Plant Diversity 47 (2025) 34–52

Contents lists available at ScienceDirect

Plant Diversity

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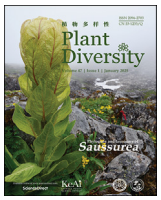
http://journal.kib.ac.cn



Research paper

A revised classification of Dryopteridaceae based on plastome phylogenomics and morphological evidence, with the description of a new genus, *Pseudarachniodes*

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ARTICLE INFO

Article history:
Received 17 September 2023
Received in revised form
24 July 2024
Accepted 25 July 2024
Available online 30 July 2024

Keywords:
Ctenitidoideae
Infra-familial
Morphological diagnostic characters
Phylogenomics
Rachis-costae architecture
Stigmatopetris

ABSTRACT

Dryopteridaceae are the largest fern family and include nearly 20% of extant fern diversity, with 24 currently recognized genera. Recognition and delineation of genera within this family have varied greatly. The three-subfamily classification of Dryopteridaceae was based primarily on molecular phylogenetic relationships but lacked morphological evidence, and the phylogenetic relationships of the subfamilies and genera of Dryopteridaceae are only partially resolved. A comprehensive and robust phylogeny is urgently needed. The heterogeneous morphology of the current members of Dryopteridaceae makes the family and its subfamilies difficult to define by single morphological characteristics or even character combinations. We carried out phylogenetic analyses to reconstruct a highly supported phylogeny of Dryopteridaceae. Our analyses recovered 24 strongly supported clades grouped into seven major clades of Dryopteridaceae. Seven morphological characters including habit, rhizome shape, frond morphology, rachis-costae architecture, appendages on stipe base and lamina, and soral arrangement were found to be informative for identifying different major clades and clades in Dryopteridaceae. Based on phylogenetic reconstruction and morphological analysis, we presented an updated infra-familial classification of Dryopteridaceae with seven subfamilies and 24 genera including four newly proposed subfamilies (Ctenitidoideae, Lastreopsidoideae, Pleocnemioideae, and Polystichopsidoideae). Morphological character combinations of each subfamily are summarized, and a key is provided. Most genera sensu PPG I are recognized, with *Stigmatopetris* reclassified into Dryopteridoideae and *Arthrobotrya* considered a synonym of *Teratophyllum*. A new genus *Pseudarachniodes* is introduced. This revised classification will serve as a foundational framework for future investigations on taxonomy, biogeography, and diversification of the most species-rich Dryopteridaceae in ferns.

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Peer review under the responsibility of Editorial Office of Plant Diversity.

https://doi.org/10.1016/j.pld.2024.07.010

1. Introduction

Lycophytes and ferns are two distinct evolutionary lineages of free-sporing vascular plants, encompassing approximately 12,000 species (PPG I, 2016). Of these plants, the shield-fern family Dryopteridaceae is the largest fern family, containing slightly more than 2100 species (Zhang et al., 2013a; PPG I, 2016). With a nearly worldwide distribution, Dryopteridaceae exhibit their highest diversity in temperate regions and tropical mountainous areas, particularly in East Asia (*Dryopteris* Adans., *Polystichum* Roth) and the New World (*Elaphoglossum* Schott ex J. Sm.) (Zhang et al., 2013a). Dryopteridaceae can be found from coastal areas to alpine tree line. Most species are terrestrial or rupestral, although some tropical taxa are root climbers (*e.g.*, *Maxonia* C. Chr., *Polybotrya* Humb. & Bonpl. ex Willd., and *Cyclodium* C. Presl; Moran et al., 2010a, b; Moran and Labiak, 2015) or hemiepiphytes (*e.g.*, one species of *Elaphoglossum*, a few species of *Bolbitis* Schott, and almost all species in *Arthrobotrya* J. Sm., *Mickelia* R.C. Moran, Labiak & Sundue, *Lomagramma* J. Sm., and *Teratophyllum* Mett. ex Kuhn) (Moran et al., 2010a; Lagomarsino et al., 2012; Nie et al., 2023).

