique annulus, trilete spores, and a perispore with well-
formed rodlets.
Biology and phylogeny.—Blechnaceae are cosmopolitan, with a wide range of growth habits, including tall arborescent species, near-annual roadside weeds, and tropical lianas. Like their sister group, the Onocleaceae, Blechnaceae have a high frequency of fertile/sterile leaf dimorphism. Many of the smaller Blechnaceae genera nest within Blechnum s.l. (Cranfill, 2001; Nakahira, 2000; Schuettpelz & Pryer, 2007). While generic circumscription remains incomplete, the family is well defined and historically stable (with the exception of Stenochlaena, which was of uncertain affinity prior to molecular data, a challenging situation further complicated by Christensen having including three widely divergent taxa in his original description of the genus; Christensen, 1906; Holttum, 1971b). Our circumscription is identical to that of Smith et al. (2006), who provide further information on this family.

2.2.10 Onocleaceae Pic. Serm., Webbia 24 (1970)

Onocleoids; sensitive fern, ostrich fern, and allies. Five species in Matteuccia (1 species), Onoclea (1 species with two varieties), Onocleopsis (1 species), and Pentarhizidium (2 species). (Gastony & Ungerer, 1997; Rothfels et al., 2012a).

=Polypodiaceae: Onocleoidae sensu Christensen (1938); ="unplaced": Onocleoidae sensu Holttum (1947); =Dryopteridaceae: Onocleoidae sensu Nayar (1970); =Onocleaceae sensu Pichi Sermolli (1977); =Onocleaceae sensu Ching (1978a); =Dryopteridaceae: Onocleoidae sensu Lovis (1978); =Dryopteridaceae: Onocleaceae sensu Tryon & Tryon (1982); =Dryopteridaceae: Athyrioidae: Onocleaceae sensu Kramer et al. (1990b); =Dryopteridaceae: Onocleaceae sensu Gastony & Ungerer (1997); =Onocleaceae sensu Smith et al. (2006); =Onocleaceae sensu Christenhusz et al. (2011).

Characters.—Plants terrestrial, often in wet or seasonally wet habitats; roots blackish, wiry, inserted radially, non-proliferous; rhizomes short-creeping, unbranched, and erect (up to 1 m tall in Onocleopsis), or long-creeping, and branched (Onoclea), rhizomes sometimes stoloniferous (Matteuccia), bearing scales; rhizome scales lanceolate, non-clathrate, brown, the margins eglandular, entire or dentate, without distinct pubescence; leaves greenish and not covered in mucilage during any stage of development, dimorphic, spirally arranged, leaves usually closely spaced (sometimes
distantly spaced in *Onoclea*), sparsely to densely scaly, sometimes pubescent; petioles
greenish to stramineous, the bases not articulate to the rhizome, expanded and often
starch-filled (forming trophopods), persistent on the rhizome, sometimes for decades,
forming a massive protective sheath in *Matteuccia* and *Pentarhizidium*; petioles with two
vascular bundles, the bundles with hippocampiform-shaped xylem, distally uniting to
form a single “U”-shaped bundle; laminae herbaceous, pinnatifid to 1-pinnate-
pinnatifid, the base with or without a series of reduced pinnae, the apex pinnatifid or
non-conform, the leaf marginal cells scarious or not differentiated (*Matteuccia*); pinna
axes not articulate; the rachis axes sulcate adaxially, the sulcae not continuous onto the
next order, lacking a free central ridge; veins mostly reaching the leaf margin, or
terminating before it in *Pentarhizidium*, free or anastomosing (*Onoclea* and *Onocleopsis*),
the areoles without free included veinlets, the vein endings expanded in *Pentarhizidium*,
otherwise not differentiated; sori orbicular, terminal on the vein, indusiate (except *P.*
*intermedium*); soral receptacle raised, conical; indusia lateral, triangular, ephemeral;
sporangia with stalks more than one cell wide in the middle; spores monolete,
chlorophyllous, the perispore brown, perispore with broad folds and echinulae;
chromosome base numbers $x=37$ (*Onoclea*; Haufler & Soltis, 1986), 39 (*Matteuccia*; Kurita,
1960), or 40 (*Onocleopsis, Pentarhizidium*; Gastony & Ungerer, 1997; Tsai & Shieh, 1985).

Onocleaceae can be diagnosed by having dimorphic leaves, petioles with two
vascular bundles, and thickened petiole bases, chlorophyllous spores, and sori with
conical receptacles. Blechnaceae appear similar, but differ by having petioles with more
than two vascular bundles (except *Woodwardia areolata*, which has two) and that are not
expanded at the base, leaves that are reddish when young, and indusia that open to face
the costa.
**Biology and phylogeny.**—Onocleaceae are a small family, yet one of the most familiar to residents of the north-temperate zone. The family is noteworthy for the strong fertile/sterile leaf dimorphism of its members, their typically large size, chlorophyllous spores, and unusual distributions: *Matteuccia* is circumboreal; *Onoclea* is disjunct between eastern North America and eastern Asia; *Onocleopsis* is endemic to southern Mexico and Guatemala; and *Pentarhizidium* is limited to eastern Asia. Our circumscription is identical to that of Smith et al. (2006), who provide further information on this family.

### 2.3 Key to Eupolypod II families

1. Sori elongate, usually on one side of the vein, rarely paired back-to-back on a single vein, never curving over to the other side of the vein and forming a “U”- or “J”-shape; petioles with two vascular bundles, these united distally to form an “X” shape as seen in cross-section, vascular bundles with xylem in the shape of a “C” as seen in cross-section; rhizome scales clathrate, rarely with darkened indurate lumens; sporangial stalks one cell wide in the middle..........................**Aspleniaceae**

1. Sori elongate or round, on top of the vein, on one side, paired back-to-back, or on one side of the vein and curving over to other side and forming a “U”- or “J”-shape; petioles with more than two vascular bundles, or if two, then these distally united to form a “U”- or “V”-shape as seen in cross-section, largest vascular bundles with hippocampiform-shaped xylem; rhizome scales non-clathrate (except Rhachidosoraceae, some Cystopteridaceae); sporangial stalks more than one cell wide in the middle, usually three cells wide.

2. **Petiole with more than two vascular bundles** (two in some *Woodwardia*); sori elongate, parallel to the costa, on a sub-costular commissural vein connecting lateral veins, indusiate, with the opening facing the costa, or sori
acrostichoid (*Stenochlaena*); leaves reddish when young, not reddish at maturity

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**Blechnaceae**

2. **Petiole with two vascular bundles** (rarely more in Athyriaceae and Thelypteridaceae); sori on, along, or at the apex of a lateral vein, round or elongate, never acrostichoid, if elongate then usually at an angle to the costa, when parallel to the costa, the indusium opening to face the segment margin (away from the costa) or exindusiate; leaves green in all stages, or if reddish when young, then reddish at maturity as well (except some Thelypteridaceae and *Onoclea sensibilis* L., which are reddish only when young).

3. Fertile leaves strongly dimorphic with sori protected by contracted and inrolled segment margins; spores chlorophyllous

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**Onocleaceae**

3. Fertile leaves holomorphic or partially dimorphic (some Thelypteridaceae), the segments weakly contracted and not inrolled; spores not chlorophyllous.

4. **Leaves pubescent** (rarely lacking hairs), the hairs acicular, forked, stellate, or hamate; rhizome scales often bearing similar hairs along the margin and surfaces; indusia, when present, reniform and attached laterally; pinna base usually with a prominent aerophore, the aerophore raised, orbicular, elongate, or vermiform; leaves sometimes mucilaginous when young

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**Thelypteridaceae**

4. **Leaves glabrous or pubescent, the hairs not acicular, simple, never forked or hamate**; rhizome scales ciliate or denticulate, but not bearing hairs similar to the leaves; indusia,
when present, attached basally or laterally, if laterally then elongate or a minute scale, not reniform; pinna base without a prominent aerophore; leaves never mucilaginous.

5. Indusium attached basally, encircling the sorus, globose, or composed of multiple scale-like or filamentous segments.......................................................... Woodsiaceae

5. Indusium attached laterally, or if attached basally then not encircling the indusium, and composed of a single scale-like segment, or exindusiate.

6. Sori round, indusiate, and the receptacle slightly raised and hardened, or sori slightly elongate (not more than 2× longer than wide), exindusiate, the soral receptacle flat; veins reaching segment margin; indusium, when present, basal, a minute hood-like scale, arching over the sorus, frequently deciduous..............................Cystopteridaceae

6. Sori usually elongate, several times longer than wide, sometimes round (some Athyriaceae), indusiate, the soral receptacle flat; veins usually ending before segment margin; indusium lateral, vaulted or essentially flat, opening along the lateral margin, usually persistent.

7. Rhizome scales clathrate; vein endings undifferentiated, neither expanded, raised nor forming hydathodes..........................Rhachidosoraceae

7. Rhizome scales non-clathrate; vein endings differentiated, either thickened, raised, or forming hydathodes.

8. Veins forming a sub-marginal collecting vein; leaf margin with a broad membranaceous border; pinna bases subcordate, the basiscopic lobes overlapping the rachis.................................Hemidictyaceae
8. Veins free or anastomosing, but not forming a sub-marginal collecting vein; leaf margin scarious or undifferentiated, but not with a broad membranaceous border; pinna bases truncate, cuneate, or excavate, but not subcordate, and not overlapping the rachis.

9. Sori usually along one side of the vein, rarely paired back-to-back; roots pale, fleshy; sori vaulted, the indusium often splitting apically prior to opening laterally; veins raised and cartilaginous on the adaxial side of the lamina.

.......................................................... Diplaziopsidaceae

9. Sori usually along both sides of the vein, either paired back-to-back, or crossing over the vein and “U”- or “J”-shaped (on top of the vein in Cornopteris and some Athyrium); roots blackish, wiry; sori usually flat, sometimes vaulted, indusium never splitting apically prior to opening laterally; veins often expanded, but not raised or cartilaginous on the adaxial side of the lamina.......................................................... Athyriaceae

2.4 Acknowledgments

We thank He Hai (College of Life Sciences, Chongqing Normal University) for providing photos of Cystoathyrium, and Art Gilman, Amanda Grusz, Layne Huiet, Anne Johnson, Fay-Wei Li, Yea-Chen Liu, Erin Sigel, Alan Smith, Michael Windham, editor Libing Zhang, and two anonymous reviewers for helpful comments on earlier drafts. This work was supported by funding from a National Science and Engineering Research Council (Canada) PGSD to CJR, a Duke University Graduate School Semester Fellowship to CJR, the Swedish Research Council for Environment, Agricultural Sciences and Spatial Planning (“Formas”; to AL as part of grant 2006–429 to Petra
Korall), an NSF DDIG award to KMP and CJR (DEB-1110767), NSF DEB-1119695 to MAS, and an NSF CAREER award to KMP (DEB-0347840).
3. A plastid phylogeny of the cosmopolitan fern family Cystopteridaceae (Polypodiopsida)


3.1 Introduction

Cystopteridaceae is an enigmatic group of ferns consisting of at least three genera—Acystopteris Nakai, Cystopteris Bernh., and Gymnocarpium Newman—and possibly Cystoathyrium Ching (Rothfels et al., 2012a; Rothfels et al., 2012b). The latter includes a single species from China, which is known only from the type specimen at PE and may be extinct (Rothfels et al., 2012b). Although Cystoathyrium has not been included in any molecular phylogenetic study to date, the limited morphological information available suggest that it may be a member of Cystopteridaceae (Rothfels et al., 2012b; Sundue & Rothfels, 2012 (in prep)). The other three genera comprise approximately 35 species: three in Acystopteris, and about 25 in Cystopteris and seven in Gymnocarpium (Blasdell, 1963; Pryer, 1993; Sarvela, 1978; see Appendix E).

Cystopteris and Gymnocarpium—including the bulblet fern, bladder ferns, fragile ferns, and oak ferns (see Fig. 10A–C, E–H)—are among the most frequently encountered and familiar ferns in the northern hemisphere, occurring in most forested and rocky habitats in North America, Europe, and Asia. However, despite their familiarity, their phylogenetic relationships have been contentious. Acystopteris (Fig. 10D) and Cystopteris have long been considered close relatives, with many authors historically treating them
together under a broad concept of *Cystopteris* (e.g., Blasdell, 1963; Tagawa, 1935). Prior to the proliferation of molecular evidence, however, most taxonomists did not consider *Cystopteris s.l.* and *Gymnocarpium* to be closely allied. Instead, these two taxa usually were assigned to the dryopteroid and athyrioid fern lineages, respectively, with the caveat that they were each morphologically anomalous and their phylogenetic position was thus uncertain (e.g., Sledge, 1973). Ching (1940a) was an early exception in that he
placed the two genera together, although he mistakenly considered them to be closely related to the athyrioid ferns. This confusion regarding the affinities of *Cystopteris* and *Gymnocarpium* to other polypod ferns has continued until very recently. For example, when naming the family Cystopteridaceae, Schmakov (2001) included *Pseudocystopteris* (which belongs in the Athyriaceae; Fraser-Jenkins, 2008; Kato, 1977; Liu, 2008; Rothfels et al., 2012b; Sano et al., 2000a). Quite recently, Z. R. Wang (1997), M. L. Wang et al. (2004) and Smith et al. (2006) each advocated familial concepts that grouped *Cystopteris* and *Gymnocarpium* with very distantly related taxa (Rothfels et al., 2012b).

Wolf et al. (1994) and Hasebe et al. (1995) provided the first molecular evidence that *Cystopteris* and *Gymnocarpium* are, indeed, closely allied to one another, and that they are evolutionarily distinct from both the dryopteroid (Dryopteridaceae *sensu* Smith et al. (2006)) and the athyrioid ferns (Athyriaceae *sensu* Rothfels et al. (2012b)). Subsequent molecular phylogenetic studies—those with greater taxon and character sampling—have yielded an increasingly clear understanding of the relationships among the three genera and of the critical position of the Cystopteridaceae as sister to the rest of the large eupolypod II clade (Kuo et al., 2011; Rothfels et al., 2012a; Sano et al., 2000a; Schuettpelz & Pryer, 2007). These studies reveal that the family Cystopteridaceae is a deeply isolated lineage within eupolypod ferns—it last shared a common ancestor with other extant fern lineages approximately 100 million years ago (Rothfels et al., 2012a; Schuettpelz & Pryer, 2009).

While the placement of Cystopteridaceae within the fern tree of life and relationships among three of the four included genera are now well established, intrageneric relationships are poorly understood, and the boundaries among recognized species remain unclear. *Cystopteris s.l.* was last monographed 50 years ago (Blasdell, 1963; he included *Acystopteris* in his generic concept), and the closest we have to a global
monograph of *Gymnocarpium* is a 6-page synopsis (Sarvela, 1978). Both *Cystopteris* and *Gymnocarpium* have extremely broad geographical distributions. Populations of *Gymnocarpium dryopteris* (L.) Newman, for example, are scattered across the northern half of both North America and Eurasia (Fig. 11A). And *Cystopteris fragilis* (L.) Bernh. s.l. is perhaps the most widely distributed fern in the world, ranging from the high arctic to Tierra del Fuego in the Americas, blanketing most of Eurasia, occurring in eastern and southern Africa, eastern Australia, New Zealand, Hawai‘i, and many isolated rocky oceanic islands (Fig. 11C, D; the “*Cystopteris fragilis* complex). The small number of geographically focused biosystematic investigations undertaken to date have revealed that these widespread species include multiple independent lineages, which often differ in ploidy level, and typically involve reticulate evolutionary histories (Haufler & Windham, 1991; Haufler et al., 1990; Pryer et al., 1983; Pryer & Haufler, 1993; Vida, 1972, 1974).

In order to explore the intriguing patterns of diversification and phylogeography within these species complexes, we first need to understand their basic phylogenetic relationships. Here, we assemble a three-locus plastid dataset that encompasses most recognized species of Cystopteridaceae, including multiple accessions from across the geographic range of many taxa (especially the polyploids). Our primary goal is to establish the first robust phylogeny for the family—focusing on the branching (divergent) relationships as a necessary prerequisite for investigating the details of reticulate evolution and species boundaries within the three genera of Cystopteridaceae (Rothfels et al. in prep.).
Figure 11: Geographic ranges of the sampled Cystopteridaceae taxa. Ranges are approximated by the colored polygons, with dotted lines indicating uncertain range limits. Colored circles indicate the collection location of the vouchers used in this study; a numeral inside the colored circle indicates the number of accessions that came from that location. A: Gymnocarpium. B: Acystopteris and Cystopteris montana. C: The bulbifera clade, the sudetica clade, and Cystopteris laurentiana and reevesiana. D: The fragilis complex (excluding C. laurentiana and C. reevesiana, which are presented in panel C). Range boundaries determined from published floristic works (Bir & Trikha, 1974; Breckle, 1987; Britton et al., 1984; Crouch et al., 2011; Denk, 1998; Fraser-Jenkins, 1986; Fraser-Jenkins, 2008; Hauffer et al., 1993; Hauffer & Windham, 1991; Hauffer et al., 1990; Latorre, 2000; Lobin, 1986; Mickel, 1972; Mickel & Tejero-Díez, 2004; Moran, 1983a; Murphy & Rumsey, 2005; Prada, 1986; Pryer, 1993; Salvo & Otermin, 1986; Sarvela, 1978; Sarvela et al., 1981; Japanese Society of Plant Systematics, 2012; Tagawa, 1935; Velayos et al., 2001; Wang, 2008)

3.2 Materials and Methods

3.2.1 Taxonomic Sampling

We analyzed an ingroup sample of 75 accessions selected to maximize the inclusion of named taxa, based primarily on published taxonomic works (Bir & Trikha, 1974; Blasdell, 1963; Hauffer et al., 1993; Mickel, 1972; Mickel & Tejero-Díez, 2004; Moran, 1983a; Pellinen et al., 1998; Pryer et al., 1983; Pryer & Hauffer, 1993; Sarvela, 1978; Sarvela et al., 1981; Tagawa, 1935; Wang, 2008). For widespread species, we attempted to include multiple accessions from across their geographic ranges. To root the tree, we included an outgroup of nine species from the remainder of Eupolypods II—the sister group to Cystopteridaceae (Rothfels et al., 2012a; Schuettplz & Pryer, 2007). The total sample included 84 accessions (Appendix C).

