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A Global Phylogenomic Study of the Thelypteridaceae

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Abstract—The generic classification of the Thelypteridaceae has been the subject of much controversy. Proposed taxonomic systems have varied from recognizing the approximately 1200 species in the family within the single genus *Thelypteris*, to systems favoring upwards of 30 genera. Insights on intrafamilial relationships, especially for neotropical taxa, have been gained from recent phylogenetic studies; however, in the most recent classification, 10 of 30 recognized genera are either non-monophyletic or untested. We sequenced 407 nuclear loci for 621 samples, representing all recognized genera and approximately half the known species diversity. These were analyzed using both maximum likelihood analysis of a concatenated matrix and multi-species coalescent methods. Our phylogenomic results, informed by recently published morphological evidence, provide the foundation for a generic classification which recircumscribed 14 genera and recognized seven new genera. The 37 monophyletic genera sampled demonstrate greater geographic coherence than previous taxonomic concepts suggested. Additionally, our results demonstrate that certain morphological characters, such as frond division, are evolutionarily labile and are thus inadequate for defining genera.

Keywords—*Amauropelta*, *Christella*, ferns, phylogeny, *Pneumatopteris*, *Pronephrium*, systematics, targeted enrichment, taxonomy.

With approximately 1200 species currently recognized, the Thelypteridaceae is one of the largest families of ferns, and its species are highly diverse morphologically (Fig. 1) and ecologically (Fawcett and Smith 2021). It is a member of the Aspleniinae, one of the two major clades of the eupolypod ferns, and is sister to a subclade that includes the Athyriaceae, Blechnaceae, Onocleaceae, and Woodsiaceae (PPG I 2016). The Thelypteridaceae is widespread throughout the tropics; a few species extend into boreal latitudes. The taxonomy of this diverse family remains contentious, and several differing generic classifications have been proposed.

Christensen (1913, 1920) was the first to suggest that thelypterids represent a natural group. In his monograph of New World members of the genus *Dryopteris*, he comprehensively treated American species now considered to belong to the Thelypteridaceae as subgenera of a broadly-defined *Dryopteris* Adans. With recognition of their many differences from *Dryopteris* s. s., most species now treated in Thelypteridaceae were subsequently transferred to *Thelypteris* in Asia (e.g. by Ching 1936) and in the Americas (e.g. by Morton 1963; Reed 1968). In these publications and others, *Thelypteris* had a very broad circumscription, including all, or nearly all, of the species belonging to modern-day Thelypteridaceae, ca. 1200 species. Many of the infrageneric taxa delimited by Christensen (1913, 1920) in *Dryopteris* were later recognized as subgenera in *Thelypteris*, with essentially the same circumscription. Ching (1963), who understood the distinctness of many Chinese and eastern Asia groups, went a step further and elevated these subgroups, as well as newly defined

segregates, to generic status. Further taxonomic refinements were made by Pichi Sermolli (1970) and Holttum (1971), and later by Ching (1978) and Holttum (1982). With these more restricted circumscriptions, *Thelypteris* became a small genus with only about two species. Pichi Sermolli (1977) was the first to attempt a worldwide reclassification of the Thelypteridaceae within a phylogenetic context; he recognized 32 genera in the family. Before the availability of molecular data, intermediate solutions were also proposed (e.g. Smith 1990). The taxonomy of the group remains controversial, with some authors still favoring a single-genus system for the family, citing the difficulty of using micro-morphological features to distinguish genera (e.g. Fraser-Jenkins et al. 2017). However, there is growing support for recognition of more genera that are supported by both molecular and morphological data (PPG I 2016; Schuettelpelz et al. 2018). For a more detailed history of the classification of the Thelypteridaceae, see Fawcett and Smith (2021).

With advances in microscopy, chromosome numbers provided a powerful new source of data for understanding the evolutionary relationships among ferns, using approaches pioneered by Irene Manton (1950). Some of the first attempts to develop hypotheses pertaining to phylogenetic relationships among genera incorporated cytological data in addition to morphology (Loyal 1963; Smith 1971; Pichi Sermolli 1977).

The first phylogenetic tree to infer relationships among genera of the Thelypteridaceae using molecular data was published by Smith and Cranfill (2002), based on three chloroplast DNA regions. This study confirmed the monophyly

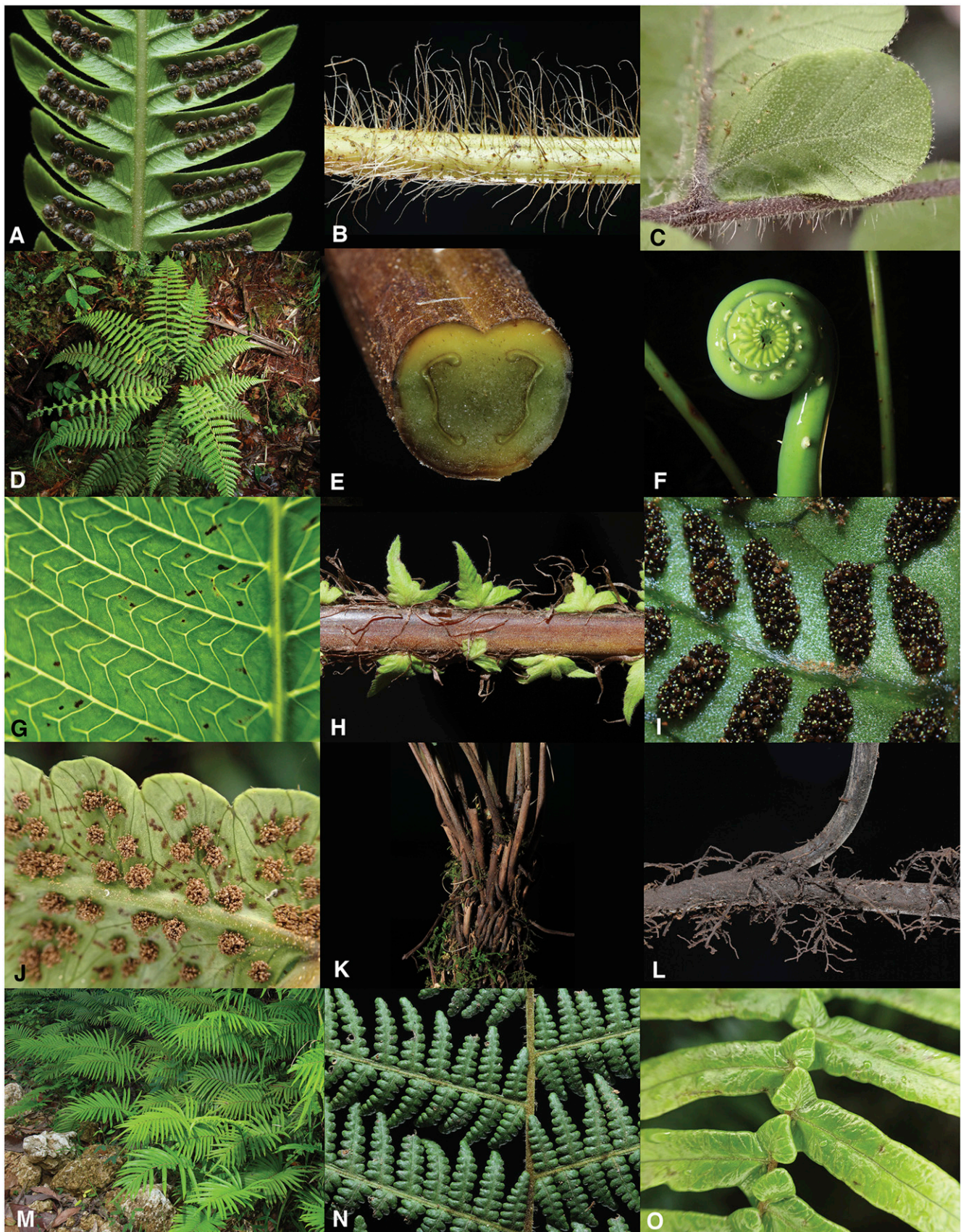


FIG. 1. Morphological diversity of the Thelypteridaceae. A. *Plesioneuron attenuatum*, indusiate sori. B. *Chingia malodora*, stipe with terete scales. C. *Goniopteris pellita*, rachis and pinna base, with both long hyaline acicular and short stellate hairs (these visible on lamina margin). D. *Coryphopteris kolombangarae*, habit. E. *Reholtumia magnifica*, stipe vasculature. F. *Pneumatopteris glandulifera*, mucilaginous crozier with peg-like aerophores. G. *Meniscium reticulatum*, venation. H. *Sphaerostephanos polycarpus*, auriculate proximal pinnae. I. *Sphaerostephanos beccarianus*, sori with spherical yellow glands. J. *Goniopteris serrulata*, exindusiate round sori. K. *Sphaerostephanos doodioides*, erect rhizome. L. *Strophocaulon invisum*, long-creeping rhizome. M. *S. unitum*, habit. N. *Macrothelypteris polypodioides*, adaxial lamina. O. *Goniopteris sagittata*, pinna bases. Photos C, G, J, O by S. Fawcett, all other photos by C.-W. Chen.

of the family and demonstrated the sister relationship of two major subclades within the family now recognized as subfamilies Phegopteridoideae and Thelypteridoideae. It also showed that the chromosome base number $x = 36$ is synapomorphic for the large lineage they named the cyclosoroid clade. The 27 taxa chosen for their study represented all major lineages within the family, and the relationships they inferred have been largely supported by all subsequent studies.

