Slowly but surely: gradual diversification and phenotypic evolution in the hyper-diverse tree fern family Cyatheaceae

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• **Background and Aims** The tremendously unbalanced distribution of species richness across clades in the tree of life is often interpreted as the result of variation in the rates of diversification, which may themselves respond to trait evolution. Even though this is likely a widespread pattern, not all diverse groups of organisms exhibit heterogeneity in their dynamics of diversification. Testing and characterizing the processes driving the evolution of clades with steady rates of diversification over long periods of time are of importance in order to have a full understanding of the buildup of biodiversity through time.

• **Methods** We studied the macroevolutionary history of the species rich tree fern family Cyatheaceae and inferred a time calibrated phylogeny of the family including extinct and extant species using the recently developed fossilized birth-death method. We tested whether the high diversity of Cyatheaceae is the result of episodes of rapid diversification associated with phenotypic and ecological differentiation or driven by stable but low rates of diversification. We compared the rates of diversification across clades and modelled the evolution of body size and climatic preferences and tested for trait-dependent diversification.

• **Key results** This ancient group diversified at a low and constant rate during its long evolutionary history. Morphological and climatic niche evolution were found to be overall highly conserved, although we detected several shifts in the rates of evolution of climatic preferences, linked to changes in elevation. The diversification of the family occurred gradually, within limited phenotypic and ecological boundaries, and yet resulted in a remarkable species richness.

• **Conclusions** Our study indicates that Cyatheaceae is a diverse clade which slowly accumulated morphological, ecological, and taxonomic diversity over a long evolutionary period and provides a compelling example of tropics as a museum of biodiversity.

**Key words:** diversification, macroevolution, tree ferns, Cyatheaceae, phylogeny, target sequencing, climatic preferences, fossilized birth-death, phenotypic evolution, gradual evolution, species richness, divergence times.
INTRODUCTION

The distribution of biodiversity is strikingly unbalanced when comparing the species richness among lineages and one of the main challenges in evolutionary biology is to understand the underlying factors that are responsible for this uneven distribution (Wiens 2017). This challenge is commonly tackled by comparing the patterns of diversification among regions and taxonomic groups (Antonelli et al. 2015; Eiserhardt et al. 2017), but this requires comparing large numbers of species to have a good understanding of the potential processes involved. Fortunately, the increasing availability of DNA sequence data coupled with advances in phylogenetic comparative methods over the last decades provides us with powerful tools to study the assembly of Earth’s biodiversity through time and space.

The heterogeneity of species richness across clades is likely the result of variations in the rates of speciation or extinction (Wiens 2017). Changes in diversification rates, measured as the difference between the estimated rates of speciation and extinction, have been attributed to a wide range of factors. Intrinsic traits such as the tank habit in bromeliads (Silvestro et al. 2014) or heterostyly in primroses (de Vos et al. 2014), have been linked with accelerated diversification. Ecological interactions, for instance clownfish mutualism with sea anemones (Litsios et al. 2012) or plant interactions with pollinators (Breitkopf et al. 2015; Serrano-Serrano et al. 2017) have also been hypothesized to play a role in species diversification. Finally, the ecological niche, can also affect the diversity between lineages, as shown in the large fern family Polypodiaceae, where lineage diversification is positively associated with the colonization of a wider elevation range (Sundue et al. 2015).

Clade age has also been proposed as an explanation for differences in species richness between groups of organisms through the “clade-age” hypothesis, which argues that older clades tend to have more species simply because they had more time to accumulate diversity (Wiens 2017). In that case, species richness results from the steady accumulation of lineages through time rather than from shifts in the rates of diversification. Stable evolutionary dynamics have been reported, for example, in Neotropical Troidini butterflies (Condamine et al. 2012) and several plant groups such as figs (Bruun-Lund et al. 2017), malagasy Angraecum orchids (Andriananjamanantsoa et al. 2016), Annonaceae (Couvreur et al. 2011), Australian Proteaceae (Cardillo and Pratt 2013) and liverworts (Wilson et al. 2007). The “clade-age” hypothesis also provides a possible scenario to explain geographic variation in species richness whereby more diverse regions, for instance the tropics, would be the result of early colonizations rather than variation in the rate of clade diversification among regions (Brown 2014). In contrast to the clade-age hypothesis, a recent study has proposed that diversification is a time-dependent process.
whereby younger clades diversify more rapidly than older clades (Henao Diaz et al. 2018). This time dependency could potentially disrupt a correlation between the age of a clade and its diversity.

Alternatively, instead of invoking a single underlying factor, some studies have proposed a more nuanced model of evolutionary dynamics where both time and rates of diversification contribute to explain diversity patterns. For instance, the palm family (Arecaceae) exhibited a constant rate of diversification during most of its long evolutionary history, but several increases in the rate of diversification have punctuated the recent evolution of this group (Couvreur et al. 2011; Baker and Couvreur 2013). Similarly, the assembly of fern diversity appears to have been shaped by a strong turnover of clades resulting from the combination of both diversity-dependent speciation and climate-driven extinction (Lehtonen et al. 2017).