3.2.2 DNA Isolation, Amplification, and Sequencing

DNA was extracted from herbarium specimens or silica-dried material in the Fern Lab Database (http://fernlab.biology.duke.edu/) using a 96-well modification (Beck et al., 2011a; doi:10.5061/dryad.11p757m0) of a standard CTAB protocol (Doyle & Dickson, 1987), or with the DNeasy kit (Qiagen, Valencia, California, USA). Three plastid loci were selected for analysis: matK, rbcL, and the trnG-trnR intergenic spacer (henceforth “trnG-R”). Primer sequences and associated data are provided in Table 3. The full lengths of matK and trnG-R were amplified and sequenced in two overlapping portions, using the primers AjmatKf1+AjmatKr3B and AjmatKf3+AjmatKr1 for matK and
Table 3: Primers used for amplification and sequencing in Chapter Three.

<table>
<thead>
<tr>
<th>Locus</th>
<th>Primer</th>
<th>Sequence (5’ – 3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>matK</td>
<td>AJmatKf1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>F GTATTACAKAAAGTGRAGRGCTTAG</td>
</tr>
<tr>
<td>matK</td>
<td>AJmatKf3&lt;sup&gt;1&lt;/sup&gt;</td>
<td>F TGGAAAAGGYAYTCA GTGYCGGTCTTGG</td>
</tr>
<tr>
<td>matK</td>
<td>AJmatKr1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>R ATYTCAATCTACGCAATCCAT</td>
</tr>
<tr>
<td>matK</td>
<td>AJmatKr3B&lt;sup&gt;1&lt;/sup&gt;</td>
<td>R CGATTTTGTATGTAATAAATTTCG</td>
</tr>
<tr>
<td>rbcL</td>
<td>ESRBCL1f&lt;sup&gt;2&lt;/sup&gt;</td>
<td>F TCAGGACTCCACTTACTAGCTTCACG</td>
</tr>
<tr>
<td>rbcL</td>
<td>ESRBCL663R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>R TACRAATARGA AAGCTCTCTCCAACG</td>
</tr>
<tr>
<td>rbcL</td>
<td>ESRBCL1361R&lt;sup&gt;2&lt;/sup&gt;</td>
<td>R TCAGAATCCACTTACTAGCTTCACG</td>
</tr>
<tr>
<td>trnG-R</td>
<td>trnGf1&lt;sup&gt;3&lt;/sup&gt;</td>
<td>F GCCGGTGATAGTTTAGTGGTAA</td>
</tr>
<tr>
<td>trnG-R</td>
<td>CRcysTRNGf1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>F GCTAYACGACCAARACGTAAGC</td>
</tr>
<tr>
<td>trnG-R</td>
<td>CRcysTRNGr1&lt;sup&gt;1&lt;/sup&gt;</td>
<td>R GTGCGATCCATAAAAATCYATGTCAG</td>
</tr>
<tr>
<td>trnG-R</td>
<td>trnR22R&lt;sup&gt;3&lt;/sup&gt;</td>
<td>R CATCCATTAGACGATGGACG</td>
</tr>
</tbody>
</table>

Notes: F = forward; R = reverse. Superscripts indicate place of first publication: 'This study; 2Schuettpelz & Pryer (2007); 3Nagalingum et al. (2007).

trnG1F+CRcysTRNGr1 and CRcysTRNGf1+trnR22R for trnG-R. Most rbcL sequences were amplified in one piece using the primer pair ESRBCL1f+ES1361r. For degraded DNA from herbarium specimens, rbcL was also amplified in two pieces, using the primer pairs ESRBCL1f+ES633R and ES645F+ES1361R. Loci were amplified in 21 µL reactions consisting of 2 µL Denville buffer (10x), 2 µL dNTPs (each 2mM), 0.2 µL BSA (10 mg/ml), 0.2 µL Denville Choice taq (5 U /µL), 1 µL of each primer (10 µM), 1 µL of DNA, and 13.6 µL of water. Our thermal cycling program for matK consisted of an initial denaturation step (94°C for 3 min), 35 denaturation, annealing, and elongation cycles (94°C for 45 sec, 50°C for 30 sec, 72°C for 1.5 min), and a final elongation step (72°C for 10 min). For rbcL and trnG-R we used the same program, except that the annealing temperature was 45°C for rbcL and 55°C for trnG-R. PCR products were purified using Shrimp Alkaline Phosphatase (USB, Cleveland, Ohio) following established protocols (Rothfels et al., 2012a) and sequenced on an ABI Prism 3700 DNA Analyzer (Applied Biosystems) at the Duke University Genome Sequencing and Analysis Core Resource, again using established protocols (Schuettpelz & Pryer, 2007). Chromatograms were assembled and edited in Sequencher 4.5 (Gene Codes Corporation) and the resulting 169 newly generated sequences are deposited in GenBank (Appendix C).
3.2.3 Sequence Alignment and Phylogenetic Analysis

Sequences for each locus were manually aligned in Mesquite v2.72 (Maddison & Maddison, 2009). Ambiguously aligned regions (limited to trnG-R) were excluded prior to analysis. A total of four datasets were analyzed: the three single-locus datasets (to test for inter-locus incongruence), and a combined three-locus dataset (Table 4). The single-locus datasets were analyzed under maximum likelihood (ML) in Garli v2.0 (Zwickl, 2006), using the best-fitting model as determined by the AICc in jModeltest v0.1.1 (Posada, 2008; see Table 4). For each locus, ML tree searches were performed on 500 bootstrap pseudoreplicate datasets, each searched from two different random-addition starting trees; other settings were left at their default values. The majority-rule consensus trees from each pool of bootstrap trees were compared for highly supported (>70% bootstrap support) incompatible splits (Mason-Gamer & Kellogg, 1996).

Table 4: Statistics for the datasets used in Chapter Three.

<table>
<thead>
<tr>
<th>Dataset</th>
<th>Taxa</th>
<th>Included Sites</th>
<th>Variable sites</th>
<th>Missing data (%)</th>
<th>Best-fitting model</th>
<th>MLBS</th>
<th>Partitions &gt;70%</th>
</tr>
</thead>
<tbody>
<tr>
<td>matK</td>
<td>74</td>
<td>1211</td>
<td>559</td>
<td>1.6</td>
<td>GTR+G</td>
<td>90%</td>
<td>85%</td>
</tr>
<tr>
<td>rbcL</td>
<td>61</td>
<td>1309</td>
<td>256</td>
<td>6.2</td>
<td>TrNef+I+G</td>
<td>81%</td>
<td>67%</td>
</tr>
<tr>
<td>trnG-R</td>
<td>84</td>
<td>1119</td>
<td>538</td>
<td>4.9</td>
<td>TVM+I+G</td>
<td>87%</td>
<td>80%</td>
</tr>
<tr>
<td>combined</td>
<td>84</td>
<td>3639</td>
<td>1353</td>
<td>17.4</td>
<td>&quot;*&quot;</td>
<td>90%</td>
<td>85%</td>
</tr>
</tbody>
</table>

Notes: Missing data includes both uncertain bases (?; N, R, Y, etc.) and gaps (\(-\)). MLBS: Maximum likelihood bootstrap support. *The combined dataset was analyzed under a partitioned model, which each locus given its own best-fitting model.

The combined three-locus dataset was analyzed under both ML and Bayesian frameworks. The ML analyses used the same settings as the single-locus analyses (above), in a single Garli (Zwickl, 2006) run with the data partitioned by locus, substitution parameters unlinked among partitions, and each partition permitted its own average rate (subsetspecific rates = 1). The search for the ML best tree started from each of 20 different random-addition starting trees, and support was assessed with 1000
bootstrap pseudoreplicates, each searched from two random-addition starting trees. The Bayesian analyses were performed in the parallel version of MrBayes v3.1.2 (Altekar et al., 2004; Ronquist & Huelsenbeck, 2003), with parameters unlinked among the three partitions. The best-fitting models for rbcL and trnG-R (see Table 4) were implemented with the nst=mixed and rates=invgamma settings; rbcL also had base frequencies fixed at .25 each (statefreqpr=fixed(equal)). The model settings for matK were more straightforward (nst=6 rates=gamma). The average rates for each partition were allowed to be different (ratepr=variable); other priors were left at their default values. Four independent runs, each with four chains (one cold, three heated), were run for 50 million generations with a sample taken every 7500 generations. The resulting sample parameter tracers were visualized in Tracer v1.5 (Rambaut & Drummond, 2007). The runs each converged (and to the same area of parameter space) well before 500,000 generations; to be very conservative, we excluded the first 5 million generations of each run as burn-in, prior to summarizing the posterior. Our final pool included 24000 samples; effective sample sizes for all parameters was greater than 300.

3.3 Results

3.3.1 Phylogenetic Analyses

Four datasets were analyzed in this study: one for each of the three individual loci and one of the combined data. While the differing numbers of sequences among the datasets making precise comparisons difficult, matK appears to be both more variable and more informative than trnG-R, which in turn outperforms rbcL. In addition, there are two well-supported conflicts among the loci (see the Discussion for more details). The combined dataset includes 3639 sites for 84 taxa; additional dataset characteristics are provided in Table 4.
3.3.2 Cystopteridaceae Phylogeny

Analysis of the combined dataset inferred strong support for the vast majority of the internodes across the tree, under both ML bootstrapping and Bayesian analysis (Fig. 12; Table 4). The monophyly of the family as a whole is maximally supported (1.0 posterior probability and 100% ML bootstrap support), as is each of the three genera. *Cystopteris* and *Acystopteris* are maximally supported as sister genera, and they, together, are sister to *Gymnocarpium* (Fig. 12).

The *Gymnocarpium* phylogeny features three deeply diverged “major clades”: the *disjunctum* clade, the *robertianum* clade, and core *Gymnocarpium*. Though each of these is strongly supported, relationships among them are uncertain. The *disjunctum* clade includes the diploid *G. disjunctum* and the widespread allopolyploid *G. dryopteris*. The *G. robertianum* clade includes all accessions of that species, as well as a Japanese accession of uncertain identity. The remainder of the genus constitutes the core *Gymnocarpium* clade, including the morphologically anomalous *G. oyamense*, the eastern North American *G. appalachianum*, the two taxa treated as subspecies of *G. jessoense* (subsp. *jessoense* and subsp. *parvulum*), and the east Asian *G. remotepinnatum*. Within the *Acystopteris* clade, each of the three recognized species is maximally supported as monophyletic. *Acystopteris japonica* and *A. tenuisecta* are sister (1.0 posterior probability and 100% ML bootstrap support), and they, together, are sister to *A. taiwaniana*.

The first split in *Cystopteris* is maximally supported, separating *C. montana* from the rest of the genus. The next branch is not well supported—similar to the situation at the base of *Gymnocarpium*. Thus, relationships among three highly supported clades (in this case, the *sudetica* clade, the *bulbifera* clade, and the *fragilis* complex) remain
uncertain. The *sudetica* clade is predominately Asian; of the three recognized species, *Cystopteris pellucida* and *C. moupinensis* are exclusively east Asian while *C. sudetica* extends across Eurasia. In contrast, the *bulbifera* clade, which includes the diploid *Cystopteris bulbifera* and related allopolyploids, is limited to North America. The bulk of *Cystopteris* species belong to the remaining clade, informally known as the *C. fragilis* complex. This complex is maximally supported as monophyletic, as is its first division, which separates *C. protrusa* from the rest of the complex. Relationships among the remaining *C. fragilis* complex species are convoluted, with many taxa (including *C. fragilis s.s.*) appearing in multiple clades.

### 3.4 Discussion

#### 3.4.1 Phylogenetic Analyses and Intralinkage Incongruence

The relative utility of the loci in our dataset (*matK* outperforming *trnG*-R, which was superior to *rbcL*) is consistent with other phylogenetic analyses of ferns across a variety of phylogenetic depths (Kuo et al., 2011; Li et al., 2011b; Rothfels et al., 2012a). An unexpected result, however, was the appearance of two well-supported conflicts among the individual loci. Since all three loci are in the plastid genome, they should be portions of a single non-recombining linkage group and be expected to share a common evolutionary history. Our data, however, reveal two cases of intralocus incongruence—situations where at least one locus has ≥70% bootstrap support for a relationship that is incompatible with a relationship supported by another locus (again, with at least 70% support; Fig. 13; Mason-Gamer & Kellogg, 1996). The first of these conflicts is relatively minor: *trnG*-R supports a large *Gymnocarpium* clade that includes...
all our accessions except for those in the *disjunctum* clade, whereas *rbcL* supports a clade of all our Gymnocarpium accessions except for those in the *robertianum* clade (Fig. 13A). In this case, the conflicting bootstrap support is only marginally greater than our 70% cut-off (70.6% for *trnG*-R and 72% for *rbcL*).

![Diagram showing support levels for conflicting relationships in Gymnocarpium and Cystopteris](image)

**Figure 13: Intralinkage incongruence.** Simplified rooted three-taxon trees showing support levels for the two conflicting relationships in our dataset, one in Gymnocarpium (A) and the other in Cystopteris (B). Numbers above branches are maximum likelihood bootstrap support values, gray branches indicate an absence of support (<70%), and thickened branches indicate supported relationships that conflict with a relationship supported by another locus in our dataset (in each case, there is a single such conflict).

The second case of intralocus incongruence is more substantial. Here, the conflicting relationships are near the base of the Cystopteris crown group (following the divergence of *C. montana*) and the discordance is much stronger. The *matK* locus supports a sister relationship between the *bulbifera* clade and the *fragilis* complex with 82.4% bootstrap support, whereas *trnG*-R supports a sister relationship between the *bulbifera* and *sudetica* clades with 92.6% bootstrap support (*rbcL* is equivocal; Fig. 13B). This incongruence is not the result of misidentification or lab error (the same extractions were used for all loci, and multiple accessions were involved in each case). Errors of alignment inference remain a possibility, but only one locus (*trnG*-R) has areas of
ambiguous alignment, and they were excluded prior to analysis. Furthermore, careful review of the alignments failed to uncover any regions that could possibly be contributing to this result. Instead, we suspect that this incongruence is due to the failure of our phylogenetic inference methods to fully capture the idiosyncrasies of molecular evolution in these taxa, as has been seen in other multi-region studies of single linkage groups. As with the mitogenomic data of Weisrock (2012), the conflicts in our data might be due to different patterns of selection operating on the individual loci, to biases in base composition across loci and taxa, to effects of slight changes in taxon representation, or to unusual variance in the stochastic substitution process.

3.4.2 Cystopteridaceae Phylogeny

Our broad results, demonstrating that both the family and constituent genera are monophyletic and that *Cystopteris + Acystopteris* are sister to *Gymnocarpium*, are consistent with the high support inferred for these relationships in earlier molecular phylogenies (Li et al., 2011a; Liu, 2008; Rothfels et al., 2012a; Sano et al., 2000a). The monophyly of the family, and its deep divergence from its closest relatives further emphasizes the need to recognize these three genera as constituting their own family, rather than including them in a broad Athyriaceae *sensu* Wang et al. (2004) or Woodsiaceae *sensu* Smith et al. (2006).

3.4.3 Gymnocarpium Phylogeny

While *Gymnocarpium* is maximally supported as monophyletic, the deepest divergence within the genus is uncertain (Fig. 12). Data from *trnG-R* support the *G. disjunctum* clade as sister to the remainder of the genus (71% bootstrap support), whereas *rbcL* places *G. robertianum* as the earliest diverging branch (with 72% bootstrap support; Fig. 13A). On its own, *matK* resolves the same relationship as *trnG-R*, but without support, which is the same result obtained from the concatenated data (Fig. 12).
Despite the addition of substantial amounts of data relative to previous studies, the early evolutionary history of Gymnocarpium remains enigmatic.

Of the three major groups that constitute Gymnocarpium, the disjunctum clade is the most straightforward to describe. In our sampling, it includes both accessions of the diploid species G. disjunctum, all samples of the cosmopolitan tetraploid G. dryopteris, and a single accession of triploid G. × brittonianum (Sarvela) K.M. Pryer & Haufler, which is hypothesized to be a hybrid between the other two taxa (Pryer & Haufler, 1993; Fig. 12). The grouping of G. disjunctum and G. dryopteris is expected, given that isozyme analyses suggest that G. dryopteris is an allopolyploid between G. disjunctum and G. appalachianum (Pryer & Haufler, 1993). Our data support this hypothesis by indicating that G. disjunctum is the maternal parent of all G. dryopteris populations sampled. Another striking feature of this clade is the genetic uniformity observed across all loci—the sequences of G. dryopteris are nearly identical regardless of whether they are from Alaska, Scandinavia, or Japan. This genetic similarity of G. dryopteris accessions across loci extends to the lone accession of G. dryopteris var. aokigaharaense, lending no support to its taxonomic recognition. However, in naming the variety, Nakaike (1969) pointed out that it was somewhat intermediate between G. dryopteris and G. jessoense. Thus, nuclear data will be necessary to corroborate or refute a possible hybrid origin of this taxon.

