Morphological innovation, ecological opportunity, and the radiation of a major vascular epiphyte lineage

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The emergence of angiosperm-dominated tropical forests in the Cretaceous led to major shifts in the composition of biodiversity on Earth. Among these was the rise to prominence of epiphytic plant lineages, which today comprise an estimated one-quarter of tropical vascular plant diversity. Among the most successful epiphytic groups is the Polypodiaceae, which comprises an estimated 1500 species and displays a remarkable breadth of morphological and ecological diversity. Using a time-calibrated phylogeny for 417 species, we characterized macroevolutionary patterns in the family, identified shifts in diversification rate, and identified traits that are potential drivers of diversification. We find high diversification rates throughout the family, evidence for a radiation in a large clade of Paleotropical species, and support for increased rates of diversification associated with traits including chlorophyllous spores and noncordiform gametophytes. Contrary to previous hypotheses, our results indicate epiphytic species and groups with humus-collecting leaves diversify at lower rates than the family as a whole. We find that diversification rates in the Polypodiaceae are positively correlated with changes in elevation. Repeated successful exploration of novel habitat types, rather than morphological innovation, appears to be the primary driver of diversification in this group.

KEY WORDS: Adaptive radiation, fern, phylogeny, trait, vascular plants.

The emergence of angiosperm-dominated forests in the Cretaceous resulted in major shifts in the composition of biological diversity on earth (Beerling and Woodward 1997; Wang et al. 2009a; Boyce and Lee 2010). Conspicuous among these was the radiation of vascular plant lineages into the epiphytic niche (Benzing 1990; Gravendeel et al. 2004; Schneider et al. 2004; Schuettpelz and Pryer 2009; Silvera et al. 2009; Watkins and Cardelús 2012). Today epiphytes constitute an estimated 10% of global vascular plant diversity and 25% of tropical plant diversity (Nieder et al. 2001). The fourth largest family of primarily epiphytic vascular plants and perhaps the most species-rich family of ferns is the Polypodiaceae (Gentry and Dodson 1987), which comprise an estimated 1500 species and exhibit the greatest ecological and morphological diversity of any fern lineage (Watkins

and Cardelús 2012; Sundue et al. 2014). Most Polypodiaceae are tropical forest epiphytes (Smith et al. 2006), however the family also includes terrestrial, epipetric, rheophytic, climbing, and hemiepiphytic members and is represented in a diverse array of habitat types. The family includes a large, monophyletic clade known as the "grammitids," which comprise about 2/3 of the family's diversity. The remaining species are referred to as the "polypods." Morphological innovations in the family are remarkable; they include multiple independent origins of humus-collecting leaves (Hennipman and Roos 1982; Roos 1985), symbioses with ants (Gomez 1974; Gay 1991, 1993; Haufler et al. 2003) associations with epibiotic fungi (Sundue 2010a, b, 2014), chlorophyll-bearing spores (Wagner 1974; Sundue et al. 2011), CAM photosynthesis (Winter et al. 1986; Rut et al. 2008), indeterminate

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gametophyte development (Nayar and Kaur 1971), disarticulating leaves (Phillips and White 1967), and desiccation tolerance (Kessler and Siorak 2007; Watkins et al. 2007a).

Several authors have proposed that current diversity of the Polypodiaceae is the result of adaptive radiations that occurred in concert with dramatic morphological innovations in certain clades, notably the humus-collecting genera *Aglaomorpha* and *Drynaria* (Janssen and Schneider 2005; Schneider et al. 2010), the myrmecophilous genus *Lecanopteris* (Haufler et al. 2003; Schneider et al. 2010), and the grammitids, a clade of dwarfed species that comprises more than half of the family's species richness (Schneider et al. 2004; Sundue et al. 2014). By contrast, a few studies have suggested that diversification in some genera has resulted from exploration of novel ecological niches, particularly in response to major mountain-building events (Kreier et al. 2008; Wang et al. 2012). To date, a lack of sufficient DNA sequence data and analytical tools has precluded a robust family-wide test of these hypotheses. Here, we provide new insights in the evolution of the family and test hypotheses regarding the drivers of diversification by modeling macroevolutionary patterns, calculating trait-dependent speciation and extinction rates, and contrasting the correlation of ecological opportunity and morphological innovation with diversification rates.

Methods

We assembled a matrix of three molecular markers (the *rbcL* gene, *rps4-trnS* IGS, and *trnL-trnF* IGS) and 417 taxa, including 410 representatives of the Polypodiaceae and seven taxa from the sister families Davalliaceae and Oleandraceae (Schuettpelz and Pryer 2009) using data from GenBank (Table S1). To assess the impact of ecological and morphological traits on diversification, we scored nine characters based on information in the literature: mean elevation, leaf area, growth habit, leaf dissection, spore color, nest-forming ability, rhizome symmetry, and presence of epibiotic *Acrospermum* fungi (Tables S2, S3). We chose these traits because they have been hypothesized to represent important evolutionary innovations in the family (Phillips and White 1967; Nayar and Kaur 1971; Schneider et al. 2004; Janssen and Schneider 2005; Watkins et al. 2007b; Schneider et al. 2010; Sundue 2010, 2014; Sundue et al. 2011; Watkins and Cardelús 2012).

DIVERGENCE TIME ESTIMATION

We estimated divergence times using a relaxed molecular clock method implemented in BEAST version 2.1.3 (Drummond et al. 2006; Drummond and Rambaut 2007). The dataset was partitioned by marker and an unlinked GTR + G substitution model was used for all regions, as determined by PartitionFinder (Lanfear et al. 2012). We implemented a birth-death speciation tree

prior and an uncorrelated lognormal clock model that was unlinked across regions. We used two calibration points: we assigned the minimum age of the family at 33.9 Ma based on the oldest known Polypodiaceae fossil (*Protodrynaria,* Van Uffelen 1991), and the MRCA of the grammitid clade at 31.2 Ma, following Schuettpelz and Pryer (2009); clades corresponding to each of these calibration points were constrained to be monophyletic. We allowed for uncertainty in these estimates by employing a lognormal distribution prior (SD = 3.39) and a normal distribution prior (SD = 3.12) on these divergence time estimates, respectively. Three analyses were run, each for 40,000,000 generations, with parameters sampled every 1000 generations. The resulting files were combined using LogCombiner version 2.1.4. Tracer version 1.5 (Drummond and Rambaut 2007) was used to examine the posterior distribution of all parameters and estimated sample sizes. TreeAnnotator version 2.1.3 (Drummond and Rambaut 2007) was used to summarize the post burn-in trees and generate a maximum clade credibility chronogram. We used mean divergence time estimates with 95% highest posterior density (HPD) intervals for all subsequent analyses.

BAYESIAN ANALYSIS OF MACROEVOLUTIONARY MIXTURES

To model patterns of diversification in the family and to identify shifts in diversification rate, mean elevation, and leaf size, we used BAMM version 2.2.0 (Bayesian Analysis of Macroevolutionary Mixtures; Rabosky 2014). This program models speciation and extinction rates within a reverse-jump Bayesian framework and allows for identification of distinct rate regimes across a phylogeny. BAMM differs from earlier approaches to modeling shifts in diversification rate (e.g., MEDUSA; Alfaro et al. 2009) in two ways: (1) it explicitly allows for speciation and extinction rates to vary within a given diversification regime; and (2) it has the ability to account for incomplete taxon sampling on a clade-by-clade basis. We accounted for incomplete sampling in our dataset by providing clade-specific sampling proportions calculated from values provided in the literature (Table S3). Our input tree was the chronogram generated from BEAST. Although BAMM does not account for phylogenetic uncertainty, relationships for clades of interest are all well supported in our input tree.

Post-run analysis of BAMM output was performed using the "BAMMtools" package in R version 3.1.1 (R Core Team 2013). Support for shifts in net diversification rate across the tree was evaluated in four ways: (1) by mapping diversification rates across all branches of the phylogeny; (2) identifying diversification regimes across the tree with high posterior probability support; (3) mapping Bayes-factor support for the occurrence of a rate shift on nodes in the phylogeny; and (4) calculating the probability for the number of distinct rate regimes across the phylogeny. We calculated clade-specific speciation, extinction, and

diversification rates for all clades from BAMM output and used these data to generate diversification through time plots. To test relationships between diversification rate and rates of change in two commonly used niche proxies, mean elevation and leaf size (Zotz et al. 2001; Kluge and Kessler 2010), we performed phylogenetic generalized least squares (PGLS) regressions using the R package "geiger" (Harmon et al. 2008) on log-transformed clade-specific values of these data, which were obtained from the BAMM output.

TRAIT-ASSOCIATED DIVERSIFICATION

We used the binary state speciation and extinction model (BiSSE; Maddison et al. 2007) and its multistate extension (MuSSE; Fitzjohn 2012) modified for incompletely sampled phylogenies (Fitzjohn et al. 2009) to evaluate trait-associated differences in diversification rate. BiSSE and MuSSE were implemented in the "diversitree" package in R (Fitzjohn 2012). Traits were scored as described previously except for leaf dissection, which was scored with three states (simple, 1-pinnate, more divided) to minimize the effects of uneven character state distribution on the analysis. Model parameters were estimated from the time-calibrated tree using a maximum likelihood search algorithm; these values were used as a starting point for subsequent Markov Chain Monte Carlo (MCMC) sampling in a Bayesian framework. Priors for each parameter used an exponential distribution and we combined results from two separate MCMC chains run for 2500 generations each. We tested for differences in diversification rates between character states as recovered from our MCMC sampling using two-tailed t-tests for binary characters and one-way analysis of variance for leaf dissection. To quantify effect size of trait-associated diversification rate differences, we calculated effect size as Cohen's D (d) for binary characters and Eta-squared (η^2) for leaf dissection.

