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ORIGINS OF THE ENDEMIC SCALY TREE FERNS ON THE GALÁPAGOS AND COCOS ISLANDS

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Premise of research. Successful long-distance dispersal is rarely observed in scaly tree ferns (Cyatheaceae). Nevertheless, recent molecular evidence has suggested that the four endemic scaly tree ferns on the Galápagos Archipelago (*Cyathea weatherbyana*) and Cocos Island (*Cyathea alfonsiana*, *Cyathea nesiotica*, and *Cyathea notabilis*), two oceanic island groups west of Central and northern South America, probably each originated from different mainland America ancestors. However, the phylogenetic relationships inferred among these endemics and their mainland relatives have been unclear. This study is aimed at better resolving the relationships and tracing the origins of these island endemics.

Methodology. Five plastid regions from 35 *Cyathea* species were analyzed to reconstruct phylogenetic relationships using parsimony, likelihood, and Bayesian approaches. We also estimated divergence times of these species, and our chronogram was used to reconstruct their biogeographical range history.

Pivotal results. Our well-resolved phylogenetic tree of *Cyathea*, which is in agreement with previous studies, shows that when the four Galápagos and Cocos endemics are included, they each belong to separate subclades. Our biogeographical study suggests that the four endemics originated from independent colonization events from mainland America and that there was no dispersal of *Cyathea* between the island groups. We reveal more detailed relationships among the endemics and their respective close mainland relatives; some of these relationships differ from previous studies. Our findings are corroborated by new morphological data from ongoing stem anatomy studies.

Conclusions. The four scaly tree ferns endemic to the Galápagos and Cocos Islands each did indeed originate as independent colonization events from separate sources in mainland America, and their closest relatives are identified here.

Keywords: *Cyathea*, Cyatheaceae, island biogeography, phylogenetic analysis, *rbcL*, *rbcL-accD*, *rbcL-atpB*, *trnG-R*, *trnL-F*.

Introduction

The origin of species endemic to islands has long fascinated scientists (Wallace 1880; Keeley and Funk 2011; Tye and Francisco-Ortega 2011; Igea et al. 2014). Recent phylogenetic studies have demonstrated that the closest relatives of some island endemics are not always from the mainland but rather from neighboring islands (Sato et al. 1999; Motley et al. 2005; Harbaugh and Baldwin 2007; Andrus et al. 2009; Namoff et al. 2010), emphasizing the contributions of both continents and islands to insular biodiversity. Trusty et al. (2012) recently investigated whether the plants of the Galápagos Archipelago and Cocos Island, which are each other's closest neighboring insular systems, show any such sister relationships. Using molecular phylogenetic data for all five genera of vascular plants

with endemics in both island locations, Trusty et al. (2012) demonstrated no evidence for any phylogeographical links between the islands. This lack of floristic affinity between Galápagos and Cocos was attributed primarily to atmospheric and ocean circulation patterns that likely limit dispersal between the two.

Here we follow up on the study by Trusty et al. (2012) by focusing on tracing the spatial and temporal origin of the scaly tree fern species endemic to the Galápagos and Cocos Islands. These are the two largest oceanic islands off the Pacific shore of tropical America. The Galápagos Archipelago is located on the equator, some 900 km west of Ecuador, whereas Cocos Island is 780 km north of Galápagos and 550 km southwest of Costa Rica. Both are relatively young volcanic islands, with Cocos estimated at approximately 2 million years old (Castillo et al. 1988) and the Galápagos at 3–5 million years old (Geist 1996; O'Connor et al. 2007). The ancient portions of the Galápagos Archipelago (including Cocos Ridge, which is now submerged in the Pacific) possibly date back to 14.5 Ma (Christie et al. 1992; Werner et al. 1999). Despite their geographical

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and geological similarities, the floras of both island formations are quite distinct, with only 16 vascular plant species shared between them (less than 8% of the total vascular flora of either island; Trusty et al. 2012). The endemic floras of both Galápagos and Cocos Islands have mostly been inferred to have their origins from Central America, the Caribbean, and northern South America (Trusty et al. 2006; Tye and Francisco-Ortega 2011).

Four scaly tree fern species inhabit the Galápagos and Cocos Islands—one is endemic to the Galápagos (*Cyathea weatherbyana*) and the other three to Cocos (*Cyathea alfonsiana*, *Cyathea nesiotica*, and *Cyathea notabilis*). From where did they originate? *Cyathea* (Cyatheaceae), a genus of about 200 species (Lehnert 2011), is primarily confined to mainland tropical America (fig. 1), with the exception of eight South Pacific species in the *decurrans* group—the earliest diverging lineage of the genus (fig. 1; Holttum 1982; Korall et al. 2007; Lehnert 2011; Korall and Pryer 2014). In previous studies, it was hypothesized that *C. nesiotica* had its origin within the *armata* group (fig. 1; Maxon 1922; Riba 1967, 1969; Barrington 1978; Gastony 1979; Trusty et al. 2012), whereas the other three species have been linked to *Cyathea multiflora* (a member of the *gibbosa* group; fig. 1), a species that is widely distributed from Mexico to northern Brazil (Tryon 1976; Moran 1995; Large and Braggins 2004).

Molecular phylogenetics has provided us with many new insights into the biogeographical origins of island floras, especially for identifying the most likely source of endemic species (Moore et al. 2006; Janssen et al. 2008; Andrus et al. 2009; Mansion et al. 2009; Salvo et al. 2010; Keeley and Funk 2011). Trusty et al. (2012) was the first molecular study to include all four species of *Cyathea* endemic to the Galápagos and Cocos Islands. Using the plastid *trnL-F* intergenic spacer, Trusty et al. (2012) established *C. nesiotica* as sister to *Cyathea stipularis* (*armata* group; fig. 1) and *C. notabilis* as sister

to *C. multiflora* (*gibbosa* group; fig. 1). However, the phylogenetic placement of *C. alfonsiana* and *C. weatherbyana* was ambiguous (Trusty et al. 2012). Using the same data set (but analyzed independently), Igea et al. (2014) confirmed Trusty's (2012) results, including the ambiguous placement of *C. alfonsiana* and *C. weatherbyana*.

