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Global biogeography of scaly tree ferns (Cyatheaceae): evidence for Gondwanan vicariance and limited transoceanic dispersal

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ABSTRACT

Aim Scaly tree ferns, Cyatheaceae, are a well-supported group of mostly tree-forming ferns found throughout the tropics, the subtropics and the south-temperate zone. Fossil evidence shows that the lineage originated in the Late Jurassic period. We reconstructed large-scale historical biogeographical patterns of Cyatheaceae and tested the hypothesis that some of the observed distribution patterns are in fact compatible, in time and space, with a vicariance scenario related to the break-up of Gondwana.

Location Tropics, subtropics and south-temperate areas of the world.

Methods The historical biogeography of Cyatheaceae was analysed in a maximum likelihood framework using Lagrange. The 78 ingroup taxa are representative of the geographical distribution of the entire family. The phylogenies that served as a basis for the analyses were obtained by Bayesian inference analyses of mainly previously published DNA sequence data using MrBayes. Lineage divergence dates were estimated in a Bayesian Markov chain Monte Carlo framework using beast.

Results Cyatheaceae originated in the Late Jurassic in either South America or Australasia. Following a range expansion, the ancestral distribution of the marginate-scaled clade included both these areas, whereas *Sphaeropteris* is reconstructed as having its origin only in Australasia. Within the marginate-scaled clade, reconstructions of early divergences are hampered by the unresolved relationships among the *Alsophila*, *Cyathea* and *Gymnosphaera* lineages. Nevertheless, it is clear that the occurrence of the *Cyathea* and *Sphaeropteris* lineages in South America may be related to vicariance, whereas transoceanic dispersal needs to be inferred for the range shifts seen in *Alsophila* and *Gymnosphaera*.

Main conclusions The evolutionary history of Cyatheaceae involves both Gondwanan vicariance scenarios as well as long-distance dispersal events. The number of transoceanic dispersals reconstructed for the family is rather few when compared with other fern lineages. We suggest that a causal relationship between reproductive mode (outcrossing) and dispersal limitations is the most plausible explanation for the pattern observed.

Keywords

Cyatheaceae, Gondwana, historical biogeography, Lagrange, long-distance dispersal, scaly tree ferns, transoceanic dispersal, vicariance.

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INTRODUCTION

The dispersal units of ferns are haploid spores, whereas in seed plants they are diploid seeds. Spores are minute, relative to most seeds, and easily transported by wind, suggesting that successful long-distance dispersal of ferns should be both easy and common. This idea, however, has been challenged by recent studies showing that the reproductive biology of ferns is more complex than previously assumed. Most diploid ferns are predominantly obligate outcrossers (Soltis

& Soltis, 1987; Haufler, 2007), requiring that the male and female gametes originate from different gametophytes that, in turn, each originated from spores from different sporophytic individuals, termed intergametophytic crossing (Klekowski, 1973), or now simply referred to as outcrossing (K.M. Pryer *et al.*, in prep.). Therefore, for a fern to migrate into a new environment, not one, but two, spores – from different individuals – are usually needed. Furthermore, the spores must land in such close proximity that the male gamete is able to swim to the female gamete in a thin film of water. This additional biological complexity confounds the presumed potential of small spore size for yielding a successful dispersal event. Hence, our understanding of fern biogeography is undergoing a paradigm shift where vicariance versus long-distance dispersal scenarios both need to be carefully evaluated in analysing the global distribution patterns of ferns (Wolf *et al.*, 2001; Haufler, 2007).

Despite the reported bias of ferns towards outcrossing, however, there are studies showing that some ferns appear to be more easily dispersed than their sister group, the seed plants. For example, the proportion of fern species on islands, relative to angiosperms, has been shown to be higher than expected when compared with mainland diversity (Kreft *et al.*, 2010; see also early work by Tryon, 1970; Smith, 1972). Furthermore, the level of fern endemism on islands is lower than that of angiosperms (Smith, 1972; Ranker *et al.*, 1994), suggesting that the dispersal of fern individuals to islands occurs frequently enough to prevent those populations from becoming genetically distinct (Ranker *et al.*, 1994).

What is the impact of long-distance dispersal in shaping the biogeography of ferns? To what extent are vicariance scenarios responsible for these patterns? Ultimately, to fully address these and other questions on fern distribution patterns from an evolutionary perspective, several comparable studies are needed across many clades of ferns. Although geography is often discussed in fern phylogenetic studies, more studies are needed that explicitly analyse the historical biogeography of a group (see e.g. Kreier & Schneider, 2006; Janssen *et al.*, 2007, 2008; Perrie *et al.*, 2007; Kreier *et al.*, 2008; Hennequin *et al.*, 2010), and that test hypotheses of vicariance versus long-distance dispersal by incorporating geological time.

Scaly tree ferns, Cyatheaceae, are a well-supported group of mostly tree-forming ferns and include approximately 500 species (Conant *et al.*, 1995). Members of Cyatheaceae are found throughout the tropics, subtropics and the south-temperate zone (Kramer, 1990), with the greatest species diversity in tropical areas of America and Malesia (Conant *et al.*, 1995). Recently, the phylogeny of scaly tree ferns received considerable attention (Conant *et al.*, 1994, 1995; Conant & Stein, 2001; Korall *et al.*, 2006, 2007; Janssen *et al.*, 2008), such that we now have a robust understanding of the broader relationships within the group. Members of the family consistently fall into four major (genus-level) groups, with *Sphaeropteris* sister to the other three, *Cyathea*,

Alsophila s.s. and *Gymnosphaera* + *Cyathea capensis* (Korall *et al.*, 2007). The basal dichotomy is supported by scale morphology, with *Sphaeropteris* having conform scales and the others marginate scales (see fig. 1 in Korall *et al.*, 2007).