Although SSE models may fail to accurately characterize association of traits and diversification for small phylogenies and/or those with unbalanced trait distribution (Davis et al. 2013) and the potential for Type-I errors has been well documented (Maddison et al. 2007; Maddison and Fitzjohn 2015; Rabosky and Goldberg 2015), they represent the most robust framework available for characterizing trait-associated diversification. Further, our dataset satisfies the criteria presented by Davis et al. (2013) with respect to sample size and trait distribution for implementation of these models. Following the suggestion of Rabosky and Goldberg (2015), we assessed the potential for Type-I errors in our dataset by testing the association of diversification with a neutral, simulated trait. We scored all taxa for a neutral binary character based on the first letter of their specific epithet (one group of A-M, the other of N-Z) and ran the analyses described above.

Results

DIVERGENCE TIME ESTIMATION

The topology of our phylogeny is generally congruent with those of previous studies of the Polypodiaceae (Ranker et al. 2004; Schneider et al. 2004; Schuettpelz and Pryer 2007; Sundue et al. 2010), including weak support for the position of the drynarioid and selligueid ferns, which our analysis recovered as nested within a large clade of Neotropical polypod genera (Fig. 1). The position of this clade has been inconsistent or unresolved in previous studies (Schneider et al. 2004, 2010; Schuettpelz and Pryer 2007). Our divergence time estimates (Fig. S1) for major clades are also similar to those reported by Schuettpelz and Pryer (2009).

BAYESIAN ANALYSIS OF MACROEVOLUTIONARY MIXTURES

Our BAMM analyses recovered a general increase in diversification rate over the family's evolutionary history (Fig. 2A). We also found strong support for a single shift in diversification rate corresponding to the Old World genus *Lepisorus* (Fig. 2B–D). Posterior probability and Bayes-factor support indicate that this increase in diversification rate coincided with early divergence events in the genus, though it is unclear at which node this shift occurred (Fig. 2). Additional patterns of diversification not associated with this single major rate shift include a general increase in diversification rate over the family's evolutionary history, minor increases in diversification relative to the background rate in the selligueids and the *Oreogrammitis* clade (Figs. 2, 3), and a reduction in diversification rate in *Platycerium* (Fig. 2). These were not, however, supported as distinct from the background rate regime.

PGLS regressions recovered a positive correlation between diversification rates and rates of elevation change (Fig. 4A, B, D, $R^2 = 0.29$, $P < 0.001$) but no association of diversification rate with rates of leaf area evolution (Fig. 4B, C, E, $R^2 = 0.01$, $P = 0.66$).

TRAIT-ASSOCIATED DIVERSIFICATION

Analysis of the neutral simulated trait found no evidence of an association between diversification rate and character state (two-tailed t-test, $t = 0.726$, $P = 0.352$), so we proceeded with our analyses.

Our results indicate that diversification rates were significantly different between states of all seven traits that were investigated (Fig. 5). Terrestrial habit was associated with higher diversification rates ($r = 0.260$) than epiphytic habit ($r = 0.147$); the associated effect size ($d = 3.65$) was the greatest among all traits examined. Diversification rate was highest among taxa with once-pinnate leaves ($r = 0.201$), followed by simple-leaved taxa
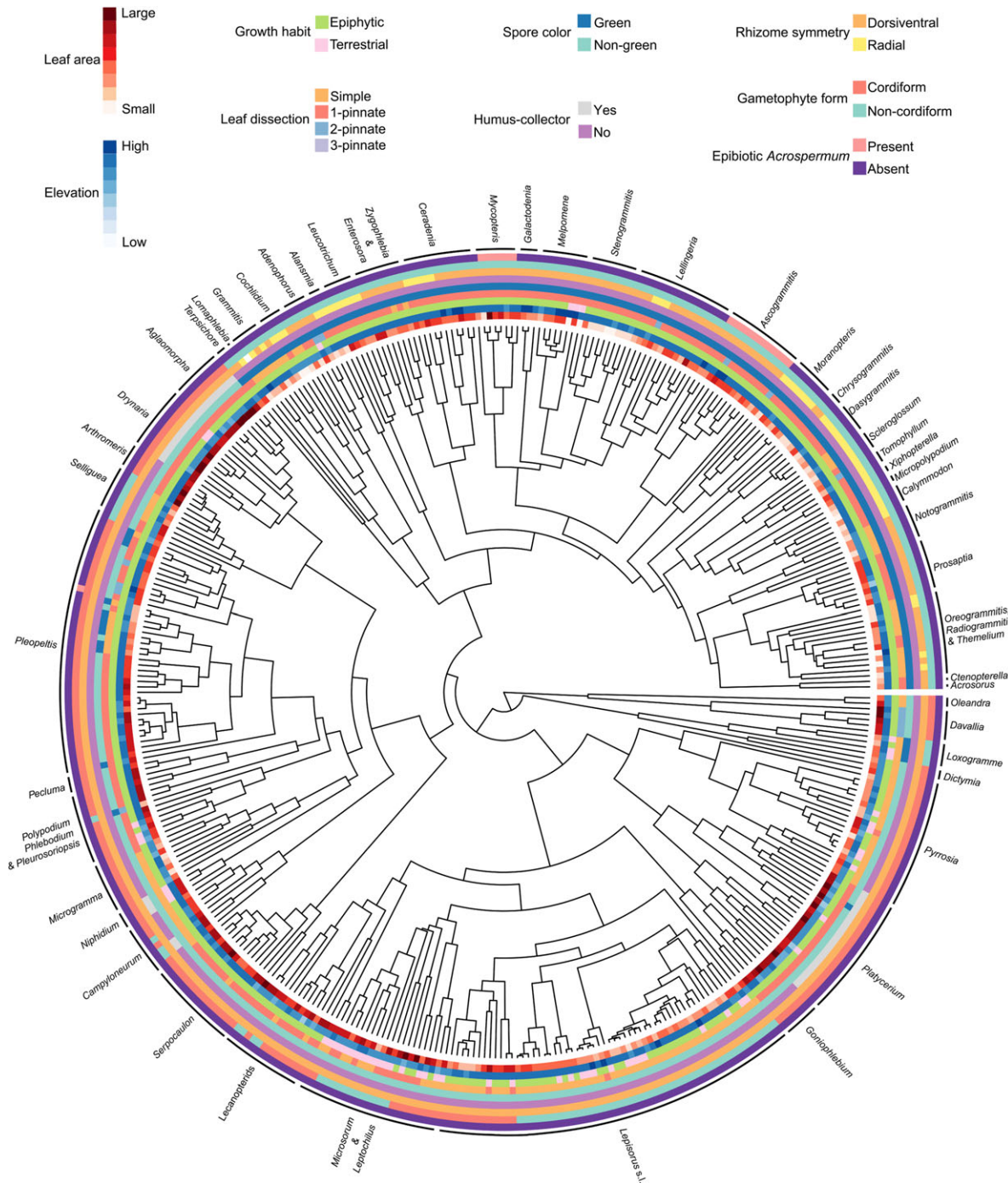
Figure 1. Polypodiaceae phylogeny with terminals scored for eight traits (from inner to outer circles): leaf area, lamina dissection, elevation, spore color, leaf nesting, rhizome symmetry, gametophyte morphology, and growth habit. Species are grouped by genus (or groups of genera in the case of polyphyletic groupings).