In this context, the long-term drivers of the diversification history of old and species-rich plant clades can give important insights into the build-up of biodiversity through time. We focused here on the scaly tree fern family Cyatheaceae, an iconic plant group of humid forests comprising about 660 known species distributed worldwide in tropical and subtropical regions. This species richness is remarkable compared to the seven other families of the Cyatheales, which contain from a single species in the Thysopteridaceae to ca. 35 species in the Dicksoniaceae (Schneider et al. 2016). Phylogenetic studies have estimated the divergence between the Cyathaceae and its sister family the Dicksoniaceae around 145 Ma and have shown that both Gondwanan vicariance and long-distance dispersal contributed to generating the current distribution of scaly tree ferns (Korall and Pryer 2014). Significant among-clade variations in the rate of diversification have been reported for the family (Janssen et al. 2008; Ramírez-Barahona et al. 2016), and a recent study also found a positive association between the rates of morphological and climatic niche evolution and the rates of diversification, suggesting that the evolutionary dynamics of Cyatheaceae followed that of an adaptive radiation (Ramírez-Barahona et al. 2016). However, only about 10% of the total number of Cyatheaceae species were represented in these studies. Such a low taxonomic sampling weakens phylogenetic comparative analyses (Heath et al. 2008; Silvestro et al. 2011) and calls into question our understanding of the evolutionary history of the group.

Here, we combined molecular data at an unprecedented level of taxonomic sampling with fossil information to reconstruct a new dated phylogenetic tree of the Cyatheaceae. The origin of the group dates back to the Jurassic and recent theory suggests that diversification in such an old group could be the result of a steady but slow accumulation of species through time (Henao Diaz et al. 2018). Alternatively, the high diversity of the family could be associated with the evolution of intrinsic or ecological traits in specific lineages driving species diversification (Ramírez-Barahona et al. 2016). We used macroevolutionary models to test several hypotheses related to species and trait diversification of the Cyatheaceae. We first tested if the build-up of species diversity in this
group followed the expectation of the clade-age hypothesis, which would imply that species richness in this family increased at a steady rate through its long evolutionary history. As an alternative hypothesis, we tested, instead, whether their great diversity originated from variations in the rate of diversification between lineages. Second, we investigated the role played by morphological and ecological traits during the evolution of the group by reconstructing the evolutionary dynamics of these traits through time and testing their impact on the rates of diversification. Our findings show that, unlike many species-rich groups, the family Cyatheaceae is an old lineage that diversified at an exceptionally low and homogeneous pace without undergoing extensive morphological evolution. Although the rate of evolution of climatic preferences accelerated in several clades, likely in response to dispersals to higher elevation habitats, this was decoupled from the diversification of the group.

MATERIAL AND METHODS

**Taxonomic sampling**

Our dataset includes 323 species of Cyatheaceae representing ca. 49% of the total extant diversity of the group. All genera were included, with sampling fractions of 49% in *Alsophila*, 47% in *Cyathea*, 72% in *Gymnosphaera* and 49% in *Sphaeropteris*. Sixty six accessions were previously published and we took them directly from Genbank. The rest of the sampled material (257) was collected and identified personally (by one of the authors); voucher material for published sequences (Korall 2000) was also revised (by one of the authors). For taxonomy we follow the recent reinstatement of *Gymnosphaera* as a separate genus from *Alsophila* (Dong and Zuo 2018). As outgroup, we included 12 species from 3 genera (*Calochlaena, Dicksonia, and Lophosoria*) of the sister family Dicksoniaceae.

**DNA extraction, amplification and sequencing**

DNA extraction was performed with the NucleoSpin® Plant II (Macherey Nagel GmbH & Co. KG, Düren, Germany) kit following the manufacturer’s protocol. We generated sequence data for three chloroplast markers: *trnL-trnF* (including the *trnL* group I intron (g1i) and the *trnL-trnF* intergenic spacer (IGS)), *trnG-trnR* (including the *trnG* group II intron (g2i) and the *trnG-trnR* IGS) and *rpl16* (*rps3-rpl16* including parts of the *rps3*-exon, as well as the *rps3-rpl16* IGS and the *rpl16* g2i). For polymerase chain reaction (PCR) and sequencing reactions of *trnG-R*, the primer set and PCR program described by Nagalingum et al. (Nagalingum *et al.* 2007) were used. The PCR and sequencing reactions of *trnL-F* and *rpl16* followed the protocol by Noben et al. (Noben *et al.* 2017). Reactions were performed either on a Biometra TProfessional TRIO thermocycler (Biometra
GmbH, Göttingen, Germany) or on an Eppendorf Mastercycler epgradient S (Eppendorf AG, Hamburg, Germany). For sequencing the services of Macrogen Europe (Amsterdam, Netherlands) and GATC Biotech AG (Konstanz, Germany) were used. All the sequences newly generated in this study are deposited in Genbank (genbank numbers will be added upon acceptance).