Relationships among the three groups with marginate scales are unclear, with DNA sequence data not strongly supporting any of the possible resolved topologies. All four groups occur in both South America and Australasia, whereas only *Alsophila* s.s. and *Gymnosphaera* + *C. capensis* are represented in Africa. Fossils that can be referred to stem lineages of Cyatheaceae and the marginate-scaled clade show that the family had its origin at least as early as in the Late Jurassic (Lantz *et al.*, 1999) and diversified in the Late Cretaceous (Mohr & Lazarus, 1994).

Given its robust phylogeny and a fossil record that suggests a Mesozoic origin for the group (i.e. dating back to the supercontinent Gondwana), the scaly tree ferns are an ideal model group with which to study fern dispersal in an evolutionary context (Salvo *et al.*, 2010). In this study we analysed the large-scale historical biogeography of Cyatheaceae by using a phylogeny that covered the broad geographical distribution of the group. We investigated the possible impact of transoceanic dispersals in shaping the biogeography of scaly tree ferns, and tested the hypothesis that some of the observed distribution patterns were in fact compatible, in time and space, with a vicariance scenario related to the break-up of Gondwana.

MATERIALS AND METHODS

Nomenclature

Taxonomy within Cyatheaceae differs among authors. We follow Korall *et al.* (2007) and use generic names corresponding to the major lineages within the family: *Alsophila*, *Cyathea* and *Sphaeropteris*. Species within the *Gymnosphaera* + *C. capensis* clade are all referred to as *Cyathea* and not *Alsophila* as in Korall *et al.* (2007) to accommodate those taxa new to this study for which *Alsophila* synonyms are not available.

Taxon sampling

The ingroup comprised a total of 78 taxa (see Appendix S1 in Supporting Information) encompassing the geographical (as well as morphological and taxonomic) variation within Cyatheaceae and includes almost all taxa from Korall *et al.* (2007), as well as 14 additional species from Janssen *et al.* (2008). The *Alsophila sinuata* specimen used in Korall *et al.* (2007) was excluded here and replaced by *A. sinuata* from Janssen *et al.* (2008) because more sequence data were available for the latter.

The outgroup includes 14 tree fern representatives from three closely related families (Korall *et al.*, 2006; Schuettpelz & Pryer, 2007): 10 representatives from Dicksoniaceae, three from Cibotiaceae, and *Thyrsopteris elegans* from the

monotypic Thyrsopteridaceae [family circumscriptions follow Smith *et al.* (2006); Appendix S1]. This sampling covers the entire geographical range for the outgroup. Representatives from Metaxyaceae were excluded to avoid analytical problems potentially associated with the elevated substitution rates previously reported for the family (Korall *et al.*, 2006).

Molecular data

Almost all the DNA sequence data used in this study were previously published in either Korall *et al.* (2007) or Janssen *et al.* (2008). GenBank accession numbers are presented in Appendix S1 for the five plastid regions analysed, comprising the protein-coding *rbc*L gene, and four non-coding, intergenic spacer (IGS) regions: *rbc*L–*acc*D [including 93 bases from the *rbc*L gene (bases not included in the *rbc*L data set) and 779 from the *acc*D gene], *rbc*L–*atp*B, *trn*G–*trn*R (*trn*G–R, includes the *trn*G intron), and *trn*L–*trn*F (*trn*L–F, includes the *trn*L intron). New sequence data is provided here for the non-coding regions of *Thyrsopteris elegans* (GenBank accession numbers HG422547–HG422550). DNA isolation, amplification and sequencing of these regions were as described in Korall *et al.* (2007). For a few taxa, sequences from all regions were not available (one sequence was missing for *rbc*L–*atp*B, two for *rbc*L–*acc*D, one for *trn*G–R, and ten for *trn*L–F; see Appendix S1); in these cases, the sequence data were coded as missing.

Sequence alignments

Each region was aligned manually using MacClade 4.08 (Maddison & Maddison, 2005). Ambiguously aligned regions were excluded from the analyses and no gap coding was performed. The sequence alignments are deposited in the Dryad data repository (see Data Accessibility below).

Phylogenetic analyses

The five single-region data sets and the combined data set were analysed with a Bayesian Markov chain Monte Carlo (BMCMC) approach using the parallel version of MrBayes 3.1.1 (Huelsenbeck & Ronquist, 2001; Ronquist & Huelsenbeck, 2003). Nucleotide substitution models for each of the regions were chosen using MrAIC 1.4 (Nylander, 2004) in combination with phyml 2.4.4 (Guindon & Gascuel, 2003). Model choice was based on the corrected Akaike information criterion (AIC$_c$) and was the GTR+I+G model for the *rbc*L and *rbc*L–*acc*D regions, and the GTR+G model for *rbc*L–*atp*B, *trn*G–R and *trn*L–F. Each analysis was run for 10 million generations, on six parallel chains, with the temperature parameter (for heating the chains) set to 0.1. Four independent analyses of each region were run simultaneously. The values sampled for different parameters were examined using the program Tracer 1.5 (Rambaut & Drummond, 2009) to determine whether the parameters had converged. We also examined the standard deviation of the split frequencies

among the independent runs as calculated by MrBayes. For each analysis, every 1000th tree was sampled and, after parameter values were analysed, 1000 initial trees were discarded as 'burn-in'. Trees from each of the independent analyses (except those discarded as burn-in) were pooled before calculating a majority-rule consensus tree for each region. All trees were rooted with the most distant outgroup taxon, *Thyrsopteris elegans* (Korall *et al.*, 2006).

Combinability of data sets

The combinability of data sets was evaluated by examining the consensus topologies from each of the five single-region analyses for potential conflicts. Incongruence supported by a Bayesian posterior probability of 0.99 or higher was considered a conflict. A single conflict was found at the branch tips, involving the position of *Alsophila lastii*; it was sister to *Alsophila dregei* in the *rbc*L topology, and in a polytomy with *Alsophila hyacinthei* and *Alsophila glaucifolia* in the *trn*G–R topology. Given the very minimal conflict detected, the five data sets were combined into a single concatenated data set including a total of 5889 characters (not counting the excluded characters).

