Systematic Botany (2014), 39(4): pp. 1042–1055
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DOI 10.1600/036364414X683921
Date of publication September 3, 2014

Phylogeny, Divergence Time Estimates, and Phylogeography of the Diploid Species of the *Polypodium vulgare* Complex (Polypodiaceae)

Erin M. Sigel,[1,3] Michael D. Windham,[1] Christopher H. Haufler,[2] and Kathleen M. Pryer[1]

[1]Department of Biology, Duke University, Box 90338, Biological Sciences Building, Durham, North Carolina 27708 U. S. A.
[2]Department of Ecology and Evolutionary Biology, University of Kansas, 1200 Sunnyside Avenue, Haworth Hall, Lawrence, Kansas 66045 U. S. A.
[3]Author for correspondence (erin.sigel@duke.edu)

Communicating Editor: Leslie Goertzen

***Abstract*—**The *Polypodium vulgare* complex (Polypodiaceae) comprises a well-studied group of fern taxa whose members are cryptically differentiated morphologically and have generated a confusing and highly reticulate species cluster. Once considered a single species spanning much of northern Eurasia and North America, *P. vulgare* has been segregated into 17 diploid and polyploid taxa as a result of cytotaxonomic work, hybridization experiments, and isozyme studies conducted during the 20th century. Despite considerable effort, however, the evolutionary relationships among the diploid members of the *P. vulgare* complex remain poorly resolved. Here we infer a diploids-only phylogeny of the *P. vulgare* complex and related species to test previous hypotheses concerning relationships within *Polypodium* sensu stricto. Using sequence data from four plastid loci (*atpA, rbcL, matK,* and *trnG-trnR*), we recovered a monophyletic *P. vulgare* complex comprising four well-supported clades. The *P. vulgare* complex is resolved as sister to the Neotropical *P. plesiosorum* group and these, in turn, are sister to the Asian endemic *Pleurosoriopsis makinoi*. Using divergence time analyses incorporating previously derived age constraints and fossil data, we estimate an early Miocene origin for the *P. vulgare* complex and a late Miocene-Pliocene origin for the four major diploid lineages of the complex, with the majority of extant diploid species diversifying from the late Miocene through the Pleistocene. Finally, we use our node age estimates to reassess previous hypotheses, and to propose new hypotheses, about the historical events that shaped the diversity and current geographic distribution of the diploid species of the *P. vulgare* complex.

***Keywords*—**Divergence dating, Hawaiian Islands, Macaronesia, plastid sequence data, Pleistocene.

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Members of the *Polypodium vulgare* L. complex (Polypodiaceae) are among the best-studied examples of ferns that combine cryptic morphology with complex patterns of reticulate evolution. For much of the 19th and 20th centuries, taxonomists considered *P. vulgare*, the type species of *Polypodium* L., to be a single species spanning much of northern Eurasia and North America. Subtle variations in morphology, including differences in leaf shape, indument type, rhizome scale shape and coloration, spore size and ornamentation, and rhizome flavor, were either dismissed or treated as taxonomic varieties. As these cryptic characters (summarized by Shivas 1961a and Hennipman et al. 1990) were examined in greater detail and their strong correlations with geography were demonstrated, researchers began to split *P. vulgare* into segregate species. Using cytotaxonomic studies and hybridization experiments, Manton (1950) and Shivas (1961a, b) were the first to implicate a history of reticulation as one source of the taxonomic confusion. Based on these studies, they assigned specific status to each of the three sexually reproducing European cytotypes of *P. vulgare* (Manton 1947, 1950, 1951; Shivas 1961a, b). Subsequent investigations have led to the recognition of about ten diploid, six allotetraploid, and one allohexaploid species, as well as numerous sterile hybrids, that constitute the *P. vulgare* complex worldwide (Lloyd 1963; Taylor and Lang 1963; Valentine 1964; Lloyd and Lang 1964; Lang 1969, 1971; Whitmore and Smith 1991; Haufler and Zhongren 1991; Haufler and Windham 1991; Haufler et al. 1993; Haufler et al. 1995b; Schmakov 2001). Today, the name *P. vulgare* sensu stricto (s. s.) is applied only to the North European and Asian allotetraploid taxon derived from the diploid progenitors *P. glycyrrhiza* D.C. Eaton and *P. sibiricum* Sipliv. (Shivas 1961b; Haufler et al. 1995b).

Despite progress in resolving the reticulate relationships and the origins of the polyploid members of the *P. vulgare* complex (Manton 1947, 1950; Shivas 1961a; Lloyd and Lang 1964; Lang 1971; Haufler et al. 1995a, b), the monophyly of the complex and phylogenetic relationships among its diploid taxa are not fully resolved. A synthesis of previous studies provides support for three major clades of diploid taxa, each united by a shared morphological character (Fig. 1; Manton 1950; Lloyd and Lang 1964; Lang 1971; Roberts 1980; Haufler and Ranker 1995; Haufler et al. 1995a, 1995b, 2000; Schneider et al. 2004; Otto et al. 2009). Two of these (clade A: *P. appalachianum* Haufler & Windham + *P. amorphum* Suksd. + *P. sibiricum* and clade G: *P glycyrrhiza* + *P. californicum* Kaulf. + *P. fauriei* Christ) are predominantly North American but extend to eastern Asia, and the third (clade C: *P. cambricum* L. + *P. macaronesicum* A.E. Bobrov) is found in Europe, primarily the Mediterranean region, and in Macaronesia. Members of the *P. appalachianum* clade (A) all have sporangiasters (see Fig. 1 i and ii; Martens 1943; Peterson and Kott 1974), which are sterile, often glandular structures within sori that are homologous to sporangia. In contrast, the *P. glycyrrhiza* clade (G) is devoid of sporangiasters, but has hairs on the adaxial surface of the leaf midrib. The *P. cambricum* clade (C) has branching, hair-like paraphyses distributed among the sporangia (Fig. 1 iii and iv; Martens 1943, 1950; Wagner 1964). Relationships among and within these three clades, as well as the placement of the Hawaiian endemic *P. pellucidum* Kaulf., were not well resolved by previous studies (Fig. 1 a–f). Perhaps the most contentious relationship among diploids is that of *P. scouleri* Hook. & Grev., a western North American coast endemic; some molecular studies suggest it is sister to *P. glycyrrhiza* (Fig. 1 d, e; Haufler and Ranker 1995; Haufler et al. 2000), whereas others show it as sister to the Hawaiian endemic *P. pellucidum* (Fig. 1 f; Otto et al. 2009).

On morphological grounds, the *Polypodium vulgare* complex has been considered closely related to the Neotropical *P. plesiosorum* Kunze group (Christensen 1928; Tryon and Tryon 1982; Haufler et al. 1995a, b). While recent phylogenetic work reveals the need for a recircumscription of the *P. plesiosorum* group (Otto et al. 2009), it comprises an estimated 20 diploid and polyploid species (A. R. Smith, pers. comm.; Tejero-Díez and Pacheco 2004; Luna-Vega et al. 2012). Whereas some molecular analyses support a sister relationship between

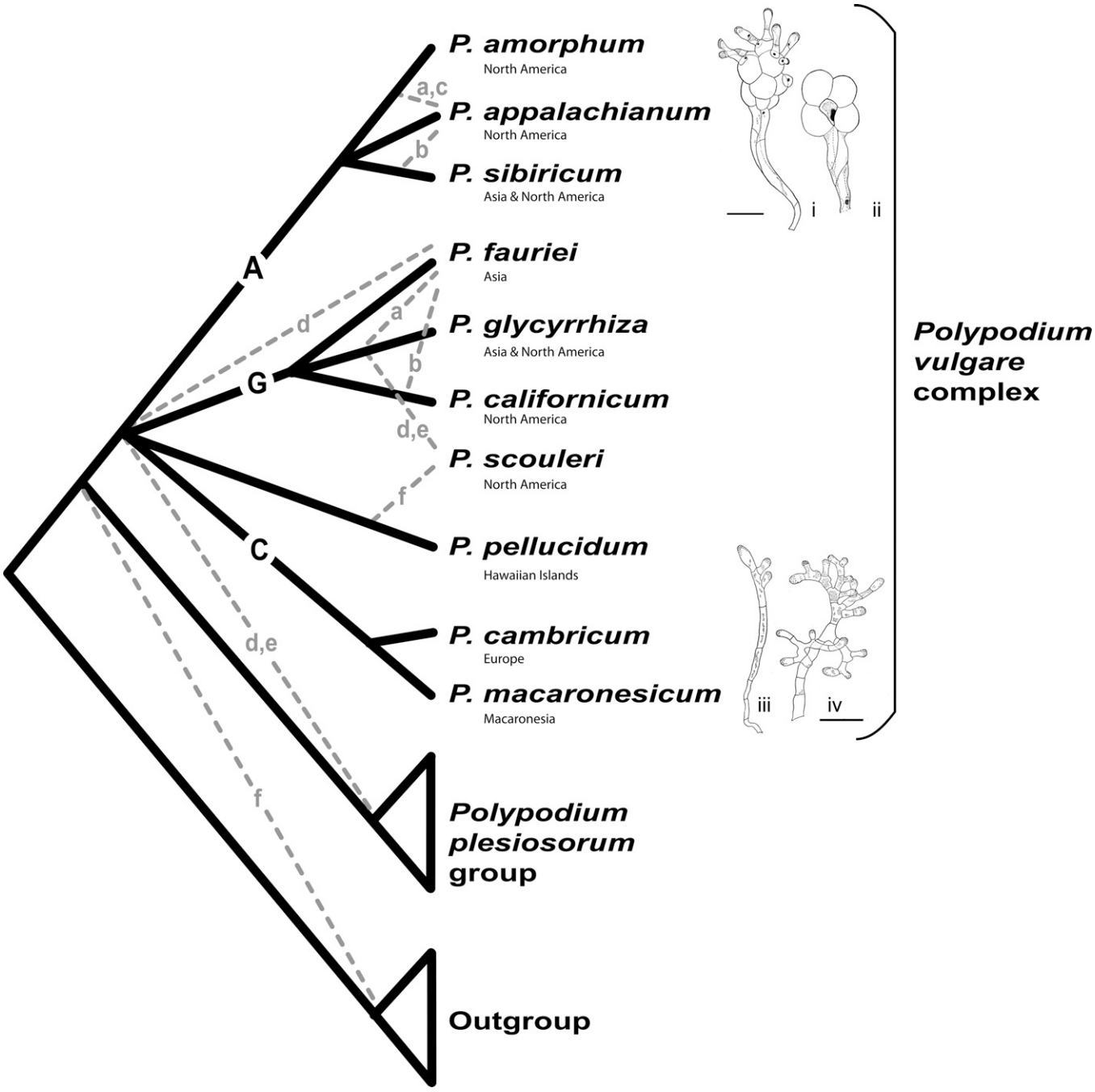

FIG. 1. Consensus topology of previously published relationships for diploid species of the *Polypodium vulgare* complex and members of the Neotropical *Polypodium plesiosorum* group. Thick black lines represent the consensus topology inferred from morphological, isozyme, chloroplast restriction site, and plastid sequence data by Haufler et al. (1995a, b) and Haufler and Ranker (1995). Small text under each taxon name indicates the generalized geographic distribution for that species. Three commonly recovered clades are marked as A (*P. appalachianum* clade), G (*P. glycyrrhiza clade*), and C (*P. cambricum* clade). Grey dashed lines and lowercase letters depict topological differences recovered by specific studies: a. Haufler et al. (1995b), based on biogeographic patterns and morphological synapomorphies; b. Haufler et al. (1995b), derived from genetic analysis of isozyme data; c. Haufler et al. (1995a), nodes with ≥70% bootstrap support in a parsimony analysis of chloroplast restriction site data; d. Haufler and Ranker (1995), nodes with ≥70% bootstrap support in a parsimony analysis of *rbcL* plastid sequence data; e. Haufler et al. (2000), relationships based on a strict consensus of 180 most parsimonious trees derived from *trnL-trnF* plastid sequence data; f. Otto et al. (2009), nodes with = 1.0 posterior probability support in a Bayesian inference analysis of *trnL-F, rbcL,* and *rps4* plastid sequence data. Only Haufler et al. (2000) had complete sampling of the ten diploid species included in this study. Inset images are modified from Martens (1943) as follows: i. sporangiasters with glandular trichomes found in *P. amorphum* and *P. appalachianum*; ii. sporangiasters without glandular trichomes found in *P. sibiricum*; iii. soral paraphyses found in *P. macaronesicum*; iv. soral paraphyses found in *P. cambricum*. Horizontal lines next to inset images represent 100 μm.

the *P. vulgare* and *P. plesiosorum* groups (Haufler et al. 1995a, Otto et al. 2009), others suggest that the *P. plesiosorum* group may be nested within the *P. vulgare* complex (Haufler and Ranker 1995; Haufler et al. 2000; Otto et al. 2009). Thus, even the monophyly of the *P. vulgare* complex has been called into question (Fig. 1).

Here we adopt a "diploids-first" approach (Beck et al. 2010; Govindarajulu et al. 2011) to investigate relationships within

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Polypodium s. s., with a particular focus on the *P. vulgare* complex. Using DNA sequence data from four plastid loci (*atpA*, *matK*, *rbcL*, and *trnG-trnR* intergenic spacer) and sampling that includes ten diploid species assigned to the *P. vulgare* complex, we infer a molecular phylogeny of these iconic ferns. In addition, we use established calibrated divergence dates for the Polypodiaceae (Schuettpelz and Pryer 2009), together with a recently described *Polypodium* fossil (Kvaček 2001), to date the divergence of *Polypodium* s. s. and the *P. vulgare* complex. Our goals are to: 1) assess the monophyly of the *P. vulgare* complex and its hypothesized sister relationship with the *P. plesiosorum* group; 2) infer evolutionary relationships among the diploid taxa of the *P. vulgare* complex; 3) provide temporal and biogeographic context for the origin of the diploid species; and 4) lay the foundation for ongoing studies detailing the reticulate history and timing of allopolyploid formation within the *P. vulgare* complex.