($r = 0.121$), whereas taxa with highly dissected (2- and 3-pinnate) leaves exhibited much lower ($r = 0.067$) rates; effect size for these differences was moderate ($\eta^2 = 0.32$). Spore color was associated with a strong effect ($d = 2.92$) on diversification, with green-spored taxa diversifying faster ($r = 0.208$) than those with nongreen spores ($r = 0.145$). Lower diversification rates were associated with nest-forming taxa ($r = 0.151$) relative to those that do not form humus-collecting nests ($r = 0.227$); the corresponding effect size was large ($d = 3.08$). Dorsiventral rhizome symmetry was associated with higher diversification rates ($r = 0.155$) than radial symmetry ($r = 0.136$); the effect size recovered for this difference was relatively weak ($d = 0.97$).
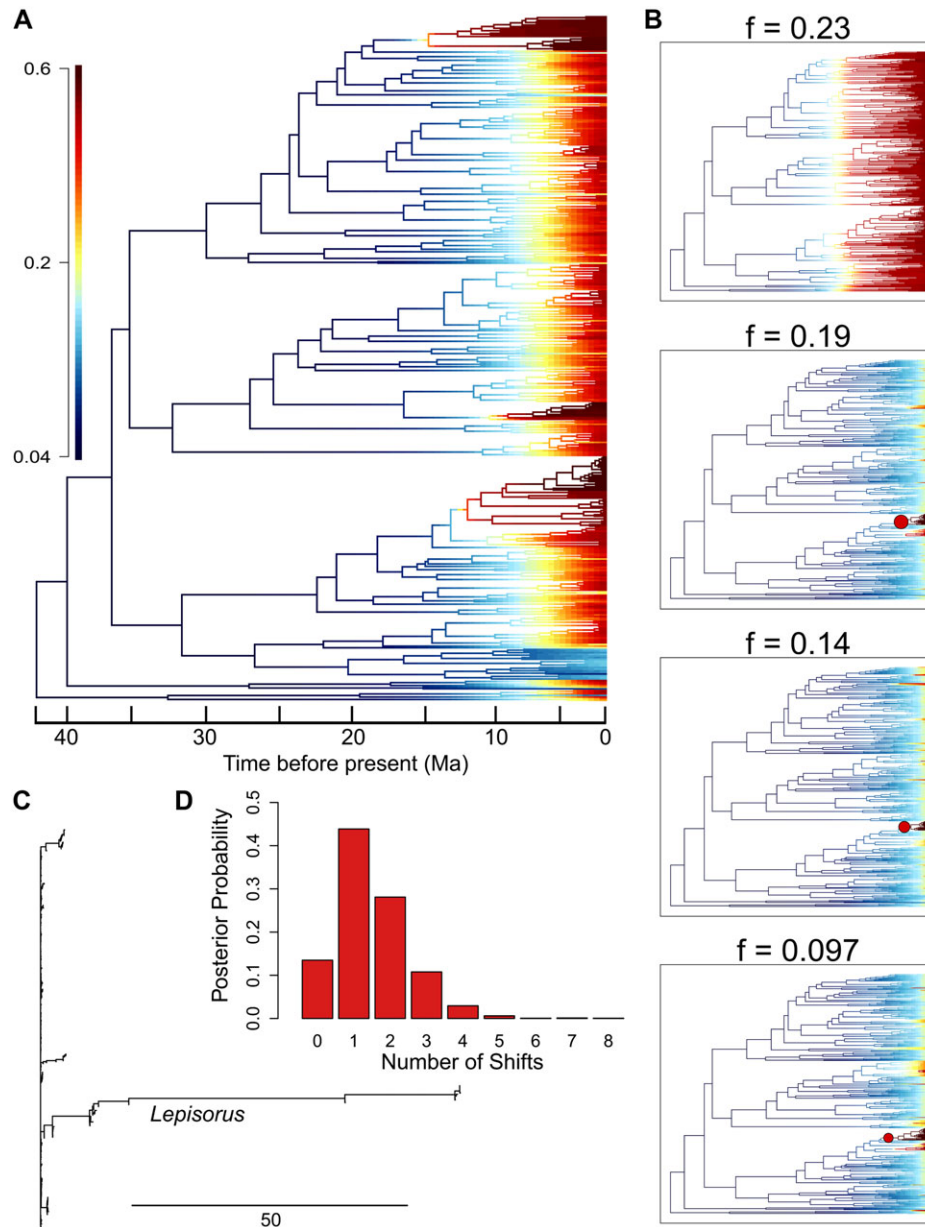
Figure 2. Diversification rate heterogeneity in the Polypodiaceae. (A) BAMM phylorate plot showing diversification rate across the Polypodiaceae where rates increase from blue to red. Rate values represent new lineages per million years. (B) Top four most credible shift sets recovered by BAMM with associated probabilities (*f*). The red circle indicates location of estimated shift. (C) Phylogeny of Polypodiaceae with branch lengths transformed to Bayes-factor support for rate shifts. (D) Posterior distribution for number of distinct diversification processes on the Polypodiaceae phylogeny recovered by BAMM.

Gametophyte morphology was associated with a strong effect ($d = 2.98$) on diversification; taxa with noncordiform gametophytes diversified faster ($r = 0.188$) than those with cordiform gametophytes ($r = 0.148$). Taxa possessing an association with epibiotic *Acrospermum* fungi were found to diversify faster ($r = 0.186$) than those lacking such associations ($r = 0.162$); the effect size of this difference was small ($d = 0.69$).

Discussion

Diversification rates in the Polypodiaceae are high compared to those of most fern lineages (Schuettpelz and Pryer 2009; Fiz-Palacios et al. 2011). Following their divergence from the Davalliaceae about 42 Ma, diversification rates in the Polypodiaceae have increased with little variation across most lineages (but see discussion below). Consistent with the conclusions of
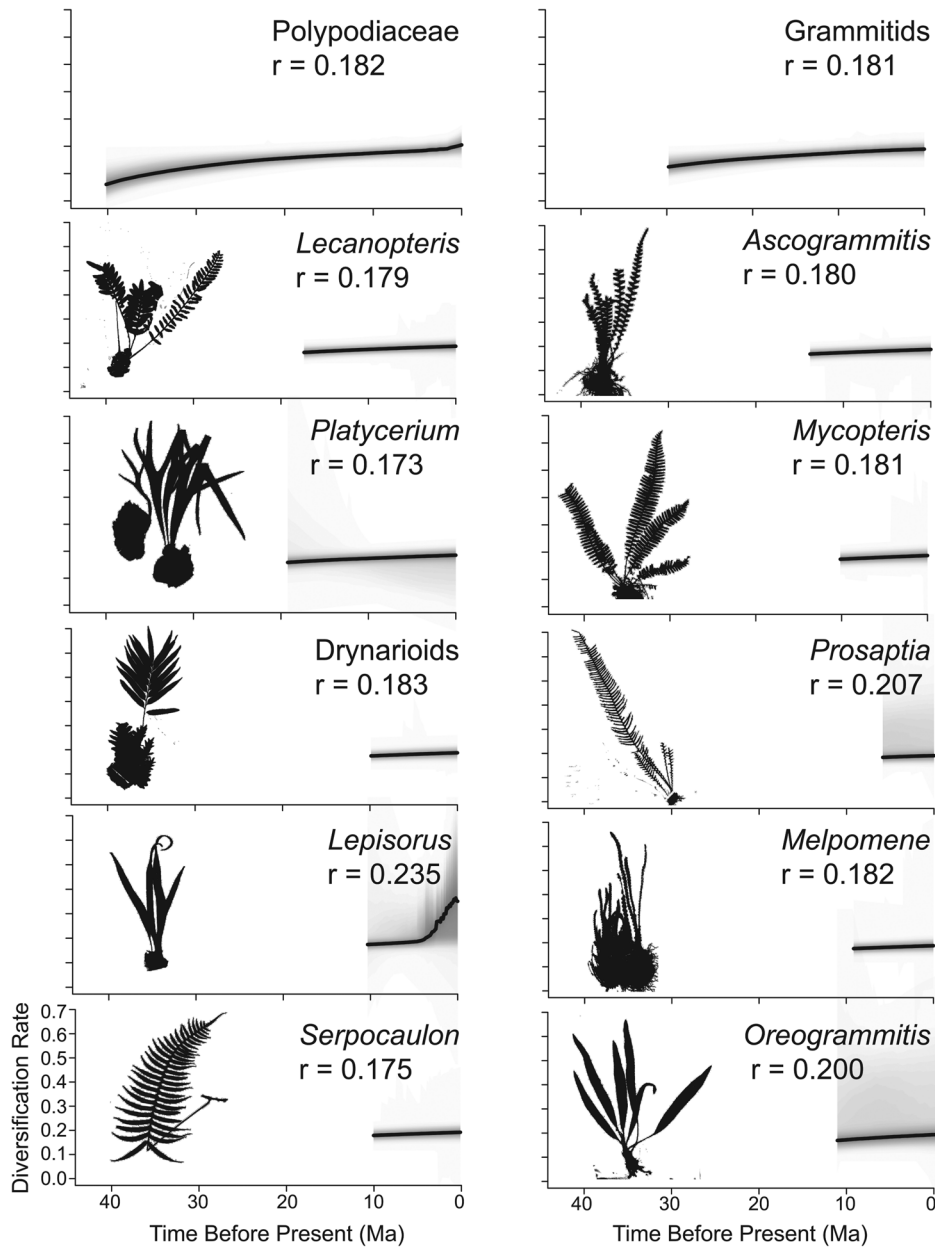
Figure 3. Estimated diversification rates through time for selected lineages of the Polypodiaceae as calculated by BAMM.

Schuettpelz and Pryer (2009), we find that the primary diversification events of the crown group of the Polypodiaceae began approximately 10–15 Ma after the Paleocene-Eocene thermal maximum (55.8 Ma), following the emergence of canopy ecosystems in newly formed angiosperm-dominated rainforests (Boyce and Lee 2010).

DIVERSIFICATION AND SHIFTS IN ELEVATION AND LEAF AREA

The roles of ecological and morphological shifts as drivers of diversification feature prominently in macroevolutionary studies of plants (Moore and Donoghue 2007; Ackerly 2009; Givnish et al. 2009; Kozak and Wiens 2010). Addressing these questions, however, has been challenging, and explicit comparisons of phenotypic and evolutionary changes are lacking in many studies. Among ferns, transitions in elevational niche and innovations in leaf morphology have been proposed as drivers of major radiations (Janssen and Schneider 2005; Kreier et al. 2008; Wang et al. 2012; McHenry and Barrington 2014; Sánchez-Baracaldo and Thomas 2014), however, few tests of these hypotheses have been made.