Later work (Schuettepelz and Pryer 2007; Ebihara 2011; He and Zhang 2012; Almeida et al. 2016; Patel et al. 2019), also relying primarily on cpDNA, increased taxon sampling and provided greater resolution and support for relationships inferred in earlier studies. However, even with limited sampling in the most diverse groups, these studies revealed that several genera were not monophyletic. In their study of Old World genera, He and Zhang (2012) demonstrated that *Christella*, *Parathelypteris*, and *Pronephrium* were non-monophyletic. Almeida et al. (2016) provided greater resolution for the monophyletic neotropical genera *Amauropelta*, *Goniopteris*, *Meniscium*, and *Steiropteris*, and published accompanying taxonomic changes (Salino et al. 2015). They also provided evidence that the paleotropical genera *Pneumatopteris* and *Sphaerostephanos* were non-monophyletic, based on very limited sampling. Most recently, Patel et al. (2019) corroborated these findings, notably the coherence of subgeneric taxa such as *Christella* sect. *Pelazoneuron* Holttum and *Pronephrium* sect. *Grypotherix* Holttum.

The Pteridophyte Phylogeny Group (PPG) was organized to develop a consensus-based classification system for all ferns and lycophytes, based on a collaborative effort of nearly 100 pteridologists from around the world, incorporating all available phylogenetic data. The resulting publication (PPG I 2016) proposed a family and genus-level taxonomy for all ferns and lycophytes, including species-number estimates, and phylogenetic status (e.g. monophyletic, non-monophyletic, or untested) for each genus. The resulting classification of the Thelypteridaceae largely followed the circumscriptions presented by Smith and Cranfill (2002), He and Zhang (2012), and Almeida et al. (2016). Although the Thelypteridaceae is by far most diverse in the Paleotropics, it was also the least studied there, with 10 of the 30 recognized genera known to be non-monophyletic or untested. In the absence of molecular data, the morphology-based taxonomy proposed by Holttum (1971, 1974, 1977, 1982) was largely adopted for these taxa.

The objectives of the present study are to: 1) infer the infra-familial and infrageneric relationships within the Thelypteridaceae using a large nuclear-DNA dataset; 2) assess the taxonomic hypotheses proposed by PPG I (2016); and 3) provide the phylogenetic foundation for a revised generic classification of the Thelypteridaceae worldwide.

MATERIALS AND METHODS

Sampling Design—Our goal was to sample broadly across the family, but with an emphasis on the Paleotropics, which hosts the greatest diversity both in species richness and number of genera and has been poorly represented in previous phylogenetic studies. In most cases we included a single sample per species, but widespread or taxonomically complex species (e.g. *Christella hispidula*) were represented by multiple accessions. We sought to include the type species for each genus, but were unable to include those of *Leptogramma*, *Pronephrium*, or *Trigonospora*. Our sample includes 621 accessions of about 500 species (including several that are probably undescribed), representing nearly half of the approximately 1200 species in the family. Our sample includes all genera recognized by PPG I (2016), including the rare, monotypic African genus *Menisorus*,

which has not previously been included in any molecular phylogenetic study. Tissue was derived from silica-dried samples and herbarium specimens, some of which were more than 100 years old. All samples collected by contributing authors were obtained under the respective collection and export permits of the collection countries, and by local landowner permission. Most tissue vouchers are at UC, TAIF, or VT, with others at BO, CMUH, CR, DUKE, F, FLAS, GH, GOET, HOXA, HUH, INB, JBSD, JEPS, KEP, KLU, LBG, LPB, MAPR, MICH, MO, NY, P, PAP, PE, PNH, PTBG, S, SBBG, TNS, and Z. (Appendix 1).

DNA Extraction and Sequencing—DNA extractions were performed at the University of Florida, Gainesville, using a cetyltrimethylammonium bromide (CTAB) extraction protocol (Doyle and Doyle 1987), with 2.5% polyvinylpyrrolidone and 0.4% beta-mercaptoethanol and two rounds of chloroform washes followed by an isopropanol precipitation and an ethanol wash. We also added 2 μ L of 10mg/mL RNase A (Qiagen, Valencia, CA, USA) to each sample between chloroform washes to remove RNA contamination. All samples yielded more than the minimum threshold of 5ng DNA required for sequencing.

We used a targeted enrichment sequencing approach with the *GoFlag 408* probe set to generate a large-scale nuclear data set (Breinholt et al. 2021). This probe set targets 408 conserved exons that are found within 229 of the single-copy nuclear genes identified by the 1KP transcriptome sequencing initiative (Leebens-Mack et al. 2019). The probes were designed from transcriptome and genomic sequences to target exons across flagellate land plants (mosses, liverworts, hornworts, lycophytes, ferns, and all gymnosperms) that were single or low-copy, at least 120 bp in length, and that had at least 65% pairwise sequence identity across land plants (see Breinholt et al. 2021). The library construction, targeted enrichment, and sequencing were all done by RAPiD Genomics (Gainesville, Florida) as part of the NSF-funded Genealogy of Flagellate Plants (GoFlag) project. After a bead-based DNA cleanup step, DNA was normalized to 250 ng and sheared to an average size of 300 base pairs (bp). Illumina-comparable libraries were constructed by repairing the ends of the sheared fragments followed by the addition of an adenine residue to the 3'-end of the blunt-end fragments (Bentley et al. 2008). Barcoded adapters suited for the Illumina sequencing platform were ligated to the libraries, and ligated fragments were PCR-amplified 9–11 cycles. Target enrichment was performed using the *GoFlag 408* probes following protocols based on Gnirke et al. (2009). After enrichment, samples were re-amplified for additional 6–12 cycles. All enriched samples were sequenced using an Illumina HiSeq 3000 with paired-end 100 bp reads. The sequence reads were deposited in the NCBI sequence read archive (SRA; BioProject 646399).

The raw reads were cleaned and assembled into sequence alignments containing both the targeted exon regions and the more variable flanking intron regions using the six-step iterative baited assembly pipeline described in Breinholt et al. (2021). In short, first adapters and bases with Phred scores less than 20 were trimmed from the paired-end raw reads with Trim Galore! v. 0.4.4 (https://www.bioinformatics.babraham.ac.uk/projects/trim_galore/), and we retained only pairs of reads in which both the forward and reverse read were at least 30 bp long. Next, raw reads with significant homology to each exonic target region based on reference transcriptome sequences were identified using USEARCH v. 7.0 (Edgar 2010), and then an iterative (3 \times) de novo assembly was performed with the set of homologous reads for each target locus with BRIDGER v. 2014-12-01 (Chang et al. 2015). The BRIDGER kmer size parameter was 25, and the minimum depth of coverage for the kmers to be included in the assembly was 10. We assessed orthology of the assembled sequences based on the best tblastx (Camacho et al. 2009) hit of the exonic target region of each assembled sequence to the coordinates of nine plant genomes representing hornworts, liverworts, mosses, lycophytes, ferns, and gymnosperms. We called an assembled sequence an ortholog of the probe region if it had no additional tblastx hits with > 95% of the best bit score, outside of a 1000 base pair flanking window around the genomic coordinates of the exonic target locus in any one of the reference genomes. Next, to filter out likely contaminants, for each assembled sequence we performed a tblastx search against the respective reference sequences for that locus. If a sequence's best hit was not a fern, that sequence was removed as a potential contaminant. Finally, we aligned the resulting sequences using MAFFT v. 7.425 (Katoh and Standley 2013). Alignments were done for the full sequences, which included the exonic target loci and flanking intron sequences. After the alignments, sequences from the same taxon with mismatches due to heterozygous sites were merged using nucleotide ambiguity codes to represent heterozygous sites. All the pipeline scripts with instructions are available on Dryad (Fawcett et al. 2021).



FIG. 2. A synoptical ML 1000 bootstrap consensus tree inferred from the 407-locus concatenated alignment illustrating relationships among genera of the Thelypteridaceae, and identifying major clades. Colors correspond to genera that underwent recent taxonomic changes. Letters indicate position in Figs. 3A–3I.

In some cases, after running the pipeline, a sample will have more than one sequence in locus alignment where the BRIDGER assembler identified potentially more than simple allelic variation. To minimize the possible effects of paralogy, in these cases, for each locus, we only retained sequences from samples with a single copy. We also removed any loci with sequences from fewer than 25 samples, and within each locus alignment, we removed any columns with fewer than 25 nucleotides. We then concatenated all the locus alignments into a supermatrix for phylogenetic analysis. One locus was inadvertently duplicated in the probe design process (Loci 91 and 92 are identical). The scripts used to perform these post-processing steps, as well as the resulting supermatrix, are available on Dryad (Fawcett et al. 2021).

Phylogenetic Analyses—The supermatrix was analyzed using maximum likelihood (ML) implemented in IQ-Tree v. 2.0.3 for Linux (Minh et al. 2020), with partitions representing each locus. We chose the edge-linked proportional substitution model, and GTR + F with separate rates for each partition was inferred by ModelFinder (Chernomor et al. 2016; Kalyaanamoorthy et al. 2017). Ultrafast bootstrap approximation with 1000 iterations (Minh et al. 2013; Hoang et al. 2017) was used to assess branch support. Ultrafast bootstrapping, which uses RELL (resampling estimated log-likelihoods) is a probabilistic approach, so we consider a minimum threshold of 95% to be “high support,” in contrast to the lower threshold typically accepted for traditional bootstraps (Minh et al. 2013).

Maximum likelihood analyses were also performed on each of the 407 single-locus alignments in IQ-Tree. Gene concordance factors (gCF) and site concordance factors (sCF) were calculated using the ML tree inferred from the concatenated alignment (Fawcett et al. 2021). These metrics complement classical measures of branch support such as bootstraps by providing detailed information about the topological variation in the underlying data, while accounting for variable taxon coverage among gene trees. Concordance factors are especially useful for large phylogenomic datasets, because unlike bootstrap support, which tends to increase

with the addition of loci, gCF and sCF are stable and informative with increasing sample size (Minh et al. 2020).

A species tree was inferred from the 407 individual ML gene trees using multi-species coalescence implemented in ASTRAL 5.7.3 (Fawcett et al. 2021; Zhang et al. 2017), and annotated with local posterior probabilities. Analyses were performed on the Vermont Advanced Computing Core at the University of Vermont and at the HiPerGator facility at the University of Florida. Raw sequence data were uploaded to the NCBI Sequence Read Archive, available at ncbi.nlm.nih.gov/bioproject/646399. The supermatrix alignment, partition file, multispecies coalescence analysis phylogeny, concordance analyses, and a spreadsheet of molecular vouchers are available on Dryad (Fawcett et al. 2021). Figures were created using FigTree v. 1.4.4. and Adobe Photoshop CC 2014.