To better pinpoint the likely relationships and source regions of the scaly tree ferns endemic to the Galápagos and Cocos Islands, we assembled a molecular data set focused specifically on *Cyathea* (already well established as a strongly supported monophyletic group; Korall et al. 2007; Janssen et al. 2008) that comprised five plastid markers: *rbcl*, *rbcl-accD*, *rbcl-atpB*, *trnG-R*, and *trnL-F*. In addition, we incorporated new data from ongoing *Cyathea* stem anatomy studies that complement our findings.

Material and Methods

Taxon Sampling

Thirty-five species of *Cyathea* were sampled from across all six major clades (fig. 1), including the Galápagos endemic *Cyathea weatherbyana* and the three Cocos endemics *Cyathea alfonsiana*, *Cyathea nesiotica*, and *Cyathea notabilis*. Three South Pacific species belonging to the *decurrans* group (*Cyathea alata*, *Cyathea howeana*, and *Cyathea robertsiana*) were chosen as the outgroup, on the basis of Korall et al. (2007) and Korall and Pryer (2014).

DNA Extraction and Sequencing

Protocols for DNA extraction, amplification, and sequencing followed Korall et al. (2007) and Schuettpelz and Pryer (2007). Genomic DNA was extracted from silica-dried or herbarium material using a DNeasy Plant Mini Kit (Qiagen). Five plastid regions (*rbcl*, *rbcl-accD*, *rbcl-atpB*, *trnG-R*, and *trnL-F*) were amplified separately using polymerase chain reaction (PCR) with primers listed in table 1. PCR products were purified using an ExoSAP-IT PCR cleanup kit (United States Biochemical), and purified PCR products were sequenced using Big Dye Terminator V3.1 Cycle Sequencing reagents (Applied Biosystems 3730xl). Sequencing reads from each species and locus were assembled and edited using Sequencher 5.0.1 (Gene Codes 2011). Sixty-six newly obtained DNA sequences were deposited in GenBank (appendix).

Phylogenetic Analyses

Sequences of five plastid regions (*rbcl*, *rbcl-accD*, *rbcl-atpB*, *trnG-R*, and *trnL-F*) for 35 *Cyathea* species were either downloaded from TreeBase (study 1789; Korall et al. 2007), GenBank, or newly sequenced here (appendix). For each species, only sequences obtained from the same voucher specimen were used. The aligned five-locus data set from Korall et al. (2007) was downloaded from TreeBase, divided by plastid region, and saved as five separate files. Sequences (new or from GenBank) were aligned to their appropriate data sets using MUSCLE (Edgar 2004) in Aliview 1.13 (Larsson 2014). Homopolymer regions with length differences among taxa (total 281 bases) were excluded from analysis (5402 bases

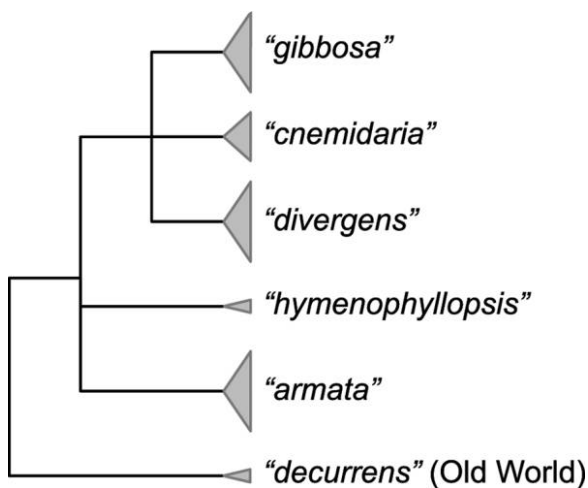


Fig. 1 Simplified phylogeny of scaly tree ferns (*Cyathea*) modified from Korall et al. (2007, p. 878, fig. 3). Six major clades are represented by nicknames that are either epithets from a species included in that clade or a genus name that was attributed to that clade. Each clade triangle representation roughly approximates its relative species richness.

Table 1
Primers Used for DNA Amplification and Sequencing

DNA region	Primer	5'-3' primer sequence	Reference
<i>rbcl</i>	ESRBCL1F	ATGTCACCACAAACGGAGACTAAAGC	Schuettpelz and Pryer 2007
<i>rbcl</i>	ESRBCL645F	AGAYCGTTTCYTATTYGTAGCAGAAGC	Schuettpelz and Pryer 2007
<i>rbcl</i>	ESRBCL663R	TACRAATARGAAACGRTCTCTCCAACG	Schuettpelz and Pryer 2007
<i>rbcl</i>	ESRBCL1361R	TCAGGACTCCACTTACTAGCTTCACG	Schuettpelz and Pryer 2007
<i>rbcl-accD</i>	RBCL1187F	GGAACYTTGGGACATCCTTGG	Korall et al. 2007
<i>rbcl-accD</i>	ACCDHIF4	GAAGATAAACGAAAATTGGGTGG	Ebihara et al. 2003
<i>rbcl-accD</i>	ACCD887R	TTATCACABCIMGCCCAATATCC	Korall et al. 2007
<i>rbcl-accD</i>	ACCD816R	CCATGATCGAATAAAGATTGAGC	Ebihara et al. 2003
<i>rbcl-atpB</i>	ESRBCL26R	GCTTTAGTCTCCGTTTGTGGTGACAT	Korall et al. 2007
<i>rbcl-atpB</i>	ATPBPACER703R	CCAATGATCTGAGTAATSTATCC	Korall et al. 2007
<i>trnG-R</i>	TRNG1F	GCGGGTATAGTTTAGTGGTAA	Nagalingum et al. 2007
<i>trnG-R</i>	TRNGR353F	TTGCTTMTAYGACTCGGTG	Korall et al. 2007
<i>trnG-R</i>	TRNG63R	GCGGGAATCGAACCCGCATCA	Nagalingum et al. 2007
<i>trnG-R</i>	TRNR22R	CTATCCATTAGACGATGGACG	Nagalingum et al. 2007
<i>trnL-F</i>	FernL1lr1	GGYAATCCTGAGCCAAATC	Li et al. 2010
<i>trnL-F</i>	TRNLC	CGAAATCGGTAGACGCTACG	Taberlet et al. 1991
<i>trnL-F</i>	TRNLE	GGTTC AAGTCCCTCTATCCC	Taberlet et al. 1991
<i>trnL-F</i>	TRNLD	GGGGATAGAGGGACTTGAAC	Taberlet et al. 1991
<i>trnL-F</i>	TRNFF	ATTTGAAC TGGTGACACGAG	Taberlet et al. 1991

