



Phylogenomic evolutionary insights in the fern family Gleicheniaceae

Lucas Vieira Lima^{a,*}, Alexandre Salino^a, Michael Kessler^b, Germinal Rouhan^c,
Weston L. Testo^d, Caio Suzart Argolo^e, GoFlag Consortium^f, Thaís Elias Almeida^g

^a Universidade Federal de Minas Gerais, Instituto de Ciências Biológicas, Departamento de Botânica, Laboratório de Sistemática Vegetal, Belo Horizonte, Minas Gerais, Brazil

^b Systematic and Evolutionary Botany, University of Zurich, Zurich, Switzerland

^c Institut de Systématique, Evolution, Biodiversité (ISYEB), Muséum national d'Histoire naturelle, CNRS, Sorbonne Université, EPHE, UA, Paris, France

^d Department of Science and Education, Negaunee Integrative Research Center, The Field Museum, Chicago, IL, USA

^e Universidade Estadual de Santa Cruz, Departamento de Ciências Biológicas, Centro de Biotecnologia e Genética, Rodovia Ilhéus-Itabuna, km 16, Ilhéus-BA, Brazil

^f GoFlag is an NSF-funded project (DEB 1541506) based at the University of Florida, Field Museum, and the University of Arizona. Project personnel include (at UF), J. Gordon Burleigh, Emily Sessa, Stuart McDaniel, Christine Davis, Pavlo Antonenko, Sarah Carey, Lorena Endara, Weston Testo; (at Field), Matt von Konrat, Eve Gaus; (at UA): Hong Cui

^g Universidade Federal de Pernambuco, Centro de Biociências, Departamento de Botânica, Avenida Professor Moraes Rego 1235, CEP 50.670-420, Recife, PE, Brazil

ARTICLE INFO

Keywords:

Gleicheniales
Ferns
Phylogeny
Plastome
Systematics

ABSTRACT

The pantropical fern family Gleicheniaceae comprises approximately 157 species. Seven genera are currently recognized in the family, although their monophyly is still uncertain due to low sampling in phylogenetic studies. We examined the monophyly of the genera through extended sampling, using the first phylogenomic inference of the family including data from both nuclear and plastid genomes. Seventy-six samples were sequenced (70 Gleicheniaceae species and six outgroups) using high throughput sequencing, including all seven currently recognized genera. Plastid and nuclear data were recovered and assembled; the nuclear data was phased to reduce paralogy as well as hybrid noise in the final recovered topology. Maximum likelihood trees were built for each locus, and a concatenated dataset was built for both datasets. A species tree based on a multispecies coalescent model was generated, and divergence time analyses performed. We here present the first genomic phylogenetic inferences concerning Gleicheniaceae, confirming the monophyly of most genera except *Sticherus*, which we recovered as paraphyletic. Although most of the extant genera of Gleicheniaceae originated during the Mesozoic, several genera show Neogene and even Quaternary diversifications, and our results suggest that reticulation and polyploidy may have played significant roles during this diversification. However, some genera, such as *Rouxopteris* and *Stromatopteris*, appear to represent evolutionary relicts.

1. Introduction

Gleicheniaceae C.Presl is a distinct leptosporangiate fern family that currently is considered to comprise approximately 157 species distributed into seven genera (Gonzales and Kessler, 2011; PPG, 2016; Liu et al., 2020). The plants have long-creeping rhizomes and pseudo-dichotomous branched fronds with indeterminate growth due to periodic apical dormancy (Holttum, 1957; Tryon and Stolze, 1989; Ostergaard Andersen and Ollgaard, 2001; Gonzales and Kessler, 2011; Lima and Salino, 2018). They are heliophytes, predominantly terrestrial, occasionally occurring on rocks, with low demands for mineral nutrients and often inhabiting disturbed areas such as roadsides (Penrod, 2000;

Walker and Sharpe, 2010).

The numbers of recognized genera in the family have changed over time (Table 1). Smith (1793) initially considered the family to be monogeneric (*Gleichenia* Sm.). Diels (1900) later maintained all species in *Gleichenia*, separating the species into subgenera and sections. This infrageneric classification was initially followed by Christensen (1905), although that author 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 but further recognized the genus *Hicriopteris* C.Presl. Ching (1940) segregated five genera from *Gleichenia* and proposed a new monotypic genus, *Gleichenella* Ching. Holttum (1947)

* Corresponding author.

E-mail addresses: lucasilima1618@gmail.com (L. Vieira Lima), salinobh@gmail.com (A. Salino), michael.kessler@systbot.uzh.ch (M. Kessler), germinal.rouhan@mnhn.fr (G. Rouhan), wtesto@fieldmuseum.org (W.L. Testo), caiosuzart@gmail.com (C. Suzart Argolo), thais.elias@ufpe.br (T. Elias Almeida).

<https://doi.org/10.1016/j.ympev.2023.107782>

Received 26 September 2022; Received in revised form 16 March 2023; Accepted 6 April 2023

Available online 10 April 2023

1055-7903/© 2023 Elsevier Inc. All rights reserved.

initially considered the genera proposed by Christensen (1938), but later (Holtum, 1957, 1959) placed them in the subgenera *Gleichenia* and *Dicranopteris*. Nakai (1950) removed *Platyzoma* from Gleicheniaceae, positioning it in Platyzomataceae, which was later placed in Pteridaceae and subsumed in the genus *Pteris* L. (PPG, 2016).

Phylogenetic inferences based on chloroplast DNA sequences, including *atpA*, *atpB*, *rbcL*, and *rps4* (Pryer et al., 2004; Perrie et al., 2007; Schuettpelz and Pryer, 2007; Li et al., 2010), suggest that the family is composed of two clades. One clade comprises *Diplopterygium* (Diels) Nakai, with scaly rhizomes, as a sister of *Dicranopteris*, while *Gleichenella* has a rhizome covered exclusively by hairs, which has been considered a possible synapomorphy. The other clade comprises *Gleichenia*, *Sticherus*, and *Stromatopteris*, all with scaly rhizomes (Gonzales and Kessler, 2011). Liu et al. (2020) segregated *Gleichenia boryi* from the other *Gleichenia* species, placing it in *Roulopteris* H.M. Liu due to its morphology and phylogenetic characteristics. In their phylogenetic topology, *Roulopteris* is recovered as sister group to a clade formed by *Gleichenella* + *Dicranopteris* + *Diplopterygium*.

Despite these studies, based on phylogenetic reconstructions from a few plastid loci, and major advances in the application of molecular tools to the taxonomy of various fern and lycophyte groups (e.g., Gasper et al., 2017; Zhang and Zhang, 2017; Almeida et al., 2017; Testo et al., 2018; Lehtonen et al., 2020; Chen et al., 2022), the monophyly of the genera of Gleicheniaceae is still questionable in light of low sampling (PPG, 2016), as exemplified by the segregation of *Roulopteris*, which was only discovered due to dense sampling in *Gleichenia* (Liu et al., 2020). Phylogenetic studies, associated with divergence time analyses, have provided valuable information for a better understanding of the evolutionary history of many groups, as they can contextualize the rise of their lineages (Testo et al., 2018; Testo and Sundue, 2016). Gleicheniaceae has previously been included in large-scale dated phylogenies (e.g., Pryer et al., 2004; Testo and Sundue, 2016), although these studies did not include all its genera. Recently, all Gleicheniaceae genera were included in a divergence-time analysis – but based on only a single plastid locus (Liu et al., 2020). Therefore, no multilocus dataset that tested all Gleicheniaceae genera has yet been utilized to estimate the divergence times of the extant lineages of the family.

In that context, we addressed the following questions: What are the phylogenetic relationships among the different Gleicheniaceae lineages? Are the genera of Gleicheniaceae, as currently circumscribed, monophyletic? Are the data recovered from the plastid and nuclear genomes congruent in terms of recounting the evolutionary history of the family? When did the main lineages of Gleicheniaceae arise?

2. Materials and methods

2.1. Sampling, sequencing, and read quality control

In the present study, 76 samples (70 Gleicheniaceae, representing about 44% of recognized species in the family, and six outgroups) from herbarium samples and tissue dried in silica gel were sequenced (Table 2). Samples of the seven genera of Gleicheniaceae sensu PPG I (2016) and Liu et al. (2020) were included (*Dicranopteris*, *Gleichenella*, *Gleichenia*, *Diplopterygium*, *Sticherus*, *Stromatopteris*, and *Roulopteris*). Type species of all genera were sampled. DNA was extracted from silica-dried tissue using the DNeasy Mini Plant Kit (Qiagen); the CTAB protocol (cetyltrimethylammonium bromide) protocol was used for herbarium samples, following Doyle and Doyle (1990). The samples were sequenced using Rapid Genomics (Gainesville, USA) using target enrichment sequencing using GoFlag 451 probes (a set of 56,989 probes that covered 451 exons from 248 single or low-copy nuclear genes) (Breinholt et al., 2021). Samples were sequenced using the Illumina HiSeq 2500 platform, generating 150 bp paired-end reads. Raw data quality was verified using FastQC (Andrews, 2010, version 0.11.9); the filtering and trimming of low quality pair-end reads was performed using Trimmomatic (Bolger et al., 2014, version 0.36) (illuminaclip 2:30:10, leading 10, trailing 40). The trimmed raw data were assembled in two separate datasets: nuclear and chloroplast. Sequence reads were deposited in the NCBI Sequence Read Archive (Table 2) and recovered gene sequences and alignments are available on GitHub (https://github.com/lucaslima1618/phylo_gleich).

2.2. Chloroplast dataset

To build a partial plastome dataset, Geneious Prime 2021 (version 2021.1.1) (<https://www.geneious.com>) was used to assemble the trimmed raw data by reference. The annotated plastome of *Diplopterygium glaucum* [deposited at GenBank (NC_024158) (Kim et al. 2014)] was used as a 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 TrimAL (Capella-Gutierrez et al., 2009, version 1.2) was used in each partition to eliminate columns with >60% gaps. The result was a matrix with 73 terminals and approximately 15,000 bp.