RESULTS

Sequence Alignment—The supermatrix alignment of the Thelypteridaceae includes 621 total samples and 407 nuclear loci. Thirty-two samples initially included in the study were excluded. Among these, 14 were excluded because they amplified fewer than 50 loci. An additional 18 samples were excluded because they were resolved among outgroup taxa in preliminary phylogenetic analyses, and/or were on extremely long branches, likely indicating low sequence quality or contamination. In the remaining 621 samples, the average number of loci per included taxon is 378 (out of a possible 407), with a minimum of 113. An average of 5% of sequences per sample were discarded as possible paralogs. We found no significant difference ($t = -0.757$, $p = 0.449$) in the number

TABLE 1. A comparison of the classification system proposed by Fawcett and Smith (2021) to that of PPG I (2016), which largely follows He and Zhang (2012) and Holttun (1974, 1977, 1982) for the Old World, and Almeida et al. (2016) for the New World. Names in bold indicate new or recircumscribed genera. An asterisk (*) indicates a resurrected synonym, (**) indicates sections elevated in rank to genera, and (***) indicates newly described genera.

| Fawcett and Smith 2021 | PPG I (2016) |
|---------------------------|--|
| <i>Abacopteris</i> * | <i>Pronephrium</i> |
| <i>Amauropelta</i> | <i>Amauropelta</i> , <i>Parathelypteris</i> |
| <i>Amblovenatum</i> | <i>Amblovenatum</i> |
| <i>Ampelopteris</i> | <i>Ampelopteris</i> |
| <i>Chingia</i> | <i>Chingia</i> , <i>Amblovenatum</i> , <i>Plesioneuron</i> |
| <i>Christella</i> | <i>Christella</i> , <i>Pelazoneuron</i> , <i>Menisciopsis</i> |
| <i>Coryphopteris</i> | <i>Coryphopteris</i> |
| <i>Cyclogramma</i> | <i>Cyclogramma</i> |
| <i>Cyclosorus</i> | <i>Cyclosorus</i> |
| <i>Glaphyopteridopsis</i> | <i>Glaphyopteridopsis</i> |
| <i>Goniopteris</i> | <i>Goniopteris</i> |
| <i>Grypothrix</i> ** | <i>Pronephrium</i> |
| <i>Hoiokula</i> *** | <i>Pneumatopteris</i> |
| <i>Leptogramma</i> * | <i>Stegnogramma</i> |
| <i>Macrothelypteris</i> | <i>Macrothelypteris</i> |
| <i>Menisciopsis</i> ** | <i>Pronephrium</i> , <i>Christella</i> |
| <i>Meniscium</i> | <i>Meniscium</i> |
| <i>Mensorus</i> | <i>Mensorus</i> , <i>Pneumatopteris</i> |
| <i>Mesophlebion</i> | <i>Mesophlebion</i> |
| <i>Mesopteris</i> | <i>Mesopteris</i> , <i>Amblovenatum</i> |
| <i>Metathelypteris</i> | <i>Metathelypteris</i> |
| <i>Oreopteris</i> | <i>Oreopteris</i> |
| <i>Pakau</i> *** | <i>Pneumatopteris</i> |
| <i>Pelazoneuron</i> ** | <i>Christella</i> |
| <i>Phegopteris</i> | <i>Phegopteris</i> |
| <i>Plesioneuron</i> | <i>Plesioneuron</i> , <i>Pneumatopteris</i> , <i>Chingia</i> |
| <i>Pneumatopteris</i> | <i>Pneumatopteris</i> |
| <i>Pronephrium</i> | <i>Pronephrium</i> , <i>Nannothelypteris</i> |
| <i>Pseudocyclosorus</i> | <i>Pseudocyclosorus</i> |
| <i>Pseudophegopteris</i> | <i>Pseudophegopteris</i> |
| <i>Reholtumia</i> *** | <i>Pneumatopteris</i> |
| <i>Sphaerostephanos</i> | <i>Sphaerostephanos</i> , <i>Pneumatopteris</i> , <i>Pronephrium</i> , <i>Strophocaulon</i> |
| <i>Stegnogramma</i> | <i>Stegnogramma</i> |
| <i>Steiropteris</i> | <i>Steiropteris</i> |
| <i>Strophocaulon</i> *** | <i>Sphaerostephanos</i> |
| <i>Thelypteris</i> | <i>Thelypteris</i> |
| <i>Trigonospora</i> | <i>Trigonospora</i> |

of loci recovered from tissue that was silica-dried in the field (mean loci = 379, N = 375) or tissue taken from herbarium specimens (mean loci = 377, N = 246), and the age of the specimen had little effect on the number of loci recovered ($R^2 = 0.03$, $p = 0.007$). The oldest specimen sequenced for this study was collected in 1889. The final supermatrix alignment (exons plus flanking regions) included 621 samples with 533,059 nucleotide sites, and is 49.6% full; 61% of these sites are parsimony-informative, 16% are singletons, and 23% are invariant. The supermatrix alignment and data organized by voucher specimen and by locus are available on Dryad (Fawcett et al. 2021).

Phylogenetic Analyses—The topologies of the phylogenetic trees inferred both through maximum likelihood analysis of the concatenated matrix (Figs. 2, 3) and multi-species coalescent analysis (Fawcett et al. 2021) are largely congruent with each other and with previously published trees based primarily on Sanger sequencing of cpDNA (Smith and Cranfill 2002; He and Zhang 2012; Almeida et al. 2016; Patel et al. 2019). Our focus on problematic taxa, a three-fold increase in taxon sampling, and a large nuclear DNA dataset enabled us to find support for previously unresolved nodes and

provides evidence for the resolution of many previously intractable taxonomic problems.

The average bootstrap support for all nodes in the ML analysis was 97%. All crown nodes corresponding to our generic classification have 100% bootstrap support except *Christella* (92%). Some upper backbone nodes are variable or have low support (e.g. the chingoid clade (Figs. 2, 3F) has 100% BS support, but low concordance factors, gCF = 0.5, sCF = 35), and a few rogue taxa are present, discussed in detail below. The two subfamilies Phegopteridoideae and Thelypteridoideae each resolve as monophyletic if the root is placed between them, as done here based on robust results of previous phylogenetic analyses (Smith and Cranfill 2002; He and Zhang 2012; Almeida et al. 2016).

To evaluate the tree, and to better understand widespread species throughout their ranges, we included multiple accessions of 77 species in the analyses. All species represented by multiple accessions resolved within the expected genus-level clade. Among these, more than half of species represented by more than one accession (N = 40) form a clade, as expected. Of the remainder, an additional 21 had a near sister relationship in the phylogeny, while 13 were resolved as less closely related. Three additional species represented by more than two accessions resolved in a combination of the above states. Some of these species include varieties that are not sister to the typical variety of that species (e.g. *Christella patens*, *Christella ovata*, and *Steiropteris glandulosa*). In other cases, currently accepted species concepts are broadly applied, and are potentially polyphyletic (e.g. *Chingia longissima* and *Coryphopteris japonica*). Other widespread and variable taxa, however, were resolved as monophyletic; for example, all three varieties of *Christella hispidula*, comprising seven accessions from its native range in North America, South America, Africa, and Malesia, constitute a clade.

Assessment of PPG I Genera—A primary goal of this study is to test the taxonomic hypotheses of the Thelypteridaceae proposed by PPG I (2016). Of the 30 genera in that classification, 17 are supported without modification. These include *Ampelopteris*, *Coryphopteris*, *Cyclogramma*, *Cyclosorus*, *Glaphyopteridopsis*, *Goniopteris*, *Macrothelypteris*, *Meniscium*, *Mesophlebion*, *Metathelypteris*, *Oreopteris*, *Phegopteris*, *Pseudocyclosorus*, *Pseudophegopteris*, *Steiropteris*, *Thelypteris*, and *Trigonospora*. The remaining 13 genera sensu PPG I (2016) are presented below, with an explanation of their phylogenetic status. To facilitate the communication of phylogenetic results, major clades have been given informal names (Fig. 2), several of which have been applied in previous molecular analyses of the family (e.g. Smith and Cranfill 2002; Almeida et al. 2016; Patel et al. 2019; Fawcett and Smith 2021) and a summary of changes is provided in Table 1. All taxonomic authorities for species included in the analyses are listed in the appendix.

AMAUROPELTA—Although *Amauropelta* is monophyletic, the genus *Parathelypteris* is not, with some species nested within *Coryphopteris* and others in three well supported clades along a basal grade terminating in all sampled species of *Amauropelta* s. s. Although each of these three clades is well supported, their relationships to one another are in conflict among individual gene trees (Fig. 3B; Fawcett et al. 2021).

AMBLOVENATUM—The members of this genus (homotypic synonym: *Amphineuron*) resolve in two distantly related clades; one includes the type species *Amblovenatum*

Cyclosoroids



FIG. 3A. The Phegopteridoideae (*Macrothelypteris*, *Phegopteris*, and *Pseudophegopteris*) and early diverging genera of the Thelypteridoideae in the maximum likelihood phylogeny of the 407-locus concatenated alignment. The nodes are annotated with ultrafast bootstrap support values, and genera are emphasized by distinct colors. Type species of genera are denoted with an asterisk (*).

opulentum, but the others resolve with the previously monotypic genus *Mesopteris tonkinensis*. The phylogenetic position of *Amblovenatum* s. s. variously resolves among sphaerostephanoids in the ML bootstrap consensus (Fig. 2), but as sister to sphaerostephanoids plus pseudocyclosoroids in the ML tree (Fig. 3G) and coalescent tree (Fawcett et al. 2021).

CHINGIA—This genus represents a well-supported, nearly exclusive clade, except that a single species (unusual in having fully bipinnate laminae), *Plesioneuron marattioides*, is nested within it.