**Phylogenetic analyses**

All sequences were aligned using the MAFFT (Katoh et al. 2002) plug-in in Geneious 6.1.8 (Biomatters Ltd). Alignments were visually inspected and ambiguous regions were excluded. The three markers were concatenated into a single alignment using SequenceMatrix 1.7.8 (Vaidya et al. 2011). We partitioned the alignment by genes and the best model of nucleotide substitution for each region was selected based on the Akaike Information Criterion using jModelTest 2.1.7 (Darriba et al. 2012). Although additional chloroplast genes are available with relatively good species sampling on Genbank, we did not use these sequences for our final analyses since they increased the proportion of missing data and adding them to our own molecular dataset resulted in a similar topology (see Sup. Mat for details), similar branch length estimates (Supplementary data Fig. S1) and did not improve substantially the resolution of the estimated phylogenetic tree (Supplementary data Figs S2 and S3). Phylogenetic inference and divergence time estimates were performed using the fossilized birth death model (Heath et al. 2014) implemented in BEAST 2.4.7 (Bouckaert et al. 2014). Unlike traditional node calibration, the fossilized birth death allows the use of all available fossil evidence and not only the oldest unequivocal fossil for a given clade. Hence, we selected a set of 41 fossils of Cyatheales, 13 of which belonged to the Cyatheaceae, and constrained their placement using taxonomic information (Supplementary data Table S1). Importantly, we differ from previous phylogenetic studies of tree ferns regarding the placement of the fossil *Kuylisporites mirabilis* (93.9-100.5 Myr, 74; Mohr and Lazarus 1994), which as been used to calibrate the crown node of Cyatheaceae based on its resemblance with spores of extant species of *Cyathea* and *Alsophila*. However, this interpretation was due to a misleading taxonomic treatment (the species *Cyathea decurrens* used to be treated as *Alsophila decurrens*) and the *Kuylisporites* spore type is actually only found in extant species of *Cyathea* and not in any other genus. Therefore, in our FBD analysis, this fossil was only allowed to be placed in the *Cyathea* lineage and we used the age intervals of the fossils to indicate their respective age. The first appearance of the fossil genus *Cyathocaulis* (Upper Jurassic: 145-165.5 Myr ago) was used to constrain the origin of the Cyatheaceae (see Sup. Mat. for details; Lantz et al. 1999). We performed two runs of 500 million Markov Chain Monte Carlo (MCMC) generations in BEAST 2.4.7, sampling every 50,000 generations. Convergence and effective sample sizes (ESS) were checked in Tracer 1.5.0 (Rambaut and Drummond 2009). The two posterior distributions of trees
were combined after removal of a burn-in of 10% using LogCombiner. We used TreeAnnotator to obtain the maximum clade credibility (mcc) tree after removing the fossil taxa from the combined posterior distributions of trees. In all subsequent macroevolutionary analyses, outgroup species were removed from the phylogenetic tree. Additionally, for comparison we performed divergence time estimation using traditional node dating using four fossil calibrations, including *K. mirabilis* to calibrate the stem node of Cyathea (see Sup. data for details). We also assessed the effect of using a single vs multiple occurrences for each fossil during the FBD analyses (Supplementary data Figs S5 and S6).

**Diversification rate analyses**

We used a variety of approaches to estimate net diversification rates across the Cyatheaceae family and to assess the presence of rate heterogeneity. First, we used BAMM 2.5 (Rabosky 2014) to infer lineage-specific diversification rates on the mcc tree from BEAST. We specified clade-specific sampling fractions to account for non-random incomplete taxon sampling. Given the recent debate on prior sensitivity of the BAMM model (Moore et al. 2016; Rabosky et al. 2017), we ran the analysis under different priors for the number of expected shifts (expectedNumberofShifts = 0.1; 1; 5; 10; 50) to assess the robustness of our result to prior parametrization. Each analysis was run for 20 million generations, sampling every 2,000 generations. Convergence and ESS values were checked using the R package coda (Plummer et al. 2008) and outputs were analyzed in the R package BAMMtools (Rabosky et al. 2014). Second, we used BayesRate (Silvestro et al. 2011) to test for different scenarios of diversification. We accounted for phylogenetic uncertainty by running the analysis over a sample of the posterior distribution of trees. Unlike BAMM, BayesRate does not estimate per-branch rate and require to specify *a priori* branches with putatively varying rates of diversification. As we did not have strong assumptions about rate variation in the family, we implemented a) a four-rates model in which each genus had its own rate and b) a two-rates model with equal speciation and extinction rates for the sister genera *Cyathea* and *Alsophila* and a second set of rates for the remaining genera *Gymnosphaera* and *Sphaeropteris*. We tested these two models against a constant-rate model by computing the marginal likelihoods of the three alternative scenarios. We then estimated speciation, extinction and net diversification rates under the best model by performing an MCMC analysis on a set of 100 trees randomly sampled from the posterior distribution of BEAST, running 10 million generations per tree. Finally, we computed the rates of net diversification (i.e. speciation – extinction) per clade (see Supplementary data Fig. S4 for clades names) using the method of moments (Magallón and Sanderson 2001; Meyer and Wiens 2018). Net diversification rates were
computed both with stem and crown ages and under zero extinction as well as high extinction ($\varepsilon=0.9$; Magallón and Sanderson 2001).