Analyses of the combined data set

The BMCMC analysis of the combined data set was performed using a single partition for the entire data set (the GTR+I+G model was selected as described above), and with the same settings as for the single-region analyses. Previous studies have shown that the relationships among *Cyathea*, *Alsophila* s.s. and *Gymnosphaera* + *C. capensis* are unclear, irrespective of analytical method used (maximum parsimony, maximum likelihood or Bayesian inference; see Korall *et al.*, 2007, and references therein). The frequency of each of the possible resolved topologies among these three groups was calculated from the pool of trees (after removal of the burn-in) resulting from the Bayesian analysis of the combined data set by using the Perl script 'seltrees.pl' (written by T. Eriksson, University of Bergen, Norway).

Molecular dating

Estimates of lineage divergence times were calculated in a BMCMC framework using beast 1.5.4 (Drummond & Rambaut, 2007). The combined data set was analysed as a single partition using the GTR+I+G model with four rate categories (model chosen as for the phylogenetic analyses above), an uncorrelated, lognormal clock model, a Yule tree prior, and the starting tree was randomly generated. The MCMC chains were run for 10 million generations, and parameters were sampled every 1000 generations.

Because the results of the phylogenetic analyses using MrBayes (above) were inconclusive with respect to the relationships of the three groups of marginate-scaled taxa (*Cyathea*, *Alsophila* s.s., and *Gymnosphaera* including *C. capensis*),

three independent BEAST analyses were performed, each with topological constraints reflecting the three possible sister relationships (i.e. with *Alsophila* + *Cyathea*, *Alsophila* + *Gymnosphaera*, and *Cyathea* + *Gymnosphaera*). This allowed us to investigate how each of the different topologies would affect our biogeographical interpretations. All analyses were also constrained so that *Thyrsopteris elegans* was designated sister to all other taxa.

Three calibration points based on fossil evidence were incorporated in each of the analyses. All three calibration points were assigned a lognormal prior distribution to allow for the possibility of very old ages, but with larger probability closer to the estimated age of the fossil. The mean and standard deviation were set to 1, and the offset was conservatively set to the estimated age (following Gradstein *et al.*, 2004) of the upper boundary of the strata where the fossil was found. Fossils referred to the genus *Cyathocaulis*, such as *Cyathocaulis naktogensis* and *Cyathocaulis yabei* (Upper Jurassic) are stem members of Cyatheaceae, as shown in a morphological phylogenetic analysis based on trunk characters of extinct and extant tree ferns (Lantz *et al.*, 1999). The upper boundary of the Upper Jurassic (145.5 Ma) therefore serves to calibrate the most recent common ancestor of Cyatheaceae and its sister group, *sensu* Korall *et al.* (2006, 2007) and Smith *et al.* (2006). The triporate spores of the fossil genus *Kuylisporites* are similar to spores of extant species in the clades *Cyathea* and *Alsophila*, but not to *Sphaeropteris* (Mohr & Lazarus, 1994; Collinson, 2001). *Kuylisporites waterbolkii* is sometimes more specifically referred to 'Cnemidaria' taxa within *Cyathea* (Mohr & Lazarus, 1994). However, this interpretation has been questioned based on similar spores observed in *Alsophila decurrens* (Collinson, 2001), suggesting a position along the stem lineage of the marginate-scaled clade. We therefore conservatively use the first appearance of these fossils (Cenomanian, Upper Cretaceous, upper boundary of strata 93.5 Ma) to calibrate the most recent common ancestor of the marginate-scaled clade + *Sphaeropteris* (i.e. Cyatheaceae). The fossils *Lophosoria cupulatus* and *Conantiopteris* (both Aptian, Lower Cretaceous) are, based on spore, leaf and/or trunk morphology, considered to be closely related to the extant species *Lophosoria quadripinnata*, either as stem or crown group members (Cantrill, 1998; Lantz *et al.*, 1999, includes a phylogenetic analysis based on trunk characters). We assign the age of the upper boundary of the Aptian, Lower Cretaceous (112 Ma) to the most recent common ancestor of Dicksoniaceae, taking into account the moderately supported relationships among the three genera in the family (*Calochlaena*, *Dicksonia* and *Lophosoria*; Korall *et al.*, 2006).

Each BEAST analysis was repeated twice and the sampled values were examined for convergence using the program TRACER 1.5 (Rambaut & Drummond, 2009). After the removal of the burn-in (1 million generations in each analysis, corresponding to 10% of the samples) the remaining samples from the two runs were summarized as a maximum clade credibility tree with mean divergence times using TREEANNOTATOR (part of the BEAST package).