CHRISTELLA—The members of *Christella* resolve in four distantly related clades (Fig. 4), with the predominantly Asian group corresponding to *Christella* sect. *Christella*, and mostly

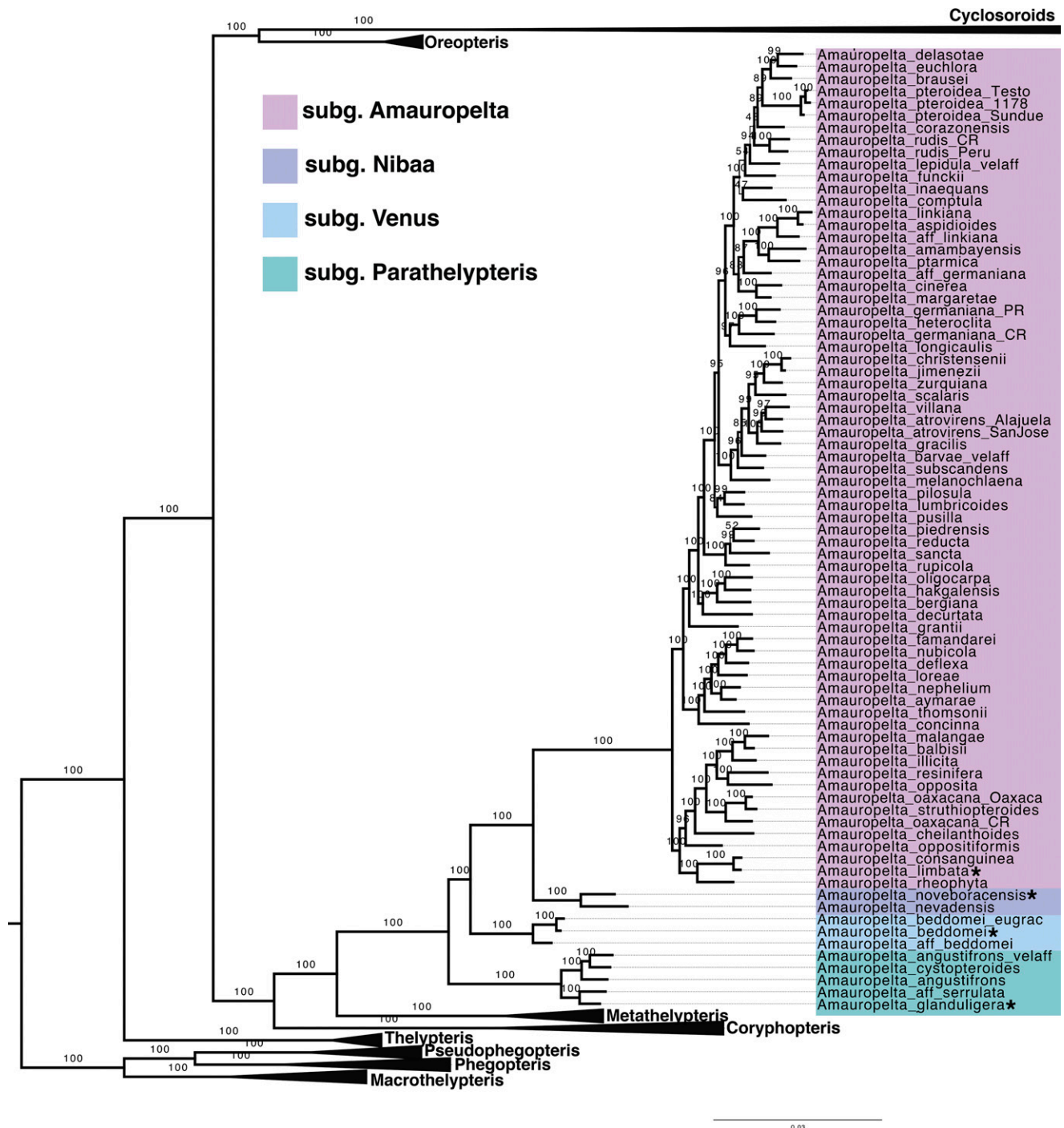


FIG. 3B. The genus *Amauropelta* in the maximum likelihood phylogeny of the 407-locus concatenated alignment. The nodes are annotated with ultrafast bootstrap support values, and colors correspond to subgenera of *Amauropelta* proposed by Fawcett and Smith (2021). Type species of genera and subgenera are denoted with an asterisk (*).

neotropical species corresponding to *Christella* sect. *Pelazoneuron*. A small group of African species including *C. chaseana* and *C. gueintziana* resolves as sister to *Christella* sect. *Christella* in most trees, but there is some conflicting support for these species as members of the pseudocyclosoroid clade. The three endemic Hawaiian *Christella* species resolve with members of *Pronephrium* s. l.

MENISORUS—This previously monotypic African genus had never been included in a molecular phylogenetic study. It is sister to another African species that also has proliferous buds, *Pneumatopteris unita* (Kunze) Holttum (Fig. 3F).

MESOPTERIS—Considered a monotypic genus endemic to China and neighboring Vietnam, this taxon is sister to two species of *Amblovenatum* from Malesia, but this clade is distantly related to the type of that genus, *A. opulentum* (Figs. 3F, 3G).

NANNOTHELYPTERIS—The type of this genus, *N. aoristisora*, is nested within a clade of *Pronephrium* that includes several species that are morphologically similar to the type of that genus, *P. lineatum* (Blume) C. Presl (Fig. 3H).

PARATHELYPTERIS—The members of this genus resolve in five distinct clades (Figs. 3A, 3B); two of these resolve



FIG. 3C. The genus *Oreopteris*, and non-christelloid members of the cyclosoroid clade, including the stegnogrammoid subclade (*Hoiokula*, *Cyclogramma*, *Stegnogramma*, and *Leptogramma*) in the maximum likelihood phylogeny of the 407-locus concatenated alignment. The nodes are annotated with ultrafast bootstrap support values, and genera are emphasized by distinct colors. Type species of genera are denoted with an asterisk (*).

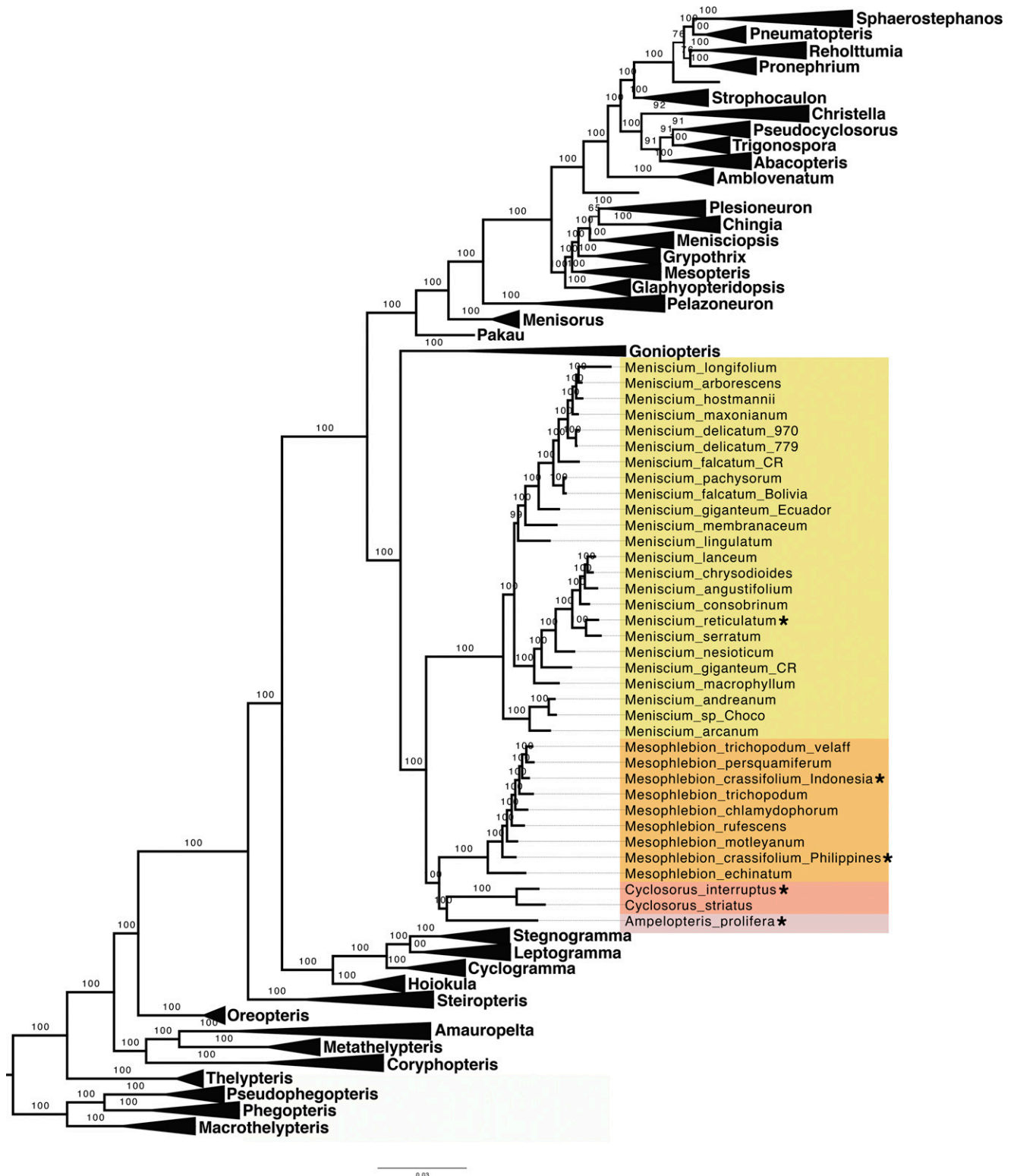


FIG. 3D. Members of the goniopteroid clade in the maximum likelihood phylogeny of the 407-locus concatenated alignment. The nodes are annotated with ultrafast bootstrap support values, and genera are emphasized by distinct colors. Type species of genera are denoted with an asterisk (*).

among species of *Coryphopteris* and the other three clades include the North American sister species *P. noveboracensis* (L.) Ching and *P. nevadensis* (Baker) Holtum, which are sister to *Amauropelta*, and two Asian clades, one including the type species *P. glanduligera* (Kunze) Ching, and the other including *P. beddomei* (Baker) Ching. Although each of these three clades is well supported as monophyletic,

the relationships among them (*Amauropelta* plus North American *Parathelypteris* and the two Asian clades) are in conflict among individual gene trees (Fawcett et al. 2021).

