Article

Cyathea Sm. (Cyatheaceae) on Trindade Island (Brazil): An integrative approach

ALLAN LAID ALKIMIM FARIA[1,6,*], ALEXANDRE SALINO[2,7], MICHELINE CARVALHO-SILVA[1,8], MICHAEL STECH[3,4,9], EDUARDO TOLEDO DE AMORIM[5,10] & PAULO EDUARDO AGUIAR SARAIVA CÂMARA[1,11]

[1] *Universidade de Brasília, UnB, Campus Universitário Darcy Ribeiro, Brasília, Brazil.*

[2] *Universidade Federal de Minas Gerais, UFMG, ICB, Departamento de Botânica, Brazil.*

[3] *Naturalis Biodiversity Center, Leiden, the Netherlands. Leiden University, Leiden, the Netherlands.*

[4] *Leiden University, Leiden, the Netherlands.*

[5] *Centro Nacional de Conservação da Flora - CNCFlora, Instituto de Pesquisas Jardim Botânico do Rio de Janeiro - JBRJ, Rio de Janeiro, Brazil.*

[6] ✉ *allanlaid@gmail.com;* ⬤ *https://orcid.org/0000-0002-9536-7547*

[7] ✉ *salinobh@gmaill.com;* ⬤ *https://orcid.org/0000-0003-0104-7524*

[8] ✉ *silvamicheline@gmail.com;* ⬤ *https://orcid.org/0000-0002-2389-3804*

[9] ✉ *michael.stech@naturalis.nl;* ⬤ *https://orcid.org/0000-0001-9804-0120*

[10] ✉ *e.amorim@live.com;* ⬤ *https://orcid.org/0000-0003-4253-1339*

[11] ✉ *pcamara@unb.br;* ⬤ *https://orcid.org/0000-0002-3944-996X*

* *Corresponding author*

Abstract

Trindade is a Brazilian oceanic island approximately 1,100 km from the American mainland. The *Cyathea* Sm. plants from Trindade were originally considered an endemic species, *Cyathea copelandii* Kuhn & Luerssen, but later included in *Cyathea delgadii* Sternb. Based on molecular data (*trnL-trnF, trnG-trnR* and *rbcL-accD*), morphometric analysis of leaf characters, scanning electron microscopy of indusia and spores, and habitat suitability modeling, the present study confirms the status of *C. copelandii* as a synonym of *C. delgadii*. Intraspecific molecular variation suggests phylogeographic structuring in *C. delgadii* and differentiation between the coastal Atlantic Forest domain and the Cerrado domain (savanna) in the interior of Brazil. The Trindade populations are more closely related to the Atlantic Forest populations, indicating a colonization of the island from the latter area.

Key words: Atlantic Forest, Cyatheaceae, Oceanic Island, Tree fern

Introduction

Trindade is a Brazilian oceanic island located in the South Atlantic Ocean (20º31'30"S, 29º19'30"W), ca. 1,200 km from the Brazilian city of Vitória (Espírito Santo state) (Fig. 1). The island originated about 3 million years ago from volcanic activity in the Atlantic abyssal zone (Alves 1998, Serafini *et al.* 2010). The ocean depths around the island reach 5,800 m deep, and the emerging part reaches 620 m high. The island has a total area of 9.28 km² (Alves 1998). Its climate is tropical Atlantic to semi-arid with almost uniform monthly average temperatures of 22–25ºC, influenced by the trade winds of the Southeast (Barros 1950; Alves 1998).

The native vegetation on Trindade was to a large extent destroyed by feral animals, especially goats that were brought to the island in the 18th century (Martins & Alves 2007, Silva & Alves 2011). The most conspicuous vegetation type on Trindade is the "*Cyathea delgadii* forest" or "giant fern forest" (floresta nebular de samambaias-gigantes, Gasparini 2004). It occurs on the slopes above 400 m on the southern side of the island (Alves 1998) and is formed by a single species of scaly tree fern (Cyatheaceae).

Cyatheaceae Kaulf. comprises about 643 species divided into three (*Alsophila* R. Br., *Cyathea* Sm., *Sphaeropteris* Bernh.) (Lehnert & Kessler 2018) or four (*Alsophila* R. Br.*, Cyathea* Sm., *Gymnosphaera* Blume, *Sphaeropteris* R. Br. ex Wall.) genus-level lineages, based on phylogenetic analyses of restriction-site data and morphology *versus* DNA sequence data, respectively (Korall *et al.* 2007 and references therein, Korall & Pryer 2014, Weigand & Lehnert 2016). Cyatheaceae is distributed throughout the wet tropics and the temperate southern hemisphere (Lehnert 2009). The

Neotropics alone harbors ca. 210 species, with centers of endemism located in the Greater Antilles, southern Mexico, Central America and the Andes (Tryon & Gastony 1975, Weigand & Lehnert 2016).

Cyathea includes about 150 species distributed from Mexico and the Caribbean to the north of Argentina and has its diversity center in the Andes, from Venezuela to Peru (Tryon & Tryon 1982, Moran 1995, Smith *et al.* 2006). The genus inhabits riversides in shaded forests and open environments with very humid soils (Barrington 1978, Fernandes 2003, Alves 1998, Labiak & Matos 2009, Windisch & Santiago 2015). In Brazil, *Cyathea* is widely distributed in the main phytogeographic domains of the Amazon, Cerrado, Campos sulinos and Atlantic Forest, the latter being the region with highest species richness (Windisch & Santiago 2015, Fiaschi & Pirani 2009). Accepted species numbers for Brazil are ca. 39, of which 14 are endemics (Prado *et al.* 2015, Windisch & Santiago 2015, Weigand & Lehnert 2016).

The taxonomy and classification of *Cyathea* was traditionally based on the morphology of scales, indusia and spores. Five species groups (*cnemidaria* group, *C. gibbosa* group, *C. divergens* group, *C. armata* group and *C. decurrens* group) previously distinguished based on these characters were resolved as monophyletic in molecular phylogenetic reconstructions (Conant *et al.* 1996, Stein *et al.* 1997, Korall *et al.* 2007). Although morphological and molecular variation in general seem to coincide at a supraspecific level in *Cyathea*, the significance of morphological variation at species level needs further investigation. For example, geographically separated populations on islands such as Trindade may already have evolved and differentiated morphologically, but the morphological variation alone may not be sufficient to decide whether island populations should be distinguished as a separate species (Clucas *et al.* 2016). Molecular tools in combination with environmental modelling (Martínez-Gordillo *et al.* 2010, Gama *et al.* 2017) may help to clarify species delimitations, infer evolutionary patterns, and understand the factors that shape species distributions.

