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ORIGIN OF HAWAIIAN *POLYSTICHUM* (DRYOPTERIDACEAE) IN THE CONTEXT OF A WORLD PHYLOGENY[1]

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A genus-wide molecular phylogeny for *Polystichum* and allied genera (Dryopteridaceae) was reconstructed to address the biogeographic origin and evolution of the three Hawaiian *Polystichum* species, all endemic there. The analysis was based on the cpDNA sequences *rbcL* and the *trnL-F* spacer from a taxonomically and geographically diverse sample. Parsimony and Bayesian phylogenetic analyses of the combined data support a monophyletic *Polystichum* and corroborate recent hypotheses as to membership and sequence of origin of the major groups within the genus. The Hawaiian *Polystichum* species are polyphyletic; two separate lineages appear to have arrived independently from the Old World. The provenance of the diploid *Polystichum hillebrandii* is continental eastern Asia, while the source of the polyploid lineage comprising tetraploid *P. haleakalense* and octoploid *P. bonseyi* is likely continental Asia. From our results, the origin of the Hawaiian species of *Polystichum,* like many Hawaiian fern genera with several species, is the result of multiple migrations to the islands, rather than single migrations yielding nearly all the local diversity as in the angiosperms. This emerging pattern provides a modern test of the premise that propagule vagility has a central role in determining pattern of evolution.

Key words: biogeography; chloroplast DNA; Dryopteridaceae; Hawaii; phylogeny; *Polystichum*; *rbcL*; *trnL-F*.

The Hawaiian Islands are a global natural treasure because of their distance from other land areas and their high natural diversity. These attributes make Hawaii a unique natural laboratory for the exploration of the forces driving the increase in diversity in native communities. For example, the islands host an extraordinarily diverse vascular-plant flora with a high proportion of endemic species: 89% of the ca. 1000 native angiosperm species (Wagner et al., 1999) and 74% of the 144 fern species (Palmer, 2003) are found only on the archipelago. Extreme geographic isolation, diverse sources of propagules, and high habitat diversity are usually included as key factors in explaining the high endemism in the Hawaiian flora. Although archipelago-level endemism is high in both Hawaiian angiosperms and Hawaiian ferns, Hawaiian fern lineages are less likely to radiate on the archipelago. Ferns have a lower percentage of generic- and species-level endemism than Hawaiian angiosperms: for every colonizing fern lineage, approximately 1.3 species have evolved in situ on the archipelago compared to 3.7 for angiosperms (Fosberg, 1948; Wagner, 1988; Wagner et al., 1999). Higher levels of gene flow between populations of Hawaiian ferns due to the high dispersability of their minute, lightweight, and easily transported spores (Tryon, 1970; Smith, 1972) have apparently led to the lower rates of speciation. The high vagility of fern spores may also explain why the proportion of species endemic to single islands within the archipelago is quite different between these two groups: 80% of angiosperms but less than 6% of ferns are single-island endemics (Ranker et al., 2000). Exploring such factors as spore dispersibility may help explain the differences in patterns of flowering plant and fern diversity and species origin and should increase our understanding of the fundamental factors in the origins of natural diversity.

Phylogenetic evidence indicates that native Hawaiian angiosperms and native Hawaiian ferns have different patterns of initial colonization and subsequent diversification. Hawaiian angiosperm species assemblages are typically the products of single colonization events and subsequent within-archipelago diversification (e.g., the Hawaiian silversword alliance, Baldwin et al., 1991; Hawaiian Lobelioideae, Givnish et al., 1995; *Tetramolopium*, Lowrey, 1995; Hawaiian mints, Lindqvist and Albert, 2002) with a couple of exceptions (*Rubus*, Howarth and Gardner, 1997; *Scaevola*, Howarth et al., 2003). Conversely, the histories of Hawaiian fern lineages are characterized by multiple colonization events followed by limited within-archipelago divergence. For instance, the Hawaiian aspleniums arrived in at least four separate introductions (Schneider et al., 2004), among which the widespread, inbreeding polyploid *Asplenium adiantum-nigrum* alone evidences at least three and as many as 17 colonizations (Ranker et al., 1994). Similarly, five colonizations appear necessary to explain the history of Hawaiian *Dryopteris* (Geiger and Ranker, 2005). However, single dispersal events have given rise to at least two groups of Hawaiian ferns—the endemic genus *Adenophorus,* with 10 species (Ranker et al., 2003), as well as the endemic genus *Diellia*, with six species (Schneider et al., 2005). In considering the origin and evolution of fern species on oceanic islands, two additional factors may be important. First, taxa that are widespread on continents are disproportionately represented among close allies of fern species on oceanic islands (Tryon, 1970). Second, apomicts and polyploids may be more common among source species of oceanic-island ferns because of their

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capacity for single-spore reproduction (Masuyama and Watano, 1990).

Comprehensive phylogenetic studies have shed light on biogeographic origins of native Hawaiian vascular-plant lineages and have allowed for the reevaluation of previous hypotheses about the source regions of Hawaiian plant lineages. Based on comparative floristics, Fosberg (1948) placed the original vascular-plant colonizers of the archipelago into six broad biogeographic categories: Indo-Pacific, Austral, American, Boreal, Pantropic, and Obscure. He proposed that the original colonizing plants came from all directions, but both Hawaiian angiosperm and Hawaiian fern colonizers migrated primarily from the Indo-Pacific region. Recent phylogenetic analyses suggest that the Hawaiian vascular-plant flora does indeed have diverse geographic origins—e.g., the Hawaiian silversword alliance, with a putative ancestor from western North America (Baldwin and Sanderson, 1998); Hawaiian *Metrosideros,* with its closest relatives in the Marquesas Islands (Wright et al., 2001); *Hesperomannia,* with its closest relatives in Africa (Kim et al., 1998); three of the five Hawaiian *Dryopteris* lineages, with Asian associations (Geiger and Ranker, 2005); and Hawaiian *Asplenium,* with at least four lineages stemming from widespread and Indo-Pacific taxa (Schneider et al., 2004). However, a rigorous test of Fosberg's hypothesis awaits a more thorough phylogenetic survey of the Hawaiian vascular-plant flora.

The homosporous fern genus *Polystichum* Roth is morphologically diverse and taxonomically complex, with about 260 species (D. Barrington, University of Vermont, unpublished manuscript). The geographic distribution of *Polystichum* is nearly cosmopolitan; centers of diversity are in Himalayan Asia (ca. 50 spp.), Yunnan and Guizhou, China (ca. 50 spp.), Sino-Japanese Asia (ca. 30 spp.), Malesia (ca. 40 spp.), and the New World tropics (ca. 70 spp.). *Polystichum* is primarily a warm-temperate and tropical genus, growing in montane regions throughout its range, often in ecologically disturbed sites. It occurs in wet forests and, at higher elevations and latitudes, in open shrubby or grassy places; a significant number are epilithic on carbonate rocks (Mickel, 1977; Kung et al., 2001). Hybridization and polyploidy have been prominent in the evolution of *Polystichum*. Eighty-two interspecific *Polystichum* hybrids have been reported, almost all of which are sterile (Knobloch, 1996). The most recent estimate of polyploids in the genus is 44% (Löve et al., 1977) with many of these identified as allopolyploids (e.g., Manton, 1950; Wagner, 1973; Barrington, 1990, 2003).

Recent phylogenetic studies have clarified the evolutionary relationships and historical biogeography of *Polystichum* and allied genera. Little and Barrington (2003), using *rbcL* sequence and morphological data for 34 species from a diversity of geographic regions, suggested that *Polystichum* is monophyletic so long as *Cyrtomium* and the earliest-diverging members of *Polystichum* sensu lato (s.l.) are recognized as distinct genera. This work also indicated that species groups within the genus tend to be confined to single continents, and morphological similarities among geographically remote taxa within the genus have likely evolved independently. Phylogenetic analyses of Chinese *Polystichum,* based on *rbcL* sequences alone (Li et al., 2004; Lu et al., 2007)) and *trnL-F* and *rps4-trnS* spacers (Li et al., in press), also support a monophyletic *Polystichum* sensu stricto (s.s.), which excludes *Cyrtomium* and the earliest diverging members of the genus. Li et al. (2004) suggested that *Polystichum* and its allies originated in its center of diversity in Yunnan and Guizhou, China and subsequently colonized other geographic regions.