As pointed out in previous studies, major geoclimatic events, including the recent Andean orogeny (Kreier et al. 2008), the Miocene intensification of the Southeast Asian monsoon climate (Wang et al. 2012), and the Pleistocene glaciation in Europe,
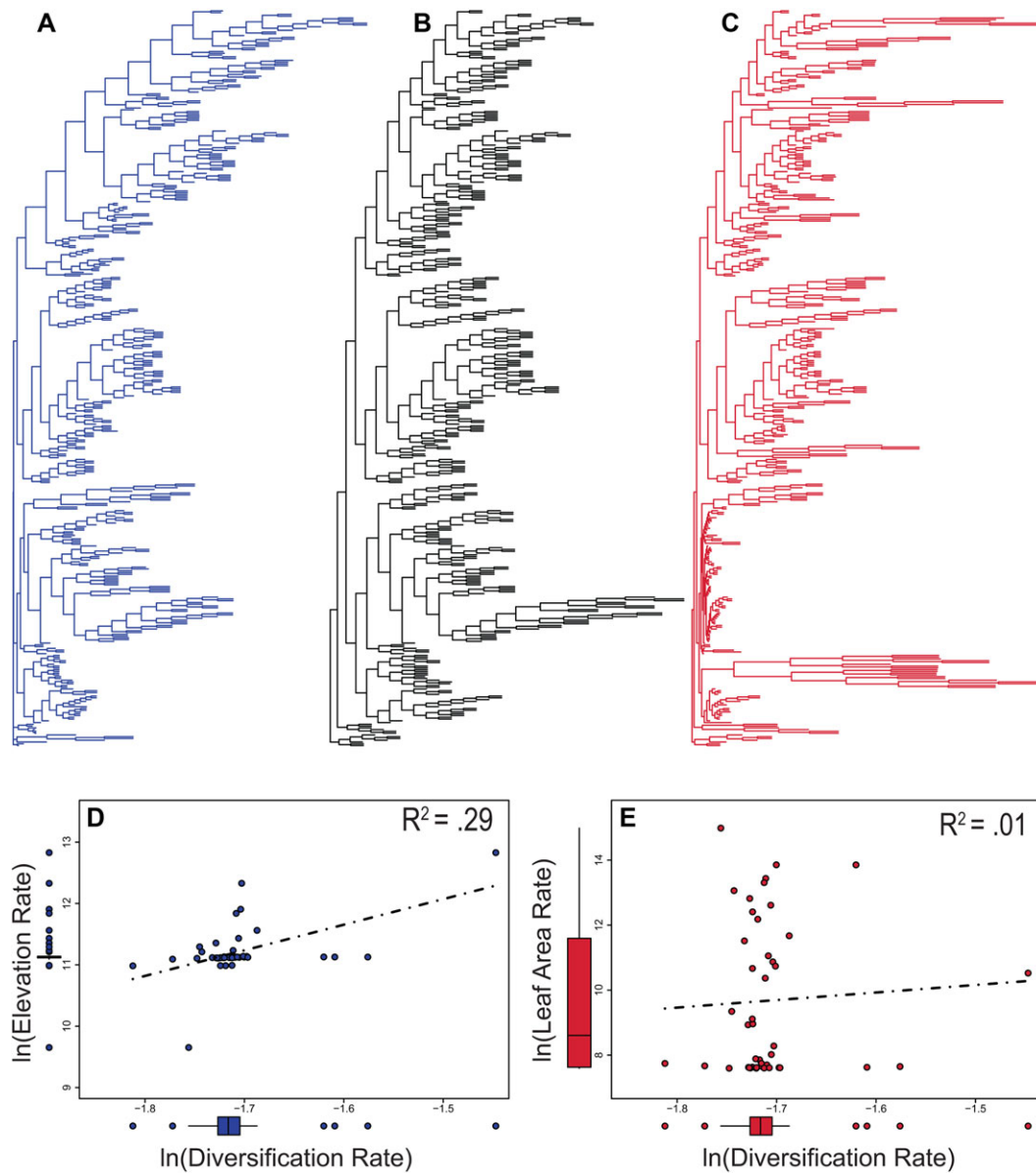
Figure 4. (A–C) Phylogeny of Polypodiaceae with branch lengths transformed to branch-specific rates of (A) elevation shift, (B) diversification, and (C) leaf size evolution. (D and E) PGLS regressions of log-transformed (D) elevation shift rate and (E) leaf size evolution rate versus diversification rates for all genera (*n* = 44).

North America, and South America (Haufler et al. 2000; Moreno and León 2003; Sigel et al. 2014a,b) appear to have played key roles as drivers of diversification in the Polypodiaceae during this period. These and similar events appear to have facilitated fern diversification either by generating novel habitat types (e.g., the appearance of cloud forests and alpine habitats following mountain building in the tropics, Janssen et al. 2008; Kreier et al. 2008; McHenry and Barrington 2014), isolating previously contiguous populations (e.g., glaciation events, Vogel et al. 1999; Trewick et al. 2002; Shepherd et al. 2007), or both (e.g., fluctuations in extent of Andean páramo since the Pleistocene, Hooghiemstra

and Van der Hammen, 2004; Sánchez-Baracaldo 2004; Sánchez-Baracaldo and Thomas 2014).

Our finding that diversification rate was positively correlated with shifts in elevation but not with shifts in leaf area indicates that habitat-related opportunities, not body size evolution, drive diversification across the Polypodiaceae. This extends the hypothesis presented by Haufler et al. (2000) that ecological differentiation along elevational gradients acts as a primary mechanism of speciation in tropical ferns and provides a potential mechanism underlying prominent patterns of generic diversity in tropical Polypodiaceae. In tropical regions, where most Polypodiaceae
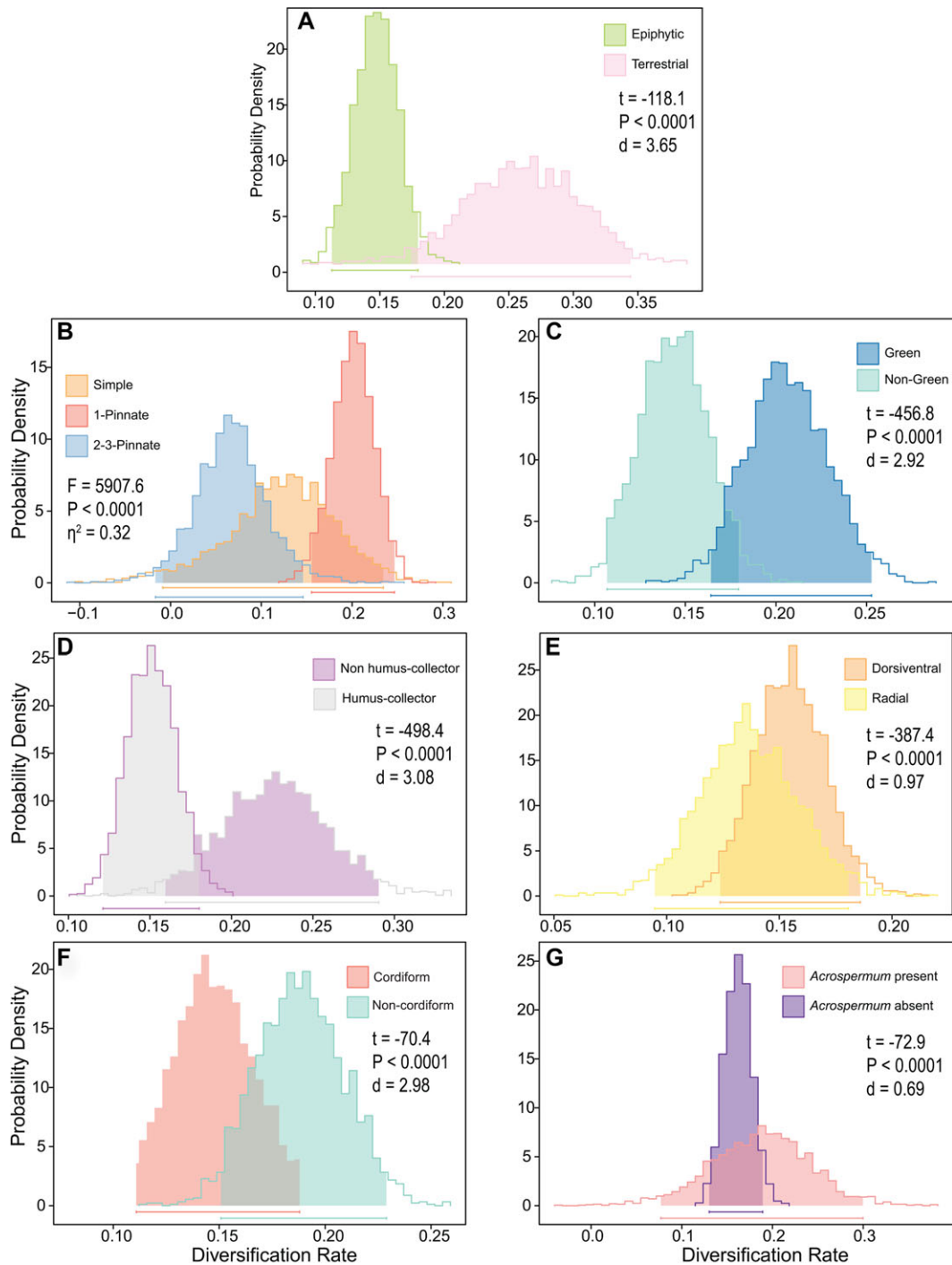
Figure 5. Marginal distribution of trait-associated diversification rates determined from Bayesian implementation of BiSSE and MuSSE models: habit (A), leaf dissection (B), spore color (C), leaf nesting (D), rhizome symmetry (E), gametophyte morphology (F), and fungal relationship (G).

occur, important eco-climatic factors such as temperature, humidity, soil type, and forest structure change much more rapidly along elevational gradients than with changes in latitude (Cardelús et al. 2006; Colwell et al. 2008). Under the model of evolution proposed by Haufler et al. (2000), tropical ferns can speciate rapidly along these gradients due to the diversity of niches available within

small geographic areas. Thus, evolutionarily successful groups should occupy a relatively high number of elevational zones in these regions. We find this pattern to be true: with few exceptions, the most species-rich genera in the family are common across broad elevational amplitudes whereas species-poor groups are restricted to narrow ranges (Fig. S3). This pattern appears to hold

for other fern lineages as well (Kessler 2001). In the absence of complex isolation mechanisms characteristic of flowering plants, allopatric ecological speciation appears to be the predominant speciation mechanism in ferns (Haufler et al. 2000), but more detailed study of sister taxa is needed to confirm this hypothesis.