**Morphological data**

We scored a matrix of three quantitative morphological traits (trunk height, lamina length and petiole length) for the species included in the phylogeny. All these variables relate to plant height and crown size, which vary with light availability (Arens and Sanchez Baracaldo 2000). Published taxonomic revisions for the Neotropics (Windisch 1977, 1978; Proctor 1989; Rojas-Alvarado 2001; Christenhusz 2009; Lehnert 2009, 2011, 2012, 2014, 2016; Lehnert and Weigand 2013, 2017; Maciel *et al.* 2017), Africa (Holttum 1981, Janssen and Rakotondrainibe 2007, 2008) and Australasia (Holttum 1959, 1963, 1964, 1965; Lehnert *et al.* 2013) were consulted for character coding, which also drew from our own field observations. We computed overall body size defined as the sum of trunk height, petiole length and lamina length. All values were then log-transformed. Body size has been shown to be one of the main drivers of Cyatheaceae diversification (Ramírez-Barahona *et al.* 2016), although, in their study, low taxonomic sampling prevented any precise modelling of the evolution of this trait through time. We therefore investigated the dynamics of morphological change through time, by modelling body size evolution on our newly inferred time-calibrated phylogenetic tree under a relaxed Brownian Motion process, using reversible-jump MCMC (Eastman *et al.* 2011) as implemented in the function *rjmcmc.bm* of the R package geiger 2.0.6 (Harmon *et al.* 2007). This Bayesian method, which is analogous to the phenotypic evolutionary rate analysis of BAMM, quantifies the heterogeneity in the rate of evolution of a continuous character across branches in the phylogeny by estimating the number and placement of rate shifts. We did not test multiple models of trait evolution but only focused on Brownian Motion because our goal here was to investigate the variation in the rate of evolution of traits through time and between lineages. We performed an analysis of 10 million MCMC generations, sampling every 1,000 generations. Convergence was checked using the R package coda (Plummer *et al.* 2008). To make sure that phylogenetic uncertainty did not bias our estimates, we repeated the analysis on 100 trees randomly extracted from the posterior distribution of BEAST, each time running 5 million MCMC generations and sampling every 1,000 generations. We then summarized the mean number of shift and the mean rate per run. Finally, we re-evaluated the hypothesis that the rates of diversification in the Cyatheaceae were positively correlated with the rate of evolution of body size using Structured Rate Permutations (STRAPP, Rabosky and Huang 2015) as implemented in the R package BAMMtools (Rabosky *et al.* 2014).
Ecological data

We collected occurrence data for all species included in our phylogeny from nine online herbaria (E, GBIF, K, NY, SING, SpeciesLink, Tropicos, US, W) as well as three personal databases from our collaborators (Marcus Lehnert, Rodrigo Cámara Leret, Wilson D. Rodríguez Duque). All records were checked for a) taxonomic correctness, using The Plant List (“The Plant List” 2013) and b) regional GPS precision to country/state borders, using the World Administrative Boarders from GADM (http://www.gadm.org/version2). We corrected the synonyms present in the databases with their accepted names and removed duplicates. Environmental data was extracted from CHELSA version 1.2 (Karger et al. 2017), recalculated to a 2x2 km resolution in R 3.4.3. We used the extract function from the raster package 2.6-7 (Hijmans and van Etten 2014) and interpolated values between the four nearest raster cells (method = bilinear) to account for local GPS errors at the scale of a few kilometers. For each occurrence, we extracted the values of annual precipitation and maximum temperature of warmest month, i.e. the two climatic variables which have been shown to have the strongest limiting effect on tree fern distributions (Bystriakova et al. 2011) and calculated their mean value for each species. The evolution of climatic preferences, along with morphological evolution, has been shown to be linked with increased diversification in the Cyatheaceae family (Ramírez-Barahona et al. 2016). Thus, we analyzed the two climatic variables following the same procedure as for body size, to re-assess the evolution of climatic preferences within the family and its impact on species diversification. We tested for heterogeneity in the rate of evolution of ecological traits by modelling the evolution of precipitation and temperature along the phylogenetic tree. We chose this single variable approach because the use of principal component scores from multivariate analyses such as PCA or OMI can potentially biased the results of macroevolutionary analyses (Uyeda et al., 2015). We performed a reversible-jump MCMC analysis of 10 million MCMC generations sampling every 1,000 generations, using the rjmcmc.bm function in R and repeated the analysis on 100 hundred phylogenetic trees from the posterior distribution. We then tested for trait-dependent diversification using STRAPP (Rabosky and Huang 2015) as implemented in the R package BAMMtools (Rabosky et al. 2014). Additionally, we recorded the mean elevation for the species included in the phylogeny using the same published taxonomic revisions that we consulted to score morphological data (see above) and visualized their altitudinal distribution by making the histogram of mean elevation and boxplots of the maximum elevation at different latitudes.
RESULTS