2.3. Nuclear dataset

HybPiper (Johnson et al., 2016) was used to assemble the nuclear sequence reads derived from the GoFlag probe set for ferns. Considering

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)
<i>Gleichenia</i>	<i>Gleichenia</i>	<i>Gleichenia</i>	—//—	—//—	—//—	—//—	—//—	—//—
—//—	Subg. <i>Eu-Gleichenia</i>	—//—	<i>Stromatopteris</i>	<i>Stromatopteris</i>	<i>Stromatopteris</i>	<i>Stromatopteris</i>	<i>Stromatopteris</i>	<i>Stromatopteris</i>
—//—	Subg. <i>Eu-Gleichenia</i>	Sect. <i>Eugleichenia</i>	<i>Gleichenia</i>	<i>Gleichenia</i>	<i>Gleichenia</i>	<i>Gleichenia</i>	<i>Gleichenia</i>	<i>Gleichenia</i>
—//—	—//—	Subsect.	—//—	—//—	—//—	—//—	—//—	—//—
—//—	—//—	<i>Gleicheniastrum</i>	—//—	<i>Calymella</i>	—//—	—//—	—//—	—//—
—//—	Subg. <i>Mertensia</i>	Subsect. <i>Calymella</i>	—//—	—//—	—//—	—//—	—//—	—//—
—//—	—//—	Sect. <i>Mertensia</i>	—//—	—//—	—//—	—//—	—//—	—//—
—//—	Sect.	Subsect.	<i>Hicriopteris</i>	<i>Hicriopteris</i>	Subg.	<i>Diplopterygium</i>	<i>Diplopterygium</i>	<i>Roulopteris</i>
—//—	<i>Diplopterygium</i>	<i>Diplopterygium</i>	—//—	—//—	<i>Diplopterygium</i>	—//—	—//—	<i>Diplopterygium</i>
—//—	Sect. <i>Holopterygium</i>	Subsect.	<i>Sticherus</i>	<i>Sticherus</i>	Subg. <i>Mertensia</i>	<i>Sticherus</i>	<i>Sticherus</i>	<i>Sticherus</i>
—//—	—//—	<i>Holopterygium</i>	—//—	—//—	—//—	—//—	—//—	—//—
—//—	Sect.	Subsect.	<i>Dicranopteris</i>	—//—	<i>Dicranopteris</i>	<i>Dicranopteris</i>	<i>Dicranopteris</i>	<i>Dicranopteris</i>
—//—	<i>Heteropterygium</i>	<i>Heteropterygium</i>	—//—	<i>Dicranopteris</i>	Subg. <i>Dicranopteris</i>	—//—	—//—	—//—
—//—	—//—	—//—	—//—	<i>Gleichenella</i>	—//—	—//—	<i>Gleichenella</i>	<i>Gleichenella</i>
—//—	Sect. <i>Acropterygium</i>	Subsect.	—//—	<i>Acropterygium</i>	Subg.	—//—	—//—	—//—
—//—	—//—	<i>Acropterygium</i>	—//—	—//—	<i>Acropterygium</i>	—//—	—//—	—//—

Table 2

Voucher information for specimens used in this study and SRA accessions. Herbarium acronyms are according to Thiers (2020 onward: <http://sweetgum.nybg.org/science/ih/>).

Taxon Name	Voucher Number	Voucher Location	Country of origin	Accession
<i>Dicranopteris dichotoma</i>	Takehara 2	Herb Inst. Biologie Tohoku	Japan	SAMN33583381
<i>Dicranopteris flexuosa</i>	Lima 220	BHCB	Brazil	SAMN33583382
<i>Dicranopteris linearis</i>	Kessler 13864	GOET	New Guinea	SAMN33583383
<i>Dicranopteris taiwanensis</i>	Wen-Liang 15282	TAIF	India	SAMN33583384
<i>Dicranopteris nervosa</i>	Lima 226	BHCB	Brazil	SAMN33583385
<i>Dicranopteris rufinervis</i>	Lima 213	BHCB	Brazil	SAMN33583386
<i>Dicranopteris seminuda</i>	Martinelli 17233	RB	Brazil	SAMN33583387
<i>Dicranopteris spissa</i>	Salino 16256	BHCB	Brazil	SAMN33583388
<i>Dicranopteris subpectinata</i>	SWK 1717	FU	Malaysia	SAMN33583389
<i>Dicranopteris tetrphylla</i>	Tobagane 4641	FU	Thailand	SAMN33583390
<i>Diplopterygium bancroftii</i>	Øllgaard 35676	AAU	Ecuador	SAMN33583391
<i>Diplopterygium brevipinnulum</i>	Karger 1441	GOET	Moluccas	SAMN33583392
<i>Diplopterygium chinensis</i>	Tobagane 76	FU	Taiwan	SAMN33583393
<i>Diplopterygium conversum</i>	Jimenez 1434	FU	Indonesia	SAMN33583394
<i>Diplopterygium glaucum</i>	Karger 597	GOET	Philippines	SAMN33583395
<i>Diplopterygium longissimum</i>	Kessler 13546	GOET	Malaysia	SAMN33583396
<i>Diplopterygium norisii</i>	Karger 1099	GOET	Malaysia	SAMN33583397
<i>Diplopterygium volubilis</i>	Jimenez 1148	FU	Indonesia	SAMN33583398
<i>Diplopterygium sp. 1</i>	T 3869	FU	Thailand	SAMN33583399
<i>Diplopterygium sp. 2</i>	T 2084	FU	Cambodia	SAMN33583400
<i>Gleichenella pectinata</i>	Lima 225	BHCB	Brazil	SAMN33583401
<i>Gleichenia dicarpa</i>	Kessler 14281	GOET	Australia	SAMN33583402
<i>Gleichenia peltophora</i>	Karger 441	GOET	Philippines	SAMN33583403
<i>Gleichenia polypodioides</i>	Kessler 13836	GOET	La Réunion	SAMN33583404
<i>Rouxopteris boryi</i>	Hennequin 254	P	La Réunion	SAMN33583405
<i>Rouxopteris boryi</i> var. <i>madagascariensis</i>	Hennequin 276	P	La Réunion	SAMN33583406
<i>Sticherus aurantiacus</i>	Øllgaard 2504	AAU	Ecuador	SAMN33583407
<i>Sticherus bifidus</i>	Lima 210	BHCB	Brazil	SAMN33583408
<i>Sticherus blepharolepis</i>	Kessler 14840	GOET	Colombia	SAMN33583409
<i>Sticherus bolanicus</i>	Karger 2626	GOET	New Guinea	SAMN33583410
<i>Sticherus brackenridgei</i>	Lehnert 3615	GOET	New Guinea	SAMN33583411
<i>Sticherus brevitomentosus</i>	Solomon 17604	MBM	Bolivia	SAMN33583412
<i>Sticherus decurrens</i>	Lima 207	BHCB	Brazil	SAMN33583413
<i>Sticherus farinosus</i>	Kluge 9255	GOET	Guadalupe	SAMN33583414
<i>Sticherus ferrugineus</i>	Kessler 14699	GOET	Colombia	SAMN33583415
<i>Sticherus flabellatus</i> var. <i>compactus</i>	Kessler 14280	GOET	Australia	SAMN33583416
<i>Sticherus flagellaris</i>	Kessler 13821	GOET	La Réunion	SAMN33583417
<i>Sticherus fulvus</i>	Kluge 9259	GOET	Guadalupe	SAMN33583418
<i>Sticherus furcatus</i>	Testo 1518	VT	Mexico	SAMN33583419
<i>Sticherus gracilis</i>	Lima 212	BHCB	Brazil	SAMN33583420
<i>Sticherus habbemensis</i>	Kessler 14121	GOET	New Guinea	SAMN33583421
<i>Sticherus hirtus</i>	Kluge s.n.	GOET	Indonesia	SAMN33583422
<i>Sticherus hypoleucus</i>	Kessler 14800	GOET	Colombia	SAMN33583423
<i>Sticherus jacha</i>	Jimenez 1615	GOET	Bolivia	SAMN33583424
<i>Sticherus lanosus</i>	Bach 1769	GOET	Bolivia	SAMN33583425
<i>Sticherus lanuginosus</i>	Lima 208	BHCB	Brazil	SAMN33583426
<i>Sticherus lechleri</i>	Jimenez 16362	GOET	Bolivia	SAMN33583427
<i>Sticherus loheri</i>	Karger 1001	GOET	Indonesia	SAMN33583428
<i>Sticherus maritimus</i>	Kessler 14839	GOET	Colombia	SAMN33583429
<i>Sticherus melanoblastus</i>	Kessler 14743	GOET	Colombia	SAMN33583430
<i>Sticherus milnei</i>	Kluge7003	GOET	Indonesia	SAMN33583431
<i>Sticherus montaguei</i>	Lehnert 3625	GOET	New Guinea	SAMN33583432
<i>Sticherus nervatus</i>	Kessler 14733	GOET	Bolivia	SAMN33583433
<i>Sticherus nigropaleaceus</i>	Lima236	BHCB	Brazil	SAMN33583434
<i>Sticherus nudus</i>	Lima 239	BHCB	Colombia	SAMN33583435
<i>Sticherus pallescens</i>	Lima 238	BHCB	Colombia	SAMN33583436
<i>Sticherus paulistanus</i>	Salino 8431	BHCB	Brazil	SAMN33583437
<i>Sticherus pruinosus</i>	Lima 325	BHCB	Brazil	SAMN33583438
<i>Sticherus remotus</i>	Jimenez 2709	GOET	Bolivia	SAMN33583439
<i>Sticherus revolutus</i>	Lima 240	BHCB	Colombia	SAMN33583440
<i>Sticherus rubiginosus</i>	Lima 237	BHCB	Colombia	SAMN33583441
<i>Sticherus salinoi</i>	Fernandes 771	BHCB	Brazil	SAMN33583442
<i>Sticherus simplex</i>	Asplund s.n.	R	Peru	SAMN33583443
<i>Sticherus squamosus</i>	Lima 233	BHCB	Brazil	SAMN33583444
<i>Sticherus tomentosus</i>	Kessler 142728	GOET	Colombia	SAMN33583445
<i>Sticherus truncatus</i>	Karger 287	GOET	Malaysia	SAMN33583446
<i>Sticherus truncatus</i>	Jimenez1388	FU	Indonesia	SAMN33583447
<i>Sticherus vestitus</i>	Jimenez1151	FU	Indonesia	SAMN33583448
<i>Stromatopteris moniliformis</i>	Munzinger 1317	P	New Caledonia	SAMN33583449
<i>Hymenophyllum pulchellum</i>	Testo 909	VT	Mexico	SAMN33583450
<i>Trichomanes ankersii</i>	Testo 1242	VT	Mexico	SAMN33583451
<i>Dipteris conjugata</i>	Knapp 1438	P	Taiwan	SAMN33583452
<i>Danaea wendlandii</i>	Testo 784	VT	Costa Rica	SAMN33583453
<i>Danaea sp. 1</i>	Testo 994	VT	Panama	SAMN33583454
<i>Danaea sp. 2</i>	Testo 1440	VT	Colombia	SAMN33583455

the HybPiper supercontig output (exons + introns), Hybphaser (Nauheimer et al., 2020, version 2.0) was used to access the quality of the sequences, exclude putative paralogs, access heterozygosity and haplotypic divergence, and to phase haplotypes, with the objective of reducing putative hybrid noise in the recovered topology. We followed Nauheimer et al. (2020) and Bloesch et al. (2022) and SNPs (single nucleotide polymorphism) were mapped and were only called when coverage was at least 10x, the allele frequency was at least 0.15, and the alternative allele occurred in at least 4 reads. All loci were removed that had an outlier value for the proportion of SNPs compared to other loci (datapoints of values that were above 1.5x the interquartile range above the third quartile were considered outliers).