Our results for the robertianum clade have three noteworthy elements. First, our single accession of G. robertianum from the southern part of its North American range (5852: USA, Minnesota; see Appendix C, Fig. 11A) is somewhat divergent from the other accessions. Second, as in tetraploid G. dryopteris, the remaining accessions in this clade have near-identical sequences across loci, whether they are from North America, Scandinavia, or Japan. And finally, one of these accessions is from far beyond the recognized range of G. robertianum (7979: Japan, Iwate; see Fig. 11A). Plants of this morphology in that region are typically treated as G. jessoense ssp. jessoense. Our two
samples of the latter from China and Pakistan are well-supported members of the core Gymnocarpium clade and thus quite divergent from the robertianum clade. Nuclear data will be needed to determine whether this Japanese accession represents an unrecognized range extension of G. robertianum or is, instead, an unnamed allopolyploid with G. robertianum as the maternal parent. Any conclusions about the identity of these plants will have important consequences for Gymnocarpium nomenclature, because G. jessoense is typified on Japanese material (Koidzumi, 1936; see Appendix E).

The third major clade of Gymnocarpium contains most of the named taxa, including G. oyamense, which is sometimes recognized as the segregate genus Currania (e.g., Copeland, 1909; Lloyd & Klekowski Jr, 1970). The nesting of this species within Gymnocarpium is surprising, given its anomalous morphology (Fig. 10C; Sundue & Rothfels, 2012 (in prep)). It seems to provide another example of a pattern seen elsewhere in Eupolypods II, where certain strongly apomorphous taxa are embedded within a group characterized by very conserved morphology (e.g., Aspleniaceae, Oncleaceae, Blechnaceae; Rothfels et al., 2012a; Sundue & Rothfels, 2012 (in prep)). Gymnocarpium oyamense also is noteworthy because it is on a much longer branch than those of other species in the genus, all of which have remarkably clock-like rates of evolution. This pattern suggests a strongly elevated rate of molecular evolution on the branch leading to G. oyamense. Such elevated rates of substitution have been seen in other groups of ferns (Des Marais et al., 2003; Rothfels et al., 2012a; Rothfels et al., 2012 (in prep); Schuettpelz & Pryer, 2006) but, in those studies, the elevated rates characterized significant portions of the phylogeny, rather than a single species.

Within the core Gymnocarpium clade, G. oyamense is the first branch to diverge. Among the remaining species, the southeastern Appalachian endemic G. appalachianum is sister to a well-supported clade of G. jessoense subsp. parvulum accessions from North
America and east Asia (Fig. 12). These two taxa are, in turn, sister to accessions of *G. jessoense* subsp. *jessoense* from mainland Asia + the east Asian *G. remotepinnatum* (Fig. 12). The placement of *G. jessoense* subsp. *jessoense* (diploid) and *G. jessoense* subsp. *parvulum* (tetraploid) in different well-supported clades with divergent diploid taxa suggests that these taxa should be treated as distinct species. The taxonomy and nomenclature of the Asian taxa of *Gymnocarpium* is particularly complex, and one problem that our dataset does not address is the uncertain identity of *G. fedtschenkoanum* Pojark., which was described from Central Asian material (Pojarkova, 1950). Fraser-Jenkins (2008) assigns the majority of Himalayan *Gymnocarpium* populations to *G. fedtschenkoanum*, restricting the name *G. jessoense* to more eastern regions.

### 3.4.4 Acystopteris Phylogeny

*Acystopteris* has long been recognized as a distinctive element, either as a subgenus of *Cystopteris* (Blasdell, 1963; Kato, 1977) or as a separate genus (Nakai, 1933; Rothfels et al., 2012b; Smith et al., 2006; Japanese Society for Plant Systematics, 2012; Wang, 2008). It shares with *Cystopteris* a base chromosome number of *x* = 42 (Mitui, 1975) and a distinctive, hood-like indusium, but differs in having catenate scales and tuberculate light-tan spores (Blasdell, 1963; Rothfels et al., 2012b; Sundue & Rothfels, 2012 (in prep)), as well as an unusual low-elevation tropical distribution (Fig. 11B; Wang, 2008). Our *Acystopteris* sampling includes two accessions from each of the three named species; each is monophyletic, as is the genus as a whole (Fig. 12). The well-supported position of *Acystopteris* as sister to *Cystopteris* permits either its continued recognition as a separate entity, or its merger into *Cystopteris* s.l. Given its ease of diagnosis, its relatively deep divergence from *Cystopteris* s.s., the absence of any reported intergeneric hybrids, and its unique ecological and biogeographical features, we favor recognizing it at the generic level.
Within the genus, *A. tenuisecta* is strongly supported as sister to *A. japonica*, and that clade is then sister to the Taiwan endemic *A. taiwaniana*. This result is surprising, given that *A. taiwaniana* is frequently considered a variety of *A. japonica* whereas these two taxa are rarely, if ever, confused with *A. tenuisecta*. Wang (2008) suggested that *A. taiwaniana* may be an allopolyplid formed by hybridization between *A. japonica* and *A. tenuisecta*; this hypothesis is not supported by our data.

### 3.4.5 Cystopteris Phylogeny

Our phylogenetic hypothesis for *Cystopteris* includes four highly supported “major” clades: *C. montana*; the *sudetica* clade; the *bulbifera* clade; and the *C. fragilis* complex (including *C. protrusa*). A clade comprising all *C. montana* accessions, including samples from Canada, Norway, and China, is strongly supported as sister to the other three. This topology would permit the recognition of the genus *Rhizomatopteris* (typified on *C. montana*; Khokhrjakov, 1985) while still retaining a monophyletic *Cystopteris*. However, in naming *Rhizomatopteris*, Khokhrjakov included *C. sudetica* (and by extension, *C. moupinensis* and *C. pellucida*) in his concept. *Rhizomatopteris sensu* Khokhrjakov, then, includes all taxa with broadly deltate-to-pentagonal leaves and widely spaced internodes on long-creeping rhizomes (see Fig. 10E, H). This assemblage is not monophyletic, and there seems little value in recognizing a monotypic *Rhizomatopteris* (containing only *C. montana*).

Blasdell’s (1963: 80) “evolutionary tendencies” diagram (an early tree-like visualization inferred using Wagner’s (1980b) “groundplan divergence scheme”) is remarkably similar to our molecular phylogeny. His diagram, based entirely on morphological characters, includes a basal division between *Acystopteris* and *Cystopteris*, followed by a branch largely corresponding to our *C. fragilis* complex clade (but excluding *C. diaphana*), and another branch with *C. montana* at its base, *C. bulbifera* next,
and *C. sudetica* and *C. pellucida* grouped together. Indeed, if the incongruent position of *C. diaphana* is ignored, and the extant species that Blasdell included on internal branches are moved to branch tips, then his diagram perfectly anticipates our four-clade (plus *Acystopteris*) result. On the other hand, Blasdell’s (1963) proposed sectional classification of *Cystopteris s.s.* is incompatible with certain aspects of our phylogeny. His section *Emarginatae* constitutes a paraphyletic grade composed of *C. montana*, the *sudetica* and *bulbifera* clades, plus *C. diaphana*. In our phylogeny, the latter is deeply nested within the *C. fragilis* complex clade (Fig. 12), rendering Blasdell’s section *Cystopteris* paraphyletic as well.

**3.4.5.1 Cystopteris montana and the sudetica and bulbifera Clades**

The position of *C. montana* as sister to the rest of the genus is very strongly supported. Within the species, there is a shallow but highly supported split separating the two high-elevation accessions from Tibet from the North American and Scandinavian samples. Blasdell (1963) reports both diploid and tetraploid cytotypes for *C. montana* (without further comment or reference); otherwise, the species is known only as a tetraploid (Haufler et al., 1993). Further investigations are necessary to determine if cryptic taxa exist within *C. montana*, and if the Chinese taxon *C. modesta* Ching is distinct.

Relationships among the three other major clades of *Cystopteris*—the *sudetica* clade, the *bulbifera* clade, and the *C. fragilis* complex—are not fully resolved in our combined analysis. This lack of support is not caused by a lack of signal in our data, but rather by conflicting signals from the different partitions (see Intralinkage Incongruence discussion, above, and Fig. 13). Our sampling includes three species from the *sudetica* clade: *C. pellucida*, *C. moupinensis*, and *C. sudetica*. These species are primarily Asian; only *C. sudetica* extends west into Europe (Fig. 11C). Members of this clade are morphologically somewhat intermediate between *C. montana* (with which they share
long-creeping rhizomes, widely-spaced leaves, and a tendency towards expanded basal
pinnules on the lowermost pinnae) and *C. bulbifera* (with which they share an elongate-
deltate leaf shape; see Fig. 10). Our analyses indicate that *C. pellucida* is the earliest
diverging lineage in the *sudetica* clade (Fig. 12). An enigmatic species with a restricted
range in central China (Fig. 11C), it differs from *C. moupinensis* in having more
membranous leaves and coarser leaf division (Wang, 2008). *Cystopteris moupinensis* was
recognized as a variety of *C. sudetica* by Blasdell (1963), and the two taxa are clearly very
closely related. Our data resolve our two accessions of *C. sudetica* as monophyletic; these
are very slightly diverged from—and form a polytomy with—our two *C. moupinensis*
accessions (Fig. 12). The two species are allopatric (or very nearly so; Fig. 11C), and
differ chiefly in the presence (*sudetica*) or absence (*moupinensis*) of glands on the indusia.

In our analysis, the *bulbifera* clade comprises all accessions of three species: *C.
bulgifera* (L.) Bernh. *C. tennesseensis* Shaver, and *C. utahensis* Windham & Haufler. A
limestone specialist of eastern North America with disjunct populations in the west,
diploid *C. bulbifera* is hypothesized to be involved in the origin of tetraploid *C.
tennesseensis* (through hybridization with *C. protrusa*; Haufler et al., 1990; Shaver, 1950)
and tetraploid *C. utahensis* (through hybridization with *C. reevesiana*; Haufler &
Windham, 1991). In our dataset, *C. bulbifera*, *C. tennesseensis*, and *C. utahensis* have nearly
identical sequences, confirming that *C. bulbifera* is the maternal parent of both
tetraploids, and that their formation is recent (as suggested by Haufler et al. (1990)). Our
only sample of *C. laurentiana*, an allohexaploid thought to contain a *C. bulbifera* genome
(Wagner & Hagenah, 1956), derived its plastid from a member of the *C. fragilis* complex
(see the section on that complex for further discussion). Molecular confirmation that *C.
bulgifera* was involved in the formation of *C. laurentiana* will require data from the
nuclear genome.
3.4.5.2 Cystopteris fragilis Complex

The Cystopteris fragilis complex has a special place in fern systematics, with Lovis (1978: 356) describing it as “perhaps the most formidable biosystematics problem in the ferns.” This species complex occurs on every continent except Antarctica (Fig. 11C, D), and includes ploidy levels ranging from diploid to octaploid (Blasdell, 1963). Our plastid data, though preliminary, make several important contributions. First, they permit a robust circumscription of the C. fragilis complex (as the inclusive clade encompassing C. fragilis and C. protrusa, but not C. bulbifera or C. sudetica; Fig. 12). And second, they strongly support the position of the eastern North American, forest-dwelling diploid C. protrusa as sister to the rest of the complex (a position anticipated by Blasdell in his groundplan divergence scheme tree; Blasdell, 1963).

The bulk of the C. fragilis complex (i.e., excluding C. protrusa) is marked by a basal dichotomy. One side of the split is significantly supported, but barely so: it has 70% bootstrap support and 0.97 posterior probability (Fig. 12). This clade contains an interesting assemblage of taxa, including the alpine Eurasian hexaploid C. alpina, the endemic Australasian tetraploid C. tasmanica, both Hawaiian endemics (C. sandwichensis and C. douglasii), an eastern North American accession of C. tenuis, and three North American accessions of C. fragilis (including two unnamed hexaploids). Most specimens with rugose spores—often called C. dickieana R. Sim. (Alston, 1951; Bir & Trikha, 1974; Nardi, 1974; Parks et al., 2000; Prada, 1986; Wang, 1983) or C. fragilis subsp. dickieana (R. Sim) Hyl. (Fraser-Jenkins, 2008)—fall in this clade. The sister clade is highly supported, and includes our only western North American accession of C. tenuis, all samples of C. reevesiana, the North American putative allohexaploid C. laurentiana, various collections of C. fragilis s.s. from Europe, western North America, and Latin America, plus single accessions of C. membranifolia, C. millefolia, and C. diaphana (Fig. 12). The latter three form...
a weak (61% bootstrap support and 0.99 posterior probability) clade with all Latin American C. fragilis, providing some support for Blasdell’s (1963) broad application of the name C. diaphana to plants from this region.

Our preliminary results for the C. fragilis clade confirm that its reputation for systematic complexity is well deserved. Except for C. protrusa, all segregate species recognized by previous authors are nested among accessions referable to C. fragilis (Fig. 12). Each of the three known diploid members—C. protrusa, C. reevesiana, and a diploid cytotype of C. diaphana (Blasdell, 1963)—lacks the morphological distinctiveness characteristic of species in the other clades, making it difficult to diagnose polyploid taxa or to infer their parentage based on morphological data alone. Though plastid DNA analyses provide critical information, they tell only part of the story. Any progress on defining species limits in this group will depend on coordinated cytological and biparental molecular analyses, which are currently underway (Rothfels et al., in prep.).

3.5 Acknowledgments

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Yatskievych (MO) for their hospitality and assistance. The U.S. National Park Service gave permission to sample populations of *Gymnocarpium appalachianum* in Shenandoah National Park (permit SHEN-2010-SCI-0019) and critical Mexican material was secured under permit SGPA/DGGFS/712/1323/09 with the generous support of Universidad Nacional Autónoma de México (UNAM) and Mark Olson. This research was supported by funding from a National Science and Engineering Research Council (Canada) PGSD to C.J.R., an NSF DDIG award to K.M.P. and C.J.R. (DEB-1110767), a Duke Biology Department Grant-in-Aid to C.J.R., and a Duke University Center for Latin American and Caribbean Studies graduate student field research grant to C.J.R. Anne Johnson provided skilled assistance in the lab.
4. *Cystocarpium* is a natural intergeneric hybrid between parents that diverged over 50 million years ago


4.1 Introduction

The formation of reproductive barriers between populations is of central importance to evolutionary biology. These barriers facilitate local adaptation, permit phenotypic and ecological divergence, and ultimately result in the subdivision of life into the diversity of species that occupy this planet (Coyne & Orr, 2004; Rieseberg & Willis, 2007). Reproductive barriers are even important when they break down—hybrid individuals may enjoy greater fitness than their parents (Arnold et al., 1999; Arnold & Martin, 2010; Grant & Grant, 1992), hybridization may introduce important genetic variation into populations (Anderson & Stebbins Jr, 1954; Larsen et al., 2010; Lewontin & Birch, 1966), and in more extreme cases, permit the formation of new species through rapid jumps across adaptive landscapes (Brasier, 2001; Brasier et al., 1999; Mallet, 2007; Rieseberg, 2003; Seehausen, 2004).

The rates at which reproductive barriers arise, and the length of time during which they remain incomplete, have understandably been the subject of considerable research effort (Coyne & Orr, 2004). Early investigations established a positive correlation between time since divergence of two lineages and the strength of the reproductive barriers between them (Coyne & Orr, 1997; Mendelson, 2003; Presgraves, 2002), and that this “incompatibility clock” (or, more generously, “speciation clock”)

105
ticks at different rates for different taxonomic groups (Coyne & Orr, 2004; Edmands, 2002; Prager & Wilson, 1975). These studies, and others utilizing robust molecular dating techniques, provide a broad picture of the pace of evolution of reproductive isolation: *Drosophila* species are generally unable to hybridize if they have diverged for more than approximately 4 million years (Carson, 1976; Coyne & Orr, 1997); Price and Bouvier (2002) report that the average time from divergence to complete postzygotic isolation in birds is approximately 10 million years; and the minimum common ancestor age for total hybrid inviability in centrarchid fishes is estimated at 25 million years (Bolnick & Near, 2005).

These studies measure only one or a few components of reproductive isolation (usually postzygotic barriers, such as hybrid inviability), and do so under controlled conditions, and thus likely underestimate the rate of evolution of total reproductive isolation in the wild. Given this background, it was particularly surprising when Fraser-Jenkins (2008) hypothesized, from morphological characters, that an unusual plant from the French Pyrenees was a natural hybrid between a fern species from the genus *Cystopteris* and one from *Gymnocarpium*; he named it in a new nothogenus, as ×*Cystocarpium roskamianum* Fraser-Jenk. (Fraser-Jenkins, 2008; Fraser-Jenkins et al., 2010). The best contemporary estimate put the divergence time of the putative parents at 72 mya (Schuettpelz & Pryer, 2009: node 160 in their Table S3), making this hypothesis of parentage, if confirmed, the most divergent natural hybridization known to science. For context, approximately comparable pairings, in terms of time since their most recent common ancestor, would include a manatee hybridizing with a hyrax, or a giraffe with a wild pig (Bininda-Emonds et al., 2007).

In this study, we use an extensive nuclear and plastid sequence dataset to test the hypothesized parentage of ×*Cystocarpium*, and confirm its ploidy level with meiotic
chromosome counts. We then integrate the best available divergence-date estimates for ferns with the most comprehensive Cystopteridaceae molecular phylogenetic dataset, using a series of nested empirical Bayesian analyses, in order to infer a robust date for the divergence of the ×Cystocarpium parental taxa.

