UNIVERSIDADE FEDERAL DE MINAS GERAIS
INSTITUTO DE CIÊNCIAS BIOLÓGICAS
Departamento de Botânica
Programa de Pós-Graduação em Biologia Vegetal

LUCAS VIEIRA LIMA

ESTUDOS SISTEMÁTICOS, FILOGENÉTICOS E BIOGEOGRÁFICOS NA FAMÍLIA GLEICHENIACEAE C. PRESL. (POLYPODIOPSIDA)

BELO HORIZONTE – MG
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“Eu sou da América do Sul
Eu sei, vocês não vão saber
Mas agora sou cowboy
Sou do ouro, eu sou vocês
Sou do mundo, sou Minas Gerais”

Para Lennon e McCartney – Autores: Fernando Brant, Márcio Borges e Lô Borges.
Intérprete: Milton Nascimento

RESUMO

A família Gleicheniaceae possui cerca de 157 espécies com distribuição pantropical. Atualmente, são reconhecidos sete gêneros na família, porém a monofilia destes é questionável, devido a insuficiência amostral dos trabalhos de filogenia molecular que os suportam. Adicionalmente, estes trabalhos têm como enforque as espécies da australásia. Semelhantemente, trabalhos que tratem a biogeografia e a evolução de caracteres a luz da filogenia são inexistentes. Ademais, a família possui uma taxonomia intrincada e mais trabalhos são necessários para preencher essa lacuna. Desta forma, o presente trabalho objetivou testar a monofilia dos gêneros de Gleicheniaceae; produzir uma filogenia molecular para família baseada em sequências de DNA; realizar estudos taxonômicos e nomenclaturais para delimitar de forma mais precisa a circunscrição das espécies, realizando atualizações nomenclaturais e tipificações; determinar os principais padrões históricos e biogeográficos relacionados à sua atual distribuição da família. Como resultado, apresenta-se um novo panorâma filogenético para família a partir de dados moleculares em escala genômica, além de novidades sobre a taxonomia, nomenclatura, anatomia e biogeografia de Gleicheniaceae.

Palavras-chave: biogeografia, filogenia molecular, Gleicheniales, pteridófitas, taxonomia

ABSTRACT

The fern family Gleicheniaceae family has about 157 species with a pantropical distribution. Currently, seven genera are recognized to the family, but their monophyly is questionable, due to insufficient sampling of the molecular phylogeny works that support them. Additionally, these works focus on species from australasia. Similarly, works dealing with biogeography and the evolution of characters in the light of phylogeny are absent. Furthermore, the family has an intricate taxonomy and more work is needed to address this gap. Thus, the present work aimed to test the monophyly of the genera of Gleicheniaceae; to produce a molecular phylogeny for the family based on DNA sequences; carry out taxonomic and nomenclatural studies to delimit the circumscription of the species, to carrying out nomenclatural updates and typifications; to determine the main historical and biogeographic patterns related more precisely to its current family distribution. As a result, a new phylogenetic overview for the family is presented based on molecular data on a genomic scale, as well as novelties on the taxonomy, nomenclature, anatomy and biogeography of Gleicheniaceae.

Keywords: biogeography, Gleicheniales, molecular phylogeny, pteridophytes, taxonomy.

SUMÁRIO

INTRODUÇÃO GERAL

Gleicheniaceae C. Presl é uma distinta família de samambaias leptosporangiadas com cerca de 157 espécies, distribuídas atualmente em seis gêneros (PPG I 2016). São plantas pioneiras que apresentam rizoma longo-reptante e frondes pseudodicotomicamente ramificadas com crescimento indeterminado devido à dormência apical periódica de suas gemas. São heliófitas, habitantes de áreas antropizadas, tais como beira de estradas, predominantemente terrícolas, podendo ocorrer sobre rochas, além de apresentarem baixa demanda por nutrientes minerais (Penrod 2000).

O número de gêneros reconhecidos na família variou ao longo do tempo, sendo considerada inicialmente monogenérica (*Gleichenia*) por Smith (1793). Posteriormente, Diels (1900) manteve todas as espécies em *Gleichenia*, porém separou as espécies em subgêneros e seções, e esta classificação foi seguida por Christensen (1905), porém Christensen (1938) reconheceu cinco gêneros (*Dicranopteris* Bernh., *Sticherus* C.Presl., *Gleichenia, Platyzoma* R. Br. e *Stromatopteris* Mett.), e Copeland (1947) adotou os cinco gêneros e ainda reconheceu *Hicriopteris* C. Presl. Ching (1940) segregou de *Gleichenia* cinco gêneros e dentre eles propõe um novo gênero monotípico, *Gleichenella* Ching. Holttum (1947) inicialmente considerou os gêneros propostos por Christensen (1938), porém, posteriormente (Holttum 1957, 1959) os colocou na posição de subgêneros de *Gleichenia* ou *Dicranopteris*. Nakai (1950) removeu *Platyzoma* de Gleicheniaceae, posicionando-o em Platyzomataceae e sugeriu que essa família possa representar alguma relação entre as Gleicheniaceae e as Polypodiales, posteriormente este gênero foi colocado em Pteridaceae, e incluído no gênero *Pteris* L. (PPG I 2016). Liu et al. (2020) segregou *Rouxopteris* de *Gleicheichenia* com base em caracteres morfológicos e sequencias de *rbcl*.

Estudos filogenéticos com base em marcadores moleculares de várias regiões de DNA plastidial, como *atpA, atpB, rbcL* e *rps4* (Pryer *et al.* 2004, Perrie *et al.* 2007, Schuettpelz & Pryer 2007, Li *et al.* 2010, Liu et al. 2020) apontam para dois clados na família, um que compreende *Diplopterigyum* (Diels) Nakai, *Dicranopteris, Gleichenella e Rouxopteris*. O primeiro gênero, que apresenta rizoma com escamas, é grupo irmão dos outros dois que, por sua vez, apresentam como possível sinapomorfia o rizoma recoberto exclusivamente por tricomas. O outro é composto por *Gleichenia, Sticherus* e *Stromatopteris*, todos com rizomas escamosos (Gonzales & Kessler 2011).

Apesar desses trabalhos, que realizaram a reconstrução filogenética da família a partir de dados de marcadores moleculares, o monofiletismo dos gêneros ainda é questionado em consequência da baixa amostragem utilizada nesses estudos (PPG I 2016). Com isso, apesar dos grandes avanços na aplicação de ferramentas moleculares na taxonomia de diversos grupos de samambaias e licófitas (*e. g.* Gasper *et al.* 2016, Zhang *et al.* 2017, Almeida *et al.* 2017), Gleicheniaceae ainda representa uma grande lacuna, e estudos filogenéticos utilizando estas ferramentas e com uma amostragem significativa devem ser realizados.

Os estudos de evolução de caracteres em samambaias têm sido cada vez mais frequentes nos estudos filogenéticos (*e.g.* Moran & Labiak 2016, Ding *et al.* 2014, Labiak *et al.* 2014, Sundue & Rothfels 2013) e tem permitido criar melhores hipóteses de sinapomorfias morfológicas. As análises de evolução de caracteres em filogenias de Gleicheniaceae são inexistentes.

Gleicheniaceae possui distribuição pantropical, com grupos restritos ao Neotrópico, como *Gleichenella*, e grupos restritos aos Paleotrópicos como *Gleichenia* e *Stromatopteris*, e grupos que ocorrem no Paleo e Neotrópicos como *Dicranopteris, Sticherus* e *Diplopterygium*, sendo que do último, apenas uma espécie ocorre no Neotrópico. Dessa forma, a família se torna um excelente grupo modelo para promover estudos de biogeografia histórica. Além disso, um outro aspecto interessante a ser explorado na biogeografia de grupos na região Neotropical, seria o soerguimento dos Andes como uma barreira geradora de eventos vicariantes na família, anteriormente já sugerido para outros grupos de samambaias (*e.g.* Moran & Labiak 2016). Semelhantemente, entender os padrões de colonização de dispersão de *Diplopterygium* pelo Neotrópico.

ORGANIZAÇÃO DA TESE

Esta tese está organizada em sete capítulos na forma de artigos, conforme descrito a seguir:

Capítulo 1 apresenta um novo panorâma filogenético para Gleicheniaceae, baseado dados moleculares em escala genômica provenientes do plastoma e do genoma nuclear a ser submetido para o periódico Frontiers in Plant Science.

Capítulo 2 aborda as barreiras migratórias de samambaias na região neotropical, utilizando *Diplopterygium bancroftii* como modelo de estudo publicado no periódico Planty Ecology and Diversity.

Capítulo 3 traz o estado da arte sobre o conhecimento citogenético em Gleicheniaceae, bem como uma hipótese de evolução cromossômica para família e novas informações sobre a quantidade de DNA de seis espécies, publicado no periódico Annals of the Brazilian Academy of Sciences.

Capítulo 4 traz uma breve descrição sobre a anatomia da câmara soral, uma estrutura atípica encontrada em algumas espécies do gênero *Gleichenia*, publicado no periódico American Fern Journal.

Capítulo 5 trata do reconhecimento de mais uma espécie para flora brasileira, Dicranopteris spissa, e discorre sobre a sua separação de *Dicranopteris rufinervis*, publicado no periódico Phytotaxa.

Capítulos 6 e 7 tratam da tipificação de dois nomes lineanos, incluindo o tipo deo gênero *Gleichenia*, publicados no periódico Phytotaxa.

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Phylogenomic and evolutionary insights in the fern family Gleicheniaceae

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Abstract

Premise of the study. Gleicheniaceae is a fern family with about 157 species and pantropical distribution. Currently, seven genera are recognized in the family; however, their monophyly is still questionable due to low sampling in phylogenetic studies. The present study aims to test the genera monophyly with an extended sampling, using the first phylogenomic inference using data from both nuclear and plastid genomes.

Methods. Seventy-six samples were sequenced (70 Gleicheniaceae and six outgroups), including all seven currently recognized genera using 57,000 probes designed for 451 loci corresponding to 250 low or single-copy genes. HybPiper and Geneious Prime were used to assemble data, and HybPhaser was used to phase nuclear data to reduce paralogy and hybrid noise at the final recovered topology. IQTREE 2 was used to build Maximum likelihood trees for each loci and also using a concatenated dataset for both nuclear and plastid datasets. A species tree based on a multispecies coalescent model was generated using ASTRAL III. Divergence time analysis were performed using TreePL.

Key results/ Conclusions. We present a phylogenetic inference for Gleicheniaceae, clarifying affinities and circunscriptions of the genera. Additionally, we discuss the congruence between nuclear and plastid datasets, hybridization, and reticulated evolution in the family. Finally, we discuss divergence times and diversification of the main lineages in the family.

Introduction

Gleicheniaceae C. Presl is a distinct leptosporangiate fern family that includes about 157 species, currently distributed in seven genera (Gonzales & Kessler 2011, PPG I 2016, Liu et al. 2016). They are plants with long-creeping rhizomes and pseudodichotomously branched fronds with indeterminate growth due to the periodic apical dormancy of their buds (Holtum 1957, Tryon & Stolze 1989, Østergaard Andersen & Øllgaard 2001, Gonzales & Kessler 2011, Lima & Salino 2018). They are heliophytes, often inhabiting anthropized areas such as roadsides, predominantly terrestrial, occasionally occurring on rocks, and having low demand for mineral nutrients (Penrod 2000, Sharpe & Mehltreter 2010).

The number of recognized genera in the family changed over time (Table 1). Initially, it was considered monogeneric (*Gleichenia Sm.*) by Smith (1793). Later, Diels (1900) maintained all species in *Gleichenia*, separating the species into subgenera and sections. This infrageneric classification was initially followed by Christensen (1905), who later (Christensen 1938) recognized five genera (*Dicranopteris* Bernh., *Sticherus* C.Presl., *Gleichenia*, *Platyzoma* R.Br., and *Stromatopteris* Mett.). Copeland (1947) adopted the five genera of Christensen's classification (Christensen 1938) and further recognized an additional genus, *Hicriopteris* C.Presl. Ching (1940) segregated five genera from *Gleichenia*, proposing a new monotypic genus, *Gleichenella* Ching. Holttum (1947) initially considered the genera proposed by Christensen (1938), but later (Holttum 1957, 1959) placed them in the position of subgenera of *Gleichenia* or *Dicranopteris*. Nakai (1950) removed *Platyzoma* from Gleicheniaceae, positioning it in Platyzomataceae, and suggested that this family may represent some relationship between Gleicheniaceae and Polypodiales. This genus was later placed in Pteridaceae and subsumed in the genus *Pteris* L. (PPG I 2016).

Phylogenetic inferences based on chloroplast DNA sequences, such as *atpA*, *atpB*, *rbcL*, and *rps4* (Pryer et al. 2004, Perrie et al. 2007, Schuettpelz & Pryer 2007, Li et al. 2010), suggested that the family is composed of two clades. One clade comprises *Diplopterigyum* (Diels) Nakai, with scaly rhizomes, as sister to *Dicranopteris*, and *Gleichenella*, which possess the rhizome covered exclusively by trichomes, a possible synapomorphy. The other clade is composed by *Gleichenia*, *Sticherus*, and *Stromatopteris*, all with scaly rhizomes (Gonzales & Kessler 2011). Liu et al. (2020) segregated *Gleichenia boryi* from the other *Gleichenia* species, placing it in *Rouxopteris* due to its morphology and phylogenetic placement.

Despite these studies based on phylogenetic reconstructions from a few plastid loci, and the major advances in the application of molecular tools in the taxonomy of several fern and lycophyte groups (*e.g*., Gasper et al. 2016, Zhang et al. 2017, Almeida et al. 2017, Testo et al. 2018, Lehtonen et. al. 2020, Chen et al. 2021), the monophyly of Gleicheniaceae genera is still questioned given the low sampling (PPG I 2016). Phylogenetic studies associated with divergence time analysis provide subsidies to better understand the evolutionary history of a given group, once it is possible to contextualize the rise of its lineages (Testo et al. 2018, Testo & Sundue 2016). Gleicheniaceae has been included in large-scale dated phylogenies (eg, Pryer et al. 2004, Testo & Sundue 2016), but those studies did not include all genera. Recently, all Gleicheniaceae genera were included in a divergence time analysis based on a single plastid locus (Liu et al. 2020). Therefore, no multi-loci dataset including all Gleicheniaceae genera has been utilized to estimate the divergence times of the family extant's lineages.

In this context, we aim to answer the following questions: What are the phylogenetic relationships among Gleicheniaceae lineages? Are Gleicheniaceae genera, as currently circumscribed, monophyletic? Does data recovered from plastid and nuclear genomes congruent in recounting the family's evolutionary history? When did the main lineages of Gleicheniaceae arise? Do morphological characters traditionally used in the taxonomy of Gleicheniaceae are phylogenetically informative?

Materials and Methods

Sampling, sequencing, and read quality control

Seventy-six samples were sequenced (70 Gleicheniaceae and six outgroups) in the present study from herbarium specimens and silica-gel dried tissue (Table 2). Samples of all seven Gleicheniaceae genera were included (*Dicranopteris*, *Gleichenella*, *Gleichenia*, *Diplopterygium*, *Sticherus*, *Stromatopteris*, and *Rouxopteris*) *sensu* PPG I (2016) and Liu et al. (2020). For silica-dried tissue, we extracted the DNA using the DNeasy Mini Plant Kit (Qiagen) and for herbarium samples, we extracted the DNA using CTAB (cetyltrimethylammonium bromide) protocol following Doyle & Doyle (1990). Samples were sequenced at Rapid Genomics (Gainsveille, USA) for target enrichment sequencing using the GoFlag 451 probes, a set of 56,989 probes covering 451 exons from 248 single or low-copy nuclear genes (Breinholt et al. 2021). Samples were sequenced using the Illumina HiSeq 2500 platform generating 150bp paired-end reads. The raw data quality was verified using FastQC (Andrews 2010, version 0.11.9), and filtering and trimming low-quality pair-end reads were made using Trimmomatic (Bolger et al. 2014, version 0.36) (illuminaclip 2:30:10, leading 10, trailing 40). The trimmed raw data was assembled in two separate datasets, nuclear and chloroplast data. The sequences reads were deposited in the NCBI Sequence Read Archive and the recovered gene sequences, and alignments are available at GitHub.

Chloroplast dataset

To build a partial plastome dataset, Geneious Prime 2021 (version 2021.1.1) (https://www.geneious.com) was used to assemble by reference the trimmed raw data. The annotated plastome of *Diplopterygium glaucum* deposited at GenBank (NC_024158) (Kim et al. 2014) was used as reference. All assembled sequences were strictly aligned to the reference sequence using MAFFT (Katoh et al. 2009, version 7.48). We extracted 40 coding regions to build a partitioned dataset, then we use TrimAL (Capella-Gutierrez et al. 2009, version 1.2) in each partition to eliminate columns with more than 60% of gaps. The result was a matrix with 73 terminals and about 15,000 bp.

Nuclear dataset

To build a nuclear dataset the HybPiper pipeline (Johnson et al. 2016) was used to assemble the sequences using the GoFlag probe set for ferns. Using the HybPiper supercontig output (exons+introns) Hybphaser (Nauheimer et al. 2020, version 2.0) was used to access the quality of the sequences, excluding putative paralogs, access heterozygosity and haplotypic divergence, and to phase haplotypes, aiming to reduce putative hybrid noise at the recovered topology.

Phylogenetic inferences

For both the nuclear and chloroplast datasets, ModelTest-NG (Darriba et al. 2020) was used to select the best-fit model of evolution for each partition (recovered loci) based on the Bayesian information criterion (BIC) (Supplementary Material 1). IQ-TREE2 was used to estimate the species tree from a concatenated partitioned matrix, defining each locus as a partition, with 1,000 ultrafast bootstrap replicates (Minh et al. 2020). IQ-TREE2 was also used to generate gene trees for the nuclear loci, estimate gene concordance factors (gCF), and site concordance factors (sCF). ASTRAL III (Zhang et al. 2018) was used in the the previously generated gene trees generated to infer the species tree based on a multi-species coalescent method. The ASTRAL tree we scored in order to obtain an estimative of incomplete lineage sorting (ILS). The trees were rooted using *Dipteris conjugata* as an outgroup. We ran all analyses in Sagarana HPC cluster housed at Universide Federal de Minas Gerais.

Divergence time analysis

To estimate the divergence times of the main extant lineages in Gleicheniaceae, TreePL (Smith & O'Meara 2012) was used. The TreePL run guide described by Maurin (2020) was followed, to optimize and conduct the analysis. The smoothing value of 10^{-6} was chosen after comparing tree runs with smoothing as 10^{-3}, 10^{-6}, and 10^{-9}. Two fossil calibrations were used: *Gleichenia chaloneri*, the most ancient *Gleichenia* fossil record (Herendeen & Skog 1998), setting *Gleichenia* crown within 99–112 mya and *Chansitheca wudaensis*, the most ancient Gleicheniaceae-like fossil (He et al. 2020, He et al. 2016), constraining the Gleicheniales crown within about 298 mya. Despite the morphological affinities, we chose to adopt a conservative path and used this fossil to calibrate the Gleicheniales crown. Further discussion regarding Gleicheniaceae fossils available at Supplementary

Material 2.

Results

For the nuclear dataset, we recovered on average 419 loci out of 451 (standard deviation = 40, median 434) (Supplementary Material 3), with 93% average loci coverage. We removed putative paralogs, samples with coverage below 50% in each locus, and loci with less than 50% of sequences (Supplementary Material 3). Our final partitioned matrix had 294 loci and approximately 150 thousand bp. Following Nauheimer et al. (2020), we found high rates of heterozygosity (LH) and haplotype divergence (HD), with 18 ingroups showing loci heterozygosity above 80% with >0% SNPs and allele divergence above 1%, including 10 *Sticherus* (55%), seven *Dicranopteris* (38%), and one *Gleichenia* (5%) (Table 3). The highest rates of haplotype divergence and heterozygosity were found in *Gleichenia peltophora* (87.88% with >0% SNPs and 3,18% of allele divergence) and the lowest in *Gleichenella pectinata* (12.07% and 0.03%) (Table 2) (Figure 2).

We used two phylogenetic approaches to recover the evolutionary relationships within Gleicheniaceae. Using maximum likelihood, we built two species trees with a partitioned matrix, one with the plastid data (PST) and the other with nuclear data (NST). We also generated a coalescence-based species tree with the nuclear dataset (MSCT).

The recovered topology of the NST showed two main clades (Figure 1). These two clades were recovered in the PST and at the MSCT as well. The first clade (here named Diplopteroid clade) is formed by *Diplopterygium* as sister to a clade formed by *Gleichenella*+*Dicranopteris*. Within *Dicranopteris,* two clades are recovered, one with *Dicranopteris tetraphylla*+*D. linearis*+ *D. taiwanensis*+ *D. dichotoma* and other formed by *D. speciosa*+*D. subpectinata*+ plus the neotropical species (*D. nervosa*, *D. rufinervis, D. seminuda, D. spissa,* and *D. flexuosa*) (Figure 1). This topology is recovered in all phylogenetic inferences; however, there is discordance regarding the relationships within the neotropical *Dicranopteris* in all recovered topologies.

In the *Diplopterygium* lineage, in all topologies, *D. bancrofitii,* the only neotropical species of the genus, is sister to the remaining species. In the NST a clade formed by *D. norrisii*+*Diplopterygium* sp.2 as sister group of *D. brevipinnulum*+*D. sordidum* is sister group of a clade formed by *D. chinensis*+*D. volubilis*+*Diplopterygium* sp.1+*D. longissimum*+*D. conversum*+*D glaucum*. This topology showed high concordance with the MSCT, except for *D. sordidum* which comes out as sister group of *D. brevipinnulum* (Figure 1). However, there is discordance between the recovered topologies of the nuclear dataset (NST and MSCT) and the plastid dataset regarding the affinities among the Asian species of *Diplopterygium*. In the PST *D. norrisii* comes out as sister group of two clades, one formed by *D. volubilis*+*Diplopterygium* sp.1 and other formed by *D. sordidum*+*D. brevipinnulum* as sister group of *D. chinensis*+*Diplopterygium* sp.1+*D. conversum*+*D. glaucum*+*D. longissimum*.

The second clade (named here Sticheroid clade) has in all recovered topologies *Rouxopteris* as sister to all other lineages. However, at the NST, *Rouxopteris* appears as sister to a clade formed by *Sticherus milnei+S. truncatus*, which is in turn sister of *Gleichenia*+*Stromatopteris* plus the remain species of *Sticherus* (Figure 1).

In the MSCT *Rouxopteris* comes out as sister group of a clade formed by *Stromatopteris* as sister group of a clade formed by *Sticherus milnei+S. truncatus* as sister group of a *Gleichenia* plus the remaining species of *Sticherus*. In the PST, *Rouxopteris* comes out as a sister group of a clade formed by *Gleichenia*+*Stromatopteris* as sister group of *Sticherus milnei*+*S. truncatus* as sister group of the remaining species of *Sticherus.* The phylogenetic placement in the MSCT of *Stromatopteris* is incongruent with the PST and NST topologies. Within *Gleichenia*, *G. polypodioides* appears as sister to *G. dicarpa*+*G. peltophora* in the PST. Only plastid sequences were recovered to *G. dicarpa*, therefore in the MSCT and the NST *G. peltophora* comes out as sister to *G. polypodioides*.

Inside clade formed by the remaining *Sticherus* species (*Sticherus l.s.*), the relationships are not well resolved and incongruent among the recovered topologies, except for early-diverging lineages and a few individual clades. At the NST, *Sticherus brackenrigdei* comes out as sister to the other species, followed by *S. montaguei* as sister to two clades, one formed by five species with distribution through Southern and Southeastern Asia and Oceania (*Sticherus flabellatus*, *S. bolanicus*, *S. loheri, S. hirtus* and *S. vestitus*), including the neotropical *Sticherus nudus* as sister to *S. bolanicus*. The other clade is formed by *Sticherus simplex* as sister to the remaining species, followed by *Sticherus flagellaris*, an African species, as sister to a clade formed by *Sticherus nervatus*, *S. pruinosus, S. lechleri*, and *S. revolutus*, which is in turn sister to the remaining species. Although the relationships among them are not well resolved, a few clades are supported, *e.g.* the one formed by *S. squamosus*, *S. lanuginosus,* and *S. paulistanus*, and another formed by *S. maritmus, S. blepharolepis*, *S. ferruginosus, S. decurrens, S. bifidus,* and *S. fulvus* (Figure 1). At the MSCT, the clade formed by *Sticherus nudus*, *S. bolanicus*, *S. flabelatus*, *S. loheri*, *S. hirtus* is recovered. However, *S. nervatus, S. pruinosus*, *S. revolutus,* and *S. lechleri* do not form a clade (Supplementary material 4). Additionally, the results of the ASTRAL III-scored tree points out a rate of 84% of quartet trees induced by the gene trees are in the species tree of our nuclear dataset.

The time-calibrated tree recovered an estimated origin of the Gleicheniaceae crown between 121 to 125 million years ago (mya) (CI= [121.77-125.03], median= 123.4), the crown of the Diplopteroid lineage originating between 105 to 115 mya (CI= [105.25-115.48], median= 110.36), and the Sticheroid clade with its crown dated between 119 and 122 mya (CI= [119.32-122.63], median= 120.97) (Figure 3). The split of *Gleichenella* from *Dicranopteris* was estimated to have occurred between 49 to 62 mya (CI= [49.87-62.61], median= 56.24). Within the *Diplopterygium* lineage, the split of *D. bancroftii*, the only neotropical species of the genera, from the other species was estimated to have occured between 47 to 68 mya (CI= [47.29-68.03], median=57.66). Within the Sticheroid clade, *Rouxopteris* splits from the other lineages around 119 and 122 mya (CI= [119.32-122.63], median= 120.97), while *Sticherus milnei* diverged from *Stromatopteris* + *Gleichenia* and *Sticherus strictu sensu* in about 114 to 117 mya (CI= [114.39-117], median= 115.85). The separation between *Stromatopteris* and *Gleichenia* ranges between 107 and 109 mya (CI= [107.38-109.46], median= 108.42). and remaining species of *Sticherus* splits from *Stromatopteris*+*Gleichenia* clade from 111 to 113 mya (CI= [111.08-113.58], median= 112.33). (Figure 3). Although the clade formed by the remaining species of *Sticherus* is an old lineage, its species have the most recent divergence, with an estimated range between 3 to 0.5 mya. However, *Diplopterygium* showed one of the most ancient species diversification dates, with an estimated split of *D. bancroftii* from the other species between 47 to 68 mya. Similarly, the diversification of *Dicranopteris* species is estimated to range from 14 to 3 mya.

Discussion

Topology

We presented the first family-level phylogenomic inference of Gleicheniaceae, recovering two main clades and supporting all the existing genera, with major circumscription adjustments needed in *Sticherus*. Our results highlight the phylogenetic and morphological uniqueness of the clade formed by *Sticheurs milnei+S. truncatus*.

In the Diplopteroid clade, all recovered topologies are congruent regarding generic relationships and circumscription. However, at the Sticheroid clade, recovered relationships were not consensual among analyses and datasets. The phylogenetic placement of the clade *S. milnei+S. truncatus* differs between the recovered topologies from the nuclear dataset (NST and MSCT) and the plastidial dataset (PST). In the NST and MSCT, *Sticherus milnei+S.truncatus* comes out as sister to clade formed by *Stromatopteris+Gleichenia*, which is in turn sister to *Sticherus l.s*. In the PST trees, *Sticherus milnei+S.truncatus* comes out as sister to *Sticherus l.s.* Additionally, we noticed discordance at the species level in the recovered topologies, such as in the neotropical species of *Dicranopteris* (*D. flexuosa*, *D. nervosa*, *D. spissa*, *D. rufinervis,* and *D. seminuda*), *Sticherus s.s.* clade and the *Diplopterygium*.

Our recovered topologies agree with Li et al. (2010) regarding the relationships of *Dicranopteris+Gleichenella* as sister to *Diplopterygium*, and *Stromatopteris+Gleichenia* as sister to *Sticherus l.s.* However, those authors did not include *Gleichenia boryi* (=*Rouxopteris boryi*). Liu et al. (2020) proposed the segregation of *Gleichenia boryi* in *Rouxopteris* due to its morphological uniqueness and placement in a phylogenetic hypothesis based on *rbcl* sequences. In their recovered topology, *Rouxopteris* comes out, with low support, as sister to a clade formed by *Dicranopteris+Gleichenella+Diplopterygium*. In our results, *Rouxopteris* is recovered with high support, in all topologies, as sister to a clade formed by *Stromatopteris+Gleichenella+Sticherus milnei+Sticherus l.s.*

Although our results support current circumscription of most of Gleicheniaceae genera, the relationships within the genera are not well resolved, especially in *Sticherus l.s.* The lack of resolution and recovered incongruences among the NST, PST, and MSC trees may be related, in different levels, to low sampling, incomplete lineage sorting, and introgression following hybridization (Rieseberg & Soltis 1991, Dorado et al. 1992, Degnan et al. 2005, Drábková et al. 2010, Xu et al. 2012, Sigel 2016, Bruun-Lund et al. 2017). Incomplete lineage sorting may also have played a role in incongruence between gene trees and species trees, especially in recently diverged species (Knowles & Carstens 2007), such as those in *Sticherus l.s.*, most of them with about 4-3 mya. The ASTRAL results with about only 84% of the quartet trees induced by the gene trees found in the species tree, indicate a significant presence of ILS in our dataset.

The high rates of heterozygosity (LH) and haplotypic divergences (HD) found in many sampled species (Table 2) may be related to hybridization, introgression, and polyploidy events (Nauheimer et al. 2020). Which suggest that such events may have been important evolutionary drivers in Gleicheniaceae and might be the source of the phylogenetic incongruence found between plastid and nuclear genomes.

Although no described hybrid was sampled in our dataset, there is evidence that some *Sticherus* species may have originated in hybridization events (Jeremy & Walker 1985). Hybrids in Gleicheniaceae are the subject of much discussion in the family, both in studies based on morphology only (Gonzales & Kessler 2001) and in studies that used cytogenetic data (Jermy & Walker 1985). Little progress has been made to test these hypotheses of reticulated evolution in the family, especially in *Sticherus,* where most of the hybrids in the family have been reported (Gonzales & Kessler 2011, Jermy & Walker 1985). Nevertheless, our results suggest the hybrid origin for some species in this lineage. Similarly, *Sticherus nigropaleaceus* showed high rates of LH and HD. This species has long-recognized morphological affinities to the *Sticherus bifidus* complex (Prado & Lellinger 1996, Gonzales & Kessler 2011, Lima & Salino 2018). Although no chromosome count has been made so far, estimates showed its DNA c-value near two to three times that of other *Sticherus* investigated (Lima et al. 2021). Regarding the *Sticherus revolutus* complex (*S. nervatus*, *S. revolutus*, and *S. pruinosus*), all species showed high rates of LH and HD (Table 2).

Most of the sampled *Dicranopteris* species showed high rates of LH and HD (above 80% and 1,0 % respectively) (Table 2), and these rates may be related to a series of autopolyploidy or allopolyploidy events (Lima et al. 20210. Currently, a broader concept of *D. linearis* has been adopted due to the lack of morphological and molecular studies (Perrie & Brownsey 2015, Chinnock & Bell 1998, Chen et al. 2017). The chromosome numbers of *D. linearis* (n=39, n=40, 78, n=80) (Lima et al. 2021) vary as much as their morphology and geographical distribution and can be used as a tool to better understand the reticulation within the complex and clarify species limits and their relationships. Therefore, this species complex may be a good study system to investigate what are the causes of high rates of LH and HD in *Dicranopteris*. Additionally, there is a significant discussion regarding the morphological distinctiveness between *D. linearis* (with Asian and African distribution) and *D. flexuosa* (neotropical distribution) (Lima & Salino 2018). So far, all the investigated specimens of *D. flexuosa* showed the base haploid number 78, and 2C = 9.16 pg (Lima et al 2021) thus they may represent polyploid populations. On the other hand, *D. linearis*, despite showing some polyploidy cases, the majority chromosome counts showed n=39 as haploid chromosome number and showed 2C = 6.41 pg (Clark et al 2016). Those species had LH above 89% >0% of SNPs and AD above 1.81%, which indicates that their origins may be related to polyploidy events. We should also consider that these LH and HD rates may be related to fixed heterozygosity in species with allopolyploid origins. This occurs when allopolyploid species retain sets of divergent gene copies inherited from each parental and may present phenotypes as products of additivity or synergy of the parental genomes in their respective ratios (Buggs et al. 2014, Soltis & Soltis 2000, Sigel 2016).