PLESIONEURON—Sequence data were available for only a single species of *Plesioneuron* when PPG I (2016) was published. This genus as recognized by PPG I (2016) corresponds to a nearly exclusive clade with the exception of *Pneumatopteris*

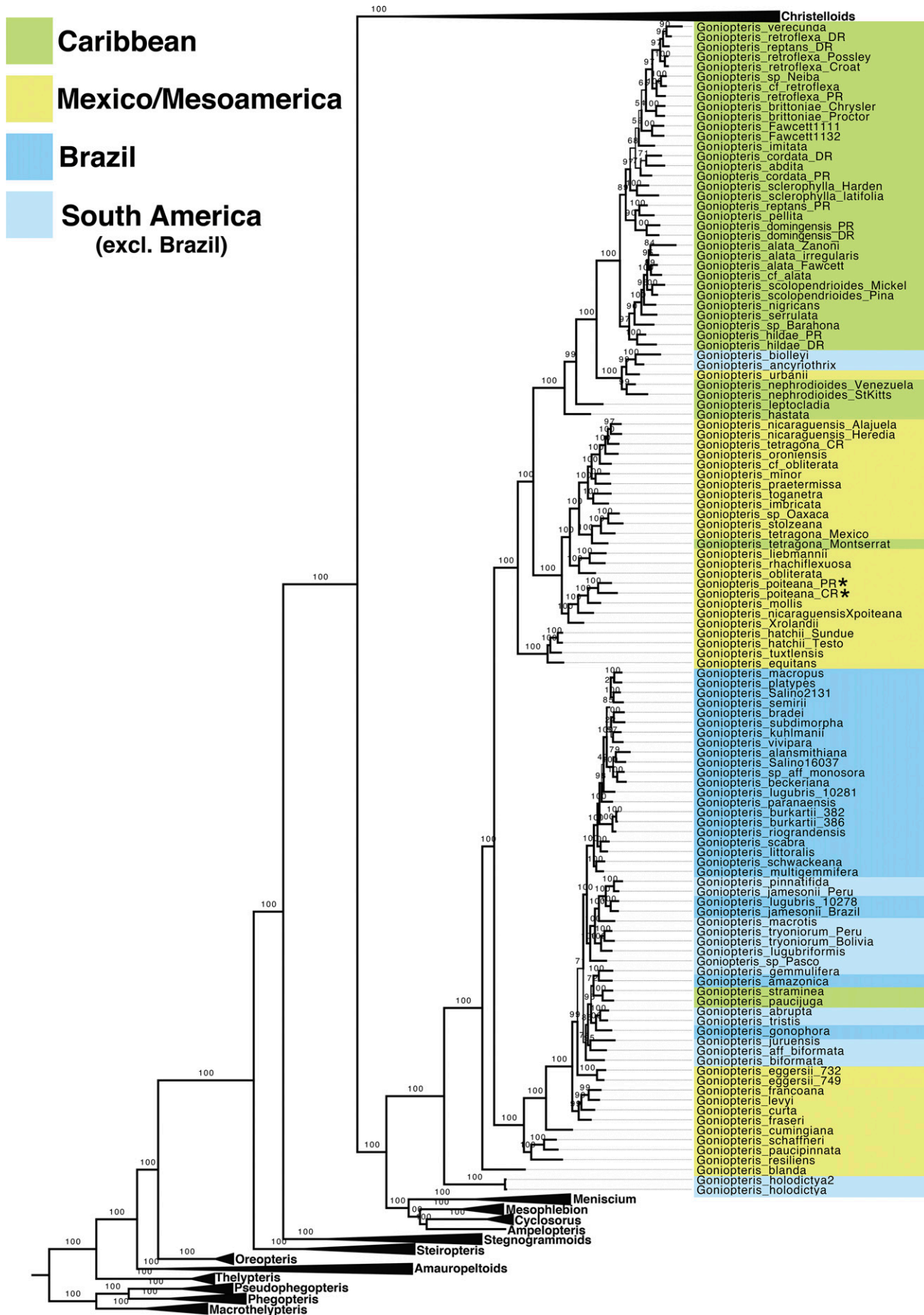


FIG. 3E. The genus *Goniopteris* in the maximum likelihood phylogeny of the 407-locus concatenated alignment. The nodes are annotated with ultrafast bootstrap support values, and the colors correspond to geographic regions where the molecular vouchers were collected. Type species of genera are denoted with an asterisk (*).



FIG. 3F. The monotypic genus *Pakau*, and the members of the chingioid clade in the maximum likelihood phylogeny of the 407-locus concatenated alignment. The nodes are annotated with ultrafast bootstrap support values, and genera are emphasized by distinct colors. Type species of genera are denoted with an asterisk (*).



FIG. 3G. The genus *Amblovenatum*, and members of the pseudocyclosoroid clade in the maximum likelihood phylogeny of the 407-locus concatenated alignment. The nodes are annotated ultrafast bootstrap support values, and colors indicate the biogeographic affinities of *Christella* s. s., and emphasize major clades. The position of several lineages (e.g. *Amblovenatum*, the clade of three African *Christella* species) differs in the ML bootstrap consensus tree and/or the multispecies coalescent tree (Fawcett et al. 2021). Type species of genera are denoted with an asterisk (*).

keysseriana Holttum, which is nested within it, and *Plesioneuron marattioides* (Alston) Holttum, which resolves in *Chingia* (Fig. 3F).

PNEUMATOPTERIS—This is by far the most polyphyletic genus recognized by Holttum (1971) and PPG I (2016) (Fig. 4). Its species are scattered in more than a dozen lineages

throughout the tree, resolving within clades corresponding to *Mensorus*, *Plesioneuron*, and *Sphaerostephanos*. Three lineages are distinct and do not fall within currently recognized genera: two Hawaiian species are sister to *Stegnogramma* plus *Cyclogramma*; the New Zealand and Australian endemic *Pneumatopteris pennigera* (G. Forst.) Holttum resolves on a



FIG. 3H. Members of the sphaerostephanoid clade in the maximum likelihood phylogeny of the 407-locus concatenated alignment. The nodes are annotated with ultrafast bootstrap support values, and genera are emphasized by distinct colors. Type species of genera are denoted with an asterisk (*).



FIG. 3I. The genus *Sphaerostephanos* in the maximum likelihood phylogeny of the 407-locus concatenated alignment. The nodes are annotated ultrafast bootstrap support values, and colors correspond to biogeographical regions illustrated by Mayr (1944), corresponding to collection localities of the molecular vouchers. Type species of genera are denoted with an asterisk (*).



FIG. 4. A phylogeny of the Thelypteridaceae illustrating the polyphyly of *Christella*, *Pneumatopteris*, and *Pronephrium* sensu PPG I (2016), and their recently proposed disposition (Fawcett and Smith 2021).

long branch at the base of the christelloid clade; and the majority of species constitute a single clade sister to *Sphaerostephanos* plus a relatively small clade of primarily Pacific Island *Pneumatopteris* species including the type species, *P. callosa*. Other taxa resolve as sister to *Trigonospora*, or to one of the four clades of *Pronephrium*, while two other taxa, *Pneumatopteris auctipinna* and an undescribed species from the Solomon Islands, resolve on long branches diverging from the backbone within the christelloid clade, but with low support.

PRONEPHRIUM—The members of this polyphyletic genus all share once-pinnate fronds with entire to shallowly incised margins, but its species resolve in four distinct, well-supported clades, while a few species are scattered throughout the *Sphaerostephanos* clade (Figs. 3H, 4). One of these four clades corresponds perfectly to *Pronephrium* sect. *Grypothrix*, and the other three correspond in part to the infrageneric taxa of *Pronephrium* proposed by Holttum (1982). Although the type species, *P. lineatum* (Blume) Presl, was not sampled, the type specimen exhibits the combination of dimorphic fronds and the presence of yellow spherical glands on the sporangia, which are characteristic of *Pronephrium* sensu Fawcett and Smith (2021) and are uncommon among other genera.

SPHAEROSTEPHANOS—Most members of this large and morphologically complex genus represent a nearly exclusive clade (Fig. 3I). Several species recognized in *Pronephrium* and *Pneumatopteris* fall within it, and two distinctive, widespread species with long-creeping rhizomes, *S. unitus* (L.) Holttum and *S. invisus* (G. Forst.) Holttum, form a clade sister to the core christelloids (the sphaerostephanoids, pseudocyclosoroids, plus *Amblovenatum*) (Fig. 3H). A few species, mainly of India and Africa, resolve variously in the christelloid or pseudocyclosoroid clades (Fig. 3G).

STEGNOGRAMMA—This genus (including *Leptogramma* and *Dictyocline* T. Moore) is monophyletic as recognized by PPG I

(2016). However, our phylogenetic results lend equal support for the recognition of two sister genera, *Stegnoqramma* (including *Dictyocline*) and *Leptogramma* (Fig. 3C).

Discordance Among Gene Trees—Congruence among gene trees, and among phylogenies inferred using concatenated and coalescent methods, was generally high, but with conflicting support for some taxa and clades (Fawcett et al. 2021). The average gene concordance factor (gCF) for crown nodes corresponding to genera is 43%. The highest gCF values corresponded to species-poor genera on long branches, exemplified by *Thelypteris* s. s. (gCF = 98%), while the lowest value was seen in *Christella* (gCF = 0.25%), a clade which contains rogue taxa (discussed below). The average site concordance factor (sCF) for generic clades is 69%, ranging from 97% in *Cyclosorus* s. s. to 32% in *Ampelopteris*.

