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Maidenhair Ferns, *Adiantum*, are Indeed Monophyletic and Sister to Shoestring Ferns, Vittarioids (Pteridaceae)

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Abstract—Across the tree of life, molecular phylogenetic studies often reveal surprising relationships between taxa with radically different morphologies that have long obscured their close affiliations. A spectacular botanical example is *Rafflesia*, a holoparasite that produces the largest flowers in the world, but that evolved from tiny-flowered ancestors within the Euphorbiaceae. Outside of parasitic lineages, such abrupt transformations are rarely seen. One exception involves the "maidenhair ferns" (*Adiantum*), which are quintessential ferns: beautifully dissected, terrestrial, and shade loving. The closely related "shoestring ferns" (vittarioids), in contrast, have an extremely simplified morphology, are canopy-dwelling epiphytes, and exhibit greatly accelerated rates of molecular evolution. While *Adiantum* and the vittarioids together have been shown to form a robust monophyletic group (adiantoids), there remain unanswered questions regarding the monophyly of *Adiantum* and the evolutionary history of the vittarioids. Here we review recent phylogenetic evidence suggesting support for the monophyly of *Adiantum*, and analyze new plastid data to confirm this result. We find that *Adiantum* is monophyletic and sister to the vittarioids. With this robust phylogenetic framework established for the broadest relationships in the adiantoid clade, we can now focus on understanding the evolutionary processes associated with the extreme morphological, ecological, and genetic transitions that took place within this lineage.

Keywords—Epiphytes, gametophytes, molecular phylogeny, rate heterogeneity.

Early molecular phylogenetic analyses of ferns (Hasebe et al. 1994, 1995) inferred several unexpected associations that had not previously been suspected. Most of these newly recognized relationships, which subsequently drew considerable attention, have stood the test of time. A prominent example is the monophyly of the heterosporous water ferns in the Marsileaceae and Salviniaceae (Hasebe et al. 1994; Rothwell and Stockey 1994; Pryer et al. 1995; Pryer 1999). Another surprise emerged within the Pteridaceae, grouping *Adiantum* (maidenhair ferns) together with the vittarioids (shoestring ferns) in a well-supported clade now referred to as the adiantoids (Schuettpelz et al. 2007).

Adiantum and the vittarioids could not be more morphologically or ecologically disparate. In coarse morphology, their conspicuous sporophytes look nothing like one another. The leaves of *Adiantum* are typically broad and finely divided, whereas those of vittarioids are almost always simple and strap-like (Fig. 1A; Tryon and Tryon 1982; Kramer 1990). Fertile *Adiantum* leaves are uniquely distinguished by their sporangia borne on, and limited to, false indusia, whereas vittarioid sori occur on the laminae (Crane et al. 1995). These groups also display major differences in the morphology of their gametophytes, although these are less obvious to the naked eye (Fig. 1B). The gametophytes of *Adiantum*, like those of most ferns, are determinate and heart-shaped, with a distinct midrib and broad wings (Nayar and Kaur 1971). They are generally ephemeral to short-lived (months) and are incapable of vegetative reproduction. Vittarioid gametophytes, on the other hand, are indeterminate and ribbon-like. They can be exceptionally long-lived (years) and can also reproduce asexually via propagules called gemmae (Atkinson and Stokey 1964; Farrar 1974, 1985). In addition, *Adiantum* and vittarioids occupy two dramatically different niches. Whereas the cosmopolitan genus *Adiantum* usually occurs on shady forest floors, vittarioids generally grow as epiphytes, colonizing tree trunks and canopies of tropical rain forests. The differences between these two groups also extend to their genomes. Although most *Adiantum* species have diploid chromosome numbers of $n = 29$ or $n = 30$ (Löve et al. 1977), nearly all vittarioids studied are $n = 60$ (Löve et al. 1977), suggesting that at least one genome duplication event occurred early in the evolutionary history of this lineage.

Because of their distinctive simplified morphology, vittarioids have until quite recently been regarded as a distinct family, the Vittariaceae (Tryon and Tryon 1982; Kramer 1990). However, in recent years, studies have not only identified a close relationship with *Adiantum*, but also suggested that perhaps vittarioids may even be nested within this genus (Prado et al. 2007; Schuettpelz and Pryer 2007). These analyses of plastid data have further revealed yet another dissimilarity between *Adiantum* and the vittarioids: a striking difference in branch lengths. Vittarioid branches are extraordinarily long relative to *Adiantum*, or to any other fern lineage, for that matter. As a consequence, branch support across the vittarioid topology is consistently robust, whereas there is mostly weak support among species of *Adiantum*, especially for the backbone nodes that lie far deeper than the tips (Schuettpelz and Pryer 2007; Schuettpelz et al. 2007).