Throughout the taxonomic history of ferns, the circumscriptions of families and genera have been highly dynamic. Over the past two centuries, numerous classifications have been proposed, reflecting different interpretations of the available evidence. Although Dryopteridaceae share some common morphological features (stipes with three or more vascular bundles arranged in a semicircle or circle, scales on rhizome and stipe, and chromosome numbers $x = 41$) with most families of the Eupolypod I (Polypodiineae), the family has been defined differently by different pteridologists due to the large difference in habit, shape of rhizome and frond, venation, and soral arrangement among different genera (Tryon and Tryon, 1982; Kramer et al., 1990; Zhang et al., 2013a) (Fig. 1). Because of extremely diverse morphology, the taxonomic position of dryopteridoid ferns has changed several times (Table 1). Most dryopteridoid ferns were historically placed in Polypodiaceae (Presl, 1836; Hooker and Baker, 1868), Aspidiaceae (*nom. illeg.* = Tectariaceae) (Christensen, 1906; Ching, 1940; Copeland, 1947; Alston, 1956; Pichi-Sermolli, 1958, 1977), or Dennstaedtiaceae (Holttum, 1947). Until the mid-19th century, Dryopteridaceae were gradually recognized and accepted (Herter, 1949; Ching, 1965a; Nayar, 1970; Pichi-Sermolli, 1970).

The circumscription of Dryopteridaceae has also changed over time. Pteridologists proposed multiple classifications of Dryopteridaceae, with varying numbers of genera accepted (Pichi-Sermolli, 1977; Ching, 1978; Tryon and Tryon, 1982; Kramer et al., 1990; Wu and Ching, 1991) (Table 1). Dryopteridaceae were divided into two subfamilies (Dryopteridoideae and Athyrioideae), five tribes, and 45 genera in the most widely accepted morphology-based classification (Kramer et al., 1990), of which three tribes (Tectarieae, Physematieae, and Onocleeae) were dealt as separate families based on molecular and morphological evidence in subsequent classifications (*e.g.*, PPG I, 2016). Smith et al. (2006), largely based on available molecular evidence at the time, included 40–45 genera within Dryopteridaceae without recognition of any subfamilies. The three genera tentatively placed in Dryopteridaceae by Smith et al. (2006) were later assigned to the other two families, respectively, *i.e.*, *Didymochlaena* Desv. in Didymochlaenaceae, *Hypodematium* A. Rich. and *Leucostegia* C. Presl in Hypodematiaceae (Zhang and Zhang, 2015). Christenhusz et al. (2011) included 34 genera in Dryopteridaceae and divided this family into two subfamilies—Dryopteridoideae B.K. Nayar and Elaphoglossoideae (Pic. Serm.) Crabbe, Jermy & Mickel, and then Zhang et al. (2013b) included 25 genera in two subfamilies of Dryopteridaceae. The circumscription of Dryopteridaceae had been confusing until recent phylogenetic studies which substantially revised it (Table 2).

The monophyly of several global large genera (more than 100 species, *e.g.*, *Ctenitis* (C. Chr.) C. Chr., *Dryopteris*, *Elaphoglossum*, and *Polystichum*) or medium genera (about 50–90 species, *e.g.*, *Bolbitis* and *Megalastrum* Holttum) has been identified by molecular phylogenetic studies (Moran et al., 2010a; Zhang et al., 2012; Le Péchon et al., 2016a, 2016b; Hennequin et al., 2017; Nie et al., 2023). Most of the small segregate genera were found to be nested within some larger genera which are closely related and are nowadays synonyms of these genera. For example, *Leptorumohra* H. Itô, *Lithostegia* Ching, and *Phanerophlebiopsis* Ching were nested in *Arachniodes* Blume (Li and Lu, 2006a; Liu et al., 2007c; He et al., 2013; Lu et al., 2019); *Ataxipteris* Holttum and *Pseudotectaria* Tardieu were nested in *Ctenitis* (Dong and Christenhusz, 2013; Wang et al., 2014; Hennequin et al., 2017); *Acrophorus* C. Presl, *Acrorumohra* (H. Itô) H. Itô, *Diacalpe* Blume, *Dryopsis* Holttum & P.J. Edwards, *Nothoperanema* (Tagawa) Ching, *Peranema* D. Don, *Revwattsia* D.L. Jones, and *Stenolepia* Alderw. were nested in *Dryopteris* (Li and Lu, 2006a, 2006b; Liu et al., 2007a; Ebihara, 2011; McKeown et al., 2012; Zhang, 2012; Zhang and Zhang 2012; Zhang et al., 2012; Kuo et al., 2016); *Cyrtogonellum* Ching, *Cyrtomidictyum* Ching, and *Sorolepidium* Christ were nested in *Polystichum* (Little and Barrington, 2003; Liu et al., 2007b; Lu et al., 2007; Li et al., 2008; Zhang et al., 2013a; Le Péchon et al., 2016a, 2016b), *Coveniella* Tindale was nested in *Lastreopsis* Ching (Labiak et al., 2014, 2015); and *Adenoderris* J. Sm. was found to be polyphyletic and its taxa were then treated in either *Dryopteris* or *Polystichum* (McHenry et al., 2013). On the other hand, several genera, *e.g.*, *Mickelia*, *Polystichopsis* (J. Sm.) C. Chr., *Parapolystichum* (Keyserl.) Ching, and *Trichoneuron* Ching were newly recognized or reinstated (Moran and Prado, 2010; Moran et al., 2010b; Labiak et al., 2014, 2015; Liu et al., 2016).