The lack of a relationship between leaf size evolution and diversification rate corroborates our findings of a lack of support for proposed radiations associated with the acquisition of large humus-collecting leaves and dwarfism (Schneider et al. 2004; Janssen and Schneider 2005). This decoupling of body size evolution and diversification rate is consistent with the findings of some (e.g., Adams et al. 2009; Venditti et al. 2011) but not all (e.g., Pfennig et al. 2010; Rabosky et al. 2013) studies of this relationship in other large clades. The extent to which phenotypic evolvability predicts diversification potential varies across and within groups and may reflect different selective pressures and speciation processes. In light of this, we emphasize that such shifts in leaf morphology correspond to adaptations to highly specialized ecologies that often lack sufficient niche breadth to support subsequent radiations. Further, although leaf area is generally considered a useful proxy for a number of ecological and physiological attributes in ferns (Zotz et al. 2001; Testo and Watkins 2012), it is possible that other traits not examined here may instead be linked to diversification in this group.

We suggest that the increasingly rapid diversification of the Polypodiaceae following their initial radiation into the epiphytic niche is linked to their capacity to colonize novel habitat types that arose during the past 20 million years. The most remarkable example supporting this pattern of opportunity and diversification is the recent rapid radiation of *Lepisorus* in southeastern Asia, which is most diverse in southern China. Our analyses date the crown group age of the genus at approximately 13 Ma and identify a single burst of diversification at 3–2 Ma. Similar hypotheses have been proposed for the diversification of other plant groups in this region (Liu et al. 2006; Wang et al. 2009b; Xu et al. 2010), including a recent study on *Lepisorus* (Wang et al. 2012). This shift in diversification rate may be linked to the large transitions in elevation that occur in the genus (Fig. 4D), suggesting that the Pliocene-Pleistocene radiation of *Lepisorus* resulted from exploration of habitats available along a broad elevational gradient at the interface of the Qinghai-Tibetan Plateau and the subtropical forests of south-central China. Our conclusions are generally similar to those of Wang et al. (2012) who inferred that the primary bursts of diversification in the genus occurred during the Miocene and early Pliocene at about 13.5 and 4 Ma, however, in our results the event occurred much more recently. We believe this disparity is driven in part by the use of a constraint on the crown group age. We did not include this secondary node calibration point in our analysis because the paper from which it was taken (Schuettpelz and Pryer 2009) had low sampling for

that clade (four of about 100 species) and employed nonparametric rate smoothing, which is sensitive to rate heterogeneity (Magallón 2010; Tamura et al. 2012). In addition, our analyses using BAMM find strong evidence for a single shift in diversification rate (Fig. 2), whereas Wang et al. (2012) did not find evidence for any significant deviation from a constant-birth pattern of diversification.

REVISITING PREVIOUSLY HYPOTHESIZED RADIATIONS

A prominent feature of the recent evolutionary history of the Polypodiaceae is the emergence of ecologically specialized groups, including the myrmecophilous genus *Lecanopteris* and humus collectors such as *Platycerium*, *Aglaomorpha*, and *Drynaria*. The conspicuous morphological features exhibited by these plants have been proposed as evidence for key innovations leading to rapid radiations in the family. For example, Schneider et al. (2010) reported a burst in diversification in the drynarioid ferns (*Drynaria* and *Aglaomorpha*) during the Miocene and another in *Lecanopteris* occurring at 1.75 Ma. Although the diversification of both clades appears to have increased over time, we find no evidence of a radiation burst in either; they have diversified at approximately the same rate as most polypod lineages (Fig. 3). Further, our BiSSE results (Fig. 5) suggest that humus-collecting plants actually diversify more slowly than those lacking this specialized morphology (Table S4); this is driven by slow-diversifying nest-forming genera such as *Platycerium* and *Niphidium*. The differences between our results and those of Schneider et al. (2010) likely stem from our family-wide approach to modeling diversification compared to their genus-oriented analyses, which result in low predictive power in BiSSE and related models (Davis et al. 2013). In light of our results, we suggest that the evolution of highly specialized morphologies in the Polypodiaceae represents innovations that allowed these lineages to colonize nutrient poor epiphytic niches. This is consistent with the findings of ecophysiological studies that have demonstrated that both myrmecophily (Gay 1993; Watkins et al. 2008) and humus collection (Turner et al. 2007) influence nutrient relations among epiphytic ferns in otherwise nutrient-poor and water-stressed epiphytic habitats. Because these lineages have diversified at the same rate or slower than the rest of the family, they should not be considered rapid radiations.

The diversification of the Andean polypod genus *Serpocaulon* was characterized as an adaptive radiation by Kreier et al. (2008), but without benefit of either a time-calibrated tree or diversification analyses. Our BEAST analyses confirm that the origin and diversification of *Serpocaulon* occurred in concert with the Andean orogeny, but we recover no evidence of a departure from the family's background rate of diversification. Thus, we suggest *Serpocaulon* should not be categorized alongside true

rapid radiations among the Andean biota, such as the explosive bursts of the angiosperm genera *Lupinus* (Hughes and Eastwood 2006; Drummond et al. 2012), *Astragalus* (Scherson et al. 2008), and *Hypericum* (Nürk et al. 2013).

The species-rich grammitid clade, comprised mostly of small plants with narrow ecological niches (Kessler 2001; Kessler and Smith 2008), has been characterized as a rapidly radiating lineage (Schneider et al. 2004; Kessler and Smith 2008). Consistent with the recently published dated phylogeny of the group by Sundue et al. (2014), we find that the grammitid ferns have diversified rather steadily, and we do not find any evidence supporting an overall increased rate of diversification relative to the rest of the family (Fig. 3).

TRAIT-ASSOCIATED DIVERSIFICATION

Few studies have attempted to explicitly examine the adaptive significance of functional traits in the family. Beyond the previously discussed study of myrmecophily in *Lecanopteris* and humus-collecting leaves in the drynarioid ferns by Schneider et al. (2010), the only study testing evolutionary significance of traits in the family was that of Weber and Agrawal (2014) who reported elevated rates of diversification among nectary-bearing species of *Pleopeltis*. We provide the first family-wide examination of trait-associated diversification in the Polypodiaceae.

Habit

Although the evolution of epiphytism is prominent in the Polypodiaceae, we found that terrestrial and epipetric species had higher diversification rates than epiphytic taxa. This pattern appears to be influenced by a number of rapidly diversifying genera that are either entirely or partly terrestrial or epipetric, such as *Lepisorus*, *Leptochilus*, *Microsorum*, *Melpomene*, *Polypodium*, and *Pyrrosia*. Testo and Sundue (2014) proposed that transitions to terrestrial growth may be linked to diversification in some Paleotropical polypod genera and that epiphytic growth may have provided these species with preadaptations suitable for occupation of xeric epipetric niches. Our findings are congruent with this hypothesis.

Leaf dissection

Polypodiaceae exhibit an exceptional variety of leaf shapes, and dramatic shifts in leaf division and size characterize some of the family's most conspicuous and diverse lineages. Simple leaves predominate in several of the most species-rich lineages in the Polypodiaceae, including *Lepisorus* and many grammitid ferns; for this reason, we hypothesized that simple leaves would be associated with faster diversification rates. However, 1-pinnate leaves were associated with the highest rates, followed by simple and then 2- and 3-pinnate leaves. Simple leaves in ferns are thought to represent an adaptation to growth in dry habitats (Holttum

1938) and this appears to be the case in the Polypodiaceae, as many representatives of xerophytic genera possess simple leaves. Our results, however, indicate that the evolution of simple leaves is characteristic of both rapidly (e.g., *Lepisorus*, *Oreogrammitis*, *Selliguea*) and slowly (e.g., *Loxogramme*, *Dictymia*, *Niphidium*) diversifying genera.

Rhizome symmetry

Polypodiaceae exhibit two principal types of rhizome symmetry. Most species possess dorsiventral rhizomes that typically creep over the substrate, sometimes for long distances, and bear leaves only dorsally. Plants with radially symmetrical rhizomes have helically arranged leaves and a suberect growth habit. Radial rhizomes arise multiple times in the grammitid genera (Sundue 2010; Sundue et al. 2010) and appear to be related to niche exploitation; these plants occupy portions of trunks and branches of trees that are inaccessible to most other epiphytic ferns. Consequently, we anticipated that radial rhizome symmetry would be associated with increased diversification. Instead, our results indicate that this character state is associated with slower rates of diversification. We conclude that changes in rhizome architecture allow these ferns to explore new habitats, but do not lead to bursts of radiation; however, more research is needed.