remained, with 478 variable sites; table 2). The five data sets were analyzed separately using maximum likelihood (ML). First, a maximum parsimony (MP) tree generated by a heuristic search was used to perform sequence-evolution model selection under the Bayesian information criterion using the automatic partitioning (for *rbcl* gene) or automatic model selection (for *rbcl-accD*, *rbcl-atpB*, *trnG-R*, and *trnL-F* intergenic spacers) function in PAUP* 4.0a136 (Swofford 2003). ML analyses were conducted in Garli 2.0 (Zwickl 2006), and ML bootstrap support (BS_{ML}) was calculated from 1000 replicates. Topologies from each individual plastid region were visually compared and inspected for conflicts supported by bootstrap values $\geq 70\%$ (Hills and Bull 1993; Mason-Gamer and Kellogg 1996). A single topological conflict that impacted only the phylogenetic position of *Cyathea nigripes* was observed for *rbcl* when compared with topologies from the other four regions. Because this incongruence had no impact on other parts

of the topology, the five data sets were combined and analyzed using MP, ML, and Bayesian Markov chain Monte Carlo (B/MCMC) approaches. MP analyses were carried out in PAUP* 4.0a136 (Swofford 2003) using heuristic searches; the starting tree was obtained by stepwise addition, branch swapping was done using TBR, and bootstrap support for MP (BS_{MP}) was calculated from 10,000 replicates. The best data partitioning scheme and sequence evolution models for ML and B/MCMC analysis were selected from 11 models (for ML) and three models (for B/MCMC; MrBayes does not support some of the models tested using ML) using the automatic partitioning function in PAUP* 4.0a136 (Swofford 2003) under Bayesian information criterion. ML analyses were carried out in Garli 2.0 (Zwickl 2006), with genthreshfortopterm set to 1,000,000. The tree with the best likelihood score among the four replicates was selected as the best ML tree; bootstrap support for ML (BS_{ML}) was calculated from 1000 replicates, with gen-

Table 2
Sequence Characteristics, Evolutionary Models, and Resulting Tree Statistics

Plastid region	<i>rbcl</i> ^a	<i>rbcl-accD</i>	<i>rbcl-atpB</i>	<i>trnG-R</i>	<i>trnL-F</i>	All combined
No. taxa	35	35	35	34	32	35
Alignment length (bp)	1309	1541	637	1099	1097	5683
Characters included (bp)	1309	1433	612	1070	978	5402
Missing data (%)	2.7	6.0	2.1	1.6	8.7	6.3
Variable sites (no. [%])	80 (6.1)	100 (7.0)	58 (9.5)	111 (10.4)	129 (13.2)	478 (8.8)
ML model:						
Separate	TrN+I+G (1st), JC+I (2nd), K81uf+I (3rd)	TrN+I	HKY	K81uf	K81uf+G	...
Combined	JC+I+G (1st, 2nd), K81uf+G (3rd)	K81uf+G	K81uf+G	K81uf+G	K81uf+G	^b
B/MCMC model:						
MrBayes	JC+I+G (1st, 2nd), GTR+G (3rd)	GTR+G	GTR+G	GTR+G	GTR+G	^b
BEAST 2	JC+I+G (1st, 2nd), TN93+G (3rd)	GTR+G	GTR+G	GTR+G	GTR+G	^b

Note. Missing data do not include indels. B/MCMC, Bayesian Markov chain Monte Carlo; bp, base pair; ML, maximum likelihood.

^a 1st, 2nd, and 3rd are the first, second, and third codons.

^b The models applied to each partition are indicated under each locus.

threshold for term set to 20,000. The B/MCMC analyses were carried out using MrBayes v3.2.3 (Ronquist et al. 2012) with two independent MCMC runs, each with four chains and 1,000,000 generations. The convergence of parameters was inspected using Tracer v1.6 (Rambaut et al. 2014). Trees were sampled every 1000 generations, and the first 25% of trees were discarded as burn-in. Data sets and phylogenetic trees are deposited in the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.5b5r1>.

Divergence Time Estimations

Lineage divergence times were estimated using BEAST 2 (ver. 2.1.3; Bouckaert et al. 2014) on the combined data set with the same sequence partitioning scheme and evolution models that were used in the B/MCMC analysis (table 2), except that the TN93 model was applied to the third codon position of the *rbcL* gene, because there were insufficient A-T transversions to apply the GTR model. A relaxed lognormal clock model and a birth-death model were used. Because there is no appropriate fossil record that we could directly apply within *Cyathea*, three strongly supported nodes (circled 1–3 in fig. 3) were secondarily calibrated with normal distribution priors (table 3) using the divergence times estimated by Korall and Pryer (2014; AlGy topology, their fig. S2). Three independent MCMC runs with 10,000,000 generations were carried out. The convergence of parameters was inspected using Tracer v1.6 (Rambaut et al. 2014). Trees were sampled every 1000 generations, and the first 10% of trees were discarded as burn-in. The maximum clade credibility tree with mean divergence times was summarized using TreeAnnotator in BEAST 2 (Bouckaert et al. 2014).

Biogeographical Analysis

A biogeographical analysis was carried out using Lagrange v.20130526 (Ree et al. 2005; Ree and Smith 2008) on the basis of the chronogram reconstructed with BEAST 2. The analysis was set up on the online Lagrange configuration tool (<http://www.reelab.net/lagrange>). Four biogeographical regions were defined: West Pacific (*decurrens* group), Cocos Island (*C. alfonsiana*, *C. nesiotica*, *C. notabilis*), Galápagos Archipelago (*C. weatherbyana*), and mainland America (all other *Cyathea* species; Tryon 1976; Barrington 1978; Holtum 1982; Korall et al. 2007). Because the ancient Galápagos Archipelago (including the now submerged portions) is no older than 14.5 Ma (Christie et al. 1992; Werner et al. 1999), dispersal events to Galápagos and Cocos Islands before that time were not permitted in the Lagrange analyses. The baseline rates of dispersal

and local extinction were estimated (the “rate parameters” option).

Stem Anatomy

Vouchers for *Cyathea* stem cross sections shown in this study are listed in the appendix (see asterisks). Stem material was available for all three Cocos Island endemics, whereas material for *C. weatherbyana* (Galápagos) was not obtainable. Stems were preserved in formalin–acetic acid–alcohol. Stem cross sections were made using a macrotome (Lucansky and White 1974; Lucansky 1976). Photographs were taken with a Canon PowerShot S3 IS digital camera.