2.4. Phylogenetic inferences

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

ModelTest-NG (Darriba et al., 2020) was used with both the nuclear and chloroplast datasets to select the best-fit model of evolution for each partition (recovered loci) based on 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 nuclear loci and to estimate gene concordance factors (gCF) and site concordance factors (sCF). ASTRAL III (Zhang et al., 2018) was used with previously generated gene trees to infer the species tree based on a multi-species coalescent method. ASTRAL trees were scored to obtain an estimate of incomplete lineage sorting (ILS). The trees were rooted using *Dipteris conjugata* as an outgroup. We ran all analyses in the Sagarana HPC cluster (housed at Universidade Federal de Minas Gerais).

2.5. Divergence time analysis

TreePL (Smith and O'Meara, 2012) was used to estimate the divergence times of the main extant lineages of Gleicheniaceae. The TreePL run was optimized and conducted following the guide described by Maurin (2020). A smoothing value of 10^6 was chosen after comparing tree runs using 10^3 , 10^6 , and 10^9 . Two fossil calibrations were used: *Gleichenia chaloneri* Herendeen & J. Skog, the most ancient known *Gleichenia* fossil (Herendeen and Skog, 1998), setting the crown age of *Gleichenia* within 99–112 million years ago (mya); and *Chansithea wudaensis* Deng, Sun and Li, the most ancient Gleicheniaceae-like fossil (He et al., 2020; He et al. 2016), constraining the crown age of Gleicheniales to within approximately 297–298 mya. Since there is no conclusive evidence (such as a pseudo-dichotomic frond structure) that unambiguously aligns these Gleicheniaceae-like fossils with Gleicheniaceae, we chose to calibrate Gleicheniales crowns conservatively. Further discussion regarding the Gleicheniaceae fossils is available in Supplementary Material 2.

3. Results

The nuclear dataset recovered an average of 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 the sequences (Supplementary Material 3). Our final partitioned matrix had 294 loci and a length of approximately 150 thousand bp. Following the thresholds suggested by 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 3) (Fig. 1). Additionally, the results of the ASTRAL III-scored tree pointed out that 84% of quartet trees induced by the gene trees are in the species tree of our nuclear dataset.

The recovered topology of NST showed two main clades (Fig. 2), which were recovered in both PST and MSCT. The first clade (here named the Diplopteroid clade) is formed by *Diplopterygium*, as sister to a clade formed by *Gleichenella* + *Dicranopteris* (bootstrap = 100). Two clades within *Dicranopteris* were recovered, one with *Dicranopteris tetraphylla* + *Di. linearis* + *Di. taiwanensis* + *Di. dichotoma* (bs = 100) and another formed by *Di. speciosa* + *Di. subpectinata* plus the neotropical species (*Di. nervosa*, *Di. rufinervis*, *Di. seminuda*, *Di. spissa*, and *Di. flexuosa*) (Fig. 2) (bs = 100). This topology was recovered in all phylogenetic inferences, although there was some discordance regarding the relationships within the neotropical species of *Dicranopteris*.

Regarding *Diplopterygium*, *Dp. bancroftii* (the only neotropical species of the genus) was sister to the remaining species in all topologies (bs = 100). In the NST, a clade formed by *Dp. norrisii* + *Diplopterygium* sp. 2 + *Dp. brevipinnulum* + *Dp. sordidum* was sister to a clade formed by *Dp. chinensis* + *Dp. volubilis* + *Diplopterygium* sp. 1 + *Dp. longissimum* + *Dp. conversum* + *Dp. glaucum* (Fig. 1) (bs = 100). This topology showed high concordance with the MSCT, except for *Dp. sordidum*, which came out as sister to *Dp. brevipinnulum* (Fig. 2, Supplementary Material 4). However, there was discordance between the recovered topologies of the nuclear dataset (NST and MSCT) and the plastid dataset regarding the affinities among Asian species of *Diplopterygium*. In the PST, *D. norrisii* emerged as a sister group of two clades, one formed by *Dp. volubilis* + *Diplopterygium* sp. 1 and the other formed by *Dp. sordidum* + *Dp. brevipinnulum* as a sister group of *Dp. chinensis* + *Diplopterygium* sp. 1 + *Dp. conversum* + *Dp. glaucum* + *Dp. longissimum* (Fig. 2, Supplementary Material 4).

In the second clade, named here as the Sticheroid clade, *Rouxopteris* was recovered as sister to all other lineages in all recovered topologies (Fig. 2) (bs = 100). However, with NST, *Rouxopteris* appeared as sister of a clade formed by *Sticherus milnei* + *S. truncatus*, which was, in turn, sister of *Gleichenia* + *Stromatopteris* plus the remaining *Sticherus* species (Fig. 2) (bs = 100). In the MSCT, *Rouxopteris* emerged as sister to *Stromatopteris* + a clade formed by *Sticherus milnei* + *S. truncatus*, which in turn is sister to *Gleichenia* + plus the remaining species of *Sticherus* (Supplementary Material 4). In the PST, *Rouxopteris* came out as sister to a clade formed by *Gleichenia* + *Stromatopteris*, which was, in turn, sister to a clade formed by *Sticherus milnei* + *S. truncatus* and the remaining species of *Sticherus* (Supplementary Material 4) (bs = 100). The phylogenetic placement of *Stromatopteris* in the MSCT was incongruent between the PST and NST topologies (Fig. 2, Supplementary Material 4). Within *Gleichenia*, *G. polypodioides* was recovered as sister to *G. dicarpa* + *G. peltophora* in the PST (Supplementary Material 4). Only plastid sequences were recovered for *G. dicarpa* (Fig. 2, Supplementary Material 4) (bs = 100).

The relationships within the clades formed by the remaining *Sticherus* species were not well resolved and incongruent among the recovered topologies, except for early diverging lineages and a few individual clades. In the NST, *Sticherus brackenridgei* was recovered as sister to the other species, followed by *S. montaguei* as sister to two clades. One clade was formed by five species with distributions throughout 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* (all node with bs = 100). The other clade was formed by *Sticherus simplex* as sister to the remaining species (bs = 100), followed by *Sticherus flagellaris*, an African species, as sister to a clade formed by *Sticherus nervatus*, *S. pruinusosus*, *S. lechleri*, and *S. revolutus* (bs = 99), which was, in turn, sister to the remaining species. Although the relationships among them are not well resolved, a few clades are well supported [e.g., the one formed by *S. squamosus*,

Table 3

Allele divergence and locus heterozygosity (showing the proportion of loci containing > 0% SNPs) rates for each sample.

Sample	Allele Divergence	Locus Heterozygosity
<i>G. peltophora</i>	3,18	87,88
<i>S. montaguei</i>	2,47	87,86
<i>D. flexuosa</i>	2,29	89,11
<i>D. klotzschii</i>	2,07	86,67
<i>D. nervosa</i>	2,05	90,62
<i>D. spissa</i>	1,93	86,41
<i>D. seminuda</i>	1,92	86,39
<i>D. rufinervis</i>	1,84	80,30
<i>D. linearis</i>	1,81	89,41
<i>D. wendlandii</i>	1,80	78,87
<i>S. truncatus</i>	1,61	66,42
<i>S. lechleri</i>	1,49	91,46
<i>S. nudus</i>	1,42	86,06
<i>S. nervatus</i>	1,40	91,50
<i>S. revolutus</i>	1,33	89,46
<i>S. pruinatus</i>	1,30	85,37
<i>S. nigropaleaceus</i>	1,15	86,70
<i>S. bolanicus</i>	1,10	81,95
<i>S. salinoi</i>	1,10	87,86
<i>H. pulchellum</i>	1,06	78,32
<i>S. vestitus</i>	0,94	78,01
<i>D. norrisii</i>	0,92	68,69
<i>Danaea sp1</i>	0,84	75,63
<i>S. simplex</i>	0,74	71,22
<i>S. remotus</i>	0,65	69,17
<i>S. milnei</i>	0,62	62,22
<i>T. ankersii</i>	0,57	26,85
<i>Diplopterygium sp2</i>	0,57	55,23
<i>D. taiwanensis</i>	0,56	28,92
<i>S. squamosus</i>	0,52	77,91
<i>D. dichotoma</i>	0,49	49,15
<i>D. sordidum</i>	0,47	62,77
<i>S. aurantiacus</i>	0,45	38,24
<i>D. glaucum</i>	0,44	53,66
<i>S. ferruginosus</i>	0,43	72,82
<i>Danaea sp2</i>	0,42	47,80
<i>R. boryi</i> var. <i>madagascariensis</i>	0,42	56,35
<i>G. polypodioides</i>	0,41	33,25
<i>S. hirtus</i>	0,41	45,63
<i>D. subpectinata</i>	0,40	53,83
<i>S. moniliformis</i>	0,39	29,97
<i>D. longissimum</i>	0,37	57,66
<i>S. truncatus</i>	0,35	29,22
<i>D. tetrphylla</i>	0,34	57,52
<i>D. conjugata</i>	0,32	50,01
<i>S. loheri</i>	0,31	45,48
<i>R. boryi</i>	0,31	19,50
<i>S. flagellaris</i>	0,29	47,80
<i>D. conversum</i>	0,28	38,54
<i>D. chinensis</i>	0,27	47,19
<i>Diplopterygium sp1</i>	0,26	40,88
<i>S. jacha</i>	0,26	44,99
<i>D. brevipinnulum</i>	0,25	47,93
<i>G. dicarpa</i>	0,25	25,13
<i>S. blepharolepis</i>	0,24	50,49
<i>S. tomentosus</i>	0,24	50,01
<i>S. hypoleucus</i>	0,23	50,98
<i>S. rubiginosus</i>	0,23	49,76
<i>D. bancroftii</i>	0,21	31,63
<i>S. brackenridgei</i>	0,21	45,63
<i>S. pallescens</i>	0,21	43,03
<i>S. paulistanus</i>	0,20	37,06
<i>D. speciosa</i>	0,19	43,50
<i>S. bifidus</i>	0,19	19,12
<i>S. melanoblastus</i>	0,19	45,12
<i>S. lanosus</i>	0,18	44,17
<i>S. habbemensis</i>	0,18	33,42
<i>S. furcatus</i>	0,16	37,84
<i>D. volubilis</i>	0,15	29,03
<i>S. lanuginosus</i>	0,15	31,28
<i>S. flabellatus</i>	0,11	21,27
<i>S. brevitomentosus</i>	0,11	20,72
<i>S. farinosus</i>	0,09	22,25