Chlorophyllous spores

Fern spores can be categorized as having chloroplasts that mature prior to germination or not; these are typically referred to as green and nongreen spores, respectively (Lloyd and Klekowski 1970; Sundue et al. 2011). Ecological convergence and rapid diversification have been demonstrated among green-spored taxa across ferns, including Hymenophyllaceae (Hennequin et al. 2008); *Elaphoglossum* (Rouhan et al. 2004); *Lomariopsis* (Rouhan et al. 2007), and may underlie the prominence of these groups in humid mid- and upper-elevation tropical forests and contribute to the peak in epiphyte species richness observed in these habitats (Bhattarai et al. 2004; Cardelús et al. 2006; Kluge and Kessler 2006; Watkins et al. 2006). Among the Polypodiaceae they are found in all grammitids and in some species of *Loxogramme*, *Pleopeltis*, *Pleurosoriopsis*, and *Platycerium*. Green spores are associated with a suite of specialized functional traits, including rapid germination and short viability (Lloyd and Klekowski 1970); for these reasons, some authors (Schneider et al. 2004, 2010) have suggested that green spores facilitated the radiation of grammitids into novel epiphytic habitats. Our data indicate this faster rate of diversification is not restricted to grammitids but instead a feature of green-spored taxa.

Gametophyte morphology

Gametophytes of leptosporangiate ferns generally have either cordiform or noncordiform morphology. Representatives of these

morphologies also differ considerably in development (Takahashi et al. 2009), reproduction (Dassler and Farrar 2001), physiology (Watkins et al. 2007b), and life-history traits (Farrar et al. 2008). Several authors (Watkins et al. 2007b; Farrar et al. 2008; Watkins and Cardelús 2012) have suggested that noncordiform gametophyte morphology may have been a key innovation underlying the radiation of ferns into the epiphytic niche because they are longer lived and more stress tolerant than cordiform gametophytes. Consistent with this hypothesis, we found that taxa with noncordiform gametophytes diversify faster than those with cordiform gametophytes (Fig. 5). The five genera with the fastest diversification rates recovered by our analyses (*Lepisorus*, *Prosaptia*, *Oreogrammitis*, *Selliguea*, and *Lemmaphyllum*) all possess noncordiform gametophytes. Whether this effect is due to an intrinsic feature of the biology of noncordiform gametophytes, improved ability to colonize novel niches, or some other factor is uncertain. We find it unlikely that this rate difference is an artifact of co-distribution with other traits, given the well-established functional significance of gametophyte form in ferns and the number of independent origins of noncordiform morphology in the most rapidly diversifying clades in the Polypodiaceae (Farrar et al. 2008).

Association with Acrospermum

Relationships with fungi are one of the major drivers of plant diversification (Brundrett 2002; Wang and Qiu 2006; Sprent and James 2007). In contrast to the well-studied fungal symbioses in angiosperms, much less is known about the distribution or evolutionary significance of such associations in ferns. The most prominent fungal association known in the Polypodiaceae is with the ascomycete *Acrospermum maxonii,* which occurs on approximately 45 species in the family. The relationship is most developed in *Ascogrammitis* and *Mycopteris*, where mycelia and fruiting bodies of the fungus are always found (Sundue 2010, 2014); *A. maxonii* is also associated with some species of *Pleopeltis* and *Campyloneurum* (Doyle et al. 2008). *Acrospermum maxonii* is apparently epibiotic, presumably deriving energy from its host, but without causing apparent harm. We find a slight increase in diversification rate among *Acrospermum*-bearing taxa (Fig. 5) suggesting the relationship may be advantageous; however, we interpret this result with caution given the small effect size associated with this pattern and linkage of this trait with a number of other diversification-associated traits, notably gametophyte morphology and spore color.

Conclusions

The wide availability of DNA sequence data and increasingly robust analytical methods have provided new insight into the patterns and drivers of large-scale radiations in many groups, especially among major animal lineages (e.g., Venditti et al. 2011; Rabosky et al. 2013; McGuire et al. 2014). Although patterns of diversification in plants as a whole have received considerable attention in recent years, relevant studies for epiphytic plant lineages are generally lacking. Here, we present one of the first explicit tests of hypotheses for diversification and trait evolution across a major lineage of epiphytic plants. We present a new paradigm for diversification in the polypod and grammitid ferns—gradually increasing rates of diversification across the family that are consistently high relative to other fern lineages. This high, constant increase in diversity suggests that the Polypodiaceae represent a single large radiation rather than an aggregate of adaptive radiations. In particular, our findings indicate that lineages which occupy broad elevational ranges—comprising diverse habitat types—diversify at faster rates than clades that are narrowly distributed. Thus, repeated successful exploration of novel habitat types, rather than morphological innovation, appears to be the primary driver of diversification in this group. Highly specialized groups such as *Platycerium* and *Lecanopteris* that had previously been proposed as evidence of adaptive radiations are instead specialists adapted to nutrient-poor conditions. Together, these distinct processes of diversification constitute an ongoing burst of diversification in one of the most derived clades of ferns, perhaps the most successful group at exploring a variety of epiphytic niches.

LITERATURE CITED
Ackerly, D. 2009. Conservatism and diversification of plant functional traits: evolutionary rates versus phylogenetic signal. Proc. Natl. Acad. Sci. 106:19699–19706.

Adams, D. C., C. M. Berns, K. H. Kozak, and J. J. Wiens. 2009. Are rates of species diversification correlated with rates of morphological evolution? Proc. R. Soc. Lond. B Biol. Sci. rspb.2009.0543.

Alfaro, M. E., F. Santini, C. Brock, H. Alamillo, A. Dornburg, D. L. Rabosky, G. Carnevale, and L. J. Harmon. 2009. Nine exceptional radiations plus high turnover explain species diversity in jawed vertebrates. Proc. Natl. Acad. Sci. USA 106:13410–13414.

Beerling, D. J., and F. I. Woodward. 1997. Changes in land plant function over the Phanerozoic: reconstructions based on the fossil record. Bot. J. Linn. Soc. 124:137–153.

Benzing, D. H. 1990. Vascular epiphytes: general biology and related biota. Cambridge Univ. Press, Cambridge.

Bhattarai, K. R., O. R. Vetaas, and J. A. Grytnes. 2004. Fern species richness along a central Himalayan elevational gradient, Nepal. J. Biogeogr. 31:389–400.

Boyce, C. K., and J.-E. Lee. 2010. An exceptional role for flowering plant physiology in the expansion of tropical rainforests and biodiversity. Proc. R. Soc. B rspb20100485.

Brundrett, M. C. 2002. Coevolution of roots and mycorrhizas of land plants. New Phytol. 154:275–304.

Cardelús, C. L., R. K. Colwell, and J. E. Watkins. 2006. Vascular epiphyte distribution patterns: explaining the mid-elevation richness peak. J. Ecol. 94:144–156.

Colwell, R. K., G. Brehm, C. L. Cardelús, A. C. Gilman, and J. T. Longino. 2008. Global warming, elevational range shifts, and lowland biotic attrition in the wet tropics. Science 322:258–261.

Dassler, C. L., and D. R. Farrar. 2001. Significance of gametophyte form in long-distance colonization by tropical, epiphytic ferns. Brittonia 53:352–369.

Davis, M. P., P. E. Midford, and W. Maddison. 2013. Exploring power and parameter estimation of the BiSSE method for analyzing species diversification. BMC Evol. Biol. 13:38.

Doyle, V. P., M. A. Sundue, and G. J. Samuels. 2008. Evaluating host specificity of epibiotic Acrospermum on neotropical polygrammoid ferns. Inoculum 59:28.

Drummond, A. J., and A. Rambaut. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. BMC Evol. Biol. 7:214.

Drummond, A. J., S. Y. W. Ho, M. J. Phillips, and A. Rambaut. 2006. Relaxed phylogenetics and dating with confidence. PLoS Biol. 4:e88.

Drummond, C. S., R. J. Eastwood, S. T. S. Miotto, and C. E. Hughes. 2012. Multiple continental radiations and correlates of diversification in *Lupinus* (Leguminosae): testing for key innovation with incomplete taxon sampling. Syst. Biol. 61:443–460.

Farrar, D. R., C. Dassler, J. E. Watkins Jr., and C. Skelton. 2008. Gametophyte ecology. Pp. 222–256 *in* C. H. Haufler & T. A. Ranker, Biology and evolution of ferns and lycophytes. Cambridge Univ. Press, Cambridge.

FitzJohn, R. G. 2012. Diversitree: comparative phylogenetic analyses of diversification in R. Methods Ecol. Evol. 3:1084–1092.

FitzJohn, R. G., W. P. Maddison, and S. P. Otto. 2009. Estimating trait-dependent speciation and extinction rates from incompletely resolved phylogenies. Syst. Biol. 58:595–611.

Fiz-Palacios, O., H. Schneider, J. Heinrichs, and V. Savolainen. 2011. Diversification of land plants: insights from a family-level phylogenetic analysis. BMC Evol. Biol. 11:341.

Gay, H. 1991. Ant-houses in the fern genus Lecanopteris Reinw. (Polypodiaceae): the rhizome morphology and architecture of *L. sarcopus* Teijsm. & Binnend. and *L. darnaedii* Hennipman. Bot. J. Linn. Soc. 106:199–208.

———. 1993. Animal-fed plants: an investigation into the uptake of ant-derived nutrients by the far-eastern epiphytic fern *Lecanopteris* Reinw. (Polypodiaceae). Biol. J. Linn. Soc. 50:221–233.

Gentry, A. H., and C. H. Dodson. 1987. Diversity and biogeography of neotropical vascular epiphytes. Ann. Mo. Bot. Gard. 74:205–233.