Originally, the *Cyathea* population from Trindade was considered an endemic species described in 1882 as *Cyathea copelandii* Kuhn & Luerssen (= *C. trindadensis* Brade), which was later synonymized with *C. delgadii* Sternb. However even with the synonymization, the name *C. copelandii* is still commonly used in lists and floras (Roskov *et al.* 2018). *Cyathea delgadii* is a species widely distributed from Costa Rica to Paraguay, and occurring in most states of Brazil (Schmitt & Windisch, 2007, Windisch & Santiago 2015, Weigand & Lehnert 2016). Tryon (1976) did acknowledge variation in the indusium between mainland Brazilian specimens of *C. delgadii* (in contrast to specimens from other parts of the distribution range), with specimens from Trindade, however, with some overlap. It is therefore not possible to separate *C. delgadii* into two species based on this character. The circumscription of *C. delgadii* by Tryon (1976) was followed in recent synopses and checklists (e.g. Lehnert 2014), also for Brazil (e.g. Windisch & Santiago 2015, Weigand & Lehnert 2016). A further small difference in the indusium between Trindade and continental *C. delgadii* plants was reported later, but also considered insignificant to distinguish the former as an endemic species (I. Fernandes in Alves 1998). Nevertheless, few morphological characters and few specimens from Trindade have been analyzed to date, and no molecular data yet exist to evaluate the synonymization of *C. copelandii* and the significance of morphological variation within *C. delgadii*.

Using molecular data (chloroplast *trnL-trnF*, *trnG-trnR*, and *rbcL-accD* sequences) in combination with morphometric analysis of leaf characters, a comparison of indusia and spores by scanning electron microscopy, and environmental niche modeling, the aims of this study are to infer (i) the status of *C. copelandii* as a distinct species or as a synonym of *C. delgadii*, (ii) the geographic origin of the *Cyathea* populations on Trindade Island and (iii) patterns of intraspecific variation in *C. delgadii* between plants from the Atlantic Forest and Cerrado (savanna) domains of Brazil.

Material and Methods

Molecular analysis
Sampling: New DNA sequences of three chloroplast markers (*trnL-trnF*, *trnG-trnR*, and *rbcL-accD*) were generated from 17 *Cyathea* specimens. These included four *C. delgadii* samples from Trindade Island, nine *C. delgadii* samples from continental Brazil as well as four samples of other *Cyathea* species (*C. gibbosa* group) from Brazil, of which two *C. phalerata* Mart. samples and two samples of species not included in earlier phylogenetic analyses (Korall *et al.* 2007, Korall & Pryer 2014), viz. *C. feeana* (C. Chr.) Domin and *C. villosa* Humb. & ex Willd. Of the *C. delgadii* samples from continental Brazil, four originated from the Atlantic Forest domain from the states of Minas Gerais (MG), Rio de Janeiro (RJ), Rio Grande do Sul (RS) and Santa Catarina (SC) and five of the Cerrado domain from Espírito

Santo (ES), Federal District (DF), Goiás (GO), and MG. DNA sequences of the newly sequenced specimens were combined with those of 21 *Cyathea* species obtained from GenBank. These included representatives from five of the six Neotropical groups included in Korall *et al.* (2007), i.e. *C. armata* group, *Cnemidaria* group, *C. decurrens* group, *C. divergens* group, and *C. gibbosa* group clades, as well as two species of *Alsophila* as outgroup representatives. DNA sequences and voucher information can be found in Appendix 1 and 2.

DNA extracting, amplification and Sequencing: Total DNA was extracted according to Doyle & Doyle (1987, 1990), with adaptations according to Borsch *et al.* (2003). Three chloroplast DNA regions (*trnL-trnF*, *trnG-trnR*, and *rbcL-accD*) were PCR-amplified using primers C, D, E and F (Taberlet *et al.* 1991) for *trnL-trnF*, TRNG1Fa / TRNR22Ra (Nagalingum *et al.* 2007) for *trnG-trnR*, and RBCL1187Fa / ACCD887R (Korall *et al.* 2007) for *rbcL-accD*. PCRs were performed in 50µl reactions containing 26.8µl ddH$_2$O, 5µl of 5X Mg free buffer, 5µl of 50mM of MgCl$_2$, 2µl at 10mg/ml of BSA (Phoneutra), 4µl of 1mM dNTP, 2.5 µl of 10 µM of each primer, 2µl of DNA template, and 0.5 µl Taq (Phoneutra). The amplification protocol comprised an initial denaturation at 95° C for 1 min, 35 cycles of 30 sec at 95°C, 45 sec at 50–57°C (50–52°C for *trnG-trnR* and *rbcL-accD*, 55–57°C for *trnL-trnF*) and 1 min at 72°C, and a final extension at 72°C for 5 min. PCR purification and bidirectional sequencing of the PCR products was done by Macrogen Inc. (Korea). Sequences were deposited in GenBank under the accession numbers listed in Appendix 1.

Phylogenetic reconstruction: Sequences were aligned with MAFFT (Katoh *et al.* 2002) as implemented in Geneious R8 (Kearse *et al.* 2012), and manually adjusted in PhyDE v0.995 (Müller *et al.* 2006). Phylogenetic analyses were performed under maximum parsimony (MP), maximum likelihood (ML) and Bayesian inference (BI), using PAUP* 4.0b10 (Swofford 2003), RaxML v7.4.2 (Stamatakis 2006) with the raxmlGUI v.1.3 (Silvestro & Michalak 2012), and MrBayes v3.2.7 (Ronquist *et al.* 2012), respectively. A comparison of analyses of each marker separately did not reveal statistically supported incongruence, therefore analyses were done using a combined matrix. Gaps were coded as informative by Simple Indel Coding (Simmons & Ochoterena 2000), as implemented in SeqState (Müller 2004). GTR+Γ was selected as best-fit evolutionary model for the ML and BI analyses of the concatenated matrix by jModeltest 2.1.1 (Guindon & Gascuel 2003, Darriba *et al.* 2012).

Heuristic searches under MP were performed using 1,000 random sequence additions and tree bisection-reconnection (TBR) branch swapping, with a maximum of 10,000 trees saved, and all characters equally weighted. Heuristic bootstrap searches under parsimony were performed with 1,000 replicates and 10 random addition cycles per bootstrap replicate. For ML, heuristic bootstrap analyses were carried out with 1,000 replicates (Felsenstein 1985). Bayesian 50% majority rule consensus trees and posterior probabilities were calculated using the Markov Chain Monte Carlo (MCMC) method. The a priori probabilities supplied were those specified in the default settings of the program. Sequence and indel data were treated as separate and unlinked partitions, employing the restriction site model ('F81') for the indel matrix. Two parallel runs with four chains each (three heated and one cold) were run for 5,000,000 generations, chains sampled at every 1,000[th] generation, and the respective trees written to a tree file. A threshold of <0.01 for the standard deviation of split frequencies and trace plots generated in Tracer 1.6 (Rambaut & Drummond 2013) were used to assess convergence of the chains. The first 25% of trees were discarded as "burn-in".