Results

Phylogeny

A summary of the six data sets analyzed for this study of *Cyathea*, including sequence characteristics, best-fit evolution models, and tree statistics, appears in table 2. The best ML tree from the analysis of the combined (five-plastid loci) data set is shown in figure 2. The four tree fern species endemic to Galápagos and Cocos Islands are shown to have independent origins from separate mainland ancestors. *Cyathea notabilis* is robustly supported as sister to *Cyathea multiflora* in the *gibbosa* group (fig. 2). *Cyathea alfonsiana* and *Cyathea weatherbyana* are both nested within the *divergens* group, with *C. alfonsiana* strongly supported as sister to *Cyathea schiedeana*, and *C. weatherbyana* forming a moderately supported clade with *Cyathea fulva*, *Cyathea caracasana*, and *Cyathea divergens* (fig. 2). *Cyathea nesiotica* is sister to *Cyathea stipularis* (fig. 2) and, together with *Cyathea bicrenata*, forms a strongly supported subclade within the *armata* group.

Divergence Time Estimations and Biogeographical Analysis

Our divergence time analysis estimates that each of the four *Cyathea* island endemics originated within a time range (fig. 3) that is roughly compatible with the estimated ages for the islands: Cocos (estimated at 2 Ma): *C. alfonsiana*, (1.4)–4.0–(6.8) Ma; *C. nesiotica*, (0.1)–1.3–(2.9) Ma; *C. notabilis*, (1.1)–3.4–(6.1) Ma; Galápagos (estimated at 3–5 Ma): *C. weatherbyana*, (3.5)–6.8–(10.4) Ma.

Our biogeographical analysis supports that the four *Cyathea* endemics were dispersed to Cocos Island and Galápagos Archipelago in four independent events, each with a closest relative on mainland America (fig. 3). Although *C. alfonsiana* and *C. weatherbyana* are both in the *divergens* group, their

Table 3

Time-Calibrated Priors for Divergence Time Estimations Using BEAST 2

Node no. in fig. 3	Model	Median	Sigma	Offset	2.5% quantile	97.5% quantile
1	Normal	54	6.4	.5	42	67
2	Normal	43	5.35	1.5	34	55
3	Normal	31	4.6	1.0	23	41

Note. The parameters were set to match the median and the 95% highest posterior density estimated by Korall and Pryer (2014; AlGy topology, their fig. S2).