On the other hand, *Gleichenella*, a monospecific genus, widespread in the Neotropics, presented the lowest allele divergence and heterozygosity rates from all sampled species (Table 2). That may be related to growth habit since this species usually occupies large areas in roadsides and steep slopes. The growth of its rhizome allows a great expansion in such a way that all *Gleichenella* patches may be formed by a single individual by clonal expansion of its rhizome. The reproductive isolation of *Gleichenella* may be related to effective pre or postzygotic barriers, preventing hybrid formations, which in turn can be connected to its divergence time, estimated to be ca. 49-62 mya. Additionally, chromosome counts point to the conservation of ploidy in the genus, with only putative dysploidy events recoded (Lima et al. 2021). Similarly, *Rouxopteris* and *Stromatopteris,* two other monospecific genera with restricted distribution, showed low rates of LH and HD. These may represent ancient relict survived lineages within the family with well-developed barriers to prevent hybrids formations.

Gleichenia had only one species, *G. peltophora*, with high rates of LH and HD, while *Diplopterygium* showed all sampled species with low rates of LH and HD. In those two cases, polyploidy and reticulation may be present, but not be a significant process in diversification as observed in *Sticherus* and *Dicranopteris* (Table 2) (Jeremy & Walker 1985, Lima et al. 2021).

It is well established that whole-genome duplication (WDG) events played a major role in ferns diversification (1KP 2019, Huang et al. 2020), and it is well established the relation of such events and reticulation with LH and HD rates (Sigel 2016). Gleicheniaceae seems to have experienced a WGD before its radiation during the Mesozoic (Hueng et al. 2019). Additionally, our results support the hypothesis that polyploidy and reticulation played a major role in the diversification of *Sticherus* and *Dicranopteris*. We also observed a gradient of LH and AD across the sampled species (Table 2), which may indicate the presence of unknown hybrids and putative introgression events (Nauheimer 2020). Although to the date, no intergeneric hybrids were recorded for the family, evidence point to several putative hybrids and allopolyploids within the genera that need further investigation.

Until recently, plastid data has been the main source of molecular information for plant phylogenetic reconstruction and classification, especially for ferns and lycophytes (*e.g.*, Schuettpelz & Pryer 2007, Pryer et al. 2004, Testo & Sundue 2016). Several premises supported its use, such as uniparental heritage, the assumed behavior of a single gene copy, a general lack of recombination, and the accessibility to universal primers (Palmer et al. 1988, Clegg & Zurawski 1992). However, recent evidence has shown that it is possible, and not so rare, the biparental inheritance of the plastid (Barnard-Kubow et al., 2017), and by structural and functional features, the plastome may not evolve or behave as a single locus (Gonçalves et al. 2019). Therefore, this may be related to the incongruence found between the nuclear and plastidial trees recovered in our results.

The present study, to the date, is the most comprehensive study dealing with Gleicheniaceae phylogeny, however, more species samples should be included for further clarifications regarding infrageneric species relationships. Further hybrid investigations should be performed, once the phylogenetic placement of some accessions and their high rates of LH and HD, are strong evidence of a possible hybrid cases. Therefore, studies with intraspecific sampling aiming to elucidate the reticulation processes in Gleicheniaceae should also be performed.

Genera monophyly and morphology

The taxonomy of Gleicheniaceae was traditionally based on the rhizome and types of bud indument (Holtum 1957, Gonzales & Kessler 2011, Tryon & Stolze 1989, Østergaard Andersen & Øllgaard 2001). The first phylogenetic works demonstrated that these characters do not reflect the evolutionary affinities of the genera, *e.g.*, *Diplopterygium* with scaly rhizome and buds as sister to *Dicranopteris*+*Gleichenella* both with hairy rhizome and buds (Liu et al 2020, Li et al. 2010).

The hairs found in some Gleicheniaceae, such as *Dicranopteris* and *Gleichenella,* can be evolutionarily interpreted as originating from reduced scales, based on their morphology and the fossil record (Liu et al. 2020). Alternatively, they may have emerged several times independently in the family. Additionally, other Gleicheniaceae species may present both hairs and scales, such as

Rouxopteris boryi and *Gleichenia microphylla*, both of which have scales with ciliated margins, and stiff stellate hairs on the rhizome (Liu et al. 2020, Holttum 1959). Several Asian *Diplopteryium* may also have rhizomes and buds covered by scales and bear stellate hairs on the abaxial surface of the rachis (Jin et al. 2013). Similarly, a few *Sticherus, e.g. S. pruinosus*, may have hairs on the abaxial surface of the segments' secondary veins (Lima & Salino 2018, Gonzales 2003). However, Gonzales (2003) did not recognize these structures of *S. pruinosus* as hairs, but as reduced scales.

The indument allied to the morphology of spores and frond ramification pattern seems to be very useful to circumscribe individually the genera (*e.g.*, *Gleichenella* has anisotomicaly branched fronds and monolete spores, while *Dicranopteris* has isotomicaly branched fronds and trilete spores).

The ramification patterns of fronds seem to be informative regarding the evolutionary history of Gleicheniaceae (Figure 4). *Diplopterygium* has bipinnate ultimate branches with linear segments; *Dicranopteris* has opposite branch pairs to the main ramifications; *Gleichenella* lacks the opposite branch pairs and it has anisotomic branches; *Gleichenia* has isotomic ramification, and bipinnate ultimate branches with rounded segments; *Rouxopteris* fronds have the main axis with or without a pseudodichotomy at its apex, and the lateral branches slightly alternate; *Stromatopteris* has fronds without pseudodichotomy and rounded segments; and *Sticherus l.s.* has isotomic ramifications with strong acute angulation (Figure 4). *Sticherus milnei* and *Sticherus truncatus*, recovered as an isolated and highly divergent lineage, has an almost orthogonal branch of each ramification, and the main axis, from which the rise the ramifications. This branching pattern clearly differentiates it from *Sticherus l.s.* In our results, the phylogenetic placement of *S. milnei* and *S. truncatus* is different in the MSCT and NST (sister to a clade formed by *Gleichenia*+*Stromatopteris*+*Sticherus s.s.*), from the PST and MSC trees (sister to *Sticherus l.s.*). Additionally, the clade of *S. milnei* and *S. truncatus* is phylogenetic distant from *Sticherus l.s.*, regarding divergence time and branch length. Only two species do not have fronds with pseudodichotomy, *Stromatopteris moniliformis*, endemic from New Caledonia, and *Sticherus simplex*, endemic from the Andes. This condition seems to be a case of morphological convergence since those species are not closely related. Alternatively, the simple fronds of *S. simplex* may represent a case of neoteny, once at the early stages of many *Sticherus* species the sporophytes blades are simple.

Our results suggest that *Dicranopteris, Diplopterygium*, *Gleichenia*, *Gleichenella*, *Stromatopteris,* and *Rouxopteris* are monophyletic, while, in its current circumscriptions, *Sticherus* is polyphyletic. Therefore, the morphological characters need to be reinterpreted and the genera circumscription should be revised, as well as more species should be sampled into large-scale phylogeny.

Divergence time analysis

Gleicheniaceae is an ancient lineage of leptosporangiate ferns with a large fossil record spanning the Mesozoic (Gandolfo et al. 1997). However, a recently described fossil from the Permian assignable to the family (*Chansitheca wudaensis*, 298 mya) (He et al, 2020) indicates an earlier rise of Gleicheniales. Our time divergence analysis corroborates previous estimates (Pryer et al. 2004, Schuettpelz & Pryer 2009, Testo & Sundue 2014, Liu et al. 2020), and support the hypothesis of establishment and diversification of most Gleicheniaceae lineages during the Mesozoic. It also agrees with the angiosperm-driven diversification hypothesis, as shown for other fern lineages (Schneider et al. 2004, Du et al. 2021).

Despite the early divergence and diversification during the Mesozoic, several genera such as *Dicranopteris*, *Diplopterygium*, and *Sticherus* have undergone recent diversification during the Miocene (Figure 3). This recent diversification may be related to polyploidy and introgression events associated with niche exploration through the mountain ranges, and response to climate changes, especially during the Pleistocene. At the middle of the Miocene, the Andean Mountain ranges were in advanced upraising (Hartley 2003), and the Panama Isthmus was formed by the end of the Miocene and the beginning of the Pliocene (O'Dea et al. 2016), which match with the period of diversification of *Sticherus* in the Neotropics. The genus, the most diverse in the family (about 90 species out of 157), has four diversity centers in the Neotropics: Central America, Andes Cordillera, the mountains of Southern and Southeastern Brazil, and the Venezuelan plateau. Speciation may have been driven in montane regions, associated with climate adaptation and niche diversification, which matches the pattern found by Suissa et al. (2021) of general fern diversification.

The early divergence of the Gleicheniaceae crown is hypothesized to have occurred connected to continental movements during the breakup of Laurasia and Gondwana during the late Mesozoic and early Cenozoic (Liu et al. 2020) (Figure 3).

Out of the three endemic genera in Gleicheniaceae, both *Rouxopteris* (Madagascar and the Mascarenes - Liu et al. 2020) and *Stromatopteris* (New Caledonia - Kramer et al. 1990) are ancient lineages and seem to be relicts from the early diversification of Gleicheniaceae genera lineage (Liu et al 2020) (Figure 3), while the neotropical *Gleichenella* (Ching 1940, Mickel & Smith 2004) diverged more recently from *Dicranopteris.*

Long-distance dispersal (LDD) is well documented in Diplopterygium, *Sticherus,* and Dicranopteris . *Diplopterygium* has an Amphi-Pacific distribution, with a single species occurring in the Neotropics and the remaining species occurring in Southern and Southeastern Asia and Oceania (Lima et al. 2021, Mickel & Smith 2004). *Sticherus* and *Dicranopteris* have a pantropical distribution (Mickel & Smith 2004, Gonzales & Kessler 2011, Lima & Salino 2018), with possible cases of LDD, such as *S. flagellaris*, an African species, comes out among other Neotropical species. (Figure 3) Therefore, these distribution patterns and species divergence time estimates suggest a strong influence of long-distance dispersal in those genera.

Conclusions

We presented the first genomic-scaled phylogenetic inference on Gleicheniaceae, confirming the monophyly of *Dicranopteris, Diplopterygium*, *Gleichenella*, *Gleichenia*, *Rouxopteris,* and *Stromatopteris*, while recovering a polyphyfiletic *Sticherus*. We clarified the relationships of *Rouxopteris* and corroborated the overall phylogenetic relationships recovered in previous works. Despite Gleicheniaceae being an ancient lineage of leptosporangiate ferns with most of its genera diverging during the Mesozoic, several genera showed recent diversification. Our results suggest that reticulation and polyploidy may play a significant role during this process. However, some genera, such as *Rouxopteris* and *Stromatopteris*, can be evolutionary relicts. Future studies with expanded sampling should focus on better understanding the relationships at the species level. Additionally, integrative approaches should be applied to elucidate the genera with reticulated evolutionary history and solve species complexes.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

All authors made a substantial contribution in the concept and design of the study, to data collection, analysis and interpretation, writing and revising the manuscript and adding intellectual content.

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Figure 1. ML species tree generated from a partionated nuclear matrix, with ultrafast bootstrap branch supports, gene concordance factor (gCF) and site concordance factor (sCF) respectively. * Indicates 100 of Ultrafast Bootstrap.

Figure 2. Graphic showing the relationship between heterozygosity rates and allele divergence rates.

Figure 3. Divergence time tree.

Figure 4. Ramifications patterns in Gleicheniaceae.

Table 1. Gleicheniaceae genera circumscription history.

Table 2. Samples vouchers.

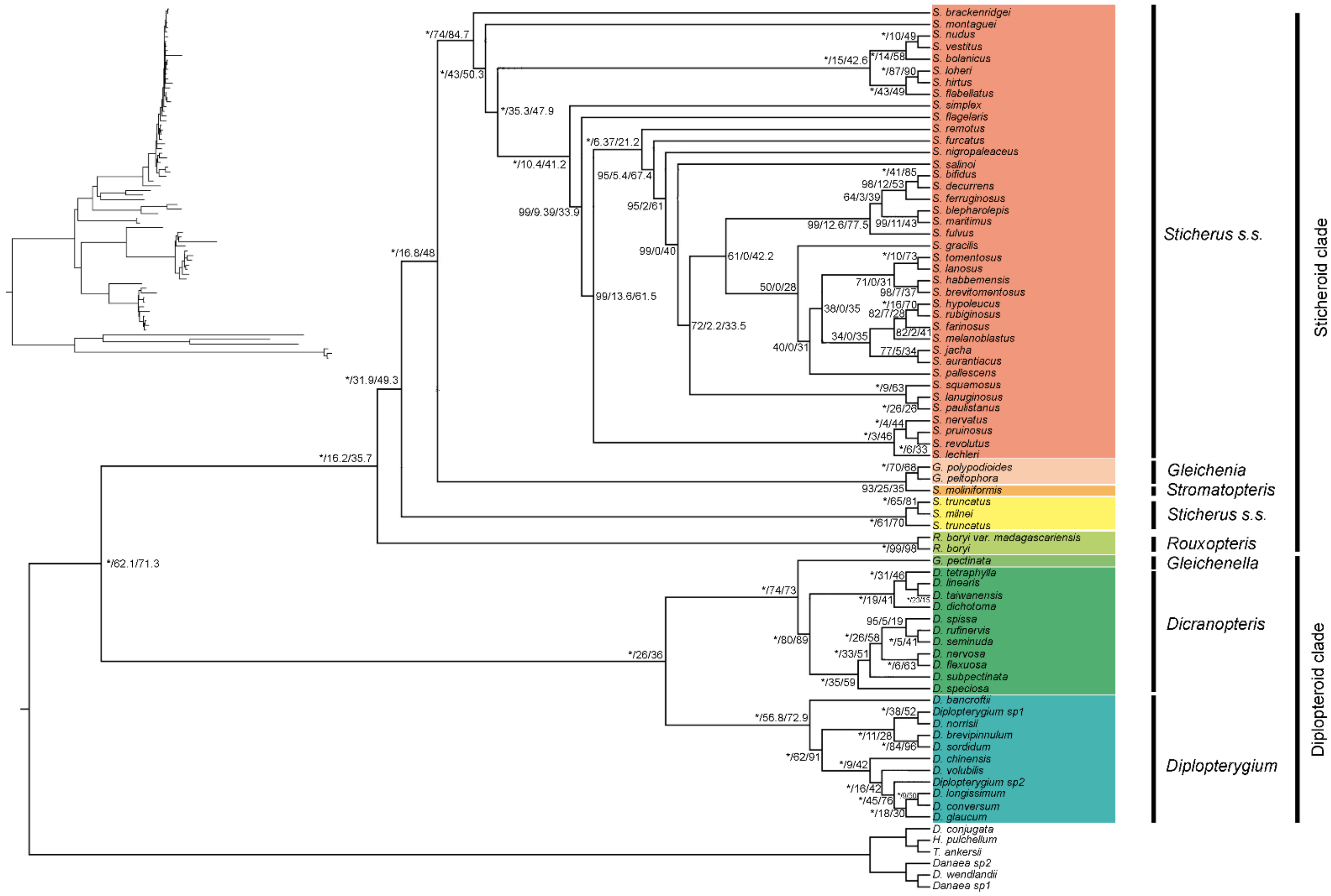
Table 3. Heterozygosity and allele divergence rates

Supplementary Material 1. Evolution models selected for the nuclear and plastidial datasets

Supplementary Material 2. Summary of fossil records in Gleicheniaceae.

Supplementary Material 3. Table showing the loci coverage and paralogs percentage.

Supplementary Material 4. Plastid and nuclear MSC.



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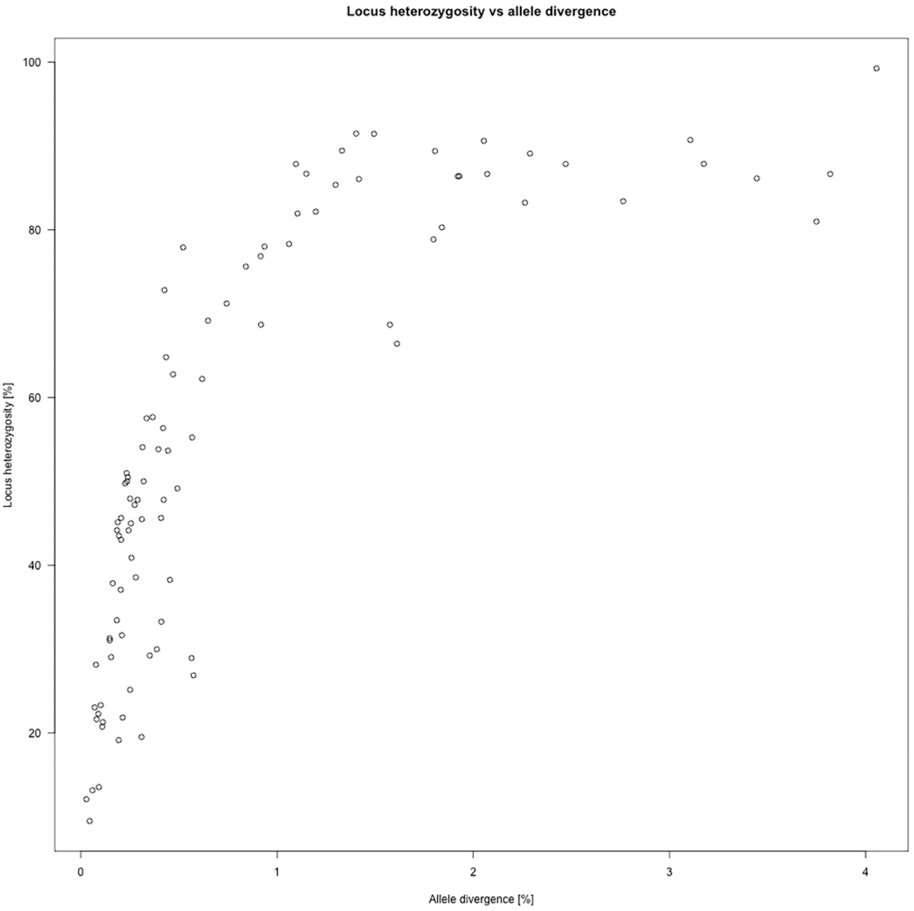

Figure 2. Graphic showing the relationship between heterozygosity rates and allele divergence rates.

Figure 3. Divergence time tree.

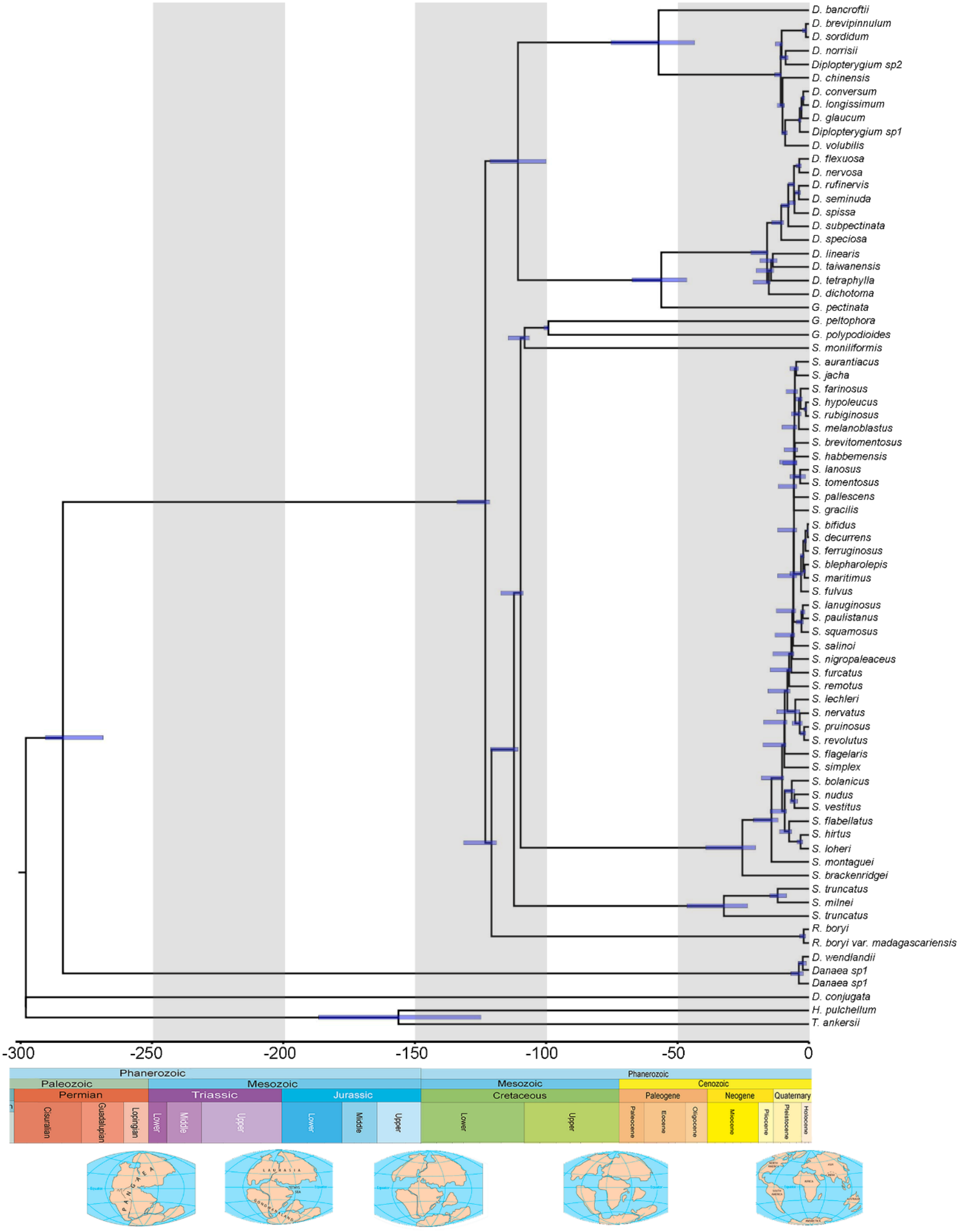

Figure 3. Divergence time tree.

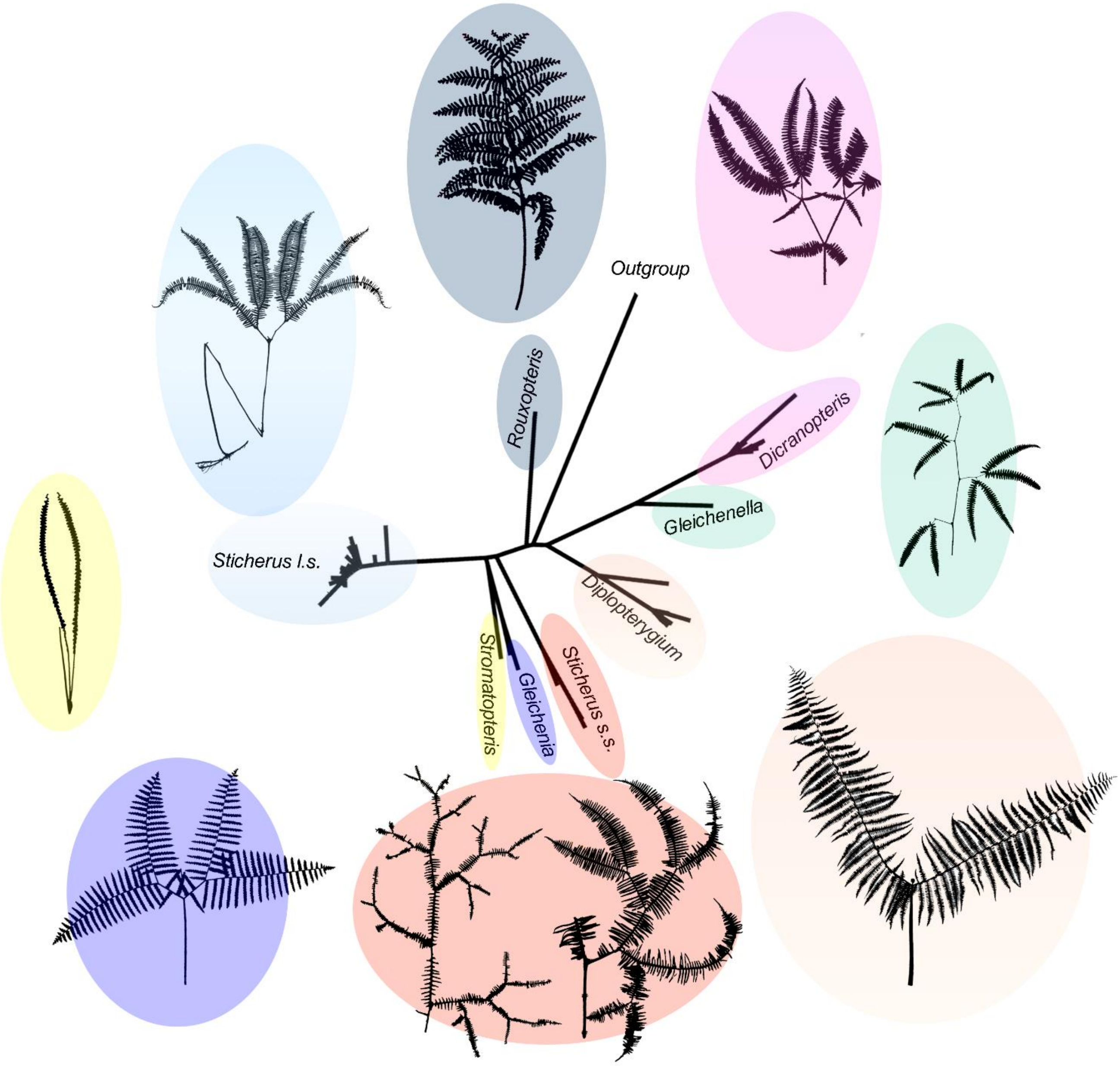

Figure 4. Ramifications patterns in Gleicheniaceae.

Table 1. Generic classification of Gleicheniaceae.

Smith (1773)	**Diels (1900)**	**Christensen (1905)**	**Copeland (1947)**	**Nakai (1950)**	**Holtum (1957)**	**Kramer (1990)**	**PPG (2016)**	**Liu et al. (2020)**
Gleichenia	*Gleichenia*	*Gleichenia*	----//-----	----//-----	----//-----	----//-----	----//-----	----//-----
----//-----	Subg. *Eu-Gleichenia*	----//-----	*Stromatopteris*	*Stromatopteris*	*Stromatopteris*	*Stromatopteris*	*Stromatopteris*	*Stromatopteris*
----//-----	Subg. *Eu-Gleichenia*	sect. *Eugleichenia*	*Gleichenia*	*Gleichenia*	*Gleichenia*	*Gleichenia*	*Gleichenia*	*Gleichenia*
----//-----	----//-----	Subsect. *Gleicheniastrum*	----//-----	----//-----	----//-----	----//-----	----//-----	----//-----
----//-----	----//-----	Subsect. Calymella	----//-----	*Calymella*	----//-----	----//-----	----//-----	----//-----
----//-----	Subg. *Mertensia*	Sect. *Mertensia*	----//-----	----//-----	----//-----	----//-----	----//-----	----//-----
----//-----	----//-----	----//-----	----//-----	----//-----	----//-----	----//-----	----//-----	*Rouxopteris*

----//-----	Sect.. *Diplopterigyum*	subsect. *Diplopterygium*	*Hicriopteris*	*Hicriopteris*	subg. *Diplopterygium*	*Diplopterygium*	*Diplopterygium*	*Diplopterygium*
----//-----	*Sect.* *Holopterigyum*	*subsect.* *Holopterygium*	*Sticherus*	*Sticherus*	*Sticherus*	*Subg. Mertensia*	*Sticherus*	*Sticherus*
----//-----	----//-----	----//-----	*Dicranopteris*	----//-----	*Dicranopteris*	*Dicranopteris*	*Dicranopteris*	*Dicranopteris*
----//-----	Sect. *Heteropterygium*	subsect. *Heteropterygium*	----//-----	*Dicranopteris*	Subg. *Dicranopteris*	----//-----	----//-----	----//-----
----//-----	----//-----	----//-----	----//-----	*Gleichenella*	----//-----	----//-----	*Gleichenella*	*Gleichenella*
----//-----	Sect. *Acropterygium*	Subsect. *Acropterygium*	----//-----	*Acropterigyum*	Subg. *Acropterygium*	----//-----	----//-----	----//-----

Table 1. Samples vouchers.

