RESEARCH ARTICLE

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The evolutionary history of ferns inferred from 25 low-copy nuclear genes[1]

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PREMISE OF THE STUDY: Understanding fern (monilophyte) phylogeny and its evolutionary timescale is critical for broad investigations of the evolution of land plants, and for providing the point of comparison necessary for studying the evolution of the fern sister group, seed plants. Molecular phylogenetic investigations have revolutionized our understanding of fern phylogeny, however, to date, these studies have relied almost exclusively on plastid data.

METHODS: Here we take a curated phylogenomics approach to infer the first broad fern phylogeny from multiple nuclear loci, by combining broad taxon sampling (73 ferns and 12 outgroup species) with focused character sampling (25 loci comprising 35 877 bp), along with rigorous alignment, orthology inference and model selection.

KEY RESULTS: Our phylogeny corroborates some earlier inferences and provides novel insights; in particular, we find strong support for Equisetales as sister to the rest of ferns, Marattiales as sister to leptosporangiate ferns, and Dennstaedtiaceae as sister to the eupolypods. Our divergence-time analyses reveal that divergences among the extant fern orders all occurred prior to ~200 MYA. Finally, our species-tree inferences are congruent with analyses of concatenated data, but generally with lower support. Those cases where species-tree support values are higher than expected involve relationships that have been supported by smaller plastid datasets, suggesting that deep coalescence may be reducing support from the concatenated nuclear data.

CONCLUSIONS: Our study demonstrates the utility of a curated phylogenomics approach to inferring fern phylogeny, and highlights the need to consider underlying data characteristics, along with data quantity, in phylogenetic studies.

KEY WORDS codon models; curated phylogenomics; divergence time dating; *Equisetum*; fern chronogram; incomplete lineage sorting; low-copy nuclear gene; model selection; monilophytes; transcriptome

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Ferns (monilophytes) are an important and ancient component of the Earth's terrestrial biodiversity—critical due to their species richness, ecological impact, and the unique role they play in the evolution and ecology of land plants (reviewed in Page, 1979, 2002; Ranker and Haufler, 2008; Sessa et al., 2014). Over the past two decades, molecular phylogenetic approaches have fundamentally altered our understanding of the evolution of ferns. These paradigm-altering results include the phylogenetic position of Equisetales and Psilotales within the ferns (Nickrent et al., 2000; Pryer et al., 2001), robust support for ferns as the sister group of seed plants (and thus phylogenetically distant from the lycophyte "fern allies"; Duff and Nickrent, 1999; Nickrent et al., 2000; Pryer et al., 2001), the recent origin of most extant fern diversity (Schneider et al., 2004b; Schuettpelz and Pryer, 2009), novel understanding of the deep relationships within ferns (Hasebe et al., 1994; Wolf et al., 1994; Pryer et al., 2004; Schuettpelz et al., 2006; Schuettpelz and Pryer, 2007; Rai and Graham, 2010; Kuo et al., 2011), and increased resolution at shallower phylogenetic depths (e.g., Wolf et al., 1999; Des Marais et al., 2003; Wang et al., 2003; Ranker et al., 2004; Schneider et al., 2004a; Korall et al., 2006b; Ebihara et al., 2007; Janssen et al., 2008; Metzgar et al., 2008; Murdock, 2008; Vasco et al., 2009; Windham et al., 2009; Sundue et al., 2010; Sigel et al., 2011; Li et al., 2012; Williams and Waller, 2012; Lóriga et al., 2014; McHenry et al., 2013; Grusz et al., 2014; Labiak et al., 2014; Moran et al., 2014; Perrie et al., 2014). These results continue to be synthesized into an emerging consensus on fern phylogeny and classification (Smith et al., 2006; Schuettpelz and Pryer, 2008; Smith et al., 2008; Christenhusz et al., 2011; Rothfels et al., 2012b) that differs greatly from premolecular hypotheses (e.g., Ching, 1940; Mickel, 1974; Smith, 1995; Stevenson and Loconte, 1996).

Much of what we know about deep divergences in fern phylogeny comes from 12 studies (Table 1) that relied almost exclusively on data from a single linkage group, the plastid genome, which is maternally inherited in ferns (Gastony and Yatskievych, 1992; Vogel et al., 1998; Guillon and Raquin, 2000). Phylogenies constructed from plastid sequences alone warrant cautious interpretation—without the inclusion of loci from other linkage groups, these studies lack the ability to identify potentially misleading idiosyncrasies in the evolution of plastid sequences (Moore, 1995). Most studies that have used nuclear markers were based on only a single locus and/or were at relatively shallow phylogenetic depths, with the goal of understanding reticulate patterns of evolution caused by hybridization and allopolyploidy (Ishikawa et al., 2002; Ebihara et al., 2005; Adjie et al., 2007; James et al., 2008; Schuettpelz et al., 2008; Shepherd et al., 2008; Chang et al., 2009; Grusz et al., 2009; Juslén et al., 2011; Nitta et al., 2011; Chao et al., 2012; Dyer et al., 2012; Li et al., 2012; Schneider et al., 2013; Sessa et al., 2012; Jaruwattanaphan et al., 2013; Metzgar et al., 2013; Chen et al., 2014; Hori et al., 2014; Rothfels et al., 2014; Sigel et al., 2014b; Zhang, W. et al., 2014; Rothfels et al., 2015). To date, there are only two analyses of divergent relationships that are based on multiple nuclear loci in ferns: (1) a recent study of Polypodiales (15 taxa) based on 20 markers across 10 distinct nuclear genes (Rothfels et al., 2013); and (2) the green-plant phylogenomics study of Wickett et al. (2014) that included over 800 loci, but only six fern species. Similarly, our understanding of the timescale of fern evolution is based entirely on plastid data (with the rare exception of largely uninformative 18S sequences; Pryer et al., 2004; Schneider et al., 2004b; Janssen et al., 2008; Pryer and Schuettpelz, 2009; Schuettpelz and Pryer, 2009; Smith et al., 2010; Lehtonen et al., 2012; Grimm et al., 2015; Sundue et al., 2014; Rothfels et al., 2015).

Here we adopt a "curated phylogenomics" approach to reconstruct the first broadly sampled multilocus nuclear phylogeny and chronogram for the fern tree of life. Instead of using probabilistic orthology assessments and building a massive character matrix as is typical in fully genome-scale phylogenetic studies (e.g., Dunn et al., 2008; Telford et al., 2014; Wickett et al., 2014), we use a dataset that is small enough to be able to manually inspect alignments, use tree-based methods to confirm orthology of individual sequences, undertake rigorous model selection, and perform analyses using computationally intensive models. Given the few available nuclear phylogenetic studies of ferns, we are more concerned with minimizing systematic error—by broad taxon sampling (Zwickl and Hillis, 2002), by focusing attention on thorough alignment inference and orthology determinations, and by rigorous model testing—than we are with minimizing stochastic error through genome-scale character sampling. Our curated approach additionally allows us to detect potential model mis-specifications (Rothfels et al., 2012a),

TABLE 1. Summary of main studies of deep fern phylogeny.

Study	Phylogenetic depth	Ferns sampled	Characters used (bp)	Data types included
Pryer et al. (1995)	All ferns	50	1206 (+77 morph. chars.)	Plastid (*rbcL*); Morphology
Hasebe et al. (1995)	All ferns	107	1206 bp	Plastid (*rbcL*)
Pryer et al. (2001)	Vascular plants	21	4072 (+136 morph. chars.)	Plastid (three loci); Nucleus (*18S*); Morphology
Pryer et al. (2004)	All ferns	53	5049	Plastid (three loci); Nucleus (*18S*)
Wikström and Pryer (2005)	All ferns	20	5697 (+138 morph. chars.)	Plastid (three loci); Nucleus (*18S*); Mitochondrion (*atp1*); Morphology
Schuettpelz et al. (2006)	All ferns	52	6113	Plastid (four loci); Nucleus (*18S*)
Schuettpelz and Pryer (2007)	Leptosporangiate ferns	400	4092	Plastid (three loci)
Qiu et al. (2007)	Land plants	36	14553	Plastid (seven loci); Nucleus (*18S*); Mitochondrion (two loci)
Rai and Graham (2010)	Land plants	34	~10000[†]	Plastid (17 loci)
Kuo et al. (2011)	All ferns	78	3876	Plastid (three loci)
Lehtonen (2011)	All ferns	2656	4406*	Plastid (four loci)*
Rothfels et al. (2012a)	Polypodiales	81	6596	Plastid (five loci)
Grewe et al. (2013)	Vascular plants	9	32547	Plastid (49 loci)
Wickett et al. (2014)	Green plants	6	1701170	Nucleus (852 loci)

[†]The total alignment was 36139 base pairs long, but much of that length was due to single-taxon regions where the sequences were staggered due to uncertain homology.
*Fewer than 10% of included taxa had all four loci; 54% were represented by only a single locus.

TABLE 2. Summary of loci used in this study.

Abbreviation	Protein name	TAIR Gn#	Chromosome	Length (aa)
NDUFS6	Complex I subunit NDUFS6	AT1G49140	1	107
GClev	Glycine cleavage protein complex	AT1G75980	1	225
DUF1077	Protein of unknown function DUF 1077	AT5G10780	5	187
Hsp40	DNAJ/Hsp40 Cysteine-rich domain superfamily protein	AT5G17840	5	154
ApPEFP_AC	Appr-1-p processing enzyme family protein	AT1G69340	1	562
ApPEFP_B	Appr-1-p processing enzyme family protein	AT1G69340	1	562
COP9	COP9 Signalosome subunit 8	AT4G14110	4	197
CRY1	Cryptochrome 1	AT4G08920	4	681
CRY2	Cryptochrome 2	AT4G08920	4	681
CRY3	Cryptochrome 3	AT1G04400	1	612
CRY4	Cryptochrome 4	AT1G04400	1	612
CRY5	Cryptochrome 5	AT4G08920	4	681
HMR	Hemera	AT2G34640	2	527
IBR3	IBA-Response 3	AT3G06810	3	824
IGPD	Imidazoleglycerol-phosphate dehydratase	AT3G22425	3	270
MCD1	Multiple chloroplast division site1	AT1G20830	1	349
SEF	Serrated Leaves and Early Flowering	AT5G37055	5	171
SQD1	UDP-Sulfoquinovose synthase	AT4G33030	4	477
TPLATE	TPLATE	AT3G01780	3	1176
DET1	De-etiolated 1	AT4G10180	4	543
GAPC	Glyceraldehyde-3-phosphate dehydrogense C subunit1	AT3G04120	3	338
gapCpLg	Glyceraldehyde-3-phosphate dehydrogense of plastid, "long" copy	AT1G79530; AT1G16300	1	422; 420
gapCpSh	Glyceraldehyde-3-phosphate dehydrogense of plastid, preduplication and "short" copy	AT1G79530; AT1G16300	1	422; 420
pgiC	Glucose-6-phosphate isomerase activity	AT5G42740	5	560
transducin	Transducin family protein/WD40 repeat family protein	AT3G21540	3	955

Notes: The TAIR accession numbers are from the *Arabidopsis* Information Resource website (www.arabidopsis.org; Lamesch et al., 2012). Location (chromosome number) and lengths refer to the corresponding homolog in *Arabidopsis thaliana*, and were retrieved from the *Arabidopsis* Information Resource website. Lengths are measured in amino acids.

i.e., with genome-scale data, even small biases from model misspecification risk overwhelming the underlying signal in the data (Philippe et al., 2011; Zhong et al., 2011). Our data comprise sequences from 25 loci for 73 ferns and 12 outgroup species, and are derived largely from transcriptomes sequenced by the One Thousand Plants Project (1KP; www.onekp.com).