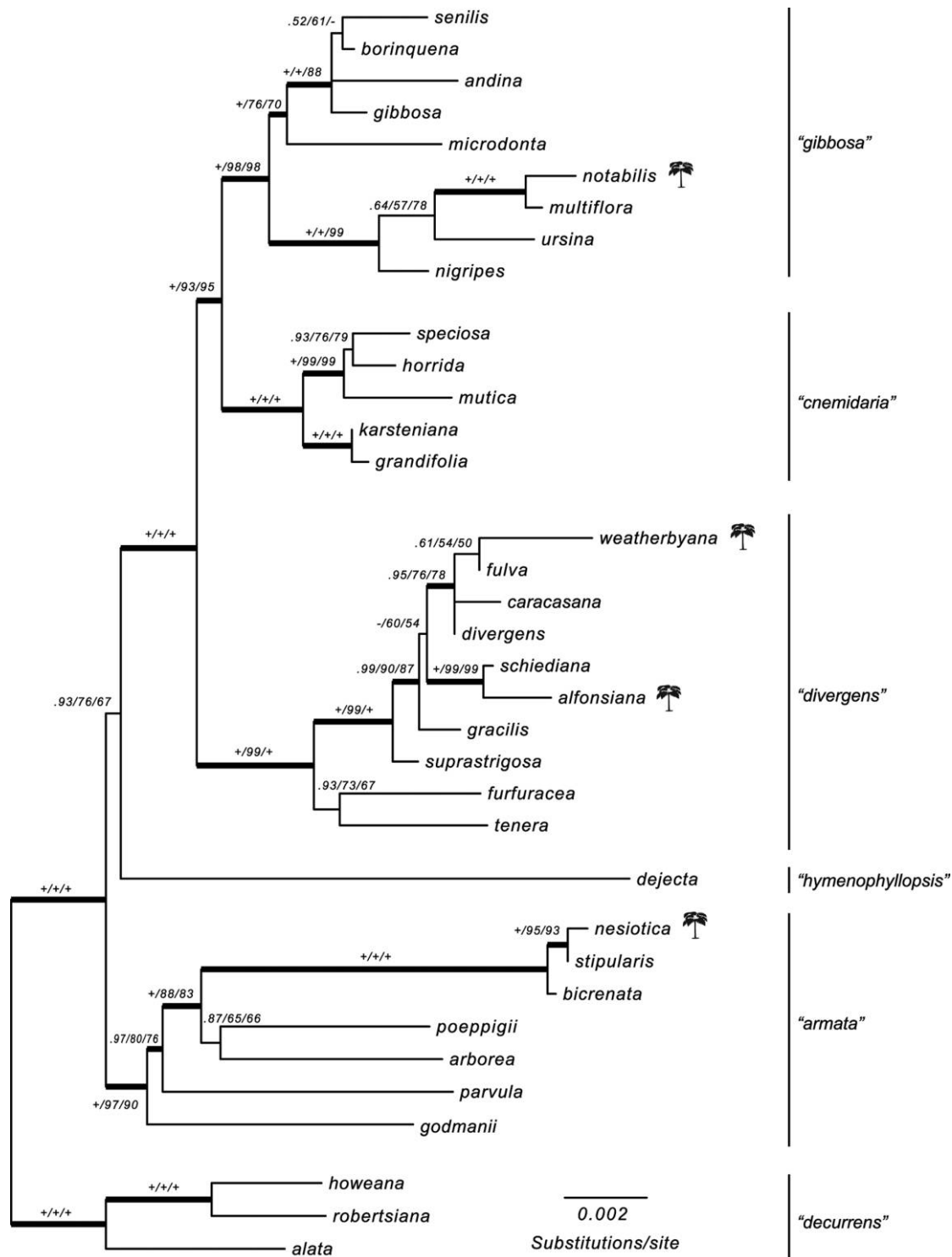


Fig. 2 Best maximum likelihood (ML) tree ($\ln L = -11,754.859$) for *Cyathea* from the combined five-locus plastid data set: *rbcl*, *rbcl-accD*, *rbcl-atpB*, *trnG-R*, and *trnL-F*. The tree is rooted with the Old World *decurrens* group. Support values shown on each branch are Bayesian posterior probability (PP)/ML bootstrap support (BS_{ML})/MP bootstrap support (BS_{MP}); a plus sign is 1.00 or 100%, and a minus sign is lower than 0.50 or 50%. All thickened branches have 0.95/70%/70% support. Branch support for all nonthickened branches is indicated where PP/ BS_{ML} / BS_{MP} support is >0.5 or 50%. Scale bar corresponds to 0.002 substitutions/site. Monophyletic groups recognized by Korall et al. (2007) are indicated on the right (cf. fig. 1). The four endemic species to Cocos and Galápagos Islands are indicated with a tree fern symbol.

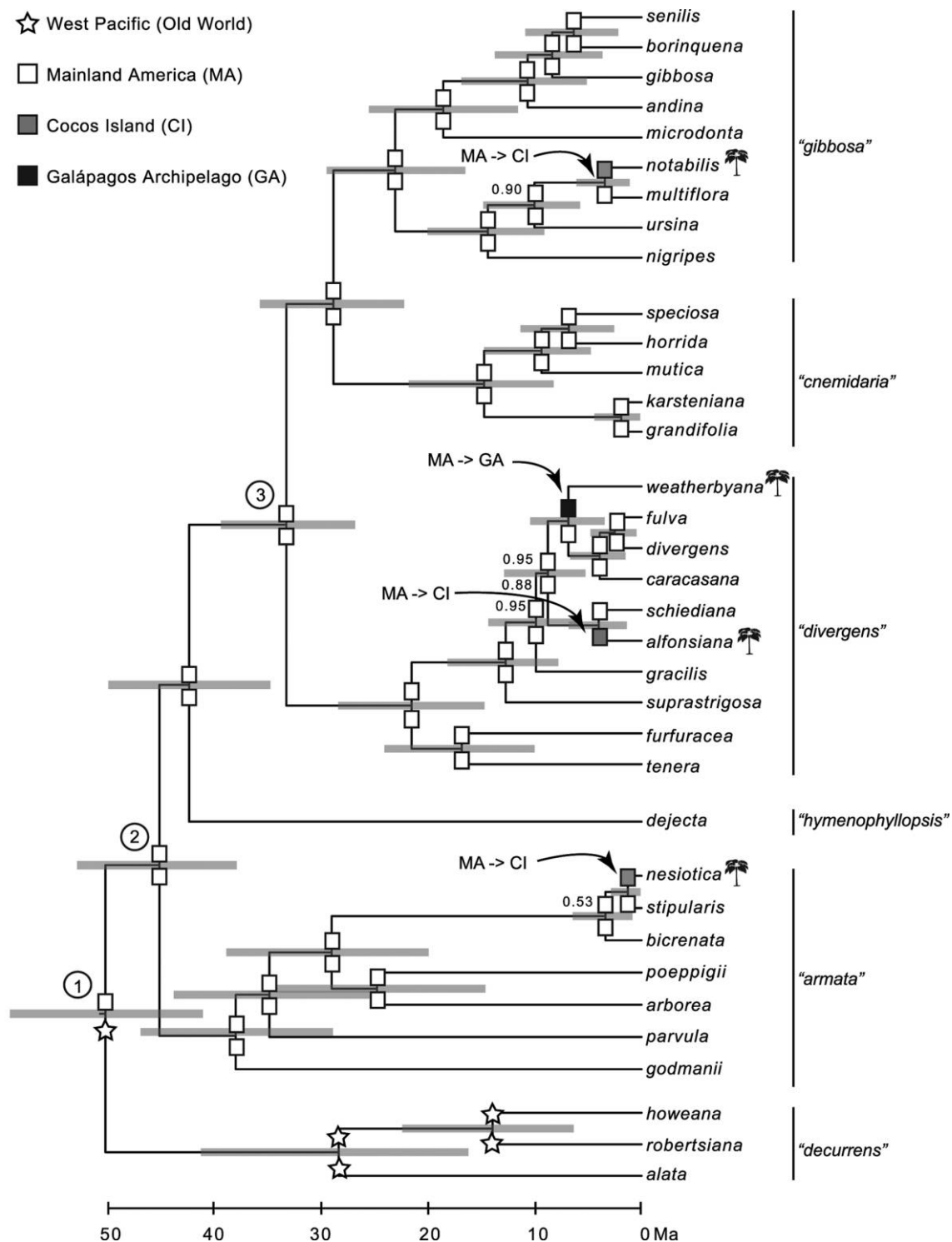


Fig. 3 Chronogram for *Cyathea*, showing results from biogeographical analysis. The 95% highest posterior density of divergence dates estimated by BEAST 2 (Bouckaert et al. 2014) are shown as gray bars. The four endemic species on Cocos and Galápagos Islands are indicated with a tree fern symbol. Three nodes that were secondarily calibrated using divergence times estimated by Korall and Pryer (2014) are denoted by circled numbers 1–3. Symbols on branches represent the most probable ancestral distributions obtained using Lagrange (Ree et al. 2005; Ree and Smith 2008); star, West Pacific; white square, mainland America; gray square, Cocos Island; black square, Galápagos Archipelago along with their probabilities if lower than 0.99. The four dispersal events from mainland America to either Cocos Island (MA → CI) or Galápagos Archipelago (MA → GA) are indicated.

phylogenetic relationships to separate subgroups of mainland American taxa are strongly supported (fig. 3).

Stem Anatomy

The stem anatomies of each of the three Cocos Island *Cyathea* species are distinct from one another (fig. 4A, 4C, 4E).