Phylogenetic reconstruction

Our concatenated alignment of the three chloroplast genes was composed of 335 individuals and had a length of 3,002 bp. For each DNA region, the best model of nucleotide substitution was GTR+G. The two runs of the fossilized birth-death analysis in BEAST2 gave congruent results and all parameters had effective sample size values above 200. The topology of the maximum clade credibility tree supported the monophyly of the four genera (Fig. 1). The topology of the marginate-scaled clade was recovered with Gymnosphaera sister to a clade formed by Alsophila and Cyathea but this relationship had a posterior probability of 0.33 and was therefore not supported (Support values provided in Supplementary data Fig. S3). Relationships among closely related species were also poorly resolved as indicated by low node posterior probabilities, especially within Cyathea and Alsophila. The Neotropical species of Alsophila formed a monophyletic clade. Divergence time estimates showed that Cyatheaceae diverged from their sister group, the Dicksoniaceae family, around 200 Myr ago (95% HPD of stem node: 184.74-217.74) and the most recent common ancestor of all extant species is 140 Myr old (95% HPD of crown node: 108.63-170.86). Sphaeropteris had a crown age of ca. 125 Myr and the remaining three genera had crown ages between 75 and 80 Myr. Overall, the node dating analysis resulted in a similar topology with constantly younger age estimates, especially in the deeper nodes of the tree (Table 1).

Diversification rates

The results of the BAMM analyses with different priors on the number of rate shift were similar, indicating that the analyses were robust to prior influence. Therefore, we present only the results from the analysis with a prior rate shift of 1. The rate of diversification slowly increased through time (mean net diversification rate of 0.039 (95% HPD: 0.004-0.055) lineages per Myr at the root and of 0.070 (95% HPD: 0.055-0.078) lineages per Myr at the tips) and BAMM detected no shift in the rate of diversification across the Cyatheaceae (Fig. 2). In BayesRate, although the model with the highest marginal likelihood was the two rates model (Supplementary data Table S2), with higher posterior net diversification rate for Alsophila and Cyathea (0.0608 lineages per Myr; 95% HPD: 0.040-0.084) than for Gymnosphaera and Sphaeropteris (0.0242 lineages per Myr; 95% HPD: 0.006-0.042), neither speciation nor extinction rates were actually significantly different in the two groups (Supplementary data Fig. S7). Per-clade rates of net diversification estimated with the methods of moments varied between 0.025 lineages per Myr and 0.142 lineages per Myr under no extinction and between 0.009 lineages per Myr and 0.072 lineages per Myr when extinction was set to 0.9 (Supplementary data Table S3). Despite some differences among rate estimates, all
methods concurred in finding low diversification rates with little evidence for strong rate heterogeneity across branches.

**Morphological evolution**

The analysis of body size evolution showed a constant background rate of 0.0038 and a single shift with a posterior probability of 0.48 towards an increased rate detected in a small clade of four species of *Cyathea* (Supplementary data Fig. S8). This result was robust to phylogenetic uncertainty as shown by the distributions of the mean number of shift and mean rate across the 100 additional runs (Supplementary data Fig. S10). The result from the STRAPP analysis indicated no significant correlation between body size and net rates of diversification (Table 2).

**Climatic niche evolution**

The analysis of trait evolution recovered a heterogeneous process for the evolution of temperature preferences, with a median posterior rate of 0.00001 and four shifts towards a rate of 0.00375 with posterior probabilities between 0.39 and 0.99 (Fig. 3), in the clades exhibiting the highest mean elevation and the widest elevational range. For annual precipitation, the median posterior rate was 0.00124 and rate acceleration was detected for the entire Neotropical *Cyathea* clade, which showed a rate of 0.00174 (Fig. 3). However, the posterior probability of this shift was only 0.14, indicating poor support (Fig. 3). The 100 replicated MCMC analyses showed that topological variation did not significantly impact the estimated rates and number of shifts of these two climatic variables (Supplementary data Fig S7). The STRAPP analysis showed that neither maximum temperature nor annual precipitation were correlated with net rates of diversification (Table 2).

**DISCUSSION**

In this study, we evaluated several hypotheses related to the evolutionary history of a diverse clade of tree ferns and investigated the impact of morphological and climatic traits on the diversification of the group. We compared whether the build-up of species diversity in this group occurred through a steady accumulation of species through time or whether their great diversity originated instead from variations in the rate of diversification between lineages. We then investigated the role played by morphological and ecological traits in their evolution and tested the impact of these traits on the rates of diversification in the group.

Based on an unprecedented sampling of nearly half of extant species diversity and integrating data from the rich fossil record of tree ferns, we estimated divergence times using the fossilized birth-death model. In agreement with published phylogenies (Janssen et al. 2008; Korall
and Pryer 2014; Ramírez-Barahona et al. 2016), we found support for four main clades within Cyatheaceae, which are here referred to as the genera Alsophila, Cyathea, Gymnosphaera and Sphaeropteris. We also recovered a monophyletic clade for the Neotropical species of Alsophila which were split between two different clades in a previous phylogeny with lower sampling (Korall and Pryer 2014). As in most previous studies (Korall 2000; Janssen et al. 2008; Korall and Pryer 2014; Ramírez-Barahona et al. 2016), we found low support for the phylogenetic relationships among Alsophila, Cyathea and Gymnosphaera, which is not surprising given that our molecular dataset is restricted to three chloroplast loci and that Cyatheaceae have a notably low rate of molecular evolution (Korall et al. 2010). Since additional plastid sequences available in public repositories did not appear to improve substantially the resolution of the phylogeny, it is likely that only the addition of nuclear genes could yield a more robust resolution of the deep and shallow phylogenetic splits in this slowing evolving group. Alternatively, the low amount of divergence at the molecular level between Alsophila, Cyathea and Gymnosphaera (Fig. 1) may result from high level of incomplete lineage sorting resulting in a “hard” polytomy in this part of the phylogenetic tree.