Among taxa with conflicting support is a small group of African species, including *Christella gueintziana* and *C. chaiseana*, which resolve as sister to the rest of *Christella* sect. *Christella* in the coalescent analysis; however, these species sometimes resolve within the pseudocyclosoroid clade in individual gene trees and in the ML tree (Fig. 3G). The conflicting position of this rogue clade contributes to the uniquely low bootstrap support of the crown node of *Christella*. There is also some conflict among gene trees for *Christella (Pelazoneuron) kunthii*, *C. (P.) schizotis*, and *C. (P.) patens* var. *dissimilis*; all three are supported as members of the *Pelazoneuron* clade, but are also inferred to be members of the *Christella* sect. *Christella* clade in some individual gene trees.

The phylogenetic position of the clade comprising three species of *Amblovenatum* s. s. resolve variously in the sphaerostephanoid clade in the ML bootstrap consensus tree, (Fig. 2) and coalescent tree (Fawcett et al. 2021), or as sister to the sphaerostephanoids plus pseudocyclosoroids (ML tree, Fig. 3G).

Two of the Hawaiian species of *Pneumatopteris* resolve as sister to *Stegnoqramma* plus *Cyclogramma* with 100% support in both concatenated and coalescent analyses; however, individual gene trees resolve these species as nested among members of *Pronephrium* or highly nested within *Leptogramma*.

Finally, a few morphologically aberrant taxa of Africa, Madagascar, and India resolve as sister to large clades with low support, and often are in conflicting positions among individual gene trees. These include *Sphaerostephanos elatus*, *Sphaerostephanos hirtisorus*, and *Pneumatopteris afra*; all three are rogue taxa within the pseudocyclosoroid clade (Fig. 3G).

DISCUSSION

Support for Taxonomic Revision—Our results support the continued recognition of 17 of the 30 genera of the Thelypteridaceae as circumscribed by PPG I (2016), and taxonomic revision of the remaining 13 genera (Table 1), as proposed by Fawcett and Smith (2021). Two of these genera, *Parathelypteris* (H.Ito) Ching, and *Nannothelypteris* Holttum, we treat in synonymy. Of the remaining 11 genera, some require only minor revisions to be monophyletic, while others, notably *Pneumatopteris* and *Pronephrium*, require more substantial changes. We recognize four recently described genera, *Hoiokula*, *Pakau*, *Reholtumia*, and *Strophocaulon*. We recommend the elevation in rank from section to genus for *Christella* sect. *Pelazoneuron*, *Pronephrium* sect. *Grypothrix*, and *Pronephrium* sect. *Menisciopsis* Holttum, and the resurrection of the genus *Abacopteris*. We recognize the expanded circumscription of *Amauropelta* to

include *Parathelypteris* pro parte, and recognize three new subgenera within *Amauropelta*. All necessary taxonomic combinations, a key to genera, and descriptions of the 37 genera here recognized are available in Fawcett and Smith (2021). These genera, which include ca. 1200 species worldwide, are recognized on the basis of both monophyly, and improved morphological diagnosability. A brief discussion of newly recognized genera and subgenera follows:

ABACOPTERIS—This genus was treated as a synonym of *Pronephrium* by Holttum (1971, 1982), but based on the morphology of the type species, *Pronephrium lineatum*, we recognize *Pronephrium* s. s. as a member of the distantly related sphaerostephanoid clade. *Abacopteris*, in the sense we apply the name, is more closely allied to *Christella* and *Pseudocyclosorus* (Fig. 3G).

AMAUIROPELTA—Following Kuo et al. (2019) we expand the concept of *Amauropelta* to include three paraphyletic clades of *Parathelypteris*, now treated as *Amauropelta* subg. *Nibaa* (the two North American species), *Amauropelta* subg. *Parathelypteris* (including the type of *Parathelypteris*, *P. glanduligera*), and *Amauropelta* subg. *Venus* (including *P. beddomei*) (Fig. 3B).

GRYPOTHRIX—This genus corresponds perfectly to *Pronephrium* sect. *Grypotherix* (Holttum 1982), a group that is defined by hooked (hamate) hairs somewhere on the body of the plant. It is in the chingoid clade (Fig. 3F).

HOIOKULA—This new genus corresponds to the two native Hawaiian species treated in *Pneumatopteris* by Holttum (1982) but are more closely related to *Leptogramma*, *Stegnogramma*, and *Cyclogramma* in our analyses (but see comments under *Discordance Among Gene Trees*) (Fig. 3C).

LEPTOGRAMMA—Our data give equal support to the recognition of *Stegnogramma* s. l. or *Stegnogramma* s. s. plus *Leptogramma*. In light of the morphological differences between these two clades, we follow Kuo et al. (2020), in which the type species was sampled, in recognizing *Leptogramma* (including *Craspedosorus* Ching & W.M.Chu) as a distinct genus (Fig. 3C).

MENISCIOPSIS—This genus includes continental Asian and Malesian members of the genus *Pronephrium* as well as the three Hawaiian endemic species of *Christella* (Fig. 3F).

PAKAU—This monotypic genus corresponds to *Pneumatopteris pennigera*, which is endemic to New Zealand and eastern Australia. It occurs on a long branch, and is seemingly distantly related to any other taxon in the family (Fig. 3F).

PELAZONEURON—In his treatment of African Thelypteridaceae, Holttum (1974) described *Pelazoneuron* as a section of *Christella* comprising several African species he believed to be allied to neotropical taxa; he designated the American species *Christella patens* as the type of this section. Based on our results, neither those African taxa nor *Christella* sect. *Christella* are closely related to *C. patens*, but following Holttum's typification, this name corresponds to a well-defined group of American species (Smith 1971) (Fig. 3F).

REHOLTTUMIA—The greatest number of *Pneumatopteris* species resolve in this clade; however, the type of *Pneumatopteris*, *P. callosa*, is in a small clade more closely related to *Sphaerostephanos*, which is the older name. Rather than expand *Sphaerostephanos*, a polymorphic but recognizable genus already containing more than 200 species, we recognize this clade as a segregate of *Pneumatopteris* (Fig. 3H). Its name honors R. E. Holttum, whose keen insights and dedication laid the groundwork that made the present study possible.

STROPHOCAULON—This genus of two species includes only *Sphaerostephanos unitus* and *S. invisus*, (Fig. 3H) both of which are widespread and have long-creeping rhizomes (Fig. 1L), distinct from the short-creeping or ascending rhizomes typical of most species of *Sphaerostephanos* (Fig. 1K).

Discordance Among Gene Trees—Various factors may contribute to conflict among gene trees, including reticulation and introgression, gene duplication and loss, and incomplete lineage sorting (Degnan and Rosenberg 2009), horizontal gene transfer (Davis et al. 2005; Li et al. 2014, 2018), and error in gene tree inference. Different patterns of discordance may indicate either lack of support or conflicting support (Pease et al. 2018). For example, among recent rapid radiations, especially for species with large populations, incomplete lineage sorting may be expected, with stochastic patterns of conflict across gene trees. For taxa of hybrid origin, in contrast, gene trees may exhibit consistent patterns of conflicting support, e.g. a taxon resolving in two distinct (and rarely, highly divergent) clades. Because of the large size of our dataset, and the use of, at most, a single sequence to represent each taxon at each locus, we expect taxa of allopolyploid origin to resolve with one progenitor, or sister to a larger clade including a progenitor, with high support, as has been previously demonstrated in phylogenetic analyses that include hybrid taxa (McDade 1992). The primary goal of this study is to resolve generic circumscription, and the great degree of congruence among our gene trees gives us confidence in our conclusions. However, we are continuing to investigate the potential of hybrid origin for the small minority of rogue taxa and lineages.

Biogeographic Patterns—The taxonomic concepts proposed by Holttum and adopted by PPG I (2016) in some cases include geographically widespread and polymorphic genera, best exemplified by *Pneumatopteris* s. l., the range of which includes Hawaii, Africa, Madagascar, East Asia, and New Zealand. Holttum (1977: 226) was clearly aware of the distinctive morphology of its most aberrant species, regarded here as *Hoiokula* (Hawai'i), *Pakau* (Australia, New Zealand), *Plesioneuron* (Malesia and Oceania), and *Reholttumia* (scattered from Madagascar to Oceania). These genera were recognized only with the benefit of a molecular dataset to guide morphological observations. Our newly segregated genera tend to be more geographically restricted, as is the case for those mentioned above, two of which are endemic to regions beyond the range of *Pneumatopteris* s. s., which is restricted to Oceania and Malesia.

Regional geographic affinities, exemplified by *Goniopteris* (Fig. 3E) and *Amauropelta* subg. *Amauropelta*, show distinct phylogeographic patterns, with major clades largely restricted to the Caribbean, Mexico/Mesoamerica, the Andes, or Brazil. Other lineages (e.g. *Sphaerostephanos*; Fig. 3I) show much less clear-cut biogeographical patterns within and between clades, suggesting extensive dispersal and possibly also extinction events. This raises the intriguing question why some genera appear to undergo limited dispersal and extensive regional diversification, whereas others have a much more dynamic biogeographical history.

Classifying the Thelypteridaceae of Africa has proven to be especially challenging, partly because the present diversity has likely been shaped by high rates of extinction (Aldasoro et al. 2004), leaving a flora of disparate relicts that are difficult to contextualize within extant diversity. For example, the four Afro-Madagascan species of *Pneumatopteris* s. l. included

in our analysis are each resolved in a distinct lineage, most closely allied to *Abacopteris*, *Menisorus*, *Reholtiumia*, and *Trigonospora*; yet each is quite morphologically distinct from the typical species in each of these nearest clades.