MATERIALS AND METHODS

***Transcriptome sequencing, assembly, and alignment*—**The majority of the data included here are from transcriptomes generated by the One Thousand Plants project (1kp; www.onekp.com). Most RNA extractions were performed with the Spectrum Total Plant RNA Kit (Sigma-Aldrich, St. Louis, Missouri, USA), although RNA extraction protocols varied. Transcriptomes were sequenced on Illumina GAIIx or HiSeq platforms at BGI-Shenzhen (2×75 bp or 2×90 bp paired-end reads, respectively). Reads for each taxon were assembled with SOAPdenovo (Luo et al., 2012) and SOAPdenovo-trans (Xie et al., 2014). For further details on RNA extractions, transcriptome sequencing, and assembly, see Johnson et al. (2012). Additional fern sequences were acquired by querying the publicly available *Pteridium aquilinum* (L.) Kuhn transcriptome and *Adiantum capillus-veneris* L. cryptochrome sequences (Kanegae and Wada, 1998; Imaizumi et al., 2000; Der et al., 2011). Outgroup sequences were acquired from 1KP transcriptomes and by querying publicly available seed-plant genomic resources (Schnable et al., 2009; Goodstein et al., 2012; Lamesch et al., 2012; *Amborella* Genome Project, 2013; *Aquilegia coerulea* Genome Sequencing Project, 2015). Our total taxon sample comprises 73 ferns, seven angiosperms, and five gymnosperms (Appendix 1), focused on capturing the deepest divergences in these groups ("deep-node sampling"; Qiu et al., 2007; Rothfels et al., 2012a; Knie et al., 2015).

Candidate single-copy loci were selected based on their utility in other plant groups or from a list of putatively single-copy markers generated by the 1KP project (Wickett et al., 2014). For each candidate locus we inferred a fern-wide alignment using the python script Blue Devil version 0.6 (Rothfels et al., 2013; Li et al., 2014a, b). Blue Devil blasts (blastn: Altschul et al., 1990; Camacho et al., 2009) a query sequence against each of the target transcriptomes, retains all transcripts satisfying a given e-value cut-off, and aligns the resulting hits with MUSCLE (Edgar, 2004). It includes the option of using CAP3 (Huang and Madan, 1999) to reassemble the blast hits prior to producing the alignment, which was particularly useful in our pipeline because it allowed the SOAPdenovo and SOAPdenovo-trans assemblies of each transcriptome to be combined into one "master" assembly.

We refined the Blue Devil alignments manually, in an iterative manner as outlined in Rothfels et al. (2013). Briefly, we first inferred a preliminary phylogenetic tree from each alignment using maximum parsimony (MP) in PAUP* version 4.0a125 (Swofford, 2002). Groups of discontinuous or slightly overlapping sequences from a given accession that appeared closely related in the resulting tree and were identical in the region of overlap were merged into a single sequence in Mesquite version 2.75 (Maddison and Maddison, 2009). We then repeated the MP analyses on this new alignment. We continued to "infer-tree, group-sequences" until no further fragments met our criteria for merging.

Despite our attempts to target single-copy genes, some of the transcriptome queries returned multiple paralogs. In the case of duplication events deep within the fern phylogeny, the sequences of one of the paralogs were excised to form their own alignment

(which was subsequently treated as an independent locus), leaving the preduplication sequences and the sequences from the other paralog in the original alignment. For shallow duplications, in those cases where paralog identity was clear, we deleted the paralog that was least represented (had fewer taxa or shorter sequences). For particularly shallow duplications, it was occasionally unclear which sequence fragments belonged to a particular paralog. In this case, we created two sequences by merging the nonconflicting fragments arbitrarily (see Fig. 5 in Rothfels et al., 2013). The resulting sequences may thus be chimeras between very closely related paralogs. We completed each alignment with a thorough manual inspection in Mesquite version 2.75 (Maddison and Maddison, 2009), and by excluding ambiguous regions and all sites that included a stop codon. The resulting single-locus alignments were merged into one master nexus file with abioscripts version 0.9.3 (Larsson, 2010; Rothfels et al., 2012a). Our final dataset comprises 25 low-copy loci (Tables 2, 3).

***Maximum likelihood tree inference*—**We analyzed our 25-locus dataset under maximum likelihood (ML) using 14 different models (Table 4) as implemented in Garli version 2.0 (Zwickl, 2006). Seven of these are codon models (Goldman and Yang, 1994) in which the likelihood of the data are computed given a certain number of site classes, each of which has an omega (ω) parameter representing the ratio of nonsynonymous to synonymous substitutions (Nielsen and Yang, 1998; Yang and Nielsen, 2000). The seven codon models incorporate different combinations of the underlying nucleotide model (HKY (Hasegawa et al., 1985) or GTR (Tavaré, 1986)), number of included site classes, method of estimating codon frequencies (empirically or from the nucleotide frequencies at the three codon positions—"F34"), and partitioning (by locus, or unpartitioned). The four nucleotide models used differ in their data partitioning (unpartitioned, partitioned by locus, partitioned by codon position, or by the optimal partitioning scheme as determined by a greedy PartitionFinder version 1.0.1 (Lanfear et al., 2012) search using the AICc). The first three analyses applied a GTR+I+G model to each subset (the best model for the codon position subsets; we did not run PartitionFinder on the individual-locus subsets). Appendix S1 (see Supplemental Data with the online version of this article) details the subsets and substitution models applied for the final partitioned analysis (partitioned according to the optimal PartitionFinder scheme). Similarly, the three amino acid models differed in their data partitioning: (1) unpartitioned; (2) partitioned by locus; or (3) by the optimal partitioning scheme as determined by a greedy PartitionFinderProtein version 1.0.1 search (Lanfear et al., 2012). The amino acid models each used JTT (Jones et al., 1992) base frequencies and exchangeability rates, with gamma-distributed site rate variation and an estimated proportion of invariant sites. For all codon, nucleotide, and amino acid partitioned analyses, parameters were unlinked across partitions, and each data subset was allowed its own average rate (refer to Table 4 for specific details of each model). For each model we employed a ML tree search ten times, from different random addition sequence starting trees.

TABLE 3. Dataset characteristics by locus used in the study.

Locus	Missing data	Included accessions	Alignment length (bp)	Pars. inf. sites	Relative rate
NDUFS6	24%	76	333	202	0.96
GClev	21%	79	507	358	0.99
DUF1077	9%	82	525	301	0.88
Hsp40	28%	72	378	273	1.17
ApPEFP_AC	19%	82	1689	1018	0.85
ApPEFP_B	60%	52	1536	817	1.06
COP9	22%	78	645	475	0.99
CRY1	29%	81	1974	1160	1.06
CRY2	46%	60	2076	1129	1.19
CRY3	43%	68	2121	1406	1.15
CRY4	54%	56	2139	1135	1.12
CRY5	52%	53	1452	741	1.14
HMR	24%	81	1299	979	1.07
IBR3	20%	82	2418	1564	0.90
IGPD	17%	79	789	486	0.95
MCD1	26%	79	972	715	1.20
SEF	18%	77	522	319	0.90
SQD1	23%	80	1482	827	1.07
TPLATE	29%	82	3426	2016	0.85
DET1	27%	79	1557	1046	0.85
GAPC	44%	59	1017	496	1.01
gapCpLg	53%	46	1242	512	1.03
gapCpSh	15%	84	1227	700	0.99
pgiC	17%	84	1719	986	0.82
transducin	28%	78	2832	1866	0.96
Total:	**32%**	**85**	**35877**	**21534**	

Notes: Locus name abbreviations follow the abbreviations in Table 2. Missing data include both missing sequence and gaps. Relative rates were inferred using a nucleotide model, with the data partitioned by locus (Model 11 in Table 4). "Pars. inf sites" refers to the number of sites that are informative under an unordered and equally weighted parsimony model.

***Bayesian divergence time estimation*—**To infer a time-calibrated phylogeny, we analyzed the data using a relaxed clock model in the parallel version of MrBayes version 3.2.2 (Huelsenbeck and Ronquist, 2001; Altekar et al., 2004; Ronquist et al., 2012). We partitioned the data by codon position, applying a GTR+I+G model (Tavaré, 1986; Yang, 1993) to each subset, with parameters unlinked among subsets and each subset permitted its own average rate ("ratepr = variable"). For the relaxed clock model, we used a uniform tree (branch lengths) prior and the "independent gamma rates" model, in which branch rates are drawn independently (no autocorrelation) from a scaled gamma distribution (Lepage et al., 2007). We applied a broad prior (a normal distribution with mean of 0.0001 and standard deviation of 0.01) on the clock rate (Rothfels and Schuettpelz, 2014) and constrained the age of 12 well-supported nodes for our main temporal information (Appendix S2; see Supplemental Data with the online version of this article). The dates for the calibrated nodes are derived from the divergence time estimates from two broadly sampled time trees—those of Schuettpelz & Pryer (2009) and Smith et al. (2010)—and were modeled as truncated normal distributions with a mean equal to that inferred in the source studies. Since Schuettpelz & Pryer (2009) provided only point estimates of divergence time, we set the standard deviation for those calibrations equal to 10% of their mean estimate (see Rothfels et al., 2012a; Sigel et al., 2014a; Sundue et al., 2014; Rothfels et al., 2015). Smith et al. (2010) presented 95% highest posterior density (HPD) intervals instead of standard deviations, so we estimated the standard deviation for those calibrations by assuming the divergence time estimates were normally distributed and thus taking one quarter of the difference between the 95% HPD maximum and minimum ages as the standard deviation. For all calibrations, we set the minimum age at the mean minus two standard deviations. Each of the 12 temporally calibrated clades were constrained to be monophyletic to assist with convergence and to ameliorate the difficulties of correctly rooting a phylogeny with relaxed clock models (e.g.,

TABLE 4. Models and model fit.

	Model descriptions										
Model	Type	Partitioning	Exchang.	Rates	Base freq.	Prop. inv.	Subsets	Param. #	lnL	AICc	Δ AICc
1	codon	none	gtr	1omeg	emp	none	1	236	−700010.7	1400496.6	54130.8
2	codon	none	hky	1omeg	emp	none	1	232	−700448.5	1401364.0	54998.2
3	codon	none	hky	3omeg	emp	none	1	236	−674109.0	1348693.1	2327.3
4	codon	locus	gtr	1omeg	emp	none	25	1916	−699199.9	1402448.2	56082.3
5	codon	none	gtr	1omeg	F34	none	1	185	−701191.4	1402754.8	56389.0
6	codon	none	gtr	3omeg	emp	none	1	240	−673727.2	1347937.7	1571.9
7	codon	none	gtr	4omeg	emp	none	1	242	−672939.3	1346365.8	0.0
8	nucleo	none	gtr	gamma	est	+I	1	177	−706716.6	1413789.0	67423.2
9	nucleo	position	gtr	gamma	est	+I	3	199	−693669.4	1387739.1	41373.3
10	nucleo	scheme	NA	NA	NA	NA	49	686	−691276.0	1383950.8	37585.0
11	nucleo	locus	gtr	gamma	est	+I	25	441	−705364.9	1411622.7	65256.9
12	AA	none	jones	gamma	jones	+I	1	169	−279343.9	559030.7	613.2
13	AA	scheme	jones	gamma	jones	+I	7	206	−278999.1	558417.5	0.0
14	AA	locus	jones	gamma	jones	+I	25	241	−279130.6	558753.2	335.7

Notes: "Scheme" indicates that the data were partitioned according to the optimal scheme as determined by PartitionFinder. Exchang.: Exchangeability parameters. Base freq.: Base frequencies. Prop. inv.: Proportion of invariant sites. Param. #: Total number of free parameters. Δ AICc: Difference in the AICc score between the focal model and the best model.

Rothfels and Schuettpelz, 2014). Calibration details are listed in Appendix S2 (see Supplemental Data with the online version of this article). The remaining priors and proposal mechanisms were left at their default values (see doi: 10.5061/dryad.62f0r and Lakner et al., 2008).

We ran four independent runs of this model, each with four chains (one cold, three heated). Two runs ran for 18.9 million generations and the other two ran for 14.5 million, consuming a total of 270 CPU days. Based on our assessment of stationarity and convergence in TRACER version 1.5 (Rambaut and Drummond, 2007b), we excluded a burnin of six million generations from each of the runs, and combined the remaining 85 640 samples for subsequent analysis. The postburnin effective sample size (ESS) for all parameters was greater than 250. Under this model configuration, MrBayes version 3.2.2 outputs ultrametric trees with branch lengths in units of expected numbers of substitution per site. To convert these branch-lengths to units of time, we ran the burntree.pl script (Nylander, 2014). We summarized our postburnin posterior sample of trees onto the maximum clade credibility tree using TreeAnnotator version 1.8.0 (Rambaut and Drummond, 2007a) and used FigTree version 1.4.1 for tree visualization and manipulation (Rambaut, 2006).