Whether we used the fossilized birth-death process or node dating, the recovered age estimates point to an older origin of the family than in most of the previous estimates (Janssen et al. 2008; Korall and Pryer 2014; Ramírez-Barahona et al. 2016; Testo and Sundue 2016; Lehtonen et al. 2017). These differences in dating can be explained by several factors, including the different taxonomic assignment of the fossil K. mirabilis, the better taxon sampling and more realistic evolutionary models used in our phylogenetic analyses. The inclusion of old fossils representing stem lineages in the FBD analyses probably explain why the ages estimates recovered by this method are older than those obtained with node calibration (Saladin et al. 2017).

Results from our diversification analyses using different methods are coherent, and consistently indicate that the rate of diversification in Cyatheaceae was low and underwent very little variation throughout their long evolutionary history. This finding is consistent with a near-stable sampled diversity in the fossil record of Cyatheales (Lehtonen et al. 2017), but contrasts strongly with previous reports of dramatic heterogeneity in the rates of diversification among Cyatheaceae (Janssen et al. 2008; Ramírez-Barahona et al. 2016). Previous studies were based on just about 10% of the extant species and we believe that the 5-fold increase in taxon sampling in our analysis and advances in the molecular dating methods are at the basis of the divergent conclusions regarding the tempo of diversification in the group. Our estimates of the net diversification rates are 10-40 times slower than those of the fastest plant radiations reported to date, such as in Dianthus (Valente et al. 2010), Lupinus (Hughes and Eastwood 2006), or Andean Campanulaceae (Lagomarsino et al. 2016). The estimates obtained for the Cyatheaceae are nevertheless among the
intermediate values for fern families, where net diversification rates range from 0.001 in Thyrsopteridaceae to 0.132 in Athyriaceae (Testo and Sundue 2018). However, apart from the Hymenophyllaceae, fern families with more than 600 species are generally younger clades that diversified at higher rates than the Cyatheaceae, whereas many of the early branching fern clades are characterized by much lower current diversity (Testo and Sundue 2016, 2018). The Cyatheaceae, therefore, exhibit an uncommon pattern of an old lineage that has successfully diversified by accumulating species at a very low rate through time without any significant among-clade variation. The exact factors that have allowed the Cyatheaceae to diversify so extensively when compared to other tree fern families remains to be investigated, but this will require a much denser taxonomic sampling in the other families than what we have in our current study.

Our analyses have also shown that the modes of evolution differed substantially between morphological and climatic traits. The rate of body size evolution was remarkably low and constant across the phylogeny. This may seem surprising given that tree ferns differ considerably in size, ranging from minute rock-hugging species with leaves 15 cm long to very large species 20 m tall with 5 m long leaves. However, dwarf species are found only in Neotropical species of *Cyathea*, where dwarf growth has repeatedly evolved as an adaptation to exposed rocky outcrop habitats. This is most conspicuous in the *Hymenophyllopsis* group with 11 species (Maciel et al. 2017), but also concerns about 25 other species, which based on morphology should be distributed in several other clades. Due to the inaccessibility of these habitats, many of these species have only recently been described (Lehnert 2006; Tejedor and Calatayud 2017; Acuña-Tarazona et al. 2018), and their diversity may be underestimated. Acceleration in the rate of body size evolution may thus be expected in dwarf *Cyathea*, but the shift detected in a clade of four of these species was not strongly supported (Supplementary data Fig. S8), which may be due to the limited sampling of these groups. Although undersampling of dwarf species may underestimate morphological evolution in Neotropical *Cyathea*, it unlikely affects the overall result of low and nearly constant rate of body size evolution in the family. Korall et al. (2010) reported exceptionally slow rates of molecular evolution in tree ferns in comparison to other fern lineages, while a recent study found a negative correlation between substitution rates and body size in tree ferns (Barrera-Redondo et al. 2018), but it is unclear whether this could actually be the underlying factor limiting the rate of phenotypic evolution. Given this result and the homogeneous rate of diversification estimated, it is thus not surprising that we did not detect any association between body size and diversification.