4.2 Methods

4.2.1 Determining Parentage

To determine the parentage of ×Cystocarpium roskamianum, we obtained 271 sequences of the low-copy nuclear marker gapCp “short” (sensu Schuettpelz et al. 2008; henceforth “gapCp”) from a sample of 29 Cystopteridaceae accessions (Appendix D; the 271 includes only those sequences retained after PCR recombinant sequences were removed). Genomic DNA was extracted from herbarium specimens or silica-dried material in the Fern Lab Database (http://fernlab.biology.duke.edu/) using a 96-well modification (Beck et al., 2011a; doi:10.5061/dryad.11p757m0) of a standard CTAB protocol (Doyle & Dickson, 1987), or with a DNeasy kit (Qiagen, Valencia, California, USA). As a precaution, we extracted ×Cystocarpium twice, in separate extractions several weeks apart. Amplifications were performed in 21 µL reactions following established protocols (Rothfels et al., 2012c) with ESGAPCP8F1 and ESGAPCP11R1 (Schuettpelz et al., 2008). PCR products were cloned following established protocols (Schuettpelz et al., 2008), and the colony PCR products visualized on agarose gels prior to sequencing with the M13 Forward and M13 Reverse primers supplied by Invitrogen. Sequencing was done on an ABI Prism 3700 DNA Analyzer (Applied Biosystems) at the Duke University Genome Sequencing and Analysis Core Resource, again using established protocols (Schuettpelz & Pryer, 2007).

The resulting pool of 271 sequences was manually aligned in Mesquite v2.75 (Maddison & Maddison, 2006); for all analyses, unambiguous indels were recoded by
simple gap recoding (Simmons & Ochoterena, 2000), using the Python script gapcode.py (Ree, 2008). This dataset contains 168 accession-unique sequences (i.e., the number of distinct sequences from each accession, summed across accessions—some sequences thus tallied are not globally distinct, because the same sequence occurs in multiple accessions). This number is far larger than would be expected even if all our accessions were tetraploid and maximally heterozygous (four alleles each); the majority of the “allelic” variants differ from each other by a small number of substitutions, and almost certain represent PCR error (see Beck et al., 2011b; Grusz et al., 2009; Li et al., 2012; Rothfels et al., 2012 (in prep)). We conservatively thinned this “all-unique” dataset to our final sequence pool in two steps. First, we generated a maximum parsimony tree of our “all-unique” dataset in PAUP* v4.0a123 (Swofford, 2002; Fig. 14), and retained, from each accession, one representative of each clade whose sequences differed from the others (of that accession) by more than one synapomorphy or more than three apomorphies. When selecting the sequence to be retained from a group, we picked the one that had the fewest apomorphies, or, if there were multiple equally diverged sequences, we selected one at random.

The resulting “thinned-1” dataset has 68 sequences (Appendix D; Fig. 15), and while much closer to the per-accession totals that we would expect, still contains sequences that are very likely PCR artifacts (occurring, for example, due to substitution errors that happened early in the reaction, and so are present in multiple sequences). We thus thinned our data a second time, this time incorporating information from the complete sample, by retaining only one representative of each exclusive clade (each
Figure 14: A most-parsimonious tree from the “all-unique” dataset. The four-digit number following the species name is that accession’s Fern Lab Database number (fernlab.biology.duke.edu). Lists of numbers following the letter “c” indicate which clones had that sequence.
Figure 15: A most-parsimonious tree from the “trimmed-1” dataset. The numbering convention follows Figure 14, with the exception that clone numbers in square parentheses indicate clones that met the criteria for being thinned in favor of the included sequence, but are not identical to it.

clade that contains sequences from only a single accession), regardless of how divergent the sequences in that clade might be. For this step we again used a maximum parsimony tree from PAUP*, including the indel characters (Fig. 15), and we interpreted “clade” as the largest exclusive pool of sequences that could be derived for the accession in question, without breaking any branches—hard polytomies involving multiple
accessions thus did not interfere with those sequences being thinned. Practically speaking, the density of our sampling meant that the sequences removed in this step tended to be very similar to a retained sequence; the only cases where divergent sequences were removed were in the sparsely sampled areas of the tree—*Acystopteris tenuisecta*, *A. taiwaniana*, and *Cystopteris montana* (only a single accession included of each)—that were peripheral to our focal question. By incorporating information from the whole thinned-1 sample we ensured that this step did not reduce our ability to make conclusions about the parentage of *×Cystocarpium*—all unique clades, for each accession, are retained. This final “thinned-2” dataset contains 52 sequences (Appendix D; Fig. 16), and is the basis for our subsequent analyses.

Our final analyses used the thinned-2 dataset, divided into four partitions: codon positions 1 and 2; codon position 3; the noncoding region; and recoded indels. For each partition, we applied the best-fitting model as determined by the small sample correction for the AIC (the AICc; Akaike, 1974; Hurvich & Tsai, 1989), using *jModelTest* (Posada, 2008), with the exception of the indels partition, which was optimized under a Mkv model (Lewis, 2001). The best-fitting models for each partition are shown in Table 5. These analyses were performed in *Garli* v2.0 (Zwickl, 2006), with the tree search repeated from 10 random-addition starting trees. To assess support, we performed 1000 ML bootstrap pseudoreplicates, again in *Garli*, under the same settings, but with each search performed from only two random-addition starting trees.
Figure 16: Maximum likelihood phylogeny of gapCp alleles from the “trimmed-2” dataset. Alleles from ×Cystocarpium are in boldface. Thickened branches have ≥ 70% bootstrap support. Numbers following the letter “c” indicate which sequence (which clone) was used to represent that allele.
<table>
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<th>Model Name</th>
<th>Exchangeability Parameters</th>
<th>State Frequencies</th>
<th>Rate Heterogeneity</th>
<th>Proportion Invariant</th>
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</thead>
<tbody>
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<td>K80+I</td>
<td>0 1 0 0 1 0</td>
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<td>None</td>
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</tr>
<tr>
<td>2</td>
<td>TPM1+G</td>
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<tr>
<td>3</td>
<td>TPM1uf+I+G</td>
<td>0 1 2 2 1 0</td>
<td>Estimate</td>
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<td>Estimate</td>
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<td>Mkv</td>
<td>1 rate</td>
<td>Equal</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

To further refine our inferences of parentage, we assessed the ploidy level of \( \times \text{Cystocarpium} \) through meiotic chromosome squashes. Young fertile leaves from living plants were placed in Farmer’s fixative (3 parts absolute ethanol : 1 part glacial acetic acid) at room temperature for 24 hours, the transferred to 70% ethanol for storage and transport. Sporangia at the proper stage of development (see Windham & Yatskievych, 2003) were transferred to a drop of 1% acetocarmine and broken open using the tip of a fine dissecting needle. When approximately 25–40 sporangia were prepared in this manner, the stain drop was mixed with Hoyer’s medium (1 : 1) and squashed following traditional methods (Manton, 1950). Slides were scanned using a Meiji MT5310L phase contrast microscope, and representative cells were photographed with a Canon EOS T3i camera.

### 4.2.2 Divergence Dating

To infer a date for the divergence of the progenitors of \( \times \text{Cystocarpium} \), we employed an empirical Bayesian approach. First, we acquired dating information from the global leptosporangiate fern chronogram of Schuettpelz & Pryer (2009). This study included three loci for 400 species spanning the leptosporangiate ferns, is calibrated with 24 fossils, and is by far the most comprehensive source of dating information available for ferns. From the Schuettpelz & Pryer (2009) results we extracted the ML divergence time estimates for 14 highly supported nodes: Dennstaedtiaceae + Pteridaceae + Eupolypods (165.6 mya); Aspleniaceae (57.7 mya); Athyriaceae (78.4 mya); Blechnaceae
+ Onocleaceae (77.8 mya); Ctenitis + Dryopteris (77.2 mya); Dennstaedtia + Pteridium (119.3 mya); Eupolypods (116.7 mya); Hemidictyum + Aspleniaceae (92.6 mya); Notholaena + Cryptogramma (110.8 mya); Polypodium + Tectaria (66.1 mya); Phegopteris + Pseudophegopteris + Macrothelypteris (45.9 mya); Thelypteridaceae (68.5 mya); Eupolypods I (98.9 mya); and Eupolypods II (103.1 mya; these nodes are marked with the black circles in Fig. 17a, b). Note that we did not use data directly from our node of interest (the crown divergence of Cystopteridaceae). Schuettpelz & Pryer (2009) included only two species from that family and inferred a surprisingly old date for that divergence, so to be conservative with respect to our “deep hybridization” hypothesis, we limited ourselves to nodes deeper in the tree, where more data were available for the original inference (Schuettpelz & Pryer, 2009).

We applied these 14 node ages as constraints to the five-locus, 81-taxon Eupolypods II dataset of Rothfels et al. (2012a); as well as providing more character data, this dataset has much denser sampling of Cystopteridaceae (eight taxa included) and its relatives (Fig. 17b). The time to most recent common ancestor (tmrca) of each of the 14 nodes was given a normal prior distribution, with a mean equal to the ML estimate from Schuettpelz & Pryer (2009), and a standard deviation equal to 10 percent of that mean; taxon groups were not constrained to be monophyletic. These analyses were performed in BEAST v1.7.2 (Drummond & Rambaut, 2007) under a lognormal uncorrelated relaxed clock model (Drummond et al., 2006), and birth-death tree prior. Each locus was permitted its own global rate, under a GTR+G substitution model with unlinked substitution parameters. Priors were left at their default values, except for the UCLD.mean parameter, which was given a lognormal distribution with a mean (in real space) of 0.0012 and a standard deviation of 1.0. This mean was selected as being slightly larger than the estimate from Rothfels et al. (2012 (in prep); larger rates implying more
recent divergence times, which is conservative with respect to our hypothesis of a wide hybridization event). Comparable runs with a lognormal prior of 0.001 or a uniform prior from 0 to 0.1 yielded indistinguishable tmrca estimates (data not shown). This dataset was run four times independently, each for 30 million generations, with parameter values logged every 6000 generations. These runs converged quickly (Rambaut & Drummond, 2007); the first 3 million generations were conservatively discarded as burnin, prior to pooling the four runs. Effective sample sizes (ESSs) for all parameters in the pooled post-burnin sample are above 300.

From this pooled run we extracted the posterior tmrca distributions for seven highly supported nodes: *Acystopteris; Cystopteris + Acystopteris; Cystopteridaceae; Cystopteris; Cystopteris fragilis + Cystopteris moupinensis; Gymnocarpium oyamense + Gymnocarpium remotepinnatum*; and *Gymnocarpium oyamense + G. remotepinnatum + G. dryopteris* (these nodes are marked with black circles in Fig. 17b). Manual inspection of these samples indicated that they could each be approximately gamma or lognormally distributed. For each node we found the best-fitting gamma and lognormal distributions by maximum likelihood, using the R (R Development Team, 2011) package *fitdistrplus* (Delignette-Muller et al., 2010), and selected the best distribution by the AIC. Six of the nodes fit a lognormal distribution best; the seventh (Cystopteridaceae) showed a slightly better fit to a gamma; in all cases, visual inspection of the posterior histograms and the best-fit distributions showed very close concordance.

These seven posterior tmrca distributions—the full, best-fitting parametric distributions from *fitdistrplus*—were incorporated as tmrca priors on their respective nodes in our final Cystopteridaceae dataset. This dataset is formed from the three-locus, 75-taxon Cystopteridaceae plastid dataset of Rothfels et al. (2012c), by the exclusion of their outgroup taxa and one sample of *Gymnocarpium disjunctum* with limited character
data, and by the inclusion of $\times$Cystocarpium. We again used BEAST 1.7.2 (Drummond & Rambaut, 2007) with a lognormal uncorrelated relaxed clock model (Drummond et al., 2006), but this time using a coalescent tree prior, which is a better match for the population-level sampling of this dataset. The three loci were given their best-fitting substitution model as determined by Rothfels et al. (2012c), with each locus permitted its own average rate. Priors were left at their default values except for the tmrca distributions (above), the rbcL.cg exchangeability parameter (given a gamma prior distribution with a shape of 4.738167 and scale of 0.000714783), and the rbcL.gt exchangeability parameter (given a gamma prior distribution with a shape of 7.009774 and scale of 0.000523609). The latter two distributions were derived from MrBayes 3.1.2 (Ronquist & Huelsenbeck, 2003) runs on the same data (Rothfels et al., 2012c) using fitdistrplus (Delignette-Muller et al., 2010; R Core Development Team, 2011), and adopted to assist with mixing; rbcL, in this dataset, has relatively little signal, and in the absence of a Dirichlet prior on exchangeability parameters (with MrBayes has), BEAST had trouble sampling these two rates under its default priors.

This analysis was run four times independently, each for 30 million generations. As before, the runs converged relatively rapidly (Rambaut & Drummond, 2007); we very conservatively removed the first 5 million generations of each run as burnin before pooling the samples. The ESSs for all parameters in the pooled data were above 1000.
Figure 17: The hybrid origin of ×Cystocarpium, and nested empirical Bayesian analysis of divergence time. Chronograms are maximum clade credibility trees from BEAST, with node heights at their median posterior estimate; Gymnocarpium is in red, Acystopteris in green, and Cystopteris in blue. a) Eupolypods II sample of Rothfels et al. (2012a), calibrated with 14 node age densities from Schuettpelz & Pryer (2009). The posterior distributions of seven node ages from that analysis were used as priors on
their respective nodes in the Cystopteridaceae dataset of Rothfels et al. (2012c) to infer the bottom chronogram (b). The star in (b) indicates the position of ×Cystocarpium, nested among accessions of Gymnocarpium dryopteris. For both chronograms, the calibrated nodes are marked with black circles. c) The posterior distribution of age estimates for the most recent common ancestor of the parents of ×Cystocarpium from each dataset, on the same time scale as the chronograms. d) Maximum likelihood phylogram of gapCp alleles. Colors follow Fig. 17a and b; black stars indicate the position of each of the four ×Cystocarpium alleles. Thickened branches in Fig. 17d have ≥70% bootstrap support.

4.3 Results and Discussion

4.3.1 Origin of ×Cystocarpium

The gapCp data provide strong evidence for the parentage of ×Cystocarpium. Each of the two ×Cystocarpium DNA extractions yielded four distinct allele sequences, two of which fall in the Gymnocarpium clade, and two in Cystopteris (Figs. 16, 17d), providing compelling corroboration of the original intergeneric hybrid hypothesis. Of the Gymnocarpium-type sequences, one allele is in a tight clade with alleles from the diploid species G. disjunctum, and the other falls with sequences from the diploid G. appalachianum. These two species have been implicated as the parental taxa of the cosmopolitan allotetraploid G. dryopteris, based on isozyme data (Pryer & Haufler, 1993) and, indeed, each of our three accessions of G. dryopteris also has gapCp alleles from each of these two clades (Fig. 16). The Gymnocarpium-type ×Cystocarpium alleles are closest to those of our European (Finland) and North American (Ontario) G. dryopteris alleles, strongly indicating that G. dryopteris is one of its parents; plastid data further show that G. dryopteris was the maternal parent (Fig. 17b). This conclusion differs slightly from the original description, where G. robertianum was the hypothesized parent on the Gymnocarpium side (Fraser-Jenkins, 2008); G. robertianum, while also appearing to be an allopolyploid, has alleles that are strongly divergent from those of G. dryopteris (Fig. 16), and thus from ×Cystocarpium, too.

Both of the Cystopteris-type ×Cystocarpium alleles fall in the Cystopteris fragilis complex (sensu Rothfels et al., 2012c). This clade is notorious for its extensive polyploidy and obscure species boundaries (Blasdell, 1963; Lovis, 1978; Rothfels et al., 2012c),
making precise determination of the ×Cystocarpium parentage difficult. However, both Cystopteris-type ×Cystocarpium alleles are tightly associated with alleles from each of our two European C. fragilis accessions, one from Sweden, and one from Switzerland (Fig. 16). These are the only two accessions to have the same allelic complement as ×Cystocarpium, strongly implicating a European member of the C. fragilis complex as the second parent of ×Cystocarpium. While we did not attempt to infer a chronogram from the gapCp data, each of the four ×Cystocarpium alleles differs at most by a single substitution from an allele from one of its parents, indicating that, while the divergence between the parents is great, the hybridization event itself was very recent.

Figure 18: Representative chromosome squashes from ×Cystocarpium roskamianum.

Chromosome squashes of spore mother cells undergoing meiosis show approximately 140 chromosomes, as a mix of univalents and bivalents, with the
bivalents being pulled to the metaphase plate, and the univalents remaining scattered in the cytoplasm (Fig. 18). If ×Cystocarpium had a complete breakdown of chromosome pairing, we would expect 164 univalents in a tetraploid (2 × 40 from Gymnocarpium and 2 × 42 from Cystopteris; Rothfels et al., 2012b). Given that we observe some bivalent formation (variable in extent) these results indicate that ×Cystocarpium is a tetraploid hybrid, between a known tetraploid species (G. dryopteris) and a tetraploid member of the Cystopteris fragilis complex.

4.3.2 Depth of Divergence

Our final mean estimate of the date of the last common ancestor of Cystopteris and Gymnocarpium—and thus the divergence bridged by the ×Cystocarpium hybridization event—is 57.9 million years ago, with the 95% highest posterior density interval of that estimate spanning 40.2 to 76.2 million years ago (Fig. 17c). The nested empirical Bayesian analyses allowed us to combine extensive broad fossil calibration data with dense taxon and character sampling within the focal clade, a combination that would not be possible in a single analysis, or under a common model. Unlike some earlier versions of “secondary calibration” analyses, this method retains the uncertainty associated with earlier estimates when those estimates are applied to the next nested dataset in the hierarchy, and thus avoids artificially precise estimates (see Graur & Martin, 2004). Nevertheless, our posterior estimates of the time of divergence of Cystopteris from Gymnocarpium became more precise as we moved through our hierarchy; as one would hope, the incorporation of additional data at each step allowed for more precise inference, even taking into account the full uncertainty of the previous level’s estimates (Fig. 17c).