There are three *Polystichum* species in the Hawaiian Islands: one diploid—*P. hillebrandii* Carruth.—and two polyploids—*P. haleakalense* Brack., a tetraploid, and *P. bonseyi* W. H. Wagner and R. Hobdy, an octoploid. All of these are endemic to the archipelago. Although each Hawaiian *Polystichum* species is morphologically distinct, the diploid *P. hillebrandii* is more strongly differentiated from both polyploid species than either polyploid is from the other. The diploid is characterized by shiny, coriaceous fronds; large indusia with nearly entire margins; weakly spinulose pinnules with cartilaginous margins; and large, amber-colored, overlapping, ovate-lanceolate petiole scales. Although each of the polyploid species has diagnostic characters, these two species also have many morphological character states in common: dull, herbaceous fronds; smaller indusia with marginal projections; strongly spinulose pinnules with noncartilaginous margins; and narrow-lanceolate petiole scales interspersed with linear hairlike scales. All species grow in upper-elevation mesic forests near the boundary with the subalpine woodland/shrubland zone. The elevational ranges of all three polystichums include, but are not limited to, this zone: tetraploid *Polystichum haleakalense* has the broadest range (1710–3230 m a.s.l.), whereas diploid *P. hillebrandii* and octoploid *P. bonseyi* have very similar elevational ranges (1400–2060 m and 1400–2000 m a.s.l., respectively). These habitat types are restricted to the volcanoes on east Maui and Hawaii, thus confining the distribution of *Polystichum* to the youngest islands of the archipelago.

According to Fosberg (1948) the Hawaiian polystichums dispersed separately to the archipelago from different biogeographic regions, but his ideas of origin for the individual species are not clear. More recently, C. Fraser-Jenkins (Kathmandu, Nepal, personal communication) has suggested that Hawaiian tetraploid *P. haleakalense* is a close relative of *P. wilsonii* H. Christ, a tetraploid widespread in Asia, and that Hawaiian octoploid *P. bonseyi* is allied with the Japanese endemic *P. tagawanum* Kurata. Fraser-Jenkins (1997) also noted the striking morphological similarity between *P. neolobatum* Nakai, an East Asian triploid, and Hawaiian diploid *P. hillebrandii*. On the other hand, the late W. H. Wagner, Jr. (University of Michigan, personal communication) suggested that *P. haleakalense* shares a common history with *P. dudleyi* Maxon, a diploid endemic to the California Floristic Province.

In this study we developed a genus-wide molecular phylogeny for *Polystichum* and allied genera from a diversity of sections and geographic regions based on two cpDNA sequences—*rbcL* and the chloroplast spacer between the *trnL* and *trnF* genes (hereafter *trnL-F*)—to address the biogeographic origin and evolution of the three Hawaiian *Polystichum* species. In particular, we sought to (1) place the three in an improved world phylogeny of *Polystichum*, (2) investigate whether the Hawaiian species are monophyletic, most likely originating from a single dispersal event, and (3) infer the provenance of the Hawaiian species from the geographic distribution of the taxa sister to Hawaiian polystichums.

MATERIALS AND METHODS

Taxon sampling—A total of 59 taxa was included in the study group. The ingroup comprised *Cyrtomium caryotideum, Cyrtomium falcatum*, *Cyrtomi-*

dictyum lepidocaulon (see Appendix for all species authorities), and 50 *Polystichum* species representing a diversity of form and geographic provenance, including members of many of the published *Polystichum* sections (Tagawa, 1940; Daigobo, 1972; Fraser-Jenkins, 1997; Roux, 2000). *Ctenitis eatonii*, two *Dryopteris* species, *Polystichopsis chaerophylloides*, and two *Phanerophlebia* species were designated as outgroups. Outgroup taxa were chosen using cladistic groupings in previous phylogenetic analyses (Hasebe et al., 1994; Little and Barrington, 2003). A complete list of taxa used in the study, with GenBank accession numbers for each marker and voucher information, is provided in the Appendix. Material for DNA extraction was of wild origin or collected from botanical gardens or herbaria. The freshly collected material was preserved in silica gel and stored at −80°C until extraction.

DNA extraction, PCR amplification, and sequencing—Total genomic DNA was extracted from leaf samples using the method of Dempster et al. (1999) based on the commonly used CTAB method (Doyle and Doyle, 1987). The chloroplast markers *rbcL* and *trnL-F* were amplified using the polymerase chain reaction (PCR) in 25-μL aliquots with the following reaction components: 50–150 ng of genomic DNA, 1 × Ex Taq buffer (TaKaRa, Madison, Wisconsin, USA), 200 μmol/L of each dNTP, 0.1 μmol/L of each primer, and 0.625 U Ex Taq (TaKaRa). A model TC-312 thermal cycler (Techne, Burlington, New Jersey, USA) was used with thermal cycling conditions as follows for *rbcL*: initial denaturation was for 5 min at 95°C; followed by 40 cycles of 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C; with a final extension of 72°C for 8 min. For *trnL-F*, initial denaturation was 5 min at 95°C; followed by 40 cycles of 1 min at 94°C, 1 min at 50°C, and 2 min at 72°C; and finishing with 72°C for 8 min. The primers used for amplification and sequencing include those published previously for the *rbcL* (Little and Barrington, 2003) and *trnL-F* regions (Taberlet et al., 1991) as well as two *rbcL* primers designed for this study (424Fnew: CTG CTT ATT CTA AAA CTT TC and 524F: ATG GTA GAG CCG TCT ACG AAT G). Before sequencing, PCR products were purified with ExoSAP-IT (USB Corp., Cleveland, Ohio, USA). The purified PCR products were sequenced in both directions using the ABI Prism BigDye Terminator Cycle Sequence Ready Reaction Kit v. 3.1 (Perkin-Elmer/Applied Biosystems, Foster City, California, USA) with the following thermal cycling parameters: initial denaturation was for 5 min at 80°C; followed by 30 cycles of 10 s at 96°C and 5 s at 50°C; and finishing with 50°C for 4 min. Sequencing products were resolved on an ABI Prism 3130xl automated sequencer (Vermont Cancer Center DNA Analysis Facility, Burlington, Vermont, USA).

Sequence alignment and indel coding—Raw forward and reverse sequences for each sample were assembled, ambiguous bases were corrected by inspection of chromatograms, and consensus sequences were edited using Sequence Navigator (Applied Biosystems, Foster City, California, USA). Consensus sequences for each region were aligned manually using MacClade v.4.06 (Maddison and Maddison, 2003) and PAUP* version 4.0b10 (Swofford, 2002). We obtained only partial *rbcL* sequence data for *Polystichum xiphophyllum* because of difficulties with PCR. The unsequenced portion of this taxon was coded as missing data. Gaps were inserted where needed for *trnL-F*. Where insertion-deletions (indels) were shared by two or more taxa and could be aligned unequivocally, they were treated as potentially phylogenetically informative. Indels were coded as independent, single, binary characters following Simmons and Ochoterena (2000). Once indels were coded, all gap regions were treated as missing data. Corrected pairwise sequence divergence per site for each chloroplast marker was calculated for the Hawaiian *Polystichum* species and their nearest allies based on the best-fit model of nucleotide sequence substitution as determined by a hierarchical likelihood ratio test in Modeltest v. 3.06 (Posada and Crandall, 1998). Aligned data for all analyses are available from the first author upon request.

Phylogenetic analyses—Separate phylogenetic analyses were conducted for each data set (*rbcL*, *trnL-F*, and the two data sets combined) employing maximum parsimony (MP) and Bayesian methods. We used PAUP* v. 4.0b10 (Swofford, 2002) for parsimony analyses. All characters were treated as unordered and equally weighted. Heuristic searches were performed on 10 000 replicates with random taxon addition, 10 trees held during tree-bisection-reconnection (TBR) branch swapping for each addition sequence, saving all trees at each step (MulTrees) with ACCTRAN character-state optimization. Bootstrap analyses (Felsenstein, 1985) were conducted to assess topological support under the following conditions for each data set: 100 bootstrap replicates with simple taxon addition, TBR branch swapping, and MulTrees option on. Only informative characters were included in bootstrap analyses. We considered bootstrap values greater than 70% to be acceptable clade support.

Bayesian phylogenetic analyses were performed with MrBayes v. 3.1 (Huelsenbeck and Ronquist, 2001). Each region was assigned its own model of nucleotide substitution, as determined by a hierarchical likelihood ratio test and Akaike information criterion (AIC) in Modeltest v. 3.06 (Posada and Crandall, 1998). For the combined data set we ran a mixed-model analysis, allowing each gene region to evolve under its own best-fit model. Posterior probabilities of the generated trees were approximated using a Markov chain Monte Carlo (MCMC) algorithm with four incrementally heated chains ($T = 0.2$) for 1 000 000 generations and sampling trees every 100 generations. Two independent runs were conducted for each data set simultaneously, the default setting in MrBayes v. 3.1. Following completion, the sampled trees from each analysis were plotted against their log-likelihood score to identify the point where log-likelihood scores reached a maximum value. All trees prior to this point were discarded as the burn-in phase, all post-burn-in trees from each run were pooled, and a 50% majority-rule consensus tree was calculated to obtain a topology with average branch lengths as well as posterior probabilities for all resolved nodes. We considered values greater than 95% to be strong support for common ancestry.