Morphometric analysis and SEM

Three quantitative leaf characters not previously compared in detail between Trindade Island and continental Brazilian populations, namely length of pinnae, length of pinnules, and width of pinnules were measured from about 200 individuals of *C. delgadii* deposited in herbaria BHCB, CEN, K, P, R, RB, SP, SPF and UB. Each character was measured on five fronds from different parts of the specimen using an optical microscope (Leica DM750) and a video camera (MC 170 HD) to capture the images to a computer Leica Application Suite software (Version 4.5.0) was used for image analysis. A matrix was constructed with the mean values obtained from the five fronds measured in each sample, and subjected to principal component analysis (PCA) in PAST 3.15 (Hammer *et al.* 2001). Discriminant Analysis (DA) was performed to verify the significance of the groups identified by PCA. This analysis determines to what extent an independent set of quantitative variables explain a grouping (Borcard *et al.* 2011). PCA was performed using the mean values of each individual. The values of the morphological characters were standardized and transformed into log10 before their use in PCA.

According to Holttum (1963), Tryon (1970), Gastony (1973), Tryon & Feldman (1975), Holttum & Edwards (1983), Lellinger (1987) and Conant *et al.* (1996) the spores and indusia are the characters considered very informative in *Cyathea*. Thus, such characters were used for comparing between samples from the island and the continent. Reproductive characters (indusia and spores) of 17 specimens of *C. delgadii* (ten Trindade island, seven continental specimens) were studied by scanning electron microscopy (SEM). Samples were dehydrated in a series of ethanol (70%), ethanol (90%), two steps of ethanol (100%), ethanol (100%) / acetone (100%) and two steps of acetone (100%).

The dehydrated material was dried to critical point with carbon dioxide as transition fluid in a CPD 030 critical point drier (Balzers). The material was assembled on metal stubs and covered with platinum using the high-vacuum sputter coater EM SCD 500 (Leica), following Castro (2001). The samples were studied using a JSM-7001F scanning electron microscope (Jeol) at the Electron Microscopy Laboratory, University of Brasília.
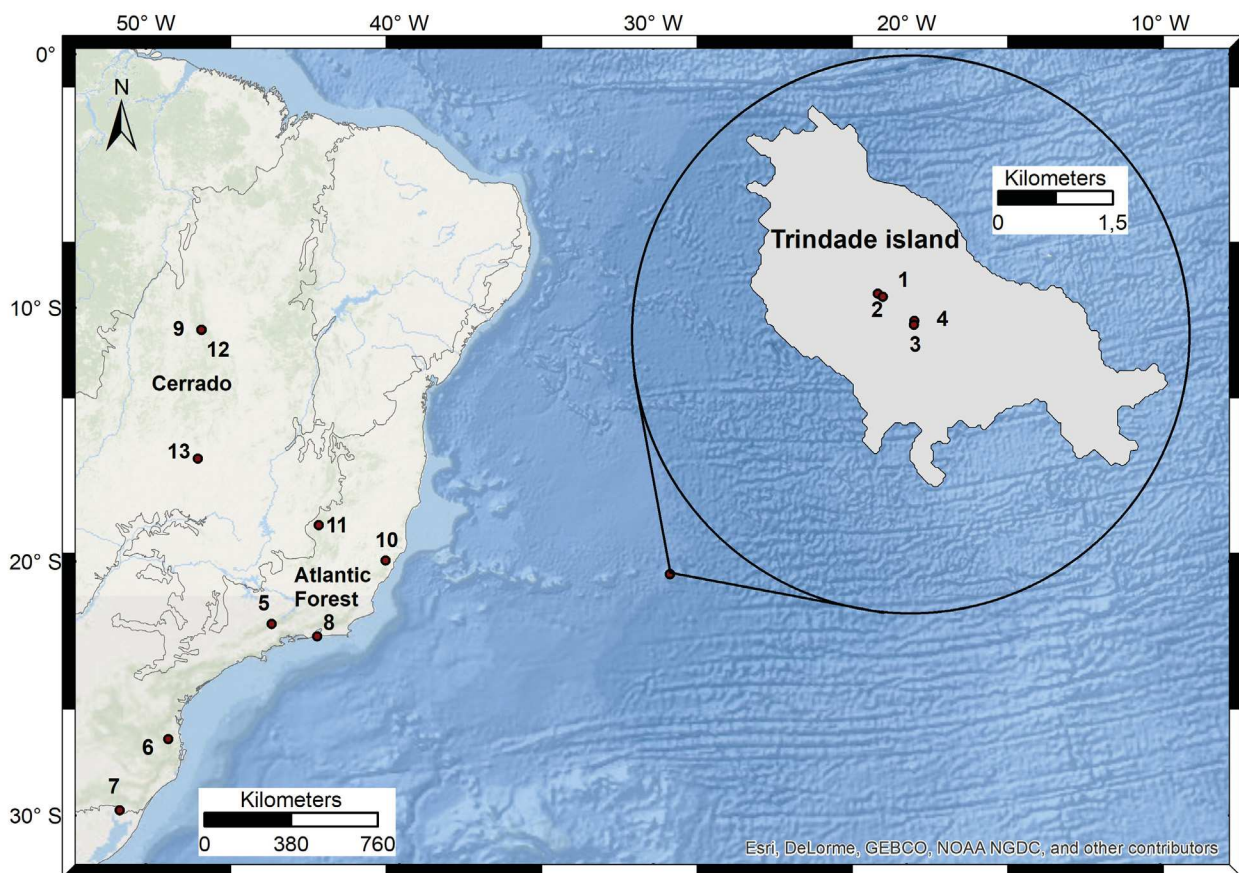
FIGURE 1. Geographic location of Trindade Island. The numbers represent the samples according to the phylogenetic tree.

Habitat suitability modeling

For the modeling analysis, voucher information from 164 specimens of *Cyathea delgadii* (92 from Atlantic Forest and Amazon, 72 from Cerrado) was compiled from the databases GBIF, SpeciesLink (splink.org.br) and Jabot (flor.jbrj. gov.br) as well as our own field collections.

Nineteen bioclimatic variables were obtained from the WorldClim version 1.4 database (Hijmans *et al*. 2005). In order to eliminate the multicollinearity of the layers, a Pearson correlation was performed between the predictive bioclimatic variables and, later, between those with high correlation, $R^2 > 0.8$ (Guisan & Thuiller 2005). The values used for the correlation were obtained from the raster files for each environmental variable. After elimination of the redundant variables, six were included in the models, with a resolution of 10 min (bio2 bio3 bio5 bio12 bio14 bio18). We used four algorithms, BioCLim, GLM, Random Forest and SVM, to generate the suitability models.