The 95% highest posterior density interval of our divergence estimate requires that the ×Cystocarpium hybridization event brought together two genomes that have
experienced a minimum of 80 million years of cumulative independent evolution. This figure puts ×Cystocarpium at the very extreme end of documented wide hybridizations. Interestingly, the other examples of extremely wide hybridizations also tend to be from plants. The parents of the Leyland Cypress (Cupressaceae) are estimated to have diverged approximately 45 mya (Garland & Moore, 2012; Mao et al., 2012: node 31 in their Fig S2). And the hybridization event, some 40 million years ago, that gave rise to the angiosperm genus Physacanthus is hypothesized to have occurred between lineages that were themselves already 40 million years diverged (Tripp et al., 2012). Animals are not without their extreme examples—in captivity guineafowl have been persuaded to successfully hybridize with chickens (and with peafowl), despite last sharing a common ancestor some 30 to 70 million years ago (depending on the study; Brown et al., 2008; Dimcheff et al., 2002; Pereira & Baker, 2006).

Examples of this magnitude are, however, extremely rare, especially outside of the laboratory or cultivation. Bolnick et al. (2005), for example, documented viable eggs produced by a centrarchid (sunfish) artificial hybridization that spanned approximately 34 million years, but the most divergent centrarchids known to produce hybrids in the wild are under 15 million years diverged (Bolnick & Near, 2005: their Fig. 7). Similarly, the majority of the intergeneric seed plant hybrids listed by Knobloch (1972) were generated artificially, and many others—like the Mahonia-Berberis hybrid ×Mahoberberis C.K. Schneid.—are based on faulty generic concepts and actually represent rather shallow, intrageneric hybridizations. Ferns are no exception to this pattern. Of the five putative intergeneric hybrids in Knobloch et al.’s (1984) thorough list of fern and lycophyte hybrids (×Asplenoceterach D. E. Meyer, ×Asplenophyllitis Alston, ×Asplenosorus Wherry, ×Ceterophyllitis R.E.G. Pichi Sermolli, ×Pleuroderris Maxon), all would be considered intrageneric under current classifications (Smith et al., 2006), as would the
treefern hybrid ×Cyathidaria Caluff & Shelton (Caluff, 2002). Aside from ×Cystocarpium, we know of only one other wide fern hybridization—the well-documented ×Dryostichum W.H. Wagner, a hybrid between Dryopteris goldiana (Hook. ex Goldie) A. Gray and Polystichum lonchitis (L.) Roth (Wagner et al., 1992). Schuettpelz and Pryer’s (2009) estimate for the Dryopteris-Polystichum divergence time is 67.8 million years ago (vs. their estimate of 72.2 million years ago for the Gymnocarpium-Cystopteris split), putting ×Dryostichum in the same league as ×Cystocarpium; further investigations are necessary to more precisely date the ×Dryostichum divergence. Pending these investigations, ×Cystocarpium stands alone, as deepest strongly documented wild hybridization event on record.

4.4 Acknowledgments

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Conclusions

The results of the research presented in this thesis contribute to the beginnings of an understanding of the evolution of the Cystopteridaceae. Perhaps the most significant weakness in our progress to date is our fragmentary understanding of the species limits in the family (see Appendix E). *Acystopteris* and *Gymnocarpium* each contain unrecognized lineages (Rothfels et al., unpublished data), but the greatest challenge is within the *Cystopteris fragilis* complex. Part of this challenge is nomenclatural—Tropicos (http://www.tropicos.org/) lists over 140 names in *Cystopteris*, most of which are in the *C. fragilis* complex (see Appendix E). The abundance of names reflects the worldwide distribution of the complex and the extensive morphological variation it contains. However, relatively few of these names are likely to apply to unique biological lineages. Sorting out which do, and which belong in synonymy, will require both a much greater understanding of the species limits in the complex, and extensive biosystematic data from the type specimens themselves (many of which are very old, fragmentary, or lost; Blasdell, 1963), in order to appropriately apply the names.

Coupled with the nomenclatural issues are phylogenetic and biological challenges. To untangle the often-cryptic lineages within this complex (named and otherwise), we need to integrate ploidy information and morphological data (including spore morphology; Bir & Trikha, 1976b; Fraser-Jenkins, 2008; Jermy & Harper, 1971; Larsen, 1952; Liu et al., 2000; Pearman, 1976; Rothfels unpublished) with phylogenetic data from low-copy nuclear markers. This approach—notably the incorporation of ploidy and low-copy nuclear data—is particularly useful where morphological discontinuities have been obscured by past reticulation events, as they have in this group (for examples of this approach see Brysting et al., 2007; Grusz et al., 2009; Guggisberg et al., 2009; Kim et al., 2008; Schuettpelz et al., 2008; Shepherd et al., 2008).
Inferences from single-locus data are susceptible to misinterpretation due to incomplete lineage sorting and other vulnerabilities (Marcussen et al., 2012; Popp et al., 2005) so multiple loci from a broad sample of plants will be necessary. Fortunately, the selection of this sample can be strongly guided by geography, ecology, and morphology, and, importantly, by ploidy level (or inferences of ploidy level from spore size data; e.g., Beck et al., 2010; Li et al., 2012; Sigel et al., 2011). The number of low-copy markers developed for use in ferns remains small (see Chen et al., 2012; Ishikawa et al., 2002; Schuettpelz et al., 2008), but recent work by myself and collaborators under the aegis of the 1KP project (www.onekp.com) has yielded approximately 50 fern transcriptomes, which we are currently using to develop primer sets for low-copy loci across leptosporangiate ferns (Rothfels et al., in prep.).

Low-copy nuclear data also provide a solution to a major outstanding issue in fern systematics in general, and Cystopteris in particular: resolving the reticulate relationships of allopolyploids. Due to reticulation, the relationships among species cannot be directly inferred by current methods or represented by a purely bifurcating tree (Linder & Rieseberg, 2004; Vriesendorp & Mort, 2005). However, low-copy nuclear markers provide the necessary information to form a species network, and models for translating such “multiply-labeled” gene trees into species networks (Huber & Moulton, 2006; Smedmark et al., 2005) are implemented in the program PADRE (Huber et al., 2006; Lott et al., 2009a; Lott et al., 2009b), allowing us to infer species networks from gene trees (Marcussen et al., 2012). In inferring such networks, it may be necessary to determine whether multiple sequences from an individual are homeologous (different copies that trace back to a polyploidy event) or homologous (different copies that segregate at a single locus, i.e., alleles). We can distinguish between these two
possibilities by assaying the gametophytes: gametophytes will show both copies if they are homeologous, but only one if they are allelic.

Such data—an understanding of the species units in *Cystopteris* and the relationships among them—would allow for a wide variety of studies of polyploid evolution. The most interesting of these, to me, are those that explore the macroevolutionary consequences of polyploidy. These consequences have been the subject of much debate among evolutionary biologists. For example, Stebbins (1950) believed polyploidy to be an evolutionary dead-end, because “extra” genomes would mask mutations from selection, and Wagner (1970) echoed those sentiments when he argued that polyploidy, and other deviations from “the main pillars of plant evolution” are mere “evolutionary noise.” However, more recently, polyploidy has been widely credited as a major contributor to organismal diversity (De Bodt et al., 2005; Mable & Roberts, 1997; Otto & Whitton, 2000; Petit & Thompson, 1999; Ricklefs & Renner, 1994; Shaw et al., 2008; Sidow, 1996; Soltis et al., 2010; Soltis et al., 2007; Stebbins, 1985; Taylor et al., 2001; Wagner et al., 2003; Werth & Windham, 1991). Indeed, polyploids form frequently (Ramsey & Schemske, 1998), and are very common among extant species (especially in plants; Otto & Whitton, 2000). Polyploidy is virtually unique among speciation mechanisms in that it can yield “nearly instantaneous reproductive isolation” (Coyne & Orr, 2004; Wood et al., 2009). However, neither the prevalence of extant polyploids nor the ease of their divergence from their diploid ancestors speaks to the greater thrust of the Stebbins-Wagner critique: the evolutionary potential of these new lineages. Do polyploid lineages themselves diversify at comparable (or greater) rates than do their diploid relatives, or are they evolutionarily stagnant dead-ends?

In a recent meta-analysis across land plants, collaborators and I provided the first quantitative estimate of the fate of polyploids in a phylogenetic context (Mayrose et al.,
We found that polyploids diversify more slowly than do their diploid relatives (polyploids both speciate more slowly and go extinct more quickly), a finding that has profound implications for our understanding of planetary biodiversity. *Cystopteris* provides an ideal model system to further explore the macroevolutionary consequences of polyploidy. Unlike the taxonomically broad but shallow sampling of our meta-analysis, *Cystopteris* can provide narrower, dense sampling, with ploidy data directly available for each terminal taxon (cryptic polyploid taxa aren’t assumed to be diploid in those cases where the diploids are the only taxonomically recognized entities).

Furthermore, *Cystopteris* is free of the confounding effects of asexuality (c.f. Beck et al., 2011b; Vamosi & Dickinson, 2006), and a robust phylogenetic history is available from both the maternal (plastid and nuclear) and paternal (nuclear) contributions.

As in the investigation by Mayrose et al. (2011), the relationships between character state (ploidy level) and extinction and speciation rates in *Cystopteris* should be inferred under the BiSSE framework (FitzJohn et al., 2009; Maddison et al., 2007), because BiSSE can co-infer rates of character change and rates of speciation/extinction, and thus avoids their conflation (Maddison, 2006). However, the basic BiSSE model prohibits character state changes and speciation or extinction events from occurring simultaneously, which violates one of the expectations of the polyploidization process (that genome doubling events directly contribute to reproductive isolation, and thus might be simultaneous with a speciation event). Fortunately, recent extensions of the BiSSE model (into “BiSSEness”; Magnuson-Ford, 2011; Magnuson-Ford & Otto, 2012; Mayrose et al., 2011) permit these two types of events to occur simultaneously, and thus are ideal for investigations of the macroevolutionary consequences of polyploidy. The application of BiSSEness to the global *Cystopteris* taxon sample amassed in the course of this thesis, using species-unit designations inferred with the aid of the low-copy markers
currently being developed, will allow for very exciting investigations of this deeply contentious issue.

*Cystopteris* is also an excellent system with which to explore many other aspects of polyploid evolution. Are there geographical or environmental correlates of polyploid incidence (e.g., latitude or habitat stability)? What is the effect of polyploidy on the rate of evolution of reproductive evolution between populations (including empirical examinations of the models of Werth and Windham (1991) and Taylor et al. (2001))? What is the relationship between polyploidy and geographic range, or between the range sizes or ecological breadths of polyploids versus their diploid progenitors? Are there short-term fitness consequences of polyploidization, or is it a neutral process (e.g., Meyers and Levin (2006))? Or, most broadly perhaps, what implications does a fuller understanding of polyploid evolution have on our picture of global biodiversity (e.g., Soltis et al. (2007))? This thesis provides the foundation for the investigation of these questions, and for research into many other exciting aspects of polyploid evolution.
## Appendix A: Voucher table for Chapter One

Voucher table and Genbank accession numbers. DB#: Pryer Lab DNA Database number (fernlab.biology.duke.edu). Shaded headings mark each “major clade,” following Figure 6. Superscripts following Genbank numbers indicate previously published sequences from: a Duffy et al. (2009); b Gastony and Ungerer (1997); c Kuo et al. (2011); d Lu & Li, unpub.; e Murikami et al. unpub.; f Nakahira (2000); g Pinter et al. (2002); h Rothfels et al. (2008); i Sano et al. (2000a); j Schuettpelz & Pryer (2007); k Schuettpelz et al. (2007); l Smith and Cranfill (2002); m Wolf et al. (1994); n Yatebe & Murikami unpub. Data missing or not applicable are indicated with a “–”.

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| Dryopteridaceae | | | |
|-----------------|-----|---------|------------|------|------|------|--------|------|
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| C. sloanei (Poep. ex Spreng.) C.V. Morton | 3607 | Schuettpelz 552 et al. (DUKE) | Cult. (Botanischer Garten München-Nymphenburg) | EF463172 | EF463383 | JF832210 | JF832202 | – |
| Didymochlaena truncatula (Sw.) J. Sm | – | RBGE 1993685 | Unknown | DQ508769 | – | – | – | – |
| D. truncatula (Sw.) J. Sm | 2435 | Schuettpelz 267 (DUKE) | Ecuador: Zamora-Chinchipe | – | EF452030 | JF832112 | JF832210 | – |
| D. truncatula (Sw.) J. Sm | – | No voucher taken. | Cult. (Dr. Cecilia Koo Bot. Cons. Center K017011) | – | – | – | – | – |

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**Note:** The table shows the species, collection information, location, and GenBank accessions for various fern species. The list includes species from various genera, with specific details on collections, locations, and GenBank accessions.
| **Diplazium bombonasae** Rosenst. | 3764 | Moran 7493 (NY) | Ecuador: Pastaza | EF463308 | EF463570 | JF832115 | JF832213 | JF832217 | EF463311 | EF463573 | JF832116 | JF832214 | – |
| **D. dilatatum** Blume | 3638 | Schuettpehl 588 et al. (GOET) | Cult. (Botanischer Garten München-Nymphenburg) | EF463311 | EF463573 | JF832116 | JF832214 | – |
| **D. dilatatum** Blume | – | Kuo 987 (TAIF) | Taiwan: Taipei | – | – | – | – | – | JF832274 | – | – | |
| **D. prollferum** (Lam.) Thouars | 3639 | Schuettpehl 590 & Schneider (GOET) | Cult. (Botanischer Garten München-Nymphenburg) | EF463311 | EF463573 | JF832116 | JF832214 | – |
| **D. wichurae** (Mett.) Diels | 2874 | Kuo 986 (TAIF) | Taiwan | – | EF463573 | JF832117 | JF832215 | – |
| **Pseudocystopteris atkinsonii** (Bedd.) Ching | 4837 | Schuettpehl 1094A et al. (DUKE) | Taiwan: Nantou | JF832083 | – | – | – | – |
| **Blechnaceae** | | | | | | | | | | | |
| **Blechnum orientale** L. | – | Kuo 827 (TAIF) | Taiwan: Taipei | – | – | – | – | – |
| **B. orientale** L. | – | Unknown | Unknown | AB040567 | – | – | – | – |
| **B. schomburgkii** (Klotzsch) C. Chr. | 2410 | Schuettpehl 242 (DUKE) | Culture (Duke U. Greenh.; Chinchipe) | EF463160 | EF463353 | JF832101 | JF832198 | JF832261 | – |
| **B. spicand (L.) J. Sm.** | 6943 | Windham 3395 (DUKE) | Canada: British Columbia | JF832059 | JF832157 | JF832102 | JF832199 | JF832262 | – |
| **Doodia media** R. Br. | 2555 | Schuettpehl 295 (DUKE) | Canada: British Columbia | JF832059 | JF832157 | JF832102 | JF832199 | JF832262 | – |
| **Sadleria cyathoides** Kaulf. | 3432 | Schuettpehl 507 (DUKE) | Cult. (Duke U. Greenh. 87-166) | EF463161 | EF463356 | JF832141 | JF832240 | JF832288 | – |
| **Stenochlaena tenuifolia** (Desv.) T. Moore | 3429 | Schuettpehl 504 (DUKE) | Cult. (Duke U. Greenh.) | EF463163 | EF463358 | JF832142 | JF832241 | JF832289 | – |
| **Woodwardia areolata** (L.) T. Moore | – | Unknown | Unknown | – | – | – | – | – | JF832297 | – |
| **W. unigemmata** (Makino) Nakai | – | Kuo 493 (TAIF) | Taiwan | – | – | – | – | JF832297 | – |
| **Cytoptepis taiwianiana** (Tagawa) Love & Love | 4870 | Schuettpehl 1127A et al. (DUKE) | Taiwan: Nantou | JF832052 | – | – | – | – | – |
| **A. taiwianiana** (Tagawa) Love & Love | – | Kuo 175 (TAIF) | Taiwan | – | – | JF832091 | JF832188 | – |
| **A. tenuisepta** (Bl.) Tagawa | – | Kuo 474 | Taiwan: Nantou | – | – | JF832217 | JF832215 | JF832250 | – |

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**Rhachidosorus**

- **Rhachidosorus mesosorus** (Makino) Ching
  - 7075 | Matsumoto s.n. (DUKE) | Cult. (TBG; origin: Japan) |
  - | Chen 441 (TNU) | Taiwan |

**Thelypteridaceae**

- **Coryphopteris seemannii** Holttum
  - 3774 | Game 95/147 (UC) | Fiji: Viti Levu |
- **Macrothelypteris torresiana** (Gaudich.) Ching
  - 6502 | Rothfels 3050 et al. (DUKE) | Mexico: San Luis Potosi |
- **M. tosasiana** (Gaudich.) Ching
  - | Kuo 826 (TAIF) | Taiwan |
- **Phegopteris connectilis** (Michx.) Watt
  - 7060 | Larsson 17 (UPS) | Sweden: Uppsal |
- **P. connectilis** (Michx.) Watt
  - | Kuo 151 (TAIF) | Taiwan |
- **Pseudophygodium cruciata** (Willd.) Holttum
  - 3559 | Janssen 2724 (P) | France: Ile de la Reunion |
- **Thelypteris dentata** (Forssk.) E.P. St. John
  - 3654 | Schuettpelz 607 et al. (B) | Cult. (Botanischer Garten Berlin-Dahlem) |
  - 3747 | Huiet s.n. et al. (UC) | Cult. (UCBG 83.0943; origin: Venezuela) |
- **T. palustris** Schott
  - 7055 | Larsson 16 (UPS) | Sweden: Uppsal |
- **T. pozoi** (Lag.) C.V. Morton
  - | Kuo 110 (TAIF) | Taiwan: Orchid Is. |
- **T. pozoi** (Lag.) C.V. Morton
  - | Unknown | Unknown |