Materials and Methods

Taxon Sampling—We sampled 34 *Polypodium* specimens representing ten recognized diploid taxa in the *P. vulgare* complex and five species belonging to the *P. plesiosorum* group (Appendix 1). These include two taxa formerly assigned to the *P. dulce* Poir. or *P. subpetiolatum* Hook. groups, but subsequently shown to be related to *P. plesiosorum* (Tryon and Tryon 1982; Moran 1995; Mickel and Smith 2004; Otto et al. 2009). Representatives of the *P. plesiosorum* group were selected without regard to ploidy because of a lack of cytogenetic data for these taxa. *Pleurosoriopsis makinoi* (Maxim.) Fomin was included in the analyses based on its position in recent molecular studies as the closest relative to a clade including the *Polypodium vulgare* + *P. plesiosorum* complexes (Schneider et al. 2004; Otto et al. 2009). Seven other outgroup taxa were selected to represent a diversity of major clades in the Polypodiaceae (Appendix 1), including *Polypodium sanctae-rosae* (Maxon) C. Chr., a species that was shown to be closely allied with the scaly-leaved genus *Pleopeltis* Humboldt & Bonpland ex Willdenow but has yet to transferred to that genus (Mickel and Smith 2004; Otto et al. 2009). Analyses were rooted with *Platycerium* Desv., the outgroup taxon most distantly related to *Polypodium* s. s. according to recent studies (Schneider et al. 2004; Otto et al. 2009).

DNA Extraction and Plastid Sequencing—For each individual sampled, total genomic DNA was isolated from herbarium, silica-dried, or fresh material using the DNeasy Plant Mini Kit (Qiagen, Valencia, California) following the manufacturer's protocol. One to four plastid regions, *atpA, matK, rbcL,* and the *trnG-trnR* intergenic spacer (henceforth *trnG-R*), were entirely or partially amplified and sequenced for each of the samples (Appendix 1). Primers used for amplification and sequencing, as well as amplification and sequencing protocols, were based on previous studies (*atpA*: ESATPA412F, ESATPA535F, ESATPA557R, and ESTRNR46F from Schuettpelz et al. 2006; *matK*: fEDR and rGLR from Kuo et al. 2011; *rbcL*: ESRBCL1F, ESRBCL628F, ESRBCL654R, and ESRBCL1361R from Schuettpelz and Pryer 2007; *trnG-R*: TRNG1F, TRNG43F1, TRNG63R, and TRNR22R from Nagalingum et al. 2007). Plastid data sets were supplemented with sequences from GenBank. The 115 newly generated sequences are available in GenBank (Appendix 1).

Sequence Alignment and Data Sets—DNA sequence chromatograms were manually edited and assembled using Sequencher 4.8 (Gene Codes Corporation 2005). Sequences for each locus were manually aligned with MacClade 4.05 (Maddison and Maddison 2005). Unsequenced portions of each locus were coded as missing data. Indels (present only in *trnG-R*) were coded as present or absent for each individual using the method of Simmons and Ochoterena (2000), as implemented in gapcode.py v. 2.1 (Ree 2008). These binary characters were appended to the alignment, and the corresponding indels were excluded from analysis. Five datasets were compiled: four single-locus datasets and one combined four-locus dataset. Sequences from multiple specimens of *P. plesiosorum, P. rhodopleuron* Kunze, *Drymotaenium miyoshianum* Makino, *Microsorum* Link (*M. varians* (Mett.) Hennipman & Hett. and *M. fortunei* (T.Moore) Ching), *Platycerium* (*P. stemaria* (Beauv.) Desv. and *P. superbum* de Jonch. & Hennipman), and *Prosaptia contigua* C. Presl. were concatenated in the combined four-locus dataset (Appendix 1).

Phylogenetic Analyses—Separate phylogenetic analyses were conducted for each of the single-locus datasets using maximum likelihood (ML) as implemented in Garli 2.0 on the CIPRES computing cluster (Zwickl 2006; Miller et al. 2010). Single-locus data sets for *atpA, matK,* and *rbcL* were analyzed using the best model as determined by the Akaike Information Criterion (AIC; Table 1) in PartitionFinder (Lanfear et al. 2012). The *trnG-R* single-locus dataset was divided into two partitions for analysis: a partition of sequence data with the best model as determined above, and a partition of binary-coded indel data with a Mkv model (Table 1; Lewis 2001). Each analysis was run for two independent searches with ten replicates each, resulting in 20 optimal maximum likelihood trees. The single "best" ML tree was identified as having the largest -ln score (tree statistics summarized in Table 1). For each dataset, tree searches were performed on 1,000 bootstrap pseudoreplicate datasets. The bootstrap majority-rule consensus tree for each dataset was compiled using PAUP* v. 4.0a123 (Swofford 2002) and compared for well supported (≥ 70% bootstrap support) incompatible clades (Mason-Gamer and Kellogg 1996). No significant incongruences were detected, allowing us to concatenate the four single-locus datasets into a single combined dataset. This combined dataset (with five data partitions: one partition for each of the four sequence loci and one partition for the *trnG-R* binary-coded indel data) was analyzed using the ML approach described above for the single-locus analyses.

The combined dataset was also analyzed using Bayesian inference as implemented in MrBayes v 3.1.2 on the CIPRES computing cluster (Ronquist and Huelsenbeck 2003; Miller et al. 2010). Parameters were unlinked among the five partitions. In order to accommodate the range of models accepted by MrBayes, the best-fitting models for *atpA, rbcL* and *trnG-R* sequence partitions were implemented with the nst = 6, statefreqpr = dirichlet(1,1,1,1) and rates = gamma settings. The best-fitting model for the *matk* partition was implemented with the nst = mixed, statefreqpr = dirichlet(1,1,1,1) and rates = gamma settings. The Mkv model for the *trnG-R* indel partition was implemented with the nst = 1, rates = equal, and coding = variable. The average rates for each partition were allowed to be different (ratepr = variable) and all other priors were left at their default values. Four analyses were run with four chains (one cold, three heated), for 10 million generations with a sample taken every 1,000 generations. The sample parameter traces were visualized in Tracer v1.5 (Rambaut and Drummond 2007). The four runs each converged around one million generations, but to be conservative, we excluded the first two million generations of each run as burn-in, resulting in a final pool of 32,000 samples. A majority-rule consensus tree was generated using PAUP* v. 4.0a123 (Swofford 2002). The combined

Table 1. Statistics for the datasets analyzed in this study. Missing data include both uncertain bases (?, N) and gaps (-). Sequence data and binary coded indels data for *trnG-trnR* were united into a single maximum likelihood (ML) analysis. MLBS: maximum likelihood bootstrap support; BIPP: Bayesian inference posterior probability; * The combined dataset was analyzed under a partition model, with each locus given its own best-fitting model.

						MLBS		BIPP	
Locus	No. of Sequences	Included Sites	Variable Sites	% Missing Data	Best-Fitting Model	Mean	% Partitions ≥ 70%	Mean	% Partitions ≥ 0.95
atpA	34	1,524	231	5.9	TrN + G	76	81	–	–
matK	24	624	232	2.7	TVM + G	79	56	–	–
rbcL	41	1,309	227	8.5	GTR + G	90	81	–	–
trnG-trnR	29	964	230	1.0	K81uf + G	83	64	–	–
trnG-trnR indels	29	31	31	0	Mkv				
Combined	42	4,452	951	24.6	–*	92	75	0.89	76

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dataset and consensus trees are deposited in TreeBASE (http://treebase.org; study 15263).

Divergence Time Estimation—Divergence times were estimated from the combined dataset using Bayesian inference as implemented in Beast v. 1.7.4 (Drummond et al. 2012) on the Duke Shared Cluster Resource (https://wiki.duke.edu/display/SCSC/DSCR). Sequence data for each locus was assigned to a separate data partition (four partitions in total) with parameters corresponding to the best nucleotide substitution model as determined by PartitionFinder (Table 1; Lanfear et al. 2012). The four data partitions were assigned a linked lognormal relaxed clock model with estimated parameters and a linked tree. Coded indels (present only for *trnG-R*) were excluded from the divergence time analyses because Beast v. 1.7.4 is unable to employ a Mkv model that is appropriate for standard ordered variable data (Lewis 2001).

Four analyses were run, each employing a unique combination of tree model priors and calibration points. Because sampling for this study included both interspecific variation (sampling across species) and intraspecific variation (multiple individuals of a single species), separate analyses were run with either a birth-death speciation prior or a constant population size coalescence prior. All analyses incorporated five previously derived age constraints (calibration points A–D, F; Appendix 2). These calibration points are best-age estimates obtained from Schuettpelz and Pryer (2009) for the Polypodiaceae. To compensate for uncertainty surrounding these estimates, each calibration point was assigned a normal distribution, with a mean equal to the best-age estimate and one standard deviation equal to ten percent of the best-age estimate (Rothfels et al. 2012). Two of the four analyses included an additional age constraint from a Polypodiaceae fossil from the Oligocene (Kvaček 2001), with a minimum age of 26.8 ± 1.2 million years ago (mya; calibration point E). Kvaček (2001) presents strong, but inconclusive, evidence that the fossilized frond fragment with visible venation, intact sporangia, and observable spores belongs to the genus *Polypodium*. This calibration point was assigned an exponential distribution with a mean of 5 and an offset of 25.6 mya, resulting in a 95% confidence interval between 25.73 and 44.03 mya. These settings closely approximate the minimum age estimate for the fossil (25.6 mya) and allow for uncertainty in the upper age estimate. In summary, the four analyses were: (1) birth-death speciation prior and calibration points A–F; (2) birth-death speciation prior and calibration points A–D, F; (3) constant population size coalescence and calibration points A–F; and (4) constant population size coalescence and calibration points A–D, F.

Each analysis was run four times for 10,000,000 generations, with parameters sampled every 1,000 generations. Tracer v. 1.5 (Rambaut and Drummond 2007) was used to assess the posterior distribution of all parameters, and mixing was considered sufficient when the effective sample size of each parameter exceeded 200. The first 2,000 (20%) trees of each run were discarded as burn-in. The program TreeAnnotator v1.5.4 (Drummond et al. 2012) was used to combine the 32,000 trees (8,000 trees/run for four runs) and produce a maximum clade credibility (MCC) chronogram with mean divergence time estimates and 95% highest posterior density (HPD) intervals.

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Results

Phylogenetic Analyses—Of the 125 DNA sequences used in this study, 115 were newly generated and all are available in GenBank (Appendix 1). The best ML tree for the combined dataset and the Bayesian inference consensus tree yielded phylogenetic hypotheses of identical topology and comparable support (see Table 1 for tree statistics). Nodes were considered well supported if maximum likelihood bootstrap support (MLBS) ≥ 70% and Bayesian inference posterior probabilities (BIPP) ≥ 0.95. In Fig. 2 , we present the best ML phylogram showing evolutionary relationships within *Polypodium* and the associated MLBS and BIPP support values.

Our phylogeny (Fig. 2) provides robust support (MLBS = 88%, BIPP = 0.95) for the monophyly of *Polypodium* s. s., encompassing two well-supported species complexes: the *P. vulgare* complex (MLBS = 97%, BIPP = 1.0) and the *P. plesiosorum* group (MLBS = 100%, BIPP = 1.0). The P. *vulgare* complex comprises four well-supported clades: the *P. appalachianum* clade, the *P. glycyrrhiza* clade, the *P. cambricum* clade, and the *P. scouleri* clade (henceforth referred to as clades A, G, C, and S, respectively; Fig. 2). Relationships among these clades are poorly resolved, but weak support for the union of clades A + G (MLBS = 60%, BIPP = 0.72) and moderate support for the union of clades C + S (MLBS = 75%, BIPP = 0.91) hint at potential relationships among clades A, G, C, and S.

Clade A (MLBS = 100%, BIPP = 1.0) includes all samples of *P. amorphum, P. appalachianum*, and *P. sibiricum*, though there is no support for differentiating among the species. Within Clade G (MLBS = 97%, BIPP = 1.0), there is strong support for *P. fauriei* as sister to *P. californicum* + *P. glycyrrhiza* (MLBS = 100%, BIPP = 1.0). Both accessions of *P. californicum* are united in a well-supported clade (MLBS = 94%, BIPP = 1.0), whereas all accessions of *P. glycyrrhiza* are united in a clade with weak support (MLBS = 80%, BIPP = 0.58). Clade C unites *P. cambricum* and *P. macaronesicum* as sister species (MLBS = 100%, BIPP = 1.0), with the two accessions of *P. cambricum* unambiguously sister to one another. Clade S provides maximal support (MLBS = 100%, BIPP = 1.0) for *P. pellucidum* being sister to two accessions of *P. scouleri* (MLBS = 100%, BIPP = 1.0).

Among members of the monophyletic *P. plesiosorum* group (Fig. 2), *P. subpetiolatum* is well supported as sister to the other four species (MLBS = 92%, BIPP = 1.0). Within the latter clade, *P. martensii* Mett. is sister to a well-supported and highly differentiated clade of *P. colpodes* Kunze + *P. rhodopleuron* + *P. plesiosorum* (MLBS = 100%, BIPP = 1.0), with *P. plesiosorum* sister to *P. colpodes* + *P. rhodopleuron* (MLBS = 95%, BIPP = 1.0). Our analyses position *Pleurosoriopsis makinoi* as closest sister to *Polypodium* s. s. with strong support (MLBS = 87%, BIPP = 1.0). In our inferred phylogeny, there is good support (MLBS = 88%, BIPP = 0.97) for a larger clade consisting of *Polypodium* s. s. + *Pleurosoriopsis makinoi*, *Prosaptia contigua*, and *Phlebodium decumanum* J.Sm. + *Pleopeltis polypodioides* (L.) E.G. Andrews & Windham + *Polypodium sanctae-rosea* (the latter with MLBS = 70%, BIPP = 0.95). The relationships among these three groups are unsupported.