Polystichum infrageneric relationships and evolution of Hawaiian polystichums were inferred from a phylogeny developed from cpDNA sequences and thus solely reflect maternal relationships for polyploid taxa.

RESULTS

Sequence characteristics—The *trnL-F* sequences of the 59 sampled members of *Polystichum* and allies varied from 286 base pairs (bp) in *Dryopteris expansa* to 457 bp in *P. lentum*. The *trnL-F* alignment, including 10 coded indels, resulted in a matrix of 412 characters, of which 165 (40.0%) were variable and 105 (25.5%) were parsimony-informative. The mean GC content of the *trnL-F* sequences was 36.6%. There was no variation in sequence length between taxa for *rbcL*. The *rbcL* alignment consisted of 1287 characters, 265 (20.6%) of which were variable and 153 (11.9%) parsimony-informative. The mean GC content of the *rbcL* sequences was 48.0%. The aligned combined *trnL-F* and *rbcL* matrix consisted of 1699 characters (including coded indels); 430 (25.3%) of these were variable and 258 (15.2%) parsimony-informative. Divergence values for the Hawaiian polystichums and their closest neighbors, which were based on the best-fit model for each data partition (*trnL-F*: K81uf+G [Kimura, 1981]; *rbcL*: TrN+I+G [Tamura and Nei, 1988]), are reported in Table 1.

Separate phylogenetic analyses—Phylogenetic analyses were conducted for each data set employing maximum parsimony (MP) and Bayesian methods. Maximum parsimony analyses of the *trnL-F* sequence data resulted in 23 091 equally parsimonious trees of 313 steps (consistency index, including all characters, CI = 0.645; retention index, RI = 0.815), while 991 most parsimonious trees of 498 steps were recovered in the phylogenetic analysis of the *rbcL* data set (CI, including all characters = 0.576; RI = 0.643). Bayesian posterior probabilities (PP) and bootstrap support (BS) for tree structure are indicated at supported nodes (PP/BS), in both *trnL-F* (Fig. 1) and *rbcL* trees (Fig. 2). In the *trnL-F* analyses, support was weak for the deepest nodes within the tree using either maximum parsimony or Bayesian methods. *Cyrtomium* and *Phanerophlebia*, however, are strongly supported as sister taxa (PP = 98). *Cyrtomium* and *Phanerophlebia* together are resolved as the sister group to *Polystichum* (PP = 100), which is monophyletic but weakly supported (PP = 69; BS < 50). Within *Polystichum*, there are four major clades of species in

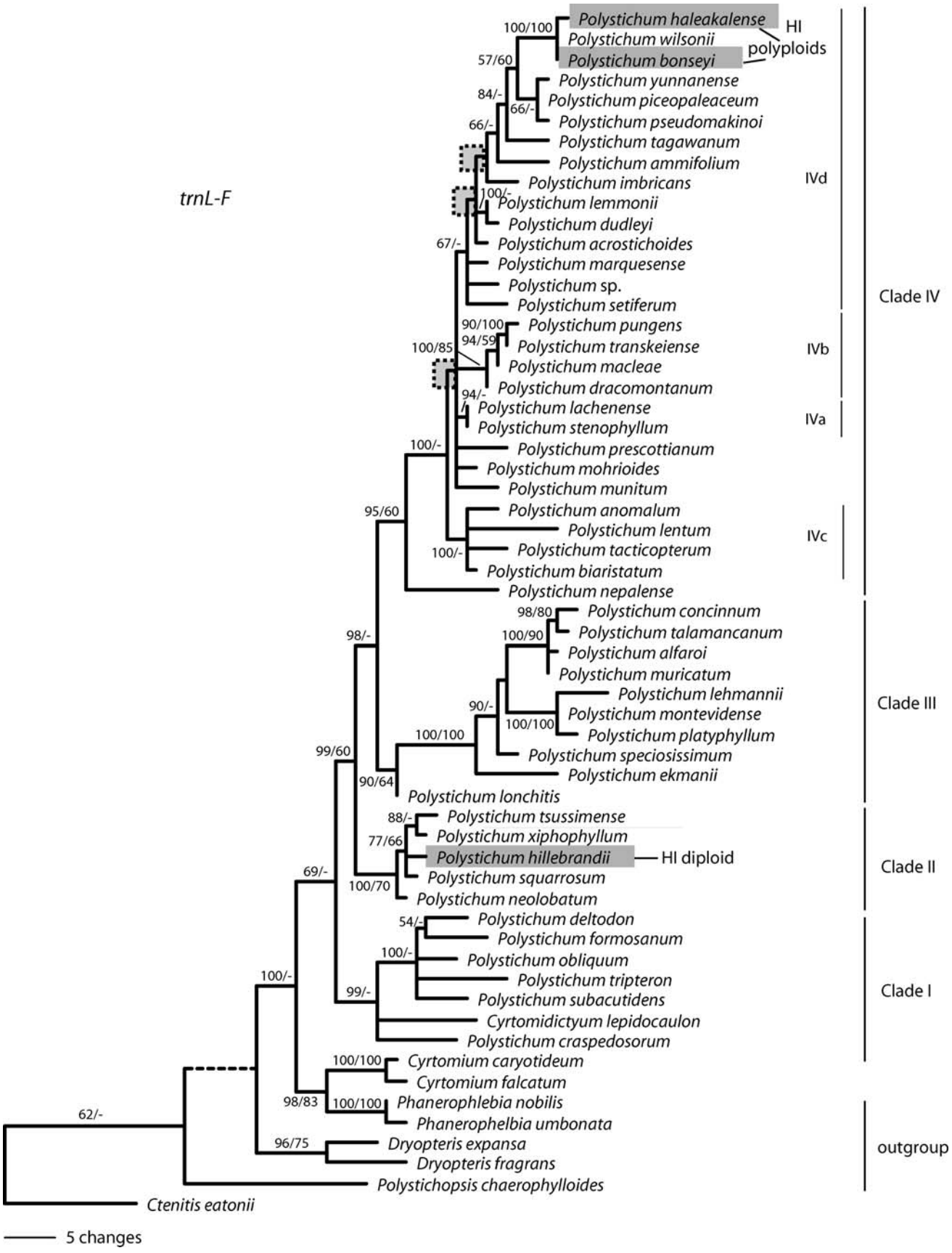
trnL-F
Polystichum haleakalense
Polystichum wilsonii
Polystichum bonseyi
HI polyploids
Polystichum yunnanense
Polystichum piceopaleaceum
Polystichum pseudomakinoi
Polystichum tagawanum
Polystichum ammifolium
Polystichum imbricans
Polystichum lemmonii
Polystichum dudleyi
Polystichum acrostichoides
Polystichum marquesense
Polystichum sp.
Polystichum setiferum
Polystichum pungens
Polystichum transkeiense
Polystichum macleae
Polystichum dracomontanum
Polystichum lachenense
Polystichum stenophyllum
Polystichum prescottianum
Polystichum mohrioides
Polystichum munitum
Polystichum anomalum
Polystichum lentum
Polystichum tacticopterum
Polystichum biaristatum
Polystichum nepalense
Polystichum concinnum
Polystichum talamancanum
Polystichum alfaroi
Polystichum muricatum
Polystichum lehmannii
Polystichum montevidense
Polystichum platyphyllum
Polystichum speciosissimum
Polystichum ekmanii
Polystichum lonchitis
Polystichum tsussimense
Polystichum xiphophyllum
Polystichum hillebrandii
HI diploid
Polystichum squarrosum
Polystichum neolobatum
Polystichum deltodon
Polystichum formosanum
Polystichum obliquum
Polystichum tripteron
Polystichum subacutidens
Cyrtomidictyum lepidocaulon
Polystichum craspedosorum
Cyrtomium caryotideum
Cyrtomium falcatum
Phanerophlebia nobilis
Phanerophelbia umbonata
Dryopteris expansa
Dryopteris fragrans
Polystichopsis chaerophylloides
Ctenitis eatonii
IVd
IVb
IVa
IVc
Clade IV
Clade III
Clade II
Clade I
outgroup
5 changes

TABLE 1. Corrected pairwise sequence divergence per site based on the best-fit model of nucleotide sequence substitution as determined by a hierarchical likelihood ratio test for each chloroplast marker. Species in the lineage containing Hawaiian diploid *Polystichum hillebrandii*. Species in the lineage containing Hawaiian polyploid taxa *P. haleakalense* and *P. bonseyi* and their nearest allies. Values below and left of the dashed line correspond to *rbcL* divergence, which was calculated using the Tamura–Nei model with invariant sites and among-site variation (TrN+I+G; Tamura and Nei, 1993) in PAUP*. Values above and right of this line correspond to *trnL-F* divergence, which was calculated using Kimura's three-parameter model with unequal base frequency and among site variation (K81uf+G; Kimura, 1981) in PAUP*.