While it is clear that *Adiantum* and the vittarioids together compose a robust clade, there are still unanswered questions regarding the monophyly of *Adiantum* and the evolutionary history of this genus, as well as that of the vittarioids. For example, are there correlates in the morphology, ecology, and life history of vittarioid ferns that may be contributing to their faster rate of molecular evolution? Here we review recent studies and analyze new plastid data, and find strong support for the monophyly of *Adiantum* and for other deep divergences within adiantoids. This robust phylogenetic framework will permit us, in future studies, to explore the evolutionary processes that resulted in this

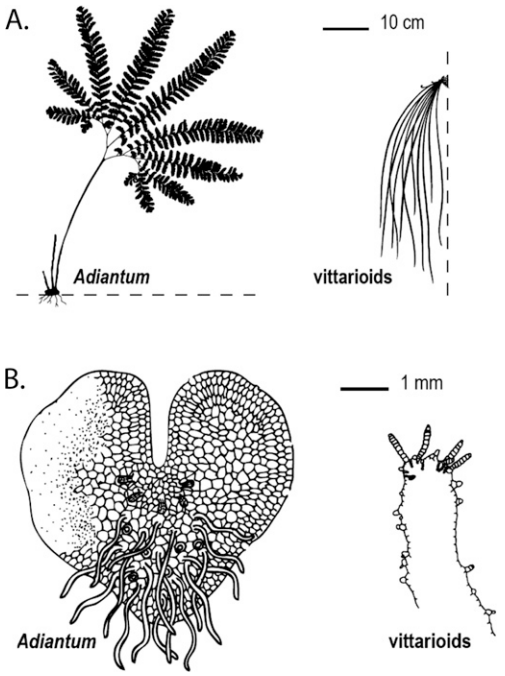

Fig. 1. Sporophyte and gametophyte comparison between *Adiantum* and the vittarioids. A. Sporophytes typical of the *Adiantum*/vittarioid assemblage: the "maidenhair ferns" (*Adiantum*) are quintessential ferns—beautifully dissected, terrestrial, and shade loving; the closely related "shoestring ferns" (vittarioids) are highly simplified, canopy-dwelling epiphytes. B. The gametophytes of *Adiantum* are determinate, heart-shaped, usually short-lived, and not capable of asexual reproduction; vittarioid gametophytes are indeterminate, ribbon-like (only small apical portion shown), exceptionally long-lived, and capable of asexual reproduction (note propagules—gemmae—at tip).

extreme morphological, ecological, and genomic makeover in ferns.

Materials and Methods

Taxon Sampling—Our taxon sample consists of 16 species of *Adiantum*, eight vittarioid species, eight species of cheilanthoid ferns (the sister group to the adiantoids), and two outgroup species (*Cryptogramma crispa* [cryptogrammoid] and *Pityrogramma austroamericana* [pteridoid]) that were selected based on Schuettpelz et al. (2007) and Rothfels and Schuettpelz (2014); see Appendix 1. While 16 of the approximately 200 species of *Adiantum* may appear to be sparse coverage, our sampling was carefully informed by our ongoing phylogenetic study focused on the bulk of *Adiantum* species. As a consequence, our study is the only one to date to capture the deepest divergences within the genus, and includes representatives from all known major clades of *Adiantum*.

DNA Extraction, Amplification, and Sequencing—DNA was isolated from either silica-dried or herbarium material. Protocols for DNA extraction, amplification, and sequencing followed Schuettpelz and Pryer (2007), Cochran et al. (2014), and Rothfels and Schuettpelz (2014). Sequences were obtained for six plastid loci (*atpA*, *atpB*, *chlN*, *rbcL*, *rpoA*, *rps4*). Primers for *atpA*, *atpB*, and *rbcL* were identical to those used in Schuettpelz et al. (2007) and Rothfels and Schuettpelz (2014); primers used for *chlN*, *rpoA*, and *rps4* are listed in Table 1.

Sequence Alignment and Data Sets—DNA sequence chromatograms were manually edited and assembled using Sequencher 4.5 (Gene Codes Corporation, Ann Arbor, Michigan). Each plastid region was aligned with AliView (Larsson 2014), which integrates MUSCLE (Edgar, 2004) as the default alignment program. Each alignment was manually inspected and edited. Although alignment was straightforward for the protein-coding loci, there were some indels in the non-protein-coding regions that rendered the alignment ambiguous; these were excluded prior to subsequent analyses. Unsequenced portions of plastid regions were coded as missing data. Six individual data sets were compiled, one for each of the plastid regions. Data set alignments and phylogenetic trees are deposited in the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.4m6s6. Eighty-one newly obtained DNA sequences were deposited in GenBank (Appendix 1).

Phylogenetic Analyses—Separate phylogenetic analyses were conducted for each of the six plastid data sets using maximum likelihood (ML) in PAUP* 4.0a136 (Swofford 2002). We first inferred a maximum parsimony tree and used the AutoModel function in PAUP* to perform model selection under the AICc. With the best-fit model selected, and the model parameters estimated for each data set, ML analyses were conducted in PAUP* with tree bisection and reconnection branch swapping and 100 random-addition-sequence replicates. Maximum likelihood trees for each individual plastid region were visually inspected for conflicts supported by bootstrap values $\geq$ 70% (Mason-Gamer and Kellogg 1996). Because no instances of mutually well-supported incongruence were detected when comparing phylogenies across different plastid regions, the six separate data sets were concatenated and analyzed together using ML and Bayesian inference (BI).