We used the cross validation, k-fold, being k the number of partitions, and in the study three partitions were established. From these, k-1 are used to train the algorithm and the remaining partition is used for the test (Giannini *et al*. 2012). Performance was assessed according to the following criteria, establishing the following cutoff limits: (1) assessment of AUC values (*area under the curve*). In this study, values of AUC> 0.7 were considered, ranging from regular to optimal (high accuracy) (Manel *et al*. 2001). The algorithms that obtained results below this number, as well as the partitions that did not reach this minimum value, were not used. (2) Use of TSS (*true skill statistic*) as a validation measure. In this study, values of TSS> 0.6, were considered good (Allouche *et al*. 2006). (3) Using the average of the TSSth value of each partition as the final cut for the model in each algorithm.

In order to increase the accuracy of the models, the Ensemble forecasting technique was performed (Araújo & New 2007), generating a consensus model. This model was generated by the average of each algorithm (general assembly), in which the areas of greater suitability are the results of the congruence that most of the models suggest as more

favorable (Giannini *et al.* 2012). A model is used together to explore a larger range of forecasts, better understanding the uncertainty of the model and obtaining a better projection on the prediction of the occurrence of the species (Franklin & Miller 2010).

Results

Phylogenetic analysis

The combined alignment comprised 2,154 nucleotide positions, of which 109 were parsimony-informative. Simple indel coding (SIC) resulted in 58 additional indel characters, of which 26 were parsimony-informative (Table 1).

TABLE 1. Alignment statistics, best-fitting models of evolution, and tree scores for maximum parsimony and maximum likelihood phylogenetic reconstructions of the separate and combined chloroplast DNA marker datasets used in the present study.

DNA marker	*trnL-trnF*	*trnG-trnR*	*rbcL-accD*	Combined
Alignment position	1-816	817-1820	1821-2154	1-2154
Taxa included	38	38	38	38
Matrix length (sites)	817	1003	334	2154
Variable sites	123	126	29	279
Parsimony-informative sites	50	77	10	109
No. of saved trees (parsimony)	10.000	10.000	10.000	10.000
Tree length	144	146	28	415
CI	0.903	0.904	0.964	0.881
RI	0.873	0.946	0.964	0.902
Model	GTR+Γ	GTR+Γ	GTR+Γ	GTR+Γ
Log likelihood	-2,062.969.923	-2,330.209.126	-647,354.332	-5,196.914.122

The maximum parsimony consensus tree, the single optimal maximum likelihood tree, and the Bayesian consensus tree had similar topologies. The Bayesian consensus tree is shown in Fig. 2, with MP/ML bootstrap support values (MP-BS/ML-BS) and Bayesian posterior probabilities (PP) shown at the nodes.

All analyzed *Cyathea copelandii/C. delgadii* specimens from Trindade and mainland Brazil were resolved in one clade with moderate to high support (67% MP-BS/85% ML-BS/0.99 PP), which was part of the highly supported *C. divergens* group clade (98%/99%/1). The *C. copelandii/C. delgadii* specimens were divided into two subclades: Subclade A (66%/70%/0.99) comprised the specimens from Trindade and the Atlantic Forest domain, whereas subclade B (72%/85%/1) comprised the specimens from the Cerrado domain, with the exception of the sample *C. delgadii* (ES) from Atlantic Forest. The other newly sequenced Brazilian *Cyathea* species were resolved in a highly supported subclade (97%/91%/1) clade within the Gibbosa clade (79%/90%/1). The *C. gibbosa* group clade and *cnemidaria* group clade formed sister clades (70%/90%/1) and together were sister to the *C. divergens* group clade (92%/97%/1) (Fig. 2).

Morphometric and SEM analyses

In the PCA analysis of leaf characters, the first component explained 61% of the variation and the second component 28%. The first component was strongly related to length of pinnae and the second component to width of pinnules. Specimens could neither be separated between Trindade Island versus continental Brazil nor between Atlantic Forest/ Amazon and Cerrado (Fig. 3). The Discriminant Analyses corroborated the PCA results regarding extent morphology among *C. delgadii* specimens and overlap between the sampled regions.

Regarding the reproductive characters studied by SEM, there was no differentiation between the sampled areas, neither by the type of indusium nor in the spores. All indusia were sphaeropteroid with variously developed apical umbo (Fig. 4a). The spores (Fig. 4b) were trilete, large (equatorial diameter 30–50 μm) heteropolar, sub-triangular (triangular in polar view) and varied as to the quantity and presence of rodlets in the outer layer, characteristics present in the whole *C. divergens* group.