Divergence Time Estimations—Age estimates from our four divergence time analyses produced nearly identical maximum clade credibility (MCC) topologies and similar divergence time estimates (mean node ages and 95% highest posterior distribution (HPD) for all four analyses are presented in Appendix 3). This suggests that estimations of topological and divergence times employed by Beast v. 1.7.4 (Drummond et al. 2012) were relatively insensitive to the choice of tree model prior (birth-death speciation or constant population size coalescence) or to the absence of the fossil constraint (calibration point E). Topological differences among the analyses were primarily confined to branch tips and did not affect the comparison of divergence times of interspecific taxa across the four analyses. Figure 3 depicts the chronogram derived from analysis 2 (node estimates for all the analyses can be found in Appendix 3). For the majority of the nodes uniting interspecific taxa (nodes 1–20, 22, 23, 25, 28, 31, 33, 35, 39), differences in mean node divergence time (mean node height) and 95% HPD were less than two million years. For most nodes uniting intraspecific individuals (nodes 21, 24, 26, 27, 29, 30, 32, 34, 36–38, 40–41), differences in mean node divergence time and 95% HPD were less than 0.2 million years.

A few nodes showed greater variation in divergence times across the analyses, most notably node 1, the designated root node of the chronogram. In this instance, mean age estimates

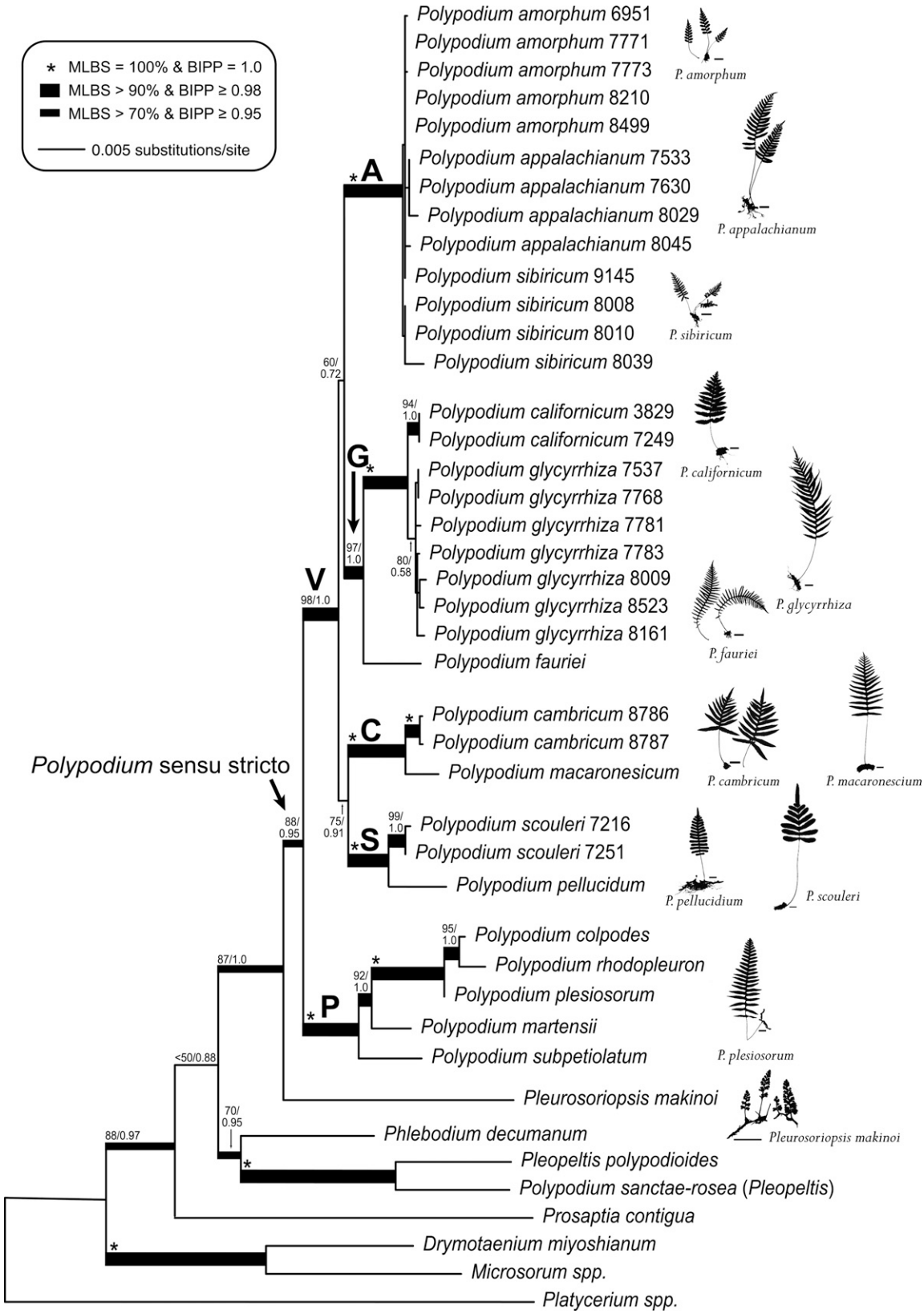

FIG. 2. The best ML phylogram from the combined analysis of *atpA*, *rbcL*, *matK*, and *trnG-trnR* sequence data for ten diploid taxa of the *Polypodium vulgare* complex, five taxa belonging to the *P. plesiosorum* complex, and eight outgroup taxa (Appendix 1). See Table 1 for tree statistics. The tree is rooted with *Platycerium*, the outgroup taxon most distantly related to the *P. vulgare* complex according to Schneider et al. (2004). ML bootstrap support values (MLBS) and Bayesian inference posterior probabilities (BIPP) are mostly given above nodes and indicated with thickened branches (see inset legend). The bolded V and P identify the monophyletic *P. vulgare* complex and monophyletic *P. plesiosorum* complex, respectively. Bolded letters A, G, C, and S indicate the major subclades of diploid species within the *P. vulgare* complex: the *P. appalachianum* clade, the *P. glycyrrhiza* clade, the *P. cambricum* clade, and the *P. scouleri* clade, respectively. Thumbnail silhouettes of members of the *P. vulgare* complex, *P. plesiosorum*, and *Pleurosoriopsis makinoi* were obtained by modifying scanned images of herbarium vouchers used in this study. Scale bars next to each silhouette represent 2.54 cm.

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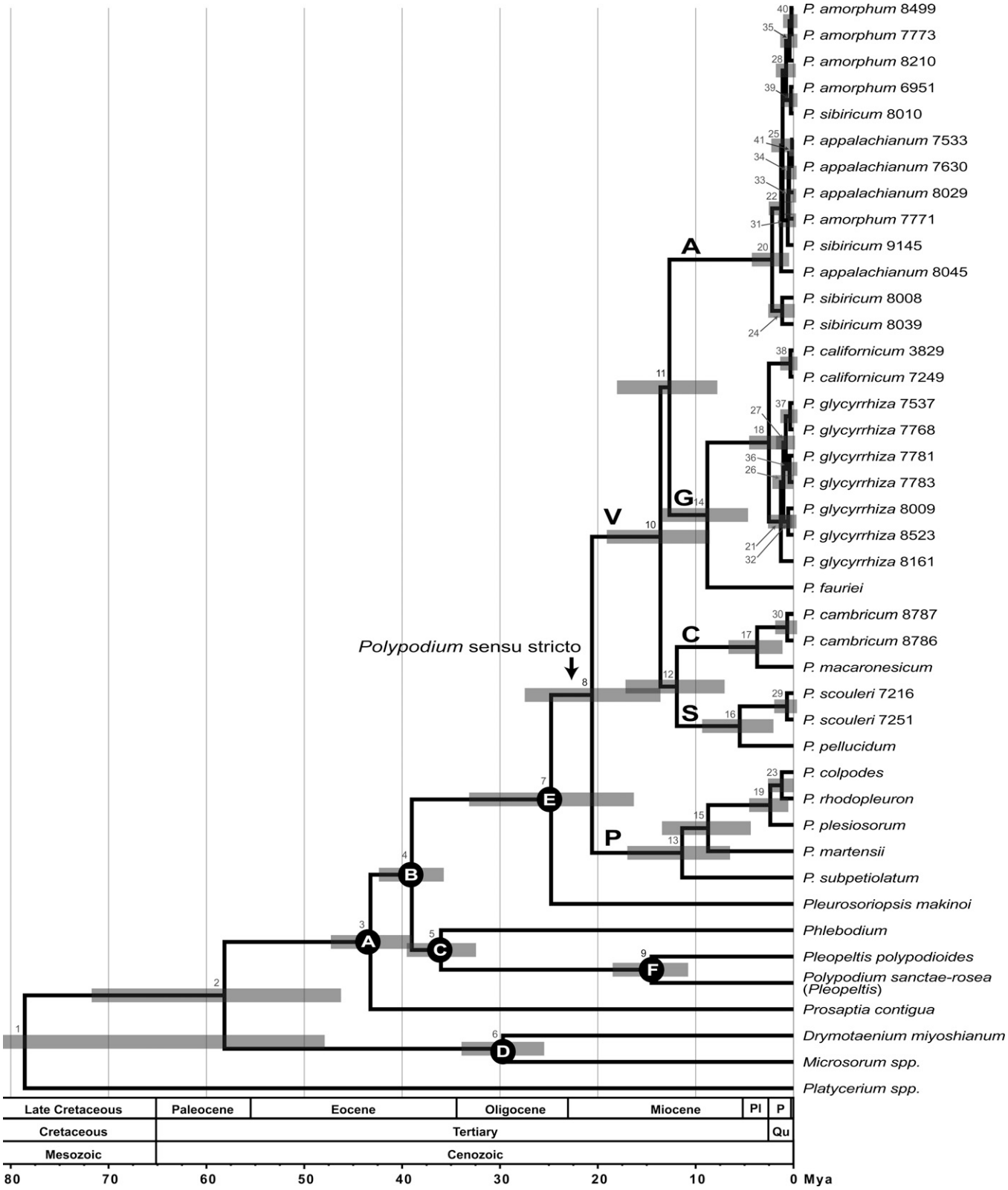

FIG. 3. Maximum clade credibility (MCC) chronogram with the divergence time estimates from analysis 2. Clades P, V, A, G, C, and S are as indicated in Fig. 2. Black circles with letters indicate the six calibration points using in this study: points A–D and F were obtained from Schuettpelz and Pryer (2009) and point E refers to a fossil reported by Kvaček (2001; Appendix 2). Small numbers above nodes indicate mean node age estimates from oldest (node 1) to youngest (node 41). Statistics corresponding to each node are reported in Appendix 3. Mean age and 95% HPD are indicated for each node by the grey bars. The lower limit of the 95% HPD for node 1 is truncated. Dates for geologic periods and epochs as represented in the bottom bar correspond to Walker and Geissman (2009).

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range from 78.57 mya (analysis 2) to 99.33 mya (analysis 3). We suspect this variation results from not placing an age constraint on the root node, but it does not affect our conclusions regarding relationships and divergence dates within *Polypodium* s. s. Greater variation was also seen for node 7, the most recent common ancestor of *Polypodium* s. s. and *Pleurosoriopsis makinoi.* Mean divergence time estimates incorporating a fossil age constraint for that node ranged from 27.64–27.80 mya (analyses 1 and 3, respectively), whereas those not incorporating the fossil age constraint ranged between 24.65 and 24.77 mya (analyses 2 and 4). Thus, it appears that the older estimated divergence times for analyses 1 and 3 reflect the minimum age constraint imposed on that node by the Oligocene fossil dated at 26.80 ± 1.2 mya (Kvaček 2001). Nevertheless, all analyses indicate a mean divergence for node 7 in the late Oligocene, with substantial overlap in the 95% HPD estimates among all four analyses.

Based on analysis 2 (Fig. 3), we infer that *Polypodium* s. s. and *Pleurosoriopsis makinoi* diverged approximately 24.77 mya (95% HPD: 16.72–32.74 mya; node 7). Cladogenetic events within *Polypodium* began approximately 20.59 mya (95% HPD: 14.01–27.06 mya; node 8), leading to the formation of the reciprocally monophyletic *P. vulgare* complex and *P. plesiosorum* group. Within the *P. plesiosorum* group, diversification is estimated to have started 11.38 mya (95% HPD: 6.90–16.57; node 13). Within the *P. vulgare* complex, divergence into the major clades A, G, C, and S occurred between approximately 13.60 mya (95% HPD: 8.98–18.67 mya; node 10) and 11.91 mya (95% HPD: 7.45–16.76 mya; node 12). Diversification within clades A, G, C, and S is estimated at about 2.18 mya (95% HPD: 0.86–3.85 mya; node 20), 8.81 mya (95% HPD: 5.06–13.08 mya; node 14), 3.72 mya (95% HPD: 1.53–6.24 mya; node 17) and 5.50 mya (95% HPD: 2.46–8.93 mya; node 16), respectively.

Discussion

The taxonomy of *Polypodium* has a long and complicated history. As originally described by Linnaeus (1753), the genus encompassed most leptosporangiate ferns with discrete, non-marginal, round sori. This circumscription has narrowed significantly over the years (see De la Sota 1973 and Hennipman et al. 1990), and many late 20th century authors limit application of the generic name *Polypodium* to a group of approximately 100–150 primarily New World species (Tryon and Tryon 1982; Moran 1995; Haufler et al. 1995b; Mickel and Smith 2004). Schneider et al. (2004, 2006) and Otto et al. (2009) provided compelling molecular evidence for the polyphyly of even this narrowed circumscription of *Polypodium*, supporting the recognition of a series of segregate genera such as *Pecluma* Price, *Phlebodium* (R. Br.) J. Sm., *Serpocaulon* A. R. Sm., and *Synammia* C. Presl (Haufler et al. 1993; Schneider et al. 2006; Smith et al. 2006).