For the concatenated data set, we used PartitionFinder (Lanfear et al. 2012) to determine the optimal data-partitioning scheme and substitution models according to the AICc (Table 2). ML tree searches and ML bootstrap (MLBS) analyses (1,000 replicates) were carried out from eight independent random-addition-starting trees in Garli 2.0 (Zwickl 2006) with "genthreshfortopoterm" set to 1,000,000 and 100,000, respectively. MrBayes 3.2 (Ronquist et al. 2012) was used to conduct BI analyses. Because it is not possible to implement some of the best-fitting models in MrBayes, another PartitionFinder analysis was run to choose more applicable models. Two independent Markov chain Monte Carlo (MCMC) runs were carried out, each with four chains (one heated and three cold) running for 20 million generations. Priors followed the default settings with a flat Dirichlet distribution for both the stationary state frequencies and the substitution rates, and trees were sampled every 1,000 generations. The substitution parameters were unlinked, and the rate prior was set to allow variation among the subsets. After the MCMC runs, the output parameters were inspected in Tracer v1.5 (Rambaut and Drummond 2009) to ensure convergence and proper mixing. The first 25% of the sample was discarded as burn-in and the remainder was used to calculate a 50% majority-rule consensus tree.

Results

Seven aligned data matrices (six single-region matrices and one concatenated) were analyzed for this study; a summary of sequence characteristics, best-fit models of sequence evolution, and tree statistics appears in Table 3. Trees resulting from the maximum likelihood and Bayesian analyses were identical in topology. The best ML tree ($\ln L = -38{,}392.964$) from the analysis of the concatenated six-plastid loci (*atpA*, *atpB*, *chlN*, *rbcL*, *rpoA*, and *rps4*) is shown in Fig. 2. Nearly all of its internal nodes (24 out of 31) are highly supported, with ML bootstrap support $\geq$ 70% and Bayesian posterior probability (PP) support $\geq$ 0.99 (Fig. 2). The monophyly of both *Adiantum* (82% MLBS, 1.0 PP) and the vittarioid clade (100% MLBS, 1.0 PP) is robustly supported, as is their sister relationship to one another (100% MLBS, 1.0 PP; Fig. 2). There is strong support for all relationships across vittarioids, whereas within *Adiantum*, the entire backbone of the clade is weakly supported (<70% MLBS, <0.97 PP).

Discussion

Phylogenetic analyses from across the tree of life have revealed that the rate of molecular evolution in closely related lineages can be very similar, or can vary dramatically (Lanfear et al. 2010). Within ferns, notable molecular rate heterogeneity has been reported from horsetails (Des Marais et al. 2003) and filmy ferns (Schuettpelz and Pryer 2006) to vittarioids (Rothfels and Schuettpelz 2014) and polygrammoid ferns (Schneider et al. 2004). Sometimes, but not always,

Table 1. Primers used for DNA amplification and sequencing of plastid *chlN*, *rpoA*, *rps4* in this study.

DNA region	Primer name	Primer sequence 5′-3′	Primer source
chlN	chlN-F2	CGWTAYGCRAYGGCVGAATYGSAAG	Schuettpelz et al. (unpubl.)
	chlN-R2	CAWATTTTTTCGATCCARGCRCGTG	Schuettpelz et al. (unpubl.)
rpoA	rpoA-F1	TRCAYGAGTATTCYACAATAACGGG	Schuettpelz et al. (unpubl.)
	rpoA-R1	AATTAAARGCTCTRGCRGGTRATTC	Schuettpelz et al. (unpubl.)
rps4	Adcvrps45' (forward)	CTCTCGGTATCGAGGACC	this study
	trnS (reverse)	TACCGAGGGTTCGAATC	Souza-Chies et al. 1997

Table 2. Optimal data-partitioning scheme and substitution models for the concatenated data set of six plastid loci; determined according to the AICc using PartitionFinder (Lanfear et al. 2012).

Subset	Model for Garli	Model for MrBayes	Subset composition
1	GTR + I + G	GTR + I + G	*atpA*, *atpB*, *rbcL* first codon position
2	TrN + I + G	HKY + I + G	*atpA*, *atpB* second codon position
3	TVM + I + G	GTR + I + G	*atpA*, *atpB*, *rbcL*, *rps4* third codon position
4	TVM + I + G	GTR + I + G	*chlN*, *rpoA*, *rps4* first and second codon position
5	TVM + I + G	GTR + I + G	*chlN*, *rpoA* third codon position
6	JC + I + G	JC + I + G	*rbcL* second codon position

this rate variation appears to correlate strongly with certain other aspects of biology. For example, Soltis et al. (2002) and Korall et al. (2010) found that an abrupt rate deceleration coincided with the evolution of the long-lived, tree-like habit at the base of the tree fern clade (i.e. tree ferns, with longer generation times, consistently have slower rates of molecular evolution).