The morphological characters of the other three newly sequenced *Cyathea* species (*C. phalerata*, *C. feeana,* and *C.villosa*), including chartaceous fronds, strong presence of spines, and sporangia devoid of indusia, are consistent with their position in the *C. gibbosa* group clade in the phylogenetic reconstructions (data not shown).
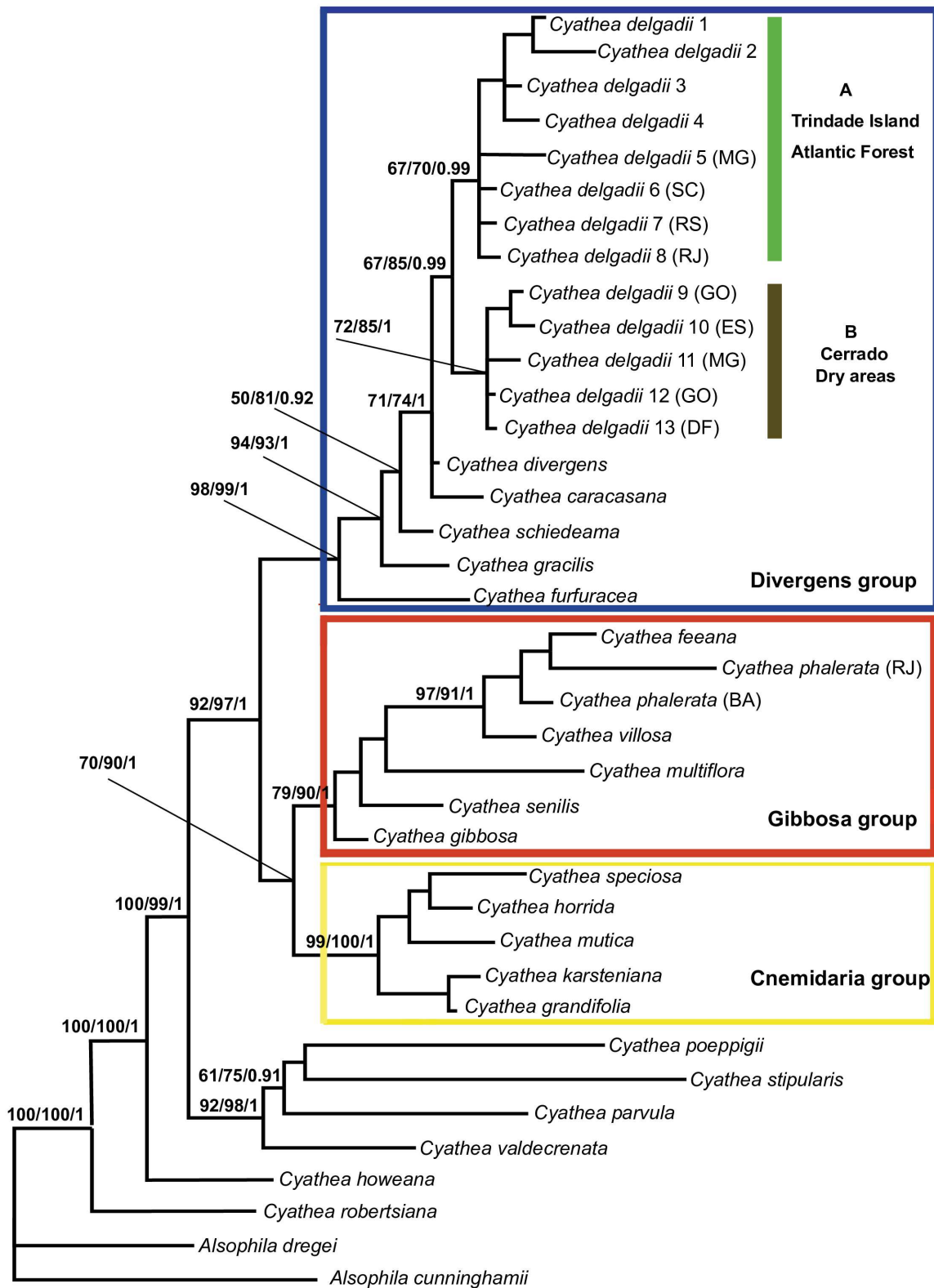
FIGURE 2. Cladogram obtained from Bayesian Inference of *Cyathea* based on all three plastid markers, and indels coded by simple indel coding. Numbers above branches are bootstrap support for maxmimum parsimony, maximum likelihood and Bayesian posterior probabilities, respectively. *Cyathea delgadii* samples 1 to 4 originate from Trindade Island. The acronyms in parentheses indicate the geographic origin (Brazilian state) of the specimens.
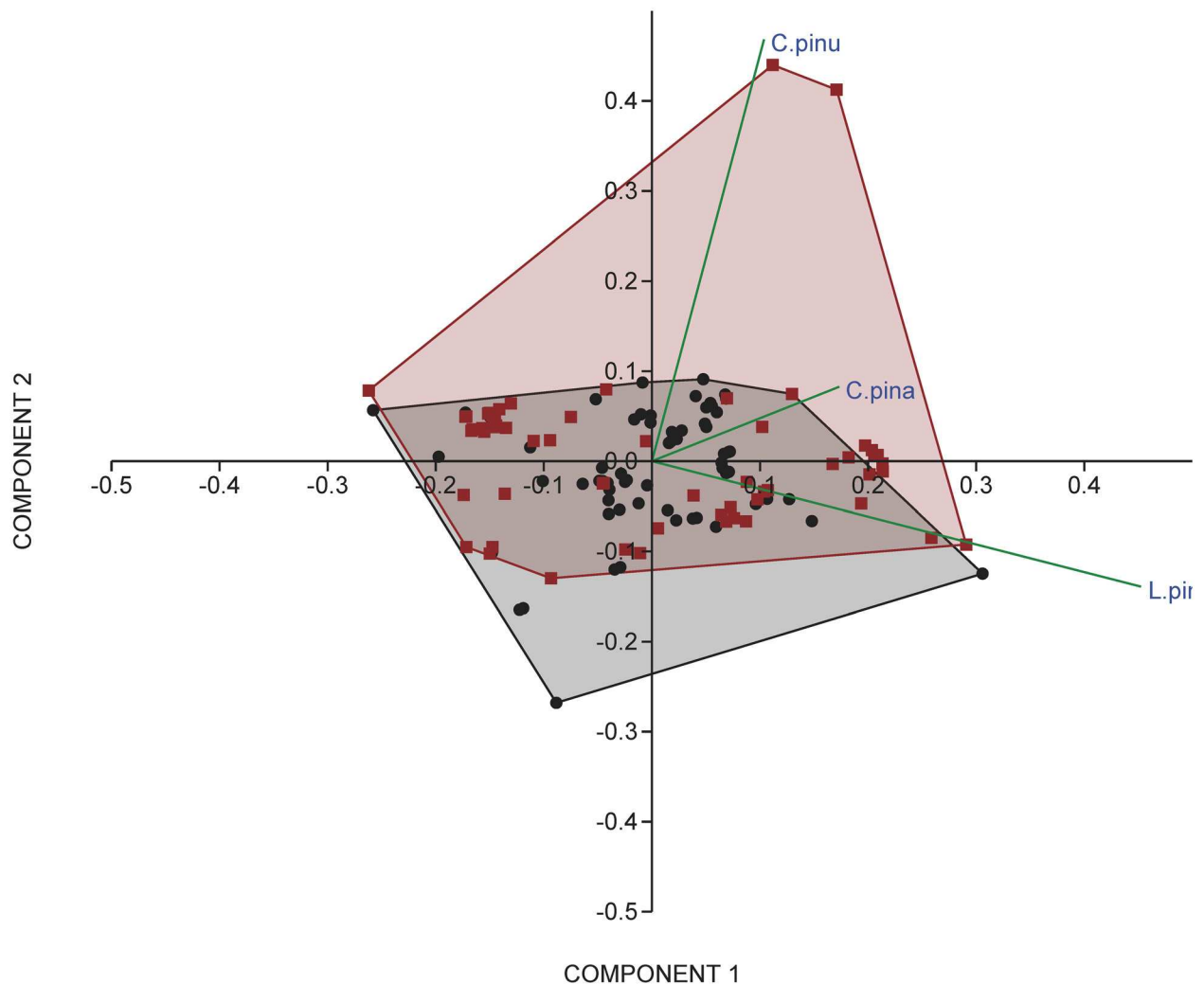
FIGURE 3. Combined PCA data between populations from Cerrado represented as squares and Atlantic Forest represented as circles. L.pina = length of pinnae; L.pinu = length of pinnules and W.pinu = width of pinnules.