Our phylogenetic analyses of sequence data from four plastid loci (Fig. 2) strongly support the monophyly of the primarily north-temperate *Polypodium vulgare* complex and its sister relationship with the Neotropical *P. plesiosorum* group (Christensen 1928; Tryon and Tryon 1982; Haufler et al. 1995 a, b). As in previous molecular studies (Schneider et al. 2004; Otto et al. 2009), the enigmatic Asian taxon *Pleurosoriopsis makinoi* is inferred to be sister to these two groups of *Polypodium.* This remains a surprising result because the diminutive, highly divided leaves (Fig. 2), sporangia following the veins, and chlorophyllous spores (Kurita and Ikebe 1977; Iwatsuki et al. 1995; Shugang and Haufler 2013) of *Pleurosoriopsis* are so different from those of typical *Polypodium*. However, it does provide for a convenient and unequivocal demarcation of *Polypodium* s. s., which can be defined as the monophyletic clade sister to *Pleurosoriopsis makinoi*.

Phylogenetic Relationships Among Diploid Members of the Polypodium vulgare Complex—Our analyses provide strong support for four clades of diploid species belonging to the *P. vulgare* complex (Figs. 2, 3; clades A, G, C, and S). Here we discuss the inferred relationships among these species in light of hypotheses proposed by previous researchers.

Polypodium appalachianum clade (A)—This clade encompasses all samples assigned to three primarily North American taxa: *P. appalachianum, P. amorphum,* and *P. sibiricum* (Fig. 2). These taxa correspond to the diploid members of Lloyd and Lang's (1964) "*Polypodium virginianum* L. group", as expanded by subsequent taxonomic revisions (Lang 1969, 1971; Siplivinski 1974; Haufler and Windham 1991). Strong support for this clade has been recovered in all previous molecular studies (Haufler and Ranker 1995; Haufler et al. 1995 a, b; Otto et al. 2009), and the presence of sporangiasters (Fig. 1 i and ii) provides an unambiguous morphological synapomorphy for the group.

Despite strong support for the monophyly of clade A, our best maximum likelihood topology for the combined dataset recovers no support (< 50% MLBS and < 0.70 BIPP) for the monophyly of individual species (Fig. 2). All included specimens of *P. appalachianum*, *P. amorphum*, and *P. sibiricum* form a polytomy with little sequence differentiation. This lack of resolution is consistent across topologies derived from individual locus datasets, with the exception of *matK* which unites the three sampled individuals of *P. appalachianum* (MLBS = 87%) and provides weak support (MLBS = 62%) for a combined clade of *P. appalachianum* + *P. amorphum* sister to one individual of *P. sibiricum* (*matK* topology not shown). This lack of interspecific resolution is surprising in light of earlier isozyme electrophoresis studies of the group. Haufler et al. (1995b) reported that genetic identity values within these taxa were much higher than those between taxa (average of 0.96 vs. 0.46), suggesting that each is genetically distinct from its close relatives. This discrepancy might be explained by differential rates of evolution between the nuclear (coding the isozyme loci) and plastid genomes (Wolfe et al.1987).

Despite the inability of plastid sequence data to resolve relationships among taxa in clade A, we do not advocate collapsing *P. appalachianum*, *P. amorphum,* and *P. sibiricum* into a single broadly circumscribed species. Each taxon has a unique suite of stable, if somewhat cryptic, morphological features and a distinct geographic distribution (Haufler and Windham 1991; Haufler et al. 1993; Haufler et al. 1995 a, b). *Polypodium appalachianum* has relatively long, narrow pinnae with acute apices, abundant glandular sporangiasters, and spores <52 μm long; its range extends from the southern Appalachians to eastern Canada. *Polypodium amorphum* has short, obtuse pinnae, relatively few (but still glandular) sporangiasters, and spores >52 μm long; it is confined to the northern Cascade Mountains of Oregon, Washington, and British Columbia. *Polypodium sibiricum* is distinguished from both of these by its eglandular sporangiasters and an arctic/boreal distribution. Further molecular studies incorporating nuclear sequence data may resolve these species relationships

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and allow for inferences of morphological evolution within this clade.

Polypodium glycyrrhiza clade (G)—This clade encompasses all samples of three species occupying the Pacific Rim of western North America and eastern Asia: *P. glycyrrhiza, P. californicum,* and *P. fauriei* (Fig. 2). Lloyd and Lang (1964) hypothesized a close relationship between *P. californicum* and *P. glycyrrhiza* because they have hairs on their adaxial leaf surfaces, lack sporangiasters, and are largely confined to coastal habitats in western North America. Based on isozyme studies indicating high levels of genetic identity with *P. glycyrrhiza* and *P. californicum*, Haufler et al. (1995a) hypothesized that the Asian species *P. fauriei* was also a member of this group. However, relationships among these species remained unresolved (Fig. 1 a, b). Similarities in isozyme electrophoresis banding profiles suggested that *P. fauriei* might be sister to *P. californicum,* whereas biogeographic distributions supported *P. fauriei* and *P. glycyrrhiza* as sister (*P. fauriei* is exclusively Asian and *P. glycyrrhiza* extends into the Kamchatka Peninsula; Haufler et al. 1995b). A subsequent study utilizing *trnL-trnF* plastid sequence data provided weak support (< 50% maximum parsimony BS) for *P. fauriei* being sister to a clade including *P. glycyrrhiza, P. californicum*, and *P. scouleri* (Fig. 1 e; Haufler et al. 2000). All other studies addressing relationships within the *P. vulgare* complex included no more than two of the three diploid species belonging to this group, limiting the ability to infer sister relationships (Haufler and Ranker 1995; Haufler et al. 1995a; Otto et al. 2009).

Our study provides strong support for the monophyly of the diploid members of the "*P. glycyrrhiza* group" and novel, unequivocal support for the sister relationship of *P. californicum* and *P. glycyrrhiza* (Fig. 2 clade G). These three species are morphologically united by the presence of multicellular hairs on the adaxial surface of the leaves (Christensen 1928; Haufler et al. 1993; Iwatsuki et al. 1995), a trait not found in other diploid members of the *P. vulgare* complex. Branch lengths indicate that *P. fauriei* is strongly differentiated from *P. glycyrrhiza* + *P. californicum* (Fig. 2), a pattern reflected in its unique morphology. Unlike other diploid members of the *P. vulgare* complex, *P. fauriei* has a distinctive curved frond when dried and long, gray adaxial rachis hairs (Christensen 1928; Iwatsuki et al. 1995). In contrast, the rachises of *P. glycyrrhiza* and *P. californicum* are puberulent (Haufler et al. 1993). The two sampled individuals of *P. californicum* form a well-supported clade, whereas *P. glycyrrhiza* is only weakly supported as monophyletic (Fig. 2, MLBS = 80% and BIPP = 0.58). This could be due to greater molecular variation in *P. glycyrrhiza* across a larger geographic range (as reflected in the sampling for this study), compared to *P. californicum* whose geographic range is largely limited to central and southern California (Haufler et al. 1993; Baldwin et al. 2012; Sigel et al. 2014).

Polypodium cambricum clade (C)—Our analysis unites the two accessions of *P. cambricum* and our only sample of *P. macaronesicum* (Fig. 2 clade C) into a well-supported clade characterized by having branched paraphyses (distinctive receptacular hairs; see Fig. 1 iii and iv; Martens 1950; Wagner 1964) scattered among the sporangia. It has long been hypothesized that these two taxa are closely allied, with some authors treating *P. macaronesicum* as a synonym of *P. cambricum*, or as a subspecies restricted to the Macaronesian Islands (summarized in Rumsey et al. 2014). Alternatively, some authors recognize populations of *P. macaronesicum* on the Azore Islands as *P. macaronesicum* subsp. *azoricum* (Vasc.) F.J. Rumsey, Carine & Robba or as a distinct species, *P. azoricum* (Vasc.) Ros. Fernandes (Haufler et al. 2000; Schäfer 2005; Rumsey et al. 2014). Roberts (1980) documented differences in lamina serration, paraphysis structure and abundance, and rhizome scale shape that support the recognition of *P. cambricum* and *P. macaronesicum* as distinct species. Our study reinforces previous phylogenetic analyses that showed significant genetic divergence between these two taxa (Haufler and Ranker 1995; Otto et al. 2009; Rumsey et al. 2014). We also are able to confirm the monophyly of *P. cambricum* from the southern and northern portions of its range (Appendix 1; Fig. 2; Spain and England, respectively), which further supports the segregation of *P. macaronesicum* from *P. cambricum*.

Polypodium scouleri clade (S)—Two species are included in this well-supported clade, *P. scouleri* and *P. pellucidum* (Fig. 2 clade S). Despite having rarely been associated with one another, they are similar in the thick, leathery texture of their leaves and their sister relationship was recovered by Otto et al. (2009; Fig. 1 f). *Polypodium scouleri* has obscure, strongly anastomosing veins and is restricted to coastal habitats in western North America (Haufler et al. 1993; Mickel and Smith 2004; Baldwin et al. 2012). *Polypodium pellucidum* has transparent (pellucid) free veins and is endemic to Hawaii (Hillebrand 1888; Li 1997; Li and Haufler 1999; Palmer 2003).

The taxonomic placement of *P. scouleri* has been a matter of much historical debate. Christensen (1928) suggested that this species, with its strongly anastomosing venation, belonged with the Neotropical goniophlebioid species of *Polypodium*, many of which have been recently segregated into *Serpocaulon* (Smith et al. 2006). This idea was dispelled, in part, by Lellinger (1981), who pointed to widespread homoplasy of anastomosing venation across *Polypodium* s. l. Nevertheless, *P. scouleri* was excluded from several phylogenetic studies of the *P. vulgare* complex because it was thought to have played little or no part in reticulate speciation (Lloyd and Lang 1964; Haufler et al.1995 a, b).

Haufler and Ranker (1995) included both *P. scouleri* and *P. pellucidum* in the first *rbcL* phylogeny of *Polypodium*, and were surprised to resolve *P. scouleri* as sister to *P. glycyrrhiza* (Fig. 1 d). A later study using *trnL-trnF* plastid sequence data recovered *P. scouleri* and *P. glycyrrhiza* united in a polytomy with *P. californicum* (Fig. 1 e; Haufler et al. 2000). The discrepancy between our results and those of earlier studies may be explained by the subsequent recognition of a triploid hybrid between *P. scouleri* and the allotetraploid *P. calirhiza* (derived from *P. californicum* and *P. glycyrrhiza*; Whitmore and Smith 1991) from Pt. Reyes and Tank Hill, California (Manton 1951; Hildebrand et al. 2002). The individuals of *P. scouleri* included in the earlier molecular studies were collected in Marin County and Pt. Reyes specifically, and it is possible that they, despite resembling *P. scouleri*, were actually triploid hybrids with maternally inherited plastid genomes from either *P. californicum* or *P. glycyrrhiza* (Gastony and Yatskievych 1992; Vogel et al. 1998; Guillon and Raquin 2000). Such a scenario would explain why previous studies resolved *P. scouleri* as sister to *P. glycyrrhiza*, rather than *P. pellucidum*.

Relationships among clades A, G, C, and S—Our inferred phylogeny of the *P. vulgare* complex hints at, but provides low support for, relationships among the four diploid clades.

Clades A and G, both predominantly North American, are united with weak support (Fig. 2; MLBS = 60% and BIPP = 0.72). Somewhat better supported (Fig. 2; MLBS = 75% and BIPP = 0.91) is the sister relationship between clade C and clade S. Despite their broad geographic separation (clade C is European/Macaronesian, whereas clade S is Pacific Basin in distribution), some previous authors have suggested affinities among the taxa belonging to these two clades. In describing *P. macaronesicum*, Bobrov (1964) noted that its rhizome scales were most similar to those of *P. scouleri*, and that these two species and *P. cambricum* are all found in persistently unglaciated regions with oceanic climates. Li (1997) also suggested an affinity between the members of clade C and *P. pellucidum* based on hybridization experiments and morphological observations. He demonstrated a higher percentage of successful crosses between *P. pellucidum* and *P. macaronesicum* (5.95%) relative to crosses between *P. pellucidum* and other diploid congeners (0–0.79%). In addition, Li (1997) noted the presence of deciduous branched paraphyses in the developing sori of *P. pellucidum*, which are similar to those found in *P. cambricum* and *P. macaronesicum.*

Divergence Time Estimates and Phylogeography Among Diploid Members of the* Polypodium vulgare *Complex—Our phylogeny provides a new framework for addressing the historical events that have shaped the evolutionary relationships and geographic distributions of the diploid members of the *P. vulgare* group. To date, phylogeographic hypotheses regarding the *P. vulgare* complex have focused on the origins of the allotetraploid taxa (Haufler and Zhongren 1991; Haufler et al. 1995b; Windham and Yatskievych 2005). By their very existence, allopolyploid species indicate that diploid species, which may be allopatric at present, had geographic contact in the past. Major geographic and climatic changes, such as the Pleistocene glaciation, have been invoked to explain these distributional changes. Less attention has been accorded the historical events that may have precipitated cladogenesis and speciation of the diploid taxa (Haufler et al. 2000). By calculating divergence date estimates on our "diploids-only" phylogeny, we are able to provide new perspective on the origin of the diploids.

Polypodium sensu stricto—The present day geographic distribution of the two large monophyletic clades in *Polypodium* s. s. are largely non-overlapping. Members of the *P. plesiosorum* group (including species formerly assigned to the *P. dulce* and *P. subpetiolatum* groups) occur solely in the Neotropics, mainly south of the Tropic of Cancer, from Mexico through Central America, northern and central South America, and Hispañola (Luna-Vega et al. 2012). In contrast, members of the *P. vulgare* complex occur, with few exceptions, in the northern temperate regions of Europe, Asia, and North America (Valentine 1964; Haufler et al. 1993; Shugang and Haufler 2013). Our divergence time analysis provides support for a late Oligocene/early Miocene origin of *Polypodium* s. s. (Fig. 3 node 7; 24.77 mya, 95% HPD: 16.72–33.52 mya), and the subsequent divergence of the *P. vulgare* complex (V) and *P. plesiosorum* group (P) during the early Miocene (Fig. 3 node 8; 20.6 mya, 95% HPD: 14.00–27.06 mya). These estimates for the divergence of the mostly epiphytic *P. plesiosorum* group and the largely terrestrial *P. vulgare* complex roughly correspond to the mid-Cenozoic radiation of epiphytic ferns that followed the rise of modern tropical rain forests (Schuettpelz and Pryer 2009).