A) Hawaiian diploid lineage	*P. hillebrandii*	*P. tsus-simense*	*P. neolobatum*	*P. squarrosum*	*P. xiphophyllum*	
Polystichum hillebrandii	—	0.01451	0.01211	0.00861	0.01156	
P. tsus-simense	0.01586	—	0.01512	0.01156	0.00861	
P. neolobatum	0.00726	0.02048	—	0.00890	0.01210	
P. squarrosum	0.00652	0.01245	0.00982	—	0.00861	
P. xiphophyllum	0.00890	0.00537	0.01246	0.00748	—	
B) Hawaiian polyploid lineage	*P. haleakalense*	*P. tagawanum*	*P. yunnanense*	*P. wilsonii*	*P. piceopaleaceum*	*P. bonseyi*
Polystichum haleakalense	—	0.02473	0.02059	0.00285	0.01844	0.00285
P. tagawanum	0.00516	—	0.02153	0.02790	0.01943	0.02793
P. yunnanense	0.00235	0.00431	—	0.02067	0.00317	0.02067
P. wilsonii	0.00078	0.00430	0.00157	—	0.01852	0.00000
P. piceopaleaceum	0.00477	0.00702	0.00237	0.00398	—	0.01855
P. bonseyi	0.00162	0.00518	0.00242	0.00081	0.00493	—

the *trnL-F* tree, each with different statistical support. Each clade, identified with Roman numerals in Fig. 1, was well supported as monophyletic in the Bayesian *trnL-F* analysis (PP $>$ 90), but there was weak or no bootstrap support for these relationships. The *trnL-F* analysis did not support Hawaiian *Polystichum* as monophyletic. The Hawaiian diploid *P. hillebrandii* is in clade II. The Hawaiian polyploid polystichums, *P. haleakalense* and *P. bonseyii*, which resolved in a trichotomy along with *P. wilsonii* (PP = 100; BS = 100), are in clade IV, a diverse evolutionary group comprised of four clades designated IVa-IVd. Clades IVa, IVb, and IVc received good support only in the Bayesian analysis (PP $>$ 93), except that clade IVb received moderate bootstrap support (BS = 85). Clade IVd was not well supported in either analysis (PP = 67; BS $<$ 50). The *trnL-F* results provide weak or no resolution of the relationships among clades IVa–IVd.

Both Bayesian and parsimony phylogenetic hypotheses of the *rbcL* data recovered the same four clades with the same membership within *Polystichum*, including the Hawaiian lineages in the same positions, with varying levels of support. Clades I and II were each strongly supported as monophyletic in both the Bayesian and maximum parsimony *rbcL* analyses (PP $>$ 99; BS = 100). Clades III and IV received strong bootstrap support (BS = 100) but were weakly supported in the Bayesian analysis (PP = 56 and 54, respectively).

There are some inconsistencies between the *rbcL* and *trnL-F* phylogenies. The structure of the relationships within clade IV on the *rbcL* tree is different from the *trnL-F* tree; each subclade of clade IV in the *trnL-F* tree had at least one difference in membership from the *rbcL* tree. In the *rbcL* analysis clade IVa and *P. prescottianum* resolve in a strongly supported trichotomy with clade IVc (PP = 98; BS = 100), while in the *trnL-F* analysis this relationship was not recovered. Also in the *rbcL* analysis, *P. yunnanense* and *P. piceopaleaceum* together were strongly supported as sister to Hawaiian polyploid species *P. haleakalense* and *P. bonseyi* and widespread *P. wilsonii* (BS = 71; PP = 99; Bayesian consensus tree not shown), a relationship not shown in the *trnL-F* analysis. The *rbcL* and *trnL*-F phylogenies are also different in the placement of *Cyrtomium* relative to *Polystichum* s.l. In the *trnL-F* analysis *Cyrtomium* is sister to *Phanerophlebia* (Fig. 1), but in the *rbcL* analysis *Cyrtomium* was positioned in *Polystichum* s.l., sister to clades II, III, and IV (Fig. 2). There were also a few notable differences in the placement of species within *Polystichum*. The positions of *Polystichum craspedosorum*, *P. nepalense*, and *P. imbricans* differed for the separate data sets (Figs. 1 and 2). *Polystichum craspedosorum* resolved in clade IV in the *rbcL* analyses (PP = 100; BS = 100), whereas in the *trnL-F* analyses it was positioned in clade I (PP = 100; BS $<$ 50). *Polystichum nepalense* also resolved in clade IV for the *rbcL* analysis, with moderate support from the Bayesian analysis (PP = 84) and strong bootstrap support (BS = 100), while in the *trnL-F* analysis *P. nepalense* was sister to the entire clade IV. *Polystichum imbricans*, which was an unresolved member of clade IVd in the *trnL-F* analysis, remained in clade IV in the *rbcL* analysis but resolved as sister to *P. munitum* outside of subclade IVd.

Because these inconsistencies did not affect the relationships of the Hawaiian taxa, the focus of this research, we did not fully explore topological incongruence and felt justified in combining *rbcL* and *trnL-F* markers for a combined analysis. To be certain that these phylogenetic discordances were not affecting relationships in other parts of the tree, we removed the species involved in the incongruent relationships and repeated the analyses. The topology of the *rbcL* and *trnL-F* phylogenies produced from these reduced taxa sets did not differ from the topologies presented here.

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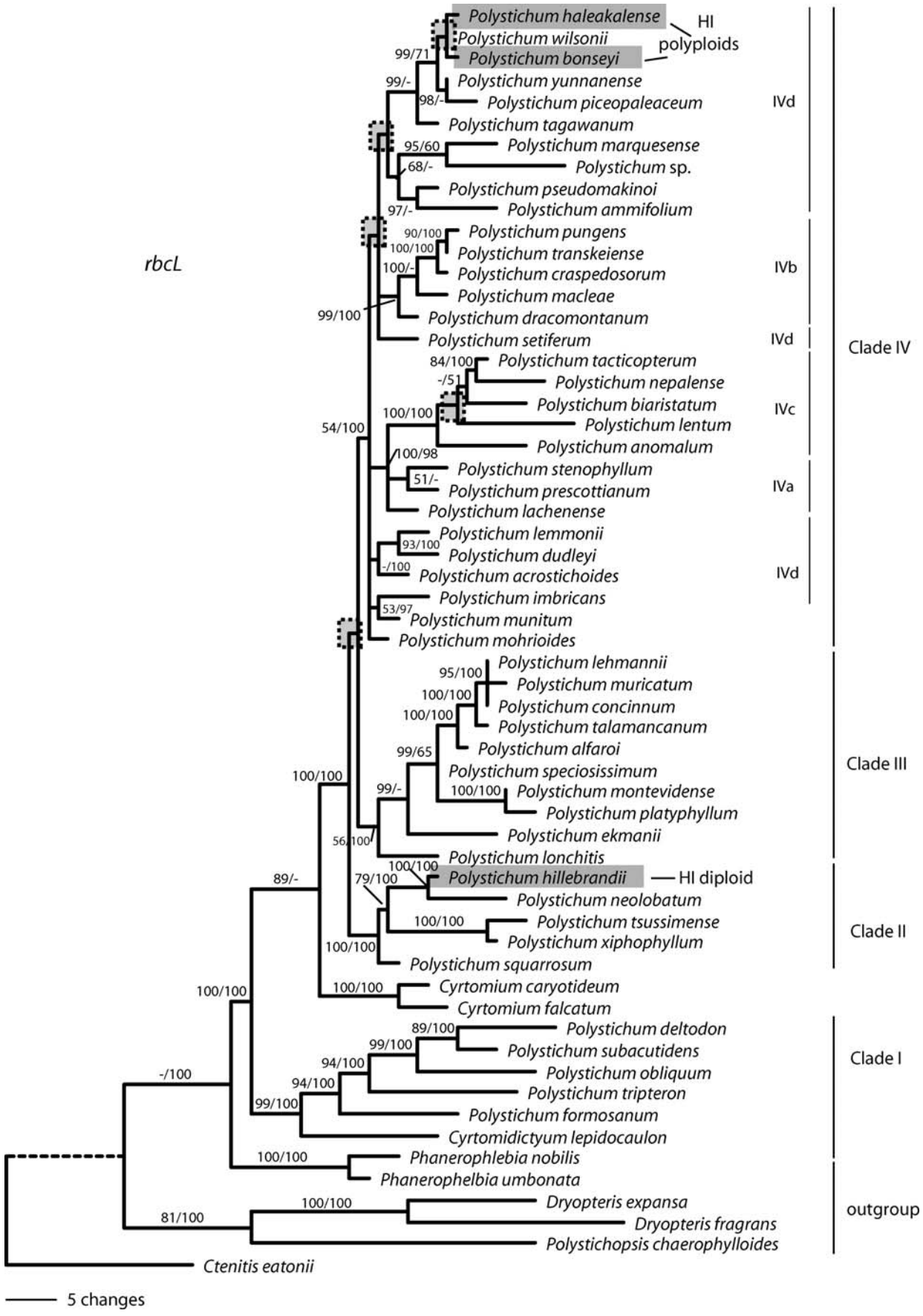
Fig. 1. One of 23 091 minimum-length trees (tree length = 313 steps; CI = 0.645; RI = 0.815) inferred from parsimony analysis of the *trnL-F* data set. Gray shading on species highlights taxa from Hawaii (HI). Branches that collapse in the 50% majority-rule consensus tree in the parsimony analysis are represented by dashed lines and short branches by gray dash-edged boxes. Measures of support are given at the nodes: Bayesian posterior probability (PP)/ maximum parsimony bootstrap support (BS). Support values less than 50 are shown as hyphens (-).