In contrast, the evolution of climatic preferences followed a more heterogeneous process. For precipitation, a small rate increase was detected in the clade of Neotropical *Cyathea*, whereas for temperature, several shifts occurred independently within four clades in three different genera (Fig. 3). These four clades correspond to a primarily Andean clade in *Cyathea*, and primarily New
Guinean clades in *Alsophila* and *Sphaeropteris*. We interpret these patterns as the results of the topo-climatic heterogeneity of these mountains: The Andes and New Guinea are the most extensive and the highest tropical mountain systems, allowing for niche shifts along the elevational gradient. However, whereas New Guinea mostly has humid climatic conditions in the mountains, the Andes have a much wider range of precipitation regimes, ranging from xeric to hyper-humid, which probably explains why only a single shift was detected for precipitation in the Neotropical clade of *Cyathea*. However, these shifts in the evolution of climatic preferences likely triggered by the colonization of mountains ranges were decoupled from species diversification, as shown by the lack of association between climatic traits and the rates of net diversification. This result was unexpected given that tropical mountains probably played an important role during the evolutionary history of Cyatheaceae. Indeed, these ecosystems harbour most of the global fern diversity and colonization of montane habitats has been hypothesized to be the primary driver of diversification in several fern lineages (Kessler *et al.* 2016). For example, in Polypodiaceae, it has been shown that shifts in elevation but not in leaf area, were positively associated with the rates of diversification, supporting the idea that exploration of new habitats rather than morphological innovation drove the diversification of the group (Sundue *et al.* 2015). Environmental factors were also associated with diversification in a study using palaeontological data to investigate the drivers of diversification in all ferns (Lehtonen *et al.* 2017), but the effect was correlated with extinction rates and not origination rates, which were largely diversity-dependent. In Cyatheaceae, tropical mountains likely provided new suitable habitats in which topographical complexity triggered allopatric speciation. Indeed, geographic isolation favoured by geological events has been suggested as an important factor in promoting differentiation in tree ferns (Ramírez-Barahona and Luna-Vega 2015). Although the distribution of Cyatheaceae reaches high latitudes, their altitudinal range is greatest closer to the Equator and decreases towards higher latitudes (Supplementary data Fig. S9). The appearance of montane habitats in tropical regions may have compensated the range contraction that Cyatheaceae underwent during their evolutionary history, as shown by the latitudinal range of their fossil record exceeding their extant distribution (Collinson 2001). For example, Late Cretaceous fossil spores (*Kuylisporites*) similar to the spores of extant *Cyathea* are found in Siberia. Records of Cyatheaceae are also known from Antarctica in the Eocene, but are restricted to Central America by the Pliocene (Collinson 2001). Therefore, rather than triggering a sharp biome shift that could increase net diversification rates, dispersals into higher elevations in the tropics may have simply represented an opportunity for Cyatheaceae to track or slightly expand their preferred climatic conditions. This is coherent with the fact that tree ferns are restricted to warm and humid environments with low seasonality and exhibit strong niche conservatism (Bystriakova *et al.* 2011; Sosa *et al.* 2016).
Our findings regarding morphological and climatic niche evolution disagree with the results of Ramírez-Barahona et al. (Ramírez-Barahona et al. 2016) who reported a positive correlation between the rates of body size and climatic niche evolution with the rates of diversification. Although our phylogeny is neither complete nor fully supported, we argue that our modelling of trait evolution, which took into account phylogenetic uncertainty, is more realistic than the approach used by Ramírez-Barahona et al. (Ramírez-Barahona et al. 2016), who circumvented a poor taxonomic sampling by modelling trait evolution on simulated trees, a procedure that has been shown to yield biased estimates (Rabosky 2015). Conversely, and in line with our own results, a recent study across all ferns found a lack of association between the rates of diversification and the rates of body size evolution, a result that held true when looking at the Cyatheaceae in particular (Testo and Sundue 2018).

CONCLUSIONS

Our study provides a new perspective on the evolution of one of the most conspicuous vegetation elements of tropical montane forests. Rather than interpreting the present-day diversity of the Cyatheaceae as the result of adaptive bursts of diversification as previously suggested (Ramírez-Barahona et al. 2016), our findings indicate that their diversity steadily accumulated over time. Colonization of mountain habitats seems to have triggered a small acceleration of niche evolution but it was not followed by rapid radiation, unlike what has been observed in several plant groups, particularly in the Andes (Hughes and Atchison 2015; Uribe-Convers and Tank 2015; Pérez-Escobar et al. 2017; Pouchon et al. 2018; but see Wagner et al. 2013; Salaríto et al. 2016). Many empirical examples support the view that changes in diversification rates linked to trait evolution are the primary driver of species diversity (Matuszak et al. 2016; Onstein et al. 2017), but counterexamples have shown that it is not a ubiquitous pattern (Vamosi and Vamosi 2011; Rabosky et al. 2012). The present study provides further evidence that a species-rich clade can result from the slow and steady accumulation of species over long evolutionary times and that rapid morphological differentiation, the evolution of key innovations, and niche divergence are not a prerequisite for a clade to thrive for hundreds of millions of years.