Polypodium vulgare complex—Within the *Polypodium vulgare* complex (V), an initial diversification occurred during the mid-Miocene, with all four major extant clades (Fig. 3 clades A, G, C, and S) diverging within a span of approximately two million years. These divergences show a topological pattern characteristic of ancient rapid radiations (Whitfield and Lockhart 2007; Whitfield and Kjer 2008). Although the specifics are unclear, this burst of diversification most likely was driven by changing environmental conditions that provided novel ecological opportunities (Rundell and Price 2009). Subsequent diversification of extant species within each of the four major clades did not begin until the late Miocene-Pliocene, with the majority of the North American taxa diversifying near the Pliocene-Pleistocene boundary. Two geographic patterns emerge among the resulting clades: 1) both clades A and G are composed of a taxon occurring in Asia together with North American taxa; 2) both clades C and S have only two species, one with a primarily maritime or Mediterranean mainland distribution, and one endemic to volcanic islands.

Polypodium appalachianum clade (A)—The three diploid species in clade A inhabit rocky outcrops in the temperate forests of North America and eastern Asia, each with its own distinct geographic range. *Polypodium sibiricum* has a wide boreal distribution in central and western Canada, northern Japan, China, and Siberia (Haufler et al. 1993; Shugang and Haufler 2013). *Polypodium appalachianum* and *P. amorphum* have more limited and southern distributions. *Polypodium amorphum* is confined to southern British Columbia, the Cascade and Olympic Mountains in Washington, and northern Oregon along the Columbia River Valley (Lang 1969; Haufler et al. 1993). *Polypodium appalachianum* is found in eastern North America extending from Newfoundland along the Appalachian Mountains south to Alabama (Haufler and Windham 1991; Haufler et al. 1993). Their geographic distributions prompted Haufler et al. (2000) to hypothesize that these diploid taxa resulted from allopatric speciation precipitated by glaciation events in North America. Our study lends credence to this hypothesis by showing little divergence at the plastid loci analyzed and providing a relatively recent estimated divergence date for the crown group of clade A (Fig. 3 node 20; 2.18 mya, 95% HPD: 0.86–3.85 mya). This corresponds closely to the Pliocene-Pleistocene boundary, the beginning of repeated cycles of glaciation (Walker and Geissman 2009).

Most of the present-day range of *Polypodium sibiricum* in North America is above 40°N latitude, which marks the southern edge of the Laurentide Ice Sheet during the last glacial maximum (LGM), 21–18 thousand years ago (Dyke and Prest 1987; Pielou 1991; Haufler et al. 2000). We hypothesize that the common ancestor of *P. sibiricum*, *P. appalachianum,* and *P. amorphum* was broadly distributed across boreal Canada prior to the Pleistocene, but its range shifted south and became restricted to montane or coastal refugia during glacial advances. The Appalachian Mountains in the east and the Olympic Peninsula and Columbia River Valley in the west are well known Pleistocene refugia for many plant and animal species (Detling 1958; Soltis et al. 1997; Steele and Storfer 2006; Soltis et al. 2006; Barrington and Paris 2007; Zeisset and Beebee 2008; Walker et al. 2009) and are likely locations for the allopatric speciation of *P. appalachianum* and *P. amorphum*, respectively. Geographic isolation in multiple Pleistocene refugia has been invoked as a mechanism of speciation in numerous other plant and animal lineages (Avise and Walker 1998; Comes and Kadereit 1998; Willis

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and Whittaker 2000; Knowles 2001; Hewitt 2004). As the glaciers retreated, *P. sibiricum* likely expanded into its present range by dispersal from Asia, Beringia, and/or southern refugia. While it is impossible to rule out the speciation of *P. appalachianum* and *P. amorphum* occurring just prior to the beginning of the Pleistocene glaciation (as early as 3.8 mya; Fig. 3 node 20), it is evident that cycles of glaciation shaped their current distribution in North America and, likely, reinforced genetic differentiation among these three taxa (Hewitt 1996, 2001).

Polypodium glycyrrhiza clade (G)—The three members of clade G are distributed in a nearly continuous arc along the Pacific Rim from Japan to Baja California (Whitmore and Smith 1991; Haufler et al. 1993; Iwatsuki et al. 1995). *Polypodium fauriei* occurs on Cheju Island of Korea, all major islands of Japan except Okinawa, and the Kuril Islands of Russia (Iwatsuki et al. 1995). The Kamchatka Peninsula, situated just northeast of the Kuril Islands, marks the western extent of *P. glycyrrhiza*. This species arcs across the Aleutian Islands, then continues south along the coast of mainland Alaska, British Columbia, and the Pacific Northwest of the United States (Haufler et al. 1993). The San Francisco Bay area marks the southern limit of *P. glycyrrhiza* and the northern limit of *P. californicum,* which extends south along the coast into the Baja California peninsula (Haufler et al. 1993; Baldwin et al. 2012; Sigel et al. 2014).

Polypodium fauriei diverged from *P. glycyrrhiza* + *P. californicum* in the Late Miocene (Fig. 3 node 14; 8.81 mya, 95% HPD: 5.06–13.08 mya). This divergence time marks the establishment of distinct Asian and North American lineages, and corresponds to when the Bering Land Bridge was submerged and boreal forests with dissimilar species compositions were forming in northeastern Asia and northwestern North America (Hultén 1968). Similar to the species in clade A, the estimated Pliocene-Pleistocene divergence between P. *glycyrrhiza* and *P. californicum* (Fig. 3 node 18; 2.52 mya, 95% HPD: 1.14–4.12 mya), together with their geographic distributions, suggest a history of allopatric speciation that was precipitated and reinforced by the climatic cycles of the Pleistocene. The current geographic range of *P. glycyrrhiza* encompasses numerous Pleistocene coastal refugia, including Vancouver Island, the Queen Charlotte Islands, and the Columbia River Valley (Pielou 1991; Soltis et al. 1997). The present range of *P. californicum* includes the Pacific coast of the Baja California peninsula and Guadalupe Island (Mickel and Smith 2004; Sigel et al. 2014), areas also thought to harbor northern species during the Pleistocene glacial advances (Lindsay 1981; Arbogast et al. 2001; Maldonado et al. 2001; Oberbauer 2005). We hypothesize that prior to the Pleistocene, the common ancestor of *P. glycyrrhiza* and *P. californicum* spanned a coastal range from Alaska through the Pacific Northwest. Isolation in separate northern and southern refugia during repeated cycles of glaciation may have triggered and reinforced the allopatric speciation of *P. glycyrrhiza* and *P. californicum* (Hewitt 1996, 2001, 2004). Following the Last Glacial Maximum, these species likely expanded into their present ranges, coming into secondary contact in the San Francisco coastal basin (Baldwin et al. 2012).

Polypodium cambricum clade (C)—*Polypodium cambricum* and *P. macaronesicum* are the only diploid members of the *P. vulgare* complex to have a European-Macaronesian distribution. *Polypodium cambricum* is most abundant on calcareous rocks near the Mediterranean, but it ranges as far north as the British Isles (Valentine 1964; Page 1997). *Polypodium macaronesicum* is endemic to Macaronesia where it is a common epiphyte in Madeira, the Azores, and the Canary Islands (Press and Short 1994; Schäfer 2005; Vanderpoorten et al. 2007).

Polypodium cambricum and *P. macaronesicum* conform to a well-established Mediterranean-Atlantic floristic distribution pattern, whereby approximately 75% of Macaronesian plant species have a sister group found in the Mediterranean (Carine et al. 2004; Carine 2006). The dominant vegetation type of the Macaronesian islands, the laurel forest (laurisilva), is a relic of the humid, subtropical evergreen forests that were most extensive and diverse in southern Europe and northwestern Africa during the Tertiary (2.6–65.5 mya; Walker and Geissman 2009; Rodríguez-Sánchez and Arroyo 2011). Clade C is estimated to have diverged during the middle to late Miocene (Fig. 3 node 12; 11.92 mya, 95% HPD: 7.45–16.76 mya), suggesting that the most recent common ancestor of *P. cambricum* and *P. macaronesicum* may have evolved in and was broadly distributed within the Tertiary laurisilva. The divergence of these two species in the Pliocene (Fig. 3 node 17; 3.72 mya, 95% HPD: 1.53–6.24 mya) corresponds to a time of greatly diminished laurisilva on continental Europe, with isolated patches persisting in only the Mediterranean and Black Sea Basins (Sunding 1979; Mai 1987; Emerson 2002; Rodríguez-Sánchez and Arroyo 2011). Thus, both allopatric (insular) divergence and fragmentation of the ancestral laurisilva habitat may have contributed to the evolution of *P. macaronesicum* and *P. cambricum*.

Alternatively, it is possible that *P. macaronesicum* evolved on Macaronesia following a long distance dispersal event from North America, with subsequent dispersal to Europe or North Africa. If true, *Polypodium cambricum* would have likely evolved in the Mediterranean and then expanded northward. Such a scenario is consistent with a previously proposed hypothesis that the Macaronesian archipelagos acted as a stepping-stone for the spread of American taxa eastward (Sim-Sim et al. 2005), and would help explain the apparent sister relationship between clades C and S (Figs. 2 and 3). Numerous Macaronesian bryophytes, lycophytes, and ferns are phylogenetically nested within Neotropical and temperate North American lineages (summarized in Vanderpoorten et al. 2007).

Polypodium scouleri clade (S)—Much like clade C, clade S is composed of taxa primarily found in temperate oceanic or foggy forest habitats. *Polypodium scouleri* is endemic to western North America, growing on rocks or as an epiphyte on the branches and trunks of coniferous trees (Sillett and Bailey 2003). It is restricted to the salt-spray coastal zones and high rainfall coastal ranges from southern British Columbia to the San Francisco Bay area, with a disjunct population reported from high elevation sites on Guadalupe Island west of Baja California (Oberbauer 2005; Haufler et al. 1993; Baldwin et al. 2012). The other species of clade S is *P. pellucidum*, a Hawaiian endemic with four described morphological varieties occupying distinct habitats (Li 1997; Li and Haufler 1999; Palmer 2003). *Polypodium pellucidum* var. *pellucidum* occurs almost exclusively as an epiphyte living on tree trunks in rainforests. In contrast, *P. pellucidum* var. *vulcanicum* is terrestrial and inhabits barren lava flows and cinder (Li and Haufler 1999; Palmer 2003). Using genetic distance estimates derived from isozyme electrophoresis, Li (1997) hypothesized a single introduction of an epiphytic ancestor to Hawaii, followed by

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subsequent adaptations to volcanic habitats (Li and Haufler 1999). However, the geographic provenance of this ancestor was not established.

Our phylogeny shows a well-supported sister relationship between *P. scouleri* and *P. pellucidum*, suggesting the possibility of a North American origin for the ancestor of *P. pellucidum*. Because the Hawaiian Islands are of volcanic origin (approximately 80–0.5 mya; Clague and Dalrymple 1994; Geiger et al. 2007) and have never been connected to a larger landmass, all non-endemic biota must have immigrated by long-distance dispersal via wind or water. The estimated divergence time between *P. pellucidum* and *P. scouleri* (Fig. 3 node 16; 5.50 mya, 95% HPD: 2.46–8.93 mya) closely corresponds to the emergence of Kauai, the oldest of the current eight main Hawaiian Islands (Price and Clague 2002). We hypothesize that *P. pellucidum* originated from a single, long distance dispersal of a common ancestor with *P. scouleri* from the Pacific Coast of North America during the late Miocene, followed by subsequent dispersal events to all of the eight main Hawaiian Islands. The shared epiphytic habit of *P. scouleri* and *P. pellucidum* var. *pellucidum*, as well as evidence for the North American origins of several endemic Hawaiian ferns and angiosperms lends support to this hypothesis (Barrington 1993; Ranker et al. 2003; Geiger et al. 2007; Nagalingum et al. 2007; Baldwin and Wagner 2010; Vernon and Ranker 2013). Alternatively, we cannot exclude the possibility that the common ancestor of *P. pellucidum* and *P. scouleri* is of Hawaiian origin, perhaps following dispersal from Mediterranean Europe or Macaronesia. Under this scenario, the common ancestor would have dispersed from Hawaii to the Pacific coast of North America, likely aided by the jetstream from tropical latitudes in the Pacific to the west coast of North America (Gillespie and Clark 2011).

The results of this study contradict a previous hypothesis that *P. scouleri* evolved from a maritime-adapted population of *P. glycyrrhiza* (Haufler and Ranker 1995), but they do not readily evoke an alternative hypothesis to explain the origin, unique morphology, or distribution of *P. scouleri*. As with clades A and G, the present day geographic distribution of *P. scouleri* in North America could very well reflect a history involving Pleistocene refugia. *Polypodium scouleri* is part of an assemblage of northwestern plant species with disjunct distributions in the coastal islands west of California and the Baja California peninsula (Raven 1965). It is possible that the relatively cool, foggy northern highlands of Guadalupe Island served as a refugium during the southward displacement of the flora during Pleistocene glacial advances (Oberbauer 2005). However, it is equally possible that *P. scouleri* persisted in unglaciated coastal areas of the Pacific Northwest, and that its distribution was relatively unaffected by the LGM. Additional population genetic data will be required to address this and other biogeographic hypotheses presented herein.

Note added in proof: *Polypodium sanctae-rosae* has been transferred to the genus *Pleopeltis* and assigned the new combination *Pleopelis sanctae-rosae* (Maxon) A. R. Sm. & Tejero (Smith and Tejero-Díez 2014).