Table 3 (continued)

Sample	Allele Divergence	Locus Heterozygosity
<i>S. decurrens</i>	0,08	21,62
<i>S. fulvus</i>	0,08	28,12
<i>S. gracilis</i>	0,07	23,02
<i>S. maritimus</i>	0,06	13,14
<i>G. pectinata</i>	0,03	12,07

S. lanuginosus, and *S. paulistanus* (bs = 100), and another formed by *S. maritimus*, *S. blepharolepis*, *S. ferruginosus*, *S. decurrens*, *S. bifidus*, and *S. fulvus* (Fig. 2) (bs = 100). The clade formed by *S. nudus*, *S. bolanicus*, *S. flabellatus*, *S. loheri*, and *S. hirtus* was also recovered in the MSCT, although *S. nervatus*, *S. pruinatus*, *S. revolutus*, and *S. lechleri* did not form a clade (Supplementary Material 4).

The time-calibrated tree recovered an estimated origin of the Gleicheniaceae crown approx. 121–125 mya (CI = [121.77–125.03], median = 123.4), of the Diplopteroid lineage crown at 105–115 mya (CI = [105.25–115.48], median = 110.36), and of the Sticheroid clade crown at 119–122 mya (CI = [119.32–122.63], median = 120.97) (Fig. 3). The split between *Gleichenella* and *Dicranopteris* was estimated to have occurred 49–62 mya (CI = [49.87–62.61], median = 56.24). Within the *Diplopterygium* lineage, the split of neotropical *D. bancroftii* from the other *Diplopterygium* species was estimated to have occurred 47–68 mya (CI = [47.29–68.03], median = 57.66). Within the Sticheroid clade, *Rouxopteris* diverged from the other lineages about 119–122 mya (CI = [119.32–122.63], median = 120.97), while *Sticherus* s.s. diverged from *Stromatopteris* + *Gleichenia* and the remaining *Sticherus* species about 114–117 mya (CI = [114.39–117], median = 115.85). The separation between *Stromatopteris* and *Gleichenia* occurred 107–109 mya (CI = [107.38–109.46], median = 108.42), while the remaining *Sticherus* diverged from *Stromatopteris* + *Gleichenia* clade about 111–113 mya (CI = [111.08–113.58], median = 112.33). (Fig. 3). Although the clade formed by the remaining species of *Sticherus* represents an old lineage, its species showed recent divergences approximately 3–0.5 mya (Fig. 3). In contrast, *Diplopterygium bancroftii* has an estimated separation from the other *Diplopterygium* species of about 47 to 68 mya. Finally, it is estimated that the diversification of *Dicranopteris* species took place between 14 and 3 mya (Fig. 3).

4. Discussion

4.1. Topology

Here we present the first family-level phylogenomic inference of Gleicheniaceae based on both nuclear and plastid data. Our analyses recovered two main clades, the Diplopteroid and Sticheroid clades, and clarified the phylogenetic relationships among all Gleicheniaceae genera. Our results support the current circumscription of the genera with the exception of *Sticherus*, which was recovered as paraphyletic, and highlight the phylogenetic and morphological uniqueness of the clade formed by *Sticherus milnei* + *S. truncatus*. However, the phylogenetic placement of the *S. milnei* + *S. truncatus* clade differs among the topologies recovered from the nuclear (NST and MSCT) and plastid datasets (PST). Therefore, the morphological characters of *Sticherus* will need to be reinterpreted and its circumscription revised. Importantly, *Sticherus laevigatus* (= *Sticherus truncatus*), the type species of *Sticherus*, appears as a clade independent of the majority of remaining *Sticherus* species. All recovered topologies in the Diplopteroid clade are congruent with respect to generic relationships and circumscriptions (Fig. 2, Fig. 4, Supplementary Material 4).

Our recovered topologies agree with Li et al. (2010) in terms of *Dicranopteris* + *Gleichenella* being sister to *Diplopterygium* and *Stromatopteris* + *Gleichenia* being sister to the remaining species of *Sticherus*, although these authors did not include *Rouxopteris boryi*. Liu et al. (2020) proposed the segregation of *Rouxopteris boryi* from *Gleichenia* due

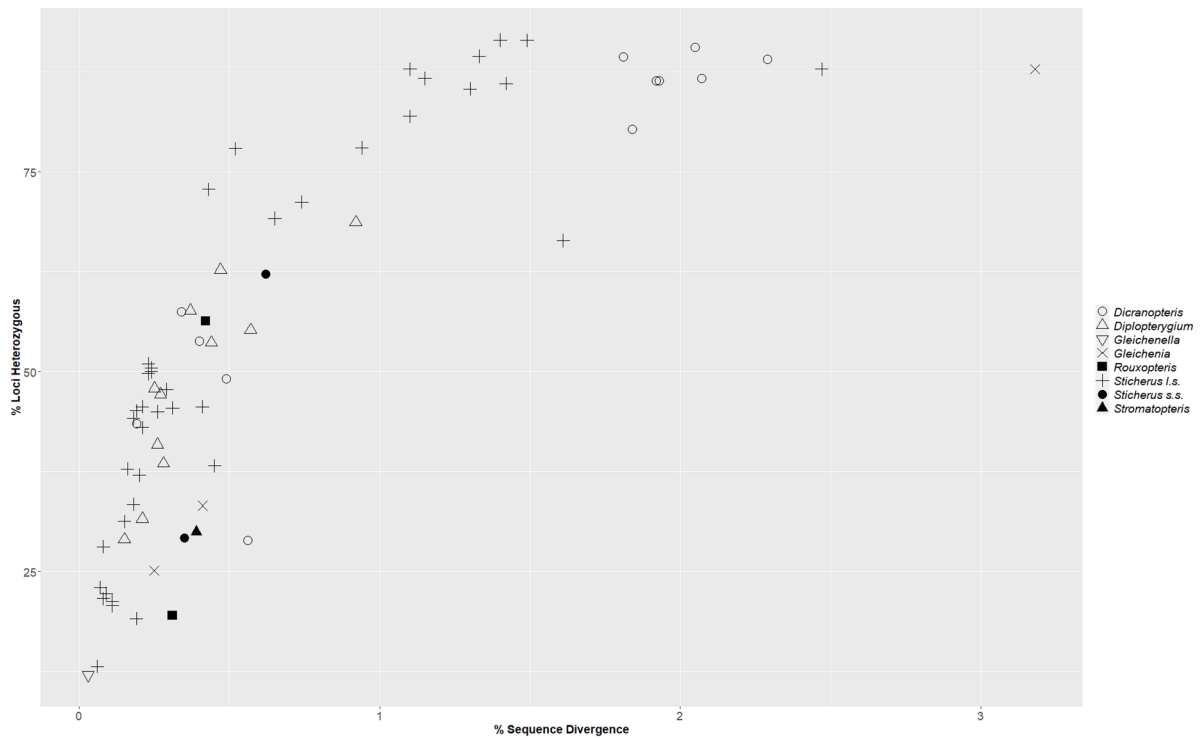


Fig. 1. Relationship between locus heterozygosity and allele divergence. Symbols correspond to representative of the following genera: white hexagon = *Dicranopterus*; white triangle = *Diplopterygium*; inverted white triangle = *Gleichenella*; multiplication sign = *Gleichenia*; black squares = *Rouxopterus*; cross = remaining species of *Sticherus*; black hexagon = *Sticherus s.s.*; black triangle = *Stomatopterus*.