Habitat suitability modeling

Consensus maps of habitat suitability areas are shown in Fig. 5B/C. These are based on specimens of Atlantic Forest/ Amazon, which includes species from Trindade Island, and Cerrado (Fig. 5A), which correspond to subclades A and B, respectively, in the molecular phylogeny. In general, the models showed congruences in the areas of habitat suitability for the two molecular clades (Atlantic Forest and Cerrado). For Cerrado specimens, the maps showed areas of greater transition adequacy value in the areas of the central Atlantic Forest corridor and in the mid-south regions of the Cerrado (Fig. 5B). The maps for the Atlantic Forest specimens presented areas with greater suitability in the central corridor and Serra do Mar, and also with areas of suitability of the Cerrado domain (Fig. 5C). We highlight the areas of congruent suitability between the two morphotypes, in the coastal northeast region of the Atlantic Forest, in the western region of the Cerrado and in the east and central Amazon. The validation measure values according to each partition and algorithm are presented in Table 2.

TABLE 2. The values of the validation tests represent the average of the partitions and are separated by the algorithms respectively. NP = partitioning number; BC = BioClim; GLM = General Linear Models, RF = Random Forest; SVM = Support vector machine; AUC = area under the curve; TSS = true skill statistic; TSSth = presence threshold.

| NP	Algorithm	Forest AUC	TSS	TSSth	Dry areas AUC	TSS	TSSth
1	BioClim	0.946	0.865	0.049	0.871	0.646	0.027
2		0.875	0.752	0.024	0.916	0.725	0.028
3		0.907	0.764	0.024	0.75	0.441	0.054
1	GLM	0.971	0.88	0.148	0.816	0.526	0.19
2		0.94	0.753	0.148	0.878	0.706	0.152
3		0.972	0.922	0.192	0.887	0.662	0.094
1	RF	0.99	0.97	0.244	0.93	0.739	0.047
2		0.929	0.761	0.208	0.969	0.847	0.073
3		0.99	0.949	0.163	0.953	0.812	0.062
1		0.979	0.919	0.046	0.772	0.551	0.027
2	SVM	0.904	0.774	0.024	0.926	0.748	0.07
3		0.97	0.901	0.067	0.901	0.793	0.021

Discussion

The present phylogenetic reconstructions were congruent with those in Korall *et al.* (2007) and clearly supported *C. copelandii*/*C. delgadii* as part of the *C. divergens* group, which is characterized by a sphaeropteroid type indusium and warty spores (Holttum 1963, Korall *et al.* 2007).

   Our results furthermore corroborate the synonymization of *C. copelandii* with *C. delgadii*, as proposed by Tryon (1976), since both taxa are inseparable based on both molecular and morphological data. Molecular variation within *C. delgadii* does show a geographic pattern, however, it does not correspond to the separation of Trindade Island from continental Brazil, but rather separates Trindade Island together with Atlantic forest populations from Cerrado populations. The molecular data may suggest a broader species concept of *C. copelandii* (including Atlantic Forest plants), as an alternative to a broadly circumscribed *C. delgadii*. However, neither molecular clades could be distinguished by the spores or indusia nor by the morphometric analyses of leaf characters. Considering these results together with the earlier conclusions that small differences in indusia is of no taxonomic value (Tryon 1976, Alves 1998), and the fact that one of the included Atlantic Forest specimens displayed the Cerrado genotype (cf. Fig. 2), supports the broad species concept of *C. delgadii*. Consequently, we conclude that there is no endemic *Cyathea* species in Trindade Island.

   The intraspecific genetic structure of *Cyathea delgadii* allows some inference of the possible origin of the Trindade Island populations. Since the coastal Atlantic Forest is geographically closer to Trindade than the Cerrado savanna in the interior of Brazil, a geographic origin from the former could be hypothesized, which is supported by the present phylogenetic analyses. Connections between Southeast Brazil (the area closest to Trindade Island) and the island have recently been observed for the moss species *Campylopus introflexus* (Hedw.) Brid. and *C. occultus* (Mitt.), based on molecular data (Gama *et al.* 2016). Furthermore, although the composition of airborne diaspores collected in Trindade Island (Alves *et al.* 2018) is dominated by *Cyathea* spores that are probably of local origin, other taxa identified in that study support the presence of long-range intercontinental dispersal to the island.