Acknowledgments. We thank the herbarium curators and staff at ALA, DUKE, E, and UC for their generosity in lending their collections and for allowing the removal of leaf material for DNA extraction. Many thanks to UCBG and RBGE for allowing access to their live collections, and to A. Ebihara, L. Huiet, and A. L. Vernon who collected specimens for this project. The U.S. National Forest Service Region 6 kindly granted a Forest Products Free Use Permit (number 2010-7) to E. M. S. to collect several specimens used in this study. Our gratitude goes to P. Manos for his biogeographic musings and A. Gryganskyi for his help with Russian translation. We are grateful to A. Grusz, L. Huiet, A. Johnson, T.-T. Kao, F.-W. Li, C. Rothfels, and two anonymous reviewers for their comments, edits, and criticism of earlier versions of this manuscript. This work was funded in part by a NSF Doctoral Dissertation Improvement Grant to K. M. P. and E. M. S. (DEB-1110775), a Sigma Xi Grant-in-Aid of Research to E. M. S., and a Graduate Student Research Grant to E. M. S. from The American Society of Plant Taxonomists. This article is part of E.M.S.'s doctoral dissertation in Biology at Duke University.

Literature Cited

Arbogast, B. S., R. A. Browne, and P. D. Weigel. 2001. Evolutionary genetics and Pleistocene biogeography of North American tree squirrels. *Journal of Mammalogy* 82: 302–319.

Avise, J. C. and D. Walker. 1998. Phylogeographic effects on avian populations and the speciation process. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 265: 457–463.

Barrington, D. S. 1993. Ecological and historical factors in fern biogeography. *Journal of Biogeography* 20: 275–279.

Barrington, D. S. and C. A. Paris. 2007. Refugia and migration in the Quaternary history of the New England flora. *Rhodora* 109: 369–386.

Baldwin, B. G. and W. L. Wagner. 2010. Hawaiian angiosperm radiations of North American origin. *Annals of Botany* 105: 849–879.

Baldwin, B. G., D. H. Goldman, D. J. Keil, R. Patterson, T. J. Rosatti, and D. H. Wilken, editors. 2012. *The Jepson manual: Vascular plants of California*. Ed. 2. Berkeley: University of California Press.

Beck, J. B., M. D. Windham, G. Yatskievych, and K. M. Pryer. 2010. A diploids-first approach to species delimitation and interpreting polyploid evolution in the fern genus *Astrolepis* (Pteridaceae). *Systematic Botany* 35: 223–234.

Bobrov, A. E. 1964. A comparative morphological and taxonomic study of the species of *Polypodium* L. of the flora of the U.S.S.R. *Botanicheskii Zhurnal* 49: 534–545. (Moscow and Leningrad).

Carine, A. M. 2006. Spatio-temporal relationships of the Macaronesian endemic flora: a relictual series or window of opportunity? *Taxon* 54: 895–903.

Carine, A. M., S. J. Russell, A. Santos-Guerra, and J. Francisco-Ortega. 2004. Relationships of the Macaronesian and Mediterranean floras: molecular evidence for multiple colonizations into Macaronesia and back-colonization of the continent in *Convolvulus* (Convolvulaceae). *American Journal of Botany* 91: 1070–1085.

Christensen, C. 1928. On the systematic position of *Polypodium*. *Udgivet Af Dansk Botanisk Forening* 22: 1–10.

Clague, D. A. and G. B. Dalrymple. 1994. Tectonics, geochronology, and origin of the Hawaiian-Emperor Volcanic Chain. Pp. 5–40 in *A natural history of the Hawaiian Islands. Selected readings II*, ed. E. A. Kay. Honolulu: University of Hawai'i Press.

Comes, H. P. and J. W. Kadereit. 1998. The effect of Quaternary climatic changes on plant distribution and evolution. *Trends in Plant Science* 3: 432–438.

De la Sota, E. R. 1973. On the classification and phylogeny of the Polypodiaceae. Pp. 229–244 in *The phylogeny and classification of the ferns*, eds. A. C. Jermy, J. A. Crabbe, and B. A. Thomas. London: Academic Press.

Detling, L. E. 1958. Peculiarities of the Columbia River Gorge flora. *Madroño* 14: 160–172.

Drummond, A. J., M. A. Suchard, D. Xie, and A. Rambaut. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and Evolution* 29: 1969–1973.

Dyke, A. S. and V. K. Prest. 1987. Late Wisconsinan and Holocene history of the Laurentide Ice Sheet. *Géographie physique et Quaternaire* 41: 237–263.

Emerson, B. C. 2002. Evolution on oceanic islands: Molecular phylogenetic approaches to understanding pattern and process. *Molecular Evolution* 11: 951–966.

Gastony, G. J. and G. Yatskievych. 1992. Maternal inheritance of the chloroplast and mitochondrial genomes in cheilanthoid ferns. *American Journal of Botany* 79: 716–722.

Geiger, J. M. O., T. A. Ranker, J. M. Ramp Neale, and S. T. Klimas. 2007. Molecular biogeography and origins of the Hawaiian fern flora. *Brittonia* 59: 142–158.

Gene Codes Corporation. 2005. Sequencher version 4.8 sequence analysis software. Ann Arbor, Michigan, U. S. A. http://www.genecodes.com.

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Gillespie, A. and D. H. Clark. 2011. *Glaciations of the Sierra Nevada, California, USA*. Pp. 447–462 in *Developments in Quaternary Science,* vol. 15, *Quaternary Glaciations: Extent and Chronology: A Closer Look*. St. Louis: Elsevier.

Govindarajulu, R., C. E. Hughes, and C. D. Bailey. 2011. Phylogenetic and population genetic analyses of diploid *Leucaena* (Leguminosae; Mimosoideae) reveal cryptic species diversity and patterns of divergent allopatric speciation. *American Journal of Botany* 98: 2049–2063.

Guillon, J.-M. and C. Raquin. 2000. Maternal inheritance of chloroplasts in the horsetail *Equisetum variegatum* (Schleich.). *Current Genetics* 37: 53–56.

Haufler, C. H. and T. A. Ranker. 1995. *RbcL* sequences provide phylogenetic insights among sister species of the fern genus *Polypodium*. *American Fern Journal* 85: 361–374.

Haufler, C. H. and M. D. Windham. 1991. New species of North American *Cystopteris* and *Polypodium*, with comments on their reticulate relationships. *American Fern Journal* 81: 7–23.

Haufler, C. H. and W. Zhongren. 1991. Chromosomal analyses and the origin of allopolyploid *Polypodium virginianum* (Polypodiaceae). *American Journal of Botany* 78: 624–629.

Haufler, C. H., E. A. Hooper, and J. P. Therrien. 2000. Mode and mechanisms of speciation in pteridophytes: Implications of contrasting patterns in ferns representing temperate and tropical habitats. *Plant Species Biology* 15: 223–236.

Haufler, C. H., D. E. Soltis, and P. S. Soltis. 1995a. Phylogeny of the *Polypodium vulgare* complex: Insights from chloroplast DNA restriction site data. *Systematic Botany* 20: 110–119.

Haufler, C. H., M. D. Windham, and E. W. Rabe. 1995b. Reticulate evolution in the *Polypodium vulgare* complex. *Systematic Botany* 20: 89–109.

Haufler, C. H., M. D. Windham, F. A. Lang, and S. A. Whitmore. 1993. *Polypodium*. Pp. 356–357 in *Flora of North America north of Mexico* vol. 2, ed. Flora of North America Editorial Committee. New York and Oxford: Oxford University Press.

Hennipman, E., P. Veldhoen, K. U. Kramer, and M. G. Price. 1990. Polypodiaceae. Pp. 203–230 in *The families and genera of vascular plants* vol. 1, ed. K. Kubitzki. Berlin: Springer-Verlag.

Hewitt, G. M. 1996. Some genetic consequences of ice ages, and their role in divergence and speciation. *Biological Journal of the Linnean Society. Linnean Society of London* 58: 247–276.

Hewitt, G. M. 2001. Speciation, hybrid zones and phylogeography—or seeing genes in space and time. *Molecular Ecology* 10: 537–549.

Hewitt, G. M. 2004. Genetic consequences of climatic oscillations in the Quaternary. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences* 349: 183–195.

Hildebrand, T. J., C. H. Haufler, J. P. Therrien, and C. Walters. 2002. A new hybrid *Polypodium* provides insights concerning the systematics of *Polypodium scouleri* and its sympatric congeners. *American Fern Journal* 92: 214–228.

Hillebrand, W. 1888. *Flora of the Hawaiian Islands: A description of their phanerogams and vascular cryptogams*. London: Williams and Norgate.

Hultén, E. 1968. *Flora of Alaska and neighboring territories: A manual of the vascular plants*. Stanford: Stanford University Press.

Iwatsuki, K., T. Yamazaki, D. E. Boufford, and H. Ohba. 1995. *Flora of Japan. Vol I. Pteridophyta and Gymnospermae*. Tokyo: Kodansha Ltd.

Knowles, L. L. 2001. Did the Pleistocene glaciations promote divergence? Tests of explicit refugial models in montane grasshoppers. *Molecular Ecology* 10: 691–701.

Kuo, L.-Y., F.-W. Li, W.-L. Chiou, and C.-N. Wang. 2011. First insights into fern *matK* phylogeny. *Molecular Phylogenetics and Evolution* 59: 556–566.

Kurita, S. and C. Ikebe. 1977. On the systematic position of *Pleurosoriopsis makinoi* (Maxim.) Fomin. *Journal of Japanese Botany* 52: 39–49.

Kvaček, Z. 2001. A new fossil species of *Polypodium* (Polypodiaceae) from the Oligocene of northern Bohemia (Czech Republic). *Feddes Repertorium* 112: 159–177.

Lanfear, R., B. Calcott, S. Y. W. Ho, and S. Guindon. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Molecular Biology and Evolution* 29: 1695–1701.

Lang, F. A. 1969. A new name for a species of *Polypodium* from northwestern North America. *Madroño* 20: 53–60.

Lang, F. A. 1971. The *Polypodium vulgare* complex in the Pacific Northwest. *Madroño* 21: 235–254.

Lellinger, D. B. 1981. Notes on North American ferns. *American Fern Journal* 71: 90–94.

Lewis, P. O. 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology* 50: 913–925.

Li, J. 1997. *Biosystematic analyses of the Hawaiian endemic fern species* Polypodium pellucidum *(Polypodiaceae) and related congenerics.* Ph. D. thesis. Lawrence, Kansas: University of Kansas.

Li, J. and C. H. Haufler. 1999. Genetic variation, breeding systems, and patterns of diversification in Hawaiian *Polypodium* (Polypodiaceae). *Systematic Botany* 24: 339–355.

Lindsay, S. L. 1981. Taxonomic and biogeographic relationships of Baja California chickarees (*Tamiasciurus*). *Journal of Mammalogy* 62: 673–683.

Linnaeus, C. 1753. *Species Plantarum*. Holmiae: Imprensis Laurentii Salvii.

Lloyd, R. M. 1963. New chromosome numbers in *Polypodium* L. *American Fern Journal* 53: 99–101.

Lloyd, R. M. and F. A. Lang. 1964. The *Polypodium vulgare* complex in North America. *British Fern Gazette* 9: 168–177.

Luna–Vega, I., J. D. Tejero-Díez, R. Contreras-Medina, M. Heads, and G. Rivas. 2012. Biogeographical analysis of two *Polypodium* species complexes (Polypodiaceae) in Mexico and Central America. *Biological Journal of the Linnean Society. Linnean Society of London* 106: 940–955.

Maddison, D. R. and W. P. Maddison. 2005. *McClade 4: Analysis of phylogeny and character evolution. v. 4.08*. Sunderland: Sinauer Associates.

Mai, D. H. 1987. Development and regional differentiation of the European vegetation during the Tertiary. *Plant Systematics and Evolution* 161: 79–91.

Maldonado, J. E., C. Vilà, and R. K. Wayne. 2001. Tripartite genetic subdivisions in the ornate shrew (*Sorex ornatus*). *Molecular Ecology* 10: 127–147.

Manton, I. 1947. Polyploidy in *Polypodium vulgare*. *Nature* 159: 136.

Manton, I. 1950. *Problems of cytology and evolution in the Pteridophyta*. Cambridge: Cambridge University Press.

Manton, I. 1951. Cytology of *Polypodium* in America. *Nature* 167: 37.

Martens, P. 1943. Les organes glanduleux de *Polypodium virginianum* (*P. vulgare* var. *virginianum*) I. Valeur systématique et répartition géographique. *Bulletin du Jardin botanique de l'État a Bruxelles* 17: 1–14.

Martens, P. 1950. Les paraphyses de *Polypodium vulgare* et la sous-espèce *serratum*. *Bulletin de la Sociètè Royale de Botanique de Belgique* 82: 225–262.

Mason-Gamer, R. J. and E. A. Kellogg. 1996. Testing for phylogenetic conflict among molecular datasets in the tribe Triticeae (Gramineae). *Systematic Biology* 45: 524–545.

Mickel, J. T. and A. R. Smith. 2004. *The Pteridophytes of Mexico*. New York: The New York Botanical Garden Press.

Miller, M. A., W. Pfeiffer, and T. Schwartz. 2010. Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Pp. 1–8 in Proceedings of the Gateway Computing Environments Workshop (GCE). New Orleans, Louisiana.

Moran, R. C. 1995. *Polypodium* L. Pp. 349–365 in *Flora Mesoamericana* vol. 1, eds. M. Sousa Sanchez, S. Knapp, G. Davidse, R. C. Moran, and R. Riba. St. Louis: Missouri Botanical Garden.

Nagalingum, N. S., H. Schneider, and K. M. Pryer. 2007. Molecular phylogenetic relationships and morphological evolution in the heterosporous fern genus *Marsilea*. *Systematic Botany* 32: 16–25.

Oberbauer, T. 2005. A comparison of estimated historic and current vegetation community structure of Guadalupe Island, Mexico. *Proceedings of the Sixth California Islands Symposium* 6: 143–153.

Otto, E. M., T. Janßen, H.-P. Kreier, and H. Schneider. 2009. New insights into the phylogeny of *Pleopeltis* and related Neotropical genera (Polypodiaceae, Polypodiopsida). *Molecular Phylogenetics and Evolution* 53: 190–201.