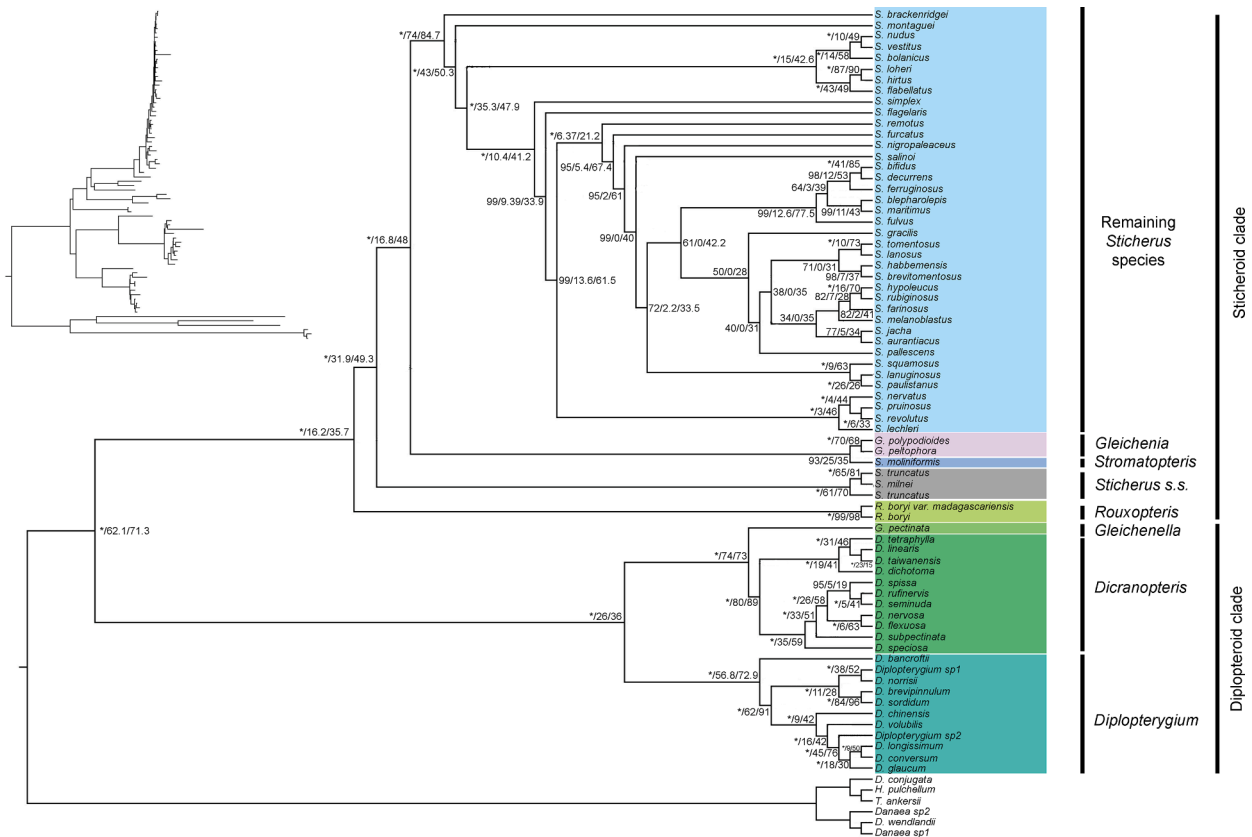


Fig. 2. Maximum Likelihood species tree generated from a partitioned nuclear matrix, with ultrafast bootstrap branch supports, gene concordance factor (gCF), and site concordance factor (sCF), respectively. * Indicates Ultrafast Bootstrap = 100.

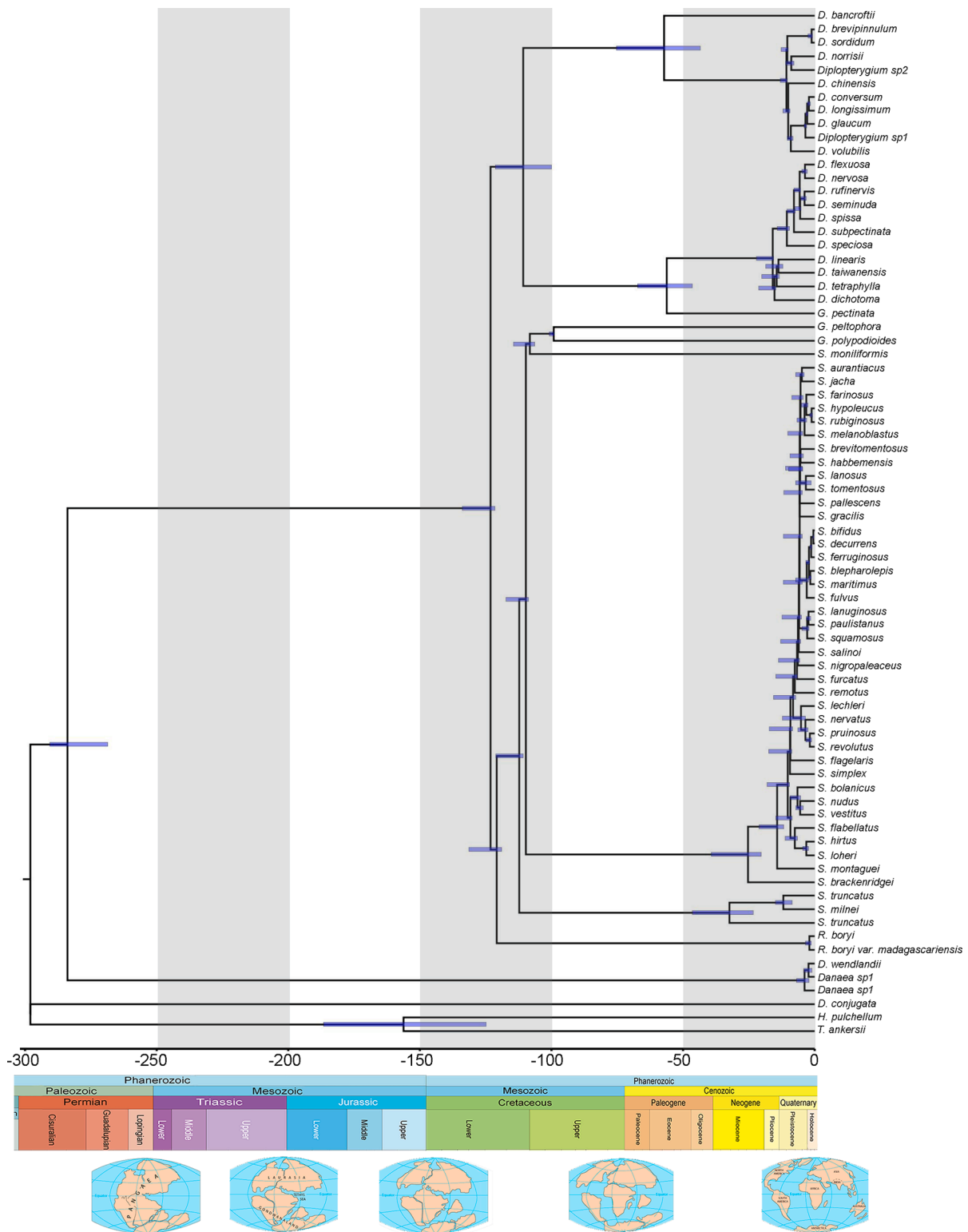


Fig. 3. Divergence time estimates for Gleicheniaceae based on the nuclear dataset. Blue lines at the nodes indicate 95% highest probability density intervals. Dates are given in million years (mya). Chronostratigraphic bar follows the International Chronostratigraphic Chart v2022/02 (www.stratigraphy.org). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

to its morphological uniqueness and its placement in a phylogenetic hypothesis based on *rbcL* sequences. In their topology, *Rouxopteris* comes out with low support as sister to the Diplopteroid clade. In contrast, in our results, *Rouxopteris* is recovered with high support in all topologies as sister to the Sticheroid clade (Fig. 4).

The taxonomy of Gleicheniaceae has traditionally been based on the rhizome and bud indument types (Holttum, 1957; Tryon and Stolze, 1989; Østergaard Andersen and Øllgaard 2001; Gonzales and Kessler, 2011). However, the first phylogenetic inferences focusing on the family demonstrated that these characters do not reflect the evolutionary

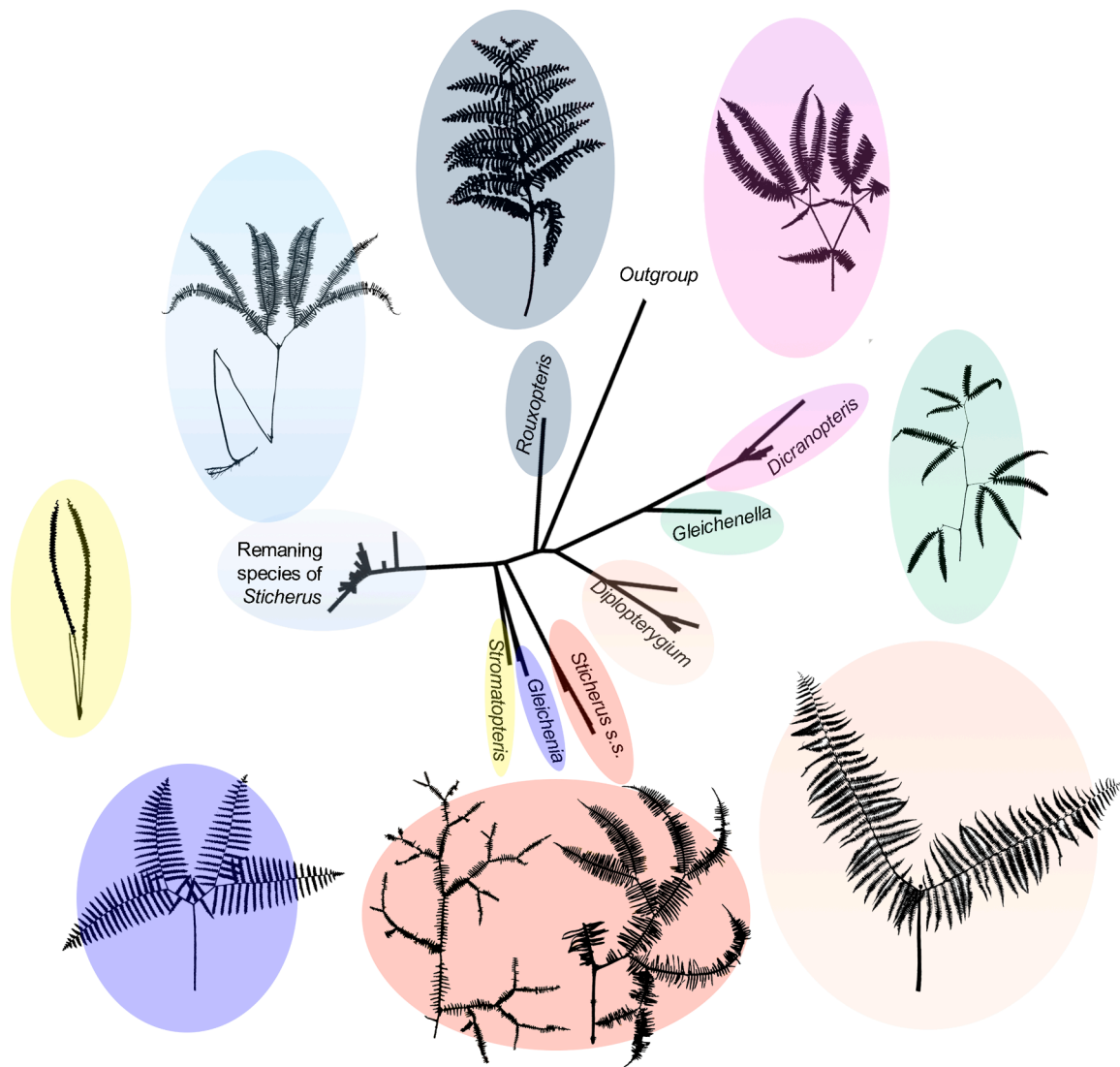


Fig. 4. Unrooted collapsed tree based on nuclear ML tree depicting generic relationships and illustrations of ramifications patterns observed in each genus of Gleicheniaceae.