One of the challenges posed by molecular evolutionary rate heterogeneity has been the associated difficulty of recovering phylogenetic topologies that reflect accurate relationships (Schuettpelz and Pryer 2006; Rothfels et al. 2012). Although all molecular analyses to date bring *Adiantum* together with the vittarioids in a strongly supported clade (adiantoids), they have also repeatedly struggled to find support for the monophyly of *Adiantum* with respect to the morphologically and ecologically highly dissimilar vittarioids. Three conflicting topologies have been recovered for relationships within adiantoids. The first places *Adiantum* sister to vittarioids, but with weak support for the monophyly of *Adiantum* (Fig. 3A; Schuettpelz et al. 2007, their Fig. 1): 67 taxa (16 adiantoids), *atpA/atpB/rbcL*; Lu et al. 2012 (their Fig. 2): 98 taxa (74 adiantoids), *atpA/atpB/rbcL*). A second topology suggests a paraphyletic *Adiantum*, with *A. raddianum* sister to a weakly supported clade of vittarioids + the rest of *Adiantum* (Fig. 3B; Schuettpelz et al. 2007, their Fig. 3): 147 taxa (36 adiantoids), *rbcL*). The third topological option is again for a paraphyletic *Adiantum*, but this time with *A. raddianum* sister to vittarioids, and the rest of *Adiantum* sister to that clade (Fig. 3C; Schuettpelz and Pryer 2007, their Fig. 1B): 400 taxa (15 adiantoids), *atpA/atpB/rbcL*). The last two topologies both suggest that the vittarioids may actually be derived from *within Adiantum* (Fig. 3B, C), a hypothesis that is difficult to reconcile with the extraordinary degree of morphological conservatism within *Adiantum* compared to other large fern genera.

The most significant phylogenetic result from our study of six-plastid loci (*atpA/atpB/chlN/rbcL/rpoA/rps4*) from 34 taxa (24 adiantoids) is strong support for a monophyletic *Adiantum*, with 82% ML bootstrap and 1.0 posterior probability support (Figs. 2, 3D). The only other study to date to convincingly demonstrate the monophyly of *Adiantum* was a six-locus (from across all three genome compartments) data set for 26 taxa (16 adiantoids, Rothfels and Schuettpelz 2014, their Fig. 1C: *atpA/atpB/rbcL* [plastid] + *gapCp* [nuc] + *atp1/nad5* [mt]). Also noteworthy—although only three *Adiantum* species (but including *A. raddianum*) and two vittarioids out of 73 ferns were included in their study—the fern phylogeny inferred by Rothfels et al. (2015) from 25 low-copy nuclear genes robustly refutes the hypothesis that vittarioids are nested within *Adiantum*.

In their combined three-plastid marker (*atpA/atpB/rbcL*) analysis of 98 taxa (74 adiantoids), Lu et al. (2012, see their Fig. 2) found strong maximum parsimony bootstrap support (87%) for the monophyly of *Adiantum*, but significant Bayesian support at that node was lacking (PP = 0.81). While bootstrap support and posterior probabilities measure different things and are thus not directly comparable, posterior probability support values almost universally exceed support

Table 3. Sequence characteristics for six plastid loci, best-fit sequence evolution models, and resulting tree statistics. Missing data does not include indels.

	atpA	atpB	chlN	rbcL	rpoA	rps4	All combined
Alignment length (bp)	1,500	1,257	621	1,308	613	612	5,911
Characters included (bp)	1,500	1,257	543	1,308	559	612	5,779
Missing data (%)	0.184	20.974	38.029	0.175	26.471	44.829	15.526
Variable sites (%)	37.600	30.469	45.304	35.627	55.993	50.980	39.522
Model used	GTR + I + G	GTR + I + G	GTR + I + G	GTR + I + G	TVM + I + G	TVM + I + G	Mixed
Best lnL	−10,846.500	−6,783.828	−3,980.225	−9,419.414	−4,610.626	−4,091.730	−38,392.964