Givnish, T. J., K. C. Millam, A. R. Mast, T. B. Paterson, T. J. Theim, A. L. Hipp, J. M. Henss, J. F. Smith, K. R. Wood, and K. J. Sytsma. 2009. Origin, adaptive radiation and diversification of the Hawaiian lobeliads (Asterales: Campanulaceae). Proc. R. Soc. B 276:407–416.

Gomez, L. D. 1974. The biology of the potato-fern, *Solanopteris brunei*. Bresnia 4:37–61.

Gravendeel, B., A. Smithson, F. J. W. Slik, and A. Schuiteman. 2004. Epiphytism and pollinator specialization: drivers for orchid diversity? Phil. Trans. R. Soc. Lond. B 359:1523–1535.

Harmon, L. J., J. T. Weir, C. D. Brock, R. E. Glor, and W. Challenger. 2008. GEIGER: investigating evolutionary radiations. Bioinformatics 24:129–131.

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

Haufler, C. H., W. A. Grammer, E. Hennipman, T. A. Ranker, A. R. Smith, and H. Schneider. 2003. Systematics of the ant-fern genus *Lecanopteris* (Polypodiaceae): testing phylogenetic hypotheses with DNA sequences. Syst. Bot. 28:217–227.

Hennipman, E., and M. C. Roos. 1982. A monograph of the fern genus *Platycerium* (Polypodiaceae). Ver Kon Ned Ak Wet Afd Nat Tweede Reeks 80:1–162.

Hennequin, S., E. Schuettpelz, K. M. Pryer, A. Ebihara, and J. Dubuisson. 2008. Divergence times and the evolution of epiphytism in filmy ferns (Hymenophyllaceae) revisited. Int. J. Plant Sci. 169:1278–1287.

Holttum, R. E. 1938. The ecology of tropical pteridophytes. Pp. 420–450 *in* F. Verdoorn, A. H. G. Alston, I. Andersson-Kottö, L. R. Atkinson, H. Burgeff, H. G. D. Buy, C. Christensen, W. Döpp, W. M. D. V. Leeuwen, H. Gams, et al., eds. Manual of pteridology. Springer, the Netherlands.

Hooghiemstra, H., and T. vander Hammen. 2004. Quaternary Ice-Age dynamics in the Colombian Andes: developing an understanding of our legacy. Philos. Trans. R. Soc. B Biol. Sci. 359:173–181.

Hughes, C., and R. Eastwood. 2006. Island radiation on a continental scale: exceptional rates of plant diversification after uplift of the Andes. Proc. Natl. Acad. Sci. USA 103:10334–10339.

Janssen, T., and H. Schneider. 2005. Exploring the evolution of humus collecting leaves in drynarioid ferns (Polypodiaceae, Polypodiidae) based on phylogenetic evidence. Plant Syst. Evol. 252:175–197.

Janssen, T., N. Bystriakova, F. Rakotondrainibe, D. Coomes, J. N. Labat, and H. Schneider. 2008. Neoendemism in Madagascan scaly tree ferns results from recent, coincident diversification bursts. Evolution 6:1876–1889.

Kessler, M. 2001. Patterns of diversity and range size of selected plant groups along an elevational transect in the Bolivian Andes. Biodivers. Conserv. 10:1897–1921.

Kessler, M., and Y. Siorak. 2007. Desiccation and rehydration experiments on leaves of 43 pteridophyte species. Am. Fern J. 97:175–185.

Kessler, M., and A. R. Smith. 2008. New species of grammitid ferns (Polypodiaceae, Polypodiopsida) from Bolivia. Org. Divers. Evol. 8:167.e1–167.e18.

Kluge, J., and M. Kessler. 2006. Fern endemism and its correlates: contribution from an elevational transect in Costa Rica. Divers. Distrib. 12:535–545.

———. 2010. Phylogenetic diversity, trait diversity and niches: species assembly of ferns along a tropical elevational gradient. J. Biogeogr. 38:394–405.

Kozak, K. H., and J. J. Wiens. 2010. Accelerated rates of climatic-niche evolution underlie rapid species diversification. Ecol. Lett. 13:1378–1389.

Kreier, H.-P., M. Rex, K. Weising, M. Kessler, A. R. Smith, and H. Schneider. 2008. Inferring the diversification of the epiphytic fern genus *Serpocaulon* (Polypodiaceae) in South America using chloroplast sequences and amplified fragment length polymorphisms. Plant Syst. Evol. 274:1–16.

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

Liu, J.-Q., Y.-J. Wang, A.-L. Wang, O. Hideaki, and R. J. Abbott. 2006. Radiation and diversification within the *Ligularia–Cremanthodium–Parasenecio* complex (Asteraceae) triggered by uplift of the Qinghai-Tibetan Plateau. Mol. Phylogenet. Evol. 38:31–49.

Lloyd, R. M., and E. J. Klekowski Jr. 1970. Spore germination and viability in Pteridophyta: evolutionary significance of chlorophyllous spores. Biotropica 2:129–137.

Maddison, W. P., and R. G. FitzJohn. 2015. The unsolved challenge to phylogenetic correlation tests for categorical characters. Syst. Biol. 64:127–136.

Maddison, W. P., P. E. Midford, and S. P. Otto. 2007. Estimating a binary character's effect on speciation and extinction. Syst. Biol. 56:701–710.

Magallón, S. 2010. Using fossils to break long branches in molecular dating: a comparison of relaxed clocks applied to the origin of angiosperms. Syst. Biol. 59:384–399.

McGuire, J. A., C. C. Witt, J. V. Remsen Jr., A. Corl, D. L. Rabosky, D. L. Altshuler, and R. Dudley. 2014. Molecular phylogenetics and the diversification of hummingbirds. Curr. Biol. 24:910–916.

McHenry, M. A., and D. S. Barrington. 2014. Phylogeny and biogeography of exindusiate Andean *Polystichum* (Dryopteridaceae). Am. J. Bot. 101:365–375.

Moore, B. R., and M. J. Donoghue. 2007. Correlates of diversification in the plant clade Dipsacales: geographic movement and evolutionary innovations. Am. Nat. 170:S28–S55.

Moreno, P. I., and A. L. León. 2003. Abrupt vegetation changes during the last glacial to Holocene transition in mid-latitude South America. J. Quat. Sci. 18:787–800.

Nayar, B. K., and S. Kaur. 1971. Gametophytes of homosporous ferns. Bot. Rev 37:295–396.

Nieder, J., J. Prosperí, and G. Michaloud. 2001. Epiphytes and their contribution to canopy diversity. Plant Ecol. 153:51–63.

Nürk, N. M., C. Scheriau, and S. Madrinan. 2013. Explosive radiation in high Andean *Hypericum*–rates of diversification among New World lineages.Front. Genet. 4:1–14.

Pfennig, D. W., M. A. Wund, E. C. Snell-Rood, T. Cruickshank, C. D. Schlichting, and A. P. Moczek. 2010. Phenotypic plasticity's impacts on diversification and speciation. Trends Ecol. Evol. 25:459–467.

Phillips, D. A., and R. A. White. 1967. Frond articulation in species of Polypodiaceae and Davalliaceae. Am. Fern J. 57:78–88.

R Core Team. 2013. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at www.R-project.org.

Rabosky, D. L. 2014. Automatic detection of key innovations, rate shifts, and diversity-dependence on phylogenetic trees. PLoS ONE 9:e89543.

Rabosky, D. L., and E. E. Goldberg. 2015. Model inadequacy and mistaken inferences of trait-dependent speciation. Syst. Biol. 64:340–355.

Rabosky, D. L., F. Santini, J. Eastman, S. A. Smith, B. Sidlauskas, J. Chang, and M. E. Alfaro. 2013. Rates of speciation and morphological evolution are correlated across the largest vertebrate radiation. Nat. Commun. 4:1–18.

Ranker, T. A., A. R. Smith, B. S. Parris, J. M. O. Geiger, C. H. Haufler, S. C. K. Straub, and H. Schneider. 2004. Phylogeny and evolution of grammitid ferns (Grammitidaceae): a case of rampant morphological homoplasy. Taxon 53:415–428.

Roos, M. C. 1985. Phylogenetic systematics of the Drynarioideae (Polypodiaceae). Tweede Sect. 85:1–318.

Rouhan, G., J.-Y. Dubuisson, F. Rakotondrainibe, T. J. Motley, J. T. Mickel, J.-N. Labat, and R. C. Moran. 2004. Molecular phylogeny of the fern genus *Elaphoglossum* (Elaphoglossaceae) based on chloroplast noncoding DNA sequences: contributions of species from the Indian Ocean area. Mol. Phylogenet. Evol. 33:745–763.

Rouhan, G., J. G. Hanks, D. McClelland, and R. C. Moran. 2007. Preliminary phylogenetic analysis of the fern genus *Lomariopsis* (Lomariopsidaceae). Brittonia 59:115–128.

Rut, G., J. Krupa, Z. Miszalski, A. Rzepka, and I. Ślesak. 2008. Crassulacean acid metabolism in the epiphytic fern *Platycerium bifurcatum*. Photosynthetica 46:156–160.

Sánchez-Baracaldo, P. 2004. Phylogenetics and biogeography of the neotropical fern genera *Jamesonia* and *Eriosorus* (Pteridaceae). Am. J. Bot. 91:274–284.