rbcL
Polystichum haleakalense
Polystichum wilsonii
Polystichum bonseyi
HI polyploids
Polystichum yunnanense
Polystichum piceopaleaceum
Polystichum tagawanum
Polystichum marquesense
Polystichum sp.
Polystichum pseudomakinoi
Polystichum ammifolium
Polystichum pungens
Polystichum transkeiense
Polystichum craspedosorum
Polystichum macleae
Polystichum dracomontanum
Polystichum setiferum
Polystichum tacticopterum
Polystichum nepalense
Polystichum biaristatum
Polystichum lentum
Polystichum anomalum
Polystichum stenophyllum
Polystichum prescottianum
Polystichum lachenense
Polystichum lemmonii
Polystichum dudleyi
Polystichum acrostichoides
Polystichum imbricans
Polystichum munitum
Polystichum mohrioides
Polystichum lehmannii
Polystichum muricatum
Polystichum concinnum
Polystichum talamancanum
Polystichum alfaroi
Polystichum speciosissimum
Polystichum montevidense
Polystichum platyphyllum
Polystichum ekmanii
Polystichum lonchitis
Polystichum hillebrandii
HI diploid
Polystichum neolobatum
Polystichum tsussimense
Polystichum xiphophyllum
Polystichum squarrosum
Cyrtomium caryotideum
Cyrtomium falcatum
Polystichum deltodon
Polystichum subacutidens
Polystichum obliquum
Polystichum tripteron
Polystichum formosanum
Cyrtomidictyum lepidocaulon
Phanerophlebia nobilis
Phanerophelbia umbonata
Dryopteris expansa
Dryopteris fragrans
Polystichopsis chaerophylloides
Ctenitis eatonii
IVd
IVb
IVd
IVc
IVa
IVd
Clade IV
Clade III
Clade II
Clade I
outgroup
5 changes

Combined phylogenetic analyses—The parsimony analysis of the combined *trnL-F* and *rbcL* data recovered 1175 shortest trees of 842 steps (CI, including all characters = 0.581; RI = 0.704). A 50% majority-rule consensus tree of 8993 trees obtained from a Bayesian analysis with average branch lengths (shown in Fig. 3) resulted in nearly the same topology as the maximum-parsimony analyses of the combined data set (not shown). As in the *trnL-F* phylogeny, *Polystichum* s.l. was monophyletic in the combined phylogeny (PP = 100; BS < 50) with *Cyrtomium* and *Phanerophlebia* together strongly supported as the sister group to *Polystichum*. Consistent with both individual data-set topologies, the combined analyses recovered four major clades in *Polystichum* (clades I–IV), all of which were strongly supported in the Bayesian analysis (PP > 93). Bootstrap support for clade II was strong (BS = 100), whereas support for clades I, III, and IV was moderate (BS = 75–79). The relationships among these clades were clearly resolved in the Bayesian analysis: clade III and clade IV are sister (PP = 99) with clade II sister to clades III and IV together (PP = 100). Clade I is sister to clades II, III, and IV (PP = 100). Clades IVa–IVd are present in the combined analysis with minimal change in membership, except that clade IVa together with *P. prescottianum* form a well-supported sister relationship with clade IVc.

The phylogenetic placement of the Hawaiian polystichums in the combined tree is consistent with the hypotheses from the individual data sets; Hawaiian *Polystichum* is paraphyletic. The combined data resolve the Hawaiian polyploids (*P. haleakalense* and *P. bonseyi*) together with *P. wilsonii* in a lineage (PP = 100; BS = 100) that is part of the large clade IV, and Hawaiian diploid *P. hillebrandii* is sister to *P. neolobatum* (PP = 100; BS = 84), which is in clade II (PP = 100; BS = 100).

The result from the combined analysis is taken here as the best estimate of the phylogenetic relationships of the genus because it is the best summary of the data to date. Therefore, the discussion is based on the phylogeny obtained from this analysis unless otherwise noted.

DISCUSSION

Phylogeny and biogeography of Polystichum—The evolutionary relationships presented here are largely congruent with those found in recent world phylogenetic studies of *Polystichum* based exclusively on *rbcL* (Li et al., 2004; Lu et al., 2007), *rbcL* and morphology (Little and Barrington, 2003), and on Chinese *Polystichum* and allies based on pairs of chloroplast markers (*rbcL* and *trnL-F*, Lu et al., 2005; *trnL-F* and *rps4-trnS*, Li et al., in press). However, unlike the results of the previous research with *rbcL* alone (Little and Barrington, 2003; Li et al., 2004; Lu et al., 2007) or *rbcL* and *trnL-F* (Lu et al., 2005), our phylogenetic hypothesis with a substantially larger sample suggests, albeit with limited support, that *Polystichum* s.l. as commonly circumscribed is monophyletic (Fig. 3). The present results also suggest that *Cyrtomium* is more closely related to *Phanerophlebia* than to *Polystichum* and that *Cyrtomium* and *Phanerophlebia* together are the sister group to *Polystichum*. Hence, a clade we took to be part of the study group at the outset of this study is better construed as a member of the sister group to *Polystichum* s.l. The results of our molecular analyses also depart from those of Little and Barrington (2003) in that *Polystichopsis* is not sister to *Phanerophlebia* but rather more closely related to *Dryopteris*, with *Ctenitis* sister to the *Dryopteris-Polystichopsis* clade, a relationship hypothesized by Pichi Sermolli (1977) based on morphological characters alone.

Four clades of *Polystichum* were resolved in the present analysis (Fig. 3). We have chosen names for these clades from existing *Polystichum* section names, using the oldest name for which one of our sampled species in the clade was the type of the taxon. Clade I, hereafter called the *Cyrtomiopsis* clade, represents the earliest diverging group of *Polystichum* in this study as in others (Little and Barrington, 2003; Li et al., 2004; Lu et al., 2007). Here, the *Cyrtomiopsis* clade contains seven species that have once-pinnate frond dissection and occur on carbonate rocks in eastern Asia. Species with restricted geographic distributions in this clade occur in southwest China, specifically in the provinces of Yunnan (eastern portion only), Guizhou, and adjacent regions (Kung et al., 2001).

Clade II, the *Xiphopolystichum* clade, is an east-Asian and mostly Himalayan alliance with lustrous-coriaceous fronds, prominent spinules on the pinnules, and oblique pinnule attachment. All of the known apogamous species in *Polystichum* (*P. neolobatum, P. tsus-simense, and P. xiphophyllum*) are in this clade (data from Fraser-Jenkins, 1997).

Clade III, the *Polystichum* clade, resolves the circumboreal *Polystichum lonchitis* (the type species of the genus), sister to all sampled neotropical polystichums. Within this neotropical clade, the Antillean *P. ekmanii*, a once-pinnate species that is epilithic on carbonate rocks, is sister to a clade comprising all the continental neotropical species. Among the continental neotropical species, most with Mexican affinities are indusiate, but species endemic to the Andes are all exindusiate (for further detail on the biogeography and ecology, see Barrington, 2005). The exception is exindusiate *Polystichum speciosissimum* of alpine Mexico and Costa Rica, type of the segregate genus *Plecosorus*. Given the Antillean and boreotropical distribution of the near-basal taxa in the *Polystichum* clade, a northern origin for the neotropical clade seems likely, followed by migration into the Andes and accompanied by loss of the indusium.