SUPPLEMENTARY DATA

Supplementary data are available online at https://academic.oup.com/aob and consist of the following. Fig S1: Correlation of branch length of the two RaxML trees. Fig S2: Boxplots of bootstrap values for the two phylogenetic trees. Fig S3: Clades used to compute net diversification rates with the methods of moments. Fig S4: Maximum clade credibility tree from the BEAST2
analyses under the FBD process. Fig S5: Comparison of parameter estimates from the FBD run with several fossil per taxon and a single fossil per taxon. Fig S6: Correlation of clade age estimates between the FBD run with a single fossil per taxon and the FBD run with several fossil per taxon. Fig S7: Differences between the posterior rates of the two groups for the best model in the Bayesrates analysis. Fig S8: Posterior rates of body size evolution modelled under relaxed Brownian motion. Fig S9: Altitudinal distribution of the Cyatheaceae. Fig S10: Histograms of the mean rate and mean number of shifts across the 100 replicates rjmcmc analysis for body size, annual precipitation and maximum temperature of warmest month. Table S1: Fossils included in the Fossilized Birth-Death analysis. Table S2: Model comparison in BayesRates and parameter estimation for the best model with two rates. Table S3: Net diversification rate estimates from the methods of moments.

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Figure captions

**Figure 1.** Time calibrated phylogeny of the Cyatheaeeae. Numbers indicate million years.

**Figure 2.** Results from BAMM analysis. Best configuration (left) and net diversification rate through time (right).

**Figure 3.** Posterior rates of the evolution of maximum temperature (left) and precipitation (right) modelled under a relaxed Brownian motion.
Table 1. Clade age estimates from the node dating (ND) and Fossilized-Birth-Death (FBD) analyses.

|                         | ND<sub>min</sub> | ND<sub>max</sub> | ND<sub>mean</sub> | FBD<sub>min</sub> | FBD<sub>max</sub> | FBD<sub>mean</sub> |
|-------------------------|------------------|------------------|-------------------|-------------------|-------------------|-------------------|
| stem Cyatheaceae        | 147.48           | 172.40           | 157.75            | 184.74            | 217.74            | 199.47            |
| crown Cyatheaceae       | 101.06           | 136.83           | 117.80            | 108.63            | 170.86            | 139.42            |
| crown Alsophila         | 55.41            | 86.86            | 71.02             | 52.32             | 99.08             | 75.15             |
| A. abbottii             | 25.41            | 47.56            | 40.04             | 18.32             | 42.81             | 30.08             |
| A. cuspidata            | 15.63            | 35.05            | 26.01             | 18.48             | 47.31             | 34.60             |
| A. australis            | 42.01            | 76.82            | 60.28             | 39.61             | 85.98             | 62.84             |
| A. humilis              | 11.20            | 32.00            | 20.66             | 9.99              | 32.00             | 20.30             |
| A. manniana             | 11.19            | 51.15            | 29.47             | 10.42             | 52.12             | 28.94             |
| A. smithii              | 36.30            | 65.77            | 50.78             | 33.83             | 73.20             | 52.78             |
| crown Cyathea           | 57.56            | 85.42            | 71.45             | 60.34             | 105.78            | 81.30             |
| C. cneumidaria          | 19.99            | 41.69            | 30.51             | 18.96             | 45.59             | 31.69             |
| C. armata               | 36.85            | 64.63            | 50.46             | 39.26             | 77.19             | 57.70             |
| C. decurrens            | 33.26            | 70.88            | 51.71             | 30.65             | 80.23             | 55.42             |
| C. divergens            | 32.13            | 54.68            | 44.74             | 29.41             | 59.08             | 48.44             |
| C. gibbosa              | 26.64            | 45.34            | 35.76             | 29.14             | 57.03             | 37.56             |
| crown Gymnosphaera      | 54.79            | 91.34            | 73.18             | 53.63             | 110.77            | 81.35             |
| G. asian                | 33.29            | 60.83            | 46.51             | 31.95             | 68.28             | 48.79             |
| G. malagasy             | 4.37             | 31.66            | 16.04             | 2.94              | 28.04             | 14.06             |
| crown Sphaeropteris     | 81.45            | 126.48           | 106.72            | 89.13             | 160.38            | 124.53            |
| S. brunei               | 10.97            | 35.11            | 22.58             | 10.59             | 37.27             | 22.76             |
| S. fourniera            | 16.27            | 56.28            | 35.18             | 15.34             | 60.81             | 35.35             |
| S. glauca               | 29.08            | 56.79            | 49.33             | 27.97             | 62.17             | 50.82             |
| S. sarcopholis          | 17.27            | 42.57            | 29.39             | 15.98             | 44.65             | 29.77             |
| S. schizocaena          | 7.90             | 30.03            | 18.38             | 7.08              | 30.13             | 18.07             |
| crown Dicksoniaceae     | 117.83           | 151.95           | 136.93            | 128.38            | 201.51            | 167.10            |
| crown Dicksonia         | 40.01            | 72.26            | 55.03             | 23.76             | 72.24             | 47.46             |
Table 2. Correlation coefficients from STRAPP analyses

|               | Body size | Max temperature | Annual precipitation |
|---------------|-----------|-----------------|----------------------|
| R             | -0.069    | 0.032           | 0.002                |
| P-value       | 0.904     | 0.940           | 0.928                |
Figure 1