Morphological Observations—The often subtle morphological characters needed to distinguish genera and identify species have contributed to the reputation of this family as taxonomically difficult (Fraser-Jenkins et al. 2017). Many of the genera can only be identified using a combination of characters, some seen only with the aid of a microscope, and the most salient features are often shared among distantly related taxa (Fawcett and Smith 2021). For example, all species within the genus *Pronephrium* s. l. share once-pinnate laminae with entire or shallowly incised pinna margins. However, our analyses demonstrate that members of *Pronephrium* s. l. resolve in distinct clades (Fig. 4), three of which are now recognized as the segregate genera *Abacopteris*, *Grypothrix*, and *Menisciopsis*, and others in multiple clades across *Sphaerostephanos*. In nearly all instances, these clades are more closely related to pinnate-pinnatifid taxa (the most common blade-division in the Thelypteridaceae) than they are to each other. These genera may be more reliably distinguished from each other by more subtle characters, including the presence of setae or glands on the sporangia and indusia, presence of proliferous buds, position of sori, and distribution and morphology of the indument (Fawcett and Smith 2021).

Conclusions—We present a densely sampled nuclear DNA phylogenomic dataset, representing half of the species diversity in the Thelypteridaceae; this broad sampling facilitates the revision of the generic classification of the family. However, species-level diversity, especially in the Paleotropics, remains poorly understood. For instance, at the time of the most recent treatment of *Sphaerostephanos* (Holttum 1982), of the 185 species recognized, 66 (36%) were known only from the type, or the type plus one or two other collections. Yet, even modern treatments of neotropical genera show the same pattern, with, e.g. 12 (22%) of the 54 Bolivian species of *Amauropelta* known from only one to three collections (Smith and Kessler 2017). This likely reflects a combination of factors, including narrow endemism, rarity, paucity of herbarium collections, and failure of general collectors to recognize subtle variations in the field. We do not doubt that many additional species remain undescribed, especially in Malesia. It is our hope that this work, and the complementary publication of a taxonomic monograph (Fawcett and Smith 2021) that provides a key to genera, detailed generic descriptions, and diagnoses, will provide a framework to facilitate the discovery, recognition, and conservation of thelypterid biodiversity.

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AUTHOR CONTRIBUTIONS

SF, ARS, GB, and EBS designed and implemented the study. GB, EBS, WLT, and GFC generated the data, SF and GB analyzed the data, and SF, MS, MK, LYK, CWC, and WLT contributed critical tissue samples and associated herbarium vouchers. SF drafted the manuscript, with contributions from all co-authors.

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APPENDIX 1. Voucher data for specimens used in molecular phylogenetic analyses. Specimens are arranged alphabetically by genus, with collector name and number, species, locality, country, year of collection, and herbaria where deposited, for tissue samples taken in the field. Many vouchers are digitized and images are hosted by the Pteridophyte Collections Consortium <pteridportal.org>.

Abacopteris Fée—Karger 1408, *Abacopteris aspera* (C.Presl) Ching, Bacan, Mauluku Utara, Indonesia, 2010 (UC 2048328, Z); Chen Wade1431, *Abacopteris birii* (R.D.Dixit & Balkr.) S.E.Fawc. & A.R.Sm., Lam Dong, Vietnam, 2010 (TAIF 409716); Kuo 857, *Abacopteris gymnoteridifrons* (Hayata) Ching, Pingtung, Taiwan, 2010 (TAIF); *Cicuzza* 693, *Abacopteris nitida* (Holtum) S.E.Fawc. & A.R.Sm., Maleo, Sulawesi, 2008 (UC, Z); Karger 1756, *Abacopteris* aff. *peltoclamys* (C.Chr.) Holtum, West Papua, Indonesia, 2008 (UC 2048607, Z); Karger 1260, *Abacopteris repanda* (Fée) S.E.Fawc. & A.R.Sm., Seram, Indonesia, 2010 (UC 2048311; UC 2048312, Z).

Sphaerostephanos archboldii (C.Ch.) Holttum, Madang, Papua New Guinea, 2014 (UC 2049100); Kessler 14130, *Sphaerostephanos archboldii* (C.Ch.) Holttum, West Papua, Wamena, Indonesia, 2008 (UC 2048587); Conn 5509, *Sphaerostephanos archboldii* (C.Ch.) Holttum, Papua New Guinea, 2010 (UC 2043794); Sundue 3848, *Sphaerostephanos atasripii* (Rosenst.) Holttum, vel aff., Madang, Papua New Guinea, 2014 (UC 2049098); Karger 1022, *Sphaerostephanos* aff. *batjanensis* (Rosenst.) Holttum, Sulawesi, Gunung Duasudara, Indonesia, 2010 (UC 2048305, Z); Fawcett 701, *Sphaerostephanos beccarianus* (Ces.) S.E.Fawc. & A.R.Sm., Taveuni, Fiji, 2016 (UC 2049798, VT); SITW11593, *Sphaerostephanos braithwaitei* Holttum, Guadalcanal, Solomon Islands, 2016 (UC 2048659, 2048660, 2048661); Kessler 13850, *Sphaerostephanos brauseanus* (Holttum) S.E.Fawc. & A.R.Sm., West Papua, Indonesia, 2008 (UC 2048589); Karger 990, *Sphaerostephanos canescens* (Blume) Holttum, Sulawesi, Gunung Duasudara, Indonesia, 2010 (UC 2048288); Alston 16718, *Sphaerostephanos canescens* (Blume) Holttum, Tidore, Maluku Utara, (MICH); Grether 3971, *Sphaerostephanos cataractorum* (W.H.Wagner & Grether) Holttum, Manus, Admiralty Islands, 1945 (UC 732987); Karger 2801, *Sphaerostephanos confertus* (Brause) Holttum, West Papua, Indonesia, 2011 (UC 2048202, Z); Sundue 3850, *Sphaerostephanos confertus* (Brause) Holttum, Madang, Papua New Guinea, 2014 (UC 2049099); Chen Wade3098, *Sphaerostephanos decadens* (Baker) Holttum, Viti Levu, Fiji, 2013 (TAIF 439697); Kuo 3656, *Sphaerostephanos dichotrichoides* (Alderw.) Holttum, Bukidnon, Philippines, 2012 (UC); Chen Wade3113, *Sphaerostephanos doodioides* (Copel.) Holttum, Guadalcanal, Solomon Islands, 2013 (TAIF); Ranker 1460, *Sphaerostephanos elatus* (Bojer) Holttum, Reunion, France, 1992 (UC 1610044); Karger 2692, *Sphaerostephanos* cf. *flavoviridis* Holttum, West Papua, Indonesia, 2011 (UC 2048193, Z); Schuettelpelz 890, *Sphaerostephanos glandulosus* (Blume) S.E.Fawc. & A.R.Sm., Malaysia, (DUKE, KEP, S); James SAJ082, *Sphaerostephanos hastatopinnatus* (Brause) Holttum, New Britain, Papua New Guinea, 2011 (UC 2043793); Fawcett 640, *Sphaerostephanos heterocarpus* (Blume) Holttum, Viti Levu, Fiji, 2016 (UC 2049794, VT); Fawcett 619, *Sphaerostephanos heterocarpus* (Blume) Holttum, Viti Levu, Fiji, 2016 (UC 2049783, VT); Cicuzza 495, *Sphaerostephanos hirsutus* (Kunze ex Mett.) Holttum, Sulawesi, Pono, Indonesia, 2008 (UC, Z); Anderson 4119, *Sphaerostephanos hirtisorus* (C.Ch.) Holttum, Chiang Mai, Thailand, 1976 (UC 1432224); Chen Wade3559, *Sphaerostephanos hochreutineri* (Christ) Holttum, Vella Lavella, Solomon Islands, 2013 (TAIF 460030); Karger 1406, *Sphaerostephanos incisus* (Copel.) S.E.Fawc. & A.R.Sm., Bacan, Maluku Utara, Indonesia, 2010 (UC 2048291, Z); Karger 1071, *Sphaerostephanos lamii* Holttum, Buru, Maluku, 2010 (UC 2048332, Z); Kessler 13559, *Sphaerostephanos larutensis* (Bedd.) C.Ch., Peninsular Malaysia, Malaysia, 2008 (GOET, UC 1951607); Chen Wade3885, *Sphaerostephanos lastreoides* (C.Presl) Holttum, Negros, Philippines, 2014 (TAIF 455400); Chen Wade3979, *Sphaerostephanos lastreoides* (C.Presl) Holttum, Negros, Philippines, 2014 (TAIF 455815, 455816); Cicuzza 219, *Sphaerostephanos latebrosus* (Kunze ex Mett.) Holttum, Sulawesi, Bariri, Indonesia, 2007 (UC, Z); Wood 14100, *Sphaerostephanos maemonensis* (W.H.Wagner & Grether) Holttum, Kosrae, Caroline Islands, 2010 (PTBG); Chen Wade3957, *Sphaerostephanos magnus* (Copel.) Holttum, Negros, Philippines, 2014 (TAIF 454834, 454835, 454836, 454837); Chen Wade3995-2, *Sphaerostephanos* aff. *microlonchus* (Christ) S.E.Fawc. & A.R.Sm., Negros, Philippines, 2014 (TAIF 454807, 454808, 454809; VT 197691); Karger 2663, *Sphaerostephanos* aff. *mundus* (Rosenst.) Holttum, West Papua, Indonesia, 2011 (UC 2048196, Z); Karger 2809, *Sphaerostephanos novoguineensis* (Brause) Holttum, West Papua, Indonesia, 2011 (UC 2048203, Z); Rothfels 4803, *Sphaerostephanos oosorus* (Baker) Holttum, Mount Silam, Borneo, 2016 (UC 2048744); Cicuzza 185, *Sphaerostephanos oppositus* Holttum, Sulawesi, Bariri, Indonesia, 2007 (Z); Chen Wade1806, *Sphaerostephanos penniger* (Hook.) Holttum, Gunung Gede Pangrango National Park, Indonesia, 2011 (TAIF 388622, 388623, UC); Chen Wade4839, *Sphaerostephanos penniger* (Hook.) Holttum, Pahang, Peninsular Malaysia, 2017 (KLU, TAIF, UC); Karger 2645, *Sphaerostephanos pentaphyllus* (Rosenst.) S.E.Fawc. & A.R.Sm., West Papua, Indonesia, 2011 (UC 2048204, Z); Karger 2859, *Sphaerostephanos pilosquamatus* (Alderw.) Holttum, West Papua, Indonesia, 2011 (UC 2048191, Z); Kessler 13709, *Sphaerostephanos polycarpus* (Blume) Copel., Sulawesi, Indonesia, 2008 (GOET, UC); Cicuzza 466, *Sphaerostephanos polyotis* (C.Ch.) Holttum, Sulawesi, Pono, Indonesia, 2007 (UC, Z); Chang 20100917-024, *Sphaerostephanos productus* (Kaulf.) Holttum, Taitung, Taiwan, 2010 (TAIF); Kluge 7448, *Sphaerostephanos sarsinorum* Holttum, Sulawesi, Korekambu, Indonesia, 2007 (BO, UC); Chen Wade2965, *Sphaerostephanos scandens* Holttum, Rendova, Solomon Islands, 2013 (TAIF 448614); Kluge 9201, *Sphaerostephanos scopulorum* (Holttum) S.E.Fawc. & A.R.Sm., West Papua, Indonesia, 2008 (UC 2048622); Karger 247, *Sphaerostephanos*