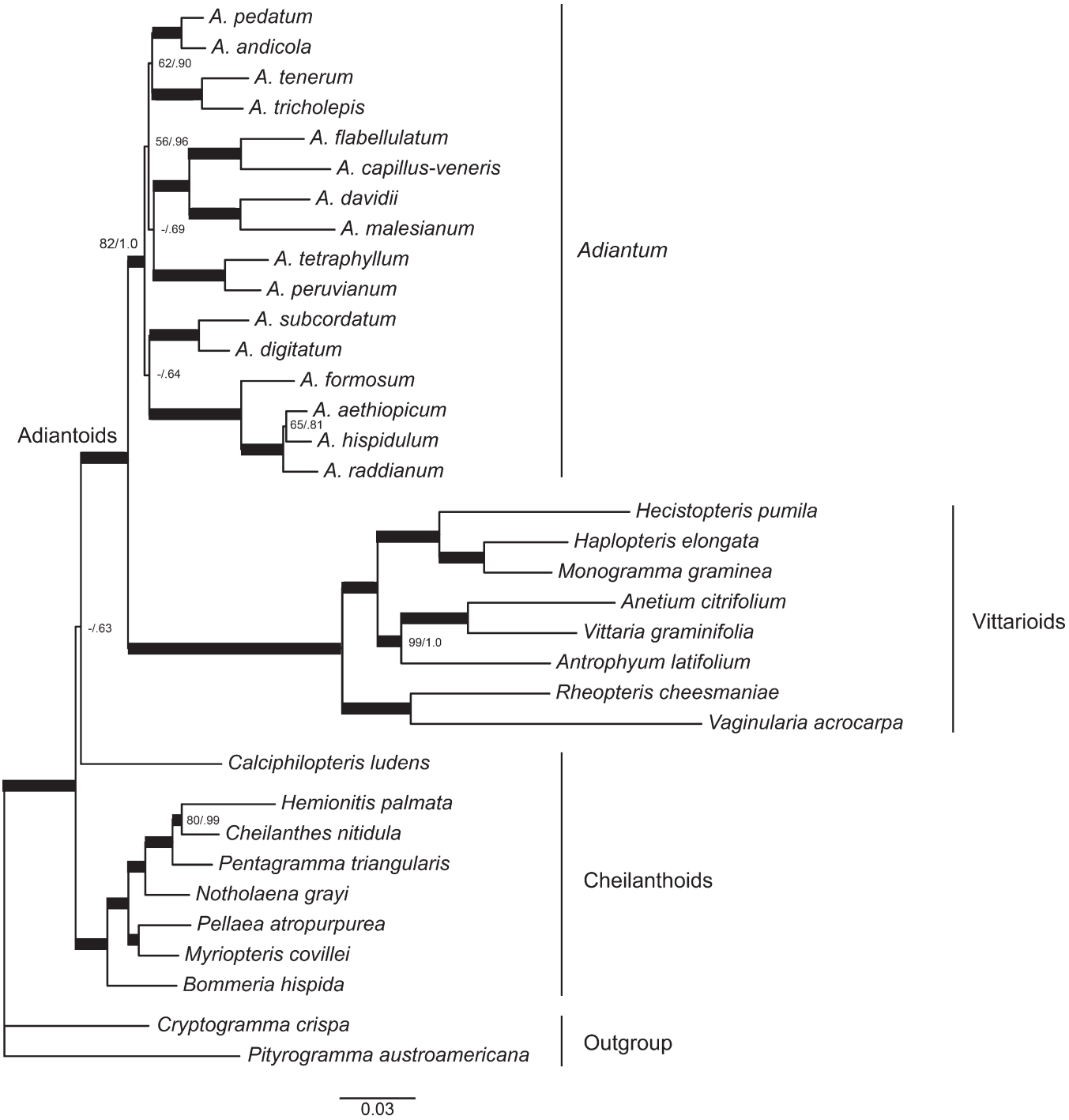

FIG. 2. The best ML tree (ln*L* = −38,392.964) from the analysis of the combined six-locus plastid data set: *atpA*, *atpB*, *chlN*, *rbcL*, *rpoA*, and *rps4*. Branch support is shown at each node as ML bootstrap support (MLBS)/Bayesian posterior probability (PP); all thickened branches have 100%/1.0 support, unless otherwise indicated. Branch support for all non-thickened branches is indicated where MLBS and PP support is >50%. Scale bar corresponds to 0.03 substitutions/site.

values from parsimony and likelihood bootstrap support (except, of course, when they are equal; Hillis and Bull 1993; Alfaro et al. 2003; Rothfels et al. 2012). The fact that the posterior probability for this particular node is so low in Lu et al. (2012), especially given that this is a situation (long branches subtended by very short internodes) that is particularly prone to long-branch attraction issues (and thus unreliable MP inference), undermines any confidence in their result. Put simply, the two methods (MP and BI) have contrasting support for a node where MP might reasonably be expected to fail. The node in question is in fact the *only* node with a Bayesian posterior probability lower than its maximum parsimony bootstrap in the entire Lu et al. (2012) study. Thus, we were not convinced that their study demonstrated the monophyly of *Adiantum*, which prompted our study using an expanded plastid sequence data set.

We believe that our increased sampling of plastid markers (including the two new loci *chlN* and *rpoA*) together with