***Species tree inference*—**To investigate whether any of the differences between inferences from our low-copy nuclear data and those of early plastid-based phylogenies might be due to incomplete lineage sorting (nuclear markers coalesce four times more slowly than do plastid markers because they are diploid and biparentally inherited; Moore, 1995), we inferred a species tree using ASTRAL version 4.4.1 (Mirarab et al., 2014). We produced 25 single-locus alignments with consistent taxon labels using the abioscripts "outputsingles" option (Larsson, 2010), and inferred a ML tree from each alignment using Garli version 2.0 (Zwickl, 2006). For this inference step, we partitioned each locus by codon position, applied a GTR+I+G model to each subset (Tavaré, 1986; Yang, 1993), and performed 10 independent tree searches from different random sequence addition starting trees, using the default termination conditions. Because ASTRAL includes in its search set only those quartets that occur in at least one of the input gene trees, we augmented the search space (-e option in ASTRAL) by including additional topologies. These topologies were inferred using Garli, under the same model settings as the individual locus best-tree searches, from reduced datasets constructed by sampling sites without replacement from the complete concatenated alignment. For 300 of the 800 searches, 33% of the sites were sampled, and for the remaining 500 searches, we sampled 50% of the sites, resulting in 800 additional, unique topologies. These 800 trees, and the 25 individual-locus best trees, were converted to newick format using a custom python script (see doi:10.5061/dryad.62f0r) and the python library dendropy (Sukumaran and Holder, 2010), for subsequent ASTRAL analysis. To assess support for the resulting species tree, we performed 160 replicates of multilocus bootstrapping (Seo, 2008), again in ASTRAL. Each replicate bootstrapped first the pool of loci, and then within each selected locus, bootstrapped the alignment sites (specifically, selected from among 200 bootstrap trees already inferred for that locus by Garli, under the same settings as for the individual-locus best-tree searches).

For all analyses, the majority of the computationally intensive work was performed in parallel on the CIPRES Science Gateway version 3.3 (http://www.phylo.org/index.php/portal/; Miller et al., 2010), supplemented by the Duke Shared Cluster Resource (https://wiki.duke.edu/display/SCSC/DSCR), the Garli Web Service (http://molecularevolution.org; Bazinet et al., 2007; Bazinet and Cummings, 2011), Compute Canada's WestGrid network (www.computecanada.ca; www.westgrid.ca), and the UBC Zoology Computing Unit cluster (www.zoology.ubc.ca).

RESULTS

Our total dataset comprises 25 loci (Table 2), most of which have not previously been used for fern phylogenetics. All newly generated sequence data are available in GenBank (Appendix 1), and the alignments for each locus are available in TreeBase (study number S17594). The 25 individual-locus alignments range in length from 333 to 3226 bp (Table 2). The final concatenated dataset is 35 877 aligned base pairs long for 85 accessions, with 30% missing data (gaps and uncertain base calls; Table 3). Much of the missing data are due to a few low coverage transcriptomes (% missing data per accession ranges from under 4% to over 90%; Appendix S3; see

TABLE 5. Selected comparisons of model fit.

Model #	Contrast	Total P	lnL	AICc	Δ AICc
Best-fitting nucleotide vs codon models					
7	codon (GTR, 4 omega, unpartitioned)	242	−672939.26	1346365.83	0.00
10	nucleotide (partitioned by scheme)	686	−691276.00	1383950.78	37584.95
Codon models: Number of omega parameters					
7	4 omegas	242	−672939.26	1346365.83	0.00
6	3 omegas	240	−673727.23	1347937.71	1571.88
1	1 omega	236	−700010.74	1400496.62	54130.79
Codon models: Effect of partitioning					
1	unpartitioned	236	−700010.74	1400496.62	0.00
4	partitioned by locus	1916	−699199.93	1402448.17	1951.56
Nucleotide models: Effect of partitioning					
10	partitioned by scheme	686	−691276.00	1383950.78	0.00
9	partitioned by codon position	199	−693669.45	1387739.13	3788.35
11	partitioned by locus	441	−705364.85	1411622.71	27671.93
8	unpartitioned	177	−706716.62	1413789.01	29838.23

Notes: Within a given comparison, the top model is the best fitting, with fit decreasing for subsequent models; Total P = total number of free parameters; delta AICc scores are calculated with respect to the best fitting model in that comparison. The full description of each model is available in Table 4.

Supplemental Data with the online version of this article) and to gene duplications within the fern clade, resulting in incomplete representation of some loci across taxa (Table 3 and Appendix S3; see Supplemental Data with the online version of this article).

The best-fitting model for the concatenated data is a codon model with four omega parameters, a GTR nucleotide substitution model, and empirical codon frequencies, applied to the unpartitioned data (Model 7; Tables 4 and 5). In general, codon models greatly outperformed nucleotide models, and additional omega parameters significantly improved codon model fit (at least up to four omega parameters; Table 5). For the nucleotide models, the unpartitioned data fit very poorly; partitioning by locus also fit relatively poorly, but partitioning by codon position resulted in strong improvements in fit. In contrast, the codon substitution models fit best on the unpartitioned data (Tables 4, 5).

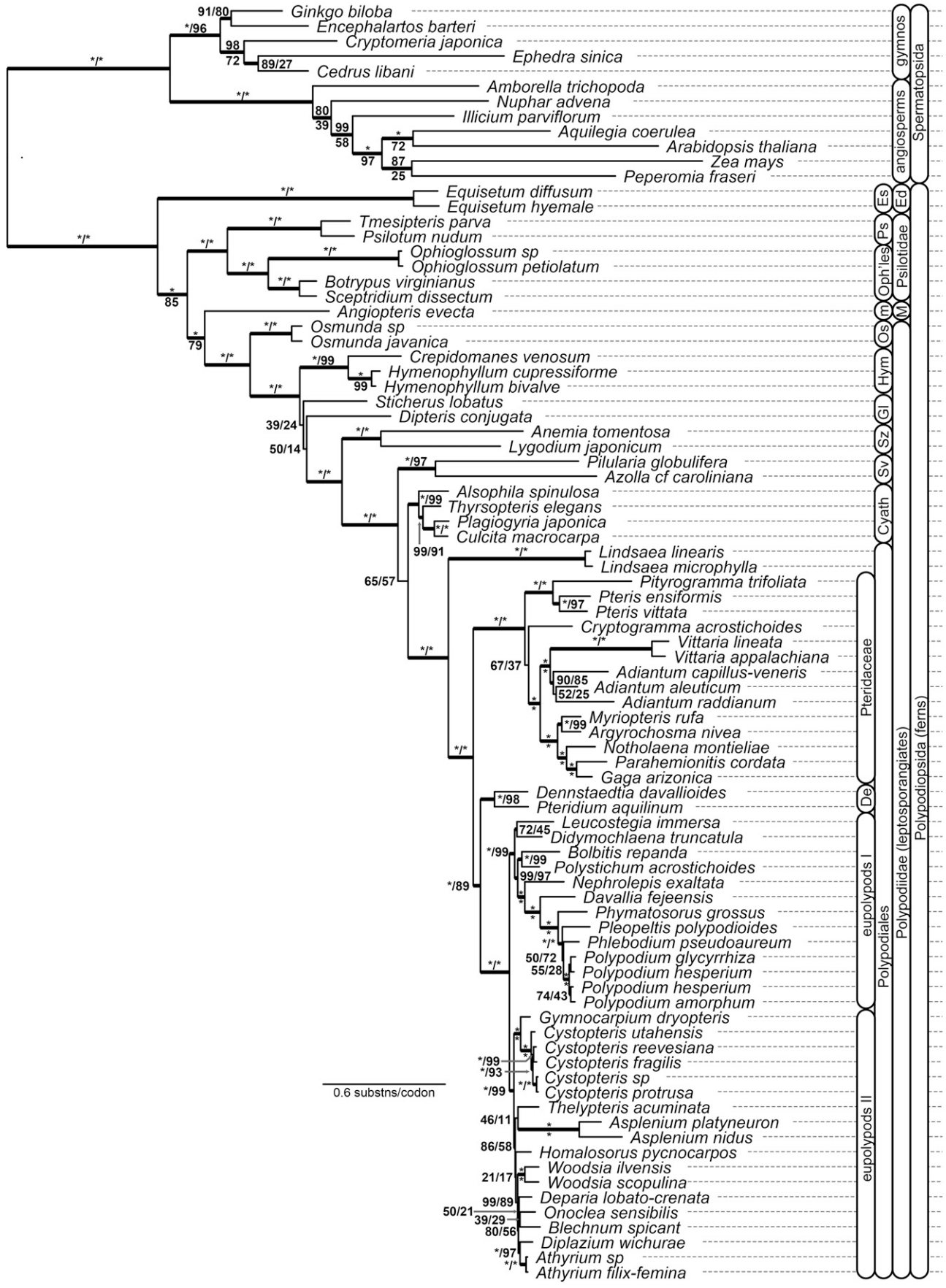
The ML tree inferred from the concatenated data is strongly supported, with 72 of the 83 total branches having bootstrap support >70% (Fig. 1). The strong support extends from the deep internodes to the more recent divergences, with areas of poor support being limited largely to the vicinity of Gleicheniales and Hymenophyllales (the taxa with the lowest-coverage transcriptomes; they have up to 92% missing data (Appendix S3; see Supplemental Data with the online version of this article)), and also within the eupolypod II radiation. Bayesian inferences of topology and divergence times (Fig. 2) are likewise strongly supported and consistent with the ML analyses. Median posterior estimates of the crown age of extant monilophytes, leptosporangiate ferns, core leptosporangiates (sensu Smith et al., 2006), and eupolypods (sensu Schneider et al., 2004b; Smith et al., 2006) are 381, 301, 232, and 112 million years, respectively.

ASTRAL species-tree inferences are largely congruent with the ML tree from the concatenated data, albeit with generally lower support (65 branches with >70% bootstrap support; Fig. 1). The bootstrap species-trees include a greater variety of topologies than do the analyses of the concatenated data, resulting in lower average support for any given clade (average ASTRAL multilocus bootstrap support across branches on the best species tree is 85.7%; average Garli bootstrap support, across branches on the best concatenated-data tree is 91.3%; t = −1.74, df = 155.5, $p = 0.042$, one-tailed t test).

DISCUSSION

***The fern tree of life*—**Perhaps the most striking outcome is the overall congruence of our nuclear-sequence based results with those inferred earlier from plastid sequence data (e.g., Pryer et al., 2001, 2004; Schuettpelz et al., 2006; Schuettpelz and Pryer, 2007; Rai and Graham, 2010; Kuo et al., 2011; Lehtonen, 2011). Most of the deep divergences inferred from nuclear and plastid data are identical, and this consistency extends to the more fine-scaled relationships (e.g., compare our tree with the corresponding plastid-derived results for tree ferns, Pteridaceae, and eupolypods II; Korall et al., 2006a; Schuettpelz et al., 2007; Rothfels et al., 2012a). While a concordant phylogenetic signal from these two very different data types has been found in several studies with more narrow taxonomic scopes (Beck et al., 2010; Chen et al., 2012; Rothfels et al., 2013; Rothfels and Schuettpelz, 2014), this is the first study to demonstrate concordance across ferns using multiple low-copy nuclear markers. Given the profound dependence of our current understanding of fern phylogeny on inferences from plastid data, this corroboration is highly reassuring. In addition, similar levels of congruence observed between plastid- and low-copy nuclear-based phylogenies within angiosperms (Zhang et al., 2012; Zeng et al., 2014) suggests that the evolutionary signal from these two data sources may be broadly consistent across the plant tree of life (although there are some important exceptions; see, e.g., our results below, and Ruhfel et al., 2014; Wickett et al., 2014; Sun et al., 2015).

FIGURE 1 Maximum likelihood (ML) phylogram from the full concatenated data, using the best-fitting model (Model 7; see Table 4). ML bootstrap support values from the analyses of the concatenated data are shown above the branches, or before the slashes; ASTRAL multilocus bootstrap support values (species-tree support values) are below the branches, or after the slashes. Taxonomy and Linnaean ranks follow the Tree of Life Web (Kenrick and Crane, 1996; Pryer et al., 2008, 2009): Cyath, Cyatheales; De, Dennstaedtiaceae; Ed, Equisetidae; Es, Equisetales; Gl, Gleicheniales; gymnos, gymnosperms; Hym, Hymenophyllales; m, Marattiales; M, Marattiidae; Oph'les, Ophioglossales; Os, Osmundales; Ps, Psilotales; Sv, Salviniales; Sz, Schizaeales.