From an ongoing and broad anatomical survey of mainland *Cyathea* species (R. A. White and M. D. Turner, unpublished data), it is clear that each Cocos Island species is more closely comparable with the other mainland members of its clade than it is to the other Cocos Island species. For example, *C. nesiotica* compares closely with other mainland *armata* group species, such as *Cyathea trichiata*, in sharing the presence of

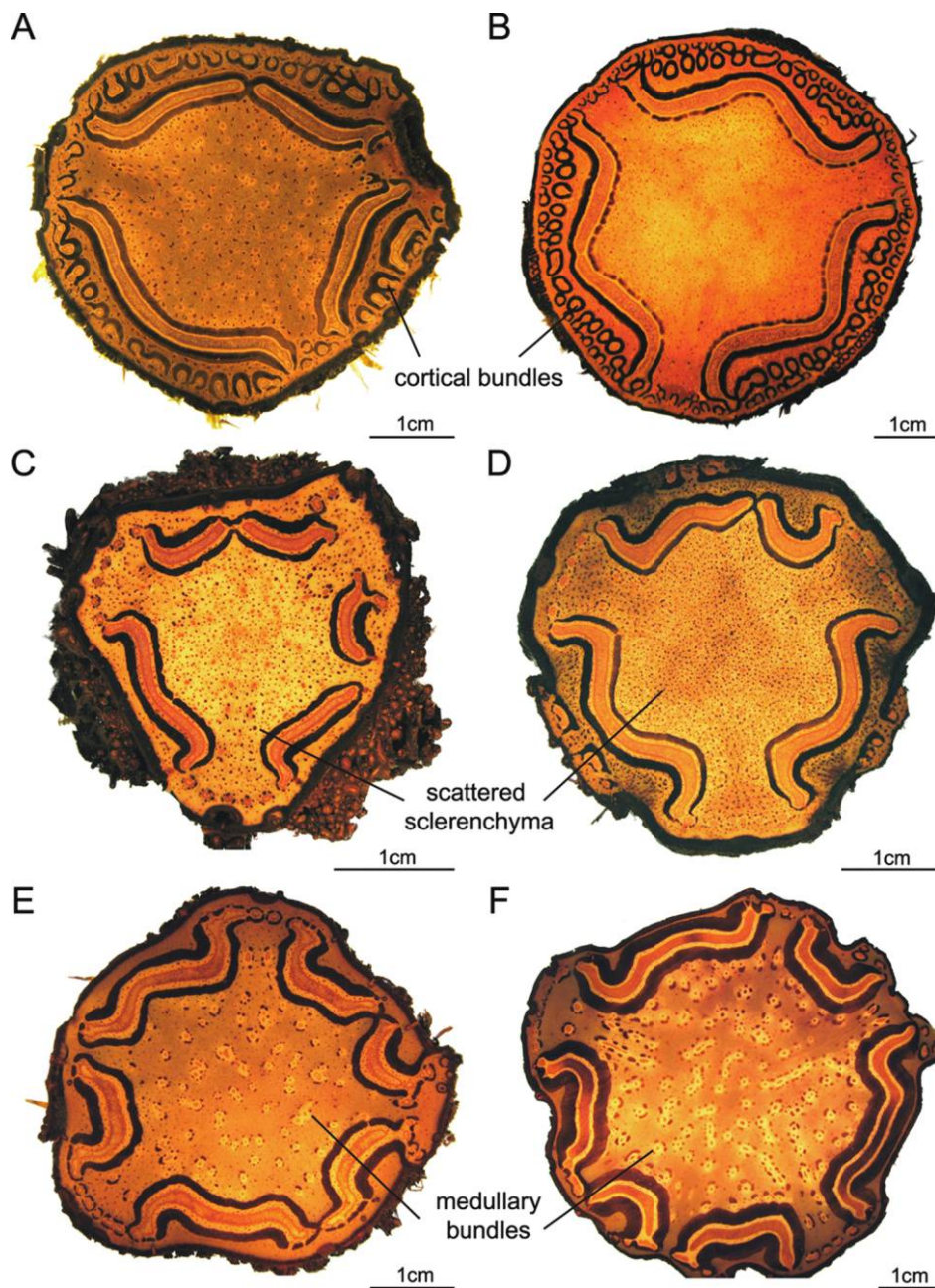


Fig. 4 Stem cross sections of Cocos Island *Cyathea* endemics (A, C, E) each paired with cross sections from a closely related Central-South American mainland species (B, D, F). A, B, Members of the *armata* group with prominent cortical bundles. A, *Cyathea nesiotica* (Trusty 527, DUKE). B, *Cyathea trichiata* (White 199904, DUKE). C, D, Members of the *gibbosa* group with scattered sclerenchyma strands throughout cortex and pith. C, *Cyathea notabilis* (Trusty 533, DUKE). D, *Cyathea multiflora* (White 200207, DUKE). E, F, Members of the *divergens* group with medullary bundles surrounded by pale parenchyma zones and sclerenchyma sheaths. E, *Cyathea alfonsiana* (Trusty 528, DUKE). F, *Cyathea caracasana* (Conant 4884, VT). Scale bars = 1 cm.

an elaborate system of cortical vascular bundles (fig. 4A, 4B). *Cyathea notabilis* is nearly indistinguishable anatomically from the mainland species *C. multiflora* and other members of the *gibbosa* group in sharing the presence of numerous scattered sclerenchyma strands throughout the cortex and pith (fig. 4C, 4D). *Cyathea alfonsiana* is closely similar in stem anatomy to other mainland *divergens* group species, such as *Cyathea caracasana*, in that they share a distinctive appearance with groups of medullary vascular bundles surrounded by sclerenchyma sheaths and confluent pale zones of parenchyma (fig. 4E, 4F).

Discussion

The dispersal units of ferns are spores, which are light, small, and easily transported by wind. Contrary to the general expectation that ferns should have high dispersal abilities and broad distribution ranges, spores more often land within the immediate neighborhood of the parent plant (Wolf et al. 2001). This is especially true for scaly tree ferns that show limited evidence for transoceanic dispersal and high levels of endemism (Tryon and Gastony 1975; Conant 1978; Korall and Pryer 2014). In the Neotropics, about 56% of scaly tree ferns are local endemics, with distribution ranges that extend less than 500 km (Tryon and Gastony 1975). This limited dispersal and restricted geographic range is primarily attributed to their outcrossing reproductive mode (Soltis et al. 1991; Chiou et al. 2003), which requires two spores to land in close proximity to establish a new population (Korall and Pryer 2014), and their adaptation to specific climate niches (Tryon and Gastony 1975; Bystrakova et al. 2011).