Clade IV is a large assemblage that has diversified throughout the world outside of tropical America. Three clades nested within clade IV are safe to name from our analysis and the other recent work. These are (1) the *Afra* clade (our clade IVb), with all species so far endemic to Africa; (2) the *Achroloma* clade (our clades IVa and IVc), including a widespread tropical Asian suite of species and a series of species that have explored the Himalayan alpine regions, some of which have been segregated in China as the genus

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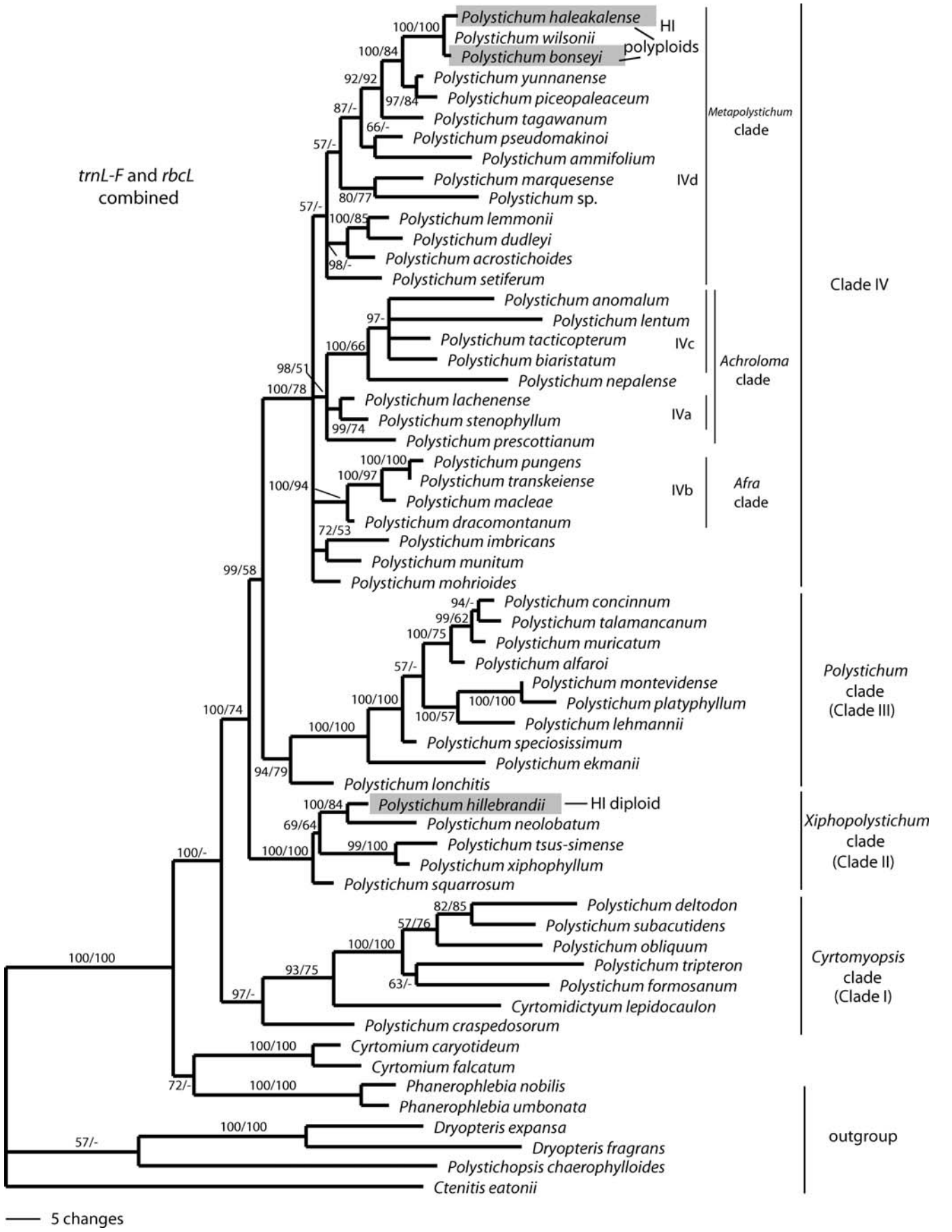
Fig. 2. One of 991 minimum-length trees (tree length = 498; CI = 0.576; RI = 0.643) inferred from parsimony analysis of the *rbcL* data set. Gray shading on species highlights taxa from Hawaii (HI). Branches that collapse in the 50% majority-rule consensus tree in the parsimony analysis are represented by dashed lines and short branches by gray dashed-edged boxes. Measures of support are given at the nodes: Bayesian posterior probability (PP)/maximum parsimony bootstrap support (BS). Support values less than 50 are shown as hyphens (-).


trnL-F and rbcL
combined
Polystichum haleakalense
Polystichum wilsonii
Polystichum bonseyi
HI
polyploids
Polystichum yunnanense
Polystichum piceopaleaceum
Polystichum tagawanum
Polystichum pseudomakinoi
Polystichum ammifolium
Polystichum marquesense
Polystichum sp.
Polystichum lemmonii
Polystichum dudleyi
Polystichum acrostichoides
Polystichum setiferum
Metapolystichum clade
IVd
Clade IV
Polystichum anomalum
Polystichum lentum
Polystichum tacticopterum
Polystichum biaristatum
Polystichum nepalense
IVc
Achroloma clade
Polystichum lachenense
Polystichum stenophyllum
Polystichum prescottianum
IVa
Polystichum pungens
Polystichum transkeiense
Polystichum macleae
Polystichum dracomontanum
IVb
Afra clade
Polystichum imbricans
Polystichum munitum
Polystichum mohrioides
Polystichum concinnum
Polystichum talamancanum
Polystichum muricatum
Polystichum alfaroi
Polystichum montevidense
Polystichum platyphyllum
Polystichum lehmannii
Polystichum speciosissimum
Polystichum ekmanii
Polystichum lonchitis
Polystichum clade (Clade III)
Polystichum hillebrandii
HI diploid
Polystichum neolobatum
Polystichum tsus-simense
Polystichum xiphophyllum
Polystichum squarrosum
Xiphopolystichum clade (Clade II)
Polystichum deltodon
Polystichum subacutidens
Polystichum obliquum
Polystichum tripteron
Polystichum formosanum
Cyrtomidictyum lepidocaulon
Polystichum craspedosorum
Cyrtomyopsis clade (Clade I)
Cyrtomium caryotideum
Cyrtomium falcatum
Phanerophlebia nobilis
Phanerophlebia umbonata
Dryopteris expansa
Dryopteris fragrans
Polystichopsis chaerophylloides
Ctenitis eatonii
outgroup
5 changes

Sorolepidium (e.g., Kung et al., 2001); and (3) the *Metapolystichum* clade (our clade IVd) of both the New and Old World, including Europe (*P. setiferum*—type species of the section), North America (*P. lemmoni*, *P. dudleyi*, and *P. acrostichoides*), Polynesia (*P. marquesense* and an undescribed species from Rapa), Africa and the Mascarene Islands (*P. wilsonii* and *P. ammifolium*), eastern China and Japan (*P. tagawanum,* and *P. pseudomakinoi*), the Himalayas and adjacent China (*P. yunnanense,* and *P. wilsonii*), and Hawaii (*P. haleakalense* and *P. bonseyi*). The well-known western North American species pair *P. munitum* and *P. imbricans* lies in clade IV, but its position is unresolved. Although there is good resolution within the clades that constitute clade IV, the relationships among these lineages lack resolution.

It is now possible to outline major evolutionary events and some morphological trends for the polystichoid ferns (which include *Cyrtomium, Phanerophlebia,* and *Polystichum* s.l.—including all candidate segregate genera) from our work and work of Little and Barrington (2003), Li et al. (2004), and Lu et al. (2005, 2007). Peltate indusia and petiole scales with marginal cilia are synapomorphies for the polystichoid ferns (Little and Barrington, 2003). A once-pinnate lineage with peltate true indusia and ciliate petiole scales appears to be ancestral in the genus *Polystichum* s.l., if *Cyrtomium* and *Phanerophlebia* are sister to the genus *Polystichum* s.l. These features are retained in the basal *Polystichum* section-*Cyrtomiopsis* clade, whose species are mostly from carbonate substrates in Yunnan, Guizhou, and adjacent regions of western China (Kung et al., 2001). (The section-*Cyrtomiopsis* clade includes species of the segregate genera *Acropelta, Ptilopteris, Cyrtogonellum*, and *Cyrtomidictyum*, as well as section *Balansae* of *Cyrtomium*—see Lu et al., 2005.) The common ancestor of the coriaceous, twice-pinnate *Xiphopolystichum* lineage diverged early in the history of *Polystichum*. Another relatively ancient event was the origin of the diverse tropical American clade. The very diverse, worldwide clade we call clade IV remains poorly resolved and is the focus of current world-level inquiry into the phylogeny of the genus.

Phylogenetic relationships and evolution of the Hawaiian species—Hawaiian *Polystichum* has long been held to be polyphyletic (Fosberg, 1948; Fraser-Jenkins, 1997; C. Fraser-Jenkins, Kathmandu, Nepal, unpublished data). A preliminary survey based on morphology and isozyme data from a small sample of the Hawaiian species conducted at the outset of this research corroborated this hypothesis. Both the distribution of allozymes and morphological characters within these taxa suggested that the evolutionary history of diploid *P. hillebrandii* was independent of the two polyploid taxa. The phylogenetic hypotheses generated from each of the three molecular data sets presented here (*rbcL*, *trnL-F* IGS, and the two combined) also strongly support the polyphyly of the endemic Hawaiian polystichums. Both maximum parsimony and Bayesian analyses of the molecular data suggest that there are two independently derived lineages of *Polystichum* in Hawaii—a diploid lineage made up of one species (*P. hillebrandii*) and a polyploid lineage with two species (tetraploid *P. haleakalense* and octoploid *P. bonseyi*).