Sánchez-Baracaldo, P., and G. H. Thomas. 2014. Adaptation and convergent evolution within the *Jamesonia-Eriosorus* complex in high-elevation biodiverse Andean hotspots. PLoS ONE 9:e110618.

Scherson, R. A., R. Vidal, and M. J. Sanderson. 2008. Phylogeny, biogeography, and rates of diversification of New World *Astragalus* (Leguminosae) with an emphasis on South American radiations. Am. J. Bot. 95:1030–1039.

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. Mol. Phylogenet. Evol. 31:1041–1063.

Schneider, H., H.-P. Kreier, T. Janssen, E. Otto, H. Muth, and J. Heinrichs. 2010. Key innovations versus key opportunities: identifying causes of rapid radiations in derived ferns. Pp. 61–75 *in* M. Glaubrecht, ed. Evolution in action. Springer, Berlin, Heidelberg.

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

———. 2009. Evidence for a Cenozoic radiation of ferns in an angiosperm-dominated canopy. Proc. Natl. Acad. Sci. USA 106:11200–11205.

Shepherd, L. D., L. R. Perrie, and P. J. Brownsey. 2007. Fire and ice: volcanic and glacial impacts on the phylogeography of the New Zealand forest fern *Asplenium hookerianum*. Mol. Ecol. 16:4536–4549.

Sigel, E. M., M. D. Windham, C. H. Haufler, and K. M. Pryer. 2014a. Phylogeny, divergence time estimates, and phylogeography of the diploid species of the *Polypodium vulgare* complex (Polypodiaceae). Syst. Bot. 39:1042–1055.

Sigel, E. M., M. D. Windham, and K. M. Pryer. 2014b. Evidence for reciprocal origins in *Polypodium hesperium* (Polypodiaceae): A fern model system for investigating how multiple origins shape allopolyploid genomes. Am. J. Bot. 101:1476–1485.

Silvera, K., L. S. Santiago, J. C. Cushman, and K. Winter. 2009. Crassulacean acid metabolism and epiphytism linked to adaptive radiations in the Orchidaceae. Plant Physiol. 149:1838–1847.

Smith, A. R., K. M. Pryer, E. Schuettpelz, P. Korall, H. Schneider, and P. G. Wolf. 2006. A classification for extant ferns. Taxon 55:705–731.

Sprent, J. I., and E. K. James. 2007. Legume evolution: where do nodules and mycorrhizas fit in? Plant Physiol. 144:575–581.

Sundue, M. A. 2010a. A monograph of *Ascogrammitis*, a new genus of grammitid ferns (Polypodiaceae). Brittonia 62:357–399.

———. 2010b. A morphological cladistic analysis of *Terpsichore* (Polypodiaceae). Syst. Bot. 35:716–729.

———. 2014. *Mycopteris*, a new neotropical genus of grammitid ferns (Polypodiaceae). Brittonia 66:174–185.

Sundue, M. A., M. B. Islam, and T. A. Ranker. 2010. Systematics of grammitid ferns (Polypodiaceae): using morphology and plastid sequence data to resolve the circumscriptions of *Melpomene* and the polyphyletic genera *Lellingeria* and *Terpsichore*. Syst. Bot. 35:701–715.

Sundue, M., Alejandra Vasco, and R. C. Moran. 2011. Cryptochlorophyllous spores in ferns: nongreen spores that contain chlorophyll. Int. J. Plant Sci. 172:1110–1119.

Sundue, M. A., B. S. Parris, T. A. Ranker, A. R. Smith, E. L. Fujimoto, D. Zamora-Crosby, C. W. Morden, W.-L. Chiou, C.-W. Chen, G. Rouhan, R. Y. Hirai, and J. Prado. 2014. Global phylogeny and biogeography

of grammitid ferns (Polypodiaceae). Mol. Phylogenet. Evol. 81:195–206.

Takahashi, N., M. Hashino, C. Kami, and R. Imaichi. 2009. Developmental morphology of strap-shaped gametophytes of *Colysis decurrens*: a new look at meristem development and function in fern gametophytes. Ann. Bot. 104:1353–1361.

Tamura, K., F. U. Battistuzzi, P. Billing-Ross, O. Murillo, A. Filipski, and S. Kumar. 2012. Estimating divergence times in large molecular phylogenies. Proc. Natl. Acad. Sci. USA 109:19333–19338.

Testo, W. L., and J. E. Watkins. 2012. Influence of plant size on the ecophysiology of the epiphytic fern *Asplenium auritum* (Aspleniaceae) from Costa Rica. Am. J. Bot. 99:1840–1846.

Testo, W., and M. Sundue. 2014. Primary hemiepiphytism in *Colysis ampla* (Polypodiaceae) provides new insight into the evolution of growth habit in ferns. Int. J. Plant Sci. 175:526–536.

Trewick, S. A., M. Morgan-Richards, S. J. Russell, S. Henderson, F. J. Rumsey, I. Pinter, J. A. Barrett, M. Gibby, and J. C. Vogel. 2002. Polyploidy, phylogeography and Pleistocene refugia of the rockfern *Asplenium ceterach*: evidence from chloroplast DNA. Mol. Ecol. 11:2003–2012.

Turner, E. C., J. L. Snaddon, H. R. Johnson, and W. A. Foster. 2007. The impact of bird's nest ferns on stemflow nutrient concentration in a primary rain forest, Sabah, Malaysia. J. Trop. Ecol. 23:721–724.

Van Uffelen, G. A. 1991. Fossil Polypodiaceae and their spores. Blumea 36:253–272.

Venditti, C., A. Meade, and M. Pagel. 2011. Multiple routes to mammalian diversity. Nature 479:393–396.

Vogel, J. C., F. J. Rumsey, J. J. Schneller, J. A. Barrett, and M. Gibby. 1999. Where are the glacial refugia in Europe? Evidence from pteridophytes. Biol. J. Linn. Soc. 66:23–37.

Wagner, W. H. Jr. 1974. Structure of spores in relation to fern phylogeny. Ann. Mo. Bot. Gard. 61:332–353.

Wang, B., and Y.-L. Qiu. 2006. Phylogenetic distribution and evolution of mycorrhizas in land plants. Mycorrhiza 16:299–363.

Wang, H., M. J. Moore, P. S. Soltis, C. D. Bell, S. F. Brockington, R. Alexandre, C. C. Davis, M. Latvis, S. R. Manchester, and D. E. Soltis. 2009a. Rosid radiation and the rapid rise of angiosperm-dominated forests. Proc. Natl. Acad. Sci. USA 106:3853–3858.

Wang, L., R. J. Abbott, W. Zheng, P. Chen, Y. Wang, and J. Liu. 2009b. History and evolution of alpine plants endemic to the Qinghai-Tibetan Plateau: *Aconitum gymnandrum* (Ranunculaceae). Mol. Ecol. 18:709–721.

Wang, L., H. Schneider, X.-C. Zhang, and Q.-P. Xiang. 2012. The rise of the Himalaya enforced the diversification of SE Asian ferns by altering the monsoon regimes. BMC Plant Biol. 12:210.

Watkins, J. E., and C. L. Cardelús. 2012. Ferns in an angiosperm world: Cretaceous radiation into the epiphytic niche and diversification on the forest floor. Int. J. Plant Sci. 173:695–710.

Watkins, J. E., C. Cardelús, R. K. Colwell, and R. C. Moran. 2006. Species richness and distribution of ferns along an elevational gradient in Costa Rica. Am. J. Bot. 93:73–83.

Watkins, J. E., M. C. Mack, T. R. Sinclair, and S. S. Mulkey. 2007a. Ecological and evolutionary consequences of desiccation tolerance in tropical fern gametophytes. New Phytol. 176:708–717.

Watkins, J. E., M. K. Mack, and S. S. Mulkey. 2007b. Gametophyte ecology and demography of epiphytic and terrestrial tropical ferns. Am. J. Bot. 94:701–708.

Watkins, J. E., C. L. Cardelús, and M. C. Mack. 2008. Ants mediate nitrogen relations of an epiphytic fern. New Phytol. 180:5–8.

Weber, M. G., and A. A. Agrawal. 2014. Defense mutualisms enhance plant diversification. Proc. Natl. Acad. Sci. USA 111:16442–16447.

Winter, K., C. B. Osmond, and K. T. Hubick. 1986. Crassulacean acid metabolism in the shade. Studies on an epiphytic fern, *Pyrrosia longifolia*, and other rainforest species from Australia. Oecologia 68:224–230.

Xu, T., R. J. Abbott, R. I. Milne, K. Mao, F. K. Du, G. Wu, G. Ciren, G. Miehe, and J. Liu. 2010. Phylogeography and allopatric divergence of cypress species (*Cupressus* L.) in the Qinghai-Tibetan Plateau and adjacent regions. BMC Evol. Biol. 10:194.

Zotz, G., P. Hietz, and G. Schmidt. 2001. Small plants, large plants: the importance of plant size for the physiological ecology of vascular epiphytes. J. Exp. Bot. 52:2051–2056.

Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's website:

Figure S1. Time-calibrated phylogeny of the Polypodiaceae.
Figure S2. Elevational ranges of Polypodiaceae genera.
Figure S3. Relationship between species richness and elevational distribution in Polypodiaceae genera.
Table S1. GenBank acession numbers.
Table S2. Values of morphological and ecological traits scored for all species in phylogeny.
Table S3. Total diversity and proportional representation in phylogeny for Polypodiaceae clades.
Table S4. Estimated evolutionary rates in the Polypodiaceae.