Taxon Name	Voucher Number	Voucher Location	Country of origin
Dicranopteris dichotoma	A. Takehara 2	Herb Inst. Biologie Tohoku	Japão
Dicranopteris flexuosa	LVL 220	BHCB	Brazil
Dicranopteris linearis	MK 13864	GOET	Nova Guiné
Dicranopteris linearis var. sebastiana	Wen-Liang 15282	TAIF	India
Dicranopteris nervosa	LVL 226	BHCB	Brazil
Dicranopteris rufinervis	LVL 213	BHCB	Brazil
Dicranopteris schomburgkiana		COL	Colombia
Dicranopteris seminuda	G. Martinelli 17233	RB	Brasil
Diplopterygium brevipinnulum	D. N. Karger 1441	GOET	Molluccas
Diplopterygium glaucum	D.N. Kager 597	GOET	Filipinas
Diplopterygium jardidum	D. Karge 2672	GOET	Molluccas
Diplopterygium laevissimum	Wang Zhong-tao 870094	MBM	China
Diplopterygium longissimum	MK 13546	GOET	Malasia
Diplopterygium norisii	D. N. Karge 1099	GOET	Malasia
Diranopteris klotzschiii	Salino 16256	BHCB	Brasil
Gleichenella pectinata	LVL 225	BHCB	Brazil
Gleichenia dicarpa	MK 14281	GOET	Australia
Gleichenia dichotoma	L. Zang 15211	GOET	China
Gleichenia peltophora	D.N. Kager 441	GOET	Filipinas
Gleichenia polypodioides	MK 13836	GOET	La Reunion
Sticherus bifidus	LVL 210	BHCB	Brazil
Sticherus blepharolepis	MK 14840	GOET	Colombia
Sticherus bolanicus	D. Karge 2626	GOET	Nova Guiné
Sticherus boliviensis	K. Bach 1462	GOET	Bolivia
Sticherus brevitomentosus	J.C. Salomon 17604	MBM	Bolívia
Sticherus chocoensis	E. Forero-R s.n.	COL	Colombia
Sticherus cryptocarpus	Nicolas s.n.	MBM	Argentina
Sticherus decurrens	LVL 207	BHCB	Brazil
Sticherus farinosus	Kluge 9255	GOET	Guadalupe
Sticherus ferrugineus	MK 14699	GOET	Colômbia
Sticherus flabelatus var. compactus	Mk 14280	GOET	Australia
Sticherus flagelaris	MK 13821	GOET	La Reunión
Sticherus fulvus	Kluge 9259	GOET	Guadalupe
Sticherus gnidioides	C. Haminton 2608		Panamá
Sticherus gracilis	LVL 212	BHCB	Brazil
Sticherus habbemensis	MK 14121		Nova Guiné
Sticherus hirtus	D. Klunge s.n.	GOET	Indonésia
Sticherus hypoleucus	MK 14800	GOET	Colômbia
Sticherus jacha	I Jimenez 1615	GOET	Bolivia
Sticherus lanosus	K Bach 1769	GOET	Bolivia
Sticherus lanuginosus	LVL 208	BHCB	Brazil
Sticherus lechleri	I. Jimenez 16362	GOET	Bolivia
Sticherus loheri	D. N. Karge 1001	GOET	Indonesia
Sticherus maritimus	MK 14839	GOET	Colombia

Sticherus melanoblastus	MK 14743	GOET	Colombia
Sticherus milnei	kluge7003	GOET	Indonesia
Sticherus nervatus	IMK 14733	GOET	Bolivia
Sticherus nigropaleaceus	LVL236	BHCB	Brazil
Sticherus nudus	LVL 239	BHCB	Colombia
Sticherus pallescens	LVL 238	BHCB	Colombia
Sticherus paulistanus	Salino8431	BHCB	Brasil
Sticherus pruinosus	LVL 325	BHCB	Brasil
Sticherus pseudoscandens	Dn Karger 2441	GOET	New Guinea
Sticherus quadripartitus	Larcen 192	MBM	Argentina
Sticherus remotus	I Jimenez 2709	GOET	Bolivia
Sticherus revolutus	LVL 240	BHCB	Colombia
Sticherus rubiginosus	LVL 237	BHCB	Colombia
Sticherus salinoi	Fernandes 771	BHCB	Brasil
Sticherus simplex	Eric Asplund s.n.	R	Peru
Sticherus squamosus	LVL 233	BHCB	Brasil
Sticherus tomentosus	MK 142728	GOET	Colombia
Sticherus truncatus	MK 14120	GOET	New Guinea
Sticherus underwoodianus	Veliz s.n.	MBM	Guatemala

Table 3. Heterozygosity and allele divergence rates

Sample	Allele Divergence	Locus Heterozygosity
G. peltophora	3,18	87,88
S. montaguei	2,47	87,86
D. flexuosa	2,29	89,11
D. klotzschii	2,07	86,67
D. nervosa	2,05	90,62
D. spissa	1,93	86,41
D. seminuda	1,92	86,39
D. rufinervis	1,84	80,30
D. linearis	1,81	89,41
D. wendlandii	1,80	78,87
S. truncatus	1,61	66,42
S. lechleri	1,49	91,46
S. nudus	1,42	86,06
S. nervatus	1,40	91,50
S. revolutus	1,33	89,46
S. pruinosus	1,30	85,37
S. nigropaleaceus	1,15	86,70
S. bolanicus	1,10	81,95
S. salinoi	1,10	87,86
H. pulchellum	1,06	78,32
S. vestitus	0,94	78,01
D. norrisii	0,92	68,69
Danaea sp1	0,84	75,63
S. simplex	0,74	71,22
S. remotus	0,65	69,17
S. milnei	0,62	62,22
T. ankersii	0,57	26,85
Diplopterygium sp2	0,57	55,23
D. taiwanensis	0,56	28,92
S. squamosus	0,52	77,91
D. dichotoma	0,49	49,15
D. sordidum	0,47	62,77
S. aurantiacus	0,45	38,24
D. glaucum	0,44	53,66
S. ferruginosus	0,43	72,82
Danaea sp2	0,42	47,80
R. boryi var. madagascariensis	0,42	56,35
G. polypodioides	0,41	33,25
S. hirtus	0,41	45,63
D. subpectinata	0,40	53,83
S. moniliformis	0,39	29,97
D. longissimum	0,37	57,66

S. truncatus	0,35	29,22
D. tetraphylla	0,34	57,52
D. conjugata	0,32	50,01
S. loheri	0,31	45,48
R. boryi	0,31	19,50
S. flagelaris	0,29	47,80
D. conversum	0,28	38,54
D. chinensis	0,27	47,19
Diplopterygium sp1	0,26	40,88
S. jacha	0,26	44,99
D. brevipinnulum	0,25	47,93
G. dicarpa	0,25	25,13
S. blepharolepis	0,24	50,49
S. tomentosus	0,24	50,01
S. hypoleucus	0,23	50,98
S. rubiginosus	0,23	49,76
D. bancroftii	0,21	31,63
S. brackenridgei	0,21	45,63
S. pallescens	0,21	43,03
S. paulistanus	0,20	37,06
D. speciosa	0,19	43,50
S. bifidus	0,19	19,12
S. melanoblastus	0,19	45,12
S. lanosus	0,18	44,17
S. habbemensis	0,18	33,42
S. furcatus	0,16	37,84
D. volubilis	0,15	29,03
S. lanuginosus	0,15	31,28
S. flabellatus	0,11	21,27
S. brevitomentosus	0,11	20,72
S. farinosus	0,09	22,25
S. decurrens	0,08	21,62
S. fulvus	0,08	28,12
S. gracilis	0,07	23,02
S. maritimus	0,06	13,14
G. pectinata	0,03	12,07

Supplementary material 1 – Nucleotide Substitution Model

Table 1. Nuclear dataset

Position	Loci	Selected model
1-355	10	TVMe+G4
356-710	100	TIM2e+G4
711-1279	103	HKY+F+G4
1280-1627	106	HKY+F+I+G4
1628-2404	108	TPM3+F+I+G4
2405-3275	11	HKY+F+I+G4
3276-3872	112	HKY+F+I+G4
3873-4815	113	TN+F+G4
4816-5391	114	HKY+F+I+G4
5392-5729	115	HKY+F+G4
5730-6346	116	TIM2+F+G4
6347-7399	118	HKY+F+G4
7400-8155	119	TPM2+F+I+G4
8156-8769	12	HKY+F+I+G4
8770-9272	120	HKY+F+I+G4
9273-9549	121	TIM3+F+G4
9550-10105	122	TN+F+I+G4
10106-10471	124	HKY+F+G4
10472-10784	127	HKY+F+G4
10785-11357	130	TIM+F+I+G4
11358-12037	131	TPM3+F+I+G4
12038-12537	132	TN+F+G4
12538-13088	133	TPM2+F+G4
13089-13722	135	HKY+F+I+G4
13723-14286	137	TN+F+I+G4
14287-14659	139	TN+F+G4
14660-15005	140	K2P+I
15006-15420	141	HKY+F+G4
15421-15776	143	HKY+F+G4
15777-16299	145	TN+F+G4
16300-16945	146	TN+F+I+G4
16946-17464	148	TPM2+F+I
17465-17928	149	HKY+F+I+G4
17929-18522	15	HKY+F+G4
18523-19139	150	HKY+F+I+G4
19140-19597	152	HKY+F+I+G4
19598-19977	153	HKY+F+G4
19978-20306	155	TIM3+F+G4
20307-20867	156	HKY+F+I+G4
20868-21385	157	HKY+F+I+G4
21386-21958	158	HKY+F+I+G4

21959-22664	159	TN+F+I+G4
22665-23269	160	TN+F+I+G4
23270-23899	161	TPM2+F+G4
23900-24357	162	TPM2u+F+G4
24358-24930	163	HKY+F+G4
24931-25238	167	TPM3+F+G4
25239-25676	169	HKY+F+G4
25677-26259	17	HKY+F+I+G4
26260-26617	170	HKY+F+G4
26618-27027	171	HKY+F+I+G4
27028-27561	175	HKY+F+I+G4
27562-28323	179	TPM3+F+I+G4
28324-28719	18	K2P+G4
28720-29213	180	HKY+F+I+G4
29214-29531	182	HKY+F+G4
29532-30043	183	HKY+F+I+G4
30044-30752	184	HKY+F+I+G4
30753-31272	185	TN+F+I+G4
31273-31814	186	TPM3+F+G4
31815-32165	188	TPM3+F+G4
32166-32646	19	TPM3+F+I+G4
32647-33316	191	HKY+F+I+G4
33317-33854	193	HKY+F+G4
33855-34596	194	HKY+F+I+G4
34597-35188	197	TIM3+F+I+G4
35189-35847	198	TN+F+G4
35848-36133	2	HKY+F+I
36134-36532	20	TPM2+F+G4
36533-37002	202	TIM2e+I+G4
37003-37268	203	HKY+F+I
37269-37612	204	HKY+F+I+G4
37613-38502	207	HKY+F+G4
38503-38921	21	TNe+G4
38922-39350	210	TN+F+G4
39351-39871	215	HKY+F+I
39872-40349	217	TN+F+G4
40350-40798	219	HKY+F+I+G4
40799-41265	220	TIM3+F+I+G4
41266-41738	222	HKY+F+G4
41739-42313	225	K3Pu+F+I+G4
42314-42986	226	TVM+F+I+G4
42987-43375	227	HKY+F+I+G4
43376-43765	228	K2P+G4
43766-44244	229	HKY+F+I+G4
44245-44846	23	HKY+F+G4
44847-45397	231	HKY+F+I
45398-45889	232	HKY+F+G4
45890-46481	234	HKY+F+G4
46482-47256	235	HKY+F+I+G4

47257-47729	237	TIM2+F+I+G4
47730-48229	238	HKY+F+I+G4
48230-48653	239	HKY+F+I+G4
48654-49355	24	TN+F+I+G4
49356-50022	240	TNe+I+G4
50023-50354	241	K2P+G4
50355-50900	242	TN+F+G4
50901-51231	243	HKY+F+G4
51232-51871	245	HKY+F+G4
51872-52516	246	TPM3+F+I+G4
52517-52855	247	HKY+F+G4
52856-53658	248	TN+F+I+G4
53659-53977	25	HKY+F+G4
53978-54422	252	TPM3+F+G4
54423-55095	253	TN+F+I+G4
55096-55518	254	TPM3+F+I+G4
55519-56028	255	HKY+F+I+G4
56029-56541	256	HKY+F+I
56542-57265	257	HKY+F+G4
57266-57623	258	HKY+F+I
57624-58052	260	K2P+G4
58053-58445	261	HKY+F+G4
58446-58941	262	TPM3+F+G4
58942-59284	266	K2P+G4
59285-59854	267	TPM3+F+I+G4
59855-60504	268	K3Pu+F+I+G4
60505-61242	269	HKY+F+G4
61243-61909	274	HKY+F+G4
61910-62634	275	HKY+F+I+G4
62635-63008	276	K3Pu+F+I+G4
63009-63678	278	TN+F+G4
63679-64149	279	K2P+I+G4
64150-64744	28	HKY+F+G4
64745-65218	280	TIM2+F+I+G4
65219-65579	283	HKY+F+G4
65580-66265	285	TIM3+F+I+G4
66266-66570	286	K2P+I
66571-66909	288	K2P+I
66910-67500	289	TN+F+G4
67501-67974	29	HKY+F+G4
67975-68280	290	TN+F+G4
68281-68683	291	TPM3+F+I+G4
68684-69302	292	K2P+G4
69303-69714	293	HKY+F+G4
69715-70175	294	K2P+G4
70176-70818	295	TPM3+F+G4
70819-71136	297	TN+F+G4
71137-71694	298	HKY+F+I+G4
71695-72426	299	HKY+F+G4

72427-72713	3	HKY+F+I+G4
72714-73199	30	TPM3+F+I+G4
73200-73727	300	HKY+F+I+G4
73728-74298	302	TPM3+F+I+G4
74299-74920	303	TN+F+G4
74921-75264	304	TPM3+F+I+G4
75265-75739	305	HKY+F+G4
75740-76100	306	HKY+F+G4
76101-76753	308	HKY+F+I+G4
76754-77169	309	TN+F+G4
77170-77938	31	TPM3+F+I+G4
77939-78462	310	TN+F+I+G4
78463-79088	311	TIM+F+G4
79089-79491	312	K3Pu+F+G4
79492-79780	313	HKY+F+G4
79781-80262	314	HKY+F+G4
80263-80956	315	TPM3+F+I+G4
80957-81650	316	HKY+F+I+G4
81651-81984	317	HKY+F+G4
81985-82291	318	HKY+F+G4
82292-82580	319	TN+F+I+G4
82581-82910	32	TN+F+I+G4
82911-83446	320	TIM3+F+G4
83447-83758	321	HKY+F+I
83759-84118	322	HKY+F+G4
84119-84574	323	TPM3+F+I+G4
84575-85053	324	HKY+F+I+G4
85054-85710	325	TPM3+F+I+G4
85711-86296	326	HKY+F+G4
86297-86868	329	TPM3+F+I+G4
86869-87188	33	TN+F+G4
87189-87927	333	HKY+F+I+G4
87928-88645	334	TPM3+F+I+G4
88646-89067	337	HKY+F+G4
89068-89334	338	HKY+F+G4
89335-89682	339	K3Pu+F+I+G4
89683-90372	34	HKY+F+I+G4
90373-90750	340	TVM+F+G4
90751-91566	344	HKY+F+I+G4
91567-91905	345	HKY+F+G4
91906-92360	346	TIM3+F+G4
92361-92872	35	K2P+I+G4
92873-93405	350	HKY+F+I+G4
93406-94155	351	TN+F+I+G4
94156-94474	355	HKY+F+I+G4
94475-94944	356	HKY+F+I+G4
94945-95850	358	TN+F+G4
95851-96580	359	TN+F+I+G4
96581-97376	36	HKY+F+G4

97377-98062	366	HKY+F+G4
98063-98930	367	HKY+F+I+G4
98931-99212	368	TN+F+G4
99213-99839	369	HKY+F+I
99840-100315	373	HKY+F+I+G4
100316-100861	375	TPM3+F+G4
100862-101204	377	TIM+F+I
101205-102022	379	TPM3+F+I+G4
102023-102564	380	HKY+F+G4
102565-103196	382	TIM3+F+G4
103197-103873	383	TPM3+F+I+G4
103874-104358	385	HKY+F+I
104359-104768	386	K2P+G4
104769-105243	387	HKY+F+G4
105244-105457	388	K2P+I
105458-106106	389	HKY+F+G4
106107-106640	39	TN+F+I+G4
106641-107426	391	HKY+F+I+G4
107427-108048	394	K3Pu+F+I+G4
108049-108754	395	HKY+F+G4
108755-109275	396	TN+F+I+G4
109276-109841	397	HKY+F+G4
109842-110265	4	TPM3+F+I+G4
110266-110591	40	TN+F+G4
110592-110958	400	TPM3+F+I+G4
110959-111248	401	K2P+G4
111249-111777	403	HKY+F+G4
111778-112151	404	TIM3+F+G4
112152-112691	405	TN+F+G4
112692-113164	406	TPM2+F+G4
113165-113886	407	TPM3+F+I+G4
113887-114536	408	TN+F+I+G4
114537-115038	409	HKY+F+I+G4
115039-115557	410	HKY+F+I+G4
115558-116057	411	TPM3+F+G4
116058-116663	412	HKY+F+I+G4
116664-117135	413	TIM3+F+G4
117136-117759	417	HKY+F+G4
117760-118305	418	TNe+G4
118306-118785	420	HKY+F+I+G4
118786-119431	421	HKY+F+G4
119432-120048	422	TPM2+F+I+G4
120049-120496	423	K2P+G4
120497-120893	424	HKY+F+G4
120894-121420	425	HKY+F+G4
121421-121787	426	TPM2+F+I+G4
121788-122095	428	K2P+I+G4
122096-122663	429	TPM3+F+I+G4
122664-123025	43	HKY+F+I+G4

123026-123604	430	TIM+F+I+G4
123605-124054	432	TN+F+G4
124055-124346	434	TNe+I
124347-125025	435	HKY+F+G4
125026-125634	436	TN+F+G4
125635-125894	438	HKY+F+G4
125895-126683	44	TN+F+G4
126684-126922	441	TIM3e+G4
126923-127467	442	TPM3+F+I+G4
127468-128354	447	HKY+F+I+G4
128355-128782	448	K2P+G4
128783-129131	449	K2P+I+G4
129132-129872	45	TN+F+G4
129873-130221	450	HKY+F+G4
130222-130527	46	TN+F+G4
130528-131268	47	TN+F+G4
131269-131710	49	TN+F+I+G4
131711-132077	5	TPM3+F+G4
132078-132646	50	HKY+F+I
132647-133178	51	HKY+F+I+G4
133179-133853	52	K3Pu+F+I+G4
133854-134355	53	TPM2+F+I+G4
134356-134864	54	TPM2+F+I+G4
134865-135448	55	TIM3e+G4
135449-136002	57	HKY+F+I+G4
136003-136405	58	TVM+F+G4
136406-137085	59	TPM2+F+G4
137086-137377	6	HKY+F+G4
137378-137803	60	HKY+F+G4
137804-138448	61	TN+F+I+G4
138449-139152	62	TIM+F+I+G4
139153-139642	64	HKY+F+G4
139643-140331	65	HKY+F+I+G4
140332-140941	66	TPM3+F+I+G4
140942-141565	68	TN+F+I+G4
141566-142302	7	HKY+F+I+G4
142303-142829	70	TPM2+F+G4
142830-143428	71	TN+F+I+G4
143429-143737	72	K3P+I+G4
143738-143989	73	HKY+F+G4
143990-144290	74	K2P+I+G4
144291-144759	75	HKY+F+I+G4
144760-145391	77	HKY+F+G4
145392-145908	78	TIM2e+G4
145909-146555	79	TN+F+G4
146556-147128	8	TPM2+F+G4
147129-147819	81	HKY+F+G4
147820-148863	83	HKY+F+G4
148864-149168	87	HKY+F+I+G4

149169-149628	89	HKY+F+G4
149629-150153	9	HKY+F+G4
150154-150508	91	K2P+G4
150509-150885	92	K2P+G4
150886-151362	93	HKY+F+G4
151363-151671	95	TPM2+F+G4
151672-152237	98	TIM3+F+G4
152238-152863	99	HKY+F+I+G4

TABLE 2. Plastid dataset

Partition Position	Loci	Selected model
1-521	1	TIM2+F+G4
522-623	12	K3P+G4
624-1034	13	K3P+G4
1035-1181	14	TN+F
1182-1271	15	TNe
1272-1376	16	K3Pu+F+G4
1377-1574	17	HKY+F+G4
1575-2997	18	TVM+F+G4
2998-4059	19	TVM+F+G4
4060-4325	2	TNe+G4
4326-4473	20	K2P+G4
4474-4989	21	HKY+F+G4
4990-5610	22	TIM+F+G4
5611-5973	23	TIM3+F+G4
5974-6355	24	K3P+G4
6356-7783	25	TIMe+G4
7784-8314	26	TIM2+F+G4
8315-8431	27	K2P+G4
8432-8551	28	HKY+F
8552-8663	30	TPM2u+F+I
8664-8833	31	HKY+F
8834-8963	33	TNe+G4
8964-9075	34	K2P+G4
9076-9439	36	HKY+F+G4
9440-9544	37	K2P
9545-9943	38	TN+F+G4
9944-10271	39	TPM2+F+G4
10272-10503	4	K2P+G4
10504-10863	40	K2P+G4
10864-11079	42	K2P+G4
11080-11668	44	TIM2+F+G4
11669-12869	45	TIM2+F+G4
12870-13213	46	TIM3+F+G4
13214-14375	47	TN+F+G4
14376-14489	5	K2P

14490-15551	6	TPM2u+F+G4
15552-15767	8	K3P+G4
15768-15944	9	TIM2e+G4

Used plastid coding regions: *atpE, atpF2, atpF, ccsA, ndhB2, ndhB, ndhC, ndhD, ndhH, ndhI, ndhJ, ndhK, petD2, petL, petN, psaJ, psbA, psbC, psbD, psbF, psbH, psbI, psbK, psbL, psbM, psbN, psbZ, rbcL, rpl14, rpl16, rpl22, rpl33, rpl36, rps122, rps123, rps14, rps162, rps8, ycf12, ycf4*.

Supplementary material 2 - The fossil record and the extinct groups

Gleicheniaceae is an ancient family of leptosporangiate ferns (Gandolfo *et al.* 1997). Some authors assigned fossil records to this family from the Paleozoic, like *Chansitheca* and *Oligocarpia* (Rege 1920, Halle 1927) from Upper Carboniferous and *Szea* from Permian (Yao & Taylor 1988). However, the relationship between Gleicheniaceae and these genera has been questioned based on the insufficient information about sori, sporangia and spores (Gandolfo et al. 1997, Bower 1926, Yao & Taylor 1988, Taylor & Taylor 1993). However, a recently discovered fossil record from China, *Chansitheca wudaensis* Deng, Sun *et* Li, is the oldest fossil assigned to Gleicheniaceae with about 298.34±0.09 Ma. (Early Permian) and it has well preserved sori, bifurcations and vascular structures.

Despite the few gleicheniaceous fossil records in Paleozoic and Permian, the family is well represented in the Mesozoic (Taylor *et al.* 2009). These records are more informative, with fronds, rhizomes with attached stipes, and fertile material (Mindell *et al*. 2006, Tidwell & Ash 1994, Collinson 1996, Skog 2001). Many genera are assigned to this era, like *Antarctipteris*, *Gleichenites*, *Gleichenioides*, *Gleichenopsis*, and *Boodlepteris*, (Gandolfo et al. 1997, Nagalingum & Cantril 2006).

Regarding the microfossils, Bolchovitina (1967) assigned to Gleicheniaceae some spores from Jurassic. *Plicifera* and *Gleicheniidites*, for example, have similar morphology to extant species by having spores with a pronounced triangular form and conspicuous trilete suture (Taylor *et al.* 2009).

Fossil record	**Geological period**	**Age (Ma)**	**Type**	**Reference**
Boodlepteris turoniana	Cretaceous	90	rhizomes, petioles, pinnules, sori, and spores	Gandolfo et al. 1997
Gleichenipteris antarcticus	Triassic	245	Sori and spores	Phipps et a. 2000
Antarctipteris sclericaulis	Middle Triassic	170	Rhizome and petioles	Millay & Taylor 1990
Gleichenia appianensis	Paleogene	45	Rhizome and petioles	Mindell et al. 2006
*Korallipteris alineae**	Miocene	21	Fronds and sori	Conran et al. 2017
Gleichenites jixiensis	Lower Cretaceous	130	Fronds	Xiao-Ju 2002
Gleichenites spp.	Lower Cretaceous	85	Fronds and sori	Tutin 1932
Plicifera spp.	Jurassic	199-150	Spores	Bolchovitina (1967)
Gleicheniidites spp.	Jurassic	199-150	Spores	Bolchovitina (1967)
Gleichenia chaloneri	Lower Cretaceous	112	Fronds and indument	Herendeen & Skog 1998
Gleichenia pulchella	Upper Cretaceous	85	Fronds	Knowlton 1994

Supplementary Material 3 – Loci recovering and quality control

Table 1. Data recovered for each sample before cleaning, of 451 target loci.

Sample	No. loci	Prop. of loci	Prop. of target length
C. integrifolia	82	0.182	0.221
D. bncroftii	434	0.964	3.594
D. brevipinnulum	438	0.973	3.012
D. chinensis	429	0.953	2.464
D. conjugata	444	0.987	4.821
D. conversum	426	0.947	2.282
D. dichotoma	450	1	2.389
D. flexuosa	421	0.936	3.15
D. glaucum	437	0.971	2.523
D. taiwanensis	429	0.953	1.819
D. lnearis	430	0.956	3.07
D. longissimum	438	0.973	3.341
D. norrisii	440	0.978	3.168
D. nervosa	421	0.936	3.474
D. rufinervis	430	0.956	3.547
D. subpectinata	418	0.929	2.407
D. seminuda	422	0.938	2.464
D. sordidum	438	0.973	3.615
D. spissa	442	0.982	3.647
D. tetraphylla	433	0.962	2.783
D. volubilis	417	0.927	1.87
D. wendlandii	372	0.827	2.287
Danaea_sp1	373	0.829	2.314
Danaea_sp2	388	0.862	2.428
Diplopterygium sp1	439	0.976	2.932
Diplopterygium sp2	434	0.964	2.487
D. dichotoma	431	0.958	2.864
G. dicarpa	418	0.929	2.396
G. pectinata	418	0.929	4.248
G. peltophora	424	0.942	1.871
G. polypodioides	429	0.953	2.96
H_pulchellum	427	0.949	2.911
R. boryi	413	0.918	3.388
R. boryi var. madagascariensis	406	0.902	3.204
S. antillensis	196	0.436	0.767
S. aurantiacus	383	0.851	1.704
S. bfidus	442	0.982	3.259
S. blepharolepis	446	0.991	3.131
S. bolanicus	437	0.971	3.1
S. boliviensis	436	0.969	2.326
S. brackenridgei	446	0.991	4.837
S. brevitomentosus	340	0.756	1.258
S. decurrens	438	0.973	3.721

S. farinosus	443	0.984	3.057
S. ferruginosus	446	0.991	3.559
S. flabellatus	436	0.969	2.905
S. flagelaris	441	0.98	2.756
S. fulvus	440	0.978	3.205
S. gracilis	426	0.947	3.525
S. habbemensis	434	0.964	3.339
S. hirtus	442	0.982	2.713
S. hypoleucus	438	0.973	3.318
S. jacha	435	0.967	2.458
S. lanosus	446	0.991	2.437
S. lechleri	437	0.971	2.266
S. lanuginosus	427	0.949	3.151
S. loheri	430	0.956	2.908
S. maritimus	442	0.982	2.601
S. melanoblastus	437	0.971	3.253
S. milnei	438	0.973	3.281
S. moniliformis	315	0.7	1.944
S. montaguei	442	0.982	3.246
S. nervatus	442	0.982	2.94
S. nigropaleaceus	433	0.962	3.132
S. nudus	442	0.982	3.047
S. pallescens	440	0.978	3.423
S. fucatus	427	0.949	2.799
S. peruvianus	393	0.873	1.486
S. pruinosus	437	0.971	3.343
S. paulistanus	383	0.851	1.425
S. rubiginosus	442	0.982	3.4
S. remotus	442	0.982	2.431
S. revolutus	434	0.964	3.277
S. salinoi	445	0.989	2.31
S. simplex	351	0.78	1.409
S. strictissimus	280	0.622	1.021
S. squamosus	445	0.989	3.389
S. tomentosus	436	0.969	3.196
S. truncatus	441	0.98	2.08
S. truncatus	431	0.958	1.765
S. vestitus	409	0.909	1.953
T_ankersii	331	0.736	1.491

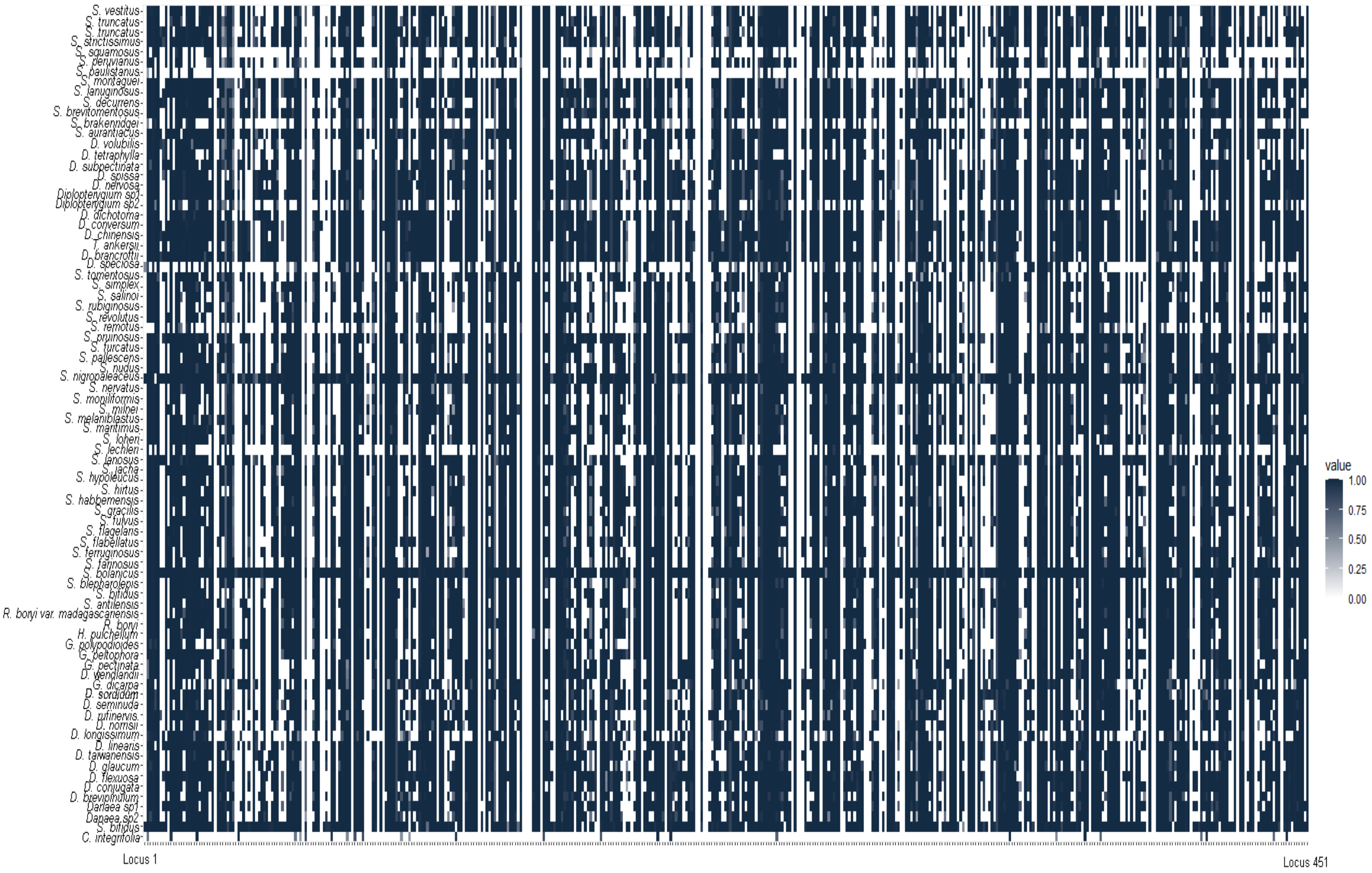

Figure 1. Heatmap showing the loci percentage covering per sample.

Table 2. Paralogs removed for each sample.