sessilipinna (Copel.) Holttum, Panay, Philippines, 2009 (Z); Chen Wade2027-1, *Sphaerostephanos stipellatus* (Blume) Holttum, West Java, Halimam, Indonesia, 2011 (TAIF 388183, 388184); Sundue 3752, *Sphaerostephanos subappendiculatus* (Copel.) S.E.Fawc. & A.R.Sm., Madang, Papua New Guinea, 2014 (UC 2049167, VT); Nitta 3025, *Sphaerostephanos subjectinatus* (Copel.) Holttum, Moorea, Society Islands, 2013 (GH, UC); Karger 2668, *Sphaerostephanos superbus* (Brause) S.E.Fawc. & A.R.Sm., West Papua, Indonesia, 2011 (UC, Z); Kuo 878, *Sphaerostephanos taiwanensis* (C.Ch.) Holttum ex C.M.Kuo, Pingtung, Taiwan, 2010 (TAIF); Karger 1586, *Sphaerostephanos urdanatensis* (Copel.) Holttum, Mindanao, Philippines, 2010 (UC 2048235; Z 104920); Chen Wade2816, *Sphaerostephanos veitchii* Holttum, Ranonga, Solomon Islands, 2013 (TAIF 445062); Grether 4202, *Sphaerostephanos vestigiatus* (Copel.) Holttum, Los Negros, Admiralty Islands, 1945 (UC 732711); Rinehart 21351, *Sphaerostephanos warburgii* (Kuhn & Christ) Holttum, Guam, U.S.A., 1991 (UC 1734677); Kessler 13952, *Sphaerostephanos womersleyi* (Holttum) S.E.Fawc. & A.R.Sm., West Papua, Indonesia, 2008 (UC 2048619); Kluge 9088, *Sphaerostephanos* sp., West Papua, Indonesia, 2008 (UC 2048624); Kluge 9071, *Sphaerostephanos* sp., West Papua, Indonesia, 2008 (UC 2048584); Karger 544, *Sphaerostephanos* sp., Luzon, Philippines, 2009 (UC); SITW11650, *Sphaerostephanos* sp., Guadalcanal, Solomon Islands, 2016 (UC 2048648); Chen Wade4923, *Sphaerostephanos* sp., Negros, Philippines, 2017 (CMUH, PNH, TAIF); Co 3474, *Sphaerostephanos* sp., Luzon, Philippines, 1991 (MICH); Karger 443, *Sphaerostephanos* sp., Mindanao, Philippines, 2009 (Z); Karger 2558, *Sphaerostephanos* sp., West Papua, Indonesia, 2011 (UC, Z).

Stegnogramma Blume—Chen Wade1902, *Stegnogramma aspidioides* Blume, Java, Indonesia, 2011 (TAIF); Yu 655, *Stegnogramma dictyoclioides* Ching, Pingtung, Taiwan, (PE, TAIF); Kuo 1885, *Stegnogramma griffithii* (T.Moore) K.Iwats., Tam Dao, Vietnam, 2010 (TAIF); Kuo 4240, *Stegnogramma mingchegensis* (Ching) X.C.Zhang & L.J.He, Fukien, China, 2015 (TAIF); Chang 20140714044, *Stegnogramma sagittifolia* (Ching) L.J.He & X.C.Zhang, Guizhou, China, 2014 (TAIF); Kuo 2368, *Stegnogramma wilfordii* (Hook.) Seriz., Taipei, Taiwan, 2011 (TAIF); Chen Wade4167, *Stegnogramma* sp., Lam Dong, Vietnam, 2014 (TAIF); WXP 149, *Stegnogramma* sp., Yunnan, China, 2010 (PE).

Steiropteris (C.Ch.) Pic.Serm.—Fuentes 10972, *Steiropteris buchtienii* (A.R.Sm.) Salino & T.E.Almeida, La Paz, Bolivia, 2006 (UC 1921877); Christenhusz 2795, *Steiropteris clypeolulata* (Desv.) Pic.Serm., Guadeloupe, 2003 (UC 1788485); Fawcett 464, *Steiropteris deltoidea* (Sw.) Pic.Serm., Adjuntas, Puerto Rico, 2016 (VT); Possley 215, *Steiropteris deltoidea* (Sw.) Pic.Serm., Jayuya, Puerto Rico, 2016 (MAPR); Meier 4771, *Steiropteris fendleri* (D.C.Eaton) Pic.Serm., Yaracuy, Venezuela, 1999 (UC 1737148); Jimenez 1061, *Steiropteris gardneriana* (Baker) Pic.Serm., La Paz, Bolivia, 2001 (GOET, LPB, UC 1764496); Hill 28047, *Steiropteris glandulosa* (Desv.) Pic.Serm., St. Patrick, Dominica, 1996 (VT 197839); Testo 1020, *Steiropteris glandulosa* (Desv.) Pic.Serm. var. *brachyodus* (Kunze) Salino & T.E.Almeida, Puntarenas, Costa Rica, 2016 (VT); Tuomisto 10697, *Steiropteris glandulosa* (Desv.) Pic.Serm. var. *brachyodus* (Kunze) Salino & T.E.Almeida, Napo, Ecuador, 1997 (UC 1788318); Gudino 1326, *Steiropteris glandulosa* (Desv.) Pic.Serm. var. *longipilosa* (A.R.Sm.) Salino & T.E.Almeida, Pastaza, Ecuador, 1991 (UC 1597601); Labiak 2993, *Steiropteris hatschbachii* (A.R.Sm.) Salino & T.E.Almeida, Parana, Brazil, 2003 (UC 1926875); Testo 1227, *Steiropteris leprieurii* (Hook.) Pic.Serm. var. *subcostalis* (A.R.Sm.) A.R.Sm., Heredia, Costa Rica, 2017 (VT); Churchil 3614, *Steiropteris leprieurii* (Hook.) Pic.Serm. var. *subcostalis* (A.R.Sm.) A.R.Sm., Cartago, Costa Rica, 1980 (VT 197835); Mexia 4904, *Steiropteris mexiae* (Copel. ex C.Ch.) Salino & T.E.Almeida, Minas Gerais, Brazil, 1930 (UC 466421); Salino 2003, *Steiropteris mexiae* (Copel. ex C.Ch.) Salino & T.E.Almeida, Minas Gerais, Brazil, 2003 (UC); Tuomisto 11636, *Steiropteris pennellii* (A.R.Sm.) Salino & T.E.Almeida, Napo, Ecuador, 1998 (UC 1788328); Holm-Nielsen 2912, *Steiropteris polyphlebia* (C.Ch.) Salino & T.E.Almeida, Cotopaxi, Ecuador, 1973 (UC 1437100); Salino 10229, *Steiropteris polypodioides* (Raddi) Salino & T.E.Almeida, São Paulo, Brazil, 2005 (UC 2047328); Nitta 2321, *Steiropteris seemannii* (J.Sm.) Salino & T.E.Almeida, Alajuela, Costa Rica, 2013 (GH, UC); Moran 3249, *Steiropteris seemannii* (J.Sm.) Salino & T.E.Almeida, Alajuela, Costa Rica, 1983 (UC 1265902); Grayum 3239, *Steiropteris valdepiolosa* (Baker) Pic.Serm., Heredia, Costa Rica, 1983 (UC 1490562); Matos 1870, *Steiropteris villosa* (Link) Salino & T.E.Almeida, 2010 (DUKE); Testo 1442, *Steiropteris* sp., Chocó, Colombia, 2017 (VT).

Strophocaulon S.E.Fawc. & A.R.Sm.—Fawcett 697, *Strophocaulon invisum* (G.Forst.) S.E.Fawc. & A.R.Sm., Taveuni, Fiji, 2016 (UC 2049790, VT.); Kluge 7791, *Strophocaulon unitum* (L.) S.E.Fawc. &

A.R.Sm., Singani, Grand Comores, 2008 (UC 1924777); *Fawcett* 641, *Strophocaulon unitum* (L.) S.E.Fawc. & A.R.Sm., Viti Levu, Fiji, 2016 (UC 2049788, VT).

Thelypteris **Schmidel**—*Rothfels* 4667, *Thelypteris confluens* (Thunb.) C.V.Morton, Kwazulu-Natal, South Africa, 2015 (UC); *Fawcett* 569, *Thelypteris palustris* Schott, Michigan, U.S.A., 2016 (UC 2049130, VT);

Arsene 1, *Thelypteris palustris* Schott, Michoacán, Mexico, 1910 (UC 477810); *Testo* 1645, *Thelypteris palustris* Schott, Florida, U.S.A., 2019 (FLAS).

Trigonospora **Holtum**—*Ballard* 1393, *Trigonospora obtusiloba* Sledge, Hedigala, Sri Lanka, 1951 (UC 1948787); *Ballard* 1522, *Trigonospora zeylanica* (Ching) Sledge, Sri Lanka, 1951 (UC 194876).