- Additional data for specific collections and accessions is also included, but the table format is not listed here.
<table>
<thead>
<tr>
<th>Species</th>
<th>Collection Code</th>
<th>Location</th>
<th>Accession Numbers</th>
</tr>
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<tr>
<td><em>T. uraiensis</em> (Rosenst.) Ching</td>
<td>Kuo 139 (TAIF)</td>
<td>Taiwan: Taipei</td>
<td>JF303972 – JF304002 – JF303934</td>
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<td><strong>Woodsia &amp; allies</strong></td>
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<td><em>Cheilanthes elongata</em> (Hook.) Copel.</td>
<td>Polunin, Sykes &amp; Williams (UPS)</td>
<td>Nepal: Maharigaon</td>
<td>JF832060 – JF832103</td>
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<td><em>Protowoodsia manchuriensis</em> (Hook.) Ching</td>
<td>Nakaike, Sakakibara &amp; Ishizuka s.n. (E)</td>
<td>Japan: Koisawa</td>
<td>JF832082 – JF832138</td>
</tr>
<tr>
<td><em>P. manchuriensis</em> (Hook.) Ching</td>
<td>Fujimoto s.n. (TNS)</td>
<td>Cult. (TBG; origin: Japan)</td>
<td>– – – – JF832284</td>
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<tr>
<td><em>Woodsia andersonii</em> Beddome</td>
<td>Ho et al. 2601 (E)</td>
<td>China: Qinghai</td>
<td>JF833268 – JF832123 – JF832279</td>
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<tr>
<td><em>W. ilvensis</em> (L.) R. Br.</td>
<td>Karis s.n. (UPS)</td>
<td>Sweden: Runmarö</td>
<td>JF832086 – JF832293</td>
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<tr>
<td><em>W. mollis</em> (Kauf.) J. Sm.</td>
<td>Larsson 103 (UPS)</td>
<td>Mexico: Hidalgo</td>
<td>JF832087 – JF832294</td>
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<tr>
<td><em>W. plummerae</em> Lemmon</td>
<td>Schuettpeitz 1235A (DUKE)</td>
<td>USA: Arizona</td>
<td>JF832088 – JF832295</td>
</tr>
<tr>
<td><em>W. polystichoides</em> D.C. Eaton</td>
<td>Li 420 (TAIF)</td>
<td>Taiwan</td>
<td>– – – – JF303930</td>
</tr>
</tbody>
</table>
Appendix B: Supplementary figures for Chapter One

Figure 19: Majority-rule consensus tree from *Phycas* with C=1. Posterior probabilities (as percents) are listed for each internode.
Figure 20: Majority-rule consensus tree from *Phycas* with $C=e$. Posterior probabilities (as percents) are listed for each internode.
Figure 21: Majority-rule consensus tree from *Phycas* with C=10. Posterior probabilities (as percents) are listed for each internode.
Figure 22: Majority-rule consensus tree from MrBayes with the branch-length prior (mu0/mu1) = 0.01. Posterior probabilities are listed for each internode.
Figure 23: Majority-rule consensus tree from MrBayes with the branch-length prior (mu0/mu1) = 0.001. Posterior probabilities are listed for each internode.
Figure 24: Majority-rule consensus tree from MrBayes with the branch-length prior (\(\mu_0/\mu_1\)) = 0.0001. Posterior probabilities are listed for each internode.
Figure 25: Majority-rule consensus tree from BEAST, with posterior probabilities listed above each internode.
Appendix C: Voucher table for Chapter Three

In the format: Species, Voucher (HERBARIUM), Fern Lab database number (fernlab.biology.duke.edu), Provenance, and Genbank numbers (and citations for previously published sequences) for trnG-R; rbcl; matK. The first instance of a taxon is in bold, with authority included. Missing data are indicated by “--”.

*Acystopteris japonica* (Luerss.) Nakai. Tsugaru & Takahashi 25409 (MO).

Schuettpelz 1127A (DUKE). 4870. Taiwan: Nantou County. JF832188 (Rothfels et al., 2012a); JF832052 (Rothfels et al., 2012a); xxx. *Acystopteris taiwaniana*. Kuo 175 (TAIF). 6137. Taiwan: Chial County. xxx; JF303968 (Kuo et al., 2011); JF303925 (Kuo et al., 2011). *Acystopteris tenuisecta* (Bl.) Tagawa. Schuettpelz 807 (DUKE).


Chittenden County. xxx; xxx; xxx. *Cystopteris utahensis* Windham & Haufler.

Rink 6566 (DUKE). 5832. USA: Arizona. Coconino County. xxx; xxx; xxx.


Rothfels & Zylinski 3914 (DUKE). 7639. USA: Virginia. Highland County. xxx; --;


Larsson 6 (DUKE). 7059. Sweden: Uppsala. JF832218 (Rothfels et al., 2012a);


This page is part of a larger text that seems to be a list of plant species, possibly for a botanical study or publication. The text includes details such as species names, authors, and geographic locations, indicating a focus on plant taxonomy or ecology. The page appears to be a continuation of a previous list, starting from page 145.

The text continues with entries like:


The OUTGROUP is mentioned as *Athyrium otophorum* (Miq.) Koidz. Smith s.n. (UC). 3744. Cult. JF832195 (Rothfels et al., 2012a); EF463305 (Schuettpelz & Pryer, 2007); --. *Athyrium otophorum*. Ebihara et al. 070210-02 (TNS). --. Japan: Shizuoka Pref. --; --; JF832258 (Rothfels et al., 2012a). *Blechnum schomburgkii* (Klotzsch) C. Chr. Schuettpelz 242 (DUKE). 2410. Ecuador: Zamora-Chinchipe. JF832198 (Rothfels et al., 2012a); EF463160 (Schuettpelz & Pryer, 2007); JF832261 (Rothfels et al., 2012a). *Deparia*
**lancea (Thunb.) R. Sano.** Schuettpelez 298 (DUKE). 2558. Cult. (Duke U. Greenhouse). JF832207 (Rothfels et al., 2012a); EF463306 (Schuettpelez & Pryer, 2007); --. *Deparia lancea*. Kuo 112 (TAIF). --. Taiwan. --; JF303940 (Kuo et al., 2011). **Diplaziopsis javanica (Blume) C.Chr.** Schuettpelez 1220A (DUKE). 4967. Taiwan: Ilan. JF832212 (Rothfels et al., 2012a); JF832066 (Rothfels et al., 2012a); --. *Diplaziopsis javanica*. Kuo 138 (TAIF). --. Taiwan. --; JF303928 (Kuo et al., 2011).

**Hemidictyum marginatum (L.) C.Presl.** Christenhusz 2476 (DUKE). 3054. French Guiana: Montagnes Tortue. JF832221 (Rothfels et al., 2012a); EF463318 (Schuettpelez & Pryer, 2007); JF303927 (Kuo et al., 2011). **Onocleopsis hintonii Ballard.** Rothfels 3360 et al. (DUKE). 6729. Mexico: Oaxaca. JF832230 (Rothfels et al., 2012a); JF832077 (Rothfels et al., 2012a); JF832281 (Rothfels et al., 2012a).

**Pseudocystopteris atkinsonii (Bedd.) Ching.** Schuettpelez 1094 (DUKE). 4837. Taiwan: Nantou County. JF832235 (Rothfels et al., 2012a); xxx; --.

**Pseudocystopteris atkinsonii.** Kuo 477 (TAIF). --. Taiwan: Nantou County. --; JF832285 (Rothfels et al., 2012a). **Pseudophegopteris cruciata (Willd.) Holttum.** Janssen 2724 (P). 3559. France: Ile de la Reunion. JF832236 (Rothfels et al., 2012a); EF463279 (Schuettpelez & Pryer, 2007); JF832286 (Rothfels et al., 2012a). **Wood sia obtusa (Spr.) Torrey.** Schuettpelez 328 (DUKE). 2973. Cult.: originally from USA: Texas. Burnet County. xxx; EF463319 (Schuettpelez & Pryer, 2007); --.
Appendix D: Voucher table for Chapter Four

Voucher table (sequences not yet entered into Genbank) for the $gapCp$ data in chapter 4. DB#: Pryer Lab DNA Database number (fernlab.biology.duke.edu). For the three reduced datasets (All-Unique, Thinned-1, and Thinned-2) the number of sequences included from each accession is listed, followed by a description of the specific clones that were selected for each sequence. For example, the entry “2 (2x4, 5)” in the All-Unique column indicates that there were two unique sequences obtained from that accession, and they are represented by clone #2 and clone #5, and further that the sequence represented by clone #2 occurred four times in the Total Seqs. dataset, whereas the sequence represented by clone #5 only occurred once. Not all clone numbers in a series will necessarily be represented in the Total Seqs. dataset—some will have been of low quality, of a locus other than $gapCp$ “short”, or a chimaera.

<table>
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<th>DB#</th>
<th>Species</th>
<th>Voucher</th>
<th>Provenance</th>
<th>Total Seqs.</th>
<th>All-Unique</th>
<th>Thinned-1</th>
<th>Thinned-2</th>
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<td>7974</td>
<td>×Cystocarpium roskumianum Fraser-Jenk.</td>
<td>Roskam s.n. (DUKE)</td>
<td>Cultivation. Originally: France.</td>
<td>18</td>
<td>11 (13, 15, 16, 20, 21, 22, 23, 25, 4x5, 5x3, 6x2)</td>
<td>4 (13, 4, 5, 6)</td>
<td>4 (13, 4, 5, 6)</td>
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<td>4870</td>
<td>Acystopteris taiwaniana (Tagawa) A.Löve &amp; D.Löve</td>
<td>Schuettpelz 1127A (DUKE)</td>
<td>Taiwan: Nantou County.</td>
<td>5</td>
<td>2 (2x4, 5)</td>
<td>2 (2, 5)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>4831</td>
<td>Acystopteris tenuisecta (Bl.) Tagawa</td>
<td>Schuettpelz 1088A (DUKE)</td>
<td>Taiwan: Nantou County.</td>
<td>4</td>
<td>2 (1x3, 3)</td>
<td>1 (1)</td>
<td>1 (1)</td>
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<tr>
<td>5836</td>
<td>Cystopteris bulbifera (L.) Bernh.</td>
<td>Windham 94-189 (DUKE)</td>
<td>USA: Arizona. Coconino County.</td>
<td>3</td>
<td>1 (1x3)</td>
<td>1 (1)</td>
<td>1 (1)</td>
</tr>
<tr>
<td>6116</td>
<td>Cystopteris bulbifera (L.) Bernh.</td>
<td>Almack s.n. (DUKE)</td>
<td>Canada: Ontario. Durham Region.</td>
<td>13</td>
<td>8 (12, 9x2,1x3, 10x3, 2, 4x2, 7, 8)</td>
<td>4 (1, 12, 9, 10)</td>
<td>1 (12)</td>
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<tr>
<td>5316</td>
<td>Cystopteris diaphana (Bory) Blasdell</td>
<td>Matos 08-147 (DUKE)</td>
<td>Costa Rica: San Jose. Canton Villa Mills.</td>
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<td>7 (1x3, 10, 12, 14, 3x5, 4, 7)</td>
<td>2 (1, 3)</td>
<td>1 (1)</td>
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<tr>
<td>Datasheet</td>
<td>Name</td>
<td>Collector</td>
<td>Location</td>
<td>Country</td>
<td>Coordinates</td>
<td>Notes</td>
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<tr>
<td>6380</td>
<td>Cystopteris diaphana (Bory) Blasdell</td>
<td>Arana 889 (DUKE)</td>
<td>Argentina: Prov. San Luis.</td>
<td>Argentina: Prov. San Luis.</td>
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<td>5 (1, 2, 4, 5, 6)</td>
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<td>Cystopteris fragilis (L.) Bernh.</td>
<td>Christenhusz 2931 (DUKE)</td>
<td>Sweden: Uppsala</td>
<td>Sweden: Uppsala</td>
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<td>5851</td>
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<td>Kelsey s.n. (DUKE)</td>
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<td>USA; Utah. Salt Lake County.</td>
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<td>Cystopteris fragilis (L.) Bernh.</td>
<td>Heidmarsson c.f.03 (DUKE)</td>
<td>Iceland: Vesturland Region</td>
<td>Iceland: Vesturland Region</td>
<td>17</td>
<td>10 (10x2, 17, 19, 2x2, 3, 4x2, 6, 7x5, 8, 9)</td>
<td>4 (10, 2, 4, 7)</td>
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<td>7248</td>
<td>Cystopteris fragilis (L.) Bernh.</td>
<td>Sigel 2010-59 (DUKE)</td>
<td>Switzerland: Bern Canton. Jungfrau Region.</td>
<td>Switzerland: Bern Canton. Jungfrau Region.</td>
<td>7</td>
<td>5 (1, 2, 3, 4x3, 7)</td>
<td>2 (2, 4)</td>
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<tr>
<td>7625</td>
<td>Cystopteris fragilis (L.) Bernh.</td>
<td>Li 1056 (DUKE)</td>
<td>Taiwan: Heping Twp, Taichung County.</td>
<td>Taiwan: Heping Twp, Taichung County.</td>
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<td>4 (1, 2x2, 3x2, 6)</td>
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<td>Cystopteris fragilis (L.) Bernh.</td>
<td>S.F.Smith 3 (DUKE)</td>
<td>USA: Colorado. Park County.</td>
<td>USA: Colorado. Park County.</td>
<td>21</td>
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<tr>
<td>5839</td>
<td>Cystopteris montana (Lam.) Berhn. Ex Desv.</td>
<td>LeBlond 6448 (DUKE)</td>
<td>Canada: Newfoundland. St. Barbe North District.</td>
<td>Canada: Newfoundland. St. Barbe North District.</td>
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<td>3 (1, 4x2, 5)</td>
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<tr>
<td>4861</td>
<td>Cystopteris moupinensis Franch.</td>
<td>Schuettpelz 1118A (DUKE)</td>
<td>Taiwan: Nantou County.</td>
<td>Taiwan: Nantou County.</td>
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<td>5 (1, 2, 3, 4, 5)</td>
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<td>Cystopteris protrusa (Weath.) Blasdell</td>
<td>Alford 2088 (DUKE)</td>
<td>USA: Mississippi. Wilkinson County.</td>
<td>USA: Mississippi. Wilkinson County.</td>
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<td>Cystopteris tenuis (Michx.) Desv.</td>
<td>Barrington 2373 (DUKE)</td>
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<td>USA: Vermont. Chittenden County.</td>
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<td>7639</td>
<td>Gymnocarpium appalachianum Pryer &amp; Haufler</td>
<td>Rothfels 3914 &amp; Zylinski (DUKE)</td>
<td>USA: Virginia. Highland County.</td>
<td>USA: Virginia. Highland County.</td>
<td>8</td>
<td>5 (1, 2x4, 5, 7, 9)</td>
<td>1 (2)</td>
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<td>7800</td>
<td>Gymnocarpium appalachianum Pryer &amp; Haufler</td>
<td>Rothfels 3897 &amp; Zylinski (DUKE)</td>
<td>USA: Virginia. Page County.</td>
<td>USA: Virginia. Page County.</td>
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<td>8 (10x2, 17, 8x2, 1, 11, 16, 2, 9)</td>
<td>3 (10, 8, 9)</td>
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<tr>
<td>4710</td>
<td>Gymnocarpium disjunctum (Michx.) Desv.</td>
<td>Metzgar 224</td>
<td>USA: Alaska. Kenai</td>
<td>USA: Alaska. Kenai</td>
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<td>4 (1, 2, 3, 4)</td>
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<td>Species</td>
<td>Collection</td>
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<td>Province/Region</td>
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<tr>
<td>7751</td>
<td>Gymnocarpium disjunctum</td>
<td>Sigel 2010-82 &amp; Miles (DUKE)</td>
<td>DUKE</td>
<td>USA: Washington</td>
<td>Snohomish County</td>
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<td>7 (1, 10, 11x2, 14, 15, 2x4, 6)</td>
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<td>Gymnocarpium dryopteris</td>
<td>Christenhusz 3758 (DUKE)</td>
<td>DUKE</td>
<td>Finland: Varsinais-Suomi, Jurmo.</td>
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<td>3 (1x2, 3, 4x4)</td>
<td>2 (1, 4)</td>
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<td>Ebihara &amp; Kadota HK2007-815</td>
<td>DUKE</td>
<td>Japan: Hokkaido Pref.</td>
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<td>11 (1, 11, 13, 15x2, 16x2, 18, 2x4, 3x2, 4, 7, 8x2)</td>
<td>2 (2, 6)</td>
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<tr>
<td>8031</td>
<td>Gymnocarpium dryopteris</td>
<td>Oldham 38191 &amp; Brinker (DUKE)</td>
<td>DUKE</td>
<td>Canada: Ontario.</td>
<td>Thunder Bay District</td>
<td>15</td>
<td>9 (1x5, 3, 4x2, 6, 13, 14, 15, 16, 8x2)</td>
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<tr>
<td>6979</td>
<td>Gymnocarpium jessoense ssp. parvulum</td>
<td>Harris 08-131 (DUKE)</td>
<td>DUKE</td>
<td>Canada: Ontario.</td>
<td>Thunder Bay District</td>
<td>3</td>
<td>2 (1, 2x2)</td>
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<tr>
<td>8120</td>
<td>Gymnocarpium jessoense ssp. parvulum</td>
<td>Legler 877 (NY)</td>
<td>DUKE</td>
<td>Russia: Sakhalin Region.</td>
<td>12</td>
<td>5 (1x6, 11, 4, 5, 6x3)</td>
<td>2 (1, 6)</td>
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<tr>
<td>4862</td>
<td>Gymnocarpium remotepinnatum</td>
<td>Schuettpeiz 1119A (DUKE)</td>
<td>DUKE</td>
<td>Taiwan: Nantou County.</td>
<td>8</td>
<td>5 (1x2, 2, 3, 4x3, 5)</td>
<td>2 (3, 4)</td>
</tr>
<tr>
<td>7945</td>
<td>Gymnocarpium robertianum</td>
<td>Larsson 282 (DUKE)</td>
<td>DUKE</td>
<td>Norway; Nordland.</td>
<td>Fauske County.</td>
<td>12</td>
<td>7 (1, 10, 3, 4x4, 5x2, 7, 9x2)</td>
</tr>
</tbody>
</table>

**Total Sequences:** 271 168 68 52
Appendix E: Tentative treatment of Cystopteridaceae taxa

The following treatment is based on an incomplete survey of the literature and on the preliminary results of this thesis. In particular, I’ve drawn heavily on secondary sources—tropicos (www.tropicos.org) and the International Plant Names Index (www.ipni.org)—in structuring this list, and providing nomenclatural data.