Sample	Threshold	Total removed	Names
D. bancroftii	0.00848	16	locus150, locus251, locus259, locus25, locus299, locus303, locus305, locus306, locus334, locus338, locus390, locus401, locus447, locus70, locus71, locus81
D. brevipinnulum	0.00979	21	locus135, locus139, locus235, locus238, locus239, locus242, locus262, locus265, locus267, locus268, locus269, locus279, locus324, locus334, locus335, locus338, locus345, locus384, locus395, locus400, locus48
D. chinensis	0.0114	18	locus123, locus125, locus127, locus128, locus12, locus160, locus170, locus265, locus334, locus335, locus340, locus344, locus400, locus401, locus407, locus425, locus448, locus58
D. conjugata	0.01438	20	locus211, locus212, locus213, locus214, locus215, locus216, locus21, locus220, locus233, locus23, locus24, locus266, locus286, locus310, locus406, locus407, locus425, locus436, locus437, locus448
D. conversum	0.01907	18	locus131, locus238, locus253, locus277, locus325, locus335, locus339, locus340, locus38, locus395, locus434, locus448, locus63, locus67, locus69, locus6, locus81, locus93
D. dichotoma	0.07529	9	locus114, locus15, locus189, locus190, locus240, locus333, locus394, locus407, locus437
D. flexuosa	0.06823	2	locus325, locus326
D. glaucum	0.01895	23	locus119, locus184, locus222, locus226, locus231, locus238, locus246, locus299, locus321, locus322, locus335, locus340, locus358, locus395, locus420, locus425, locus448, locus451, locus452, locus453, locus45, locus55, locus70
D. linearis	0.04459	16	locus169, locus181, locus182, locus187, locus189, locus18, locus235, locus244, locus249, locus24, locus325, locus326, locus329, locus350, locus421, locus57
D. longissimum	0.01327	26	locus118, locus119, locus131, locus158, locus164, locus165, locus166, locus168, locus16, locus180, locus184, locus198, locus226, locus246, locus279, locus299, locus320, locus334, locus335, locus395, locus401, locus405, locus410, locus448, locus52, locus99
D. nervosa	0.06133	2	locus379, locus401
D. norrisii	0.04003	46	locus112, locus118, locus119, locus122, locus124, locus135, locus139, locus151, locus15, locus170, locus186, locus205, locus206, locus208, locus209, locus20, locus220, locus222, locus230, locus231, locus233, locus23, locus242, locus268, locus290, locus292, locus293, locus294, locus325, locus351, locus358, locus395, locus405, locus418, locus421, locus425, locus426, locus450, locus451, locus452,

			locus453, locus45, locus52, locus55, locus73, locus78
D. rufinervis	0.04973	6	locus291, locus325, locus326, locus379, locus412, locus59
D. seminuda	0.05848	10	locus158, locus193, locus215, locus269, locus299, locus311, locus325, locus326, locus59, locus71
D. sordidum	0.0161	34	locus108, locus133, locus137, locus151, locus154, locus15, locus184, locus220, locus222, locus238, locus241, locus244, locus24, locus292, locus308, locus317, locus334, locus335, locus340, locus356, locus357, locus358, locus385, locus395, locus400, locus401, locus405, locus421, locus436, locus50, locus52, locus65, locus77, locus80
D. speciosa	0.01133	12	locus119, locus193, locus237, locus247, locus340, locus379, locus385, locus403, locus438, locus56, locus5, locus87
D. spissa	0.04914	6	locus203, locus248, locus289, locus325, locus326, locus442
D. subpectinata	0.01453	26	locus114, locus170, locus237, locus241, locus250, locus251, locus254, locus256, locus259, locus25, locus325, locus326, locus340, locus358, locus359, locus379, locus385, locus390, locus395, locus398, locus399, locus39, locus401, locus408, locus412, locus450
D. taiwanensis	0.05469	11	locus123, locus175, locus279, locus295, locus333, locus359, locus391, locus396, locus421, locus447, locus61
D. tetraphylla	0.01515	10	locus191, locus251, locus254, locus256, locus259, locus25, locus293, locus326, locus401, locus412
D. volubilis	0.00538	25	locus133, locus135, locus180, locus243, locus253, locus280, locus285, locus308, locus334, locus335, locus340, locus368, locus369, locus375, locus395, locus397, locus400, locus401, locus403, locus407, locus408, locus410, locus448, locus78, locus99
D. wendlandii	0.04921	13	locus158, locus190, locus192, locus195, locus196, locus199, locus19, locus354, locus357, locus35, locus394, locus50, locus77
Danaea sp1	0.02917	21	locus194, locus320, locus330, locus331, locus332, locus335, locus336, locus338, locus33, locus354, locus35, locus360, locus361, locus362, locus363, locus364, locus36, locus400, locus74, locus75, locus77
Danaea sp2	0.01926	18	locus101, locus105, locus109, locus131, locus194, locus354, locus35, locus360, locus361, locus362, locus363, locus364, locus36, locus400, locus401, locus74, locus75, locus77
Diplopterygium sp1	0.017	9	locus156, locus250, locus251, locus259, locus25, locus335, locus395, locus401, locus448
Diplopterygium sp2	0.02279	35	locus108, locus151, locus154, locus15, locus172, locus173, locus174, locus17, locus200, locus204, locus205, locus206, locus208, locus209, locus20, locus230, locus233, locus238, locus23, locus246, locus250, locus251, locus259, locus25, locus294, locus299, locus304, locus306, locus322, locus340, locus394, locus395, locus448, locus55, locus99
G. dicarpa	0.04085	1	locus297

G. pectinata	0.00613	2	locus297, locus70
G. peltophora	0.10229	6	locus295, locus390, locus392, locus398, locus399, locus39
G. polypodioides	0.04735	10	locus151, locus154, locus15, locus179, locus261, locus278, locus294, locus320, locus332, locus412
H. pulchellum	0.02825	28	locus125, locus202, locus207, locus215, locus228, locus230, locus231, locus240, locus245, locus250, locus257, locus267, locus292, locus294, locus295, locus29, locus314, locus334, locus348, locus350, locus382, locus383, locus398, locus410, locus420, locus421, locus69, locus99
R. boryi	0.05009	3	locus253, locus333, locus394
R. boryi var. madagascariensis	0.0133	32	locus100, locus109, locus117, locus11, locus140, locus145, locus167, locus219, locus220, locus222, locus240, locus250, locus251, locus259, locus25, locus285, locus315, locus333, locus379, locus396, locus406, locus418, locus55, locus90, locus91, locus92, locus93, locus94, locus96, locus97, locus99, locus9
S. aurantiacus	0.03516	19	locus135, locus172, locus173, locus174, locus176, locus178, locus17, locus193, locus202, locus203, locus231, locus290, locus316, locus382, locus395, locus401, locus407, locus410, locus450
S. bifidus	0.04611	7	locus151, locus154, locus15, locus220, locus350, locus407, locus447
S. blepharolepis	0.01106	14	locus125, locus128, locus129, locus12, locus237, locus293, locus300, locus320, locus340, locus382, locus401, locus405, locus425, locus429
S. bolanicus	0.03008	10	locus158, locus234, locus300, locus320, locus325, locus391, locus410, locus412, locus423, locus58
S. brackenridgei	0.01061	15	locus211, locus213, locus214, locus216, locus21, locus220, locus237, locus255, locus340, locus405, locus412, locus433, locus59, locus81, locus98
S. brevitomentosus	0.00757	13	locus159, locus163, locus203, locus240, locus241, locus243, locus244, locus249, locus24, locus366, locus375, locus382, locus429
S. decurrens	0.00575	9	locus100, locus125, locus130, locus203, locus382, locus383, locus385, locus429, locus432
S. farinosus	0.00696	14	locus106, locus125, locus130, locus151, locus154, locus15, locus237, locus312, locus31, locus320, locus337, locus356, locus382, locus429
S. ferruginosus	0.01338	19	locus116, locus123, locus128, locus129, locus12, locus130, locus139, locus149, locus171, locus184, locus203, locus227, locus237, locus300, locus359, locus386, locus388, locus429, locus58
S. flabellatus	0.00658	18	locus112, locus125, locus130, locus151, locus154, locus15, locus255, locus278, locus307, locus313, locus31, locus320, locus340, locus431, locus439, locus43, locus449, locus51
S. flagelaris	0.01369	19	locus140, locus151, locus154, locus15, locus160, locus161, locus175, locus191, locus290, locus303, locus354, locus35, locus401, locus407, locus408, locus412, locus424, locus438, locus58
S. fulvus	0.00501	14	locus125, locus130, locus255, locus265, locus293, locus320, locus401, locus429, locus431, locus434, locus437, locus439, locus43, locus47

S. furcatus	0.00827	16	locus140, locus151, locus154, locus155, locus15, locus203, locus225, locus237, locus263, locus276, locus340, locus397, locus74, locus76, locus7, locus99
S. gracilis	0.00584	5	locus139, locus203, locus237, locus262, locus382
S. habbemensis	0.00887	21	locus151, locus153, locus154, locus155, locus15, locus164, locus165, locus166, locus168, locus16, locus203, locus250, locus251, locus256, locus258, locus259, locus25, locus320, locus332, locus382, locus429
S. hirtus	0.02729	16	locus101, locus105, locus109, locus118, locus123, locus181, locus241, locus263, locus275, locus303, locus317, locus354, locus400, locus405, locus77, locus83
S. hypoleucus	0.01103	6	locus107, locus120, locus130, locus203, locus382, locus429
S. jacha	0.01525	13	locus151, locus154, locus155, locus15, locus164, locus165, locus168, locus16, locus293, locus356, locus429, locus64, locus95
S. lanosus	0.01005	11	locus124, locus125, locus140, locus225, locus262, locus291, locus298, locus304, locus382, locus429, locus449
S. lanuginosus	0.00946	19	locus170, locus172, locus173, locus174, locus176, locus178, locus17, locus210, locus211, locus212, locus213, locus214, locus216, locus218, locus21, locus293, locus320, locus340, locus429
S. lechleri	0.04055	26	locus125, locus330, locus331, locus332, locus335, locus336, locus338, locus33, locus351, locus358, locus388, locus391, locus402, locus406, locus40, locus412, locus441, locus443, locus444, locus445, locus446, locus44, locus94, locus96, locus97, locus9
S. loheri	0.0173	19	locus118, locus151, locus153, locus154, locus15, locus203, locus211, locus212, locus213, locus214, locus216, locus218, locus21, locus320, locus405, locus407, locus56, locus5, locus83
S. maritimus	0.0196	3	locus125, locus382, locus429
S. melanoblastus	0.00827	16	locus125, locus211, locus213, locus214, locus216, locus218, locus21, locus235, locus265, locus320, locus396, locus400, locus401, locus412, locus429, locus441
S. milnei	0.02573	34	locus123, locus127, locus128, locus129, locus12, locus165, locus16, locus179, locus207, locus211, locus213, locus214, locus216, locus218, locus21, locus268, locus275, locus292, locus299, locus303, locus305, locus325, locus351, locus373, locus380, locus387, locus391, locus395, locus421, locus435, locus47, locus61, locus77, locus84
S. moniliformis	0.01587	19	locus108, locus149, locus150, locus191, locus237, locus257, locus340, locus341, locus342, locus343, locus345, locus347, locus348, locus349, locus34, locus367, locus407, locus423, locus436
S. montaguei	0.07923	2	locus326, locus340
S. nervatus	0.03495	9	locus172, locus173, locus174, locus176, locus178, locus17, locus240, locus436, locus68

S. nigropaleaceus	0.02876	14	locus139, locus151, locus154, locus15, locus191, locus290, locus382, locus426, locus78, locus90, locus94, locus96, locus97, locus9
S. nudus	0.03459	12	locus125, locus150, locus158, locus234, locus235, locus240, locus391, locus403, locus412, locus59, locus68, locus83
S. pallescens	0.01332	9	locus119, locus125, locus151, locus154, locus15, locus203, locus293, locus429, locus61
S. paulistanus	0.01305	7	locus203, locus227, locus291, locus293, locus396, locus425, locus429
S. peruvianus	0.08962	3	locus237, locus386, locus447
S. pruinosus	0.03086	25	locus172, locus173, locus174, locus176, locus178, locus17, locus237, locus240, locus250, locus251, locus256, locus258, locus259, locus25, locus276, locus312, locus330, locus331, locus332, locus335, locus336, locus33, locus436, locus49, locus68
S. remotus	0.02176	15	locus122, locus131, locus145, locus146, locus167, locus175, locus185, locus238, locus274, locus298, locus320, locus350, locus359, locus410, locus57
S. revolutus	0.03327	20	locus149, locus150, locus172, locus173, locus174, locus176, locus178, locus17, locus203, locus240, locus330, locus331, locus332, locus335, locus336, locus33, locus430, locus442, locus68, locus93
S. rubiginosus	0.01295	6	locus161, locus225, locus237, locus261, locus412, locus426
S. salinoi	0.0276	17	locus123, locus125, locus176, locus293, locus321, locus388, locus396, locus410, locus430, locus441, locus65, locus73, locus90, locus94, locus96, locus97, locus9
S. simplex	0.02885	11	locus150, locus163, locus164, locus165, locus166, locus168, locus169, locus16, locus203, locus435, locus78
S. squamosus	0.01738	7	locus125, locus132, locus203, locus225, locus410, locus429, locus433
S. tomentosus	0.01047	12	locus125, locus151, locus154, locus155, locus15, locus203, locus320, locus407, locus410, locus422, locus429, locus442
S. truncatus	0.0765	7	locus114, locus191, locus207, locus285, locus305, locus356, locus406
S. truncatus	0.09667	6	locus157, locus266, locus395, locus447, locus61, locus77
S. vestitus	0.02812	16	locus121, locus123, locus125, locus128, locus129, locus12, locus150, locus203, locus303, locus313, locus31, locus386, locus410, locus412, locus423, locus83
T. ankersii	0.06899	4	locus267, locus269, locus342, locus449

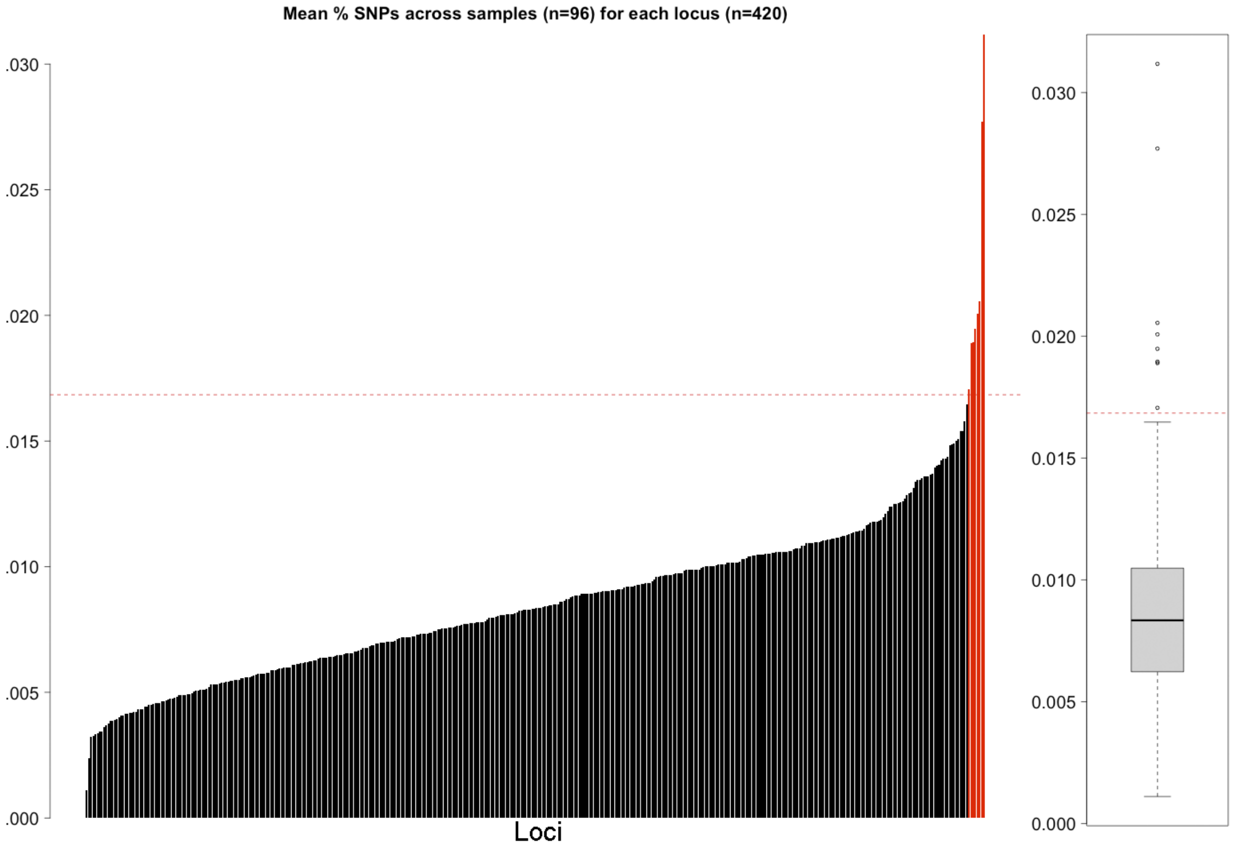

Figure 2. Mean percentage of SNPs across samples (n=76) for each loci (n=451), showing the threshold of 1.5x the interquartile range above the third quartile value, which is at 1.5% in this dataset. Loci with % mean SNPs above this threshold were removed. On the right is a box and whisker plot showing the first quartile, mean and third quartile of mean percentage of SNPs across samples for each loci.

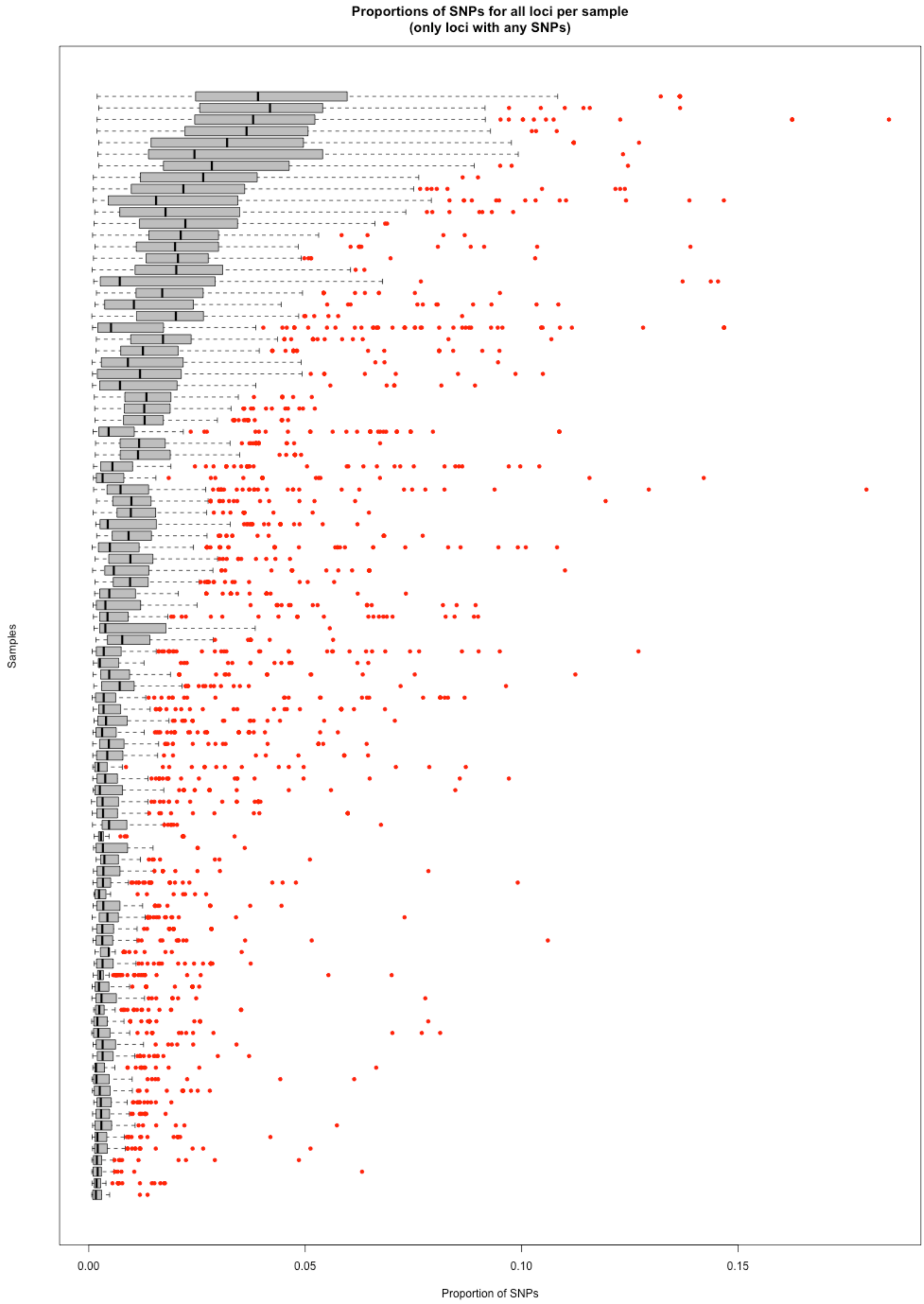

Figure 3. Box and whisker plots for each sample showing the proportion of SNPs for all loci. The first quartile, mean, third quartile and 1.5x the interquartile range above and below the interquartile range are shown. Values that were above 1.5x the interquartile range above the third quartile are marked as dots and were removed.

Supplementary material 4 – Plastid ML tree and Nuclear MSC tree

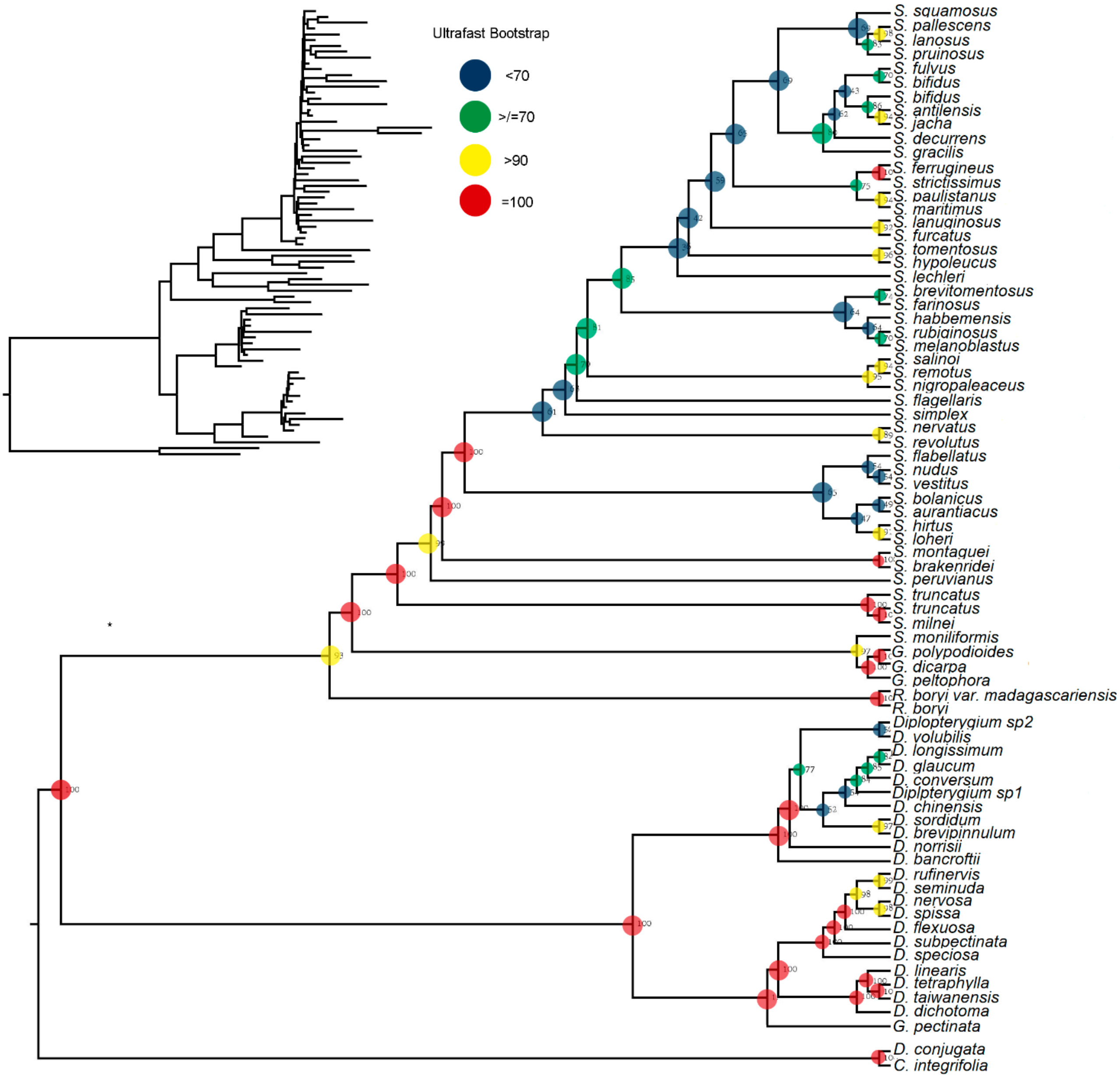

Figure 1. Plastid ML tree. Nodes with Ultrafast bootstrap support values.

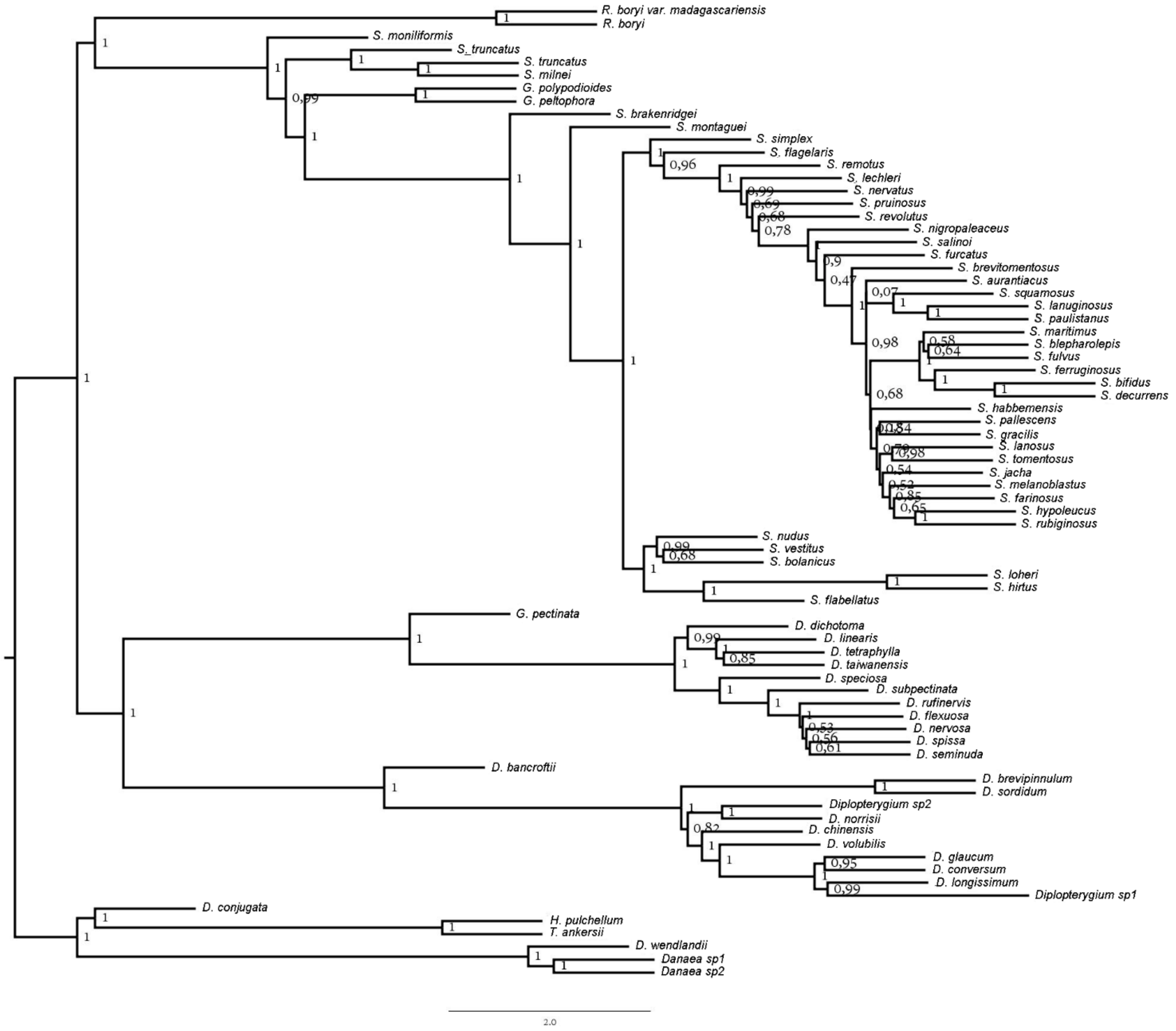

Figure 2. Nuclear Multi-species Coalescent tree. Branch lengths in coalescent units.

Capítulo 2: Artigo publicado no periódico Plant Ecology and Diversty

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Artigo publicado no periódico Plant Ecology and Diversity

Migration barriers in ferns: the case of the neotropical *Diplopterygium* (Gleicheniaceae)

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Abstract

Background: Despite the broad distribution of several species in Gleicheniaceae in the neotropical region, *Diplopterygium* is the only genus having a restricted distribution. Species of Gleicheniaceae occupy open (including anthropogenic) habitats and produce large amounts of wind-dispersed propagules – so why does *Diplopterygium bancroftii,* the only neotropical species in the genus, have a restricted distribution?

Aims: We investigated if the restricted distribution of *Diplopterygium* in the Neotropics reflected the absence of suitable areas for the establishment.

Methods: We used species distribution modelling to identify suitability areas during different periods of the Pleistocene (Last Glacial Maximum and Last Interglacial), the Holocene, and the present.

Results: The environmental suitability model at the present time corroborated the current distribution of *D. bancroftii,* and also evidenced additional suitable potential areas where the species has so far not been recorded, especially in eastern Brazil. In projections of the past, no connectivity was observed between suitable areas in the Andes and eastern Brazil.

Conclusions: As neither the dispersal ability nor the absence of suitable areas at the present time or in the past were limiting factors to the range expansion of *Diplopterygium*, we discuss possible migration barriers and propose a hypothesis for its colonization history in the Neotropics.

Keywords: Andes, biogeography, dispersion, Gleicheniales, niche modelling, pteridophytes

Introduction

The genus *Diplopterygium* is placed in the ancient family of leptosporangiate ferns, Gleicheniaceae (PPG I 2016). The genus has amphi-Pacific distribution and comprises ca. 25 species. They are pioneer species, and large plants with bipinnate forking fronds up to a few meters long, and are usually dominant in the understorey. Their long-creeping rhizomes allow them to spread and occupy large areas, forming dense thickets (Kramer 1990; Østergaard and Øllgaard 2001; Mickel and Smith 2004) (Figure 1). In the Neotropics, the genus is represented solely by *Diplopterygium bancroftii* (Hook.) A.R.Sm. occurring in Mexico, Central America, the equatorial Andes, and the Guyana Shield, mainly in open areas or in open conifer, *Alnus*, and *Quercus* forests of middle elevations (1050–2600 m) (Østergaard and Øllgaard 2001; Mickel and Smith 2004) (Figure 2).

Although some neotropical Gleicheniaceae have restricted distributions, such as *Sticherus tepuiensis* A.R.Sm. and *S. simplex* (Desv.) Ching (Gonzales and Kessler 2011), numerous species are widely distributed throughout the neotropical region and often colonise anthropogenic habitats [including *Gleichenella pectinata* (Willd.) Ching, *Dicranopteris flexuosa* (Schrad.) Underw., and *Sticherus bifidus* (Willd.) Ching] (Tryon and Tryon 1982; Mickel and Smith 2004; Gonzales and Kessler 2011; Lima and Salino 2018c). *D. bancroftii* likewise colonises disturbed habitats (Figure 1) and frequently coexists with the aforementioned widespread species. In South America, however, it is restricted to higher elevations, with its southernmost distribution reaching northern Bolivia (Figure 2) (Tryon and Tryon 1982; Tryon and Stolze 1989; Østergaard and Øllgaard 2001; Mickel and Smith 2004). Its continuous distribution throughout North, Central, and South America suggests that its ability for colonisation and establishment in different environments throughout the neotropical region may not be a limiting factor to its expansion further south. Therefore, other factors may be related to its restricted distribution in South America, such as the absence of favourable biological interactions, or dispersal limitations.

The biogeographical patterns of ferns, as well as angiosperms, are largely shaped by combinations of three main factors: long-distance dispersal (LDD), vicariance, and extinction (Kessler 2010). LDD is very frequent among ferns via their dispersal of spores (Kessler 2010) and ferns usually have wider distribution ranges than flowering plants, are more successful colonisers of oceanic islands, and have smaller numbers of endemic genera (Tryon 1985; Barrington 1993; Smith 1993; Kessler 2010). Nonetheless, even with their potential for LDD, many fern species have restricted distributions that may reflect their niche requirements and habitat specialisation, geographical isolation, or competitive interactions among species (Kessler 2010).

Special environmental limitations are more likely to have strong roles in species having highly specific habitat requirements, and therefore those species usually have only small, isolated patches of suitable habitat (Peck et al. 1990; Kessler 2010). *Diplopterygium bancroftii* is a long-lived perennial plant that produces multiple fertile fronds per year (which may be a few metres long) and presents an almost modular growth habit (Holttum 1957; Tryon and Tryon 1982; Kramer 1990). Three or four sporangia are usually formed per sorus, with 8-12 sori per segment (Tryon and Tryon 1982; Kramer 1990), and with each sporangium having the ability to produce from 128 to over 800 spores (vs. 64 or fewer spores in more derived leptosporangiate families) (Kessler and Smith 2018). The numbers of spores produced by a single frond are therefore considerably higher than those of other leptosporangiate ferns. In terms of their viability, the spores of *D. bancroftti* are non-green (Tryon and Lugardon 1990) – which usually reflects longer propagule viability as compared to green spores (Lloyd and Klekowski 1970).

The distribution of a given species is determined by its evolutionary history, associated with environmental conditions and biological interactions (Willig et al. 2003; Mittelbach et al. 2007). Understanding the dynamic interactions between species and their environments over time, through the reconstruction of past distribution projections, allows us to project evolutionary patterns as influenced by climatic changes (Gentry 1982; Gentry 1988). The significant climate changes in the

Neotropics during the Quaternary period, especially in the Pleistocene, contributed significantly to shaping current species distributions (VanDerWal et al. 2009), and the 20 cycles of glacial expansion and retraction have caused significant changes in regional biotas (VanDerWal et al. 2009; Bueno et al. 2017). As climatic changes have the potential to strongly impact species distributions (Gaston 2000; Siqueira et al. 2009), they may represent one of the principal causes of the restricted distribution of *D. bancroftti* today in South America.