In our molecular phylogeny, diploid *Polystichum hillebrandii* is a member of clade II, the *Xiphopolystichum* clade, along with *P. neolobatum*, *P. tsus-simense*, *P. xiphophyllum*, and *P. squarrosum*. The triploid apomict *P. neolobatum* is strongly supported as sister to *P. hillebrandii*. Although this relationship was predicted in light of their morphological similarity (Fraser-Jenkins, 1997), the nature of the relationship is unclear. The apogamous reproductive biology of triploid *P. neolobatum* precludes its involvement in the origin of the Hawaiian diploid *P. hillebrandii,* and it is quite unlikely that geographically restricted *P. hillebrandii* is a primary contributor to the origin of *P. neolobatum*, a broadly distributed Asian taxon (Tryon, 1970). Perhaps *P. hillebrandii* is a sexual cytotype of *P. neolobatum* that remains to be discovered in Asia or has gone extinct there. Alternatively, it may be that a sexual cytotype of *P. neolobatum* is the most recent common ancestor of *P. neolobatum* and *P. hillebrandii*. Given that we found the two to be different in leaf-dissection and indument characters, it is also possible that the Asian apomict is of hybrid origin, with one of the progenitors being in the same species lineage as *P. hillebrandii*.

The two Hawaiian polyploids, tetraploid *P. haleakalense* and octoploid *P. bonseyi*, consistently form a well-supported monophyletic group with *P. wilsonii*, which belongs to the large clade IV. However, the relationships within this polyploid lineage are unresolved. It has been hypothesized that widespread *P. wilsonii*, also a tetraploid, is conspecific with Hawaiian *P. haleakalense* because of the striking morphological similarity between these two taxa (C. Fraser-Jenkins, personal communication). Not surprisingly, levels of molecular divergence between these two tetraploids for both *rbcL* and *trnL-F* are very low (Table 1), suggesting (1) that these taxa share at least part of their histories through a maternal progenitor, (2) that they share a very recent common ancestor, or (3) that *P. wilsonii* migrated to the Hawaiian Islands and diverged from the source region plants, with colonizing populations going extinct. There are, however, morphological differences that distinguish these two species, such as angle of pinnule attachment, leaf dissection, and petiole scale shape. These morphological differences suggest that their histories are at least partially independent.

Although Hawaiian octoploid *Polystichum bonseyi* is morphologically distinct from *P. haleakalense* and *P. wilsonii*, our phylogenetic analyses indicate that there is little divergence in chloroplast sequences between *P. bonseyi* and the tetraploids in its lineage (Table 1). The polyploid history of *P. bonseyi* is unknown. It may be that *P. bonseyi* is an autopolyploid that arose in situ on the Hawaiian archipelago. It is also possible that *P. bonseyi* was derived from an ancestor of this lineage, which migrated to Hawaii and has since gone extinct there. Alternatively, *P. bonseyi* could be an allopolyploid that inherited its chloroplast genome from either tetraploid *P. haleakalense* or from a recent common ancestor of this lineage. The results of the preliminary isozyme analysis were consistent

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Fig. 3. Phylogeny of *Polystichum* and closest relatives obtained from combined cpDNA *rbcL* and *trnL-F* IGS sequences using Bayesian inference; average branch lengths are shown. Measures of support are given at the nodes: Bayesian posterior probability (PP)/maximum parsimony bootstrap support (BS). Support values less than 50 are shown as hyphens (-).

with both of these hypotheses about the origin of the Hawaiian octoploid. The isozymes revealed an affinity between the genomes of the Hawaiian polyploids, because they had six allozymes in common. However, unique allozymes distinguish them: the tetraploid had three and the octoploid had four. These differences suggest that the polyploids either (1) have a shared history (e.g., the octoploid is an autopolyploid derived from the tetraploid) and each has subsequently gained novel variation, or (2) they share one or more genomes, and that an additional taxon has contributed at least one genome to each of their genomic constitutions. A phylogenetic analysis based on a nuclear gene or other biparentally inherited markers would likely clarify the origins of both Hawaiian polyploids.

Biogeography of the Hawaiian species—Although multiple colonizations of the Hawaiian archipelago are uncommon among native Hawaiian angiosperms, the diversity of some genera of the Hawaiian fern flora have demonstrably resulted from multiple colonizations of the archipelago (Ranker et al., 1994; Schneider et al., 2004; Geiger and Ranker, 2005). Fosberg (1948) proposed that there were two separate *Polystichum* migrations to the Hawaiian Islands, which we have corroborated with our molecular phylogeny (Fig. 3).

The phylogenetic results suggest that both diploid and polyploid lineages of Hawaiian *Polystichum* arrived on Hawaii from the Old World and more specifically from high-montane regions of continental eastern Asia. The provenance of diploid *P. hillebrandii* is likely to be continental eastern Asia because it is sister to triploid *P. neolobatum*, which is geographically distributed throughout that region. *Polystichum neolobatum* prefers high-elevation habitats (1260–3000 m a.s.l. in China; Kung et al., 2001). All other taxa in the *Xiphopolystichum* clade, of which *P. hillebrandii* and *P. neolobatum* are members, are also from continental eastern Asia and prefer high-elevation habitats. It is likely that the colonizing ancestor of *P. hillebrandii* is from this region as well and shares high-elevation habitat preference with members of this lineage. Our data indicate that tetraploid *Polystichum haleakalense* and octoploid *P. bonseyi* are closely related to the widespread *P. wilsonii*. *Polystichum wilsonii* has a wide distribution ranging from Africa, northern India, Bhutan, and China to Taiwan (Roux, 2001), with a preference for high-elevation habitats throughout its range (2500–3400 m a.s.l. in China, Kung et al., 2001). Although *P. wilsonii* occurs in both Asia and Africa, its distribution in Africa is localized (Roux, 2001). Because widespread species are more likely than restricted species to migrate to oceanic islands (Tryon, 1970), we suggest that the likely source region of the Hawaiian polyploid lineage is high-montane continental Asia.

Conclusions—Origin of the Hawaiian *Polystichum* species, like most other Hawaiian fern groups, has resulted from multiple migrations: two migrations yielded the three species. This pattern reinforces the idea that propagule vagility is a key determinant of colonization history and evolutionary divergence on the species-rich, remote oceanic islands of the Hawaiian archipelago. Our investigation on the number of origins as well as the geographic provenances of the Hawaiian polystichums benefited from improved resolution of global phylogenetic relationships within *Polystichum* from combined coding (*rbcL*) and non-coding (*trnL-F*) chloroplast sequences. The phylogeny based on the combined cpDNA data set supports a monophyletic *Polystichum s.l.* and corroborates recent hypotheses as to membership and sequence of origin of the major groups within the genus. The phylogeny suggests that the two Hawaiian lineages appear to have originated from remotely related high-elevation species of *Polystichum* in the Old World. Taken together, these results demonstrate the power of phylogenetic analysis, as informed by geography, to provide insight into the sources and patterns of evolutionary diversity on oceanic islands.

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APPENDIX. Sources of material for DNA sequencing. Species included in this study; GenBank accession numbers for the two chloroplast regions studied; source (wild or, if cultivated [Cult.], location and accession number if available); and voucher information.

Taxon; GenBank accession number: *rbcL*, *trnL-F*; Source; *Voucher*, herbarium location (abbreviations for voucher locations follow Holmgren et al., 1990).

Ctenitis eatonii (Baker) Ching; U05614, EF177264; Wild; *M. Hasebe et al. 27600,* Taiwan, TI (see Hasebe et al., 1994).

Cyrtomidictium lepidocaulon (Hook.) Ching; AF537224, EF177266; Cult. NYBG 570/76; *source unknown*, VT.

Cyrtomium caryotideum (Wall. ex Hook. & Grev.) C. Presl; AF537225, —; *Terry et al. in 1984*, USA (Hawaii), IND (see Yatskievych et al., 1988). ***Cyrtomium caryotideum*** (Wall. ex Hook. & Grev.) C. Presl; —, EF177267; Wild; *H. Driscoll 313,* USA (Hawaii), VT. ***Cyrtomium falcatum*** (L. f.) C. Presl; AF537226, EF177268; Cult. *D. P. Little 342*, Univ. of VT Greenhouses; wild source unknown, VT.