   The monophyly of the Trindade specimens in Fig. 2, although without statistical support, may suggest a single colonization event. Likely, after an initial founding event, the immigrant population would have reduced genetic variation, and variation would accumulate again through mutation and recombination (Lande 1992). The slightly higher molecular divergence on Trindade compared to the Atlantic Forest samples (as inferred from the branch lengths in Fig. 2) may suggest that considerable time has passed since the colonization of Trindade. However, molecular dating analyses as well as fine-scale population genetic analyses based on a larger sampling and possibly next generation sequencing approaches would be necessary to test this hypothesis. Also, DNA sequences generated from *Cyathea* spores in air samples may provide insights into the frequency and diversity of genotypes that may arrive at the island, although the establishment difficulties must overcome the transport difficulties seen in the phenomenon of colonization and competitive exclusion (Waters 2011).
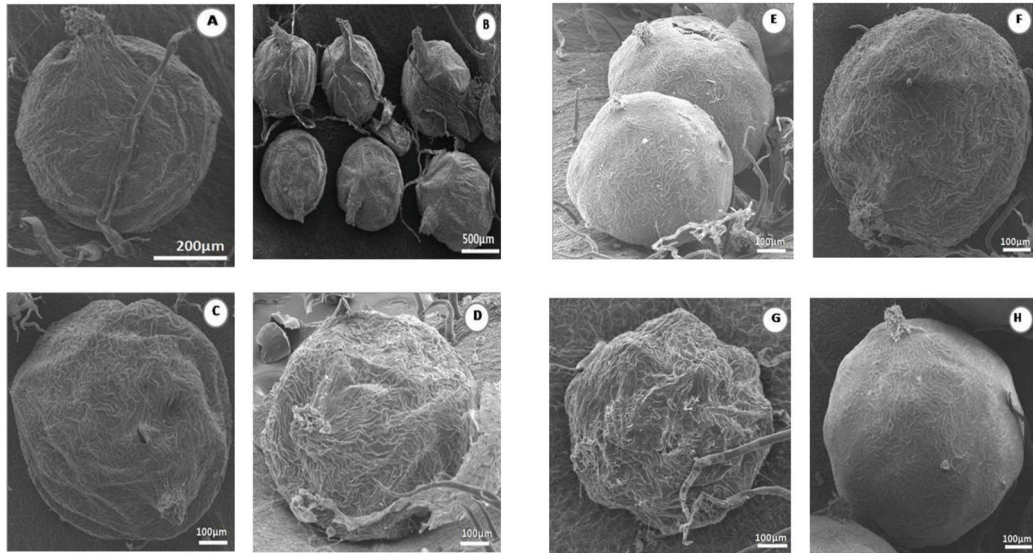
FIGURE 4a. Indusia of *Cyathea delgadii* Sternb. **A** and **B** (Carvalho-Silva, 1788 – Ilha da Trindade); **C** (Pereira, 202 – DF); **D** (Carvalho-Silva, 1146 – GO); **E** (Alkimim-Faria, 1058 – MG); **F** (Alkimim-Faria, 1076a – RS); **G** (Alkimim-Faria, 1000 – ES); **H** (Alkimim-Faria, 1062 – RJ).
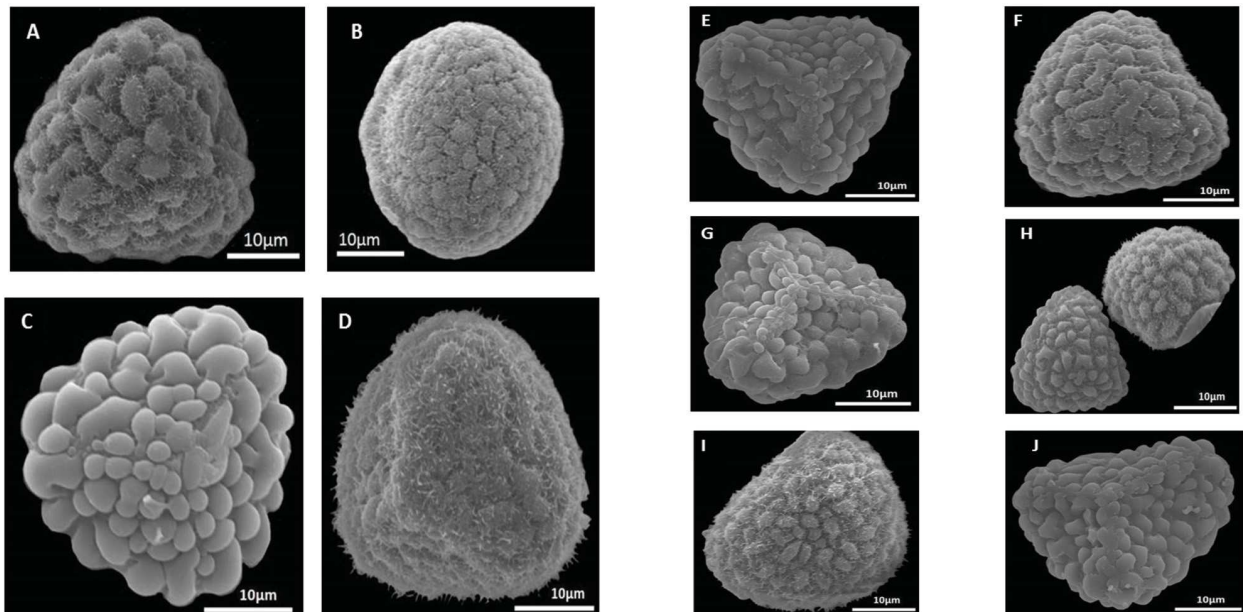
FIGURE 4b. Spores of *Cyathea delgadii* Sternb. **A** and **B** (Carvalho-Silva, 1788 –Trindade); **C** and **D** (Alkimim-Faria, 1062 – RJ); **E** (Gasper & Kassner-Filho, 3106 – SC); **F** (Alkimim-Faria, 1076a – RS); **G** (Alkimim-Faria, 1058 – MG); **H** (Carvalho-Silva, 1146 – GO); **I** (Pereira, 202 – DF); **J** (Alkimim-Faria, 1000 – ES).
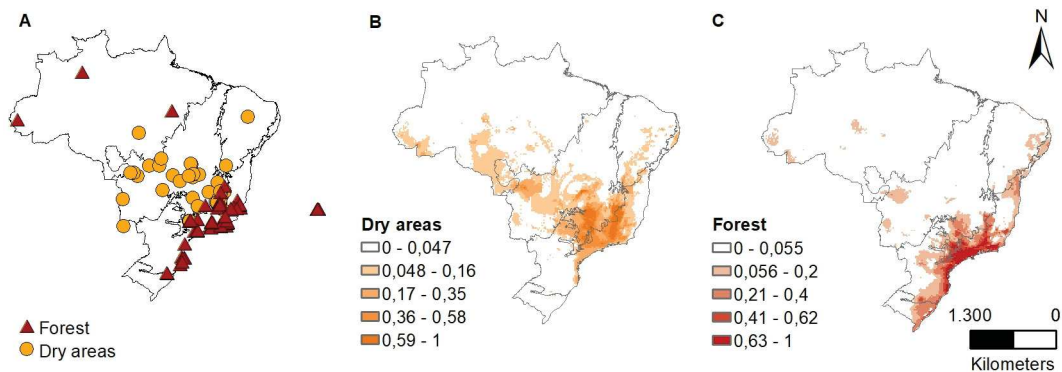
FIGURE 5. **A**. Occurrence points of *Cyathea delgadii* used in the analyses. **B**. General ensemble of suitability models for Cerrado group; **C**. General ensemble of suitability models for Atlantic Forest/Amazon group.

The molecular differentiation into Atlantic Forest and Cerrado domain clades may indicate ongoing (yet cryptic) speciation under different ecological conditions. Cryptic speciation is an important mechanism of evolution in plants (Stuessy *et al.* 2006) and with 65% predominance of anagenesis evidenced in the group of pteridophytes (Patiño *et al.* 2013). The maps of the habitat suitability models for the two groups show greater convergence between the areas of adequacy, than divergences, demonstrating that the two *C. delgadii* groups tend to occupy the same ecological niche. While knowing that niche shares can allow species coexistence (Silvertown 2004), we believe that this convergence corroborates the molecular and morphometric data, showing that both groups are in fact (still) the same species. Factors that limit species distribution, regardless of which group they belong to, involve the intersection of favorable abiotic and biotic conditions and the area they may occupy in a given period (Soberón & Peterson 2005). Thus, when these intersections converge for each group, especially in areas that do not have occurrences of the species, we understand that they are evidence for the acceptance of the circumscription of the same species. Phenotypic distinctions can be understood as the adaptations that these plants exhibit, through variations in the conditions of each habitat**.**

The Atlantic Forest houses all Brazilian endemic species of Cyatheaceae, totaling 17 species (80% of the local genus diversity), but few *Cyathea* species are more widely distributed over different domains (Weigand & Lehnert 2016, Flora do Brasil 2020). Integrative analyses of other widespread species, such as *Cyathea microdonta* (Desv.) Domin or *C. phalerata* Mart. should reveal whether the phylogeographic structuring observed in *C. delgadii*, separating Atlantic Forest populations from those of other domains, is a more general phenomenon.

Conclusions

The phylogenetic analysis did not separate the specimens of *C. delgadii* from the Trindade Island from those from the mainland, and showed the presence of two subclades with small variations between the specimens of the Atlantic Forest and those from the interior of Brazil, mainly the specimens coming from the vegetation of the Cerrado biome. The same did not happen with the studies of the PCA, where all the specimens were superimposed in the same area of the axis in the analysis, being impossible to separate them between island and continental specimens. All the sori analyzed in the study presented indusia and varied only in size, as well as the spores, but did not differentiate specimens from different areas. The congruences in the areas of habitat suitability for the two molecular clades showed that there are no differences between ecological niches.