In a morphological revision of *Cyathea*, Tryon (1976) hypothesized that the Galápagos and Cocos island endemics *Cyathea alfonsiana*, *Cyathea notabilis*, and *Cyathea weatherbyana* were likely to have been derived independently from widespread mainland species that presumably had higher dispersive ability or broader ecological tolerance, such as *Cyathea multiflora* and *Cyathea andina* (both now known to be part of the *gibbosa* group; figs. 1, 2). On the other hand, *Cyathea nesiotica* was considered to be most closely related to a group of exindusiate species (Maxon 1922; Riba 1969; Barrington 1978; Gastony 1979) that includes *Cyathea bicrenata* and *Cyathea stipularis* (part of the *armata* group; figs. 1, 2) and ranges from Mexico to South America.

In our study, we infer a well-resolved phylogeny of *Cyathea* using five plastid regions and 35 taxa (including all four island endemic species). Our tree topology is highly congruent with previous molecular phylogenetic studies of *Cyathea* (Korall et al. 2006, 2007), and the six monophyletic groups defined by Korall et al. (2007) are strongly supported here (fig. 2). Our phylogenetic and biogeographical analyses (fig. 3) confirm the hypothesis that the four endemics were independently derived from mainland America (Tryon 1976; Trusty et al. 2012; Igea et al. 2014); however, with our additional plastid data, we are now able to more clearly resolve the relationships of *C. alfonsiana* and *C. weatherbyana*. Both species are shown here to belong to the *divergens* group (fig. 2) and were independently derived from different mainland taxa (fig. 3).

Even though the phylogenetic positions of *C. alfonsiana* and *C. weatherbyana* differ from Tryon's (1976) expectation that they would be closely related to either *C. multiflora* or

C. andina, his hypothesis that the island endemics were likely to have been derived from widespread mainland species is still true. In our phylogenetic analysis (fig. 2), *C. alfonsiana* is most closely related to *Cyathea schiediana* (Mexico to Panama), and *C. weatherbyana* is closely related to *Cyathea fulva* (Mexico to Ecuador), *Cyathea caracasana* (Cuba to Bolivia), and *Cyathea divergens* (Mexico to French Guiana), which are all widely distributed species. This supports Tryon's (1976) hypothesis that there is a correlation between tree ferns having a wider distribution range and a higher probability of colonizing oceanic islands. Nevertheless, this should be confirmed with a more comprehensive sampling.

Stem Anatomy

As part of a larger and more comprehensive study that is ongoing by R. A. White and M. D. Turner (unpublished data) to investigate stem anatomy across *Cyathea*, we were able to identify patterns that clearly complement our phylogenetic findings on the basis of DNA sequence data of three separate introductions of *Cyathea* taxa to Cocos Island.

Cyathea nesiotica is closely comparable in stem anatomy to *C. bicrenata* (Riba 1967, p. 84, lamina 1, figs. 8, 9) and other species of the *armata* group (including *Cyathea armata*, *Cyathea conjugata*, *C. stipularis*, *Cyathea trichiata* [fig. 4B], and *C. tryonorum*; R. A. White and M. D. Turner, unpublished data). All these taxa share a uniquely complex system of numerous large cortical vascular bundles with thick sclerenchyma sheaths (fig. 4A, 4B). The two other Cocos Island *Cyathea* endemics (*C. alfonsiana* and *C. notabilis*) both lack cortical bundles.

Cyathea notabilis is most closely comparable in stem anatomy with *C. multiflora*, its sister species (fig. 2). Both share the distinctive characteristic of abundant slender sclerenchyma bundles or strands present throughout the otherwise histologically homogeneous ground tissue of pith and cortex (fig. 4C, 4D). Besides *C. multiflora*, numerous other taxa examined in the *gibbosa* (e.g., *Cyathea ursine*, *Cyathea senilis*, *Cyathea andina*) and *cnemidaria* groups (R. A. White and M. D. Turner, unpublished data) also share this trait of scattered sclerenchyma strands not associated with vascular bundles.

Cyathea alfonsiana is similar in detail with various members of the *C. divergens* group, such as *C. caracasana* (fig. 4E, 4F). The only sclerenchyma strands in the pith are components of the medullary bundle sclerenchyma sheaths. The pale zone of small-celled starchy parenchyma tissue immediately surrounding the stele, and each of the medullary vascular bundles, is more distinctly different from the surrounding ground tissue of the pith and cortex than that formed in the other groups. The scattered sclerenchyma strands that characterize the *gibbosa* group are never seen here. Several other members of the *divergens* group that have been examined (*C. caracasana*, *Cyathea delgadii*, *C. divergens*, and *C. fulva*; R. A. White and M. D. Turner, unpublished data) typically share a distinctive strong zonation of the ground tissue of the stem that is lacking in members of the *gibbosa* and *armata* groups.

Stems of *C. weatherbyana*, the endemic *Cyathea* to the Galápagos, have not yet been observed. Because it is also nested within the *divergens* group, we predict that its stems would be anatomically similar to stems of *C. alfonsiana*.

Conclusion

In this study, we investigated the origin of the endemic scaly tree ferns on Galápagos and Cocos Islands. We present a well-resolved phylogeny of *Cyathea*, showing that the four endemic species each originated from mainland America and none from the South Pacific *Cyathea* (the *decurrens* group; fig. 3), an expected result that fits with our current knowledge of the flora of these two areas (Trusty et al. 2006; Tye and Francisco-Ortega 2011). This is different from the situation in Madagascar, where clear evidence for the local radiation of tree ferns was shown by Janssen et al. (2008). Our results show that all four Galápagos and Cocos tree fern endemics were derived from independent colonization events from wide-ranging mainland species, a biogeographic phenomenon that is also observed for other Cocos endemics (Igea et al. 2014). We also confirm that the endemics on Galápagos and Cocos are not the closest relatives of one another, which was first observed by Trusty et al. (2012).

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Appendix

Species of *Cyathea* examined in this study, with geographical source of voucher, voucher specimens (herbarium), fern DNA database numbers (<http://fernlab.biology.duke.edu>), and GenBank accession numbers for *rbcl*, *rbcl-accD*, *rbcl-atpB*, *trnG-R*, and *trnL-F* (in that order). N/A: data not available for this taxon. Species preceded by an asterisk were examined for stem cross sections.