Page, C. N. 1997. *The ferns of Britain and Ireland*. Cambridge: Cambridge University Press.

Palmer, D. 2003. *Hawai'i's ferns and fern allies*. Honolulu: University of Hawai'i Press.

Peterson, R. L. and L. S. Kott. 1974. The sorus of *Polypodium virginianum*: some aspects of the development and structure of paraphyses and sporangia. *Canadian Journal of Botany* 52: 2283–2288.

Pielou, C. E. 1991. *After the ice age: the return of life to glaciated North America*. Chicago: University of Chicago Press.

Press, J. R. and M. J. Short. 1994. *Flora of Madeira*. London: The Natural History Museum.

Price, J. P. and D. A. Clague. 2002. How old is the Hawaiian biota? Geology and phylogeny suggest recent divergence. *Proceedings of the Royal Society of London. Series B: Biological Sciences* 269: 2429–2435.

Rambaut, A. and A. J. Drummond. 2007. Tracer v1.4. Available from http://beast.bio.ed.ac.uk/Tracer.

Ranker, T. A., J. M. O. Geiger, S. C. Kennedy, A. R. Smith, C. H. Haufler, and B. S. Parris. 2003. Molecular phylogenetics and evolution of the endemic Hawaiian genus *Adenophorus* (Grammitidaceae). *Molecular Phylogenetics and Evolution* 26: 337–347.

Raven, P. H. 1965. The floristics of the California islands. Pp. 57–67 in *Proceedings of the symposium on the biology of the California Islands*, ed. R. N. Philbrick. Santa Barbara: Santa Barbara Botanical Garden.

Ree, R. 2008. gapcode.py; Available at http://www.bioinformatics.org/~rick/software.html.

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Roberts, R. H. 1980. *Polypodium macaronesicum* and *P. australe*: a morphological comparison. *The Fern Gazette* 12: 69–74.

Rodríguez-Sánchez, F. and J. Arroyo. 2011. Cenozoic climate changes and the demise of Tethyan laurel forests: lessons for the future from an integrative reconstruction of the past. Pp. 280–303 in *Climate change, ecology and systematics*, eds. T. R. Hodkinson, M. B. Jones, S. Waldern, and J. A. N. Parnell. Cambridge: Cambridge University Press.

Ronquist, F. and J. P. Huelsenbeck. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics* 19: 1572–1574.

Rothfels, C. J., A. Larsson, L.-Y. Kuo, P. Korall, W.-L. Chiou, and K. M. Pryer. 2012. Overcoming deep roots, fast rates, and short internodes to resolve the ancient rapid radiation of eupolypod II ferns. *Systematic Biology* 61: 490–509.

Rumsey, F. J., L. Robba, H. Schneider, and M. A. Carine. 2014. Taxonomic uncertainty and a continental conundrum: *Polypodium macaronesicum* reassessed. *Botanical Journal of the Linnean Society* 174: 449–460.

Rundell, R. J. and T. D. Price. 2009. Adaptive radiation, nonadaptive radiation, ecological speciation and nonecological speciation. *Trends in Ecology & Evolution* 24: 394–399.

Schäfer, H. 2005. *Flora of the Azores: A field guide*. Weikersheim: Margraf Publishers.

Schmakov, A. 2001. A new *Polypodium* from Altai. *Turczaninowia* 7: 5–7.

Schneider, H., H.-P. Kreier, R. Wilson, and A. R. Smith. 2006. The *Synammia* enigma: Evidence for a temperate lineage of polygrammoid ferns (Polypodiaceae, Polypodiidae) in southern South America. *Systematic Botany* 31: 31–41.

Schneider, H., A. R. Smith, R. Cranfill, T. J. Hildebrand, C. H. Haufler, and T. A. Ranker. 2004. Unraveling the phylogeny of polygrammoid ferns (Polypodiaceae and Grammitidaceae): exploring aspects of the diversification of epiphytic plants. *Molecular Phylogenetics and Evolution* 31: 1041–1063.

Schuettpelz, E. and K. M. Pryer. 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. *Taxon* 56: 1037–1050.

Schuettpelz, E. and K. M. Pryer. 2009. Evidence for a Cenozoic radiation of ferns in an angiosperm–dominated canopy. *Proceedings of the National Academy of Sciences USA* 106: 11200–11205.

Schuettpelz, E., P. Korall, and K. M. Pryer. 2006. Plastid *atpA* data provide improved support for deep relationships among ferns. *Taxon* 55: 897–906.

Shivas, M. G. 1961a. Contributions to the cytology and taxonomy of species of *Polypodium* in Europe and America: I. cytology. *Botanical Journal of the Linnean Society* 58: 13–25.

Shivas, M. G. 1961b. Contributions to the cytology and taxonomy of species of *Polypodium* in Europe and America: II. taxonomy. *Botanical Journal of the Linnean Society* 58: 27–38.

Shugang, L. and C. Haufler. 2013. *Polypodium*. Pp. 838–839 in *Flora of China* vols. 2–3, ed. Flora of China Editorial Committee. St. Louis: Missouri Botanical Garden Press.

Sigel, E. M., M. D. Windham, A. R. Smith, R. J. Dyer, and K. M. Pryer. 2014. Rediscovery of *Polypodium calirhiza* (Polypodiaceae) in Mexico. *Brittonia* 66: 278–286

Sillett, S. C. and M. G. Bailey. 2003. Effects of the crown structure on biomass of the epiphytic fern *Polypodium scouleri* (Polypodiaceae) in redwood forests. *American Journal of Botany* 90: 255–261.

Simmons, M. P. and H. Ochoterena. 2000. Gaps as characters in sequence based phylogenetic analyses. *Systematic Biology* 49: 369–381.

Sim-Sim, M., M. G. Esquivel, S. Fontinha, and M. Stech. 2005. The genus *Plagiochila* (Dumort.) Dumort. (Plagiochilaceae, Hepaticophytina) in Madeira Archipelago—molecular relationships, ecology, and biogeographic affinities. *Nova Hedwigia* 81: 449–461.

Siplivinski, V. 1974. Notulae de Flora Baicalensi, II. *Novosti Sistematiki vysshikh rastenii* 11: 327–337.

Smith, A. R., H.-P. Kreier, C. H. Haufler, T. A. Ranker, and H. Schneider. 2006. *Serpocaulon* (Polypodiaceae), a new genus segregated from *Polypodium*. *Taxon* 55: 919–930.

Smith, A. R. and D. Tejero-Díez. 2014. *Pleopeltis* (Polypodiaceae), a redefinition of the genus and nomenclatural novelties. *Botanical Sciences* 92: 43–58.

Soltis, D. E., M. A. Gitzendanner, D. D. Strenge, and P. S. Soltis. 1997. Chloroplast DNA intraspecific phylogeography of plants from the Pacific Northwest of North America. *Plant Systematics and Evolution* 206: 353–373.

Soltis, D. E., A. B. Morris, J. S. McLachlan, P. S. Manos, and P. S. Soltis. 2006. Comparative phylogeography of unglaciated eastern North America. *Molecular Ecology* 15: 4261–4293.

Steele, C. A. and A. Storfer. 2006. Coalescent-based hypothesis testing supports multiple Pleistocene refugia in the Pacific Northwest for the Pacific giant salamander (*Dicamptodon tenebrosus*). *Molecular Ecology* 15: 2477–2487.

Sunding, P. 1979. Origins of the Macaronesian flora. Pp. 13–40 in *Plants and islands*, ed. D. Bramwell. London: Academic Press.

Swofford, D. L. 2002. PAUP* Phylogenetic analysis using parsimony (*and other methods), v. 4.0 beta 10. Sunderland: Sinauer Associates.

Taylor, T. M. C. and F. Lang. 1963. Chromosome counts in some British Columbia ferns. *American Fern Journal* 53: 123–126.

Tejero-Díez, J. D. and L. Pacheco. 2004. Taxa nuevos, nomenclatura, redefinición y distribución de las especies relacionadas con *Polypodium colpodes* Kunze (Polypodiaceae, Pteridophyta). *Acta Botanica Mexicana* 67: 75–115.

Tryon, R. M. and A. F. Tryon. 1982. *Ferns and allied plants with special reference to tropical America*. New York: Springer.

Valentine, D. H. 1964. *Polypodium*. Pp. 15–16 in *Flora Europaea* vol.1, eds. T. G. Tutin, N. A. Burges, A. O. Charter, J. R. Edmonson, V. H. Heywood, D. M. Moore, D. H. Valentine, S. M. Walters, and D. A. Webb. Cambridge: Cambridge University Press.

Vanderpoorten, A., F. J. Rumsey, and M. A. Carine. 2007. Does Macaronesia exist? Conflicting signal in the bryophyte and pteridophyte floras. *American Journal of Botany* 94: 625–638.

Vernon, A. L. and T. A. Ranker. 2013. Current status of the ferns and lycophytes of the Hawaiian Islands. *American Fern Journal* 103: 59–112.

Vogel, J. C., S. J. Russell, F. J. Rumsey, J. A. Barrett, and M. Gibby. 1998. Evidence for maternal transmission of chloroplast DNA in the genus *Asplenium* (Aspleniaceae, Pteridophyta). *Botanica Acta* 111: 247–249.

Wagner, W. H. 1964. Paraphyses: Filicineae. *Taxon* 13: 56–64.

Walker, J. D. and J. W. Geissman. 2009. 2009 GSA Geologic Time Scale. *GSA Today* 19: 60–61.

Walker, J. M., A. K. Stockman, P. E. Marek, and J. E. Bond. 2009. Pleistocene glacial refugia across the Appalachian Mountains and coastal plain in the millipede genus *Narceus*: Evidence from population genetic, phylogeographic, and paleoclimatic data. *BMC Evolutionary Biology* 9: 25–36.

Whitfield, J. B. and K. M. Kjer. 2008. Ancient rapid radiations of insects: challenges for phylogenetic analysis. *Annual Review of Entomology* 53: 449–472.

Whitfield, J. B. and P. J. Lockhart. 2007. Deciphering ancient rapid radiations. *Trends in Ecology & Evolution* 22: 258–265.

Whitmore, S. A. and A. R. Smith. 1991. Recognition of the tetraploid *Polypodium calirhiza* (Polypodiaceae) in western North America. *Madroño* 38: 233–248.

Willis, K. L. and R. J. Whittaker. 2000. The refugial debate. *Science* 287: 1406–1407.

Windham, M. D. and G. Yatskievych. 2005. A novel hybrid *Polypodium* (Polypodiaceae) from Arizona. *American Fern Journal* 95: 57–67.

Wolfe, K. H., W.-H. Li, and P. M. Sharp. 1987. Rates of nucleotide substitution vary greatly among plant mitochondrial, chloroplast, and nuclear DNAs. *Proceedings of the National Academy of Sciences USA* 84: 9054–9058.

Zeisset, I. and T. J. C. Beebee. 2008. Amphibian phylogeography: a model for understanding historical aspects of species distributions. *Heredity* 101: 109–119.

Zwickl, D. J. 2006. GARLI, vers. 0.951. *Genetic algorithm approaches for the phylogenetic analysis of large biological sequence datasets under the maximum likelihood criterion*. Ph.D. thesis. Austin: University of Texas.

Appendix 1. Specimens included in this study. Taxon name Fern Database accession number (http://fernlab.biology.duke.edu): collection locality; collector and collection number (herbarium); GenBank accessions in the order; *atpA, matK, rbcL,* and *trnG-R*. - = data not available. Nomenclatural authorities are given for the first specimen of each taxon.

Drymotaenium miyoshianum (Makino) Makino **4879:** TAIWAN. Hualien Co.: *E. Schuettpelz 1136A* (DUKE); KF909068, KF909023, -, -. ***Drymotaenium miyoshianum* -**: CHINA. Sichuan: *C. C. Liu DB06104* (PE); -, -, GQ256255.1, -. ***Microsorum fortune*** (T.Moore) Ching **4817:** TAIWAN. Nantou Co.: *E. Schuettpelz 1074A* (DUKE); -, KF909024, KF909054, -. ***Microsorum varians*** (Mett.) Hennipman & Hett. **3475:** In cultivation, Alter Botanischer Garten Goettingen:; *H. Schneider s. n.* (GOET); EF463832.1, -, -, -. ***Phlebodium decumanum*** (Willd.) J. Sm. **2384:**