I’ve attempted to account for each basionym that has ever been treated under one of the Cystopteridaceae genera (Acystopteris, Cystoathyrium, Cystopteris, Gymnocarpium, ×Cystocarpium), and to provide, for each, a list of nomenclatural synonyms. Where a given basionym has been named at different ranks, I list those names under the highest applied rank (with species > subspecies > varieties > forms). This decision is based both on convenience, and on the loosely held conviction that the number potentially interbreeding Cystopteridaceae populations that share a unique history and have a decent chance of a unique shared future is underestimated, and further, that such populations are most appropriately recognized at the rank of species. As such, I’m much more inclined to recognize different cytotypes as distinct species (for some supporting arguments, see, e.g., Haufler & Windham, 1991; Soltis et al., 2007) than would be Fraser-Jenkins, for example (e.g., Fraser-Jenkins, 1997). The general theme of which rank is most appropriate for Cystopteridaceae taxa has been much debated, with many taxa having names available at both the specific and subspecific (and/or varietal) ranks.

Names are presented in alphabetical order, regardless of rank. Where possible, I have included the type information for each basionym (HT=holotype; IT=isotype; LT=lectotype), and a very brief discussion, including comments on synonymy. The publication year of those names for which I have the protologue are included in
parentheses (e.g., ×Cystocarpium rosakanianum Fraser-Jenk., Taxon. Revis. Indian Subcontinental Pteridophytes 207. 2008), and the publication information is included in the References; names for which I do not have the protologue have their year of publication without parentheses. Taxa that I tentatively accept are in boldface, and include:

**Acystopteris** Nakai; **Acystopteris japonica** (Luerss.) Nakai; **Acystopteris taiwaniana** (Tagawa) Á.Löve & D.Löve; **Acystopteris tenuisecta** (Blume) Tagawa; **Cystoathyrium** Ching; **Cystoathyrium chinense** Ching; **Cystopteris** Bernh.; **Cystopteris almaatensis** Kotukhov; **Cystopteris alpina** (Jacq.) Desv.; **Cystopteris altaicensis** Gureeva; **Cystopteris bulbifera** (L.) Bernh.; **Cystopteris deqinensis** Z.R.Wang; **Cystopteris diaphana** (Bory) Blasdell; **Cystopteris douglasii** Hook.; **Cystopteris fragilis** (L.) Bernh.; **Cystopteris fragilis** subsp. **huteri** (Hausm. ex Milde) C.Prada & Salvo; **Cystopteris guizhouensis** X.Y.Wang & P.S.Wang; **Cystopteris kansuana** C.Chr.; **Cystopteris laurentiana** (Weath.) Blasdell (pro hybr.); **Cystopteris millefolia** Mickel & Tejero; **Cystopteris modesta** Ching; **Cystopteris montana** (Lam.) Bernh. ex Desv.; **Cystopteris moupinensis** Franch.; **Cystopteris pellucida** (Franch.) Ching in C.Chr.; **Cystopteris protrusa** (Weath.) Blasdell; **Cystopteris reevesiana** Lellinger; **Cystopteris sikkimensis** Ching ex Bir; **Cystopteris sudetica** A.Braun & Milde; **Cystopteris tasmanica** Hook.; **Cystopteris tennessensis** Shaver; **Cystopteris tenuis** Desv.; **Cystopteris tibetica** Z.R.Wang; **Cystopteris utahensis** Windham & Haufler; **Cystopteris × illinoensis** R.C. Moran; **Cystopteris × montserratii** Prada & Salvo; **Cystopteris × wagneri** R.C.Moran; **Gymnocarpium** Newman; **Gymnocarpium appalachianum** K.M.Pryer & Haufler; **Gymnocarpium disjunctum** (Rupr.) Ching; **Gymnocarpium dryopteris** (L.) Newman; **Gymnocarpium jessoense** (Koidz.) Koidz.; **Gymnocarpium jessoense** subsp. **parvulum**; **Gymnocarpium oyamense** Ching; **Gymnocarpium remotepinnatum** (Hayata) Ching;
Gymnocarpium robertianum (Hoffm.) Newman; Gymnocarpium × achriosporum;
Gymnocarpium × bipinnatifidum Miyam; Gymnocarpium × brittonianum (Sarvela)
K.M.Pryer & Hauser; Gymnocarpium × heterosporum W.H. Wagner (pro. sp.);
Gymnocarpium × intermedium J.Sarvela; ×Cystocarpium Fraser-Jenk.; ×Cystocarpium
roskamianum Fraser-Jenk.

Nakai. Basionym: Cystopteris japonica Luerss.

Hezukumura. Tachiro s.n. (HT: unknown).

Acystopteris japonica var. taiwaniana (Tagawa) W.C.Shieh, J. Sci. Engin. (Nation. Chung-

Sp. Fil. 3: 222. 1859. Athyrium tenuisectum (Blume) T. Moore, Index Fil. 188. 1860.
“Crecit in sylvis temperatis insulae Java. Blume” fide Blasdell (1963) (HT: L?).
Bir (1971) reports both triploid and tetraploid individuals, and argues that the triploids are “clearly … formed as a result of a cross between 4x and 2x races.”

*Cystoathyrium* Ching, Acta Phytotaxonomica Sinica 11: 23, t. 4. (1966).—TYPE:
*Cystoathyrium chinense* Ching. Basionym: *Cystoathyrium chinense* Ching.


Basionym: *Polypodium fragile* L.


= *Cystopteris fragilis* (fide IPNI). I have no information on this name.


= *Cystopteris diaphana*? I don’t have access to the protologue and haven’t seen the type, so don’t know the correct application of this name. However, it is typified on material from Veracruz, Mexico, beyond the range of *C. fragilis* as I very tentatively treated it (Rothfels et al., 2012c), and within the range, instead, of *C. diaphana*. Note, however, that *acuta* is the older name, if *diaphana* is considered conspecific.

= Davallia hymenophylloides (fide IPNI)


=Cystopteris pellucida (fide Wang et al., 2012b)

Cystopteris albescens Link, Fil. sp. 47. 1841.

= Woodsia obtusa (fide IPNI)

Cystopteris allioni Newm., Phytologist 1851. app. XXV. 1851. [Corrected from C. allioni following ICBN Art 60C.1.b]

=Cystopteris montana (fide TROPICOS).


Based on the illustration included in the protologue, this is likely a rugose-spored member of the C. fragilis complex, and this name is thus of uncertain application. The description includes the statement “Spori VIII”—does this suggest that this species has eight spores per sporangium? That would be exceedingly unusual. I’ve not seen this species, and don’t have any data from it.

This is a narrow-segmented European hexaploid in the *C. fragilis* complex. Very preliminary chloroplast data suggest that *C. alpina* is closer to the Australian/New Zealand endemic than to other members of the *C. fragilis* complex (or, given its hexaploid nature, that one of its maternal parents, at least, is close to *C. tasmanica*; Rothfels et al., 2012c).


This member of the *C. fragilis* complex is described as glandular abaxially, with typical echinate spores. I have not seen it, and don’t have any data from it.


=C*ystopteris fragilis*? This taxon is recognized, as a variety of *C. fragilis*, by Christ (1900)—his citation is: “Var. anthriscifolia. (Polypodium Hoffm. Deutsch flor. II. 9. 10.) Koch synops. Ed. II. 980.”


=C*ystopteris diaphana*. This treatment (*C. apiiformis* as a synonym of *C. diaphana*) is based entirely on geographic range; the placement of this name is very tentative.
Blasdell (1963) recognizes this as a variety of C. fragilis, due to its glandular indusium and “veins mostly not extending to margin.”

*Cystopteris aspidioides* C.Presl, Tent. Pterid. 93. 1836.

= *Microlepia speluncae* (fide IPNI); TROPICOS lists *Microlepia aspidioides* (C. Presl)

C. Presl as a nomenclatural synonym.


= *Cystopteris bulbifera* (fide IPNI, Blasdell (1963)).

*Cystopteris atrovirescens* C.Presl, Tent. Pterid. 93. 1836; Epim. 66. 1849 (atrovirens). 1836.

Basionym listed, in Presl (1836), as “*Cystopteridis* spec. Hort. bot. berol.”

= *Cystopteris fragilis*?


= *Cystopteris diaphana* (fide IPNI)


= *Cystopteris fragilis* (fide IPNI), = *Cystopteris dickieana* (fide Wang et al., 2012b)


IPNI says that this is the hybrid between *Cystopteris fragilis* and *Asplenium trichomanes*? That such a hybrid has ever formed is extraordinarily unlikely.
Cystopteris brasiliana C.Presl, Tent. Pterid. 93. 1836.

=Hypolepis (fide IPNI)

Cystopteris brevinervis Fée, Mém. Foug., 5. Gen. Filic. 300. 1850-52; 7 mem. 65 t. 26 f. 2. 1850.

=Dryopteris hirta (fide IPNI)


This is the common limestone dwelling diploid of eastern North America (with some disjunct populations in the west; Haufler et al., 1993). It has been implicated as a parent of the allotetraploids C. tennesseensis and C. utahensis (Haufler & Windham, 1991; Haufler et al., 1990; Shaver, 1950), and the allohexaploid C. laurentiana (Wagner & Hagenah, 1956). Otherwise, however, it is morphologically very distinctive, and seemingly largely uniform. Aspidium bulbiferum L. is a taxonomic, rather than nomenclatural, synonym? Aspidium bulbiferum Sw. is listed as the basionym for Cystopteris bulbifera by Presl (1836)?
Cystopteris bulbifera f. crispa H.L.Foster, Amer. Fern J. 44: 116, tab. 13. (1954).—TYPE:
U.S.A. Connecticut, ex hort, wild-collected from Housatonic River, below falls near Falls Village, Litchfield County. Foster s.n. (HT: US 2082460).

=Cystopteris bulbifera. Based on the illustration in the protologue, I suspect that this is just a “sport”—an aberrant individual of C. bulbifera. However, its unusual morphology continued across several generations of vegetative reproduction in cultivation (Foster, 1954). The relatively short stature of the plants, and their upright posture could indicate that they are actually examples of C. tennesseensis, but their abundant bulblet production (Foster doesn’t mention anything about those bulblets being misformed) argues against that possibility (Foster, 1954). An examination of the type, and particularly its spore size, is necessary to be conclusive one way or the other.

Cystopteris bulbifera var. flagelliformis G.Lawson, [publication details unknown].
Cystopteris bulbifera f. flagelliformis (G.Lawson) M.Broun, Index No. Amer. Ferns 55. 1938.

=Cystopteris bulbifera?

Cystopteris bulbifera var. horizontalis G.Lawson, [publication details unknown]. Filicula bulbifera Farw. var. horizontalis Farw. Amer. Midl. Naturalist 12: 251. (1931).—TYPE:
U.S.A. Holyoke, Massachusetts. Upson s.n. (HT: ?).

=Cystopteris bulbifera? This variety is described as having squatter leaves than typical, so might be C. tennesseensis.

Cystopteris bulbifera f. horizontalis Gilbert, [publication details unknown].

=Cystopteris bulbifera?
Cystopteris canariensis C.Presl, Tent. Pterid. 93. 1836. Cystopteris fragilis subsp. canariensis Christ, [publication details unknown].

=Cystopteris diaphana?


=Cystopteris fragilis (fide IPNI)


=Cystopteris fragilis (fide IPNI). Presuming that this name is typified on Chilean material, it probably belongs under C. diaphana.


This name is applied to the plant that Christ (1900) hypothesized to be a hybrid between C. fragilis and C. montana. Christ’s illustration (1900; his fig. 28 on page 163) does look intermediate, but could be C. alpina, or perhaps even C. sudetica. His description, unfortunately, does not say whether the plant had well-formed spores or not. I, personally, am skeptical that it’s a fragilis × montana hybrid, although given the parentage of ×Cystocarpium (Fraser-Jenkins, 2008; Chapter Four of this thesis) it is certainly possible.

Cystopteris comosa C.Presl, Tent. Pterid. 93. 1836.

= Alsophila squamulata (fide IPNI)


= Athyrium (fide IPNI)
Cystopteris dalhousiana Fée, 1857 [publication details unknown].

= Davallia hymenophylloides (fide IPNI)


Cystopteris fragilis var. dentata (Sw.) Hook., Sp. Fil. 1: 198. 1846. [IPNI has: Cystopteris fragilis var. dentata (Dicks.) Hook., Sp. Fil. 1: 198. 1846. Is this an error?]

=Cystopteris fragilis, fide Blasdell (1963). Christ (1900) recognizes this taxon, as a variety of C. fragilis, and seems to follow the IPNI authority: “(Polypodium Dickson Pl. Crypt. Brit. III 1.) Hook. spec. fil. I 198.”


Based on the illustration in the protologue (Wang, 1994), and the discussion in Wang et al. (2012b), this name looks like it applies to a large member of the sudetica clade (sensu Rothfels et al., 2012c). It may be a distinct species, or a particular large example of, e.g., Cystopteris pellucida. Fraser-Jenkins (1997), who has examined the type, believes it to be simply a large plant of Cystopteris moupinensis.

I’m not sure why *C. diaphana* seems to have priority over *C. viridula* (e.g., Fraser-Jenkins, 1991; Murphy & Rumsey, 2005; Rumsey, 2003), *C. canariensis*, or *C. azorica*.


This is one of the most hotly contested entities within the Cystopteridaceae (Dyer et al., 2000). The type material has unusual cutting (the pinnae are short and blunt, and overlap each other) and additionally has tuberculate spores (rather than echinate, as is more common in the *Cystopteris fragilis* complex). The name could, then, be circumscribed to include only those plants similar in cutting to the type material (a very narrow circumscription), or expanded to, e.g., all members of the *Cystopteris fragilis* complex with rugose spores. Generally speaking, North American workers have not recognized *dickieana* (but c.f. Setzer, 1970; Wiggins, 1954), whereas Eurasian workers are more likely to (Bir et al., 1979; Bir & Trikha, 1974; Breckle, 1987; Elven, 1984; Fraser-Jenkins, 1991; Fraser-Jenkins, 1997, 2008; Larsen, 1952; Nardi, 1974; Peroni & Peroni, 2003; Prada & Salvo, 1985; Profumo & Raggi, 1968; Rumsey, 2003; Vida, 1974; Wang, 1983). The general argument most frequently offered against the recognition of *C. dickieana* is the prevalence of populations with mixed spore types (Haufler et al., 1993), which could be taken to suggest segregating variation in this trait (perhaps due, even, to a single locus), the occurrence of apparent “transitional” spore morphologies (Blasdell, 1963), and the lack of correlation between spore type and
other morphological or isozyme markers (Haufler & Windham, 1991). The contrary argument (e.g., Fraser-Jenkins, 1997) is that mixed populations of co-occurring widespread species are not unusual in the Cystopteridaceae, and that Vida’s breeding experiments showed that the two taxa (Cystopteris fragilis and Cystopteris dickieana) have different genomic compositions (Vida, 1974). It is unfortunately unclear precisely what plants Vida used in his experiments. In my data rugose-spored plants tend to be found in one subclade of the Cystopteris fragilis complex (Rothfels et al., 2012c), but I would need much more spore and nuclear data (including incorporating Scottish dickieana material) before having any confidence in the validity of dickieana, one way or the other.


=Davallia bullata vel immersa (fide IPNI)


My preliminary data suggest that there is an endemic Hawaiian member of the C. fragilis complex that should bear this name. The status of C. sandwicensis is unclear – it is not distinct from douglasii using plastid data, but I don’t have any nuclear data for Hawaiian material. Contrary to the reports in Blasdell (1963), my very limited sample of C. douglasii does not appear to contain any octaploids.


=Sphaerostephanos elatus (Bory ex Willd.) Holttum (fide Schatz et al., 2011)

Cystopteris emarginata Hook., Sp. Fil. 1. 201. 1846.
=Cystopteris diaphana (fide IPNI)


=*Cystopteris fragilis?*

*Cystopteris emarginulata* C.Presl, Tent. Pterid. 93. 1836; Epim. 65. 1849.

=*Cystopteris diaphana* (fide IPNI)

*Cystopteris filix-femina* Coss. & Germ., Fl. Env. Paris 676. 1845.

=*Athyrium* (fide IPNI)


=*Cystopteris fragilis*. For an excellent discussion of *C. fragilis* vs. *C. filix-fragilis*, see Merrill (1935) and Weatherby (1926).

*Cystopteris formosana* Hayata, Icon. Pl. Formosan. 4: 143, f. 83. 1914.

=Acystopteris tenuisecta* (fide Tagawa, 1938)


*Cystopteris fragilis* is the oldest name in the *C. fragilis* complex, and the type of the genus, so has understandably functioned as rather a grab-bag taxon (for me, as well!). Biosystematic information from the type will be critical to the correct application of this name.