The use of different tools in this work corroborated the synonymization of *C. copelandii* from the island of Trindade with *C. delgadii* from the continent. This study showed that the small morphological differences, possibly caused by the environment, did not reflect the phylogenetic divergence between the two regions studied.

Acknowledgements

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Appendix 1. Voucher information (voucher number, herbarium, locality) and GenBank accession numbers (*trnG-trnR*, *trnL-trnF* and *rbcL-accD*) of the *Cyathea* specimens newly sequenced for the present study.

Taxon—Voucher (Herbário); Fern DNA DB no.; Collection / locality; GenBank accessions: *trnGR, trnL-F* e *rbcL-accD*.

Cyathea delgadii **Sternb**, Almeida 3381, BHCB, Serra do Caparaó, Minas Gerais, Brazil; MW357711, MW442156, MW448068; *C. delgadii*, Carvalho-Silva 1788, UB, Ilha da Trindade, Brazil; MW357701, MW442144, MW448057; C. delgadii, Carvalho-Silva 1789, UB, Ilha da Trindade, Brazil; MW357713, MW442158, MW448070; *C. delgadii*, Carvalho-Silva 1790, UB, Ilha da Trindade, Brazil; MW256436, MW442142, MW448055; *C. delgadii*, Carvalho-Silva 1792, UB, Ilha da Trindade, Brazil; MW448053, MW442143, MW448056; *C. delgadii*, Carvalho-Silva 1906, UB, Goiás, Brazil; MW357712, MW442157, MW448069; *C. delgadii*, Carvalho-Silva 1146, UB, Goiás, Brazil; MW357702, MW442145, MW448058; *C. delgadii*, Faria 1000, UB, Espírito Santo, Brazil; MW451297, MW442146, MW448059; *C. delgadii*, Faria 1058, UB, Minas Gerais, Brazil; MW357703, MW442147, MW448060; *C. delgadii*, Faria 1062, UB, Parque Nacional da Tijuca, Rio de Janeiro, Brazil; MW448054, MW442148, MW448061; *C. delgadii*, Faria 1076a, UB, Nova Hamburgo, Rio Grande do Sul, Brazil; MW357704, MW442149, MW448062; *C. delgadii*, Gaspar 3106, BHCB, Santa Catarina, Brazil; MW357705, MW442150, MW448063; *C. delgadii*, Pereira 202, UB, Distrito Federal, Brazil; MW357706, MW442151, MW448064; *C. feeana* **(C. Chr.)Domin**, Salino, 15687, BHCB, Serra do Caraça, Minas Gerais, Brazil; MW357709, MW442154; *Cyathea phalerata* **Mart.**, Faria, 882, UB, Parque Nacional Pau Brasil, Bahia, Brazil; MW357707, MW442152, MW448065; *C. phalerata*, Carvalho-Silva, 1969, UB, Serra dos Órgãos, Rio de Janeiro, Brazil; MW357708, MW442153, MW448066; *Cyathea villosa* **Kunth**, Salino, 15695, BHCB, Serra do Caraça, Minas Gerais, Brazil; MW357710, MW442155, MW448067.

Appendix 2. GenBank accession numbers (*trnG-trnR*, *trnL-trnF*, and *rbcL-accD*) of the specimens taken from Korall *et al.* (2007).

Ingroup: *Cyathea alata* Copel.- AM410363, AM410436, NA**.** *Cyathea arborea* (L.) Sm. - Conant 4344(LSC); 2491; AM410396, NA; Puerto Rico. *Cyathea caracasana* (Klotzsch) Domin - Conant 4412 (LSC); 3114; AM410396, NA; Puerto Rico. *Cyathea caracasana* (Klotzsch) Domin - Conant 4412 (LSC); 3114; AM410422, AM410351, AM410470, AM410493; Costa Rica. *Cyathea divergens* Kunze - Conant 4384 (LSC); 2332; AM410386, AM410321, AM410460; Costa Rica. *Cyathea furfuracea* Baker - Conant 4325 (LSC); 3115; AM410423, AM410352, AM410494; Puerto Rico. *Cyathea gibbosa* (Klotzsch) Domin - Conant 4462 (LSC); 2492; AM410397, AM410330, AM410471; Venezuela. *Cyathea gracilis* Griseb. - Conant 4415 (LSC); 3108; AM410416, AM410345, AM410487; Costa Rica. *Cyathea grandifolia* Willd. - Conant 4488 (LSC); 2309; AM410367, AM410302, AM410440; Venezuela. *Cyathea horrida* (L.) Sm. - Conant 4343 (LSC); 2331; AM410385, AM410320, AM410459; Puerto Rico. *Cyathea howeana* Domin - Conant 4665 (LSC); 2317; AM410372, AM410308, AM410446; Lord Howe Island. *Cyathea karsteniana* (Klotzsch) Domin - Conant 4471 (LSC); 3112; AM410420, AM410349, AM410491; Venezuela. *Cyathea multiflora* Sm. - Conant 4425 (LSC); 2333; AM410387, AM410322, AM410461; Costa Rica. *Cyathea mutica* (Christ) Domin - Conant 4385 (LSC); 3111; AM410419, AM410348, AM410490; Costa Rica. *Cyathea parvula* (Jenman) Domin - Conant 4332 (LSC); 2330; AM410384, AM410319, AM410458; Puerto Rico. *Cyathea poeppigii* Domin - Conant 4410 (LSC); 2367; AM410394, AM410328, AM410468; Costa Rica. *Cyathea robertsiana* (F. v. Muell.) Domin - Shirley 12(LSC); 3107; AM410415, AM410344, AM410486; Australia. *Cyathea schiediana* (C. Presl) Domin - Conant 4367 (LSC); 3109; AM410417, AM410346, AM410488; Honduras. *Cyathea senilis* (Klotzsch) Domin - Conant 4479(LSC); 2496; AM410399, AM410332, AM410203; Venezuela. *Cyathea speciosa* H. & B. ex Willd. - Conant 4476(LSC); 2493; AM410398, AM410331, AM410472; Venezuela. *Cyathea stipularis* (Christ) Domin - Conant 4395(LSC); 3110; AM410418, AM410347, AM410219; Costa Rica. *Cyathea valdecrenata* Dominc - Conant 4376 (LSC); 3113; AM410421, AM410350, AM410492; Honduras.

Outgroups

Alsophila cunninghamii (Hook. f.) R. Tryon - Shirley 06 (LSC); 3102; AM410410, AM410339, AM410482; Australia. *Alsophila dregei* (Kunze) R. Tryon - Shirley 13 (LSC); 2325; AM410380, AM410315, AM410454; Africa.