The question, therefore, arises of why *Diplopterygium bancroftii*, a ruderal plant, with significant long-distance dispersal potential, has a such restricted distribution in South America as compared to other Gleicheniaceae in the Neotropics. We used species distribution modelling tools to investigate (i) if the restricted distribution of *D. bancroftii* reflected the absence of suitable areas in South America outside its current distribution area, (ii) how the distribution of *D. bancroftii* likely to have responded to climatic fluctuations during the Pleistocene. We expected to evaluate the biogeographic and ecological factors that could be related to the current distribution of the species, identifying newly suitable areas for the occurrence of *Diplopterygium* in South America, and propose a new hypothesis for its historical colonisation of the Neotropics.

Materials and methods

Model construction and validation

We built species distribution models for current and past periods of *Diplopterygium bancroftii* using the BioDinamica toolset (Oliveira et al. 2019) implemented in Dinamica-EGO software (Soares-Filho et al. 2013). We used the Species Distribution Modeling (SDM) function of BioDinamica to generate models of the suitable climatic niche of *D. bancroftii* in the Neotropics. The following seven algorithms, with different logics, were used to generate the models: GLM—

Generalised Linear Models (McCullagh and Nelder 1989); MaxEnt—Maximum Entropy (Phillips et al. 2006); RF—Random Forests (Breiman 2001); FDA—Flexible Discriminant Analysis (Hastie et al. 1994); GBM—Generalised Boosted Models (Friedman et al. 2000); MARS—Multiple Adaptive Regression Splines (Friedman 1991), and SVM—Support Vector Machines (Vapnik 1998).

Since the algorithms selected use presence-only occurrence data, we chose to generate pseudo-absence points in places where there has been intense sampling for the Gleicheniaceae group, and where there were no records of *D. bancroftii*. This was done based on the premise that the most sampled sites for the study group that does not have records of that species' presence are sites with greater evidence of its absence. We adopted the strategy of building pseudo-absences available in BioDinamica (Oliveira et al. 2019) and generated pseudo-absence points based on evidence of historical collections of Gleicheniaceae in the Neotropics. We used 8348 records of the family obtained from GBIF (https://doi.org/10.15468/dl.1h63kg) and randomised 300 pseudoabsence points, maintaining a distance radius of at least 100 km from confirmed presence points. To calibrate and evaluate the quality of the models, we divided the species data into a training set (75% of the records) and a test or validation set (25% of the occurrences). We evaluated the models using the AUC (area under the curve) values of the ROC (receiver operating characteristic) and the TSS (true skill statistic). The AUC value measures the discriminatory ability of the model, with values above 0.9 being considered here of high accuracy (Manel et al. 2001); values lower than that threshold were discarded. The TSS was used as a validation measure, as it responds independently of species prevalence where sensitivity and specificity are calculated. TSS values above 0.6 were considered good, and values lower than that threshold were discarded (Allouche et al. 2006).

We then defined a threshold value above which the grid cells could be considered to have environmental characteristics suitable for the maintenance of viable populations of the species (Pearson et al. 2007). We used the ‘minimum ROC plot distances’ as the threshold selection method as it uses a threshold that ensures maximum AUC values (Fawcett 2006).

Species dataset and environmental data

Records of *Diplopterygium bancroftii* (Figure 2) were obtained from GBIF (https://doi.org/10.15468/dl.pp7haf) and checked for misidentifications or imprecise geographic information, resulting in a 260-record dataset. Focusing on the neotropical region, we used 19 bioclimatic variables with 30 arc-second resolution (ca. 1 km), covering temperature and precipitation (Hijmans et al. 2005), plus high-quality DEM (digital elevation models) data obtained from the NASA Shuttle Radar Topography Mission (SRTM) (Jarvis et al. 2008).

To avoid over-parameterisation and overfitting the models with redundant variables (Dormann et al. 2013) and remove collinearity, we ran a Principal Component Analysis (PCA) using the BioDinamica toolset (Oliveira et al. 2019) implemented in Dinamica-EGO software (Soares-Filho et al. 2013) of the 19 bioclimatic variables plus elevation. Dimensionality was reduced by using the five components with most explanatory value, corresponding to about 85% of the information, to build the species distribution model. Principal Component Analysis is particularly useful for dealing with over-parameterisation as it allows the use of only the first few principal components of a given dataset, and the explanatory axes are linearly uncorrelated (Jolliffe 2002).

To produce models and infer the current and past distributions of *D. bancroftii*, we modelled species distribution based on environmental suitability climatic simulations (www.worldclim.org; Hijmans et al. 2005) during the Current (0 ka pre-industrial), Mid-Holocene (6 ka Before Present - BP), Last Glacial Maximum (LGM – 21 ka BP), and Last Interglacial (LIG – 130 ka BP) periods from Community Climate System Model 4 (Gent et al. 2011), MIROC-ESM (Watanabe et al. 2011), and MPI-ESM-MR Max-Planck-Institut für Meteorologie Earth System Model (MPI). Ensemble maps based on the model runs with different algorithms were made by weighted average, using the AUC criterion as a weight to each algorithm projection on ArcGIS ver. 10 (ESRI 2011). The ensemble maps are presented here by periods as well as the different Global Circulation Models used for the Holocene and LGM. The final maps were prepared in ArcGIS ver. 10 (ESRI 2011).

Results

The SDM of the present period for *D. bancroftii* corroborated our current knowledge of its distribution and revealed additional suitable areas where the species has not yet been recorded (Figure 3, Table 1). Those areas with environmental suitability, given the present model, include southern, south-eastern, and central Brazil, including the mountain ranges of Serra do Caparaó, Serra do Itatiaia, Quadrilátero Ferrífero, Chapada dos Guimarães, Chapada Diamantina, as well as the northern border with Venezuela in the Guiana Shield region (Figure 3). Additionally, areas of high suitability were identified in central and southern Bolivia, with areas of median suitability in Chile and Argentina (Figure 3). Those newly recognised suitable areas were present in all tested scenarios and projections, although no connectivity was observed between the current extent of occurrence of *D. bancroftii* and those areas during any moment of the Pleistocene or in the present time frame.

When compared with the model for the present time, suitable areas estimated for the Holocene (10.000 BP) showed a small reduction in the northern region of Mexico and increases in the Guiana Shield and in south-eastern and central Brazil (Figure 3, Table 1). Suitable areas in the CCSM4 projections during the Last Glacial Maximum (LGM) were closer to the present model prediction, about 25,000 BP, when the climate was colder and drier (Figure 4A, Table 1). The MIROC projections showed some differences and indicated suitable areas in the central and southern Amazon region in Brazil and Bolivia; the MPI projection showed areas of median to high suitability in the northern Amazon (Figures 4-5).

The models for the Last Interglacial period (LIG), ca. 120,000-140,000 BP, showed similar distributions to those estimated for the Holocene regarding suitable areas in north-eastern and south-eastern Brazil (Figure 4B, Table 1). There was a smaller suitable area in northern Mexico and a larger one in southern Bolivia in comparison with the projection using today’s climate, as well suitable areas in the north-eastern Brazilian Amazon, and smaller suitable areas of in southern and south-eastern Brazil.

We identified areas such as Central America, southern Mexico, and the Ecuadorian Andes as constantly suitable for species occurrences through all simulated current and past scenarios. Only a few portions of these areas showed reductions in suitable area (e.g., the Guiana Shield and southern Bolivia) in response to climatic fluctuations in the Pleistocene. The suitable areas were validated by the current distribution of the species. Suitable areas were also observed further south of the current range in South America.

Discussion

During the Upper Pleistocene and early Holocene, stable suitability areas for *D. bancroftii* were available across the Neotropics, even with climatic fluctuations between warmer (LIG and Holocene) and cooler (LGM) scenarios. Few differences were observed in comparisons with the CCSM4, MIROC, and MPI projections, although the CCSM4 projection was slightly more restrictive than MPI and MIROC in terms of suitable areas in South America. Moreover, stable suitable areas, where *D. bancroftii* has not been observed, were identified in both present and past models in southern South America, with areas of high environmental suitability in eastern Brazil. This reinforces that the availability of suitable areas was not a limiting factor to the range expansion of *D. bancroftii*. The models generated for past scenarios, however, support the rejection of the hypothesis of connections between the current extent of occurrence of *D. bancroftii* and the remaining suitable areas found in South America. We, therefore, recognise a disjunct area of apparently suitable climatic conditions for *D. bancroftii* beyond its current distribution. Either this plant occurs in all possible suitable areas and has not yet been collected in eastern Brazil, or its absence is related to other factors besides the presence of suitable habitat areas.

Despite gaps and biases in the collection efforts for several different groups of living organisms in Brazil (Oliveira et al. 2016), including ferns and lycophytes in southern and south-eastern Brazil (Almeida and Salino 2016), it is unlike that a large and easily identifiable plant such as *D. bancroftii* would have passed unnoticed; we did, however, identify suitable areas in the Guiana Shield region, including the extreme northern portion of Brazil, regions that have been very poorly sampled (Almeida and Salino 2016). The absence of records of *D. bancroftii* in northern Brazil could be the result of collection gaps and the need for expensive logistics to collect in those areas (Almeida and Salino 2016; Barbosa-Silva et al. 2016). Two new records of Gleicheniaceae for Brazil from the northernmost part of the country (near the Serra do Acará, Amazonas state) were recently published (Lima and Salino 2018a; Barbosa-Silva et al. 2016), showing that collection efforts in this region have a high potential for new records (Carvalho et al. 2012).

Since long-distance dispersal appears not to be a limiting factor for ferns (Kessler 2010), why does *D. bancroftii* not occur in suitable, but more southern, areas in South America? Given that the climatic conditions for the occurrence of *D. bancroftii* are potentially satisfied, two other aspects must be considered as possible explanations: the biotic component (Wisz et al. 2013; Guisan and Thuiller 2005; Leach et al. 2016; Meier et al 2010; Van der Putten et al. 2010) and the requirements for species migration.

The range expansion of a given species via long-distance dispersal will depend on the numbers of propagules produced, their viability, propagule transportation to suitable sites, and the establishment of reproductive individuals and stable populations (Wolf et al. 2001). There are several species shared among the mountains in south-eastern Brazil and the Andes (Brade 1956; Safford 1999a, 1999b; Ribeiro et al. 2007), including Gleicheniaceae, such as *Dicranopteris nervosa* (Kaulf.) Maxon, *Sticherus nigropaleaceus* (J.W.Sturm) J.Prado & Lellinger, and *Sticherus lanuginosus* (Fée) Nakai. Those species occur mainly in the south-eastern and southern mountains of Brazil, with disjunct populations in Bolivia and Peru. Likewise, *D. nervosa*, *S. lanuginosus,* and *S. nigropaleaceus* occur on the eastern slopes of the Andes (Tryon and Stolze 1989; Gonzales and Kessler 2011; Kessler and Smith 2018; Lima and Salino 2018c). Spore transport to suitable areas could, it seems, be one of the limiting factors to the range expansion of *D. bancroftii*, as neither the numbers of propagules produced, nor the occurrence of suitable climatic areas appear to be limiting factors. Puentha (1991) has pointed out the importance of wind currents to fern propagule dispersal at distances over 1000 km, suggesting that inter-continental winds and air currents are important factors to be considered in their long-distance dispersal. Munoz et al. (2004) likewise have demonstrated the importance of those winds to understanding the patterns of distribution of several fern taxa in South America. James (1939) has examined the present wind directions in South America and determined that wind directions in both the austral summer and winter seasons mainly flow from east to west – that is the opposite that would ensure the dispersal of fern spores from the current areas of fern distribution - which could help explain the disjunct distribution of some of the species already mentioned here, such as *D. nervosa* and *S. nigropaleaceus.*

The main physical barrier in South America is the Andes, and that mountain chain directly affects the continent's climate by affecting atmospheric circulation in the Southern Hemisphere (Gregory-Wodzicki 2000; Garreaud 2009). Insel et al. (2009) have demonstrated the large influence on wind patterns and air humidity transportation of the Andean range through simulations with or without the Andes. Therefore, the Andes ranges acting as a an orographic barrier can influence not only climatic conditions (temperature, precipitation, and humidity – variables used in the modelling of the present study) but also the transportation and dispersal of propagules by directly influencing wind direction and atmospheric circulation. Therefore, most wind currents that flow from west to east in South America tend to be barred by the Andes, not reaching the south-eastern, southern, or central portions of Brazil where high suitability areas for *D. bancroftii* were identified. Nearly 25% of the fern species in Colombia and Ecuador (on the western side of the Andes) do not occur on the eastern side (Moran 1995; 1996), suggesting the roles of those mountains as barriers to dispersal, although environmental contrast between the slopes may also play a significant role in shaping those floristic differences (Kessler 2010). However, winds interactions are complex, and even though most of the winds in the west-east direction are blocked by the Andes, it is still possible for some to extend in that direction. As such, further attention should be paid to test the affinities of wind dispersal of other South American species.

An alternative hypothesis is that despite the great number of spores produced, they are only scattered at short distances from the mother plant (Wolf et al. 1991; Penrod and McCormick 1996). That hypothesis was tested experimentally by Conant (1978) and Peck et al. (1990); Conant (1978) and Raynor et al. (1976) have found that it was likely that spores of herbaceous mid-sized terrestrial ferns were more quickly deposited in the humid forest understorey than released into longer-distance wind currents. Additionally, Gómez-Noguez et al. (2016) have measured spore density and demonstrated that the spores of *Gleichenella pectinata*, one the most widespread species of Gleicheniaceae, have about half of the weight of the spores of *D. bancroftii*, the genus with the narrowest range in the family in the Neotropics. Cousens et al. (2008) have demonstrated that, according to Stoke’s equation, spore mass is directly related to their buoyancy and terminal velocity. Therefore, spore mass may influence the ability of spores to join the wind currents – and therefore limit colonisation of suitable areas in the mountains of southeastern Brazil. Horn et al. (2001) have additionally pointed out that wind characteristics may play a greater role in the probability of a propagule being transported long distances than intrinsic propagule traits.

The establishment of individuals and populations also plays a crucial role in determining the distribution range of fern species, although biological factors such as competition and fungal associations have only been poorly investigated. Page (2002) has pointed out several features that could limit the ecological success of the long-distance colonisation by ferns, including the handicap of an independent gametophyte stage, slow plant growth rates, intolerance of widely fluctuating conditions. Page (2002) has also noted features favourable to long-distance colonisation, such as diverse phytochemical defences, high tolerance of substrates with acute nutrient disequilibrium, exploitation of mycotrophy, and the potential of polyploidy. Unfortunately, the lack of information about the biological and ecological interactions of Gleicheniaceae, associated with the absence of those variables in the different models, represent limitations for evaluating and determining the crucial conditions that shape the ability of ferns to successfully establish following spore dispersal.

The projected historical and present-day distributions of *D. bancroftii* included suitable areas across the Neotropics, including in the mountain ranges of eastern and northern Brazil, suggesting that a lack of suitable areas would not be a limiting factor to the range expansion of *D. bancroftii*. Additionally, past projections showed larger areas of suitability when compared to the present, although without any suitable areas connecting the Andes and the mountains of Brazil. The absence of connections may therefore not be the factor limiting its expansion, as LDD is rather common in ferns, with other neotropical genera of Gleicheniaceae having some widely distributed taxa. Paleopalynological data from the suitable areas identified in the present study are quite scarce, however, making it currently impossible to closely determine the exact past distribution of *D. bancroftii*. More comprehensive phylogenetic and biogeographic studies will be needed to investigate the possible origin area of the genus and to test the colonisation hypothesis of *Diplopterygium* in the neotropical region. More intensive collection efforts in the Brazilian Guiana Shield and surrounding regions will also be needed to validate the projections of the models presented here and uncover possible new records of the genus in Brazil.

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All authors made a substantial contribution in the concept and design of the study, to data collection, analysis and interpretation, writing and revising the manuscript, and adding intellectual content.

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Disclosure statement

No potential conflict of interest was reported by the authors.

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Table 1. Differences in the extent of modelled climatically suitable areas of *Diplopterygium bancroftii* in the last interglacial, last glacial maximum and in the Holocene, relative to the area projected in today's climate.

	Area (1000km^2)		
	Holocene	**LGM**	**LIG**
No occupancy	8,681	8,544	8,518
No change	6,226	7,704	7,392
Range reduction	5,276	3,799	4,110

Note. – Holocene = Mid-Holocene (6 ka Before Present - BP), LGM = Last Glacial Maximum (LGM – 21 ka BP), and IG = Last Interglacial (LIG – 130 ka BP). Total of suitability area in the modelled scenario to current (1960-1990) is 6,632 (1000 km^2). No occupancy corresponds to the areas in which the distribution model showed no climatic suitability areas to *D. bancroftii* occurrence; Range reduction presents how much of the suitability areas retracted when compared to the present, and No change represent how much of those areas had no change when compared to the present.

Figure 1. *Diplopterygium bancroftii*. A–C. Habit. D. Abaxial surface. E. Bud scales. F. Sori, detail. G. Vascular bundle. Photographs: Mattos, F.B.

Figure 2. The neotropical occurrence records used in the analyses.

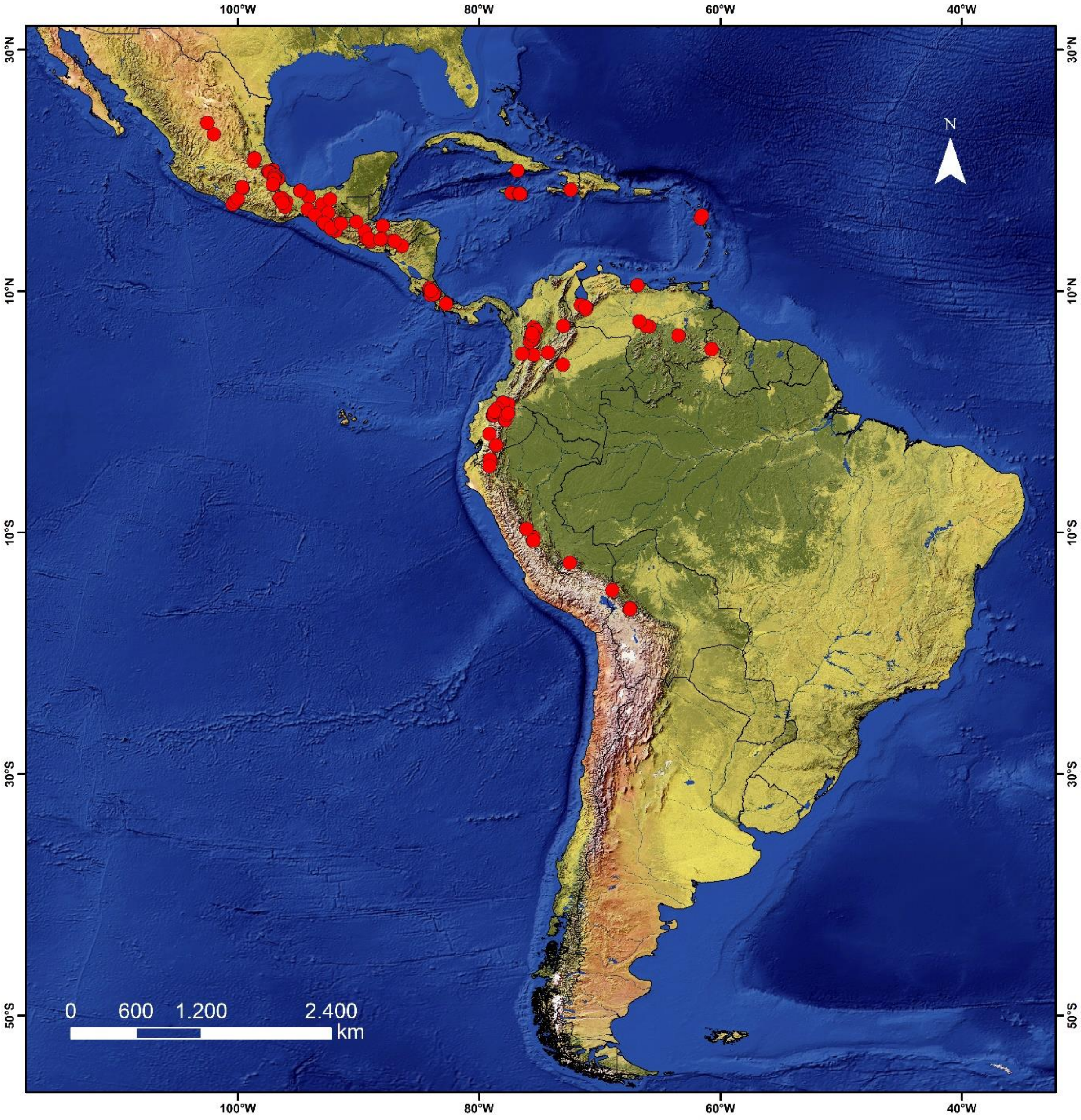

Figure 3. Map of climatic suitability for the current distribution of *Diplopterygium bancroftii* to the present, with a detail of the Guiana Shield showing suitable areas for its occurrence in Brazil.

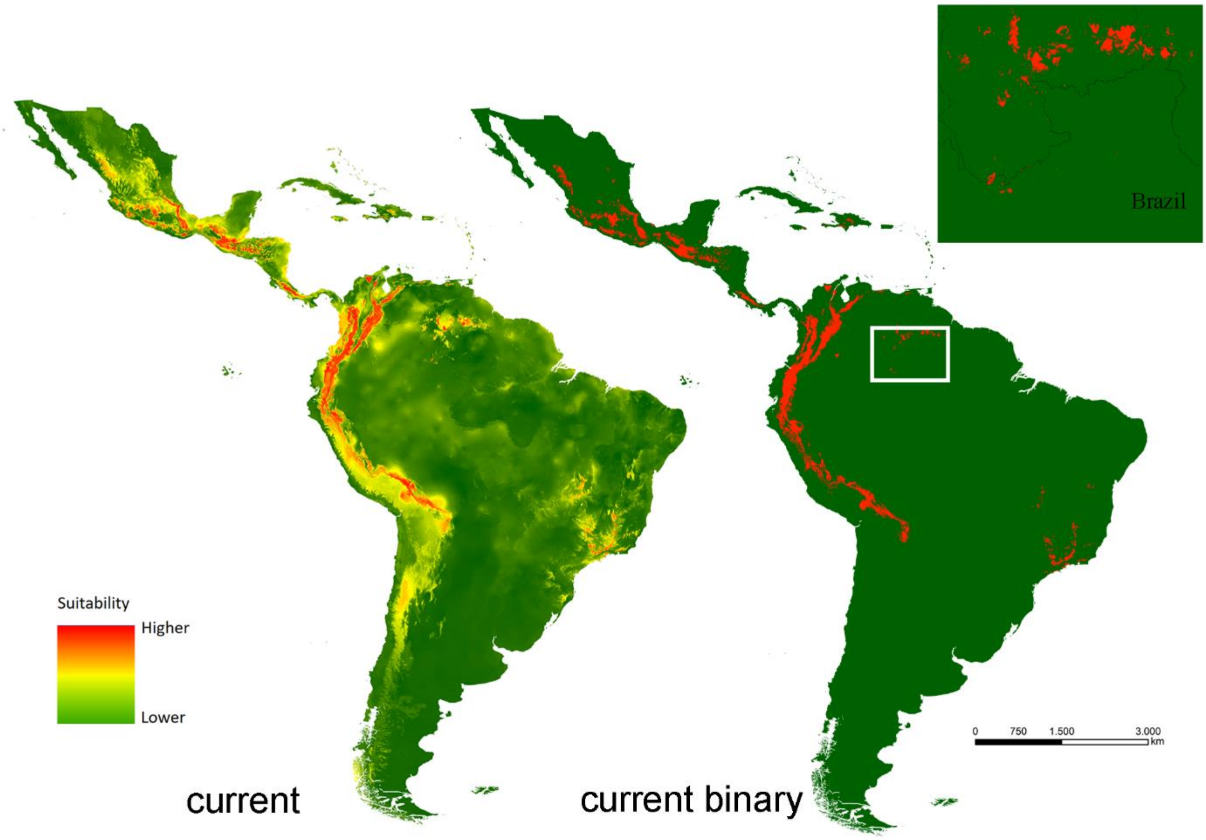

Figure 4. Ensemble maps showing variations in projected climatically suitable areas among algorithms and over time periods considered for *Diplopterygium bancroftii*.

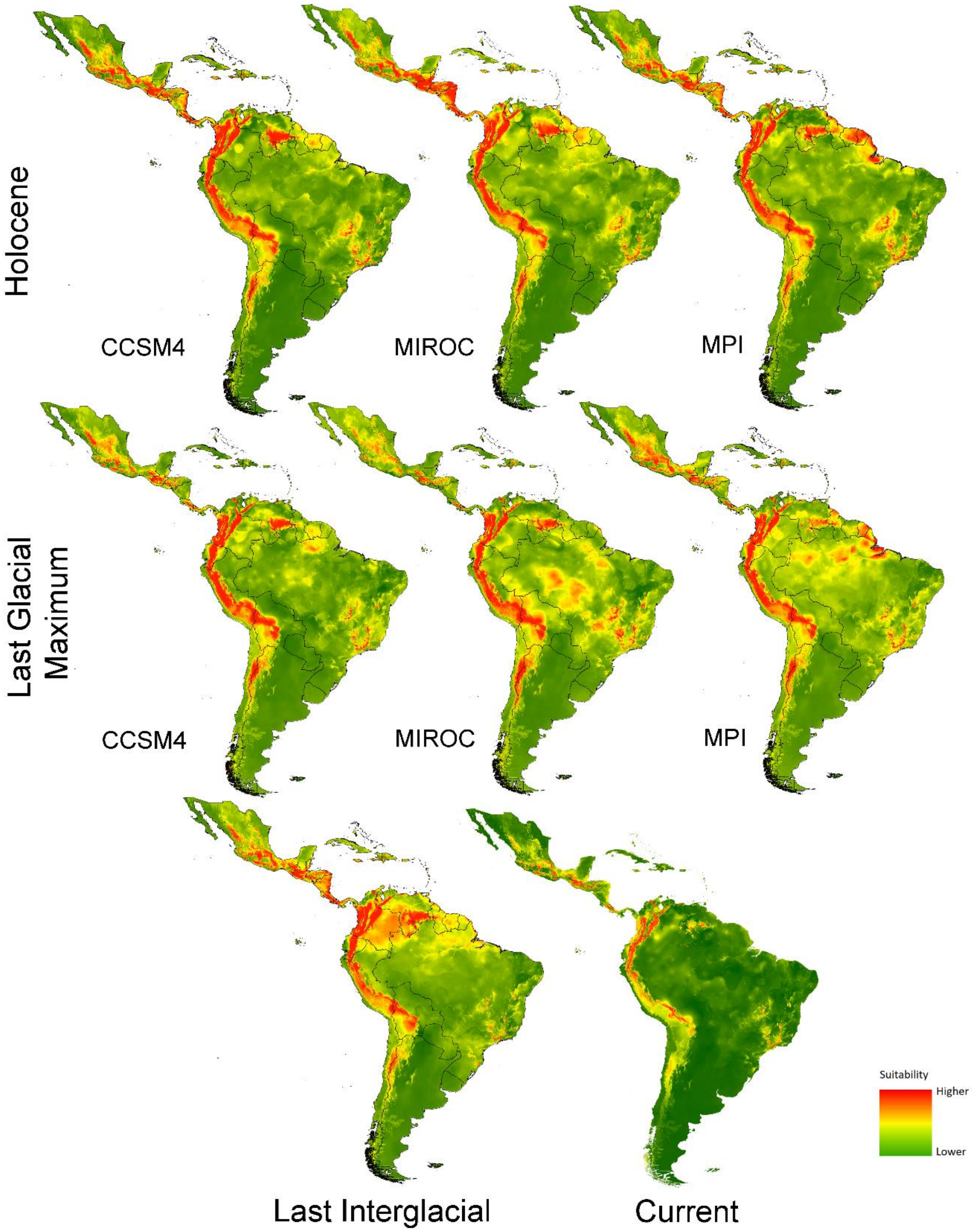

Figure 5. Binary maps showing the areas of climatic suitability for *Diplopterygium. bancroftii* at different times in different projections. **No change**: stable environmental suitability.

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State of the art in cytogenetics, insights into chromosome number evolution, and new C-value reports for the fern family Gleicheniaceae

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Abstract

Studies concerning the cytogenetics of Gleicheniaceae have been scarce, especially those employing evolutionary approaches. Two chromosome number evolutionary models have been hypothesized for Gleicheniaceae. One proposes that ancestral haploid numbers were small and that the chromosome numbers of extant species evolved through polyploidy. The other model proposes that, at the genus level, fern chromosome evolution occurred from ancestors with essentially the same high chromosome numbers seen in living lineages. Neither of those hypotheses has been tested based on phylogenetic frameworks. We sought to (i) present the state of the art of Gleicheniaceae chromosome numbers; (ii) test the two evolutionary models of chromosome numbers within a phylogenetic framework; (iii) test correlations between DNA contents and chromosome numbers in the family. We report here DNA C-values for five species, which increases the number of investigated taxa nearly twofold and report two new genera records. Ancestral state chromosome reconstruction corroborates the hypothesis that ancestral chromosome numbers in Gleicheniaceae were as high as those of extant lineages. Our results demonstrate the important role of dysploidy in the evolutionary chromosome history of Gleicheniaceae at the genus level and suggest that the relationship between chromosome number and DNA content does not appear to be linear.

INTRODUCTION

Ferns and lycophytes stand out among vascular plants for their distinct genomic evolutionary histories, with the conservation of high chromosome numbers in taxa with diploid gene expressions (Haufler 1987, 2002, 2014). Evidence shows that those plants underwent multiple cycles of polyploidy (whole-genome duplications - WGDs) (1KP 2019, Huang et al. 2020), with subsequent diploidization involving gene silencing, but without apparent chromosome losses, so that high chromosome numbers were retained (Haufler 2002, 2014). Some putative gene loss is possible, however, as Liu et al. (2019) demonstrated that the range of genome sizes in ferns arose not only from repeated cycles of polyploidy but also through clade-specific constraints governing DNA accumulation and/or loss.

Whole Genome Duplication played a major role in fern and lycophyte speciation (Wood et al. 2009) and influenced not only chromosome numbers but also genome sizes (*e.g.,* Klekowski & Baker 1966, Leitch & Leitch 2012, 2013, Barker, 2013, Henry et al. 2015). The DNA content showed high variability in ferns (Obermayer et al. 2002), ranging from 1C = 0.25 pg in *Salvinia cucullata* Roxb. ex Bory (Li et al. 2018) to 1C = 150.61 pg in *Tmesipteris obliqua* Chinnock (Hidalgo et al. 2017). Although a few fern lineages show exceptionally large (or very small) genomes, ferns are typically characterized by medium-sized genomes. They are distinctive as compared to other land plants, however, as the only group with a correlation between genome size and chromosome numbers (Nakazato et al. 2008, Clark et al. 2016).

Even with significant advances in molecular studies of ferns and lycophytes, our knowledge concerning DNA C-values and the genome sizes of those plants remains incipient when compared to angiosperms. The Plant DNA C-values Database (Leitch et al. 2019), for example, contains DNA C-value data for 12,273 species but cites only 246 ferns (about 0.2% of all fern species) and 57 lycophytes (about 4% of all lycophyte species).

Although recent studies have shed light on the evolution of fern genome sizes (Clark et al. 2016, Liu et al. 2019), sampling data is still scarce, and it will be important to expand fern genome size information to better understand their genomic evolution (Bennett & Leitch 1995, Bennett *et al.* 2000).

The first interpretation of the evolutionary significance of C-values in ferns was made by Obermayer *et al.* (2002), based on a well-supported phylogenetic hypothesis of vascular plants (Pryer *et al.* 2001). Clark et al. (2016) subsequently significantly increased the number of fern species with documented C-values, providing evolutionary significance to a well-supported phylogeny of the group. A positive correlation between the genome sizes of ferns and lycophytes and their chromosome numbers was observed (Nakazato et al. 2008, Obermayer *et al.* 2002). The fern family Gleicheniaceae was included in previous works, but it was represented only by five species distributed in two genera (Kuo & Li 2019, Clark et al. 2016).