Biogeographical analyses

We performed biogeographical analyses in a maximum likelihood framework using LAGRANGE v.20100721 (Ree *et al.*, 2005; Ree & Smith, 2008). Three analyses were performed, one for each of the three possible resolved topologies, and based on the time-calibrated trees resulting from the BEAST analyses. We defined eight biogeographical regions, mainly following Sanmartín & Ronquist (2004), but with a few exceptions where the aim of our study was better addressed using more inclusive units. These regions are: (A) South America (including Mexico and Central America); (B) Atlantic (including St. Helena); (C) Africa; (D) Madagascar, Comoros, Réunion and Mauritius; (E) India (including Sri Lanka); (F) Southeast Asia (including the Malaysian Peninsula, Philippines, Sumatra and Borneo); (G) Australasia including Australia, New Zealand, New Caledonia and New Guinea, as well as Lord Howe and Norfolk Islands (this is the greatest departure from Sanmartín & Ronquist (2004), who treated this area as four separate regions); and (H) Southwest Pacific (including Fiji and Hawaii). Information on the geographical distribution of the terminal taxa was gathered from the literature (mainly Holttum, 1963, 1964, 1965; Conant, 1983; Conant *et al.*, 1995; Conant & Stein, 2001; Large & Braggins, 2004). The distribution ranges for each of the extant taxa (the terminals) are almost always restricted to a single region (86 out of 91 taxa, the remaining five taxa occur in two regions, Appendix S1). Therefore, when running the LAGRANGE analyses, the ancestral ranges were set to include a maximum of two of the eight defined regions. All possible combinations of regions were allowed. Because our aim was to test whether vicariance could explain some of the patterns observed, we used a conservative approach with no dispersal constraints. The rate parameters were estimated. Analyses were set up using the online configuration tool (http://www.reelab.net/lagrange). The Python script 'Output.py' in LAGRANGE was manually modified to report all relative posterior probabilities (default setting is to report up to a cumulative probability of 0.95). This allowed us to calculate, for each daughter lineage, the sum of the relative probabilities for each possible ancestral area; in each case, the area yielding the highest relative probability is presented here.

RESULTS

Phylogenetic relationships

The phylogenetic analysis using MRBAYES shows a robustly supported topology (see Fig. S1 in Appendix S2). All relationships discussed below are supported by a posterior probability (PP) of 0.99, unless otherwise stated. Within Cyatheaceae, *Sphaeropteris* is sister to the marginate-scaled clade, which includes three lineages: *Cyathea*, *Alsophila* s.s. and *Gymnosphaera* + *C. capensis* (the two latter groups are hereafter referred to as *Alsophila* and *Gymnosphaera*, respectively). The relationships among these groups, however, are unclear, with none of the

three possible topological resolutions receiving support above PP 0.5. Our calculations suggest that the *Alsophila + Gymnosphaera* ('AlGy') topology is slightly more common (present in 39% of the pool of trees, after the removal of the burn-in) than the other two topologies, *Alsophila + Cyathea* ('AlCy', present in 31% of the trees) and *Cyathea + Gymnosphaera* ('CyGy', present in 30% of the trees). All other ingroup relationships are the same as in previously published studies (Korall *et al.*, 2007; Janssen *et al.*, 2008). It should be noted that, besides the single-partition analysis presented here, an analysis with five partitions representing each of the different plastid regions was tried in order to address differences in evolutionary constraints. The analysis, however, failed to converge (data not shown). Because the topology resulting from our single-partition analysis is congruent with a previous study that used a five-partition scheme in the analysis (Korall *et al.*, 2007), we consider it to be a reasonable estimate of the phylogeny. The topologies resulting from the BEAST analyses were congruent with the MRBAYES analyses (except when certain nodes were constrained) (Fig. 1 and Figs S1–S4 in Appendix S2).

Divergence date estimates and biogeographical patterns

Divergence date estimates calculated for the three differently constrained topologies resulted in very similar estimates, and differed by only a few million years across the analyses (Figs S2–S4). These differences are minor when compared to the relative uncertainty inherent in each analysis, as reflected in the 95% highest posterior density of the age estimates (grey horizontal bars in Fig. 1e and Figs S2–S4). The data set is also rather robust to different analytical schemes. Preliminary analyses that changed the prior distributions of the fossil constraints or that analysed the data using penalized likelihood (Sanderson, 2002) showed mostly very minor differences (data not shown).

The estimated dates from the BEAST analysis of the most frequent topology (i.e. with *Alsophila + Gymnosphaera* being constrained as monophyletic) are presented below. Age estimates from the analyses based on the other two constrained topologies are found in Figs S3 and S4. The results of the three separate biogeographical analyses were congruent across most nodes (detailed results, including all possible reconstructions, from the three analyses are deposited in the Dryad data repository, see Data Accessibility). The major exceptions are early divergences in the marginate-scaled clade – an obvious consequence of the three different topologies constrained for the possible relationships among the *Alsophila*, *Cyathea* and *Gymnosphaera* lineages (Fig. 1b–d). The phylogenetic reconstruction of biogeographical patterns that is presented below focuses mainly on large-scale, transoceanic events.

Origin of Cyatheaceae – the scaly tree ferns

Cyatheaceae diverged from its sister lineage Dicksoniaceae 150 Ma in the Late Jurassic (Fig. S2), with the crown group

originating 96 Ma in the mid-Cretaceous (Fig. 1e, Fig. S2). The geographical range of the ancestor to the crown group is inconclusive, however, with two areas estimated as possible: the areas that today are South America or Australasia (Fig. 1b–d). Neither of these geographical alternatives receives strong support under any of the three differently constrained scenarios; the ancestral area assigned the highest relative probability even differs among these topologies (Fig. 1b–d).

Sphaeropteris

Within Cyatheaceae, the crown group of *Sphaeropteris* dates back to 90 Ma in the Late Cretaceous (Fig. 1e, Fig. S2), with a very high probability (0.95–0.98) of having originated in Australasia (Fig. 1b–d). Within *Sphaeropteris*, range expansion into Southeast Asia occurred at three different time intervals from the Late Cretaceous to the present, whereas its expansion into South America was a single event in the Eocene (43–36 Ma, range expansion event number 1 in Fig. 1e). A single species, *S. medullaris*, occurs in Australasia and the Southwest Pacific. Its range expansion occurred in the stem lineage of the species, either in, or subsequent to, the Oligocene.