Ginkgo biloba
Encephalartos barteri
Cryptomeria japonica
Ephedra sinica
Cedrus libani
Amborella trichopoda
Nuphar advena
Illicium parviflorum
Aquilegia coerulea
Arabidopsis thaliana
Zea mays
Peperomia fraseri
Equisetum diffusum
Equisetum hyemale
Tmesipteris parva
Psilotum nudum
Ophioglossum sp
Ophioglossum petiolatum
Botrypus virginianus
Sceptridium dissectum
Angiopteris evecta
Osmunda sp
Osmunda javanica
Crepidomanes venosum
Hymenophyllum cupressiforme
Hymenophyllum bivalve
Sticherus lobatus
Dipteris conjugata
Anemia tomentosa
Lygodium japonicum
Pilularia globulifera
Azolla cf caroliniana
Alsophila spinulosa
Thyrsopteris elegans
Plagiogyria japonica
Culcita macrocarpa
Lindsaea linearis
Lindsaea microphylla
Pityrogramma trifoliata
Pteris ensiformis
Pteris vittata
Cryptogramma acrostichoides
Vittaria lineata
Vittaria appalachiana
Adiantum capillus-veneris
Adiantum aleuticum
Adiantum raddianum
Myriopteris rufa
Argyrochosma nivea
Notholaena montieliae
Parahemionitis cordata
Gaga arizonica
Dennstaedtia davallioides
Pteridium aquilinum
Leucostegia immersa
Didymochlaena truncatula
Bolbitis repanda
Polystichum acrostichoides
Nephrolepis exaltata
Davallia fejeensis
Phymatosorus grossus
Pleopeltis polypodioides
Phlebodium pseudoaureum
Polypodium glycyrrhiza
Polypodium hesperium
Polypodium hesperium
Polypodium amorphum
Gymnocarpium dryopteris
Cystopteris utahensis
Cystopteris reevesiana
Cystopteris fragilis
Cystopteris sp
Cystopteris protrusa
Thelypteris acuminata
Asplenium platyneuron
Asplenium nidus
Homalosorus pycnocarpos
Woodsia ilvensis
Woodsia scopulina
Deparia lobato-crenata
Onoclea sensibilis
Blechnum spicant
Diplazium wichurae
Athyrium sp
Athyrium filix-femina
0.6 substns/codon
gymnos
angiosperms
Spermatopsida
Es
Ed
Ps
Oph'les
Psilotidae
m
M
Os
Hym
Gl
Sz
Sv
Cyath
Pteridaceae
De
eupolypods I
eupolypods II
Polypodiales
Polypodiidae (leptosporangiates)
Polypodiopsida (ferns)

Building on earlier broad molecular studies that demonstrated that seed plants and ferns are reciprocally monophyletic (Duff and Nickrent, 1999; Nickrent et al., 2000; Pryer et al., 2001; Qiu et al., 2006; Ruhfel et al., 2014; Wickett et al., 2014), our nuclear dataset provides new insights into fern evolution, most notably with respect to relationships among the leptosporangiate ferns and the major eusporangiate lineages. For example, we find strong support for horsetails as the sister group to all other extant ferns (Fig. 1). This result contradicts most studies based on plastid loci (Pryer et al., 2001, 2004; Schuettpelz et al., 2006; Lehtonen, 2011) that instead find high support for Psilotidae (sensu Pryer et al., 2009) as the sister group to the rest of ferns. There is some indication that the earlier result—Psilotidae sister to the rest of the ferns—may be due to sparse taxon sampling and characteristics of the specific plastid loci analyzed, rather than to plastid genome-wide signals. For example, analyses based on whole chloroplast genomes were unable to consistently resolve these basal nodes (e.g., Grewe et al., 2013; Ruhfel et al., 2014). Also, the three-gene dataset of Kuo et al. (2011) and the 17-gene plastid dataset of Rai and Graham (2010) resolved the same deep relationships for ferns that we find here, but with weak support for the earliest divergence.

Furthermore, the relationship that we infer—Equisetales sister to the rest of ferns—is arguably more compatible with some hypotheses of land plant morphological evolution, especially those that include fossils, than was the consensus from earlier molecular phylogenies (Equisetales embedded within the rest of ferns; Pryer et al., 2001, 2004). See, for example, Fig. 7.10 in Kenrick and Crane (1997). The placement of Equisetopsida as sister to Filicopsida in this tree requires fewer transitions from the ancestral pattern of protoxylem at the lobes of the xylem strand than would be required if *Equisetum* evolved from within the ferns. In addition, this result is consistent with the conclusions of the only other study of deep fern relationships to use low-copy nuclear loci (Wickett et al., 2014), as well as with a recent analysis of a combined mitochondrial and plastid dataset (Knie et al., 2015).

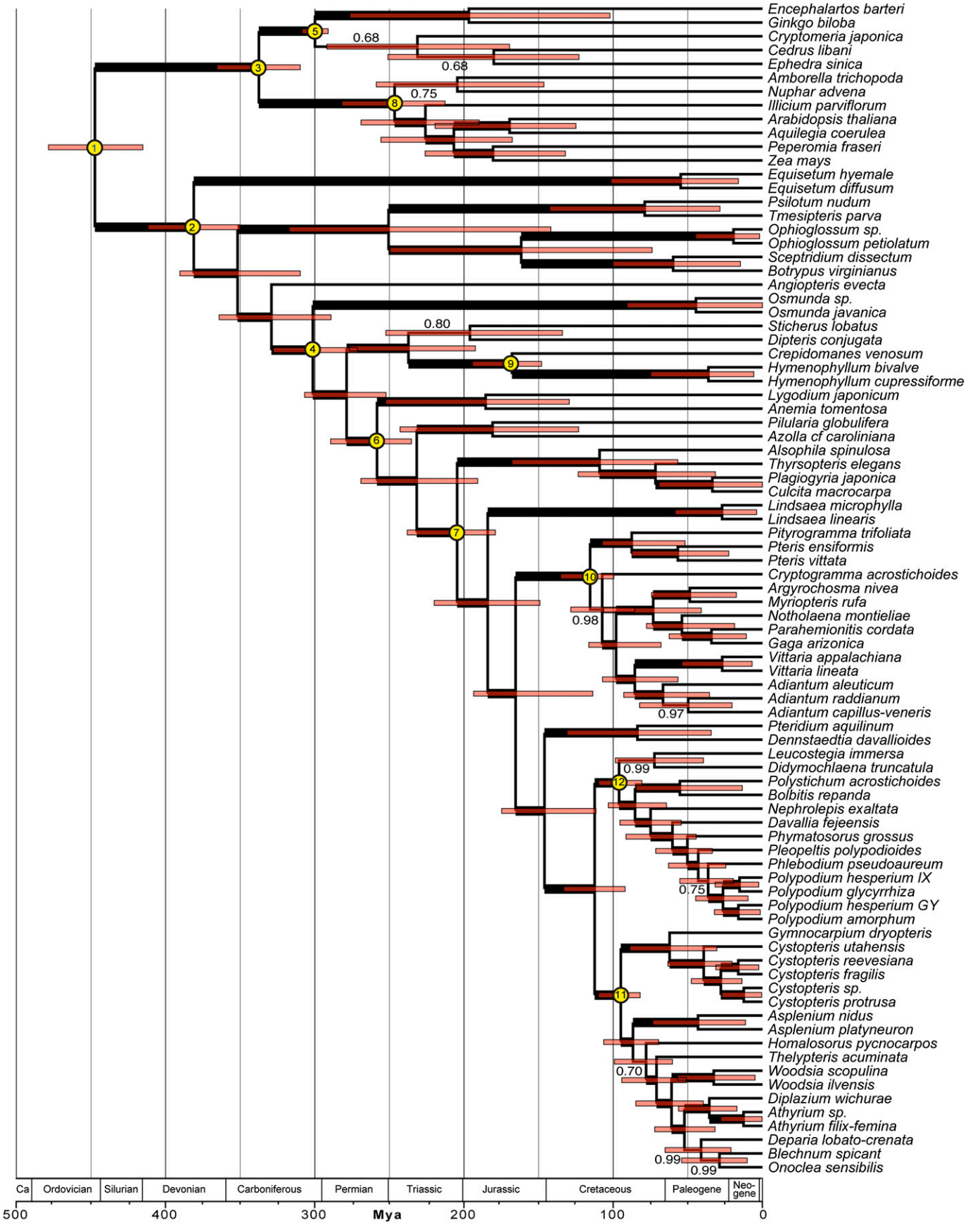
Our finding that Psilotales (whisk ferns) are sister to Ophioglossales (adder's-tongues, grape ferns, and their allies) confirms one of the most surprising results from early plastid-based studies (Pryer et al., 2001, 2004; Wikström and Pryer, 2005; Schuettpelz et al., 2006). Previous morphology-based hypotheses of land plant evolution tended to view Psilotales as the vestige of an ancient group entirely outside the ferns (Wagner, 1977; Stevenson and Loconte, 1996; Rothwell, 1999; but see Bierhorst, 1977). The perspective of Psilotales as "living fossils" with a primitive land plant morphology is contradicted not only by our results, but also by the presence of shared developmental and micromorphological characters between Psilotales and the rest of ferns (Bierhorst, 1977; Lugardon and Piquemal, 1993; Renzaglia et al., 2000; Renzaglia et al., 2001), and by other recent molecular phylogenies (Rai and Graham, 2010; Kuo et al., 2011; Lehtonen, 2011; Grewe et al., 2013; Wickett et al., 2014; Zhong et al., 2014; Knie et al., 2015). Instead of being an instance of morphological stasis in ferns (e.g., Phipps et al., 1998; Sundue and Rothfels, 2014), the dramatically reduced morphology of Psilotales is a prime example of secondary simplification (Bierhorst, 1977).

Determining the sister group to leptosporangiate ferns has been a formidable challenge. Earlier molecular studies consistently showed leptosporangiates to be embedded within eusporangiate ferns, but were unable to determine with confidence which lineage among these was their closest relative (Pryer et al., 2001, 2004; Wikström and Pryer, 2005; Schuettpelz et al., 2006; Qiu et al., 2007; Lehtonen, 2011; Grewe et al., 2013). Here, we find strong support for the marattioid ferns as sister to leptosporangiates (Fig. 1). This result contradicts the conclusions of Wickett et al. (2014) who found strong support for marattioids as sister to the Psilotidae; however, they had a very small fern taxon sample. Our study agrees with some studies based on plastid sequence data (Rai and Graham, 2010; Kuo et al., 2011; Grewe et al., 2013), with patterns of mitochondrial intron loss (Wikström and Pryer, 2005), with some traditional interpretations of morphological evolution (e.g., Kenrick and Crane, 1997), and with a recent analysis of four mitochondrial and five plastid loci (Knie et al., 2015).

Within leptosporangiate ferns, our results generally corroborate earlier studies as to the composition of the seven leptosporangiate fern orders (sensu Smith et al., 2006) and their relationships to one another (Pryer et al., 2001, 2004; Schuettpelz et al., 2006; Schuettpelz and Pryer, 2007; Pryer et al., 2008; Rai and Graham, 2010; Kuo et al., 2011; Lehtonen, 2011). As with the majority of previous studies, we show a lack of support for the relationships immediately following the divergence of Osmundales. This phylogenetic uncertainty—whether Gleicheniales, Hymenophyllales, or a combined clade of the two is sister to the remaining leptosporangiates—probably reflects our relative lack of data for these particular taxa. Our taxon sample does not include Matoniaceae, an important Gleicheniales lineage, and our Hymenophyllales and Gleicheniales transcriptomes unfortunately have much missing data (Appendix S3—see Supplemental Data with the online version of this article). Only two studies thus far found strong support among these branches (Rai and Graham, 2010; Lehtonen, 2011); both inferred Hymenophyllales sister to Gleicheniales plus the remaining leptosporangiates. Additional nuclear data may help to corroborate this hypothesis.