affinities of the genera. For example, *Diplopterygium*, with scaly rhizomes and buds, is sister to *Dicranopteris* + *Gleichenella*, both with hairy rhizomes and buds (Liu et al., 2020; Li et al., 2010). The hairs found on some Gleicheniaceae, such as *Dicranopteris* and *Gleichenella*, can be interpreted evolutionarily, based on their morphology and the fossil record, as originating from reduced scales (Liu et al., 2020). Alternatively, they may have emerged independently several times in the family. Other Gleicheniaceae species, such as *Rouxopteris boryi* and *Gleichenia microphylla*, have scales (with ciliated margins) and hairs, as well as stiff stellate hairs on their rhizomes (Holtum, 1959; Liu et al., 2020). Several Asian species of *Diplopterygium* also have rhizomes and buds covered by scales, and bear stellate hairs on the abaxial surface of the rachis (Jin et al., 2013). Similarly, some species of *Sticherus* (e.g., *S. pruinosus*) have hairs on segments of the abaxial surfaces of secondary veins (Gonzales and Kessler, 2011; Lima and Salino, 2018). Gonzales (2003), however, did not recognize those structures as hairs but rather as reduced scales. Clearly, the evolution of scales and hairs, and their taxonomic significance, need to be explored in more detail in light of the new phylogenetic evidence.

4.2. Hybridization and polyploidization

In contrast to the well-resolved relationships between genera, in many cases in our study the relationships within genera are not well-resolved, especially in *Sticherus*. The lack of resolution and the recovered incongruences among the NST, PST, and MSC trees may be related, at different levels, to incomplete taxonomic sampling, incomplete lineage sorting, and introgression following hybridization (Rieseberg and Soltis, 1991; Dorado et al., 1992; Degnan and Salter, 2005; Drábková and Vleck, 2010; Xu et al., 2012; Sigel, 2016; Bruun-Lund et al., 2017). Incomplete lineage sorting may also play a role in the incongruence between gene trees and species trees, especially in recently diverged species (Knowles and Carstens, 2007), such as those in *Sticherus* (most of them with divergence estimated to have occurred ca. 3–4 mya). The ASTRAL results (with about 84% of the quartet trees induced by the gene trees found in the species tree) indicate a significant amount 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). High LH and HD values may also be related to fixed heterozygosity in species of allopolyploid origin. This situation occurs when allopolyploid species retain sets of divergent gene copies inherited from

each parental species and may present phenotypes reflecting the additivity or synergy of the parental genomes in their respective ratios (Buggs et al., 2014; Soltis and Soltis, 2000; Sigel, 2016). Further, Ohlsen et al. (2022) recovered a topology of *Gleichenia dicarpa* as a polyphyletic taxon using chloroplast *rbcl* and *trnL-trnF* sequences, which may also be related to reticulation and hybrid formation. Thus, all these data suggest that hybridization and polyploidization may have been important evolutionary drivers in Gleicheniaceae.

It has long been proposed that some *Sticherus* species may have originated through hybridization events, based on evidence from both morphology (Gonzales and Kessler, 2011) and cytogenetic data (Jermy and Walker, 1985). Unfortunately, little progress has been made in testing these hypotheses of reticulate evolution in *Sticherus*. Although our analyses do not include described hybrids, our results support a hybrid origin for some species in this lineage. For example, *Sticherus nigropaleaceus*, one of the species that shows morphological intermediacy between two species groups in *Sticherus* (Prado and Lellinger, 1996; Gonzales and Kessler, 2011; Lima and Salino, 2018) showed high rates of LH and HD. Although no chromosome count has been performed so far for *S. nigropaleaceus*, its DNA c-value has been estimated at about 2–3 times that of other *Sticherus* species that have been investigated (Lima et al., 2021), supporting an allopolyploid origin. Similarly, all species in the taxonomically complex group of species of *Sticherus revolutus* (*S. nervatus*, *S. revolutus*, and *S. pruinosus*) showed high rates of LH and HD (Table 2). In the taxonomically equally complex genus *Dicranopteris*, most of the sampled species also 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., 2021). Traditionally, a broad taxonomic concept has been adopted for *Di. linearis* due to the lack of morphological and molecular studies (Chinnock and Bell, 1998; Perrie and Brownsey, 2015; Chen et al., 2017). However, the reported chromosome numbers of *Di. linearis* ($n = 39$, $n = 40$, 78 , $n = 80$) (Lima et al., 2021) vary as much as their morphology and geographical distribution – and could be used to better understand the reticulation within the complex as well as to clarify species limits. The complex was recently the focus of molecular and morphological studies, and a few taxa were segregated from it (Wei et al., 2021), although further work using integrative tools is still needed. Furthermore, there has been discussion about the morphological distinctiveness of *Di. linearis* (with Asian and African distribution) and *Di. flexuosa* (with neotropical distribution) (Lima and Salino, 2018). All the investigated samples of *Di. flexuosa* have so far shown a base haploid number of 78, and 2C values of 9.16 pg (Lima et al., 2021), and therefore may represent polyploid populations. On the other hand, most *Di. linearis* chromosome counts have shown $n = 39$ as the haploid chromosome number, with $2C = 6.41$ pg (despite some cases of polyploidy) (Clark et al., 2016). In the present study, these species had LH values above 89%, SNPs > 0%, and AD above 1.81%, which also suggests that their origins may be related to polyploidy events. In the genus *Gleichenia*, *G. peltophora* was the only species that showed high rates of LH and HD, and all samples of *Diplopterygium* showed low rates of LH and HD (Table 3). Therefore, it would appear that polyploidy and reticulation might not have played such significant roles in the diversification of these genera as proposed in *Sticherus* and *Dicranopteris* (Table 2) (Jermy and Walker, 1985; Lima et al., 2021).

In contrast, the monospecific genus *Gleichenella*, widespread in the Neotropics, showed the lowest rates of allele divergences and heterozygosity among all species sampled (Table 2). This might be related to its growth habit, as it usually occupies large areas along roadsides and on steep slopes. The extensive clonal expansion of its rhizome allows for great expansion, so that *Gleichenella* patches may be formed by a single individual. The reproductive isolation of *Gleichenella* may be related to effective pre- or postzygotic barriers, preventing hybrid formation (Roe et al., 2014), which, in turn, could be related to its early divergence time (estimated at around 49–62 mya). Additionally, chromosome counts point to the conservation of ploidy in the genus, with only putative

dysploidy events being recorded (Lima et al., 2021). Similarly, *Rouxopteris* and *Stromatopteris*, two other monospecific genera with restricted distributions, showed low rates of LH and HD and may represent ancient relict lineages within the family with well-developed reproductive barriers to prevent hybrid formation.

It is well established that whole-genome duplication (WGD) events have played major roles in fern diversification (IKP, 2019; Huang et al., 2020), as have WGD and reticulation with high LH and HD rates (Sigel, 2016). Gleicheniaceae seem to have experienced a WGD before its radiation during the Mesozoic (Huang et al., 2020). Our results also support the hypothesis that polyploidy and reticulation played a major role in the diversification of *Sticherus* and *Dicranopteris* (Table 3). A gradient of LH and AD across the sampled species was also observed (Table 2), which may indicate the presence of unknown hybrids and putative introgression events (Nauheimer et al., 2020). Although no intergeneric hybrids have yet been recorded for the family, there is evidence that several putative hybrids and allopolyploids within the genera will require further investigation (Table 3).

4.3. Divergence time analysis

Gleicheniaceae is an ancient lineage of leptosporangiate ferns with an extensive fossil record spanning the Mesozoic (Gandolfo et al., 1997), although a recently described fossil from the Permian assignable to the family (*Chansitheca wudaensis*, 298 mya) indicates an even earlier origin (He et al. 2020). Our time divergence estimates corroborate previous estimates (Pryer et al., 2004; Schuettpelz and Pryer, 2007; Testo and Sundue, 2016; Liu et al., 2020), and support the hypothesis of the establishment and diversification of most Gleicheniaceae lineages during the Mesozoic (Schneider et al., 2004). It is hypothesized that the early divergence of the Gleicheniaceae crown occurred in connection with continental movements during the breakup of Laurasia and Gondwana during the late Mesozoic and early Cenozoic (Liu et al., 2020) (Fig. 3). Of the three geographically restricted genera in Gleicheniaceae, *Rouxopteris* (Madagascar and the Mascarenes - Liu et al., 2020) and *Stromatopteris* (New Caledonia - Kramer, 1990) are ancient lineages, and seem to be relicts from the early diversification of Gleicheniaceae lineages (Liu et al., 2020) (Fig. 3), whereas the neotropical *Gleichenella* (Ching, 1940; Mickel and Smith, 2004) diverged more recently from *Dicranopteris*.

Despite their early divergence and diversification during the Mesozoic, several early-diverged genera (such as *Dicranopteris*, *Diplopterygium* and *Sticherus*) underwent more recent diversification during the Miocene (Fig. 3). This pattern has also been observed in other lineages of seedless plants, such as *Phlegmariurus* (Testo et al., 2019) and *Isoetes* (Pereira et al., 2017). This agrees with the angiosperm-driven diversification hypothesis, as presented for other fern lineages (Schneider et al., 2004; Du et al., 2021), in that much of the current species diversity is of Neogene or even Quaternary age. This diversification may have been associated with climate adaptation and niche diversification in montane regions and matches the pattern reported by Suissa et al. (2021) for general fern diversification.