### Origin of the marginate-scaled clade

The ancestral range of the marginate-scaled clade probably included both Australasia and South America. This is the most likely scenario irrespective of topology (0.46/0.76/0.79 for the AlGy, AlCy, CyGy constrained topologies, respectively, see Fig. 1b–d). Therefore, despite the ambiguity of the ancestral range of the family as a whole, we can deduce that a range expansion occurred in the stem lineage of the family, either from Australasia to South America or from South America to Australasia (Fig. 1b–d, event 2 in Fig. 1e), in the Late Jurassic–Early Cretaceous (150–96 Ma, Fig. S2). The crown group age of the marginate-scaled clade is estimated to be 82 Ma (Late Cretaceous; Fig. 1, Fig. S2) with the three major lineages (*Alsophila*, *Cyathea* and *Gymnosphaera*) all having crown group origins around the Palaeocene–Eocene boundary (54, 56 and 55 Ma, respectively; Fig. 1e, Figs S2–S4).

Biogeographical patterns in the marginate-scaled clade: when Alsophila and Gymnosphaera are constrained to be sister-groups

Implementing the AlGy topological constraint (Fig. 1b,e) reconstructs both the *Cyathea* and the *Alsophila + Gymnosphaera* lineages to have originated in Australasia (albeit with low relative probability, 0.53 and 0.40, respectively); i.e. with their common ancestor experiencing a local extinction in South America. In *Cyathea*, this is followed by a range expansion back into South America in its stem lineage (82–54 Ma; event 3 in Fig. 1e, Fig. S2). Because the relative

probability for a local extinction in the common ancestor of the marginate-scaled clade is rather low (0.53), it is possible that *Cyathea* inherited the full ancestral range of the ancestor, including both South America and Australasia (relative probability of 0.31). Within the crown group of *Cyathea*, a basal split suggests a vicariance scenario, whereby the Australasian and South American species diverged sometime in the Late Cretaceous–Palaeocene (82–54 Ma; Fig. 1e, Fig. S2).
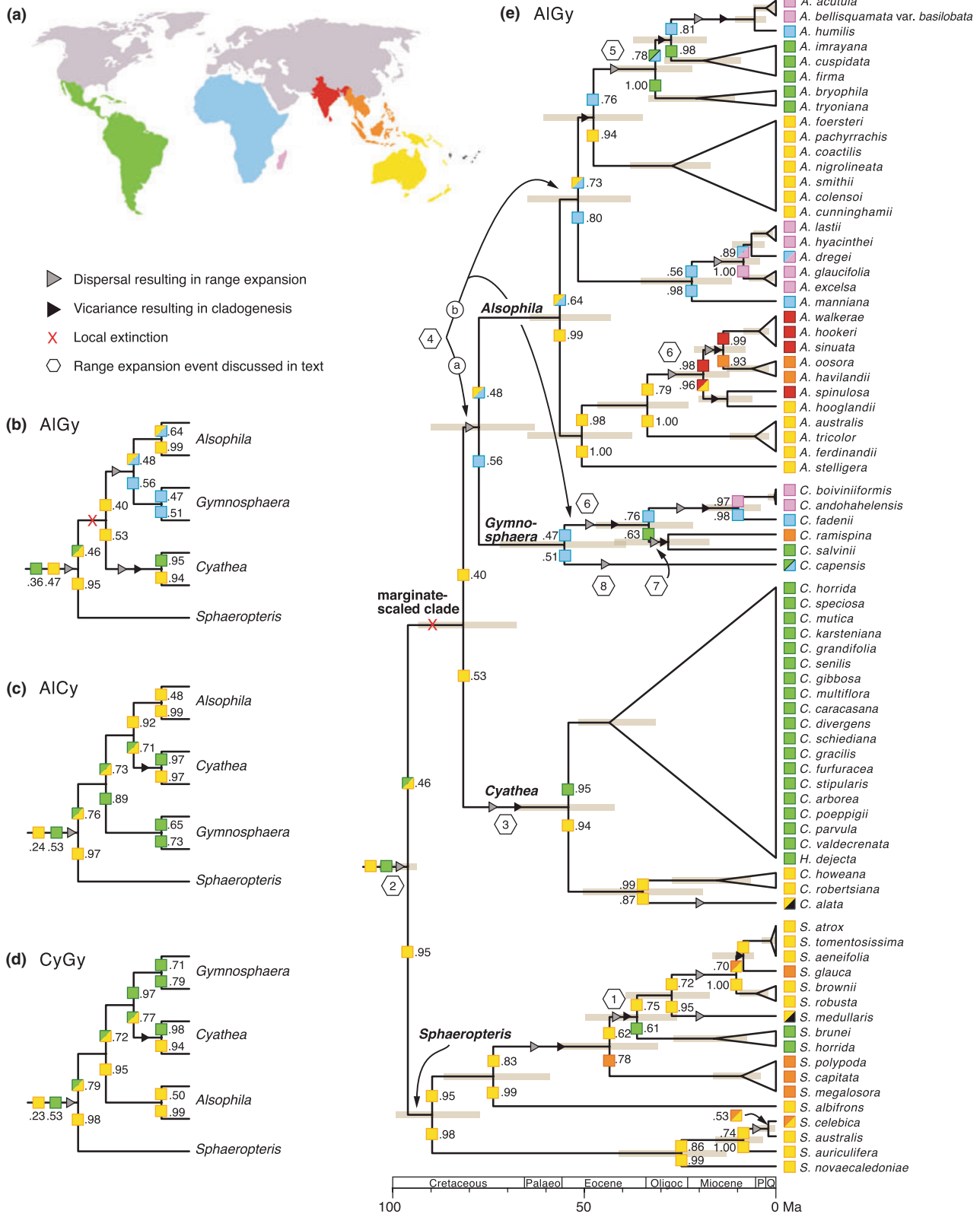
A single colonization from Australasia to the Southwest Pacific is found in *Cyathea alata*.