=C*ystopteris fragilis*? Christ (1900) recognizes this variety.


=C*ystopteris fragilis*?


These are both invalid names? See Weatherby (1936).

=Cystopteris fragilis? See Weatherby (1936).


=Cystopteris dickieana based on spore morphology (=C. fragilis). However, Bir recognizes C. dickieana (e.g., Bir & Trikha, 1974), so apparently felt that this form was distinct.


=Cystopteris fragilis, fide Fraser-Jenkins (1997)


I haven’t seen any specimens of this taxon, and don’t have any data from it, but tentatively accept it based on Prada (1986). Prada and Selva (1985) suggest that it might be octaploid, based on spore size.

Cystopteris fragilis var. laciniata Davenp., Cystopteris filix-fragilis Farw. var. laciniata Farw., C. fragilis f. laciniata (Davenport) Clute [publication details missing.]
Cystopteris fragilis? I don’t have publication information for any of these names.

See, also, Cystopteris laciniatus.


Cystopteris fragilis var. lobulato-dentata Koch [publication details missing]


Cystopteris fragilis (L.) Bernh. var. mackayi G.Lawson, Fern Fl. Canada 233. 1889.

Cystopteris filix-fragilis Farw. var. mackayi (G.Lawson) Farw.


Cystopteris fragilis f. magnasora Clute, Fern Bull. 9: 65. 1901.


=Cystopteris fragilis?


=Athyrium palmense (Christ) Lellinger, fide Mickel & Smith (2004), etc.

Cystopteris fragilis var. pubescens Phil., Anales Univ. Chile 43: 582. (1873).—TYPE: CHILE. Western Patagonia. Simpson ? (HT: ?). No type is explicitly designated—is this a valid name?

=Cystopteris fragilis?


=Cystopteris fragilis?


=Cystopteris tennesseensis, based on the character description in McGregor (1950).

Listed as invalid in TROPICOS.

Cystopteris fragilis var. vulgaris Lange, Meddel. Gronland 3: 306. 1887.

=Cystopteris fragilis?

Cystopteris fragilis subsp. eu-fragilis Asch. & Graebn., Synopsis der Mitteleuropaischen Flora 1. 1879.

Invalid name “as this is the type subspecies” (fide IPNI).


=Cystopteris fragilis (fide Stolze et al., 1994). These two C. fumarioides names are homotypic (I think), but I don’t know which has priority.

Cystopteris gigantea C.Presl, Tent. Pterid. 93. 1836.

= Acrophorus stipellatus, fide IPNI.

Cystopteris grandis C.Chr., Cat. Pl. Yun-Nan 100. 1916.

= Athyrium atkinsonii, fide Wang et al. (2012a).

Based on the illustration in the protologue, this is a strange-looking member of the *Cystopteris fragilis* complex, and a close match (at least superficially) to one of the accessions in my pool of samples (LY Kuo 2250, database #8657). This taxon is accepted by Wang et al. (2012b).


= *Dryopteris* (fide IPNI)


= *Cystopteris fragilis?*


= *Acystopteris japonica*


= *Acystopteris taiwaniana*


= *Dryopteris punctata* (fide IPNI). Libing Zhang sent me a digital photo of the type, with the comment that it looked like a *Hypolepis* (Zhang, pers. comm. Feb. 16, 2012).

La Chang K’ou, near Sining, Kansu; alt. 3060 m.; on densely shaded rocky cliff, by a stream. R.C. Ching 631 (HT: US?).

The spores on the type collection are immature, making the application of this name to either *C. fragilis* or *C. dickieana* tricky. Blasdell (1963) considered this taxon to be a hybrid between *C. fragilis* and *C. moupinensis* (the characters allying it the latter being vein position and clathrate rhizome scales). I’m skeptical of this hypothesis; if confirmed, it would be the only example of hybridization between the *sudetica* clade and the *fragilis* complex.

*Cystopteris laciniata* Col., Trans. New Zealand Inst. 31. 265. (1898) [or should this be 1899?]. TYPE: NEW ZEALAND. North Canterbury, 1898. T. Keir s.n. (HT: ?).

This name is unrelated to either of the two *C. fragilis* var. *laciniata* names? This taxon is tricky, given the title of the protologue, and the fact that the only known native *Cystopteris* in New Zealand is *C. tasmanica* (which is not the taxon to which this name applies). Presumably, this name refers to populations of the introduced “*Cystopteris fragilis*”?


This is a hexaploid putative allopolyploid between *Cystopteris bulbifera* and a North American member of the *Cystopteris fragilis* complex. I have good material of it from Michigan, and scattered collections from other areas in the greater Great Lakes region, so should be able to confirm or refute this hypothesis of parentage.
Cystopteris leptophylla C.Presl, Tent. Pterid. 93. 1836.

=Hypolepis incisa, fide IPNI.


=Cystopteris sudetica fide Wang et al. (2012b).


=Cystopteris fragilis?


=Cystopteris moupinensis fide Wang et al. (2012b).


=Cystopteris diaphana? This is certainly an extraordinary looking plant, with its membranous leaves, broad pinnae, and long-creeping rhizome. Preliminary molecular data don’t show any differentiation from C. fragilis accessions in the vicinity of the C. membranifolia type location, so my suspicion is that this is an environmentally induced morphology (caused from the spray of the waterfalls that C. membranifolia is restricted to).

172

=Cystopteris diaphana?


My samples of C. millefolia are not clearly distinguished (at plastid loci), from nearby samples of C. diaphana, but given their morphological distinctiveness, I recognize this species, pending further data from the nucleus.

CHINA. Yunnan: Salwin-Chiuijiang divide, Tsukuai, on rocks by the side of stream, 5600 m, 19 Oct. 1938. T.T. Yü 20777 (HT: PE?).

This species is recognized by Wang et al. (2012b), although they note that it is “very similar to C. montana, but distinct from it in the revolute segments and foveolate or finely reticulate perispore.”


Chloroplast data show slight differentiation between low-elevation North American/European C. montana accessions, and those from high elevations in Tibet; further research is necessary to see if more than one taxon is involved (Rothfels et al., 2012c). See also C. modesta, C. myrrhidifolia.


Certainly, this species is very close to *C. sudetica*, but worthy of recognition based on its geographic distinctness, and very slight morphological and chloroplast sequence divergence (Rothfels et al., 2012c). The current *Flora of China* treatment (Wang et al., 2012b) is spelling this species “*Cystopteris mouupperensis,*” for some reason.


=*Cystopteris montana*? TROPICOS treats *C. myrrhidifolia* as a synonym of *C. montana*, citing the *Flora of China*. However, the current *Flora of China* treatment does not include *myrrhidifolia*, either as a species or a synonym (Wang et al., 2012b).


= *Cystopteris fragilis*, fide Fraser-Jenkins (1997).

=Acrophorus stipellatus (fide IPNI)


=Cystopteris tasmanica? This epithet has been frequently misspelled as “novae-zealandicae.”

Cystopteris obovata C.Presl, Tent. Pterid. 93. 1836.

=Asplenium lanceolatum (fide IPNI).

Cystopteris obtusa C.Presl, Tent. Pterid. 93. 1836.

=Woodsia (fide IPNI).


TROPICOS lists this name as a synonym for Hypodematum crenatum (Forssk.) Kuhn, citing the Flora of China. However, the current Flora of China Hypodematum treatment does not include this name (Zhang et al., 2012).


=Cystopteris fragilis? TROPICOS treats C. orientalis as a synonym of C. fragilis, citing the Flora of China. However, the current Flora of China treatment does not include orientalis, either as a species or a synonym (Wang et al., 2012b).

Dryopteris pellucida (Franch.) C. Chr., Index Filic. 5: 283. 1905.—TYPE: CHINA.


The first-diverging species of the Cystopteris sudetica clade, at least according to plastid data (Rothfels et al., 2012c).

Cystopteris perriniana Link, Hort. Berol. 2. 131. 1833.

=Woodsia obtusa (fide IPNI)

Cystopteris polymorpha Bubani, Fl. Pyren. (Bubani) 4: 431. [Dec 1901-Feb 1902]

=Cystopteris fragilis? TROPICOS treats C. polymorpha as a synonym of C. fragilis, citing the Flora of China. However, the current Flora of China treatment does not include polymorpha, either as a species or a synonym (Wang et al., 2012b).

Cystopteris pontederae Link, Fil. sp. 45. 1841.

=Cystopteris fragilis? IPNI treats this as C. fragilis

The diploid member of the *C. fragilis* complex of southeastern North America; it forms autotriploids at the northern extent of its range (Haufler et al., 1985).


=C*ystopteris fragilis*


The diploid member of the *Cystopteris fragilis* complex of southern North America.


=C*ystopteris alpina*? Christ (1900) recognizes this taxon, as a subspecies of *C. fragilis*, with *alpina* as a variety within it. It is my impression that *regia* is typically considered a synonym of *alpina*, for the linear-segmented European hexaploid. But I need to look into this nomenclature further.


*Cystopteris alpina* f. *latiloba* Milde Milde
Cystopteris regia? (=C. alpina)


=Athyrium filix-femina (fide IPNI).

Cystopteris rhaetica Link, Hort. Berol. 2. 131. 1833.


Cystopteris rufescens Fée, Mém. Foug., 5. Gen. Filic. 300. 1850-52; 6 mem. 22 t. 6 f. 5. 1850.

=Dryopteris hirta (fide IPNI).


=Cystopteris douglasii, fide Blasdell (1963) (See entry under that species).


=Athyrium (fide IPNI)

Cystopteris setosa Bedd., Ferns br. Ind. t. 312. 1869. Chr. 281. 1869.

=Acystopteris tenuisecta (fide Tagawa, 1938)

Fraser-Jenkins (1997) places this name in synonym with *C. dickieana*, based on its rugose spores. He notes that, based on spore size, it is likely a hexaploid—the common ploidy level for Himalayan “*C. dickieana*” (Fraser-Jenkins, 1997). Bir (1971) however, reports this species as an octaploid. *Cystopteris sikkimensis* is also treated as a synonym of *dickieana* in the *Flora of China* (Wang et al., 2012b).


= *Cystopteris moupinensis* (fide Wang et al., 2012b).


= *Athyrium* (fide IPNI). This is the basionym for *Pseudocystopteris spinulosa* (Maxim.) Ching (= *Athyrium spinulosum* (Maxim.) Milde).


= *Davallia* (fide IPNI). This is the basionym for *Araiostegia squamata* (Decne.) Fraser-Jenk.


= *Acrophorus* (fide IPNI). This name is based on an invalid basionym?

One of the members, with *C. pellucida* and *C. moupinensis*, of the *C. sudetica* clade.

Very close (in chloroplast sequence) to *C. moupinensis* (Rothfels et al., 2012c).


=C*ystopteris sudetica*?


=C*ystopteris sudetica*?


=C*ystopteris sudetica*?


=C*ystopteris moupinensis* (fide Wang et al., 2012b)


The small, high-elevation tetraploid of Australia and New Zealand.

*Cystopteris taygetensis* Heldr. & Sart. in Heldr., Herb. norm. n. 35; Salom Nom. 147. 1883.

I don’t know anything about this name.


The allotetraploid between *C. bulbifera* and *C. protrusa* (Haufler et al., 1990; Shaver, 1950).


I don’t know anything about this name. (I can’t find the protologue, as listed, in the Biodiversity Heritage Library, even though this publication is there).


*Cystopteris filix-fragilis* Farw. var. *tenuis* Farw.

This is the taxon long known as *Cystopteris fragilis* var. *mackayi*. It’s an allotetraploid between *C. protrusa* and something else in the *C. fragilis* complex (Moran, 1983a).


= *Acystopteris tenuisecta*


[“*Cystopteris termale*”]

= *Cystopteris fragilis* (fide Fraser-Jenkins, 1997)

The Flora of China recognizes this species due to the short glandular hairs on the lamina and indusia (Wang et al., 2012b), and the Fraser-Jenkins treats it under C. moupinensis: “The type in PE (!) is merely a small specimen of C. moupinensis” (Fraser-Jenkins, 1997).


= *Cystopteris fragilis* (fide Stolze et al., 1994)


= *Alsophila* fide IPNI


I don’t know anything about this name.


The tetraploid between *Cystopteris bulbifera* and *C. reevesiana*.

*Cystopteris vestita* C.Presl, Tent. Pterid. 93. 1836.

= *Woodsia obtusa* fide IPNI

=Dryopteris cruciata fide IPNI


Invalid name? (Later homonym of _C. villosa_ Desv.)


= _Cystopteris diaphana_ (fide Murphy & Rumsey, 2005, etc.). I’m not sure why _C. diaphana_ seems to have priority over _C. viridula_.


The sterile (presumably triploid) hybrid between _Cystopteris protrusa_ and _C. tenuis_ (Moran, 1982).


The sterile hybrid between a European smooth-sспорed _Cystopteris (“Cystopteris dickieana”)_ and classic (echinate-sспорed) _Cystopteris fragilis_.

183

The sterile (or largely so) hybrid between *C. tennesseensis* and *C. tenuis* (Moran, 1983b).


= *Gymnocarpium robertianum* (fide Wang et al., 2012b)


A diploid endemic of the Appalachian Mountains; one of the progenitors of *Gymnocarpium dryopteris* (Pryer & Haufler, 1993).

**Gymnocarpium continentale** (Petrov) Pojark., Soobshch. Tadzh. Fil. Akad. Nauk SSSR. 22: 10. (1950 [This may be the wrong citation]). *Dryopteris pulchella var. continentalis* Petrov,
=Gymnocarpium jessoense subsp. parvulum (fide Pryer, 1993).


*Phegopteris dryopteris* var. *disjuncta* (Rupr.) Trel., Harriman Alaska Exped. 5: 382. 1904.


The diploid of western North American and northern Eurasia; one of the progenitors of *Gymnocarpium dryopteris* (Pryer & Haufler, 1993).


A cosmopolitan allotetraploid (Pryer & Haufler, 1993).

=Gymnocarpium dryopteris? Described as being somewhat intermediate between G. dryopteris and G. jessoense (Nakaike, 1969). My one accession of this variety is identical in chloroplast sequences to typical G. dryopteris (Rothfels et al., 2012c).


This name could refer to very lightly glandular Gymnocarpium dryopteris (as sometimes occur), or to Gymnocarpium jessoense subsp. parvulum (unlikely, because at the time G. jessoense parvulum was considered part of G. robertianum rather than G. dryopteris; Pryer, 1992), or (most likely?) to a hybrid between G. dryopteris and either G. jessoense parvulum or G. robertianum (i.e., G. × intermediate or G. × achriosporum, respectively).


=Gymnocarpium robertianum (fide TROPICOS)

I don’t know the appropriate application of this name. Fraser-Jenkins (1992; 2008) argues that this is the common species of the Himalayas, rather than G. jessoense (which he says also occurs there, but is much rarer; Fraser-Jenkins, 2008).


=Gymnocarpium oyamense (fide Wang et al., 2012b).


This taxon, as I understand it, is a Eurasian diploid (based on a count from Japanese material: Mitui, 1970). However, the taxonomy and nomenclature of
Asian *Gymnocarpium* needs much further work (see *G. fedtschenkoanum*, above, and Fraser-Jenkins (2008), and Rothfels et al. (2012c)).


A cosmopolitan tetraploid (Sarvela et al., 1981), which is differentiated at plastid loci from *Gymnocarpium jessoense* (2012c), so likely deserves recognition at the specific level (as *G. continentale*?).


=G*Gymnocarpium jessoense* (fide Wang et al., 2012b).


My German isn’t very good, but Google Translate and I think that Schwarz believes this name to refer to what I’m here calling *G. robertianum*.


A highly distinctive species, but includes multiple cytotypes (Nakato et al., 2007), and might include multiple independent evolutionary units (e.g., G. gracilipes?).


This is a very interesting plant, strongly reminiscent of *Gymnocarpium × bipinnatifidum*, but described as having well-formed (nonabortive) spores. Further research is necessary to determine if this is an allopolyploid between *G. oyamense* and *G. jessoense*.

**Gymnocarpium phegopteris** Newman, Phytologist 4. app. XXIII. 1851.

= *Phegopteris connectilis* (Michx.) Watt.


=Gymnocarpium remotepinnatum (because the basionym of G. remotum was replaced by the basionym of G. remotepinnatum?)


A widespread north-temperate species of North America and Eurasia.


Tetraploid (presumably); a sterile hybrid between G. dryopteris and G. robertianum (Sarvela, 1978).


The very cool sterile hybrid between G. oyamense and G. jessoense (the latter parent reported as G. robertianum—the identity of these glandular Japanese plants is unclear; Rothfels et al., 2012c)

The sterile triploid hybrid between G. disjunctum and G. dryopteris (Pryer & Haufler, 1993).


The sterile triploid hybrid between G. appalachianum and G. robertianum (Pryer, 1992).


The sterile tetraploid hybrid between G. dryopteris and G. jessoense subsp. parvulum (Pryer, 1992; Pryer et al., 1984).

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Biography

I was born on March 3, 1977, in North York, Ontario, Canada. I pursued my undergraduate education at McMaster University, Hamilton, Ontario, culminating in a Bachelor of Arts & Science, combined Honours Biology, 2001. I have published a variety of “semiformal” articles, typically in local naturalist club journals. My formal publications (in peer-reviewed journals) are:

Scholarships, fellowships, and academic honors that I have received since my undergraduate education include: NSERC Postdoctoral Fellowship (2012; for two years); NSF Doctoral Dissertation Improvement Grant (2011; for two years); NSERC PGS D Research Scholarship (2008; for three years); Julie Payette-NSERC Research Scholarship (2006; for one year); Duke University Scholars Fellowship (2006; for one year); Duke University James B. Duke Fellowship (2006; for four years).