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ECUADOR. Napo: *E. Schuettpelz 216* (DUKE); EF463836.1, -, EF463256.1, -. ***Platycerium stemaria*** (Beauv.) Desv. **3544:** In cultivation, Alter Botanischer Garten Goettingen: *H.-P. Krier s. n.* (GOET); EF463837, KF909025, EF463256, -. ***Platycerium superbum*** de Jonch. & Hennipman **-:** -: *E. B. Sessa s. n.* (WIS); -, -, -, JN189024. ***Pleopeltis polypodioides*** (Weath.) E.G. Andrews & Windham **6489:** MEXICO. Jalisco: *C. J. Rothfels 3031* (DUKE); KF909086, KF909027, KF909056, KF909115. ***Pleurosoriopsis makinoi*** (Makim.) Fomin **8723**: JAPAN. Fukui: *A. Ebihara 2972* (DUKE); -, KF909028, KF909057, KF909116. ***Polypodium amorphum*** Sudsk. **6951:** CANADA. British Columbia: *M. D. Windham 3404* (DUKE); KF909071, KF909009, KF909034, KF909094. ***Polypodium amorphum*** **7771:** U. S. A. Washington: *E. M. Sigel 2010-125* (DUKE); KF909072, -, KF909032, KF909095. ***Polypodium amorphum*** **7773:** U. S. A. Oregon: *E. M. Sigel 2010-104* (DUKE); KF909073, KF909010, KF909033, KF909096. ***Polypodium amorphum*** **8210:** U. S. A. Washington: *E. M. Sigel 2010-75* (DUKE); KF909069, KF909007, -, -. ***Polypodium amorphum*** **8499:** CANADA. British Columbia: *C. J. Rothfels 4065* (DUKE); KF909070, KF909008, KF909031, -. ***Polypodium appalachianum*** Haufler & Windham **7533:** U. S. A. North Carolina: *E. M. Sigel 2010-6*1 (DUKE); KF909074, KF909011, KF909035, KF909097. ***Polypodium appalachianum*** **7630:** U. S. A. Virginia: *C. J. Rothfels 3905* (DUKE); KF909075, KF909012, KF909036, KF909098. ***Polypodium appalachianum*** **8029:** CANADA. Nova Scotia: *M. J. Oldham 38496* (DUKE); KF909076, KF909013, KF909037, KF909099. ***Polypodium appalachianum*** **8045:** U. S. A. New York: *M. J. Oldham 27283* (DUKE); -, -, KF909038, KF909100. ***Polypodium californicum*** Kaulf. **3829:** U. S. A. California: *J. Metzgar 176* (DUKE); KF909082, KF909014, KF909039, -. ***Polypodium californicum*** **7249:** U. S. A. California: *L. Huiet 138* (DUKE); KF909083, -, KF909040, KF909101. ***Polypodium cambricum*** L. **8786:** ENGLAND. *H. S. McHaffie s. n.* (E); KF909065, -, KF909041, KF909102. ***Polypodium cambricum*** **8787:** SPAIN. Valencia: *Patrick James Blyth Collection Number: 8-61* (E); KF909066, KF909015, KF909042, KF909103. ***Polypodium colpodes*** Kunze **7254:** MEXICO. Chiapas: *L. Huiet 144* (DUKE); KF909067, -, KF909044, KF909104. ***Polypodium fauriei*** Christ **8722**: JAPAN. Fukui: *A. Ebihara 2973* (DUKE); KF909077, KF909016, KF909045, KF909106. ***Polypodium glycyrrhiza*** D.C. Eaton **7537:** U. S. A. California: *D. Toren 9399* (UC); -, -, KF909046, -. ***Polypodium glycyrrhiza*** **7768:** U. S. A. Oregon: *E. M. Sigel 2010-89* (DUKE); KF909080, KF909021, KF909047, KF909107. ***Polypodium glycyrrhiza*** **7781:** U. S. A. Washington: *E. M. Sigel 2010-101* (DUKE); KF909078, -, KF909048, KF909108. ***Polypodium glycyrrhiza*** **7783:** U. S. A. Washington: *E. M. Sigel 2010-85* (DUKE); KF909079, KF909018, KF909049, KF909109. ***Polypodium glycyrrhiza*** **8009:** U. S. A. Alaska: *M. H. Barker BG04-129* (ALA); -, KF909019, KF909050, KF909110. ***Polypodium glycyrrhiza*** **8161:** U. S. A. Alaska: *R. Lipkin 04-286* (ALA); -, KF909020, KF909051, KF909111. ***Polypodium glycyrrhiza*** **8523:** CANADA. British Columbia: *C. J. Rothfels 4046* (DUKE); KF909081, KF909017, KF909052, -. ***Polypodium macaronesicum*** A.E.Bobrov **8688:** SPAIN. Canary Islands: *A. Larsson 47* (DUKE); KF909084, KF909022, KF909043, KF909112. ***Polypodium martensii*** Mett. **7540:** MEXICO. Querétaro: *J. Rzedowski 53471* (UC); -, -, KF909053, KF909113. ***Polypodium pellucidum*** Kaulf. **8778:** U. S. A. Hawaii: *A. L. Vernon s. n.* (DUKE); KF909085, -, KF909055, KF909114. ***Polypodium plesiosorum*** Kunze **7160:** MEXICO. Jalisco: *J. B. Beck 1160* (DUKE); KF909087, -, -, -. ***P. plesiosorum* -:** MEXICO. *J. Lautner 01-39* (GOET); -, -, FJ825696.1, -. ***Polypodium rhodopleuron*** Kunze **610:** MEXICO. Vera Cruz: *C. H. Haufler 926* (KANU); -, -, U21145.1, -. ***Polypodium rhodopleuron*** **7255:** MEXICO. Vera Cruz: *L. Huiet 145* (DUKE); KF909088, -, -, KF909105. ***Polypodium sanctae-rosea*** (Maxon) C. Chr. **3580:** In cultivation, Alter Botanischer Garten Goettingen, originally from Mexico: *E. Schuettpelz 525* (GOET); EF463840, KF909026, EF463258, -. ***Polypodium scouleri*** Hook. & Grev **7216:** U. S. A. Washington: *M. D. Windham 94-115* (DUKE); KF909089, -, KF909058, KF909117. ***Polypodium scouleri*** **7251:** U. S. A. California: *L. Huiet 141* (DUKE); KF909090, KF909029, KF909059, KF909118. ***Polypodium sibiricum*** Sipliv. **8008:** U. S. A. Alaska: *C. L. Parker 9347* (ALA); KF909091, -, KF909063, KF909119. ***Polypodium sibiricum*** **8010:** U. S. A. Alaska: *P. Caswell 98-109* (ALA); -, -, KF909061, -. ***Polypodium sibiricum*** **8039:** CANADA. Ontario: *S. R. Brinker 1708* (DUKE); -, KF909030, KF909062, KF909120. ***Polypodium sibiricum*** **9145:** JAPAN. *C. H. Haufler s. n.* (DUKE); KF909092, -, KF909060, -. ***Polypodium subpetiolatum*** Hook. **6485:** MEXICO. Hidalgo: *C. J. Rothfels 3026* (DUKE); KF909093, -, KF909064, KF909121. ***Prosaptia contigua*** C. Presl **293:** TAIWAN. *W. L. Chiou 97-09-12-05* (TAIF); -, -, AY362345.1, -. ***Prosaptia contigua*** **4204:** MALAYSIA. Pahang: *E. Schuettpelz 786* (DUKE); EF463842, -, -, -.

Appendix 2. Node age constraints obtained from Schuettpelz and Pryer (2009) and as depicted in Fig. 3. Age constraint: node number in Fig. 3 and Appendix 3; corresponding node number from Schuettpelz and Pryer (2009, Fig. S1); best age estimate from Schuettpelz and Pryer (2009) ± one standard deviation.

A: node 3; node 357; 43.00 ± 4.30 mya. **B:** node 4; node 358; 39.20 ± 3.92 mya. **C:** node 5; node 359; 37.00 ± 3.70 mya. **D:** node 6; node 352; 30.00 ± 3.00 mya. **F:** node 9; node 361; 14.20 ± 1.42 mya.

Appendix 3. Divergence time estimates in MYA for nodes in Figure 3. Node number: Analysis 1 mean node age (95% HPD); Analysis 2 mean node age (95% HPD); Analysis 3 mean node age (95% HPD); Analysis 4 mean node age (95% HPD). NA = Node not recovered.

Node 1: 80.9093 (49.8643-116.7018); 78.5680 (48.3207-113.3601); 99.3296 (56.7785-148.1528); 94.2568391 (51.9024427-144.3365683). **Node 2**: 59.0926 (47.5398-73.5632); 58.1556 (46.6538-71.3184); 61.5822 (48.2060-77.2235); 60.8147904 (47.6548397-77.0995208). **Node 3**: 43.2737 (39.5811-47.0155); 43.2360 (39.3988-46.8713); 43.3403 (39.5048-47.0098); 43.284612 (39.4220265-46.9229102). **Node 4**: 39.1587 (36.2754-41.9505); 39.0101 (36.1520-41.9606); 39.2372 (36.3638-42.0730); 39.0308866 (36.0497452-41.9501179). **Node 5**: 36.0998 (33.0286-39.2506); 36.0304 (32.8590-39.1146); 36.1174 (32.9915-39.2393); 36.0076033 (32.8910391-39.154408). **Node 6**: 29.7660 (25.9940-33.5855); 29.6928 (25.8864-33.5223); 29.7374 (25.8946-33.4966); 29.6668509 (25.856626-33.4618959). **Node 7**: 27.8021 (25.6005-31.4888); 24.7731 (16.7244-32.7388); 27.6381 (25.5001-31.2645); 24.6547965 (17.1705195-32.3178474). **Node 8**: 22.2375 (16.5627-27.5515); 20.5928 (14.0057-27.0553); 22.0444 (16.7382-27.2800); 20.4979249 (14.6166243-26.8414717). **Node 9**: 14.6571 (11.2182-18.0584); 14.5090 (11.1799-18.0744); 14.6256 (11.2866-18.1274); 14.5497633 (11.0786795-17.97002). **Node 10**: 14.4076 (9.9477-19.3999); 13.5960 (8.9830-18.6721); 14.4694 (10.3315-19.2985); 13.6208218 (9.8967262-10.098109). **Node 11**: 13.4419 (9.0761-18.4595); 12.6786 (8.1951-17.6302); 13.4673 (9.2312-18.0843); 12.6441798 (8.489163-17.1542618). **Node 12**: 12.6070 (8.0990-17.1256); 11.9199 (7.4503-16.7618); 12.6647 (8.5236-17.1183); 11.8900939 (7.7775471-16.2409955). **Node 13**: 12.2370 (7.5440-17.4243); 11.3832 (6.8946-16.5744); 9.3523 (5.5573-13.4450); 11.3841674 (6.845854-16.1018793). **Node 14**: 9.3332 (5.3521-13.4785); 8.8067 (5.0561-13.0767); 9.2201 (5.4489-13.4990); 8.8091156 (5.1362716-12.8036577). **Node 15**: 9.3008 (5.2464-13.8106); 8.7273 (4.7765-13.0323); 5.8812 (2.8599-9.2974); 8.7155285 (4.8365912-12.8247527). **Node 16**: 5.7479 (2.4742-9.1701); 5.4933 (2.4591-8.9299); 4.0109 (1.7563-6.4851); 5.5721493 (2.5218709-9.076938). **Node 17**: 3.9132 (1.5731-6.5035); 3.7170 (1.5281-6.2363); 2.7677 (1.3417-4.4725); 3.8198199 (1.6698643-6.4611226). **Node 18**: 2.6588 (1.2852-4.3195); 2.5169 (1.1450-4.1212); 2.6264 (1.0553-4.4121); 2.6533937 (1.2691906-4.2842092). **Node 19**: 2.5202 (0.9812-4.2770); 2.3787 (0.9421-4.1207); 2.4402 (1.0302-4.2018); 2.491035 (0.9935786-4.2383673). **Node 20**: 2.2995 (0.9574-4.0619); 2.1815 (0.8579-3.8495); 1.4515 (0.6213-2.4462); 2.3593704 (0.9464934-4.084822). **Node 21**: 1.3733 (0.6031-2.3371); 1.2912 (0.5162-2.1958); 1.4353 (0.6289-2.3351); 1.3994 (0.6166255-2.3069672). **Node 22**: 1.3384 (0.5642-2.1744); 1.2661 (0.5335-2.1206); 1.3546 (0.5990-2.2961); 1.3897871 (0.5851661-2.3502674). **Node 23**: 1.2622 (0.4176-2.2920); 1.1960 (0.3919-2.2016); 1.3331 (0.4292-2.3980); 1.2580641 (0.3823619-2.3047871). **Node 24**: 1.2200 (0.3471-2.2985); 1.1538 (0.2884-2.1737); 1.3046 (0.2998-2.3730); 1.2503524 (0.2954099-2.3214561). **Node 25**: 1.1598 (0.4983-1.8718); 1.1152 (0.4810-1.8584); 1.2080 (7.7165-16.9081); 1.1977087 (0.519568-1.9518167). **Node 26**: 1.1057 (0.4685-1.8332); 1.0339 (0.4333-1.7530); 1.1774 (0.4971-1.9486); 1.1294496 (0.4548203-1.8930163). **Node 27**: 0.9822 (0.4331-1.6731); 0.7914 (0.2565-1.3793); -; -. **Node 28**: 0.7304 (0.1427-1.3746); 0.7586 (0.1806-1.3963); 0.8214 (0.2796-1.5034); 0.7808707 (0.202273-1.468254). **Node 29**: 0.6885 (0.0406-1.6038); 0.6668 (0.0498-1.5583); 0.7821 (0.2437-1.4460); 0.7665087 (0.122964-1.4402922). **Node 30**: 0.6818 (0.0350-1.5430); 0.6425 (0.0428-1.4611); 0.7303 (0.0470-1.6873); 0.7077625 (0.1580288-1.3681133). **Node 31**: -; 0.5845 (0.1547-1.1289); -; 0.7018873 (0.0393405-1.6279652). **Node 32**: 0.6314 (0.1576-1.2059); 0.5465 (0.1052-1.1135); -; 0.6913805 (0.044884-1.5962671). **Node 33**: 0.6120 (0.0770-1.3073); 0.5105 (0.1347-1.0108); 0.6801 (0.1325-1.3343); 0.6384346 (0.1611844-1.2026506). **Node 34**: 0.5883 (0.1066-1.1952); 0.4497 (0.0904-0.9022); -; 0.5976591 (0.0971099-1.2048526). **Node 35**: -; 0.4024 (0.0131-0.9512); 0.5012 (0.1174-1.0147); 0.4863264 (0.0987683-0.9673032). **Node 36:** -; 0.3992 (0.0165-0.8916); -; 0.4373267 (0.0102414-1.0037977). **Node 37**: -; 0.3428 (0.0000-0.9362); -; -. **Node 38**: 0.2690 (0.0065-0.6118); 0.3085 (0.0000-0.9507); 0.3028 (0.0002-0.7849); 0.3333279 (0.0000075-1.0251555). **Node 39**: -; 0.2737 (0.0000-0.8115); -; -. **Node 40**: 0.2301 (0.0000-0.6688); 0.2202 (0.0001-0.6561); -; 0.2150414 (0.0000212-0.6317393). **Node 41**: 0.1551 (0.0000-0.4443); 0.1473 (0.0000-0.4155); 0.1634 (0.0000-0.4753); 0.1590446 (0.0000142-0.4614606).