Under the AlGy topological constraint, a range expansion is also reconstructed for the common ancestor of *Alsophila* and *Gymnosphaera*, but from Australasia into Africa, during the Late Cretaceous (82–77 Ma; event 4a in Fig. 1e, Fig. S2). The ancestor of *Alsophila* is reconstructed as having a wide biogeographical range in both Australasia and Africa, a distribution inherited by the *A. acutula–A. manniana* group, one of the two lineages resulting from the earliest divergence within *Alsophila* (Fig. 1e). This lineage splits into one African clade (*A. lastii–A. manniana*) that is later dispersed into Madagascar, and into another clade (*A. acutula–A. cunninghamii*) that diverges into an Australasian group and an African/Madagascan–South American group. The colonization of South America is consistently resolved as a single dispersal event from Africa in the Eocene or possibly Oligocene (48–31 Ma; event 5 in Fig. 1e, Fig. S2). The other lineage to result from the basal split in *Alsophila* (Fig. 1e), *A. walkerae–A. stelligera*, has an ancestral range that is restricted to Australasia, with later colonization of India (during the Oligocene–Miocene, 34–19 Ma) and Southeast Asia (Miocene, 19–14 Ma).

The ancestral range of the *Gymnosphaera* lineage, under the AlGy constraint, is restricted to Africa, with subsequent range expansion into South America in the Eocene (55–33 Ma; event 6 in Fig. 1e, Fig. S2). This was followed by further colonizations of Madagascar (from Africa) and Southeast Asia (from South America) (Oligocene, 33–28 Ma; event 7 in Fig. 1e, Fig. S2). *Cyathea capensis* occurs in both South America and Africa and its range expansion from Africa is estimated to have occurred along the terminal branch (55 Ma–present; event 8 in Fig. 1e, Fig. S2).

Biogeographical patterns in the marginate-scaled clade: when Alsophila and Gymnosphaera are NOT constrained to be sister-groups

Implementing either the AlCy or CyGy topological constraints (Fig. 1c,d) yields ancestral range reconstructions that are identical to one another for the three major lineages within the marginate-scaled clade. These results, however, differ to some extent from those resulting from the AlGy topology presented above.

The ancestral range for *Cyathea* is in both South America and Australasia in the AlCy and CyGy topologies (Fig. 1c,d), supporting the reconstruction with the next to highest relative probability in the AlGy topology (see above). From this, it follows that range expansion event 3, which is invoked by the AlGy topology (Fig. 1e), is absent in these reconstructions. Within the crown group of *Cyathea*, the ancestral range reconstructions are identical under all three topological constraints (described above, Fig. 1e), with minor differences in relative probabilities.

Under either the AlCy or CyGy topological constraint, a range expansion into Africa for the *Alsophila* and *Gymnosphaera* lineages is delayed when compared to the AlGy topology, where Africa was reconstructed as part of the ancestral area for their common ancestor. In the AlCy and CyGy topologies, colonizations of Africa are found in the stem lineage of the *Alsophila acutula–A. manniana* group in *Alsophila* and in the stem lineage of the *Cyathea boiviniiformis–C. salvinii* group in *Gymnosphaera* (event 4b in Fig. 1e). The ancestral area reconstructed for the *Alsophila* stem lineage is Australasia, whereas it is South America for the *Gymnosphaera* stem lineage (Fig. 1c,d).

As with the *Cyathea* crown group, the ancestral areas reconstructed for the more derived divergences within the *Alsophila* and *Gymnosphaera* crown groups are identical under all three topological constraints, with the exception of range expansion event 8 that is invoked for *C. capensis* (here it is from South America to Africa, i.e. the reverse direction that was reconstructed for the AlGy topology).

DISCUSSION

Evolutionary history of scaly tree ferns (Cyatheaceae)

The phylogenetic relationships we obtained for scaly tree ferns (Fig. 1, Figs S1–S4) are congruent with those from recently published studies (Korall *et al.*, 2007; Janssen *et al.*,

Figure 1 Global biogeographical patterns for scaly tree ferns, Cyatheaceae. (a) Map showing eight biogeographical regions as defined in this study: South America (green), Africa (blue), Madagascar and neighbouring islands (pink), India and Sri Lanka (red), Southeast Asia (orange), Australasia (yellow), and Southwest Pacific (black) (the Atlantic region has been omitted in this figure since no ingroup taxa occur in the region). Map modified from http://commons.wikimedia.org/wiki/File:Blank_map_of_world_no_country_borders.PNG under the terms of the GNU Free Documentation License, version 1.2. (b–d) Schematic cladograms showing the three possible topologies among the marginate-scaled groups, and how these different topologies impact biogeographical reconstructions in early divergences of the family. (b) AlGy topology, i.e. with *Alsophila* and *Gymnosphaera* constrained as monophyletic. (c) AlCy topology, i.e. with *Alsophila* and *Cyathea* constrained as monophyletic. (d) CyGy topology, i.e. with *Cyathea* and *Gymnosphaera* constrained as monophyletic. (e) Full historical biogeographical reconstruction (using LaGrange) on the AlGy topology, i.e. the most common topology. Divergence dates were estimated using beast. Grey bars indicate 95% highest posterior density of the age estimates. Coloured squares indicate reconstructed ancestral ranges and mirror map colours in (a). Two-coloured squares denote ancestral ranges that include two of the regions defined in (a). Numbers adjacent to squares denote the relative probability of the ancestor having that specific ancestral range. The two squares at the root nodes denote the two scenarios with the highest relative probability. Hexagons (1–8) denote range expansion events.

2008). The conclusions we draw below regarding vicariance versus long-distance dispersal take into account the uncertainty inherent in our age estimates.

Cyatheaceae originated 150 Ma in the Late Jurassic (Figs S2–S4), a time marking the start of the rifting between the western and eastern parts of Gondwana (McLoughlin, 2001, and references therein). The ancestral geographical distribution of the family was in areas that today are either South America or Australasia; our analyses failed to provide an unambiguous reconstruction (Fig. 1b–d). When the Cyatheaceae crown group began to diversify 96 Ma in the mid-Cretaceous (Figs S2–S4; this age estimate agrees with a study including all leptosporangiate ferns: Schuettpelz & Pryer, 2009), South America and Australasia were still connected via Antarctica, whereas the African, Madagascan and Indian landmasses had already separated from the rest of Gondwana. The range expansion reconstructed for the group – either from Australasia to South America, or from South America into Australasia (event 2 in Fig. 1e) – is therefore compatible with migration across the Gondwanan continent, indicating that a transoceanic dispersal event does not need to be inferred for either scenario. It is interesting that the reconstructed ancestral distribution did not include Africa, lending support to the notion that the expansion occurred after Africa had drifted away.