Our within-order taxon sampling is sparse, limiting our ability to resolve relationships at finer scales. Nevertheless, we do find novel support for *Thyrsopteris* (a monotypic genus endemic to the Juan Fernández Islands) as sister to the Culcitaceae + Plagiogyriaceae clade, rather than with the core tree ferns (sensu Korall et al., 2006a). This relationship was resolved by earlier molecular studies (Korall et al., 2006a; Schuettpelz and Pryer, 2007), but without support, and contradicts the historical tendency to place *Thyrsopteris* in Dicksoniaceae (a core tree fern family). The alliance of *Thyrsopteris* with the morphologically dissimilar Culcitaceae and Plagiogyriaceae further emphasizes the complex patterns of morphological evolution within the Cyatheales radiation (Korall et al., 2006a; Smith et al., 2006).

FIGURE 2 Phylogenetic chronogram obtained from Bayesian inference. Thickest branches indicate an estimated posterior probability of 1.0; posterior probability of all other branches is provided in the figure (either above or below the branch). Calibrated nodes (corresponding to those listed in Appendix S2—see Supplemental Data with the online version of this article) are indicated with open circles. Geological timescale follows Walker and Geissman (2009): Ca, Cambrian.


Encephalartos barteri
Ginkgo biloba
Cryptomeria japonica
Cedrus libani
Ephedra sinica
Amborella trichopoda
Nuphar advena
Illicium parviflorum
Arabidopsis thaliana
Aquilegia coerulea
Peperomia fraseri
Zea mays
Equisetum hyemale
Equisetum diffusum
Psilotum nudum
Tmesipteris parva
Ophioglossum sp.
Ophioglossum petiolatum
Sceptridium dissectum
Botrypus virginianus
Angiopteris evecta
Osmunda sp.
Osmunda javanica
Sticherus lobatus
Dipteris conjugata
Crepidomanes venosum
Hymenophyllum bivalve
Hymenophyllum cupressiforme
Lygodium japonicum
Anemia tomentosa
Pilularia globulifera
Azolla cf caroliniana
Alsophila spinulosa
Thyrsopteris elegans
Plagiogyria japonica
Culcita macrocarpa
Lindsaea microphylla
Lindsaea linearis
Pityrogramma trifoliata
Pteris ensiformis
Pteris vittata
Cryptogramma acrostichoides
Argyrochosma nivea
Myriopteris rufa
Notholaena montieliae
Parahemionitis cordata
Gaga arizonica
Vittaria appalachiana
Vittaria lineata
Adiantum aleuticum
Adiantum raddianum
Adiantum capillus-veneris
Pteridium aquilinum
Dennstaedtia davallioides
Leucostegia immersa
Didymochlaena truncatula
Polystichum acrostichoides
Bolbitis repanda
Nephrolepis exaltata
Davallia fejeensis
Phymatosorus grossus
Pleopeltis polypodioides
Phlebodium pseudoaureum
Polypodium hesperium IX
Polypodium glycyrrhiza
Polypodium hesperium GY
Polypodium amorphum
Gymnocarpium dryopteris
Cystopteris utahensis
Cystopteris reevesiana
Cystopteris fragilis
Cystopteris sp.
Cystopteris protrusa
Asplenium nidus
Asplenium platyneuron
Homalosorus pycnocarpos
Thelypteris acuminata
Woodsia scopulina
Woodsia ilvensis
Diplazium wichurae
Athyrium sp.
Athyrium filix-femina
Deparia lobato-crenata
Blechnum spicant
Onoclea sensibilis
1
2
3
4
5
6
7
8
9
10
11
12
0.68
0.68
0.75
0.80
0.98
0.97
0.99
0.75
0.70
0.99
0.99
Ca
Ordovician
Silurian
Devonian
Carboniferous
Permian
Triassic
Jurassic
Cretaceous
Paleogene
Neo-gene
500
400
300
Mya
200
100
0

Also noteworthy is the position of Dennstaedtiaceae as sister to the eupolypods (Fig. 1). The eupolypod clade contains more than two-thirds of extant fern species, and determining its sister group has been difficult. Earlier studies typically resolved Pteridaceae in that position, but without support (Pryer et al., 2004; Schuettpelz et al., 2006; Schuettpelz and Pryer, 2007; Kuo et al., 2011; Lehtonen, 2011; Rothfels et al., 2013). The only previous study to support the Dennstaedtiaceae-eupolypods sister relationship is that of Qiu et al. (2007) (79% bootstrap support; a single Dennstaedtiaceae species included).

Finally, we find support for two recalcitrant relationships within the large and disparate family Pteridaceae. First, our analyses support a monophyletic *Adiantum*, corroborating the predominantly plastid-based results of Rothfels and Schuettpelz (2014) and Pryer et al. (in review). Despite the morphological cohesiveness of *Adiantum*, previous studies have struggled to find support for its monophyly with respect to the morphologically and ecologically highly dissimilar vittarioids (Schuettpelz et al., 2007; Lu et al., 2011; Lu et al., 2012). Only three *Adiantum* species are included in our study, but they include *A. raddianum* C.Presl, a member of the subclade that has often been resolved as sister to the vittarioids (e.g., Schuettpelz et al., 2007). In addition, we find support for the monophyly of the large genus *Pteris*. This relationship—specifically the monophyly of the *Pteris longifolia* L. clade (represented in our sample by *P. vittata* L.) + the bulk of *Pteris* (represented here by *P. ensiformis* Burm.f.)—has long resisted elucidation (Schuettpelz et al., 2007; Chao et al., 2014), and has only recently been supported by inferences from plastid data (Schuettpelz and Pryer, 2007; Zhang, L. et al., 2014).

Within a few genera (namely, *Cystopteris* and *Polypodium*), our taxon sampling includes several closely related species. Relationships among these species in our study should be interpreted with caution, because some are allopolyploids (Haufler and Windham, 1991; Rothfels et al., 2014; Sigel et al., 2014a, b), and transcript assemblies of allopolyploids may be chimeras of homeologs inherited from different diploid ancestors, potentially compounded by our sequence-merging methodology. However, most of the allopolyploids are isolated representatives of larger clades (e.g., *Gymnocarpium dryopteris* (L.) Newman; Pryer and Haufler, 1993; Rothfels et al., 2014) and the fact that there might be chimeras among homeologs within such taxa should not affect tree inference. However, within *Cystopteris* and *Polypodium*, this methodological issue could mislead inference of relationships.

***Lineage-specific rate heterogeneity*—**In both seed plants and ferns, we infer generally increased rates of evolution (longer root-to-tip branch lengths) in clades where we have more species sampled (i.e., moving from top to bottom in Fig. 1). In seed plants, this pattern is largely due to the longer branches in angiosperms compared with gymnosperms, although within the angiosperms the eudicots, monocots, and magnoliids also have faster rates than the ANA-grade taxa (*Amborella* (Amborellaceae), *Nuphar* (Nymphaeaceae), and *Illicium* (Schisandraceae or Illiciaceae)). Similarly, in ferns the pattern is largely due to elevated rates in Polypodiales, especially in comparison to Osmundales, Marattiales, Hymenophyllales, and Gleicheniales (Fig. 1). Given that our taxon sampling is loosely correlated with extant species richness, there are at least three potential explanations for this pattern. First, it might be entirely artifactual, i.e., densely sampled areas of the tree result in long branches being divided into more, smaller branches, providing the substitution models with greater power to uncover otherwise hidden reverse substitutions (the "node density effect"; Venditti et al., 2006). A second possible explanation is that speciation events drive bursts of molecular evolution (the "punctuated evolution" model; Barraclough and Savolainen, 2001; Pagel et al., 2006), as we might expect if speciation tends to occur in small isolated populations. However, this explanation would require that the total number of speciation events in the history of a lineage (including those that resulted in lineages that subsequently went extinct) is correlated with extant species richness, which seems unlikely; many currently species-poor fern lineages were formerly species-rich (Rothwell, 1987). Finally, the causation could be in the opposite direction, i.e., taxa with fast rates of evolution might diversify at greater rates (Lancaster, 2010; Lanfear et al., 2010), either because mutation rates increase the rate of both substitution and diversification, or because substitution and diversification rates are both correlated with another factor, for example, generation time (Bromham et al., 1996; Barraclough and Savolainen, 2001; Lanfear et al., 2013; Bromham et al., 2015).

Within the ferns, there are also strong finer-scaled patterns of substitution rate heterogeneity, which largely agree with previous studies: (1) Marattiales are slow (Soltis et al., 2002); (2) within Hymenophyllales, the trichomanoid genera are faster than *Hymenophyllum* (Schuettpelz and Pryer, 2006); (3) there is an apparent slow-down at the base of Cyatheales (Korall et al., 2010; Zhong et al., 2014); and (4) accelerations are evident at the base of Aspleniaceae and the vittarioids (Rothfels et al., 2012a; Rothfels and Schuettpelz, 2014). Of the examples previously reported in the literature, the only one not also apparent in our data is the within-*Equisetum* rate difference between subgenus *Hippochaete* (represented here by *E. hyemale* L.) and subgenus *Equisetum* (Des Marais et al., 2003). All but one (the vittarioid increase; Rothfels and Schuettpelz, 2014) of these earlier results were based entirely on plastid data, and thus could be due to compartment-specific processes (e.g., a polymerase mutation: Parkinson et al., 2005). Our results suggest instead that these are multigenome-wide phenomena, potentially driven by life history traits (Smith and Donoghue, 2008; Korall et al., 2010; Lanfear et al., 2013) or by genome-wide mutation rate differences (Rothfels and Schuettpelz, 2014; Bromham et al., 2015). In addition, our results highlight additional fern lineages warranting further investigation regarding rates: (1) *Ophioglossum* appears to be much faster than the other members of Ophioglossales; (2) Osmundaceae are very slow (and are reported to also have slow rates of morphological and karyotypic evolution; Phipps et al., 1998; Bomfleur et al., 2014; Schneider et al., 2015); and (3) within Polypodiales, there appears to be a slowdown along the branch leading to extant Dennstaedtiaceae (Fig. 1).

***Concatenated data, species trees, and the power of plastid data*—**Studies that incorporate "deep-node sampling" like ours (e.g., those of Qiu et al., 2007; Rothfels et al., 2012a; Knie et al., 2015) might be expected to be largely immune to deep coalescence problems, i.e., extinction and sparse taxon sampling will remove the short internodes that are prone to incomplete lineage sorting (Degnan and Rosenberg, 2009). However, comparing the results of our concatenated-data and species-tree analyses (see Fig. 1), we find two patterns that warrant discussion. First, as has been previously reported (Meredith et al., 2011; Song et al., 2012; Folk and Freudenstein, 2014; Wickett et al., 2014), we find generally lower levels of support from our species-tree analyses than from their concatenated-data counterparts (see Results, and Fig. 1). This is likely due to our study design, which sought to maximize the total amount of data

available, rather than minimize the amount of missing data, with the consequence that for some loci the number of missing taxa may be considerable (Appendix S3; see Supplemental Data with the online version of this article). Many of the individual-locus gene trees thus have no information with which to resolve a subset of the relationships, which should add greater variance to the multilocus bootstrap, thereby reducing power. In contrast, each of the concatenated-dataset bootstrap replicates always has some data available for all taxa.

The second pattern involves a small subset of nodes—specifically those nodes that earlier studies, using much smaller amounts of plastid data, were able to infer with strong support, yet which our larger concatenated dataset only weakly supports (Table 6). For these nodes, our species-tree support is *higher* than expected. For example, our concatenated-data analyses weakly support (67% bootstrap support) the pteridoid ferns (sensu Schuettpelz et al., 2007; Rothfels, 2008) as sister to the rest of Pteridaceae (Fig. 1), whereas earlier plastid-based studies strongly supported the cryptogrammoids in that position (Schuettpelz et al., 2007; Kuo et al., 2011). However, 23% of our bootstrap species trees do place *Cryptogramma* sister to the rest of Pteridaceae, compared to only 0.2% of our concatenated-data trees that do so (Table 6). Plastid data coalesce four times more quickly than nuclear data (Moore, 1995) and species-tree analyses are based on the multispecies coalescent model, rendering both of these approaches less sensitive to incomplete lineage sorting (ILS). The fact that both outperform (relatively) our concatenated nuclear data on the same restricted set of nodes, all of which involve very short branches of the sort potentially vulnerable to ILS, suggests that ILS may be misleading our nuclear-sequence based concatenated-data analyses.