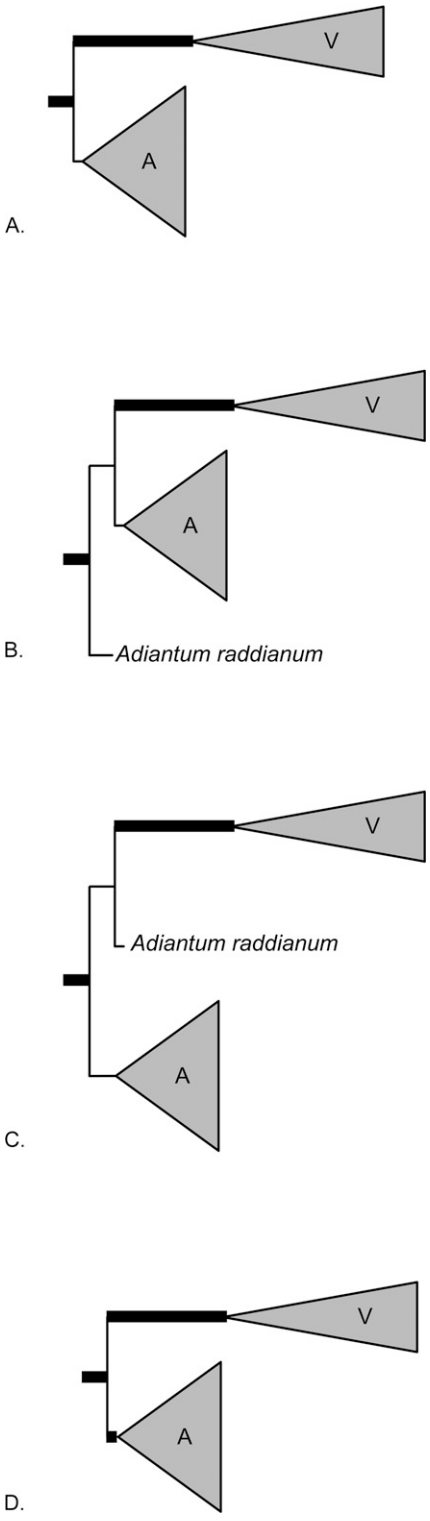

FIG. 3. Conflicting phylogenetic topologies recently hypothesized for relationships between *Adiantum* and the vittarioid ferns. A. Schuettpelz et al. (2007, their Fig. 1): 67 taxa (16 adiantoids), *atpA/atpB/rbcL*, support for *Adiantum* monophyly is Bayesian posterior probability PP < 0.95 *and* maximum likelihood bootstrap support MLBS < 70%; Lu et al. (2012, their Fig. 2): 98 taxa (74 adiantoids), *atpA/atpB/rbcL*, support for *Adiantum* monophyly is Bayesian posterior probability PP = 81 *and* maximum parsimony bootstrap support MPBS = 87%. B. Schuettpelz et al. (2007, their Fig. 3): 147 taxa (36 adiantoids), *rbcL*. C. Schuettpelz and Pryer (2007, their Fig. 1b): 400 taxa (15 adiantoids), *atpA/atpB/rbcL*. D. Rothfels and Schuettpelz (2014, their Fig. 1c): 26 taxa (16 adiantoids), *atpA/atpB/rbcL* + nuc + mt; this study: 34 taxa (24 adiantoids), *atpA/atpB/chlN/rbcL/rpoA/rps4*. Abbreviations: A=*Adiantum*; V=vittarioid ferns.

model-based approaches, such as maximum likelihood and Bayesian analyses (Swofford et al. 1996; Ronquist and Huelsenbeck 2003), as well as informed taxon sampling from our parallel large-scale analysis of *Adiantum*, allowed us to succeed in achieving credible support, comparable across both measures, for the monophyly of *Adiantum*. This level of confidence is requisite to proceeding further with our ongoing within-clade studies; we are thus now moving forward with an appropriate sampling of vittarioids as outgroup for a nearly complete phylogenetic study based on four plastid markers (*rbcL*, *atpA*, *chlN*, *rpoA*) focused on the bulk of *Adiantum* species (Huiet et al. unpubl.), and a comparable study for the vittarioid clade (Schuettpelz et al. unpubl.).

Most other relationships within the adiantoids (Fig. 2) are in agreement with earlier studies (Schuettpelz et al. 2007; Lu et al. 2012), including strong support for a *Rheopteris* + *Vaginularia* clade (Ruhfel et al. 2008; Rothfels and Schuettpelz 2014). Broad relationships within the cheilanthoid ferns also mirror those found in recent studies (Prado et al. 2007; Schuettpelz et al. 2007; Rothfels et al. 2008; Windham et al. 2009; Eiserhardt et al. 2011); however, strong support for *Calciphilopteris ludens* as a member of this clade, which was recovered by Rothfels and Schuettpelz (2014) using data from all three genomic compartments, was not achieved here. To date, analyses using only plastid data typically resolve *C. ludens* (without strong support) as either sister to the rest of the cheilanthoids (Schuettpelz et al. 2007) or the adiantoids (this study).

An extreme makeover occurred during the evolutionary history of the vittarioid ferns, involving morphological transformations in both the sporophyte and gametophyte phases of their life cycle, the evolution of epiphytism, genome duplication(s), and molecular rate acceleration. With a robust phylogenetic framework now in place, we aim to identify, in future studies, the underlying causal mechanisms that may have contributed to this rather spectacular transformation.

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Appendix 1. Vouchers and GenBank accession numbers for taxa used in our molecular phylogenetic analysis. Taxon, collection locality; voucher specimen collector and no. (herbarium acronym), Fern DNA database number (fernlab.biology.duke.edu), GenBank accession, citation for previously published data for *atpA*; *atpB*; *chlN*; *rbcL*; *rpoA*; and *rps4* (in that order). A dash (–) indicates not applicable; asterisk (*) indicates only 71 bp of sequence data were obtained for the *chlN* gene of *Adiantum hispidulum*, which is too short for GenBank to accession, therefore, the sequence is provided here: CGGCAACTACATTAATGCGTCGAAGGAAATGCCAGT TAGTTGGAGCACCTTTCCCAATTGGTCCAGATGGG

Adiantum aethiopicum L., Australia, New South Wales; *N. S. Nagalingum 24* (DUKE), 3895, KC984436, Rothfels and Schuettpelz 2014; KC984441, Rothfels and Schuettpelz 2014; KU147256, This study; KC984519, Rothfels and Schuettpelz 2014; KU147288, This study; NA (missing data), -; ***Adiantum andicola*** Liebm., Costa Rica, San José; *C. J. Rothfels 2641* (DUKE), 5549, KU147243, This study; NA (missing data), -; KU147251, This study; KU147272, This study; KU147280, This study; NA (missing data), -; ***Adiantum capillus-veneris*** L., U. S. A., California; *L. Huiet 104* (UC), 4609, KU147244, This study; NA (missing data), -; KU147252, This study; KU147273, This study; KU147281, This study; KU147305, This study; ***Adiantum davidii*** Franch., from cultivation; *L. Huiet 116* (UC), 2500, KU147245, This study; KU147250, This study; KU147253, This study; EF452136, Schuettpelz et al. 2007; KU147282,