In *Sphaeropteris*, although several range expansions are reconstructed to have taken place from its ancestral distribution in Australasia into Southeast Asia, there is only a single colonization of South America (43–36 Ma, during the Eocene, event 1 in Fig. 1e, Figs S2–S4). The timing of this range expansion coincides with the separation of South America and Antarctica from Australia and New Guinea, dated at 52–35 Ma (Sanmartín & Ronquist, 2004), suggesting that the break-up of Gondwana may be responsible for this vicariance pattern (albeit if the 95% highest posterior density is taken into account, the timing may have post-dated the separation of the continents).

Following the origin of the marginate-scaled clade crown group in South America and Australasia during the Late Cretaceous, its divergence into three different lineages is estimated to have happened during a very short time frame of approximately five million years (82–77 Ma, Fig. 1e). Such rapid radiations are notoriously difficult to resolve (Whitfield & Lockhart, 2007), and so the lack of support for the phylogenetic sequence in which these divergences took place is not surprising. Unfortunately, as a consequence, our most ambiguous biogeographical reconstructions relate to these particular cladogenesis events.

For *Cyathea*, this ambiguity affects the overall size of the ancestral distribution: was it restricted to Australasia (due to an ancestral extinction event in South America; Fig. 1b), or did it include South America as well (Fig. 1c,d)? Under the first scenario, a range expansion into South America is invoked in the stem lineage of *Cyathea* (event 3 in Fig. 1e). The estimated timing of the split of the ancestral range into

South America and Australasia at the earliest divergence of the group (82–54 Ma; Fig. 1e) is compatible with a vicariance interpretation, because the final separation of these Gondwanan elements occurred later, around 50–35 Ma (Sanmartín & Ronquist, 2004).

The inconclusive ancestral reconstructions of the biogeographical scenarios possible for the earliest lineages of *Alsophila* and *Gymnosphaera* can mainly be reduced to a single question: when did the groups spread from Australasia to Africa? The reconstructions suggest that this happened either in the common ancestor of the two groups during the Late Cretaceous (82–77 Ma; Fig. 1b, event 4a in Fig. 1e), or once within each group in the early Eocene (56–52 Ma for *Alsophila* and 55–33 Ma for *Gymnosphaera*, event 4b in Fig 1e). Irrespective of which scenario is correct, it is clear that the range expansion(s) into Africa can only be explained by transoceanic dispersal(s), because the separation of Africa from Australasia (which was then still united with South America) occurred some 135 Ma (Sanmartín & Ronquist, 2004), at least 50 Myr before any of the reconstructed range expansions.

A second transoceanic dispersal event is invoked within *Alsophila* – a range expansion from Africa to South America (48–31 Ma; event 5 in Fig. 1e). The paraphyly of the South American taxa observed in this reconstruction could be explained if the ancestor was in both Africa and South America for a relatively short time prior to cladogenesis. However, because the paraphyly is only moderately supported (PP = 0.89; Fig. S1), the New World species may actually be monophyletic. The two African *Alsophila* lineages include subsequent range expansions and cladogenesis events in Madagascar and neighbouring islands (Fig. 1e). Within the scaly tree ferns, most large-scale range expansions occurred within *Gymnosphaera*, and all of these need to be explained by long-distance dispersal: two from Africa to South America or vice versa (events 6 and 8 in Fig. 1e) and one from South America to Southeast Asia (event 7 in Fig. 1e).

Our reconstruction results for scaly tree fern colonization of Madagascar and neighbouring islands differ slightly from those of Janssen et al. (2008), whose study was based on mainly the same data set but with focus on Madagascan diversification. Within the *Alsophila* lineage, both studies show a range expansion from Africa to Madagascar in the *A. lastii*–*A. manniana* lineage. However, within *Alsophila*, we also reconstruct a second colonization of Madagascar from Africa, contradicting the reconstruction of a New World origin by Janssen et al. (2008) for these same taxa. In their discussion, however, they refer to their result as 'improbable', and suggest that an African origin is more likely (in line with our results). The ambiguity we reveal regarding the ancestral area of *Gymnosphaera* (Fig. 1) is further emphasized by Janssen et al. (2008). Their reconstruction supports an African origin of the group (as in Fig. 1b in our study), but their topology shows *Alsophila* and *Cyathea* as sister (corresponding to Fig. 1c in our study).

Vicariance and long-distance dispersal in ferns

The large-scale patterns that we reconstruct in our biogeographical analysis of the scaly tree ferns, support eight to ten range expansions over areas that today are large oceans. Three of these are expansions between Australasia and South America: a range expansion in the stem lineage of the family (event 2 in Fig. 1e), in the *Sphaeropteris* lineage (event 1 in Fig. 1e), and a possible expansion in the *Cyathea* lineage (event 3 in Fig. 1e). We estimate the timing of these events to have occurred when South America, Antarctica and Australasia were still interconnected, but the African, Madagascan and Indian landmasses had already separated. This suggests that migration from Australasia to South America via Antarctica was possible and, therefore, transoceanic dispersal does not need to be invoked. In addition, the ancestral distribution patterns that followed the range expansions in *Cyathea* and *Sphaeropteris* are compatible with vicariance due to the timing of the break-up of the South American and Australasian landmasses. All other range expansions (in *Alsophila* and *Gymnosphaera*) are too young to correspond to a Gondwanan break-up scenario, and long-distance, transoceanic dispersals are most probably responsible for the patterns seen.