***The timescale of fern evolution*—**Our divergence time analyses provide the first evolutionary timescale for a dense sample spanning both eusporangiate and leptosporangiate ferns. Supporting the conclusions of earlier studies (Pryer et al., 2004; Schneider et al., 2004b; Pryer and Schuettpelz, 2009; Schuettpelz and Pryer, 2009), these analyses confirm that ferns are an ancient group—the earliest divergence among ancestors of extant ferns predates the divergence of the angiosperm ancestor from that of the extant gymnosperms, and the crown age of the leptosporangiate ferns is approximately the same as that of extant gymnosperms (Fig. 2, Appendix S4; see Supplemental Data with the online version of this article). Within the ferns, there are a number of smaller groups (including Psilotidae, a possible Gleicheniaceae + Hymenophyllaceae clade, and the Schizaeales + core-leptosporangiates clade) whose crown ages are approximately the same as that of the angiosperms (Fig. 2, Appendix S4; see Supplemental Data with the online version of this article). However, in agreement with other time-calibrated fern phylogenies (Schneider et al., 2004b; Schuettpelz and Pryer, 2009) we find that the bulk of extant fern diversity—especially in the species-rich Polypodiales—originated relatively recently, since the Cretaceous.

***Curated phylogenomics: Model selection and moderate data*—** With the rapid decline in sequencing costs, there has been a strong increase in the quantity of sequence data available for phylogenetic questions, such that "phylogenomic" studies are becoming common (e.g., Dunn et al., 2008; Telford et al., 2014; Wickett et al., 2014). These datasets are often huge, reducing stochastic error, but at the potential expense of an increased risk of cryptic systematic error due to model mis-specification (Philippe et al., 2011). Our complementary "curated phylogenomics" approach (e.g., Qiu et al., 2007; Parfrey et al., 2010; Rothfels et al., 2012a), which used considerably less data and focused on minimizing systematic error, was successful in finding strong support for the majority of our target relationships; at least for these groups, moderate amounts of data are sufficient to overcome stochastic error (for a similar conclusion, see Knie et al., 2015).

There is still a risk, even under a curated phylogenomics approach, of systematic error caused by model violations, but the risk is reduced compared to larger datasets, and can be at least partially alleviated through model selection. Poorly fitting models—even those that are relatively parameter rich—can result in different inferences of topology and support, in comparison to better-fitting alternatives (e.g., Rothfels et al., 2013). For our data, the best fitting of the models we investigated was a codon model with four dn/ds rate categories (omega parameters), applied to the unpartitioned data (Model 7; see Tables 4 and 5); this model dramatically outperformed the best nucleotide model (Table 5). Our curated phylogenomics approach made it computationally feasible to run the best-fitting model, rather than obliging us to limit ourselves to nucleotide or amino acid models, or to simpler codon models (e.g., fewer omega parameters).

CONCLUSIONS

Our study is the first to use multiple nuclear markers for a broad taxon sample spanning all major lineages of extant ferns. Our results show a reassuring consistency with earlier conclusions derived from plastid sequence data, including extensive agreement on both the pattern and timeline of fern diversification. Our greatly increased taxon and character sample in comparison to most previous studies allowed us to obtain increased support for historically recalcitrant relationships, most notably those among the eusporangiate lineages, and specifically the position of Equisetales as the sister group to the rest of ferns. For a small number of nodes, our concatenated-data analyses weakly supported relationships that have been strongly supported previously by much

TABLE 6. Differences between species-tree and concatenated-data support values.

Clade	Bootstrap Support		p*
	Species Tree	Concat. Data	
Cyatheales + Polypodiales	56.9%	64.7%	0.0685
non*Cryptogramma* Pteridaceae	**23.1%**	0.2%	<0.0001
non*Didymochlaena* eupolypods I	**25.0%**	4.1%	<0.0001
Homalosorus + *Asplenium*	4.4%	**28.5%**	<0.0001
Athyriaceae	**7.5%**	1.5%	<0.0001
Thelypteridaceae + Woodsiaceae + Blechnaceae + Onocleaceae + Athyriaceae	**68.1%**	53.5%	0.0007

Notes: The clades listed are those that our concatenated-data analyses did not strongly support, but which have been previously supported by plastid data. Bold values indicate significantly higher support; in four of the five cases, the significantly higher support is from the species-tree rather than gene-tree analyses. *Significance values were calculated with two-tailed tests using the "prop.test" command in R (R Development Core Team, 2011), with number of bootstrap replicates (160 and 1000 for the species and gene tree inferences, respectively) counted as the number of trials.

smaller amounts of plastid data. These relationships involve very short internodes, and tend to be more strongly supported by our species-tree than by our concatenated-data analyses (Table 4), despite the generally reduced power of the latter. This pattern suggests that our concatenated-data support levels may be reduced by gene-tree conflict caused by incomplete lineage sorting, and that the strong support derived from plastid data is due to the reduced sensitivity of such data to deep coalescence.

These results highlight the importance of choosing datasets (in terms of taxon and character coverage) suited to the particular goals of a study. In our case, huge quantities of character data were not necessary to resolve many relationships of interest. Instead, our focus on taxon sampling, character homology assessments (sequence alignment and gene orthology determination), and rigorous model testing was sufficient and effective for resolving fern phylogeny. This revised phylogeny will spur reconsideration of patterns of land-plant evolution—especially with respect to the divergences among Equisetales, Psilotales, and "ferns" as classically construed, and the evolution of the leptosporangiate ferns from an ancestor more closely related to Marattiales than to any other group of extant eusporangiate species. In addition, our results provide a reinvigorated foundation for future investigations of evolution within ferns and provide a critical point of comparison for understanding evolutionary processes such as gene-family evolution and paleopolyploidy events in seed plants, and across land plants more broadly.

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APPENDIX 1

Voucher table. Accessions included in this study, listed alphabetically by order and then species, with outgroup taxa at the end. The four-letter 1KP accession code precedes the species name. GenBank numbers are listed in the following order: ApPEFP_A_C; ApPEFP_B; COP9; CRY1; CRY2; CRY3; CRY4; CRY5; DUF1077; Gclev; HMR; Hsp40; IBR3; IGPD; MCD1; NDUFS6; SEF; SQD1; TPLATE; det1; gapC; gapCpLg; gapCpSh; pgiC; transducin. Regions without data are indicated by a dash ("–").

Cyatheales GANB *Alsophila spinulosa* (Wall. ex Hook.) R.M. Tryon. Chen et al. 20081210004 (SZG) KR825471; KR825420; KR826320; KR826446; KR825548; KR826255; KR825604; KR826903; KR826025; KR825275; KR826748; KR825658; KR826597; KR826674; KR825726; KR825349; KR825952; KR826521; KR825875; KR825800;–; KR826954; KR826176; KR826824; KR826103. PNZO *Culcita macrocarpa* C.Presl. RBGE 19922977 F (E) KR825486; KR825427; KR826334; KR826461; KR825558; KR826270; KR825616; KR826911; KR826041; KR825290; KR826763; KR825670; KR826611; KR826689; KR825740; KR825363; KR825968; KR826537; KR825891; KR825814; KR826401; KR826961; KR826192; KR826840; KR826118. UWOD *Plagiogyria japonica* Nakai. RBGE 20061485 A (E) KR825528; KR825456; KR826371; KR826501; KR825587; KR826301; KR825642; KR826940; KR826083; KR825328; KR826804; KR825708; KR826653; KR826727; KR825781; KR825399; KR826005; KR826576; KR825932; KR825854;–; KR826986; KR826234; KR826882; KR826156. EWXK *Thyrsopteris elegans* Kunze. RBGE 19925041A (E: E00183382) KR825540; KR825466; KR826384; KR826513; KR825597; KR826312; KR825651; KR826948; KR826096; KR825341; KR826816; KR825719; KR826666; KR826740; KR825793; KR825412; KR826018; KR826589; KR825944; KR825867;–; KR826996; KR826247; KR826895; KR826168. **Equisetales** CAPN *Equisetum diffusum* D.Don. Stevenson 1352/77 (NY) KR825500; KR825439; KR826347; KR826475; KR825570; KR826277; KR825627;–; KR826055; KR825302; KR826777; KR825683; KR826625; KR826703; KR825754; KR825376;–; KR826551; KR825904; KR825827;–;–; KR826206; KR826854; KR826131. JVSZ *Equisetum hyemale* L. Rothfels 4137 (DUKE) KR825501; KR825440; KR826348; KR826476; KR825571; KR826278; KR825628;–; KR826056; KR825303; KR826778; KR825684; KR826626; KR826704; KR825755; KR825377; KR825980; KR826552; KR825905; KR825828;–;–; KR826207; KR826855; KR826132. **Gleicheniales** MEKP *Dipteris conjugata* Reinw. RBGE 20021886 A (E: E00269760) KR825497; KR825438; KR826345; KR826472; KR825569; KR826275;–; KR826922; KR826052; KR825301; KR826774; KR825681; KR826622; KR826700; KR825751; KR825374; KR825978; KR826548; KR825901; KR825825;–;–; KR826203; KR826851; KR826129. XDVM *Sticherus lobatus* N.A.Wakef. Burge 1380 (NSW)–;–; KR826382;–;–;–;–;–; KR826094; KR825339;–;–; KR826664; KR826738;–; KR825410; KR826016; KR826587; KR825942; KR825865;–;–; KR826245; KR826893;–. **Hymenophyllales** TWFZ *Crepidomanes venosum* (R.Br.) Bostock. Burge 1382 (NSW)–;–; KR826332;–;–; KR826267;–;–; KR826038; KR825287;–;–;–;–;–; KR825361; KR825965; KR826534; KR825888;–;–;–; KR826189; KR826837;–. QIAD *Hymenophyllum bivalve* (G.Forst.) Sw. Burge 1356 (NSW) KR825506;–; KR826353; KR826480;–; KR826283;–;–; KR826061; KR825308; KR826783; KR825688; KR826631; KR826708; KR825760; KR825381; KR825984; KR826557; KR825910; KR825833;–;–; KR826212; KR826860;–. TRPJ *Hymenophyllum cupressiforme* Labill. Burge 1383 (NSW) KR825507;–;–;–;–;–;–;–;–; KR826062;–;–; KR825689; KR826632;–;–; KR825382;–; KR826558; KR825911;–;–;–; KR826213; KR826861;–. **Marattiales** NHCM *Angiopteris evecta* (G.Forst.) Hoffm. Stevenson 1296/78 (NY) KR825473;–;–; KR826448;–; KR826257; KR825606;–; KR826027; KR825277; KR826750;–; KR826599; KR826676; KR825728; KR825351; KR825954; KR826523; KR825877; KR825802;–;–; KR826178; KR826826; KR826105. **Ophioglossales** BEGM *Botrypus virginianus* (L.) Michx. Rothfels 4159 (DUKE) KR825482;–; KR826330; KR826457; KR825556; KR826265; KR825613;–; KR826036; KR825286; KR826759; KR825667; KR826607; KR826685; KR825737; KR825360; KR825963; KR826532; KR825886; KR825811;–;–; KR826187; KR826835; KR826114. QHVS *Ophioglossum petiolatum* Hook. Graham 2014-01 (UBC) KR825519;–; KR826363; KR826492;–; KR826294;–;–; KR826074; KR825319; KR826795; KR825700; KR826644; KR826720; KR825772; KR825391; KR825996;–; KR825923; KR825845; KR826422;–; KR826225; KR826873; KR826147. WTJG *Ophioglossum petiolatum* Hook. Deyholos 2013-09 (ALTA) KR825518;–; KR826362; KR826491;–; KR826293;–;–; KR826073; KR825318; KR826794; KR825699; KR826643; KR826719; KR825771;–; KR825995;–; KR825922; KR825844;–;–; KR826224; KR826872; KR826146. EEAQ *Sceptridium dissectum* (Spreng.) Lyon. Rothfels 4102 & 4129 (DUKE) KR825538;–; KR826381; KR826511; KR825596; KR826311; KR825650;–; KR826093; KR825338; KR826814; KR825717; KR826663; KR826737; KR825791; KR825409; KR826015; KR826586; KR825941; KR825864; KR826439;–; KR826244; KR826892; KR826166. **Osmundales** UOMY *Osmunda japonica* Thunb. Stevenson 2080/93 (NY) KR825521;–; KR826365; KR826494;–; KR826296;–; KR826935; KR826076; KR825321; KR826797;–; KR826646; KR826721; KR825774; KR825392; KR825998; KR826569; KR825925; KR825847; KR826424;–; KR826227; KR826875; KR826149. VIBO *Osmunda javanica* Blume. RBGE 19933718 A (E) KR825520;–; KR826364; KR826493;–; KR826295;–; KR826934; KR826075; KR825320; KR826796; KR825701; KR826645;–; KR825773;–; KR825997;–; KR825924; KR825846; KR826423;–; KR826226; KR826874; KR826148. **Polypodiales** WCLG *Adiantum aleuticum* (Rupr.) C.A.Paris. Rothfels 4090 (DUKE) KR825469; KR825418; KR826318; KR826444; KR825546; KR826253; KR825602; KR826901; KR826023; KR825273; KR826746; KR825656; KR826595; KR826672; KR825724; KR825347; KR825950; KR826519; KR825873; KR825798; KR826390; KR826953; KR826174; KR826822; KR826101. BMJR *Adiantum raddianum* C.Presl. Deyholos 2012-17 (ALTA) KR825470; KR825419; KR826319; KR826445; KR825547; KR826254; KR825603; KR826902; KR826024; KR825274; KR826747; KR825657; KR826596; KR826673; KR825725; KR825348; KR825951; KR826520; KR825874; KR825799; KR826391;–; KR826175; KR826823; KR826102. XDDT *Argyrochosma nivea* (Poir.) Windham. Reeb 26-V-02/12 (DUKE) KR825474;–; KR826322; KR826449; KR825550; KR826258; KR825607; KR826904; KR826028; KR825278; KR826751; KR825660; KR826600; KR826677; KR825729; KR825352; KR825955; KR826524; KR825878; KR825803; KR826392; KR826955; KR826179; KR826827; KR826106. PSKY *Asplenium nidus* L. DeGironimo 1396/76-A (NY) KR825475;–; KR826323; KR826450;–;–;–; KR826905; KR826029; KR825279; KR826752; KR825661;–; KR826678; KR825730; KR825353; KR825956; KR826525; KR825879; KR825804;–;–; KR826180; KR826828; KR826107. KJZG *Asplenium platyneuron* (L.) Britton, Sterns & Poggenb. Stewart/Ell80 1051 (UTIA) KR825476; KR825421; KR826324; KR826451; KR825551; KR826259; KR825608; KR826906; KR826030; KR825280; KR826753; KR825662; KR826601; KR826679; KR825731; KR825354; KR825957; KR826526; KR825880; KR825805; KR826393;–; KR826181; KR826829; KR826108. AFPO *Athyrium filix-femina* (L.) Roth. Stevenson 941/89 (NY) KR825478; KR825423; KR826326; KR826453; KR825553; KR826261; KR825610; KR826908; KR826032; KR825282; KR826755; KR825664; KR826603; KR826681; KR825733; KR825356; KR825959; KR826528; KR825882; KR825807; KR826395; KR826957; KR826183; KR826831; KR826110. URCP *Athyrium filix-femina* (L.) Roth. DeGironimo 941/89-C (NY) KR825477; KR825422; KR826325; KR826452; KR825552; KR826260; KR825609; KR826907; KR826031; KR825281; KR826754; KR825663; KR826602; KR826680; KR825732; KR825355; KR825958; KR826527; KR825881; KR825806; KR826394; KR826956; KR826182; KR826830; KR826109. VITX *Blechnum spicant* (L.) Sm. 1746/2008-A NYBG KR825480; KR825424; KR826328; KR826455; KR825554; KR826263; KR825611; KR826909; KR826034; KR825284; KR826757; KR825666; KR826605; KR826683; KR825735; KR825358; KR825961; KR826530; KR825884; KR825809;–; KR826958; KR826185; KR826833; KR826112. JBLI *Bolbitis repanda* Schott. Stevenson 1474/96 (NY) KR825481; KR825425; KR826329; KR826456; KR825555; KR826264; KR825612; KR826910; KR826035; KR825285; KR826758;–; KR826606; KR826684; KR825736; KR825359; KR825962; KR826531; KR825885; KR825810; KR826397; KR826959; KR826186; KR826834; KR826113. WQML *Cryptogramma acrostichoides* R.Br. Rothfels 4060.2 (DUKE) KR825484; KR825426; KR826333; KR826459; KR825557; KR826268; KR825614;–; KR826039; KR825288; KR826761; KR825669; KR826609; KR826687; KR825739;–; KR825966; KR826535; KR825889; KR825812; KR826399; KR826960; KR826190; KR826838; KR826116. LHLE *Cystopteris fragilis* (L.) Bernh. Sigel 2011-49 (DUKE) KR825487; KR825428; KR826335; KR826462; KR825559;–; KR825617; KR826912; KR826042; KR825291; KR826764; KR825671; KR826612; KR826690; KR825741; KR825364; KR825969; KR826538;