This study; KU147306, This study; ***Adiantum digitatum*** C. Presl, Bolivia, Chuquisaca; *J. R. I. Wood 14432* (UC), 4673, KU147246, This study; NA (missing data), -; KU147254, This study; KU147274, This study; KU147283, This study; KU147307, This study; ***Adiantum flabellulatum*** L., Taiwan, Nantou Co.; *E. Schuettpelz 1016A* (DUKE), 4759, KU147247, This study; NA (missing data), -; NA (missing data), -; KU147275, This study; KU147284, This study; NA (missing data), -; ***Adiantum formosum*** R. Br., from cultivation; *A. R. Smith s.n.* (UC), 4602, KC984437, Rothfels and Schuettpelz 2014; KC984442, Rothfels and Schuettpelz 2014; KU147257, This study; KC984520, Rothfels and Schuettpelz 2014; KU147289, This study; KU147309, This study; ***Adiantum hispidulum*** Sw., from cultivation; *L. Huiet 101* (UC), 4603, KC984438, Rothfels and Schuettpelz 2014; KC984443, Rothfels and Schuettpelz 2014; *, This study; KC984521, Rothfels and Schuettpelz 2014; KU147290, This study; NA (missing data), -; ***Adiantum malesianum*** J. Ghatak, from cultivation; *L. Huiet 111* (UC), 2506, EF452068, Schuettpelz et al. 2007; EF452011, Schuettpelz et al. 2007; KU147258, This study; EF452132, Schuettpelz et al. 2007; KU147291, This study; KU147310, This study; ***Adiantum pedatum*** L., from cultivation; *L. Huiet 117* (UC), 2499, EF452069, Schuettpelz et al. 2007; EF452012, Schuettpelz et al. 2007; NA (missing data), -; KU147276, This study; KU147285, This study; KU147308, This study; ***Adiantum peruvianum*** Klotzsch, from cultivation; *L. Huiet 103* (UC), 2507, EF452070, Schuettpelz et al. 2007; EF452013, Schuettpelz et al. 2007; KU147259, This study; EF452133, Schuettpelz et al. 2007; KU147292, This study; KU147311, This study; ***Adiantum raddianum*** C. Presl, from cultivation; *P. G. Wolf 717* (UTC), 638, EF452071, Schuettpelz et al. 2007; U93840, Wolf 1997; NA (missing data), -; KC984522, Rothfels and Schuettpelz 2014; KU147293, This study; KU147312, This study; ***Adiantum subcordatum*** Sw., Brazil, Minas Gerais; *E. Schuettpelz 1406* (DUKE), 8340, KU147248, This study; NA (missing data), -; NA (missing data), -; KU147277, This study; KU147286, This study; NA (missing data), -; ***Adiantum tenerum*** Sw., from cultivation; *L. Huiet 107* (UC), 2504, EF452072, Schuettpelz et al. 2007; EF452014, Schuettpelz et al. 2007; KU147260, This study; EF452134, Schuettpelz et al. 2007; KU147294, This study; KU147313, This study; ***Adiantum tetraphyllum*** Humb. & Bonpl. ex Willd., from cultivation; *L. Huiet 105* (UC), 2505, EF452073, Schuettpelz et al. 2007; EF452015, Schuettpelz et al. 2007; KU147261, This study; EF452135, Schuettpelz et al. 2007; KU147295, This study; KU147314, This study; ***Adiantum tricholepis*** Fée, Mexico, Jalisco; *C. J. Rothfels 3116A* (DUKE), 6549, KU147249, This study; NA (missing data), -; KU147255, This study; KU147278, This study; KU147287, This study; NA (missing data), -; ***Anetium citrifolium*** (L.) Splitg., Guadeloupe, Etang l'As de Pique; *M. J. M. Christenhusz 4076* (TUR), 3339, EF452075, Schuettpelz et al. 2007; EF452017, Schuettpelz et al. 2007; KU147262, This study; KC984523, Rothfels and Schuettpelz 2014; KU147296, This study; NA (missing data), -; ***Antrophyum latifolium*** Blume, Papua New Guinea; *T. Ranker 1774* (COLO), 3078, EF452076, Schuettpelz et al. 2007; EF452018, Schuettpelz et al. 2007; KU147263, This study; EF452138, Schuettpelz et al. 2007; KU147297, This study; KU147315, This study; ***Bommeria hispida*** (Kuhn) Underw., U. S. A., Arizona; *E. Schuettpelz 467* (DUKE), 3174, EU268725, Rothfels et al. 2008; EF452022, Schuettpelz et al. 2007; KU147264, This study; EF452142, Schuettpelz et al. 2007; KU147298, This study; KU147316, This study; ***Calciphilopteris ludens*** (Wall. ex Hook.) Yesilyurt & H. Schneid., from cultivation; *H. Schneider s.n.