Cyathea alata (E. Fourn.) Copel., New Caledonia, Swenson et al. 613 (S), DB2245, AM177335, AM410436, AM410227, AM410363, N/A; *Cyathea alfonsiana* L.D. Gómez, Cocos Island, Gomez 25867 (CR), DB5326, KT235813, KT235826, KT235839, KT235852, KT235864; **Cyathea alfonsiana*, Cocos Island, Trusty 528 (DUKE); *Cyathea andina* (H. Karst.) Domin, Puerto Rico, Worthington 35211 (DUKE), DB10162, KT235814, KT235827, KT235840, N/A, N/A; *Cyathea arborea* (L.) Sm., Puerto Rico, Conant 4344 (LSC), DB2491, AM177336, AM410470, AM410261, AM410396, KT235865; *Cyathea bicrenata* Liebm., Honduras, Conant 4346 (LSC), DB4350, KT235815, KT235828, KT235841, KT235853, KT235866; *Cyathea borinquena* (Maxon) Domin, Puerto Rico, Conant 4864 (LSC), DB4399, KT235816, KT235829, KT235842, KT235854, KT235867; *Cyathea caracasana* (Klotzsch) Domin, Costa Rica, Conant 4412 (LSC), DB3114, AM410223, AM410493, AM410286, AM410422, AM410351; **Cyathea caracasana*, Conant 4884 (LSC); *Cyathea dejecta* (Baker) Christenh., Venezuela, Milleron s.n. (UC), DB397, AF101301, AM410435, AM410226, AM410362, AM410299; *Cyathea divergens* Kunze, Costa Rica, Conant 4384 (LSC), DB2332, AM177337, AM410460, AM410251, AM410386, AM410321; *Cyathea fulva* (M. Martens & Galeotti) Fée, Costa Rica, Conant 4397 (LSC), DB4356, KT235817, KT235830, KT235843, KT235855, KT235868; *Cyathea furfuracea* Baker, Puerto Rico, Conant 4325 (LSC), DB3115, AM410224, AM410494, AM410287, AM410423, AM410352; *Cyathea gibbosa* (Klotzsch) Domin, Venezuela, Conant 4469 (LSC), DB2492, AM177354, AM410471, AM410262, AM410397, AM410330 and KT235869; *Cyathea godmanii* (Hook.) Domin, Honduras, Conant 4376 (LSC), DB3113, AM410222, AM410492, AM410285, AM410421, AM410350; *Cyathea gracilis* Griseb., Costa Rica, Conant 4415 (LSC), DB3108, AM410217, AM410487, AM410280, AM410416, AM410345 and KT235870; *Cyathea grandifolia* Willd., Venezuela, Conant 4488 (LSC), DB2309, AM177332, AM410440, AM410231, AM410367, AM410302 and KT235871; *Cyathea horrida* (L.) Sm., Puerto Rico, Conant 4343 (LSC), DB2331, AM410196, AM410459, AM410250, AM410385, AM410320; *Cyathea howeana* Domin, Lord Howe Island, Conant 4665 (LSC), DB2317, AM410188, AM410446, AM410237, AM410372, AM410308; *Cyathea karsteniana* (Klotzsch) Domin, Venezuela, Conant 4471 (LSC), DB3112, AM410221, AM410491, AM410284, AM410420, AM410349; *Cyathea microdonta* (Desv.) Domin, Honduras, Conant 4357 (LSC), DB4352, KT235818, KT235831, KT235844, KT235856, KT235872; *Cyathea multiflora* Sm., Costa Rica, Conant 4425 (LSC), DB2333, AM410197, AM410461, AM410252, AM410387, AM410322; **Cyathea multiflora*, Costa Rica, White 200207 (DUKE); *Cyathea mutica* (Christ) Domin, Costa Rica, Conant 4385 (LSC), DB3111, AM410220, AM410490, AM410283, AM410419, AM410348; *Cyathea nesiotica* (Maxon) Domin, Cocos Island, Gomez 25869 (CR), DB5328, KT235819, KT235832, KT235845, KT235857, KT235873; **Cyathea nesiotica*, Cocos Island, Trusty 527 (DUKE); *Cyathea nigripes* (C. Chr.) Domin, Costa Rica, Conant 4417 (LSC), DB4360, KT235820, KT235833, KT235846, KT235858, KT235874; *Cyathea notabilis* (Maxon) Domin, Cocos Island, Gomez 25868 (CR), DB5327, KT235821, KT235834, KT235847, KT235859, KT235875; **Cyathea notabilis*, Cocos Island, Trusty 533 (DUKE); *Cyathea parvula* (Jenman) Domin, Puerto Rico, Conant 4332 (LSC), DB2330, AM177338, AM410458, AM410249, AM410384, AM410319; *Cyathea poeppigii* (Hook.) Domin, Costa Rica, Conant 410 (LSC), DB2367, AM410201, AM410468, AM410259, AM410394, AM410328; *Cyathea robertsiana* (F. Muell.) Domin, Australia, Shirley 12 (LSC), DB3107, AM410216, AM410486, AM410279, AM410415, AM410344; *Cyathea schiedeana* (C. Presl) Domin, Honduras, Conant 4367 (LSC), DB3109, AM410218, AM410488, AM410281, AM410417, AM410346; *Cyathea senilis* (Klotzsch) Domin, Venezuela, Conant 4479 (LSC), DB2496, AM410203, AM410473, AM410264, AM410399, AM410332; *Cyathea speciosa* Humb. & Bonpl. ex

Willd., Venezuela, Conant 4476 (LSC), DB2493, AM177339, AM410472, AM410263, AM410398, AM410331; *Cyathea stipularis* (Christ) Domin, Costa Rica, Conant 4395 (LSC), DB3110, AM410219, AM410489, AM410282, AM410418, AM410347; *Cyathea suprastrigosa* (Christ) Maxon, Costa Rica, Conant 4402 (LSC), DB4357, KT235822, KT235835, KT235848, KT235860, KT235876; *Cyathea tenera* (Hook.) T. Moore, Guadeloupe, Christenhusz 3919 (TUR), DB3239, KT235823, KT235836, KT235849, KT235861, KT235877; **Cyathea trichiata* (Maxon) Domin, Costa Rica, White 199904 (DUKE); *Cyathea ursina* (Maxon) Lellinger, Costa Rica, Conant 4423 (LSC), DB4362, KT235824, KT235837, KT235850, KT235862, KT235878; *Cyathea weatherbyana* (C.V. Morton) C.V. Morton, Galápagos, Uno 1520 (S), DB10216, KT235825, KT235838, KT235851, KT235863, N/A.

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