KR825892; KR825815; KR826402; KR826962; KR826193; KR826841; KR826119. YOWV *Cystopteris protrusa* (Weath.) Blasdell. Rothfels 3842 (DUKE) KR825488; KR825429; KR826336; KR826463; KR825560;–; KR825618; KR826913; KR826043; KR825292; KR826765; KR825672; KR826613; KR826691; KR825742; KR825365; KR825970; KR826539; KR825893; KR825816; KR826403; KR826963; KR826194; KR826842; KR826120. RICC *Cystopteris reevesiana* Lellinger. Li 1213 (DUKE) KR825489; KR825430; KR826337; KR826464; KR825561;–; KR825619; KR826914; KR826044; KR825293; KR826766; KR825673; KR826614; KR826692; KR825743; KR825366; KR825971; KR826540; KR825894; KR825817; KR826404; KR826964; KR826195; KR826843; KR826121. XXHP *Cystopteris sp.* Stewart/Ell80 1052 (UTIA) KR825490; KR825431; KR826338; KR826465; KR825562;–; KR825620; KR826915; KR826045; KR825294; KR826767; KR825674; KR826615; KR826693; KR825744; KR825367; KR825972; KR826541; KR825895; KR825818; KR826405; KR826965; KR826196; KR826844; KR826122. HNDZ *Cystopteris utahensis* Windham & Haufler. Rink 6566 (DUKE) KR825491; KR825432; KR826339; KR826466; KR825563;–; KR825621; KR826916; KR826046; KR825295; KR826768; KR825675; KR826616; KR826694; KR825745; KR825368; KR825973; KR826542; KR825896; KR825819; KR826406; KR826966; KR826197; KR826845; KR826123. OQWW *Davallia fejeensis* Hook. Stevenson 3330/78 (NY) KR825492; KR825433; KR826340; KR826467; KR825564; KR826271; KR825622; KR826917; KR826047; KR825296; KR826769; KR825676; KR826617; KR826695; KR825746; KR825369; KR825974; KR826543; KR825897; KR825820; KR826407; KR826967; KR826198; KR826846; KR826124. MTGC *Dennstaedtia davallioides* T.Moore. Burge s.n. ACC16132 (RBG) KR825493; KR825434; KR826341; KR826468; KR825565; KR826272; KR825623; KR826918; KR826048; KR825297; KR826770; KR825677; KR826618; KR826696; KR825747; KR825370; KR825975; KR826544; KR825898; KR825821; KR826408; KR826968; KR826199; KR826847; KR826125. FCHS *Deparia lobato-crenata* (Tagawa) M. Kato. Rothfels 4117 (DUKE) KR825494; KR825435; KR826342; KR826469; KR825566; KR826273; KR825624; KR826919; KR826049; KR825298; KR826771; KR825678; KR826619; KR826697; KR825748; KR825371; KR825976; KR826545; KR825899; KR825822;–; KR826969; KR826200; KR826848; KR826126. RFRB *Didymochlaena truncatula* (Sw.) J.Sm. RBG 20001061 (NSW) KR825495; KR825436; KR826343; KR826470; KR825567;–;–; KR826920; KR826050; KR825299; KR826772; KR825679; KR826620; KR826698; KR825749; KR825372;–; KR826546;–; KR825823;–; KR826970; KR826201; KR826849; KR826127. UFJN *Diplazium wichurae* (Mett.) Diels. Rothfels 4107 (DUKE) KR825496; KR825437; KR826344; KR826471; KR825568; KR826274; KR825625; KR826921; KR826051; KR825300; KR826773; KR825680; KR826621; KR826699; KR825750; KR825373; KR825977; KR826547; KR825900; KR825824; KR826409; KR826971; KR826202; KR826850; KR826128. DCDT *Gaga arizonica* (Maxon) Fay W.Li & Windham. Li 1290 (DUKE) KR825502; KR825441; KR826349; KR826477; KR825572; KR826279; KR825629; KR826923; KR826057; KR825304; KR826779; KR825685; KR826627;–; KR825756; KR825378; KR825981; KR826553; KR825906; KR825829; KR826412; KR826972; KR826208; KR826856; KR826133. HEGQ *Gymnocarpium dryopteris* (L.) Newman. Rothfels 4068 (DUKE) KR825504; KR825442; KR826351; KR826478; KR825573; KR826281; KR825630; KR826924; KR826059; KR825306; KR826781; KR825686; KR826629; KR826706; KR825758; KR825379; KR825982; KR826555; KR825908; KR825831;–; KR826973; KR826210; KR826858; KR826135. OCZL *Homalosorus pycnocarpos* (Spreng.) Pic. Serm. Rothfels 4119 (DUKE) KR825505; KR825443; KR826352; KR826479; KR825574; KR826282; KR825631; KR826925; KR826060; KR825307; KR826782; KR825687; KR826630; KR826707; KR825759; KR825380; KR825983; KR826556; KR825909; KR825832; KR826414; KR826974; KR826211; KR826859; KR826136. WGTU *Leucostegia immersa* C.Presl. RBGE 20040613 D (E: E0029474) KR825509; KR825444; KR826354; KR826482; KR825575; KR826284; KR825632; KR826926; KR826064; KR825309; KR826785; KR825691; KR826634; KR826710; KR825762; KR825383; KR825986; KR826560; KR825913; KR825835;–; KR826975; KR826215; KR826863; KR826137. NOKI *Lindsaea linearis* Sw. Burge 1381 (NSW) KR825510; KR825445; KR826355; KR826483; KR825576; KR826285; KR825633; KR826927; KR826065; KR825310; KR826786; KR825692; KR826635; KR826711; KR825763; KR825384; KR825987; KR826561; KR825914; KR825836; KR826416;–; KR826216; KR826864; KR826138. YIXP *Lindsaea microphylla* Sw. Burge 1377 (NSW) KR825511; KR825446; KR826356; KR826484; KR825577; KR826286; KR825634; KR826928; KR826066; KR825311; KR826787; KR825693; KR826636; KR826712; KR825764; KR825385; KR825988; KR826562; KR825915; KR825837;–; KR826976; KR826217; KR826865; KR826139. GSXD *Myriopteris rufa* Fée. Rothfels 3903 (DUKE) KR825513; KR825448; KR826358; KR826486; KR825579; KR826288; KR825635; KR826930; KR826068; KR825313; KR826789; KR825695; KR826638; KR826714; KR825766; KR825387; KR825990; KR826564; KR825917; KR825839; KR826417; KR826977; KR826219; KR826867; KR826141. NWWI *Nephrolepis exaltata* (L.) Schott. DeGironimo 214/94 (NY) KR825514; KR825449; KR826359; KR826487; KR825580; KR826289; KR825636; KR826931; KR826069; KR825314; KR826790; KR825696; KR826639; KR826715; KR825767; KR825388; KR825991; KR826565; KR825918; KR825840; KR826418; KR826978; KR826220; KR826868; KR826142. YCKE *Notholaena montieliae* Yatsk. & Arbel**á**ez. Rothfels 4098 (DUKE) KR825515; KR825450; KR826360; KR826488; KR825581; KR826290; KR825637; KR826932; KR826070; KR825315; KR826791; KR825697; KR826640; KR826716; KR825768; KR825389; KR825992; KR826566; KR825919; KR825841; KR826419; KR826979; KR826221; KR826869; KR826143. HTFH *Onoclea sensibilis* L. Stewart/Ell80 1056 (UTIA) KR825517; KR825451; KR826361; KR826490; KR825582; KR826292; KR825638; KR826933; KR826072; KR825317; KR826793; KR825698; KR826642; KR826718; KR825770; KR825390; KR825994; KR826568; KR825921; KR825843; KR826421; KR826980; KR826223; KR826871; KR826145. ZXJO *Parahemionitis cordata* (Hook. & Grev.) Fraser-Jenk. DeGironimo 3.13.11 (NY) KR825522;–; KR826366; KR826495; KR825583;–; KR825639; KR826936; KR826077; KR825322; KR826798; KR825702; KR826647;–; KR825775; KR825393; KR825999; KR826570; KR825926; KR825848; KR826425; KR826981; KR826228; KR826876; KR826150. ZQYU *Phlebodium pseudoaureum* (Cav.) Lellinger. Deyholos s.n. (DUKE) KR825524; KR825452; KR826367; KR826497; KR825584; KR826298; KR825640; KR826937; KR826079; KR825324; KR826800; KR825704; KR826649; KR826723; KR825777; KR825395; KR826001; KR826572; KR825928; KR825850; KR826427; KR826982; KR826230; KR826878; KR826152. ORJE *Phymatosorus grossus* (Langsd. & Fisch.) Brownlie. Shaw s.n. KR825525; KR825453; KR826368; KR826498; KR825585;–;–; KR826938; KR826080; KR825325; KR826801; KR825705; KR826650; KR826724; KR825778; KR825396; KR826002; KR826573; KR825929; KR825851; KR826428; KR826983; KR826231; KR826879; KR826153. UJTT *Pityrogramma trifoliata* (L.) R.M.Tryon. Rothfels 4109 (DUKE) KR825527; KR825455; KR826370; KR826500; KR825586; KR826300; KR825641; KR826939; KR826082; KR825327; KR826803; KR825707; KR826652; KR826726; KR825780; KR825398; KR826004; KR826575; KR825931; KR825853; KR826430; KR826985; KR826233; KR826881; KR826155. UJWU *Pleopeltis polypodioides* (L.) E.G.Andrews & Windham. Stewart/Ell80 1053 (UTIA) KR825529; KR825457; KR826372; KR826502; KR825588; KR826302; KR825643;–; KR826084; KR825329; KR826805; KR825709; KR826654; KR826728; KR825782; KR825400; KR826006; KR826577;–; KR825855; KR826431; KR826987; KR826235; KR826883; KR826157. YLJA *Polypodium amorphum* Suksd. Sigel 2010-125 (DUKE) KR825530; KR825458; KR826373; KR826503; KR825589; KR826303; KR825644; KR826941; KR826085; KR825330; KR826806; KR825710; KR826655; KR826729; KR825783; KR825401; KR826007; KR826578; KR825933; KR825856; KR826432; KR826988; KR826236; KR826884; KR826158. CJNT *Polypodium glycyrrhiza* D.C.Eaton. Rothfels 4086 (DUKE) KR825531; KR825459; KR826374; KR826504; KR825590; KR826304; KR825645; KR826942; KR826086; KR825331; KR826807; KR825711; KR826656; KR826730; KR825784; KR825402; KR826008; KR826579; KR825934; KR825857; KR826433; KR826989; KR826237; KR826885; KR826159. GYFU *Polypodium hesperium* Maxon. Sigel 2011-04A (DUKE) KR825533; KR825461; KR826376; KR826506; KR825592; KR826306; KR825647; KR826944; KR826088; KR825333; KR826809; KR825713; KR826658; KR826732; KR825786; KR825404; KR826010; KR826581; KR825936; KR825859; KR826435; KR826991; KR826239; KR826887; KR826161. IXLH *Polypodium hesperium* Maxon. Rothfels 3889 (DUKE) KR825532; KR825460; KR826375; KR826505; KR825591; KR826305; KR825646; KR826943; KR826087; KR825332; KR826808; KR825712; KR826657; KR826731; KR825785; KR825403; KR826009; KR826580; KR825935; KR825858; KR826434; KR826990; KR826238; KR826886; KR826160. FQGQ *Polystichum acrostichoides* (Michx.) Schott. Rothfels 4160 (DUKE) KR825534; KR825462; KR826377; KR826507; KR825593; KR826307; KR825648; KR826945; KR826089; KR825334; KR826810;–; KR826659; KR826733; KR825787; KR825405; KR826011; KR826582; KR825937; KR825860; KR826436; KR826992; KR826240; KR826888; KR826162. FLTD *Pteris ensiformis* Burm. f. Soltis & Miles 3001 (FLAS) KR825536; KR825463; KR826379; KR826509; KR825594; KR826309;–;–; KR826091; KR825336;