* (GOET), 3510, EU268741, Rothfels et al. 2008; EF452031, Schuettpelz et al. 2007; NA (missing data), -; EF452150, Schuettpelz et al. 2007; NA (missing data), -; KU147318, This study; ***Cryptogramma crispa*** (L.) R. Br. ex Hook., U. K., Scotland; *M. J. M. Christenhusz 3871* (DUKE), 2949, EU268740, Rothfels et al. 2008; EF452027, Schuettpelz et al. 2007; NA (missing data), -; EF452148, Schuettpelz et al. 2007; NA (missing data), -; NA (missing data), -; ***Haplopteris elongata*** (Sw.) E. H. Crane, from cultivation; *L. Huiet 112* (UC), 2546, EF452096, Schuettpelz et al. 2007; EF452035, Schuettpelz et al. 2007; KU147265, This study; EF452153, Schuettpelz et al. 2007; NA (missing data), -; KU147319, This study; ***Hecistopteris pumila*** (Spreng.) J. Sm., Guadeloupe, Sofaia; *M. J. M. Christenhusz 3976* (TUR), 3278, EF452097, Schuettpelz et al. 2007; EF452036, Schuettpelz et al. 2007; KU147266, This study; KC984524, Rothfels and Schuettpelz 2014; KU147303, This study; NA (missing data), -; ***Hemionitis palmata*** L., from cultivation; *E. Schuettpelz 297* (DUKE), 2557, EU268743, Rothfels et al. 2008; EF452037, Schuettpelz et al. 2007; NA (missing data), -; KC984525, Rothfels and Schuettpelz 2014; NA (missing data), -; NA (missing data), -; ***Cheilanthes nitidula*** Wall ex Hook., from cultivation; *H. Schneider s.n.* (GOET), 3513, EF452085, Schuettpelz et al. 2007; EF452025, Schuettpelz et al. 2007; NA (missing data), -; EF452146, Schuettpelz et al. 2007; NA (missing data), -; NA (missing data), -; ***Monogramma graminea*** (Poir.) Schkuhr, France, Ile de la Reunion; *T. Janssen 2692* (P), 3548, EF452102, Schuettpelz et al. 2007; EF452040, Schuettpelz et al. 2007; KU147268, This study; EF452157, Schuettpelz et al. 2007; KU147304, This study; NA (missing data), -; ***Myriopteris covillei*** (Maxon) Á. Löve & D. Löve, U. S. A., Arizona; *E. Schuettpelz 443* (DUKE), 3150, EU268733, Rothfels et al. 2008; KC984444, Rothfels and Schuettpelz 2014; NA (missing data), -; EU268782, Rothfels et al. 2008; KU147299, This study; KU147317, This study; ***Notholaena grayi*** Davenp., U. S. A., Arizona; *E. Schuettpelz 480* (DUKE), 3187, EU268749, Rothfels et al. 2008; JF832173, Rothfels et al. 2012; NA (missing data), -; EU268794, Rothfels et al. 2008; NA (missing data), -; NA (missing data), -; ***Pellaea atropurpurea*** (L.) Link, from cultivation; *E. Schuettpelz 312* (DUKE), 2957, JQ855925, Johnson et al. 2012; KC984440, Rothfels and Schuettpelz 2014; KU147269, This study; EF452162, Schuettpelz et al. 2007; KU147300, This study; KU147321, This study; ***Pentagramma triangularis*** (Kaulf.) Yatsk., Windham & Wollenw., U. S. A., Arizona; *E. Schuettpelz 445* (DUKE), 3152, EU268768, Rothfels et al. 2008; EF452049, Schuettpelz et al. 2007; NA (missing data), -; EF452165, Schuettpelz et al. 2007; NA (missing data), -; NA (missing data), -; ***Pityrogramma austroamericana*** Domin, from cultivation; *E. Schuettpelz 301* (DUKE), 2561, EU268769, Rothfels et al. 2008; EF452050, Schuettpelz et al. 2007; NA (missing data), -; EF452166, Schuettpelz et al. 2007; NA (missing data), -; KU147322, This study; ***Rheopteris cheesmaniae*** Alston, Papua New Guinea; *J. Croft 1749* (A), 3373, EF452126, Schuettpelz et al. 2007; EF452063, Schuettpelz et al. 2007; KU147270, This study; EF452176, Schuettpelz et al. 2007; KU147301, This study; KU147323, This study; ***Vaginularia acrocarpa*** Holttum, Papua New Guinea; *T. Ranker 1778* (COLO), 3375, KC984435, Rothfels and Schuettpelz 2014; KC984439, Rothfels and Schuettpelz 2014; KU147267, This study; EF452156, Schuettpelz et al. 2007; NA (missing data), -; KU147320, This study; ***Vittaria graminifolia*** Kaulf., Ecuador, Zamora-Chinchipe Province; *E. Schuettpelz 227* (DUKE), 2395, EF452128, Schuettpelz et al. 2007; EF452064, Schuettpelz et al. 2007; KU147271, This study; KU147279, This study; KU147302, This study; NA (missing data), -.