In that context, genome size studies, together with the analysis of chromosome numbers represent important steps for genetic variation studies, phylogenetics, taxonomy, and evolution, and for understanding genome structure and diversity (*e.g.*, ploidy levels and expression, and nuclear architecture). Studies dealing with the cytology of Gleicheniaceae have been scarce (*e.g.*, Walker 1966, 1973, 1990, Mickel et al. 1966, Löve 1976, Tindale & Roy 2002), especially those employing evolutionary approaches. Sorsa (1968) proposed an evolutionary model for chromosome numbers in Gleicheniaceae and hypothesized two ancestor haploid numbers in the family (n=17 and n=11) from which all extant species evolved by polyploidy. A different point of view about chromosome number evolution in ferns, however, was proposed by Duncan & Smith (1978). Those authors hypothesized that, at the generic level, fern evolution occurred from ancestors that had essentially the same high chromosome numbers observed in living ferns. Neither of those hypotheses has been tested within a phylogenetic framework, and more studies are therefore needed to increase our knowledge of fern cytogenetics and test possible evolutionary patterns within a phylogenetic framework.

We, therefore, sought to (i) review what is already known about chromosome numbers in Gleicheniaceae; (ii) test the hypotheses of Sorsa (1968) and Duncan & Smith (1978) regarding ancestral chromosome numbers at the genus level in Gleicheniaceae; and (iii) report new DNA C-values for the family, increase taxa sampling, and evaluate the relationships between DNA content and chromosome numbers.

MATERIALS AND METHODS

Chromosome numbers and ancestral state reconstructions

Chromosome numbers for Gleicheniaceae were obtained from the EyeChrom online database (Rivero et al. 2019) and through an extensive literature review. The phylogenetic hypothesis used to infer chromosome ancestral state reconstructions was generated based on a data matrix of three plastid genome regions (*atpA*, *atpB*, and *rbcL*) available at GenBank (Table I). The data was assembled and aligned using MUSCLE, implemented in MEGA X (Kumar et al. 2018), and the best-fitting model of molecular evolution was determined using jMODELTEST v.2.1.4 (Darriba et al. 2012) based on Bayesian information criterion (Schwarz 1978). Bayesian inference was used to estimate a tree using MRBAYES v.3.2 (Ronquist et al. 2012), treating each region as a separate partition. The analysis consisted of two independent runs, with four simultaneous Markov chains running three million generations, with a random starting tree, at a temperature of 0.2, and sampling one tree every 100 generations. Convergence was verified by examining ESS (effective sample size) and PSRF (potential scale reduction factor) using TRACER v.1.6 (Rambaut *et al.* 2014), with a 10% burn-in. The remaining trees were used to assess topology in a strict consensus.

The basal chromosome number was inferred using ancestral state reconstruction in CHROMEVOL v.2.0 (Glick & Mayrose 2014). Character states were optimized using a model assuming a constant rate of chromosome gain, loss, and duplication, along with an estimated rate of no duplication (as that model was selected based on the output of the initial analyses with 10 models of chromosome evolution and chosen using Akaike information criteria).

DNA C-values

Details and vouchers of the five species studied in the present work are presented in Table II. We used flow cytometry to estimate the DNA C-values of the species. Approximately 20 to 30 mg of young and fresh leaves of each species studied and the same amount of young leaf tissue from the internal reference standard (*Pisum sativum*, 9.09 pg) was chopped into ice containing 1 mL WPB buffer solution (0·2 M Tris.HCl, 4 mM $MgCl_2.6H_2O$, 2 mM EDTA $Na_2·2H_2O$, 86 mM NaCl, 10 mM sodium metabisulfite, 1 % PVP-10, 1 % (v/v) Triton X-100, pH 7·5) (Galbraith et al. 1983, Dolezel et al. 1998, Loureiro et al. 2007). The suspension was filtered through a 50-µm mesh and stained with 25 µL propidium iodide (10 mg L−1) (Sigma-Aldrich, USA) supplemented with 2.5 µL RNAse (20 mg L−1). For each run, at least 10,000 nuclei were analyzed per sample on a CytoFLEX cytometer (Beckman Coulter, USA). Histograms and statistical analyses were obtained using CytExpert Software version 2.0.1. DNA content was estimated using the G1 peak position of the internal standard as a reference following Dolezel & Bartos (2005). A Pearson correlation test between chromosomal numbers and DNA contents was performed using RStudio (2020).

RESULTS

Gleicheniaceae comprises approximately 120 species (PPG 2016), although chromosome numbers have been counted for only 37 species (23%) (Table III). Of the seven genera currently accepted for the family, six have at least one species with a known chromosome count (except *Rouxopteris* H.M. Liu). The lowest haploid number found in the family was n=20 (in some *Gleichenia* species), and the highest was n= 80 (*Dicranopteris linearis*) (Table III). *Sticherus*, a genus comprising approximately 94 species, has chromosome data available for only nine species (9.5%), although representing 42% of all chromosome counts recorded for the family (Figures 1 and 2). Data is available for five species of *Gleichenia* (40% of the recognized species in the genus), four species of *Diplopterygium* (16%), and three species of *Dicranopteris* (15%), as well as for the monotypic genera *Gleichenella* and *Stromatopteris* (Table III). The haploid number for most of the studied species of *Sticherus* is n=34; some species (*e.g., S. tenera, S. urceolatus, S. interjectus, S. jamaicensis*, and *S. revolutus*) showed n=68. *Gleichenia* showed two different haploid counts among the species investigated. The most common was n=20 (17 specimens), followed by n=22 (3 specimens). *Gleichenia microphylla* showed both chromosome counts (n=22 and n=20) in different populations

(Figure 1, Table III). Only three species of *Diplopterygium* have had their chromosome numbers investigated: *D. bancroftii*, *D. farinosum*, and *D. glaucum* all showed n=56, while *D. longissimum* showed n=20. *Dicranopteris* showed different haploid counts, including n=78 (45% of the counts), n=39 (34%), n=68 (7%), n=80 (7%), and n=40 (3%) (Table III, Figure 1). *Gleichnella pectinata* showed two different haploid counts in the specimens investigated (n=43 and n= 44). *Stromatopteris* (a lineage represented by a single species confined to New Caledonia) showed n=39, although by only a single chromosome count (Table III, Figure 1).

The tree resulting from phylogenetic inference agrees with the topology recovered by Liu et al. (2020) and PPG I (2016) (Figure 2). Two different clades were recovered, one with *Rouxopteris* as the sister group of a clade formed by *Diplopterygium* as the sister group of *Dicranopteris*+*Gleichenella*. The other clade is composed of *Sticherus* as sister to the clade including *Stromatopteris*+*Gleichenia*. The basal node of Gleicheniaceae had its ancestral chromosome number recovered as n=46, while the clade including *Rouxopteris*, *Diplopterygium*, *Dicranopteris*, and *Gleichenella* was recovered with n=48. In the clade including *Diplopterygium*, *Dicranopteris*, and *Gleichenella* the ancestral number recovered was n=51, while the clade *Dicranopteris*+*Gleichenella* showed n=45 (Figure 2). In the clade including *Sticherus*, *Stromatopteris*, and *Gleichenia* the ancestral number recovered was n=42, while in *Stromatopteris+Gleichenia* the number recovered was n=40.

Regarding DNA contents in Gleicheniaceae, we increased here the sampled species in the family by eight, reporting five new c-values: *Gleichenella pectinata* (2C=4.49), *Sticherus bifidus* (2C=10.90), *Sticherus gracilis* (2C=6.48), *Sticherus lanuginosus* (2C=10.77), and *Sticherus nigropaleaceus* (2C=18.32) (Table II). We, therefore, report the C-values for two genera for the first time: *Sticherus* and *Gleichenella* (Table II). Despite the low sampling of C-values in Gleicheniaceae, the correlation coefficient between chromosome numbers and DNA content was 0.47 (Supplementary Material 1).

DISCUSSION

Chromosome counts

Polyploidy events are common in ferns and have likewise been observed in Gleicheniaceae, as was hypothesized by Sorsa (1968), Duncan & Smith (1978), and later by Haufler (2002, 2014). *Sticherus* has straightforward examples of polyploidy. That family showed only two haploid numbers among the species studied (n=34 and n=64) (*e.g.*, Walker & Ortega 1992, Walker 1966, 1990, Brownlie 1958, 1963, 1961, Tindale & Roy 2002). *Sticherus tenera* showed different haploid numbers in different populations (34 and 68) (Tindale & Roy 2002, Thrower 1963), and it may represent a species with different diploid and polyploid cytotypes.

In addition to polyploidy, other events can induce chromosome number variations (either increasing or decreasing them), including aneuploidy and dysploidy, which may have played important roles in Gleicheniaceae chromosomal evolution. When one or more chromosomes are lost or gained by aneuploidy, there will presumably be deletions or duplications of many genes – resulting in unbalanced, lethal, or sub-vital constitutions, so that those types of chromosome number variations have no apparent evolutionary meaning (Guerra 2008). Dysploidy, on the other hand, can induce increases or decreases in haploid chromosome numbers without resulting in unbalanced or lethal constitutions (Friebe et al. 2005).

Polyploidy seems to be rather common in *Dicranopteris*, as it showed counts of n = 39 and n = 78 (Mehra & Singh 1956, Roy & Singh 1975, de Lange et al. 2004, Löve 1976, Walker 1973). Other haploid counts, however, have been found in the genus, such as n=40 (Manton & Sledge 1954). Both dysploidy and polyploidy events may have played roles in evolutionary changes in the chromosome numbers of that genus of Gleicheniaceae. One population of *Dicranopteris linearis* investigated showed n=40, and two others showed n=80. As n=39 is one the most frequent haploid number found in the genus, ascending dysploidy followed by polyploidization could explain those haploid numbers. *Dicranopteris flexuosa* also showed a possible case of dysploidy, with a chromosome decrease, with two specimens from different populations showing n=68 (Araujo in Love

1976). We hypothesize that from n=39, a dysploidy event occurred, resulting in a chromosome decrease and a count of n=34, followed by a polyploidy event resulting in individuals with n=68.

Another possible dysploidy series was observed in *Gleichenia*, with n=20 and n=22 (*e.g.*, Walker & Ortega 1992, Brownlie 1958, Tindale & Roy 2002), especially in *G. microphylla*, which shows populations with both haploid counts (Brownlie 1961, 1963). Similarly, *Gleichenella pectinata*, a widespread neotropical species, may also present cases of dysploidy series. Sorsa (1968) observed 44 chromosomes in four specimens from three different localities in Porto Rico, but also found populations with n=43. Those different chromosome counts were similarly reported in specimens from different populations in Jamaica (Walker 1966), Trinidad (Jermy & Walker 1985), and Mexico (Smith & Mickel 1977).

In addition to dysploidy events, another possible explanation for the variations seen in *Gleichenella* and *Gleichenia* would be the presence of B chromosomes – which are supernumerary, usually with preferential heritage, deviating from the usual Mendelian segregation (Houben 2017). There is no evidence to date, however, which could confirm the existence of B chromosomes in Gleicheniaceae, and more cytogenetics studies will be needed to test that possibility.

Additional cases of haploid numbers in Gleicheniaceae remain unexplained, such as in *Diplopterygium*. That genus has had only four species investigated, with three showing n=56, and *D. longissimum* showing n=20 (Mickel et al. 1966, Mehra & Singh 1956), which could be explained by dysploidy and polyploidy events (or may represent chromosome miscounts). Further attention should therefore be paid to *D. longissimum,* as its chromosome count is quite discrepant when compared to the other species analyzed.

Ancestral state reconstruction

The ancestral state reconstruction (Figure 2) recovered by the best-fitting model corroborates the hypothesis of Duncan & Smith (1978) that the ancestral chromosome numbers in Gleicheniaceae were as high as those of extant lineages. The ancestral chromosome number recovered in the first clade was 51 (Figure 2) in the node of *Diplopterygium* and *Dicranopteris*+*Gleichenella*. In that case, we hypothesize that ascendant dysploidy events resulted in a lineage with a basic chromosome number n=56, represented by the genus *Diplopterygium*. Despite low sampling in that genus, the chromosome counts were constant (n=56) among the investigated species, which may represent stability through the chromosomal evolutionary history of the genus.

The ancestral chromosome number recovered in other genera in that clade (*Dicranopteris* and *Gleichenella*) was n=45. *Dicranopteris* showed five different chromosome number counts (n=39, n=40, n=68, n=78, and n=80). Subsequent chromosome decreases would have to be assumed in a scenario with an ancestral number of n=45. In both cases, populations with n=80 and n=78 may have arisen through polyploidy. The second and less frequent count was n=68 (Table III), which could have resulted from an autopolyploidization event in a population having n=34. No population of *Dicranopteris* has yet been found with n=34, but that does not exclude the possibility of additional chromosome losses followed by subsequent autopolyploidization. More species and populations need to be sampled to construct a better panorama of the evolutionary history of chromosome numbers in *Dicranopteris*. As mentioned above, *Gleichenella* showed two different chromosome counts (n=44 and n=43). The ancestral chromosome number recovered (n=45) suggests a trend of chromosome loss in the lineage.

Regarding the second clade, ancestral character reconstruction showed ancestral numbers as high as those of extant lineages. A significant reduction in chromosome numbers (from n=42 to n=34) was observed in *Sticherus* as compared to the ancestral number recovered; additionally, no evidence of dysploidy was observed in the genus, only cases of autopolyploidy.

The clade formed by *Stromatopteris*+*Gleichenia* has a hypothetical ancestral chromosome number n=40, which implies a reduction of one chromosome in the former genus. Two haploid numbers have been reported in *Gleichenia* (n=20 more frequently, and n=22 less frequently). We hypothesize that there was a reduction by half of the total number of ancestral chromosomes, in this case, resulting in n=20; the chromosome count of n=22 might be the consequence of a subsequent event of ascending dysploidy, as has been documented in other fern genera (*e.g.*, by Wang et al. 2010 in *Lepisorus* [Polypodiaceae], and by Bellefroid et al. 2010 in *Asplenium* [Aspleniaceae]).

The same chromosome number may have independently appeared twice in different genera. Although the haploid number of *Stromatopteris* is the same as one of the haploid numbers of *Dicranopteris* (n=39) (Bierhorst 1968), it may not represent a homologous condition, as *Stromatopteris* is placed in a different clade with *Gleichenia* (n=20 and n=22) and *Sticherus* (n=34 and n=64). Thus, additional studies will be required focusing on fern cytology, and evolutionary patterns will need to be examined in the light of phylogenetic studies. Further attention must also be paid to *Gleichenia,* as its monophyly is still questionable due to low sampling in phylogenetic analyses.

C-values

Chromosome numbers alone are not sufficient to fully understand the evolutionary cytogenetics of ferns. Liu et al. (2019) demonstrated, using *Asplenium* (Aspleniaceae) as a model, that the evolution of fern genome sizes is not shaped solely by chromosome number changes arising from polyploidy, but also by constraints on the average quantity of DNA per chromosome. The differences in DNA contents observed in different lineages may be related to chromosome size, and not necessarily to ploidy levels. We, therefore, examined the DNA contents of five Gleicheniaceae species and present here, for the first time, C-values for two Gleicheniaceae genera, *Sticherus* and *Gleichenella*, and likewise increased the number of sampled species in the family to eight by reporting new c-values for five species (Table II, Figure 3).

The differences in DNA contents observed among *Sticherus* species could be related to chromosome size, and not just ploidy levels. We observed 3-fold variations in the DNA contents of the four *Sticherus* species examined, which ranged from 6.48 pg in *Sticherus gracilis* to 18 pg in *Sticherus nigropaleaceus*. The DNA contents of *Sticherus lanuginosus* (10.77 pg) and *S. bifidus* (10.9 pg) were similar and may be good examples of the chromosome number stability observed in the genus. The difference in the DNA content of *S. nigropaleaceus,* as compared with the other species of the genus so far investigated, may represent a case of polyploidy. Although no chromosome counts have so far been made for *S. gracilis,* its DNA content may be related to chromosome size, as the chromosome numbers in *Sticherus* usually are stable (Table III), with few cases of polyploidy (18%).

Despite the low sampling of C-values in Gleicheniaceae, our results indicate that chromosome numbers and DNA contents in Gleicheniaceae may be uncorrelated. *Gleichenella* showed the lowest DNA content in the family (4.49 pg) and has n=44, while *Sticherus,* which usually shows n=34, had the highest DNA content values, ranging from 6.48 pg to 18.32 pg. The DNA contents of *Diplopterygium bancroftii* (n=56) and *Dicranopteris linearis* (n=39) are similar (6.51 pg), which may be related to the lack of correlation between DNA content and chromosome numbers in the family; further attention must be given to *Stromatopteris, Rouxopteris,* and *Gleichenia*. Despite low sampling in the family, our results are close to the projections made by Clark et al. (2016), who estimated the mean of DNA content of Gleicheniales to be 10 pg.

CONCLUSIONS

Our chromosome ancestral state reconstructions corroborate the hypothesis that the ancestral chromosome numbers in Gleicheniaceae were as high as those now seen in extant lineages. The duplication of whole chromosome numbers (polyploidy), as well as the dysploidy series, appear to have played important roles in Gleicheniaceae chromosome evolution. We emphasize here the importance of cytogenetic studies as well as the need for more chromosome counts and DNA content data for the Gleicheniaceae (together with better-resolved phylogenetic inferences) to elucidate chromosome evolution in the group. The analysis of DNA C-values suggests that chromosome numbers and DNA contents may not be correlated in Gleicheniaceae, but an expanded sampling of DNA C-values and chromosome counts will be needed to verify that hypothesis.

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Author contributions

L.V. Lima: Contributions to the conception and design of the study, data collection, data analysis and interpretation, preparation of the manuscript draft, contribution to the critical revision, and the addition of intellectual content.

S. M. Souza: Contributions to data collection, analysis and interpretation, manuscript preparation, critical revision, and the addition of intellectual content.

T. E. Almeida: Contributions to manuscript preparation, critical revision, and the addition of intellectual content.

A. Salino: Contribution to manuscript preparation, critical revision, and the addition of intellectual content.

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Figure 1. Distribution of the chromosome numbers in the sampled species.

Figure 2. Phylogenetic inference with ancestral chromosome number reconstructions. Bayesian strict consensus tree, inferred from three plastid markers (*atpA*, *atpB*, and *rbcL*) (* indicates posterior probability equals 1.0; ** *Rouxopteris* is recently segregated genus from *Gleichenia* and it has no chromosome counts to the date). Pie charts showing the frequency of chromosome numbers in each genus.

Figure 3. Cytometry Histograms. **a.** *G. pectinata* (CV=4.47, Sd= 0.3). **b.** *S. bifidus* (CV= 5.01, Sd= 0.3). **c.** *S. gracilis* (CV= 4.7%, Sd= 0.4). **d.** *S. lanuginosus* (CV= 4.3, Sd= 0.4). **e.** *S. nigropaleaceus* (CV= 4.7, Sd= 0.2) *Internal control (*Pisum sativum*). Sd= Standard deviation.

Table I. GenBank accessions for the phylogenetic framework.

Table II. Gleicheniales species with C-values reports. Family names follow PPG I (2016).

Table III. Chromosome numbers in Gleicheniaceae. CN= Chromosome number.

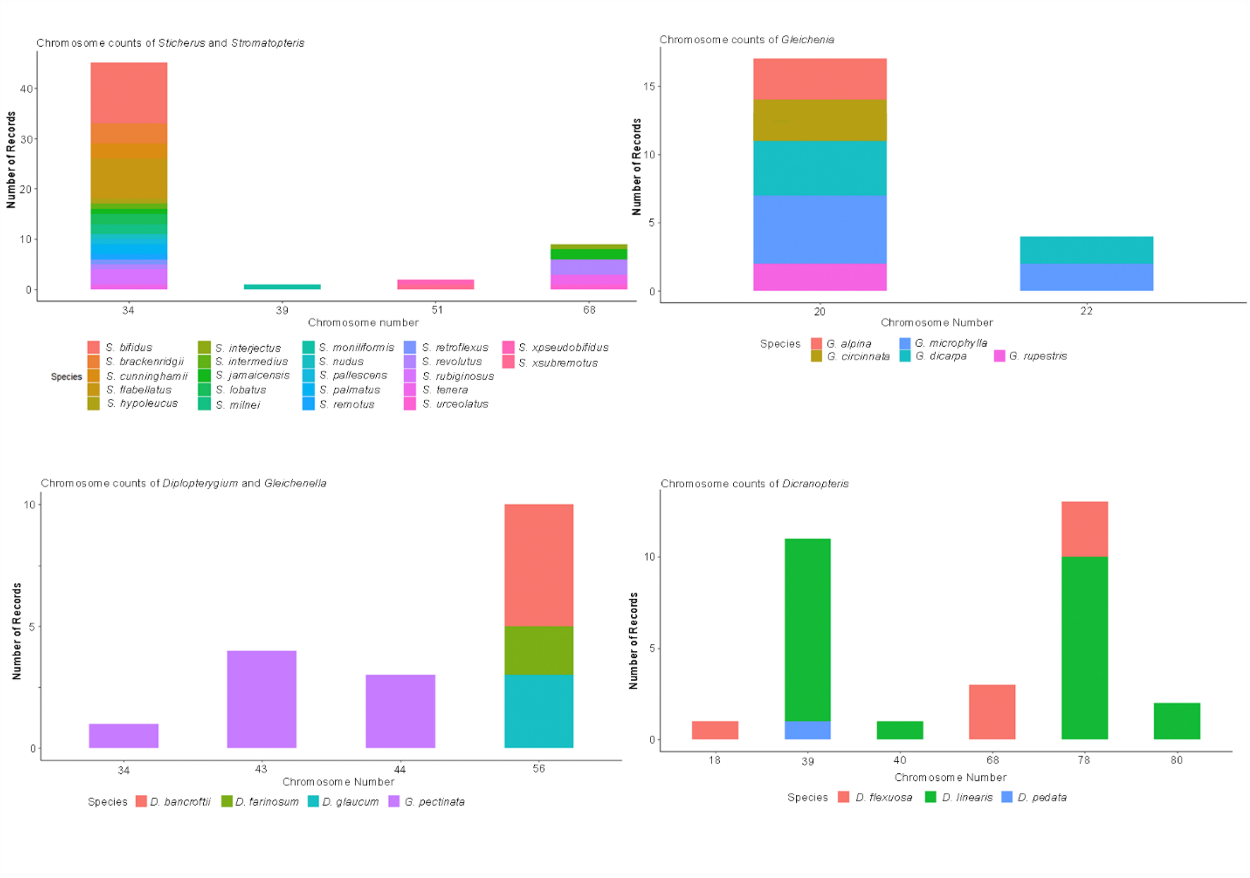
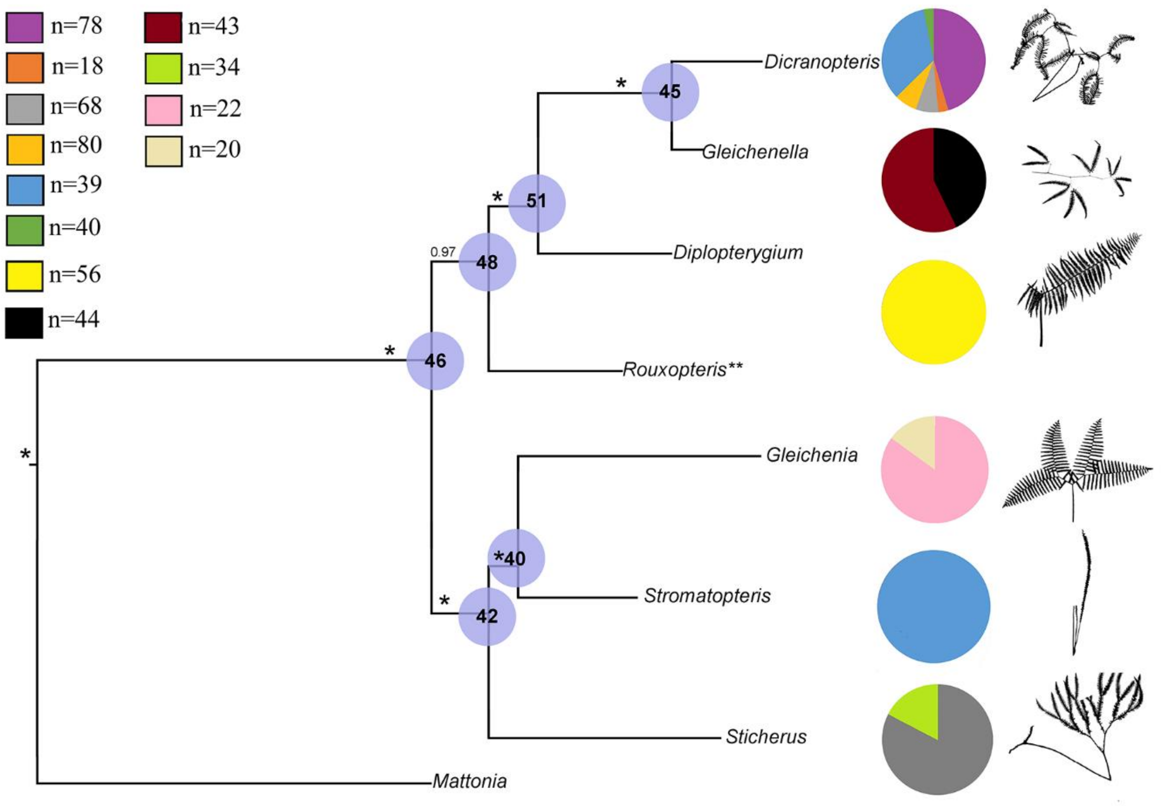


Figure 1. Distribution of the chromosome numbers in the sampled species.



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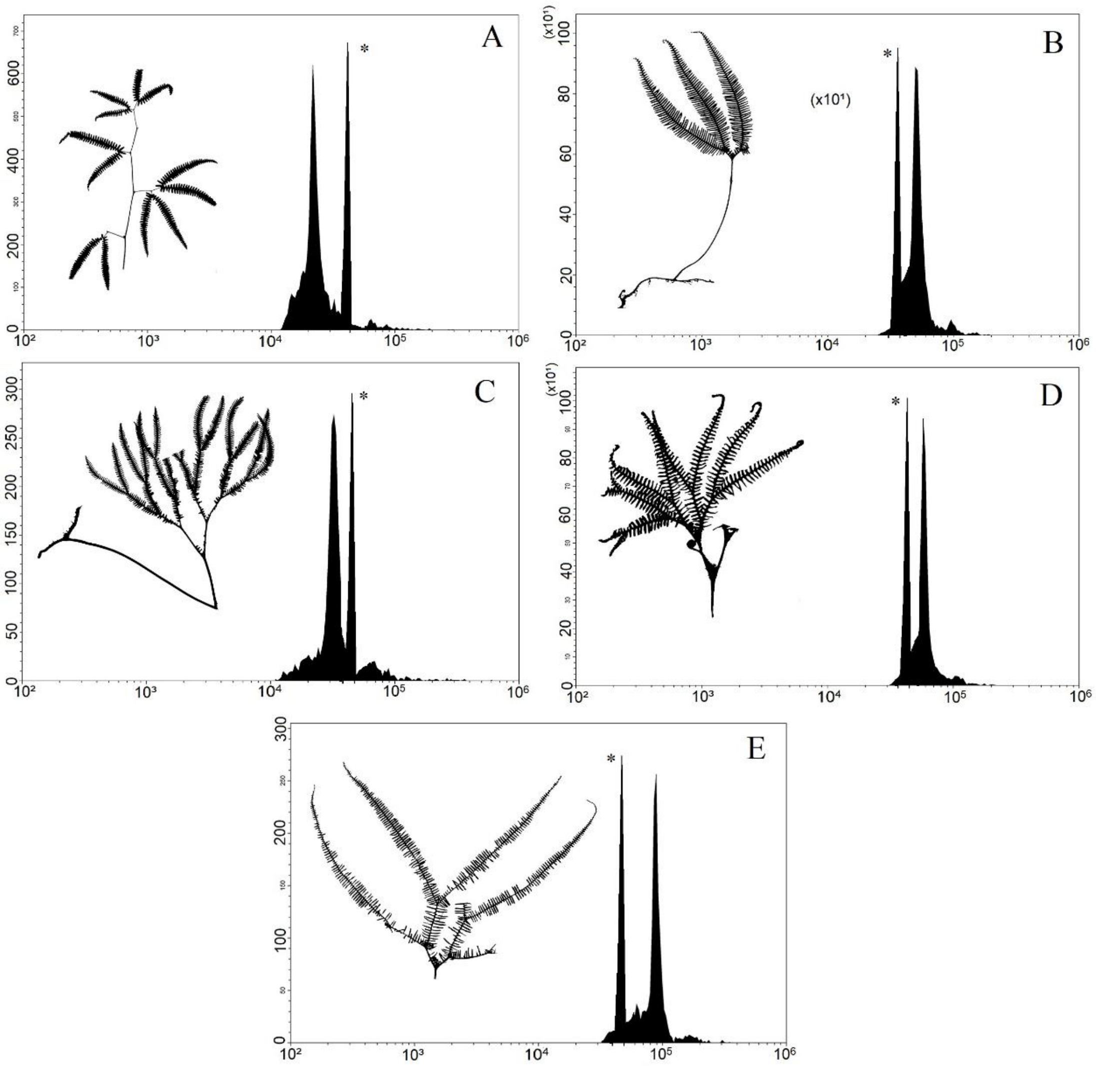

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Table I. GenBank accession for phylogenetic framework.

Taxa	***rbcl***	***atpA***	***atpB***
Matonia pectinata R. Br.	EU352307	EF463789	EF588716
Dicranopteris linearis (Burm. f.) Underw.	KU936634	DQ390557	AY612694
Diplopterygium bancroftii (Hook.) A.R. Sm.	EF463224	DQ390558	EF588713
Gleichenella pectinata (Wild.) Ching	EF588693	EF588671	AY612697
Gleichenia dicarpa R. Br.	AF313584	EF463736	AF313550
Sticherus bifidus (Wild.) Ching	EF463226	EF463737	EF463447
Stromatopteris moniliformis Mett.	AY612685	DQ390578	EF463448
Rouxopteris boryi (Kunze) H.M. Liu	KF992488	-	-

Table II. Gleicheniales species with C-values reports. Family names follow PPG I (2016).