Numerous phylogenetic studies have attempted to elucidate the infra-familial relationships of Dryopteridaceae (Li and Lu, 2006a; Liu et al., 2007c, 2016) as well as the phylogenetic relationships of different lineages (Moran et al., 2010a; Zhang et al., 2012; Labiak et al., 2014; Moran and Labiak, 2015; Le Péchon et al., 2016b; Hennequin et al., 2017). Among these studies, Liu et al. (2016) implemented the comprehensive phylogenetic study of Dryopteridaceae with the most extensive generic representatives, recognized three main clades and two subclades within the family, and proposed a three-subfamily (Dryopteridoideae, Elaphoglossoideae, and Polybotryoideae Hong M. Liu & X.C. Zhang) classification. However, there are still several remaining issues that need to be further resolved. Firstly, the phylogenetic relationships of the three subfamilies of Dryopteridaceae were not fully resolved; secondly, the placement of *Ctenitis* and *Stigmatopteris* C. Chr. was only tentatively settled; and thirdly, several small genera were not sampled in their study (Liu et al., 2016).

Twenty-six genera were recognized in Dryopteridaceae in the currently most widely accepted PPG I (2016) classification. In PPG I (2016) classification, Bolbitidaceae, Elaphoglossaceae, and Peranemataceae were treated as synonyms of Dryopteridaceae, and some genera assigned earlier in Lomariopsidaceae (*e.g.*, *Lomagramma*) and Tectariaceae (*e.g.*, *Ctenitis* and *Pleocnemia* C. Presl) were moved in Dryopteridaceae (PPG I, 2016). PPG I (2016) recognized three subfamilies in Dryopteridaceae, primarily followed Liu et al. (2016) (Table 2). Given the unresolved relationships of *Ctenitis* and *Stigmatopteris*, PPG I (2016) tentatively placed these two genera in Dryopteridoideae and Polybotryoideae, respectively. Furthermore, the relationships between *Arthrobotrya* and *Teratophyllum* were unsettled (Moran et al., 2010a; Liu et al., 2016). *Aenigmopteris* Holttum and *Dryopolystichum* Copel., not placed in any subfamilies

Fig. 1. Photographs of representative Dryopteridaceae taxa. A, *Elaphoglossum yoshinagae* (Yatabe) Makino; B, *Bolbitis subcordata* (Copel.) Ching; C, *Olfersia cervina* (L.) Kunze; D, *Lomagramma sorbifolia* (Willd.) Ching; E, *Polybotrya altescandens* C. Chr.; F, *Stigmatopteris heterophlebia* (Baker) R.C. Moran; G, *Cyrtomium fortunei* J. Sm.; H, *Dryopteris wallichiana* (Spreng.) Hyl.; I, *Ctenitis decurrenti-pinnata* (Ching) Tardieu & C. Chr.; J, *Lastreopsis subrecedens* Ching; K, *Trichoneuron microlepioides* Ching; L, *Pleocnemia winitii* Holttum. Photo credit: C, E, & F: R. C. Moran; J: S-Y Dong; the remaining: Z-Y Zuo.

of Dryopteridaceae by PPG I (2016), were later shown to be members of Tectariaceae and Lomariopsidaceae, respectively (Chen et al., 2017a, 2017b).