Because the early divergences among the major lineages of ferns occurred prior to the break-up of the Gondwanan continent (Schuettpelz & Pryer, 2009), one might expect that the resulting distribution patterns could be explained by vicariance. The timing of at least some of the early divergences and distribution patterns in these lineages are compatible with a Gondwanan vicariance scenario (Dubuisson *et al.*, 2003; Hennequin *et al.*, 2008), but more recent long-distance dispersal events post-dating the break-up of Gondwana have most probably also affected the distributions we see today (Perrie *et al.*, 2007).

Polypods, which include some 80% of extant fern species diversity (Pryer *et al.*, 2004), diverged from their closest relatives in the Triassic; however, many subgroups did not diversify until the Eocene or later (Schuettpelz & Pryer, 2009). In other words, these radiations occurred after the Gondwanan break-up, requiring long-distance dispersal to be invoked for all transoceanic dispersals. Extrapolating from some studies that either analyse, discuss and/or map distribution patterns without explicitly analysing the biogeographical data (Ranker *et al.*, 2004: grammitids, Polypodiaceae; Rouhan *et al.*, 2004: *Elaphoglossum*, Dryopteridaceae; Schneider *et al.*, 2004: Aspleniaceae; Kreier & Schneider, 2006: *Platycerium*, Polypodiaceae; Kirkpatrick, 2007: cheilanthoids, Pteridaceae; Rouhan *et al.*, 2007: *Lomariopsis*, Lomariopsidaceae; Kreier *et al.*, 2008: microsoroids, Polypodiaceae; Hennequin *et al.*, 2010: *Nephrolepis*, Lomariopsidaceae) as well from some studies on early diverging lineages (Wikström *et al.*, 2002: Schizaeaceae; Nagalingum *et al.*, 2007: *Marsilea*, Marsileaceae; Hennequin *et al.*, 2008: Hymenophyllaceae), we note that the number of transoceanic dispersal events in scaly tree ferns (calculated as the number of long-distance

dispersals per species and million years) are found at the lowest end of the scale. Being very conservative in the estimates, we can conclude that a few of these other fern groups show similar numbers, whereas most of the groups show rates of transoceanic dispersals that are 1.5–3.5 times higher, with extremes showing rates that are 20 times (or more) higher than what we observed for the scaly tree ferns in this study.

Scaly tree fern dispersability

The biological factor with the greatest impact on a fern's ability to disperse is most likely to be the mode of reproduction. Some diploid ferns are extreme outcrossers (see e.g. Soltis & Soltis, 1992); therefore, for a dispersal event to be successful, haploid spores from two different sporophytic individuals would have to disperse to the exact same place to effect fertilization (sporophytic outcrossing; K.M. Pryer *et al.*, in prep.). Polyploids, on the other hand, often seem to reproduce by gametophytic selfing (empirical studies are rather few, but see e.g. Masuyama & Watano, 1990; Soltis & Soltis, 1990; Chiou *et al.*, 2002; Haufler, 2007; K.M. Pryer *et al.*, in prep.), an extreme form of inbreeding where the sperm and egg come from the same haploid gametophyte (i.e. the progeny are homozygous at all loci). For species capable of gametophytic selfing, only a single spore is needed to effect a successful fertilization event, which should strongly increase its chances for dispersal (see empirical examples in e.g. Trewick *et al.*, 2002; Wubs *et al.*, 2010).

Scaly tree ferns are mostly functionally diploid. Although the group has a haploid chromosome number of $n = 69$, it is probably of an ancient polyploid origin. Only three polyploid tree ferns are known (Nakato, 1989; Conant *et al.*, 1994). The breeding system in scaly tree ferns has only been investigated in two species, as far as we are aware: *Alsophila firma* and *Cyathea stipularis*. Both taxa were included in this study, and have been shown to be predominantly outcrossers (Soltis *et al.*, 1991). Although the data are sparse, this suggests that sporophytic outcrossing may be the more common breeding system within the group. The relatively few transoceanic dispersal events that we reconstructed here are likely to be due to the reproductive limitations posed by outcrossing in scaly tree ferns.

Our hypothesis of a causal relationship between breeding system (and indirectly ploidy level) and dispersability success in scaly tree ferns is further strengthened by a comparison among lineages *within* the scaly tree ferns. We observe seemingly different rates of dispersal success within the family. Three out of the four to six long-distance dispersal events inferred are in the *Gymnosphaera* group, yet this lineage has the fewest taxa (approximately 30 species; Holttum, 1963, 1964, 1981; Janssen, 2007). The fact that two of the three known tetraploids in the family, *Cyathea metteniana* (Hance) C. Chr. & Tard.-Blot. and *Cyathea hancockii* Copel., belong to this group (Holttum, 1965; Nakato, 1989) indicates that

the cause of this potential shift in the dispersal trend may be explained by a shift in reproduction mode, from sporophytic outcrossing to gametophytic selfing. To further test this hypothesis a better understanding of the biology, and particularly the breeding system(s), of scaly tree ferns is critical. This is also true in the broader context of the evolutionary history of all ferns.

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SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article:

Appendix S1 Taxa examined in this study: collection locality, voucher information, fern DNA database numbers and GenBank accession numbers for each sequenced region.

Appendix S2 Additional figures showing phylogenetic relationships and lineage divergence times based on Bayesian Markov chain Monte Carlo (BMCMC) analyses (Figs S1–S4).

DATA ACCESSIBILITY

The combined (*rbc*L, *rbc*L–*acc*D IGS, *rbc*L–*atp*B IGS, *trn*G–R and *trn*L–F) dataset and detailed results, including all possible reconstructions, from the three separate biogeographical analyses using Lagrange are deposited in the Dryad data repository (doi: 10.5061/dryad.0q08k).

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