5. Conclusions

Here we present the first genomic scale phylogenetic inferences concerning Gleicheniaceae, confirming the monophyly of *Dicranopteris*, *Diplopterygium*, *Gleichenella*, *Gleichenia*, *Rouxopteris*, and *Stromatopteris*, while recovering *Sticherus* as paraphyletic. We clarify the relationships of *Rouxopteris* as belonging to the *Sticheroid* clade, and corroborate the overall phylogenetic relationships recovered in previous works. Although Gleicheniaceae is an ancient lineage (with most of its extant genera diverging during the Mesozoic), several genera show more recent diversification, and our results suggest that reticulation and polyploidy have played significant roles during that process. However, some genera, such as *Rouxopteris* and *Stromatopteris*, appear to represent

evolutionary relicts. Future studies with expanded sampling should focus on a better understanding of species-level relationships. Additionally, integrative approaches should be applied to elucidate genera with reticulate evolutionary histories and to define species complexes.

Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

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

Funding

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 thank CNPq for the research grant (313981/2020-5) awarded to A. Salino and the research grant (317091/2021-2) awarded to T.E. Almeida and the International Association for Plant Taxonomy (IAPT) for a grant awarded to L.V. Lima. This research received support from the SYNTHESYS+ project <https://www.synthesys.info/>, which is financed by the European Community Research Infrastructure Action under the H2020 Integrating Activities Program, Project number 823827. W.L. Testo received support from NSF under Grant DEB- 2045319.

CRediT authorship contribution statement

Lucas Vieira Lima: Conceptualization, Methodology, Data curation, Formal analysis, Writing – original draft, Writing – review & editing. **Alexandre Salino:** Supervision, Funding acquisition, Writing – review & editing. **Michael Kessler:** Funding acquisition, Writing – review & editing. **Germinal Rouhan:** Writing – review & editing. **Weston L. Testo:** Data curation, Methodology, Writing – review & editing. **Caio Suzart Argolo:** Data curation, Methodology, Writing – review & editing. **GoFlag Consortium:** Funding acquisition, Methodology, Data curation. **Thaís Elias Almeida:** Supervision, Conceptualization, Methodology, Data curation, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

The authors thank Marcus Lehnert and Suichiro Tobagane for sending us silica samples and Joel Nitta for his help to obtain Asian samples. The first author thanks Nils Köster and Robert Vogt for all the support provided during his time at the BGBM. We thank Prof. Yahara (Kyushu University) for leaf materials collected from many areas in SE Asia and supported by the Environment Research and Technology Development Fund (S9 and 4-1601) of the Ministry of the Environment, Japan and MEXT/JSPS KAKENHI (Grant Number JP15H02640). 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 thank CNPq for the research grant (313981/2020-5) awarded to A. Salino and the research grant (317091/2021-2) awarded to T.E. Almeida, and the International Association for Plant Taxonomy (IAPT) for a grant awarded to L.V. Lima. This study received support from the SYNTHESYS + project (<http://www.synthesys.info/>) which is financed by European Community Research

Infrastructure Action under the H2020 Integrating Activities Programme, Project number 823827, awarded to L.V. Lima.

Appendix A. Supplementary material

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ympev.2023.107782>.

References

- Almeida, T.E., Salino, A., Dubuisson, J.-Y., Hennequin, S., 2017. *Adetogramma* (Polypodiaceae), a new monotypic fern genus segregated from *Polypodium*. *PhytoKeys* 78, 109–131.
- Zhang, L., Zhang, L.B., 2017. A Classification of the Fern Genus *Tectaria* (Tectariaceae: Polypodiales) Based on Molecular and Morphological Evidence. *Annals of the Missouri Botanical Garden* 103 (2), 188–199.
- Andrews, S., 2010. FastQC: a quality control tool for high throughput sequence data. <https://www.bioinformatics.babraham.ac.uk/projects/fastqc/>.
- Bloesch, Z., Nauheimer, L., Almeida, T.E., Crayn, D., Field, A.R., 2022. HybPhaser identifies hybrid evolution in Australian Thelypteridaceae. *Mol. Phylogenet. Evol.* 173, 107526 <https://doi.org/10.1016/j.ympev.2022.107526>.
- Bolger, A.M., Lohse, M., Usadel, B., 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. 30, 2114–2120. <https://doi.org/10.1093/bioinformatics/btu170>.
- Breinhold, J.W., Carey, S.B., Tiley, G.P., Davis, E.C., Endara, L., McDaniel, S.F., Neves, L. G., Sessa, E.B., et al., 2021. A target enrichment probe set for resolving the flagellate land plant tree of life. *Appl. Plant Sci.* 9 (1), e11406.
- Bruun-Lund, S., Clement, W.L., Kjellberg, F., Ronsted, N., 2017. First plastid phylogenomic study reveals potential cyto-nuclear discordance in the evolutionary history of *Ficus* L. (Moraceae). *Mol. Phylogenet. Evol.* 109, 93–104.
- Buggs, R.J., Wendel, J.F., Doyle, J.J., Soltis, D.E., Soltis, P.S., Coate, J.E., 2014. The legacy of diploid progenitors in allopolyploid gene expression patterns. *Philosophical Transactions of the Royal Society B: Biological Sciences* 369, 20130354.
- Capella-Gutierrez, S., Silla-Martinez, J.M., Gabaldon, T., 2009. TrimAl: a tool for automated alignment trimming in large-scale phylogenetic analyses. *Bioinformatics*. 25, 1972–1973.
- Chen, C.W., Perrie, L.R., Glenny, D., Chiou, W.L., 2017. Sol amazing: lycophytes & ferns of the Solomon Islands. National Museum of Natural Science.
- Chen, D.K., Zhou, X.M., Rothfels, C.J., Shepherd, L.D., Knapp, R., Zhang, L., Zhang, L.B. A., 2022. Global phylogeny of Lycopodiaceae (Lycopodiales; lycophytes) with the description of a new genus, *Brownseyia*, from Oceania. *Taxon*. 71 (1), 25–51.
- Ching, R.C., 1940. On the genus *Gleichenia* Smith. *Sunyatsenia*. 5, 269–289.
- Chinnock, R.J., Bell, G.H., 1998. Gleicheniaceae in: *Flora of Australia* Volume 48, Ferns, Gymnosperms and Allied Groups. Australia: Melbourne.
- Christensen, C., 1905. *Index Filicum*, Vol. 1. H. Hagerup, Copenhagen.
- Christensen, C., 1938. Filicinae. In: Verdoorn, F. (Ed.), *Manual of pteridology*. Nijhoff, The Hague, pp. 522–550.
- Clark, J., Hidalgo, O., Pellicer, J., Liu, H., Marquardt, J., Robert, Y., Christenhusz, M., Zhang, S., Gibby, M., Leitch, I.J., Schneider, H., 2016. Genome evolution of ferns: evidence for relative stasis of genome size across the fern phylogeny. *New Phytol* 210, 1072–1082.
- Copeland, E.B., 1947. *Genera Filicum*. Chronica Botanica, Waltham, Mass.
- Darriba, D., Posada, D., Kozlov, A.M., Stamatakis, A., Morel, B., Flouri, T., 2020. ModelTest-NG: a new and scalable tool for the selection of DNA and protein evolutionary models. *Mol. Biol. Evol.* 37 (1), 291–294. <https://doi.org/10.1093/molbev/msz189>.
- Degnan, J.H., Salter, L.A., 2005. Gene tree distributions under the coalescent process. *Evolution* 59, 24–37.
- Diels, F.L.E., 1900. Gleicheniaceae. In: Engler, A., Prantl, K. (Eds.), *Die Natürlichen Pflanzenfamilien*. W. Engelmann, Leipzig.
- Dorado, O., Rieseberg, L.H., Arias, D., 1992. Chloroplast DNA introgression in southern California sunflowers. *Evolution* 46, 566–572.
- Drábková, L.Z., Vlček, C., 2010. Molecular phylogeny of the genus *Luzula* DC. (Juncaceae, Monocotyledones) based on plastome and nuclear ribosomal regions: a case of incongruence, incomplete lineage sorting and hybridization. *Mol. Phylogenet. Evol.* 57, 536–551.
- Du, X.Y., Lu, J.M., Zhang, L.B., Wen, J., Kuo, L.Y., Mynssen, C.M., Schneider, H., Li, D.Z., 2021. Simultaneous diversification of Polypodiales and angiosperms in the Mesozoic. *Cladistics*. 37 (5), 518–539.
- Gandolfo, M.A., Nixon, K.C., Crepet, W.L., Ratcliffe, G.E., 1997. A New Fossil Fern Assignable to Gleicheniaceae from Late Cretaceous sediments of New Jersey. *Am. J. Bot.* 84, 483–493.
- Gasper, A.L., Almeida, T.E., Dittrich, V.A.O., Smith, A.R., Salino, A., 2017. Molecular phylogeny of the fern family Blechnaceae (Polypodiales) with a revised genus-level treatment. *Cladistics*. 33 (4), 429–446.
- Gonzales, J., 2003. A taxonomic revision of the genus *Sticherus* (Gleicheniaceae – Pteridophyta) in the Neotropics. Univ. Göttingen, Germany. PhD thesis.
- Gonzales, J., Kessler, M., 2011. A synopsis of the Neotropical species of *Sticherus* (Gleicheniaceae), with descriptions of nine new species. *Phytotaxa* 31 (1), 1–54.
- He, X.Z., Wang, S.J., Wang, J., 2016. *Chansitheca wudaensis* (Gleicheniaceae, fern) from the early Permian Wuda Tuff Flora, Inner Mongolia. *Palaeoworld* 25 (2), 199–211.