Families	***Taxa***	***2C***	***1C***	***Voucher***	***Reference***
Dipteridaceae	*Dipteris chinensis* Christ	4.85	2.43	HM Liu s.n.	Clark et al. 2016
Dipteridaceae	*Dipteris conjugata* Reinw.	4.90	2.45	Schuettpelz 770	Clark et al. 2016
Gleicheniaceae	*Diplopterygium blotianum* (C.Chr.) Nakai	3.90	1.95	Kuo 4408	Kuo & Li 2019
Gleicheniaceae	*Diplopterigyum glaucum* (Thunb. Ex Houtt.) Nakai	4.50	2.25	Kuo 4408	Kuo & Li 2019
Gleicheniaceae	*Diplopterygium chinensis* (Rosesnt.) DeVol	3.90	1.95	Kuo 4410	Kuo & Li 2019
Gleicheniaceae	*Diplopterygium bancroftii* (Hook.) A.R. Sm.	6.51	3.26	HM Liu s.n.	Clark et al. 2016
Gleicheniaceae	*Dicranopteris linearis* (Burm. f.) Underw.	6.41	3.21	M. Christenhusz 7200	Clark et al. 2016
Gleicheniaceae	*Dicranopteris flexuosa* (Schrad.) Underw.	9.16	4.58	LV Lima 66	Perez et al. 2021
Gleicheniaceae	*Gleichenella pectinata* (Willd.) Ching	4.49	2.24	LV Lima 62	Present study
Gleicheniaceae	*Sticherus bifidus* (Wild.) Ching	10.90	5.45	LV Lima 90	Present study
Gleicheniaceae	*Sticherus gracilis* (Mart.) Copel.	6.48	3.24	LV Lima 212	Present study
Gleicheniaceae	*Sticherus lanuginosus* (Fée) Nakai	10.77	5.38	LV Lima 64	Present study
Gleicheniaceae	*Sticherus nigropaleaceus* (Sturm) J. Prado & Lellinger	18.32	9.16	LV Lima 94	Present study

Table III. Chromosome numbers in Gleicheniaceae. CN= Chromosome number

Species	**CN(n)**	**Reference**
Dicranopteris linearis (Burm. f.) Underw.	39,78, 80	Mehra & Singh (1956), Roy & Singh (1975), de Lange et al. (2004)
Dicranopteris flexuosa (Schrad.) Underw.	18, 68, 78	Löve (1976), Walker (1973), Sorsa (1966), Sorsa (1968)
Dicranopteris pedata (Houtt.) Nakaike	78	Nakato (1988)
Diplopterygium bancroftii (Hook.) A.R.Sm	56	Mickel et al. (1966)
Diplopterygium farinosum (Kaulf.) Nakai	56	Mickel et al. (1966)
Diplopterygium glaucum (Thunb. ex Houtt.) Nakai	56	Mehra & Singh (1956)
Diplopterygium longissimum (Blume) Nakai	20	Fabbri (1963)
Gleichenia alpina R.Br.	20	Tindale & Roy (2002)
Gleichenella pectinata (Willd.) Ching	43, 44	Walker & Ortega (1992), Jermy & Walker (1985), Sorsa (1968)
Gleichenia circinata (Sw.) C.Chr.	20	Brownlie (1958)
Gleichenia dicarpa R.Br.	22	Brownlie (1963)
Gleichenia microphylla (R.Br.) C.Chr.	20, 22	Brownlie (1961, 1963)

Gleichenia rupestris R.Br.	20	Tindale & Roy (2002)
Sticherus bifidus (Willd.) Ching	34	Walker & Ortega (1992)
Sticherus brittonii (Maxon) Nakai	34	Walker & Ortega (1992)
Sticherus brackenridgii (E.Fourn.) H.S.John	34	Brownlie (1965)
Sticherus cunninghami (Hew ex Hook.) Ching	34	Brownlie (1958)
Sticherus flabellatus (R.Br.) H.St.John	34	Brownlie (1961)
Sticherus furcatus (L.) Ching	34	Walker (1966, 1990)
Sticherus hypoleucus (Sodiro) Copeland	34	Walker (1990)
Sticherus interjectus (Jermy & T.G.Walker) J.Gonzales	68	Jermy & Walker (1985)
Sticherus intermedius (Baker) Chrysler	34	Walker (1990)
Sticherus jamaicensis (Underw.) Nakai	68	Walker (1966, 1990)
Sticherus lobatus N.A.Wakef.	34	Tindale & Roy (2002)
Sticherus nudus (Moritz) Nakai	34	Walker & Ortega (1992)
Sticherus pallescens (Mett.) Vareschi	34	Walker & Ortega (1992)
Sticherus remotus (Kaulf.) Chrysler	34	Jermy & Walker (1985)
Sticherus retroflexus (J.Bommer ex Christ) Copeland	34	Walker (1990)

Sticherus revolutus (Kunth) Ching	68	Walker (1990), Walker & Ortega (1992)
Sticherus rubiginosus (Mett.) Nakai	34	Walker & Ortega (1992)
Sticherus strictissimus (Christ) Copeland	34	Walker (1990)
Sticherus tenera (R. Br.) Ching	34, 68	Tindale & Roy (2002), Thrower (1963)
Sticherus urceolatus M. Garrett, Kantvilas & Laws	68	Tindale & Roy (2002)
Sticherus milnei (Baker) Ching	34	Holttum & Roy (1965)
Sticherus × *pseudobifidus* (Jermy & T.G.Walker) J.Gonzales	51	Jermy & Walker (1985)
Sticherus × *subremotus* (Jermy & T.G.Walker) J.Gonzales	51	Jermy & Walker (1985)
Stromatopteris moniliformis Mett.	39	Bierhorst (1968)

Capítulo 4: Artigo publicado no periódico American Fern Journal.
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SHORTER NOTES

Nesting Spores in a Cave: First Anatomical Characterization of *Gleichenia polypodioides* (Gleicheniaceae) Soral Chamber.—The taxonomy of Gleicheniaceae has passed through several changes recently (Gonzales and Kessler, Phytotaxa 31(1):1–54. 2011). The monophyly of some genera has not been elucidated yet due to poor sampling, and rare phylogenetic studies were made so far (PPG I, Journal of Systematic and Evolution 54(6):563–603. 2016). Currently, six genera are accepted in the family, including the paleotropical *Gleichenia* Sm. Initially, this genus was circumscribed in a large concept, including all species currently placed in *Diplopterygium* (Diels) Nakai, *Dicranopteris* Bernh, *Gleichenella* Ching, and *Sticherus* C. Presl (Gonzales and Kessler, 2011). However, molecular and morphological studies supported the segregation of those genera from *Gleichenia* (Copeland, Annales Cryptogamici et Phytopathologici 5:1–2471. 1947); Nakai, Bulletin of the National Science Museum 29: 1–71. 1950; Pryer *et al.*, American Journal of Botany 91:1582–1598. 2004; Li *et al.* Acta Palaeontologica Sinica 49:64–72. 2010). Nowadays, *Gleichenia* comprises about 11 species with paleotropical distribution, from which three occur in Africa, and the others are distributed through South East Asia, Malesia, Australia, New Caledonia, and New Zealand (Perrie *et al.*, New Zealand Journal of Botany 50(4):401-410. 2012). *Gleichenia* is characterized by buds and rhizomes covered with scales, pinnate-pinnatifid ultimate branches with reduced rounded segments, and tetrahedral, trilete spores. In addition, two species of *Gleichenia* have the sori arranged in deep depressions, a rare character among the ferns: the African *Gleichenia polypodioides* (L.) Sm., and *Gleichenia inclusisora* Perrie, L.D.Sheph. & Brownsey, from New Zealand (Perrie *et al.*, 2012). Those species have the sori usually composed by three sporangia, rarely four, enclosed in a deep depression (named here as soral chamber), convex toward the abaxial leaf surface, with the sporangia stomia facing the chamber opening. Moreover, some other ferns have sori in depressions, such as

Lellingeria depressa (C.Chr.) A.R.Sm. & R.C.Moran - Polypodiaceae (Labiak & Prado, Revista Brasileira de Botânica 28: 1–22. 2005) and *Amauropelta soridepressa* (Salino & V.A.O. Dittrich) Salino & T.E.Almeida -Thelypteridaceae (Salino & Dittrich, American Fern Journal 98: 199–201. 2008). However, these depressions are diminute and the sporangia are totally exposed. Although the presence and general morphology of the soral chamber has been previously reported, not much is known about the anatomy and development. Here, we provide the first anatomical diagnosis of this unique sori morphology in Gleicheniaceae. Regarding the Gleicheniaceae leaf terminology, we followed Andersen and Øllgaard (American Fern Journal 86: 52–57. 1996), with modifications. The anatomical studies were performed on sterile (Fig. 1A) and fertile ultimate branches of *Gleichenia polypodioides* (Fig. 1J) (BHCB 189155 - South Africa, Cape City, *M. Lima s.n.*). The sterile (n=3) and fertile branches (n=3) were hydrated in hot water, followed by a two-hour immersion in 2% potassium hydroxide, and 2-3 times washing to complete the reversion process (Smith and Smith, American Journal of Botany 29:464–471. 1942, modified). The branches were dehydrated in an ethanol series, before embedding in Historesin (Leica Microsystems GmbH, Wetzlar, Germany). Transverse sections (6-8 µm) were produced using a Reichert Jung Leica® biocut rotary microtome. The histological sections were stained with 0.05% toluidine blue in acetate buffer, pH 4.7 (O'Brien *et al*. Protoplasma 59(2):368–373. 1964), and mounted on slides with water. All samples were analyzed and photographed on a light microscope (Leica ICC50 HP). The sterile segments (Fig. 1A-B) are glabrous, hypostomatic with dorsiventral organization, and the margins of the segments are slightly revolute. The epidermis is uniseriate constituted of thick-walled periclinally elongated cells, covered by thin cuticle, both on the abaxial and adaxial surfaces (Fig. 1C-D). The abaxial epidermal cells are smaller than the adaxial epidermal cells, and at the midrib region, epidermal cell walls are lignified (Fig. 1E). The chlorenchyma has 1-2 layers of palisade-like parenchyma with polygonal juxtaposed cells, and 3-4 layers of spongy parenchyma (Fig. 1F-G). The vascular tissues in the midrib vein have amphicribral arrangement and are surrounded by 4-5 layers of pericyclic fibers (Fig. 1H-I). At the fertile segment (Fig. 1J-K), the epidermis is uniseriate, and similar to the sterile segment on the adaxial surface. The unique peculiarity is the formation of the soral chamber in the fertile segment by the invagination of the blade tissue. The abaxial surface have the epidermis and the 2-3 layered spongy parenchyma underline the soral chamber (Fig. 1L). The soral chamber usually bears two to three sporangia (Fig. 1J-K). The sporangia have a typical gleichenious leptosporangia morphology, with no morphological peculiarity. The base of the sporangia is connected to the secondary veins by a short multicellular stalk with polygonal cells (Fig. 1M-N). The annulus is oblique, with thick-walled cells and a discrete stomium, lined by thin-walled cells. The annulus cells are distal and thick-walled, while the stomium has thin-walled cells and usually face the soral chamber opening (Fig. 1F-G). Numerous trilete spores develop inside the chamber (Fig. 1O-P). The overall leaf morphology of *G. polypodioides* observed in the present study is in accordance with the previous anatomic studies performed in Gleicheniaceae, especially in *Gleichenia s.s.* (Boodle, Annals of Botany 15(60):703–747. 1901; Chrysler, American Journal of Botany 30(9): 735-74. 1943: Chrysler, American Journal of Botany 31(8):483–491. 1944; Ogura, Comparative anatomy of vegetative organs of the Pteridophytes, p. 336. 1972). It should be noted that all species of Gleicheniaceae, with the exception of *G. polypodioides* and *G. inclusisora*, have superficial sori. Therefore, the soral chamber represents an important taxonomic feature, and it is a very useful character to readily recognize these two species. A third species, *G. dicarpa* var. *alpina* (R. Br.) Hook. f. [≡*Gleichenia alpina* R. Br.], has chamber-like organization in the sori area formed by the revolute margin of the ultimate segments and the scales borne around the sori. This chamber is dissimilar of those of *G. polypodioides* and *G. inclusisora*, but has a supposed function of sori protection (Boodle, 1901). The arrangement of the scales around the sori is rather common and observed in other groups of leptosporangiate ferns, such as in the species of *Pleopeltis* (Polypodiaceae). The presence of scales and trichomes is usually related to water loss control, once dense indument may act by reflecting the light, reducing the leaf temperature, and thus reducing the transpiration (Hietz, Fern Ecology, p. 144. 2010). Some other Gleicheniaceae species have scales or trichomes across the abaxial surface, such as *Sticherus bifidus* (Wild.) Ching and *Sticherus lanuginosus* (Fée) Nakai, yet other species, such as *Sticherus* *brevitomentosus* Østergaard & Øllgaard, have scales restricted to the sori area. The soral chamber seems to be an adaptation of the fertile portion of the blade to sori protection and to drought avoidance, and may be ontogenetically related with the sporangia development. The drought avoidance strategies favor the Gleicheniaceae species, which are usually sunny plants, and occupy open and disrupted open habitats and forests edges (Gonzales and Kessler, 2011; Lima and Salino, Phytotaxa 358(3):199–234. 2018). Such areas are characterized by higher light levels and lower relative air humidity than the understory (Hietz, 2010). Additionally, stomata on the abaxial surface (Ogura, 1972) associated with the abaxial indument may play a role on drought avoidance for Gleicheniaceae species. However, *G. polypodioides* have glabrous abaxial leaf surface, and, supposedly, the soral chamber may act as an alternative strategy to avoid dissection, reduce water loss and protect against the excessive light radiation. Regarding *G. inclusisora,* besides the soral chamber, some sparse scales and a white farina on the abaxial surface of leaflet segments (Perrie and Brownsey, Flora of New Zealand: ferns and lycophytes. Gleicheniaceae p. 22 .2015) may also be related to drought avoidance and sori protection. Further anatomical and ontogenetic studies on *Gleichenia inclusisora* will elucidate the origin and development of its soral chamber. Additionally, phylogenetic studies with morphological character optimization may investigate the evolution of the sori chamber in *Gleichenia*. We thank Thaís Elias Almeida for notes and comments. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 (88887.19244/2018-00) to L.V. Lima, (888882.184385/2018-01) to E.C. Costa; (888877.199702/2018-00) and Í.A. Arriola. We also thank CNPq for the research grant to A. Salino (307115/2017-8) and R.M.S. 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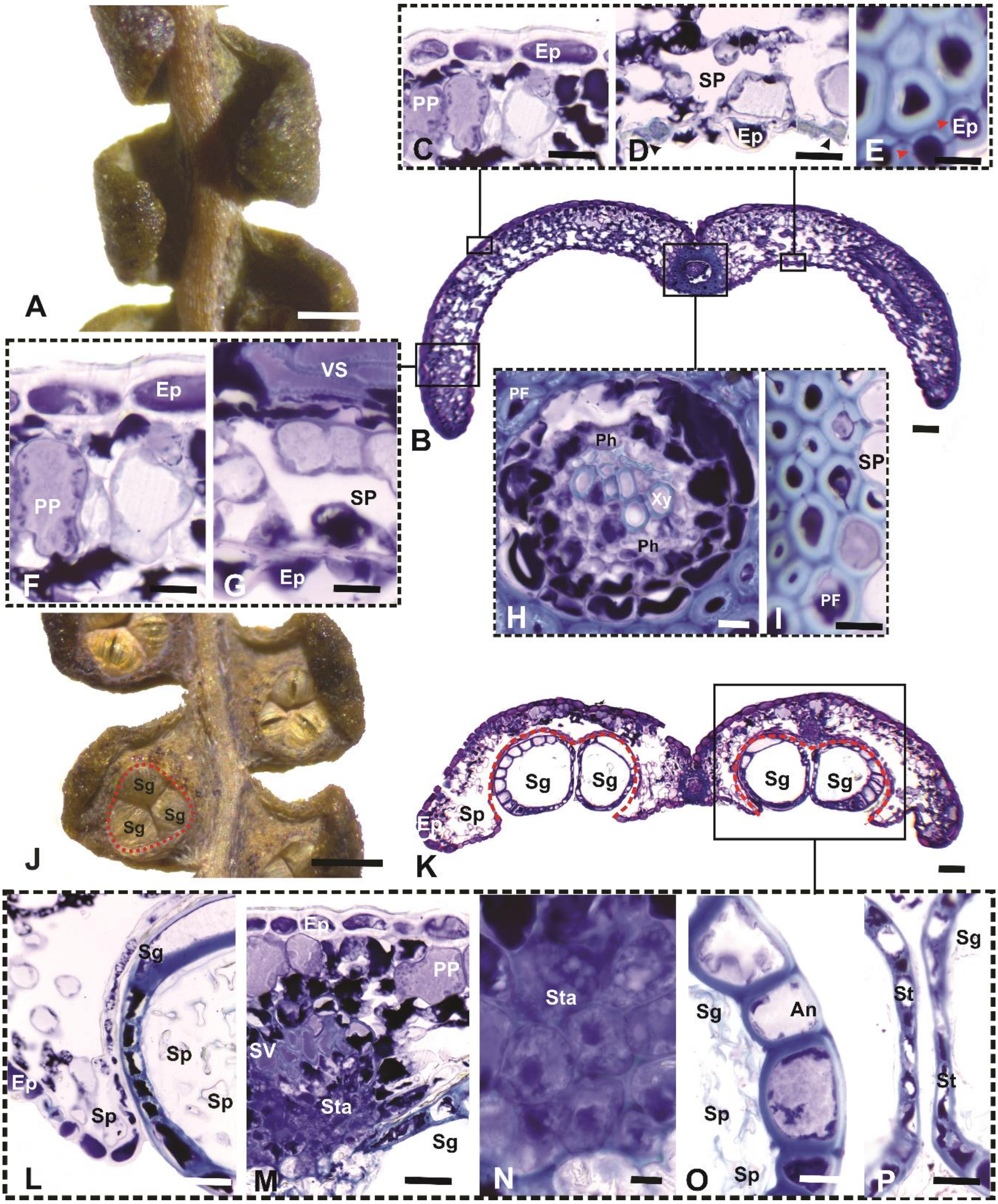

Figure 1. Anatomy of sterile and fertile ultimate branches of *Gleichenia polypodioides*. A. General aspect of a sterile branch; B. General view of a transverse anatomical section of a sterile branch; C-E. Details of epidermal cells. C. Adaxial epidermal surface with periclinally elongated cells, and palisade parenchyma adjacent cells; D. Abaxial epidermal surface and spongy parenchyma adjacent cells; E. Epidermal and adjacent cortical cells at the midrib region with lignified walls (red arrow); F. Palisade-like parenchyma in detail; G. Spongy parenchyma cells in detail; H. Detail of the midrib

vein evidencing phloem, xylem, and pericyclic fibers; I. Pericyclic fibers in detail; J. General aspect of a fertile branch evidencing soral chambers (red dotted), and sori with three sporangia; K. Transverse anatomical section of a fertile branch evidencing dermal and ground systems. The midvein is diminute Soral chamber with sori (limited by red dotted lines); L. Detail of the epidermis and spongy parenchyma underlining the soral chamber; M. Detail of the insertion of the sporangia to the secondary vein. N. Detail of cells of the multicellular stalk wit conspicuous nucleus and dense cytoplasm. O. Detail of the annulus; P. Detail of the stomium. An. Annulus; Ep. Epiderm; Pf. Pericyclic fibers; Ph. Phloem; PP. Palisade-like parenchyma; SP. Spongy parenchyma; Sg. sporangia; Sp. Sporus; Sta. Stalk; St. Stomium; VS. Vascular system; Xy. Xylem. Bars: A and J 1cm; Bars: B and K 200 μm; Bars: C, D, E, F, G, I, L, M, O and P 50 μm; Bars: H and N 20 μm.

Capítulo 5: Artigo publicado no periódico Phytotaxa.

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Reestablishing *Dicranopteris spissa* (Gleicheniaceae, Polypodiopsida) from synonymy, an endemic species from Brazil

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Abstract:

We recognized *Dicranopteris spissa* (Fée) L.V.Lima & Salino *comb. nov.* as a segregate species distinct from *Dicranopteris rufinervis* and other gleichenioid ferns occurring in Brazil. Besides the taxonomic background the study provides descriptions, images, maps, and comments regarding *D. spissa* morphology, ecology, and distribution, as well as an identification key for all Brazilian species of *Dicranopteris*.

Key words: Espinhaço range, Serra do Mar, ferns, Flora of Brazil, Gleicheniales

Introduction

Dicranopteris Bernhardi (1805: 38) is a terrestrial fern genus belonging to the leptosporangiate fern family Gleicheniaceae (PPG I 2016). Five species out of the ca. 20 currently recognized species occur in the Neotropics (Lima & Salino 2018). Current taxonomic research focusing on this genus has to resolve two key-questions. Firstly, monophyly of the genus has not been confirmed due to the lack of comprehensive phylogenetic studies (PPG I 2016, Li et al. 2010).However, the currently employed morphological diagnostics enable unambiguous identification via rhizomes and laminar buds covered exclusively by hairs, by having a pair of opposite branches at the main ramifications, and tetrahedral spores with trilete aperture (Mickel & Smith 2004, Lima & Salino 2018). This evaluation has to be taken into context that recent study recovered evidence for the distinct genus *Rouxopteris* Liu (2020: 9) separated from the genus *Gleichenia* Sm. (1793: 419) (Liu et al. 2020). The most needed question to be addressed is determination of all species. The taxonomy of this genus is very intricate, mostly due to several cases of morphological convergence, species with high morphological plasticity, and putative semi-cryptic species. For example, Holttum (1957) recognized nine varieties for the widespread paleotropical *D. linearis* (Burm. f.) Underwood (1907: 250).

Usually, *Dicranopteris* species, like *D. flexuosa* (Schrad.) Underwood (1907: 254) and *D. dichotoma* (Thunb.) Bernhardi (1805: 38), are widely distributed and often occupy anthropogenic habitats. However, other species have more restricted distribution, such as *D. nervosa* (Kaulf.) Maxon (1922: 49), which occurs in forest edges at high elevations in southern and southeastern Brazil, Bolivia, and Peru (Tryon & Stolze 1989, Kessler & Smith 2018, Lima & Salino 2018). Similarly, *D. rufinervis* occurs in the highlands of the Cerrado and Atlantic Forest open formations, like Campos Rupestres and Campos de Altitude. These are restricted and endangered habitats containing many endemic taxa. Therefore, it is paramount to improve the species delimitation as a prerequisite to manage the consideration of putatively threatened species, the basic units for conservation acts (Rojas 1992).

Due to highly improved sampling and a reinterpretation of type materials, we have evidence that supports the separation of *Dicranopteris spissa* from *D. rufinervis*. Therefore we providedescriptions, images, maps, and comments regarding *D. spissa* morphology, ecology, and distribution, *to* clarify *D. spissa* distinctiveness from other species of the genus in Brazil.

Materials & Methods

We studied specimens of all species of *Dicranopteris* mentioned below, including type materials from the following herbaria: B, BHCB, BM, K, P, and RB (acronymous according to Thiers 2021). Descriptive terms used followed Lellinger (2002) with the exception of the terminology applied to describe the complex leaves of Gleicheniaceae, to which the definition of Andersen & Øllgaard (1996) were applied with modifications as used in Lima & Salino (2018). The distribution map was generated using ArcGIS (ESRI 2011) based on voucher specimens with reliable location information. The IUCN Red List Status was evaluated using the algorithms implemented in Geocat (http://geocat.kew.org/http://geocat.kew.org/, Bachman et al 2011) to access the conservation status of *D. spissa* and *D. rufinervis*.

Taxonomic treatment

Dicranopteris spissa (Fée) L.V.Lima & Salino, *comb. nov.* (Figure 1-2)

Mertensia spissa Fée (1869: 200). Type:—BRAZIL. Rio de Janeiro. Nova Friburgo. Macaé de Cima. *Glaziou 2468* (lectotype designated by Lima & Salino 2018, P [P00633241] photo!, isolectotypes P [P00633243], [P00623242] photos!).

Plants terrestrial or epipetric. **Rhizomes** 1.5–5.20 mm thick, with reddish-brown rigid simple or branched hairs, glabrescent to tomentose. **Fronds** scrambling, 2–4–(6)-forked, ultimate branches 12.5–30.5 cm × 2.8–5.5 cm, lanceolate, apex pinnatifid, base asymmetric, inner side with reduced segments, external side with conform segments, ultimate segments linear 3–16 cm × 2–2.5 cm, margins revolute, abaxial surface green or pruinose, pubescent, rare glabrescent, with reddish, rarely whitish, multicellular hairs, on the segments midrib, bacilliform glandular hairs on the midrib, secondary veins and laminar tissue. **Buds** covered by reddish multicellular hairs, pseudostipule present, accessory branches simple to pinnatisect. **Veins** 3–4-forked. **Sori** median, with paraphyses, spores tetrahedral.

Distribution and habitat: — *Dicranopteris spissa* is endemic to southeastern Brazil occurring usually in edges of high montane forests of the south part of the Espinhaço Range, and Serra de Ibitipoca in Minas Gerais state as well as in the Serra do Mar in the states of Rio de Janeiro and São Paulo, between 900 – 1800 m of elevation (Fig. 2). While *Dicranopteris rufinervis* has a restricted distribution to the mountains of the Espinhaço range in the north of Minas Gerais and Bahia , between 600– 1500m of elevation. .

Notes: — *Dicranopteris spissa* is morphologically related to *D. rufinervis* and it is mainly differentiated from that species by the following characters. Accessions of *D. spissa* have sparse pale-yellow to reddish hairs occurring only at the base of the segment on midrib (Fig. 1 D-G) versus red hairs densely spread through the segment's midrib in *D. rufinervis* (Fig. 3 C-E). Secondary veins usually are deeply immersed at the laminar tissue with bacilliform glandular hairs versus secondary veins usually not sunk in the laminar tissue, usually with sparse tector red hairs in *D. rufinervis*. Finally, the rachis is glabrescent in *D. spissa* but pubescent in *D. rufinervis*.

Conservation status: — *Dicranopteris spissa* and *D. rufinervis* are endemic to Brazil. Both species were categorized as endangered species according to IUCN Red List criteria. However, the recording of those two species may be subsampled due to their resemblance with *D. flexuosa*, the most widely distributed species of the genus in Brazil. In contrast to the later, these two species do not occur in anthropogenic habitats and thus they are more sensible to environmental change. They occur in highland formations, usually near rock outcrops or in forest edges.

Specimens examined:—BRAZIL. Minas Gerais: Lima Duarte, Parque Estadual de Ibitipoca, 21º42'33"S 43º53'38"W, 1300m, 7 February 2017, *Lima et al. 213* (BHCB, CESJ); Lima Duarte, Parque Estadual de Ibitipoca, 21º40'18"S 43º52'22"W, 1484m, 22 June 2007, *Almeida et al. 1221* (BHCB); Catas Altas, Parque Natural do Caraça, 20º06'31"S 4327'31"W, 1800m, 19 May 2001, *Salino et al. 6835* (BHCB); Catas Altas, Parque Natural do Caraça, 20º05'44"S 43º29'03"W, 1349m, 13 October 2016, *Lima 199* (BHCB); Catas Altas, Parque Natural do Caraça, 20º05'44"S 43º29'03"W, 1349m, *Lima 200* (BHCB); Catas Altas, Parque Natural do Caraça, 07 October 2000, *Salino 5755* (BHCB); São Gonçalo do Rio Preto, Parque Estadual do Rio Preto, 18°12'53"S

43º20’06”W, 1600-1700m, 07 August 2003, *Salino et al. 9350* (BHCB); São Gonçalo do Rio Preto, Parque Estadual do Rio Preto, 19 October 2000, *Salino 5801* (BHCB); Itamarandiba, Parque Estadual da Serra Negra, 18º00’55”S 42º45’14”W, 1570-1600m, 04 July 2006, *Salino et al. 10899* (BHCB); Felício dos Santos, 18º 12’37”S 43º17’05”W, 1150-1350m, 31 October 2004, *Salino et al. 90406* (BHCB); Santo Antônio do Itambé, Parque Estadual do Pico do Itambé, 18º24’05”S 43º18’57”W, 1357m, 07 October 2006, *Almeida et al. 596* (BHCB). Rio de Janeiro: Nova Friburgo, Macaé de Cima, *Glaziou 4456* (RB); Rio de Janeiro, Parque Nacional da Tijuca, Pico da Tijuca, 14 June 1933, *Brade 12555* (RB); Santa Maria Madalena, Serra da Forquilha, 4 February 1935, *Brade 14354* (RB); . São Paulo: Natividade da Serra, Parque Estadual da Serra do Mar, núcleo Santa Virgínia, 23º26’56”S 45º11’35”W, 900-1150m, 01 November 2001, *Salino et al. 7830* (BHCB); Ubatuba, Parque Estadual da Serra do Mar, Núcleo de Picinguaba, Trilha do Pico do Cuscuzeiro, próximo ao marco da divisa entre Rio de Janeiro e São Paulo, 23°21’33.7”S, 44°50’53.0”W, 07 August 2001, *Salino et al. 7317* (BHCB).

Key to the Brazilian species of *Dicranopteris*

1. Laminae glabrous abaxially, only with unicellular globose glandular hairs on the secondary veins ... *D. flexuosa*

- Laminae pubescent abaxially, with multicellular tector hairs or, when present, unicellular bacilliform glandular hairs on secondary veins ... 2

2. Plants erect; fronds forked once (rarely twice); ultimate branches with caudate apex; accessory branches entire to slightly lobed at the base, dissimilar to the ultimate branches ... *D. nervosa*

- Plants scrambling; fronds forked two to several times; ultimate branches with pinnatifid apex; accessory branches pinnatisect, similar to the ultimate branches ... 3

3. Segments patent to strongly retroflex (northern Brazil, Guiana Shield) ... *D. seminuda*

- Segments ascendant, occasionally almost patent (southeast and northeast Brazil) ... 4

4. Segments with tector hairs restrict to the midrib base; secondary veins glabrous or with bacilliform glandular hairs … *D. spissa*

- Segments with tector hairs all through the midrib; secondary veins with tector hairs … *D. rufinervis*

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Figure 1. *Dicranopteris spissa* (*Lima et al. 213*). **A.** Habit. B. Fiddlehead. **C.** Detail of the main bifurcation showing the dormant bud covered with hairs. **D.** Abaxial surface showing the sori. **E.** Bud hair. **F.** Rhizome hair. **G.** Detail of a segment midrib.

Figure 2. Map showing the distribution of *Dicranopteris spissa* and *Dicranopteris rufinervis*, endemic species of Brazil.

Figure 3. *Dicranopteris rufinervis* (*Salino 16256*). **A.** Abaxial surface. **B.** Detail of the main bifurcation showing the dormant bud covered with hairs. **C.** Bud hairs. **D.** Detail of the abaxial surface showing the indument. **F.** Detail of a rhizome hair. **G.** Rhizome.

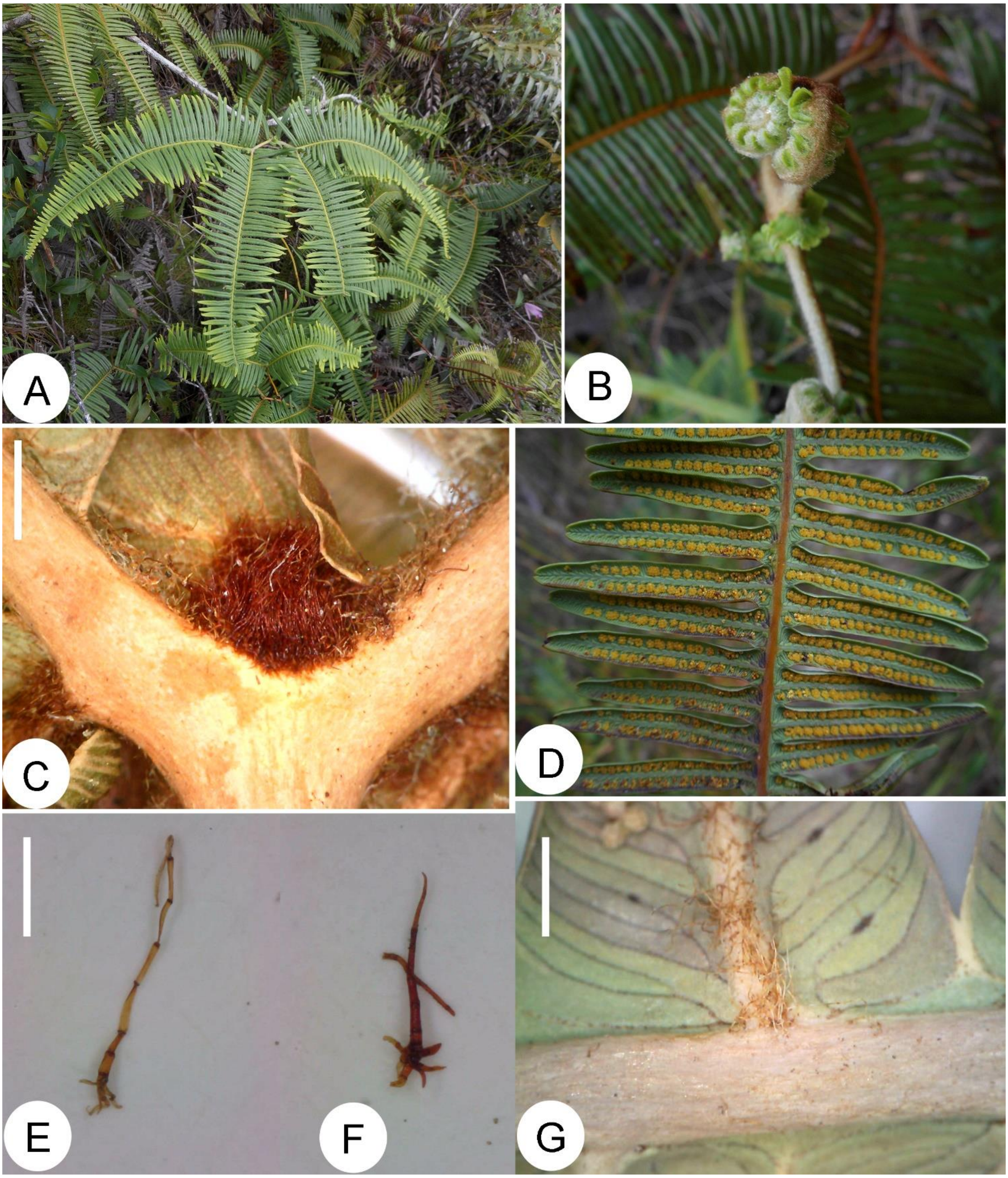

Figure 1. *Dicranopteris spissa* (*Lima et al. 213*). **A.** Habit. B. Fiddlehead. **C.** Detail of the main bifurcation showing the dormant bud covered with hairs. **D.** Abaxial surface showing the sori. **E.** Bud hair. **F.** Rhizome hair. **G.** Detail of a segment midrib.