Dryopteris expansa (C. Presl) Fraser-Jenk. & Jermy; AY268844, AY268775; Wild; *Nelson 7921*, without locality, COLO (see Geiger and Ranker, 2005). ***Dryopteris fragrans*** (L.) Schott; AY268865, AY268800; Wild; *Kelso 83–221*, USA (Alaska), COLO (see Geiger and Ranker, 2005).

Phanerophlebia nobilis (Schltdl. & Cham.) C. Presl var. ***nobilis***; AF537231, EF177269; Wild; *G. Yatskievych et al. 85–211*, Mexico, IND (see Yatskievych et al., 1988). ***Phanerophlebia umbonata*** Underw.; AF537233, EF177270; Wild; *G. Yatskievych and E. Wollenweber 83–87*, Mexico, IND (see Yatskievych et al., 1988).

Polystichopsis chaerophylloides (Poir.) C.V. Morton; EF177319, EF177265; Wild; *D. S. Conant 435*, Puerto Rico, LSC.

Polystichum acrostichoides (Michx.) Schott; AF537235, —; Wild; *D. P. Little 343*, USA (Vermont), VT. ***Polystichum acrostichoides*** (Michx.) Schott; —, EF177286; Wild; *H. Driscoll 336*, USA (Vermont), VT. ***Polystichum alfaroi*** (H. Christ) Barrington; AF537236, EF177271; Wild; *D. Barrington 1978*, Costa Rica, VT. ***Polystichum anomalum*** (Hook. & Arn.) J. Smith; EF177321, EF177277; Wild; *D. Barrington 2084* China (Yunnan), VT. ***Polystichum ammifolium*** (Poir.) C. Chr.; AF537237, EF177287; Wild; *T. Ranker, Strasberg, & Adsersen 1537*, La Reunion Island, VT.

Polystichum biaristatum (Blume) T. Moore; EF177342, EF177312; Wild; *T. Ranker 2078,* Taiwan, COLO. ***Polystichum bonseyi*** W.H. Wagner & Hobdy; EF177341, EF177311; Wild; *H. Driscoll 319,* USA (Maui, Hawaii), VT.

Polystichum concinnum Lellinger ex Barrington; EF177320, EF177276; Wild; *J. Kluge 1441*, Costa Rica, VT. ***Polystichum craspedosorum*** (Maxim.) Diels; AF537238, EF177288; Wild; *M. Kato s.n.,* Japan, VT.

Polystichum deltodon (Baker) Diels; AF537239, EF177289; Wild; *Sun Weibang s.n.*, China (Yunnan), VT. ***Polystichum dracomontanum*** Schelpe; AF537240, EF177290; Wild; *J. Roux 2715,* South Africa, NBG and VT. ***Polystichum dudleyi*** Maxon; AF537241, —; Wild; *W. A. Born s.n.,* USA (California), VT. ***Polystichum dudleyi*** Maxon; —, EF177291; Wild; *D. Barrington 1328,* USA (California), VT.

Polystichum ekmanii Maxon; AF537242, EF177272; Wild; *P. Wieczorek 215,* Dominican Republic, VT.

Polystichum formosanum Rosenst.; EF177337, EF177307; Wild; *T. Ranker 2073,* Taiwan, COLO.

Polystichum haleakalense Brack.; EF177322, EF177278; Wild; *H. Driscoll 301,* USA (Maui, Hawaii), VT. ***Polystichum hillebrandii*** Carruth.; EF177323, EF177279; Wild; *H. Driscoll 315,* USA (Maui, Hawaii), VT.

Polystichum imbricans (D.C. Eaton) D.H. Wagner; AF537262, EF177313; Wild; *D. H. Wagner 9112*, USA (Oregon), VT.

Polystichum lachenense (Hook.) Bedd.; AF537244, EF177292; Wild; *D. Boufford 27954*; China (Szechuan), HUH. ***Polystichum lehmannii*** Hieron.; AF537245, EF177273; Wild; *D. P. Little & D. Barrington 300*, Costa Rica, VT. ***Polystichum lemmonii*** Underw.; EF177324, EF177280; *P. Zika 18982,* USA (Washington) VT. ***Polystichum lentum*** (D. Don) T. Moore; AF537246, EF177293; Cult. NYBG 474/77; *source unknown*, VT. ***Polystichum lonchitis*** (L.) Roth; AF537247, —; Wild; *D.P. Little 344,* USA (Alaska), VT. ***Polystichum lonchitis*** (L.) Roth; —, EF177274; Wild; *P. Zika 19085,* USA (Washington), VT.

Polystichum macleae (Baker) Diels; AF537249, EF177294; Wild; *J. Roux 2561,* South Africa, NBG AND VT. ***Polystichum marquesense*** E. Brown; EF177325, EF177281; Wild; *K. Wood 10236*, Marquesas Islands, PTBG. ***Polystichum mohrioides*** (Bory ex Willd.) C. Presl; AF537250, EF177314; Wild; *B. Connolly 2,* Chile, VT. ***Polystichum montevidense*** (Spreng.) Rosenst.; EF177326, EF177282; Wild; *M. Sundue 621*, Bolivia, VT. ***Polystichum munitum*** (Kaulf.) C. Presl; EF177343, EF177315; Wild; *P. Zika 18932*, USA (Washington), VT. ***Polystichum muricatum*** (L.) Fée; AF537251, EF177275; Wild; *D.P. Little 349*, Costa Rica, VT.

Polystichum neolobatum Nakai; EF177331, EF177301; Wild; *D. Boufford 27183,* China (Sichuan), VT. ***Polystichum nepalense*** (Spreng.) C. Chr.; AY545499, AY534748; Wild; *S.G. Lu K49,* China (Yunnan), PYU.

Polystichum obliquum (D. Don) T. Moore; EF177328, EF177284; Wild; *D. Barrington 2090*, China (Yunnan), VT.

Polystichum piceopaleaceum Tagawa; EF177338, EF177308; Wild; *T. Ranker 2030*, Taiwan, VT. ***Polystichum platyphyllum*** (Willd.) C. Presl; EF177329, EF177285; Wild; *D. Barrington 2099,* Costa Rica, VT. ***Polystichum pseudmakinoi*** Tagawa; our EF177327, EF177283; Wild; *D. Barrington 2083,* China (Jiang Xi), VT. ***Polystichum prescottianum*** (Wall. ex Mett.) T. Moore; EF177336, EF177306; Wild; *Viane 9263*, China (Yunnan), VT. ***Polystichum pungens*** (Kaulf.) Presl; AF537253, EF177295; Wild; *J. Roux 2370,* South Africa, NBG and VT.

Polystichum setiferum (Forssk.) Moore ex Woyn.; AF537254, EF177316; Cult. NYBG; *source unknown,* Europe, VT. ***Polystichum sp. nov.;*** EF177330, EF177300; Wild; *K. Wood 9480,* Rapa, NY. ***Polystichum speciosissimum*** (A. Braun ex Kunze) Copel.; AF537255, EF177317; Wild; *Little & Barrington 297,* Costa Rica, VT. ***Polystichum squarrosum*** (D. Don) Fée; EF177339, EF177309; Wild; *D. Little 623,* Nepal (District Kaski), VT. ***Polystichum stenophyllum*** H. Christ; AF537256, EF177296; Wild; *D. Boufford 27327,* China (Sichuan), HUH, VT. ***Polystichum subacutidens*** Ching ex L.L. Xiang; AY545488, DQ150418; *S.G. Lu D8*, China (Yunnan), PYU.

Polystichum tacticopterum (Kunze) T. Moore; EF177340, EF177310; Wild; *T. Ranker 2041*, Taiwan, COLO. ***Polystichum tagawanum*** Sa. Kurata; EF177332, EF177302; Wild; *S. Tsugaru & T. Takahashi 20914,* Japan, HUH. ***Polystichum talamancanum*** Barrington; EF177335, EF177335; Wild; *Little & Barrington 299*, Costa Rica, VT. ***Polystichum transkeiense*** N. Jacobsen; AF537257, EF177297; Wild; *J. Roux 2493,* South Africa, NBG and VT. ***Polystichum tripteron*** (Kunze) C. Presl; U30832, —; Wild; *Yokoyama 5171*, Japan, TI (see Hasebe et al., 1994). ***Polystichum tripteron*** (Kunze) C. Presl; —, EF177298; Cult in Tokyo Botanical Gardens; Japan, Aomori Prefecture, *Kato s.n.,* VT. ***Polystichum tsus-simense*** (Hook.) J. Sm.; AF537258, EF177299; Wild; *Sun Weibang s.n.,* China (Yunnan), VT.

Polystichum wilsonii Christ; EF177334, EF177304; Wild; *D. Boufford 27808,* China (Sichuan), VT.

Polystichum xiphophyllum (Bak.) Diels; EF177344, EF177318; Wild; *D. Boufford 24010*, China (Dujiangyan Municipality), NY.

Polystichum yunnanense Christ; EF177333, EF177303; Wild; *D. Barrington 2087,* China, VT.