- He, X., Zhou, W., Li, D., Wang, S., Hilton, J., Wang, J., 2020. A 298-million-year-old gleicheniacean fern from China. *Rev. of Palaeob. Palynol.* 294, 104355.
- Herendeen, P.S., Skog, J.E., 1998. *Gleichenia chaloneri*-a new fossil fern from the lower Cretaceous (Albian) of England. *Int. J. Plant Sci.* 159 (5), 870–879.
- Holttum, R.E., 1947. A revised classification of leptosporangiate ferns. *Bot. J. Linn. Soc.* 53, 123–158.
- Holttum, R.E., 1957. Morphology, growth-habit, and classification in the family Gleicheniaceae. *Phytomorphology* 2, 168–184.
- Holttum, R.E., 1959. Gleicheniaceae. *Flora Malesiana-Series 2. Pteridophyta* 1 (1), 1–36.
- Huang, C.H., Qi, X., Chen, D., Qi, J., Ma, H., 2020. Recurrent genome duplication events likely contributed to both the ancient and recent rise of ferns. *J. Integr. Plant Biol.* 62, 433–455.
- Jin, X.F., Ding, B.Y., Iwatsuki, K., 2013. Gleicheniaceae, in: Wu, Z.Y., Raven, P.H., Hong, D.Y. (Eds.), *Flora of China*, Vol. 2–3 (Pteridophytes). Missouri Botanical Garden Press, St. Louis, pp. 110–115.
- Johnson, M.G., Gardner, E.M., Liu, Y., Medina, R., Goffinet, B., Shaw, A.J., Zerega, N.J., Wickett, N.J., 2016. HybPiper: Extracting coding sequence and introns for phylogenetics from high-throughput sequencing reads using target enrichment. *App. Plant Sci.* 4(7), apps.1600016. <https://doi.org/10.3732/apps.1600016>.
- Jermey, A.C., Walker, T.G., 1985. *Cytotaxonomic studies of the ferns of Trinidad*. British Museum (Natural History).
- Katoh, K., Asimenos, G., Toh, H., 2009. Multiple alignment of DNA sequences with MAFFT. In: *Bioinformatics for DNA sequence analysis*. Humana Press, pp. 39–64.
- Kim, H.T., Chung, M.G., Kim, K.J., 2014. Chloroplast genome evolution in early diverged leptosporangiate ferns. *Mol. Cells.* 37 (5), 372.
- Knowles, L.L., Carstens, B.C., 2007. Delimiting species without monophyletic gene trees. *Syst. Biol.* 56, 887–895.
- Kramer, K.U. 1990. Gleicheniaceae. in: *The families and genera of vascular plants. Vol. 1. Pteridophytes and Gymnosperms*. (Vol. eds. Kramer, K.U. & Green, P.S.). Springer-Verlag, Berlin. Pp. 145–152.
- Lehtonen, S., Poczei, P., Sablok, G., Hyvönen, J., Karger, D.N., Flores, J., 2020. Exploring the phylogeny of the marattiacean ferns. *Cladistics.* 36 (6), 569–593.
- Li, C.-X., Lu, S.-G., Ma, J.-Y., Y, q., 2010. Phylogeny and divergence of Gleicheniaceae inferred from three plastid genes. *Acta Palaeontol. Sin.* 49, 64–72.
- Lima, L.V., Salino, A., 2018. The fern family Gleicheniaceae (Polypodiopsida) in Brazil. *Phytotaxa* 358 (3), 199–234.
- Lima, L.V., Sousa, S., Almeida, T.E., Salino, A., 2021. State of the art in cytogenetics, insights into chromosome number evolution, and new C-value reports for the fern family Gleicheniaceae. *An. Acad. Bras. Ciênc.* 93 (Suppl. 3), e20201881.
- Liu, H., Rakotondrainibe, F., Hennequin, S., Schneider, H., 2020. The significance of *Rouxopteris* (Gleicheniaceae, Polypodiopsida): a new genus endemic to the Madagascar region. *Plant Syst. Evol.* 306 (2), 1–11.
- Maurin, K. J. 2020. An empirical guide for producing a dated phylogeny with treePL in a maximum likelihood framework. *arXiv preprint arXiv:2008.07054*.
- Mickel, J.T., Smith, A.R., 2004. *The Pteridophytes of Mexico* (Memoirs of The New York Botanical Garden 88). The New York Botanical Garden, Bronx, New York, USA.
- Minh, B.Q., Schmidt, H.A., Chernomor, O., Schrempf, D., Woodhams, M.D., Haeseler, A., Lanfear, R., 2020. IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol. Biol. Evol.* 37 (5), 1530–1534. <https://doi.org/10.1093/molbev/msaa015>.
- Nakai, T., 1950. A new classification of Gleicheniales. *Bull. Nat.l Sci Mus.* 29, 1–71.
- Nauheimer, L., Weigner, N., Joyce, E., Crayn, D., Clarke, C., Nargar, K., 2020. HybPhaser: a workflow for the detection and phasing of hybrids in target capture datasets. *Appl Plant Sci.* 9 (7), e11441.
- Ohlsen, D.J., Perrie, L.R., Shepherd, L.D., Bayly, M.J., 2022. Chloroplast phylogenies of Australasian *Gleichenia* ferns (Gleicheniaceae) reveal incongruence with current taxonomy, and frequent long-distance dispersal. *Plant Syst. Evol.* 308 (4), 1–16.
- Østergaard Andersen, E., Øllgaard, B., 2001. Gleicheniaceae. In: Harling, G. & Andersson, L. (Eds.), *Flora of Ecuador* 66: 107–169.
- Penrod, K.A., 2000. Ecology of hay-scented fern: spore production, viability and germination. Dissertation, Pennsylvania State University, University, Park, PA, USA.
- Pereira, J., Labiak, P.H., Stützel, T., Schulz, C., 2017. Origin and biogeography of the ancient genus *Isoetes* with focus on the Neotropics. *Bot. J. Linn. Soc.* 185 (2), 253–271.
- Perrie, L.R., Brownsey, P.J. 2015. Gleicheniaceae. In: Breitwieser, I., Heenan, P.B., Wilton, A.D. (eds.), *Flora of New Zealand - Ferns and Lycophytes. Fascicle 12*. Manaaki Whenua Press, Lincoln. Pp. 1–56.
- Perrie, L.R., Bayly, M.J., Lehnebach, C.A., Brownsey, P.J., 2007. Molecular phylogenetic and molecular dating of the New Zealand Gleicheniaceae. *Brittonia* 59 (2), 129–141.
- PPG, I., 2016. A community-derived classification for extant lycophytes and ferns. *J. Syst. and Evol.* 54 (6), 563–603.
- Prado, J., Lellinger, D.B., 1996. Observations on the nomenclature and taxonomy of *Gleichenia nigropaleacea*. *Am. Fern J.* 98–101.
- Pryer, K.M., Schuettpezel, E., Wolf, P.G., Schneider, H., Smith, A.R., Cranfill, R., 2004. Phylogeny and evolution of ferns (monilophytes) with a focus on early-diverging lineages. *Am. J. Bot.* 91, 1582–1598.
- Rieseberg, L.H., Soltis, D.E., 1991. Phylogenetic consequences of cytoplasmic gene flow in plants. *Evol. Trends Plants.* 6, 65–84.
- Roe, A.D., MacQuarrie, C.J.K., Gros-Louis, M.C., 2014. Fitness dynamics within a poplar hybrid zone: I. Prezygotic and postzygotic barriers impacting a native poplar hybrid stand. *Ecol. Evol.* 4, 1629–1647.
- Schneider, H., Schuettpezel, E., Pryer, K.M., Cranfill, R., Magallon, S., Lupia, R., 2004. Ferns diversified in the shadow of angiosperms. *Nature* 428, 553–557.
- Schuettpezel, E., Pryer, K.M., 2007. Fern phylogeny inferred from 400 leptosporangiate species and three plastid genes. *Taxon* 56, 1037–1050.
- Sigel, E.M., 2016. Genetic and genomic aspects of hybridization in ferns. *J. Syst. Evol.* 54 (6), 638–655.
- Smith, S.A., O'Meara, B.C., 2012. TreePL: divergence time estimation using penalized likelihood for large phylogenies. *Bioinformatics* 28 (20), 2689–2690.
- Soltis, P.S., Soltis, D.E., 2000. The role of genetic and genomic attributes in the success of polyploids. *Proceedings of the National Academy of Sciences USA* 97, 7051–7057.
- Suissa, J.S., Sundue, M.A., Testo, W.L., 2021. Mountains, climate and niche heterogeneity explain global patterns of fern diversity. *J. Biogeog.* 48 (6), 1296–1308.
- Testo, W., Øllgaard, B., Field, A., Almeida, T., Kessler, M., Barrington, D., 2018. Phylogenetic systematics, morphological evolution, and natural groups in neotropical *Phlegmariurus* (Lycopodiaceae). *Mol. Phylogenet. Evol.* 125, 1–13.
- Testo, W.L., Sessa, E., Barrington, D.S., 2019. The rise of the Andes promoted rapid diversification in Neotropical *Phlegmariurus* (Lycopodiaceae). *New Phytol.* 222 (1), 604–613.
- Testo, W.L., Sundue, M., 2016. A 4000-species dataset provides new insight into the evolution of ferns. *Mol. Phylogenet. Evol.* 105, 200–211.
- Tryon, R.M., Stolze, R.G., 1989. Pteridophyta of Peru. Part II. 13. Pteridaceae – 15. Dennstaedtiaceae. *Field. Bot.* 22, 1–122.
- Walker, L.R., Sharpe, J.M., 2010. Ferns, disturbance and succession. In: Mehltrater, K., Walker, L.R., Sharpe, J.M. (Eds.), *Fern ecology*. Cambridge University Press, New York, pp. 177–219.
- Wei, Z., Xia, Z., Shu, J., Shang, H., Maxwell, S.J., Chen, L., Yan, Y., 2021. Phylogeny and Taxonomy on Cryptic Species of Forked Ferns of Asia. *Front. Plant Sci.* 12, 748562 <https://doi.org/10.3389/fpls.2021.748562>.
- Xu, B., Wu, N., Gao, X.F., Zhang, L.B., 2012. Analysis of DNA sequences of six chloroplast and nuclear genes suggests incongruence, introgression, and incomplete lineage sorting in the evolution of *Lepedeza* (Fabaceae). *Mol. Phylogenet. Evol.* 62 (1), 346–358.
- Zhang, C., Rabiee, M., Sayyari, E., Mirarab, S., 2018. ASTRAL-III: Polynomial Time Species Tree Reconstruction from Partially Resolved Gene Trees. *BMC Bioinformatics* 19 (S6), 153. <https://doi.org/10.1186/s12859-018-2129-y>.