KR826812; KR825715; KR826661; KR826735; KR825789; KR825407; KR826013; KR826584; KR825939; KR825862;–; KR826993; KR826242; KR826890; KR826164. POPJ *Pteris vittata* L. Stewart/Ell80 1055 (UTIA) KR825537; KR825464; KR826380; KR826510; KR825595; KR826310; KR825649; KR826946; KR826092; KR825337; KR826813; KR825716; KR826662; KR826736; KR825790; KR825408; KR826014; KR826585; KR825940; KR825863; KR826438; KR826994; KR826243; KR826891; KR826165. MROH *Thelypteris acuminata* (Houtt.) C.V.Morton. DeGironimo 477/77-A (NY) KR825539; KR825465; KR826383; KR826512;–;–;–; KR826947; KR826095; KR825340; KR826815; KR825718; KR826665; KR826739; KR825792; KR825411; KR826017; KR826588; KR825943; KR825866; KR826440; KR826995; KR826246; KR826894; KR826167. NDUV *Vittaria appalachiana* Farrar & Mickel. Li 1568 (DUKE) KR825542;–; KR826386; KR826515; KR825598; KR826314; KR825652; KR826949;–; KR825343; KR826818;–; KR826668; KR826742;–; KR825414;–; KR826591; KR825946; KR825869;–;–; KR826249; KR826897; KR826170. SKYV *Vittaria lineata* (L.) Sm. Rothfels 4120 (DUKE) KR825543;–; KR826387; KR826516; KR825599; KR826315; KR825653; KR826950; KR826098; KR825344; KR826819; KR825721; KR826669; KR826743; KR825795; KR825415; KR826020; KR826592; KR825947; KR825870;–;–; KR826250; KR826898; KR826171. YQEC *Woodsia ilvensis* (L.) R. Br. Larsson 79 (UPS) KR825544; KR825467; KR826388; KR826517; KR825600; KR826316; KR825654; KR826951; KR826099; KR825345; KR826820; KR825722; KR826670; KR826744; KR825796; KR825416; KR826021; KR826593; KR825948; KR825871; KR826442; KR826997; KR826251; KR826899; KR826172. YJJY *Woodsia scopulina* D.C.Eaton. Sigel 2011-42 (DUKE) KR825545; KR825468; KR826389; KR826518; KR825601; KR826317; KR825655; KR826952; KR826100; KR825346; KR826821; KR825723; KR826671; KR826745; KR825797; KR825417; KR826022; KR826594; KR825949; KR825872; KR826443; KR826998; KR826252; KR826900; KR826173. **Psilotales** QVMR *Psilotum nudum* (L.) P.Beauv. Stevenson 696/90 (NY) KR825535;–; KR826378; KR826508;–; KR826308;–;–; KR826090; KR825335; KR826811; KR825714; KR826660; KR826734; KR825788; KR825406; KR826012; KR826583; KR825938; KR825861; KR826437;–; KR826241; KR826889; KR826163. ALVQ *Tmesipteris parva* N.A.Wakef. RBG 923285 (NSW) KR825541;–; KR826385; KR826514;–; KR826313;–;–; KR826097; KR825342; KR826817; KR825720; KR826667; KR826741; KR825794; KR825413; KR826019; KR826590; KR825945; KR825868; KR826441;–; KR826248; KR826896; KR826169. **Salviniales** CVEG *Azolla cf. caroliniana* Willd. Rothfels 4138 (DUKE) KR825479;–; KR826327; KR826454;–; KR826262;–;–; KR826033; KR825283; KR826756; KR825665; KR826604; KR826682; KR825734; KR825357; KR825960; KR826529; KR825883; KR825808; KR826396;–; KR826184; KR826832; KR826111. KIIX *Pilularia globulifera* L. RBGE 20040025 A (E) KR825526; KR825454; KR826369; KR826499;–; KR826299;–;–; KR826081; KR825326; KR826802; KR825706; KR826651; KR826725; KR825779; KR825397; KR826003; KR826574; KR825930; KR825852; KR826429; KR826984; KR826232; KR826880; KR826154. **Schizaeales** CQPW *Anemia tomentosa* (Savigny) Sw. Rothfels 4111 (DUKE) KR825472;–; KR826321; KR826447; KR825549; KR826256; KR825605;–; KR826026; KR825276; KR826749; KR825659; KR826598; KR826675; KR825727; KR825350; KR825953; KR826522; KR825876; KR825801;–;–; KR826177; KR826825; KR826104. PBUU *Lygodium japonicum* (Thunb.) Sw. Rothfels 4110 (DUKE) KR825512; KR825447; KR826357; KR826485; KR825578; KR826287;–; KR826929; KR826067; KR825312; KR826788; KR825694; KR826637; KR826713; KR825765; KR825386; KR825989; KR826563; KR825916; KR825838;–;–; KR826218; KR826866; KR826140. **Seed plants** GGEA *Cedrus libani* A.Rich. Zhuang & Gallant bg102/7617 (UBC) KR825483;–; KR826331; KR826458;–; KR826266;–;–; KR826037;–; KR826760; KR825668; KR826608; KR826686; KR825738;–; KR825964; KR826533; KR825887;–; KR826398;–; KR826188; KR826836; KR826115. DSXO *Cryptomeria japonica* (Thunb. ex L. f.) D.Don. Deyholos 2013-04 (ALTA) KR825485;–;–; KR826460;–; KR826269; KR825615;–; KR826040; KR825289; KR826762;–; KR826610; KR826688;–; KR825362; KR825967; KR826536; KR825890; KR825813; KR826400;–; KR826191; KR826839; KR826117. GNQG *Encephalartos barteri* Carruth. ex Miq. Stevenson 915/06 (NY) KR825498;–; KR826346; KR826473;–; KR826276; KR825626;–; KR826053;–; KR826775; KR825682; KR826623; KR826701; KR825752; KR825375; KR825979; KR826549; KR825902; KR825826; KR826410;–; KR826204; KR826852; KR826130. VDAO *Ephedra sinica* Stapf. Deyholos 2012-26 (ALTA) KR825499;–;–; KR826474;–; –;–;–; KR826054;–; KR826776;–; KR826624; KR826702; KR825753;–;–; KR826550; KR825903;–; KR826411;–; KR826205; KR826853;–. SGTW *Ginkgo biloba* L. Stevenson 76163 (NY) KR825503;–; KR826350;–;–; KR826280;–;–; KR826058; KR825305; KR826780;–; KR826628; KR826705; KR825757;–;–; KR826554; KR825907; KR825830; KR826413;–; KR826209; KR826857; KR826134. ROAP *Illicium parviflorum* Michx. ex Vent. Soltis & Miles 2799 (FLAS) KR825508;–;–; KR826481;–;–;–;–; KR826063;–; KR826784; KR825690; KR826633; KR826709; KR825761;–; KR825985; KR826559; KR825912; KR825834; KR826415;–; KR826214; KR826862;–. WTKZ *Nuphar advena* (Aiton) W.T.Aiton. Soltis & Miles 2783 (FLAS) KR825516;–;–; KR826489;–; KR826291;–;–; KR826071; KR825316; KR826792;–; KR826641; KR826717; KR825769;–; KR825993; KR826567; KR825920; KR825842; KR826420;–; KR826222; KR826870; KR826144. XSZI *Peperomia fraseri* C.DC. Deyholos 2013-10 (ALTA) KR825523;–;–; KR826496;–; KR826297;–;–; KR826078; KR825323; KR826799; KR825703; KR826648; KR826722; KR825776; KR825394; KR826000; KR826571; KR825927; KR825849; KR826426;–; KR826229; KR826877; KR826151. Note: sampling was completed with previously published sequences from the following taxa: **Polypodiales** *Adiantum capillus-veneris* L. (Kanegae and Wada, 1998); *Pteridium aquilinum* (L.) Kuhn (Der et al., 2011). **Seed plants** *Amborella trichopoda* Baill. (Amborella Genome Project, 2013); *Aquilegia coerulea* E.James (Aquilegia coerulea Genome Sequencing Project, 2015); *Arabidopsis thaliana* (L.) Heynh. (Lamesch et al., 2012); *Zea mays* L. (Schnable et al., 2009).