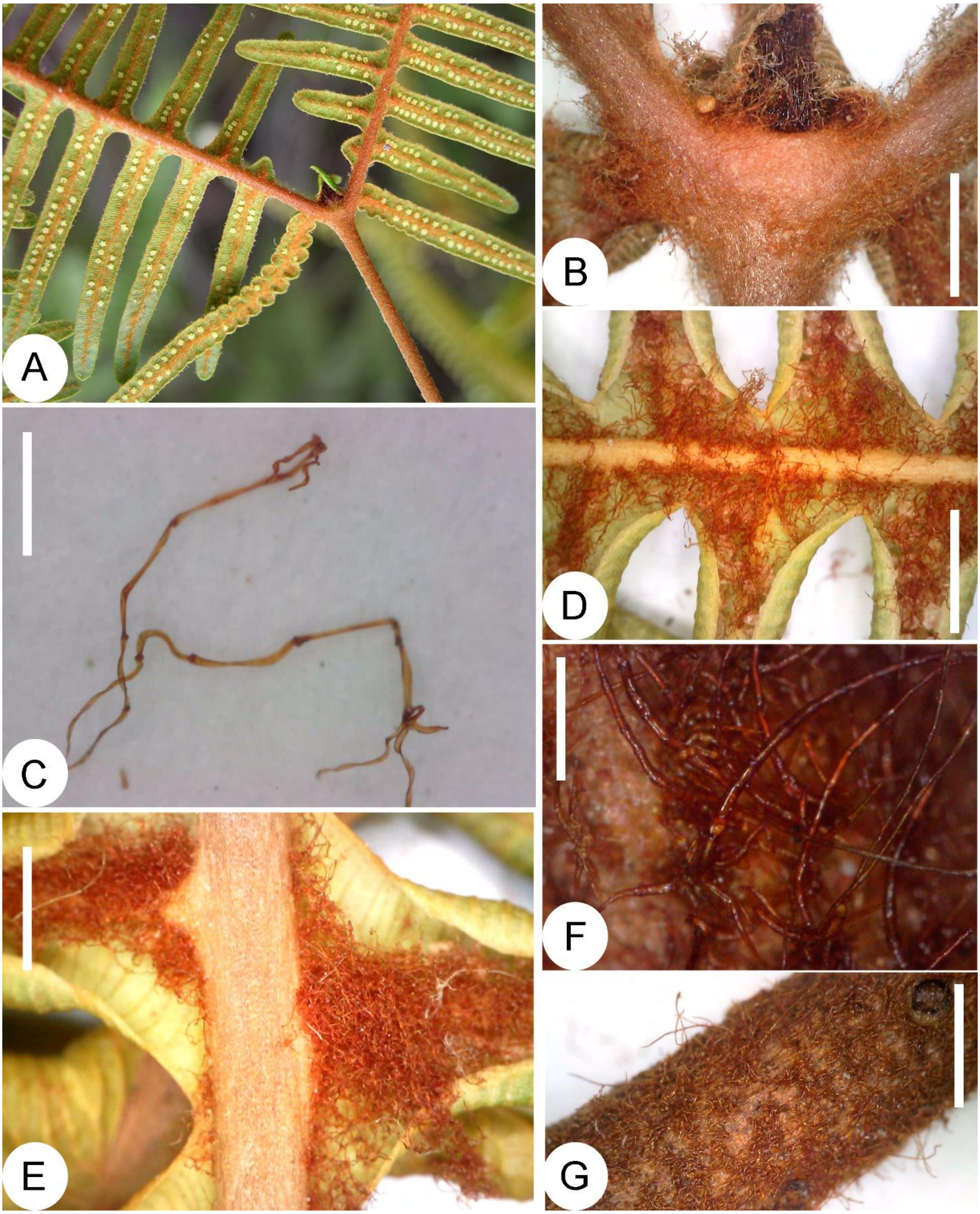

Figure 2. Map showing the distribution of *Dicranopteris spissa* and *Dicranopteris rufinervis*, endemic species of Brazil.

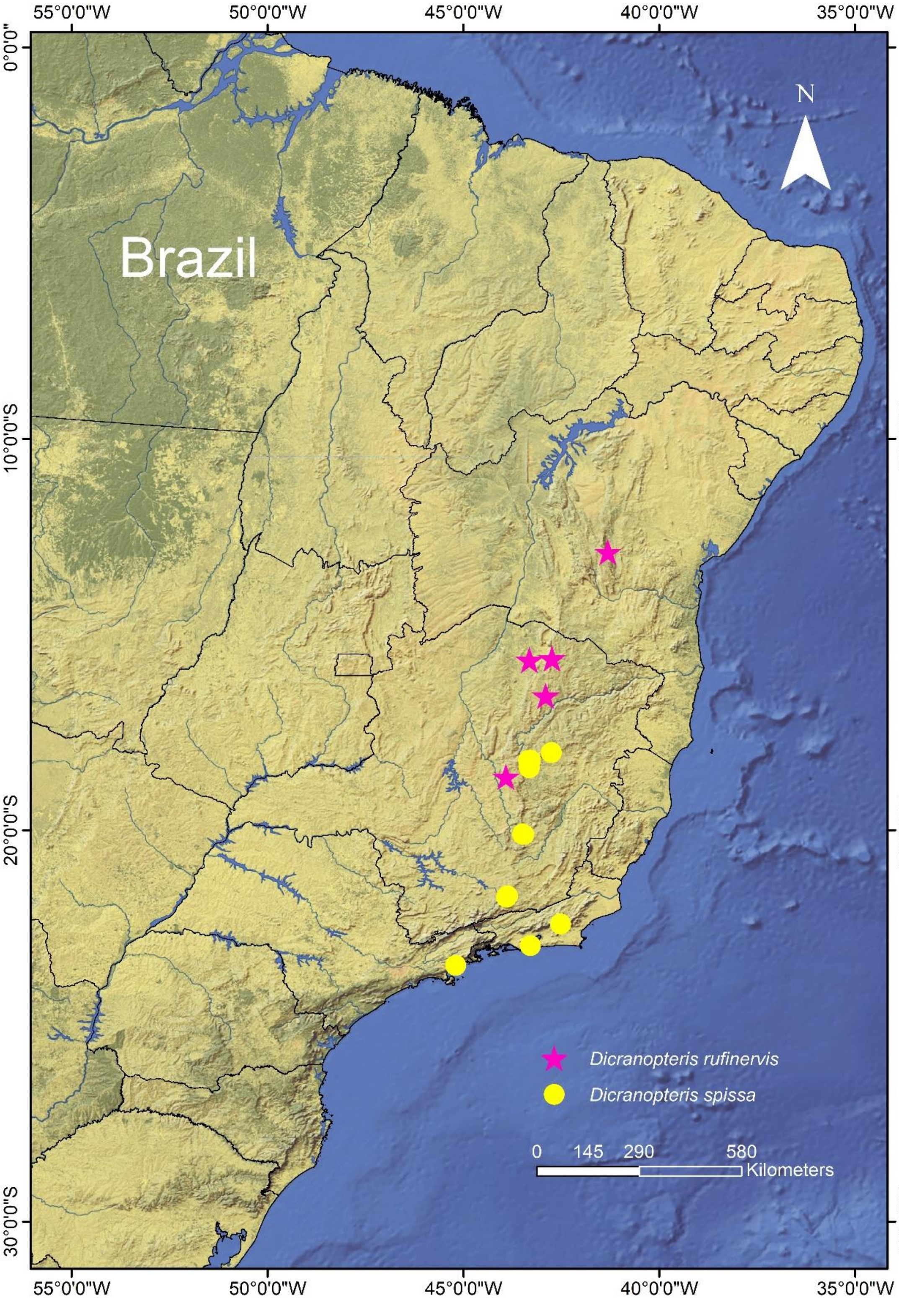

Figure 3. *Dicranopteris rufinervis* (*Salino 16256*). **A.** Abaxial surface. **B.** Detail of the main bifurcation showing the dormant bud covered with hairs. **C.** Bud hairs. **D.** Detail of the abaxial surface showing the indument. **F.** Detail of a rhizome hair. **G.** Rhizome.

Capítulo 6: artigo publicado no periódico Phytotaxa.

(DOI: 10.11646/phytotaxa.429.3.5)

Typification of the Linnaean name *Onoclea polypodioides*, type of the fern genus *Gleichenia* (Gleicheniaceae, Polypodiopsida)

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The fern family Gleicheniaceae was established by Presl (1825: 10) from the order Gleicheneae created by Brown (1810: 160) to accommodate two genera: *Platyzoma* Brown (1810: 160) and *Gleichenia* Smith (1793: 419). However, the first species described of what later would become Gleicheniaceae was *Acrostichum furcatum* Linnaeus (1759: 1321) and the genus *Gleichenia* was established by Smith (1793: 419) to include only *G. polypodioides*, later the genus concept was used in a broader sense to include all Gleicheniaceae species known until the early 20th century (Diels 1900, Christensen 1905). The name proposed by Smith is a later homonym conserved against *Gleichenia* Necker (1790: 314) (Dryopteridaceae) (Nomenclature Committees 1954), and *Gleichenia* Smith is currently used with a narrow circumscription, with about 10 species (PPG I 2016). Nowadays, Gleicheniaceae is divided into six genera, although their monophyly is still questionable (PPG I 2016). The nomenclature of the family still needs attention due to the existence of a few papers dealing with this subject as noted by Lima *et al.* (2018) and Lima & Salino (2018). Along taxonomic studies in Gleicheniaceae, we realized that another Linnaean name, *Onoclea polypodioides,* the type species of *Gleichenia*, was not properly typified, as we explain in the next paragraphs.

Smith (1793) cited only *G. polypodioides* when he described the genus, based on *O. polypodioides*. In the original protologue of this species, Linnaeus provided a very detailed description, citing material from Koenig, to which he attributed the number 44. Johann Gerhard

Koenig sent many specimens to Linnaeus, however some of his collections were not numbered. The same holds true of the specimens he sent to Anders Retzius, some of which were described as new taxa by Retzius (1779, 1781, 1783, 1786, 1789, 1791) (Fischer 1932). Such specimens are housed in Retzius' herbarium, now incorporated at LD (acronym according to Thiers 2019, continuously updated). Fischer (1932) examined those specimens and observed that those collections have no signature, nor any information that could be directly related to Koenig. Additionally, there is also a specimen in Retzius' herbarium of *Gleichenia polypodioides*, although it was not used by him to describe any taxa, neither it was cited in his publications, there is a possibility that it may be one of Koenig materials sent to Retzius. In fact, despite the date of Retzius publication is not close to the Linnaeus', Koenig may have sent duplicates of the same collection to both Linnaeus and Retzius, however there is no evidence that this material housed at Retzius herbarium is an duplicate of the material cited in *G. polypodioides* protologue, nor that Linnaeus saw this material at the time of his publication.

During the revision of Linnaeus' materials, we found one sheet of *G. polypodioides* housed at LINN (Herb. Linn. No. 1242.3) (Fig. 1). This sheet carries few annotations but no signature to associate the material with Koenig, nor the number "44" cited by Linnaeus in the protologue. Unlike the other fern species described by Linnaeus (1771) based on Koenig collections housed at LINN, which have at least his name on the sheet. Instead, there is the name '*Onoclea trilocularis*' written by Linnaeus on the sheet, which had subsequently the epithet 'trilocularis' crossed out and added 'polypodioides' by Linnaeus filius (Jarvis 2007).

Additionally, on the *G. polypodioides* sheet there is an inscription ['Spar 31'], which seems to be a reference to Anders Sparrman, a onetime student of Linnaeus who sent to his former teacher many specimens. Therefore, it seems most unlikely that this material can be linked in any way with Koenig. Moreover, there is conflict regarding the chronology between this specimen and the species publication date. Sparrman apparently was not at the Cape of Good Hope before 1772, and then he was there again in 1775-1776 (Jarvis 2007). Furthermore, his correspondence with Linnaeus started only in December 1771. Thus, all these evidences suggest strongly that Linnaeus could not have had the Sparrman specimen available to him when he described *Onoclea polypodioides* in October 1771. Therefore, it cannot be an original material for the name, which makes it ineligible for lectotype designation. Aiming to contribute to the nomenclatural elucidation within Gleicheniaceae, in accordance with the International Code of Nomenclature (Turland *et al.* 2018), here we propose a neotype for *Onoclea polypodioides*. We choose the material housed at P, once it is well-documented recent material, with silica sample to be included in the phylogenetic studies in course (Lima *et al. in prep*). Moreover, this specimen matches perfectly into Linnaeus description of the species and Smith's description of the genus *Gleichenia*.

Typification

Gleichenia polypodioides (L.) Smith (1793: 419).

Basionym:—*Onoclea polypodioides* Linnaeus (1771: 306).

Neotype (**designated here**):— TERRES AUSTRALES ET ANTARCTIQUES FRANÇAISES. Ile Saint-Paul, crète de la Novara, 38° 43' 0.984" S 77° 31' 9.984" E, 256 m, *G. Rouhan et al. 1747*, (P [P02434554!]). (Fig. 2-3).

Acknowledgements

We thank Marcos Sobral by the guidance during the preparation of this manuscript, the anonymous reviewers that helped to improve substantially this manuscript, Germinal Rouhan who provided the detailed high-resolution image of the selected neotype and the Herbarium at Muséum National d'Histoire Naturelle, Paris, for the permission to publish the neotype image. We also thank The Linnaen Society of London for allowing the use of *Onoclea polypodioides* image. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 (88887.19244/2018-00). We also thank CNPq for the research grant and scholarship (307115/2017-8) to A. Salino and the scholarship to R.S. Viveros (154846/2018-0).

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FIGURE 1. *Onoclea polypodioides* L. housed at LINN (1242.3). This image is © copyright of The Linnaean Society of London.

FIGURE 2. Neotype of *G. polypodioides*: *G. Rouhan et al. 1747* (P P02434554). This image is © copyright of the Muséum National d'Histoire Naturelle (MNHN)—Paris Herbarium (P).

FIGURE 3. Neotype of *G. polypodioides*: *G. Rouhan et al. 1747* (P P02434554). **A-B.** Habit of *G. polypodioides*. **C.** Frond primary branch, showing the bud with pseudostipule. **D.** Secondary branch showing the bud without the pseudostipule. **E.** Ultimate branch adaxial surface. **F.** Ultimate branch abaxial surface. **G.** Detail of the ultimate branch abaxial surface showing the soral chamber. **H.** Rhizome detail showing the stellate scales. Scale bars = 5 mm.

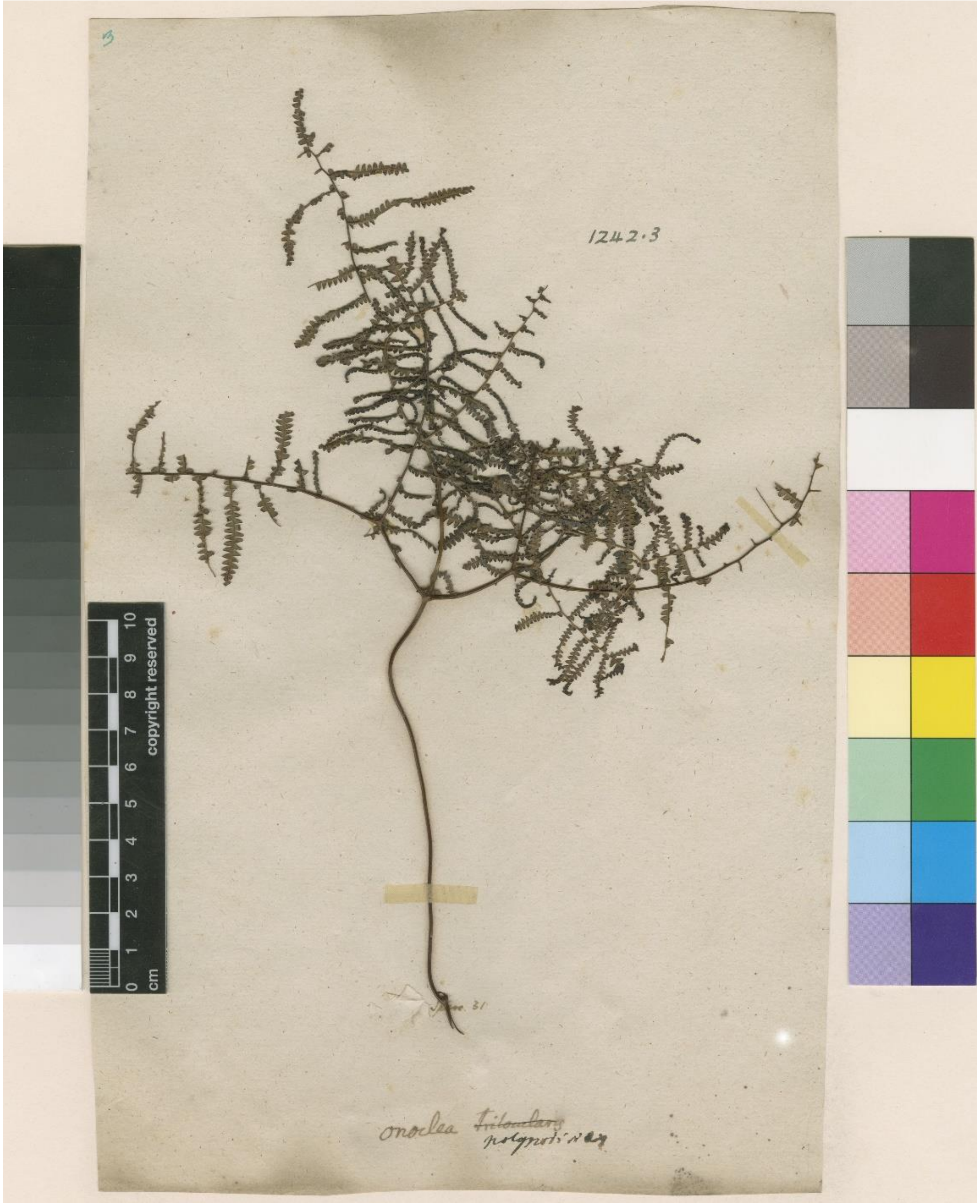
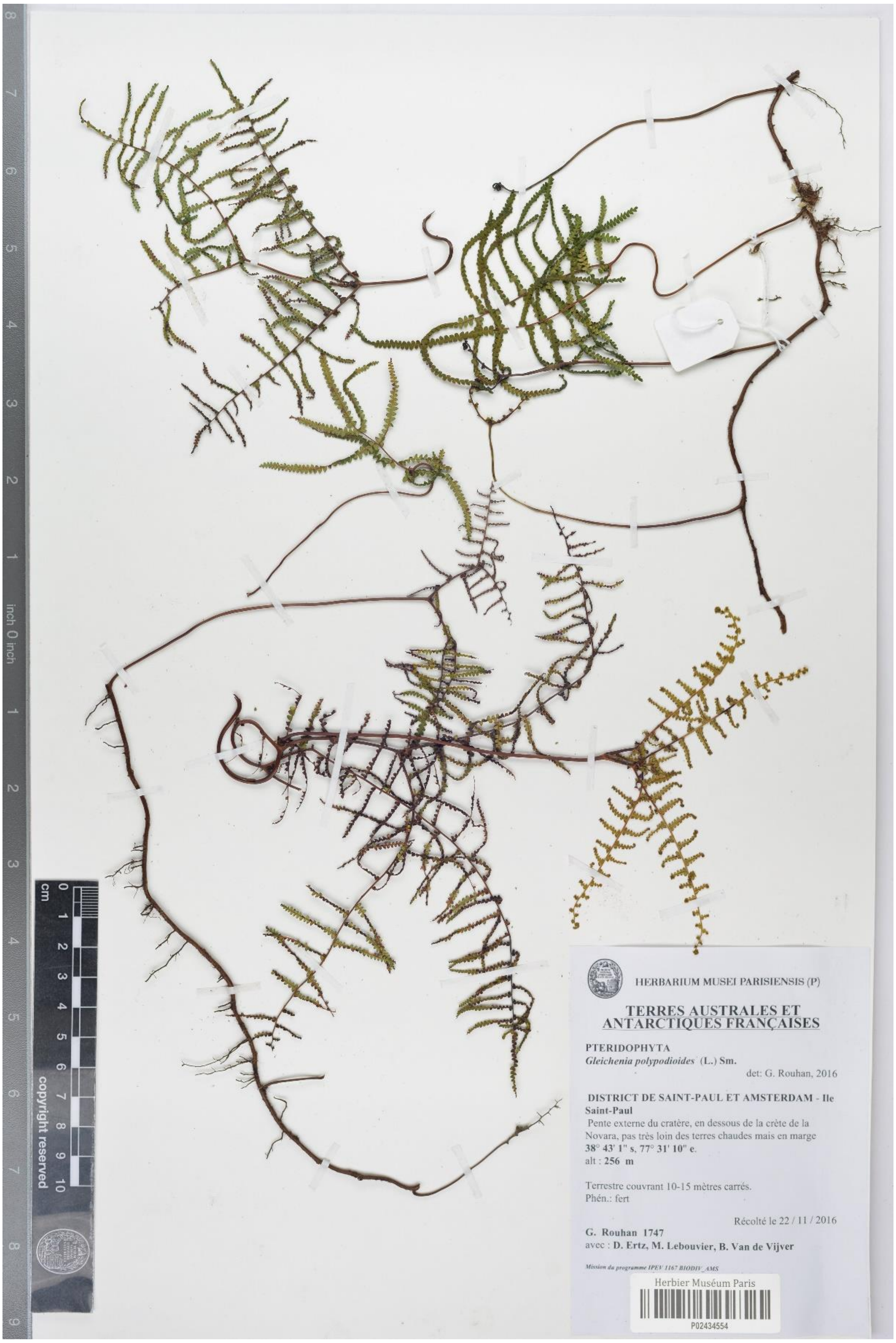


FIGURE 1. *Onoclea polypodioides* L. housed at LINN (1242.3). This image is © copyright of The Linnaean Society of London.



HERBARIUM MUSEI PARISIENSIS (P)

TERRES AUSTRALES ET ANTARCTIQUES FRANÇAISES

PTERIDOPHYTA
Gleichenia polypodioides (L.) Sm.
det: G. Rouhan, 2016

DISTRICT DE SAINT-PAUL ET AMSTERDAM - Ile Saint-Paul
Pente externe du cratère, en dessous de la crête de la Novara, pas très loin des terres chaudes mais en marge
38° 43' 1" s, 77° 31' 10" e.
alt : **256 m**

Terrestre couvrant 10-15 mètres carrés.
Phén.: fert

Récolté le 22 / 11 / 2016
G. Rouhan 1747
avec : **D. Ertz, M. Lebouvier, B. Van de Vijver**

Mission du programme IPEV 1167 BIODIV_AMS

Herbier Muséum Paris
P02434554

FIGURE 2. Neotype of *G. polypodioides*: *G. Rouhan et al. 1747* (P P02434554). This image is © copyright of the Muséum National d'Histoire Naturelle (MNHN)—Paris Herbarium (P).

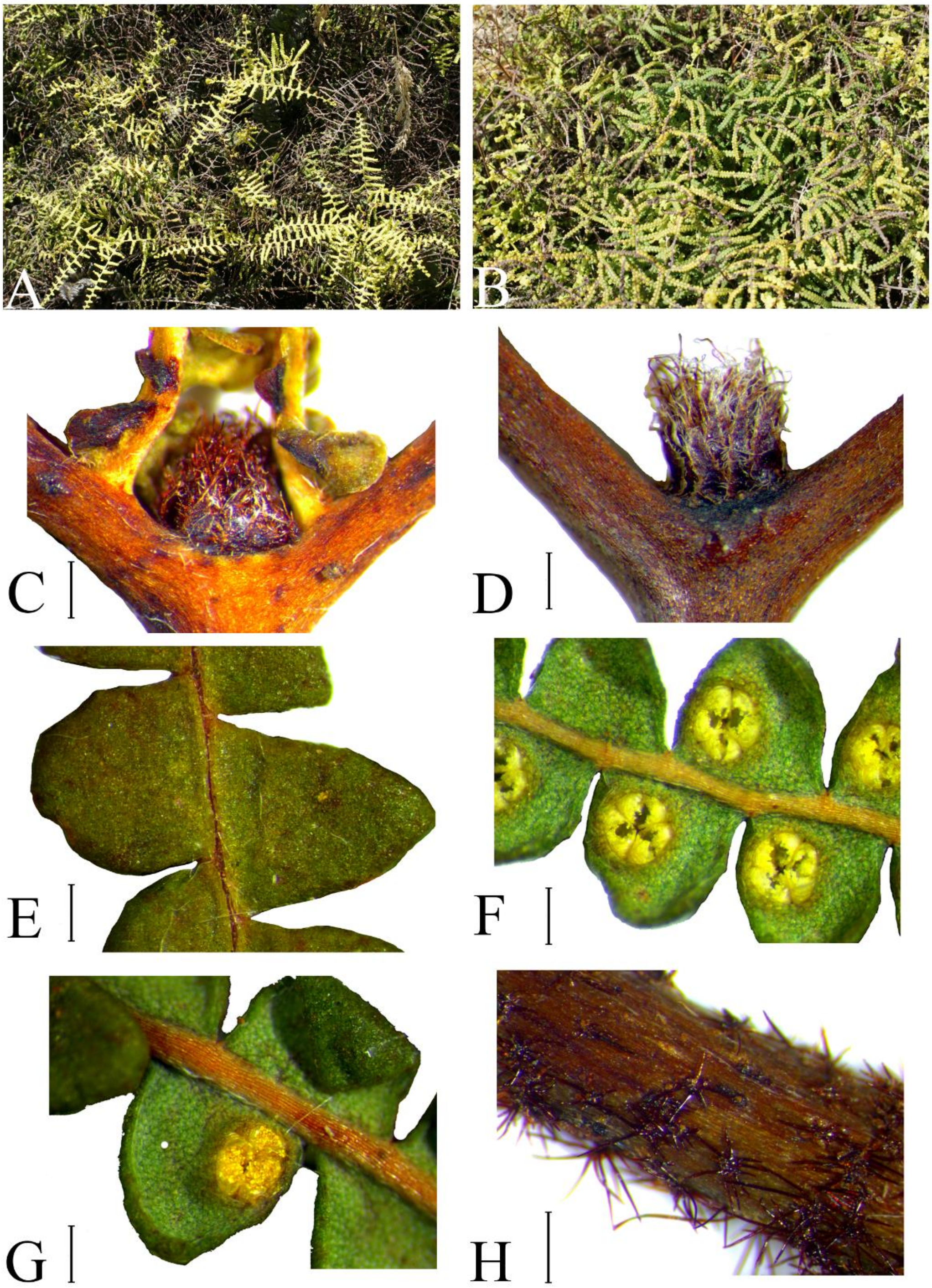


FIGURE 3. Neotype of *G. polypodioides*: *G. Rouhan et al. 1747* (P P02434554). **A-B.** Habit of *G. polypodioides*. **C.** Frond primary branch, showing the bud with pseudostipule. **D.** Secondary branch showing the bud without the pseudostipule. **E.** Ultimate branch adaxial surface. **F.** Ultimate branch abaxial surface. **G.** Detail of the ultimate branch abaxial surface showing the soral chamber. **H.** Rhizome detail showing the stellate scales. Scale bars = 5 mm.

Capítulo 7: Artigo publicado no periódico Phytotaxa.
(DOI: 10.11646/phytotaxa.351.2.9)

Typification of a Linnaean name in Gleicheniaceae (Polypodiopsida)

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Gleicheniaceae are an ancient fern family with about 157 species and six genera (PPG I 2016). Although its taxonomy has been through several changes in the last years (Gonzales & Kessler 2011), a great gap remains in both taxonomy and nomenclature. For the Neotropics, there are several national or regional taxonomic treatments of the family (*e.g.* Kessler & Smith 2018, Lellinger 1989, Tryon & Stolze 1989, Moran 1995, Mickel & Smith 2004, Proctor 1985) and a revision of the Neotropical members of the genus *Sticherus* Presl (1836: 51) (Gonzales & Kessler 2011), but only few of them are dedicated to solve nomenclatural issues.

In preparation of taxonomic studies of Neotropical Gleicheniaceae, we realized that the typification of a Linnaeus name was insufficient. This name is *Acrostichum furcatum* Linnaeus (1759: 1321), which was the first species described of what later would become Gleicheniaceae. In the original description, Linnaeus cited one of Plumier's plates (Plumier 1693). The circumscription of Gleicheniaceae and its genera has changed over time, so that eventually *A. furcatum* was transferred to the genus *Sticherus* and the species came to be called *Sticherus furcatus* (L.) Ching (1940: 283). This species forms dense thickets in the mountains of Mexico, Guatemala, Belize, Honduras, El Salvador, Nicaragua, Costa Rica, Cuba, Jamaica, and Martinique. In the taxonomic treatment of the Lesser Antilles, Proctor (1977) designated as lectotype the same plate of Plumier cited in the protologue by Linnaeus (1759). The illustration represents a fragment of a frond (Fig. 1). However, the taxonomy of Gleicheniaceae is intricate, especially regarding the genus *Sticherus*, which is mainly based on scales morphology. Since the lectotype has no representation of any scale of the plant, it is not sufficient by itself to circumscribe the taxon. Therefore, a complement of the type should be selected (article 9.8 of the ICN—McNeill *et al.* 2012).

To solve this issue, we have searched specimens that could represent uncited original material at P and we found an exsiccate of herbarium Turnerfort that corresponds to Plumier's plate. It also bears the remark "Filix furcata, pinnulis longiusculis, non dentatis" in Plumier's writing, the polyonomy created by him to address the plant. Additionally, the collection location on the label matches with the one cited in the original description (Fig. 2).

Other plates by Plumier, especially the ones of the Tournefort herbarium including Linnaean names, were already properly typificated by Cremers & Aupic (2008). However, *A. furcatum* was not included in their work, and it was also not cited in the list of Plumier's plants storage in P published by Cremers & Aupic (2007). Additionally, Cremers *et al.* (2016) cited the above-mentioned material of the Tournefort herbarium as an epitype of *A. furcatum*, but they did not state "designated here" or some equivalent sentence, which makes this type designation not effectively achieved (art. 7.10 of the ICN—McNeill *et al.* 2012). Therefore, aiming to contribute to the nomenclatural elucidation within Gleicheniaceae, here we rectify the situation and propose an epitype for *Acrostichum furcatum*.

Typification

Sticherus furcatus (L.) Ching (1940: 283).

Acrostichum furcatum Linnaeus (1759: 1321). *Mertensia furcata* (L.) Willdenow (1804: 166). *Gleichenia furcata* (L.) Sprengel (1827: 26).

Lectotype (designated by Proctor 1977):—Plumier (1693: tab. 20).

Epitype (**designated here**):—MARTINIQUE. Sur le Morne de la Calebasse, *Plumier s.n.* [Herbarium Tournefort 5232] (P-TRF [P00322140, image!]). (Fig. 2).

Acknowledgements

We thank Sandra Knapp and Marcos Sobral by the guidance for preparation of this manuscript and Germinal Rouhan who provided the high-resolution image of the selected epitype and to the Herbarium at Muséum National d'Histoire Naturelle, Paris, for permission to publish the image. We also thank CNPq for the research grant and scholarship (201414/2017-1 and 307115/2017-8) to A. Salino and the scholarship to R.S. Viveros (380069/2018-0).

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FIGURE 1. Lectotype of *Acrostichum furcatum* L. (Plumier 1693: tab. 20).

FIGURE 2. Epitype of *Acrostichum furcatum*: Plumier *without number* (P00322140). This image is © copyright Muséum National d'Histoire Naturelle (MNHN) – Paris Herbarium (P).

FIGURE 1. Lectotype of *Acrostichum furcatum* L. (Plumier 1693: tab. 20).

FIGURE 2. Epitype of *Acrostichum furcatum*: Plumier *without number* (P00322140). This image is © copyright Muséum National d'Histoire Naturelle (MNHN) – Paris Herbarium (P).