The three-subfamily classification of Dryopteridaceae is only based on molecular phylogeny. The heterogeneous morphology of the current members of Dryopteridaceae makes the family and its subfamilies difficult even to define by morphological character combinations. Various states of morphological characters generally used to identify different fern lineages (families), such as habit, rhizome shape, frond morphology, rachis-costa architecture, and sorus shape, can be observed in Dryopteridaceae (Smith et al., 2006). For example, terrestrial/epiphytic habit, erect/long-creeping rhizome, monomorphic/dimorphic fronds (with different sterile and fertile fronds), simple/1- or more pinnate lamina division, and discrete/acrostichoid sori can be found in Elaphoglossoideae and Polybotryoideae. More comprehensive morphological examination is needed to better understand and circumscribe the subfamilies and genera of Dryopteridaceae.

To determine the phylogenetic placement of *Ctenitis* and *Stigmatopteris* and establish an "practical" infra-familial classification of Dryopteridaceae supported by a robust phylogenetic framework and morphological evidence, a phylogeny of Dryopteridaceae was reconstructed using complete chloroplast genomes and morphological characteristics were examined and coded for each genus. Based on phylogenetic relationships and optimizations of morphological characters on the resulting trees, we updated a more informative infra-familial classification for Dryopteridaceae here.

2. Material and methods

2.1. Taxon sampling, DNA extraction, sequencing, and plastome assembly

A total of 94 plastomes from 86 species, representing all 24 recognized genera of Dryopteridaceae (Liu et al., 2016; PPG I, 2016), and three outgroups were gathered (Du et al., 2021), among which 53 plastomes were newly generated and 41 plastomes accessed via GenBank (Table S1). Total genomic DNA of silica-gel-dried leaf materials was extracted using a modified CTAB procedure (Doyle and Doyle, 1990), and DNA extraction of specimens was followed the protocol of Zeng et al. (2018). Library preparation was conducted at the Germplasm Bank of Wild Species, Kunming Institute of Botany (CAS, China), and Illumina sequencing was conducted at BGI Genomics Co., Ltd (Shenzhen, China). More than 2 Gb of clean data of 150 bp pair-end reads were generated for each sample. *De-novo* assemblies were constructed using GetOrganelle v.1.7.0 (Jin et al., 2020), and connection and annotation were subsequently conducted using Bandage 0.8.1 (Wick et al., 2015) and Geneious 8.0.2 (Kearse et al., 2012). Collinearity among the plastomes was evaluated through the progressiveMauve algorithm and default

Table 1
Historical treatment of genera of Dryopteridaceae (DR.) in the morphology-based classifications.

Genera in PPG I[a]	Ching (1978); Wu and Ching (1991)	Pichi-Sermolli (1977)	Tryon and Tryon (1982)	Kramer et al. (1990)
Subfamily Dryopteridoideae				
Arachniodes	DR.	Aspidiaceae	DR. (Tribe Dryopterideae, = *Dryopteris*)	DR. (Subfamily Dryopteridoideae, Tribe Dryopterideae)
Dryopteris	DR.	Aspidiaceae	DR. (Tribe Dryopterideae)	DR. (Subfamily Dryopteridoideae, Tribe Dryopterideae)
Polystichum	DR.	Aspidiaceae	DR. (Tribe Dryopterideae)	DR. (Subfamily Dryopteridoideae, Tribe Dryopterideae)
Cyrtomium	DR.	Aspidiaceae	DR. (Tribe Dryopterideae)	DR. (Subfamily Dryopteridoideae, Tribe Dryopterideae, =*Polystichum*)
Phanerophlebia	DR.	Aspidiaceae	DR. (Tribe Dryopterideae, =*Cyrtomium*)	DR. (Subfamily Dryopteridoideae, Tribe Dryopterideae, =*Polystichum*)
Ctenitis	Aspidiaceae	Aspidiaceae	DR. (Tribe Dryopterideae)	DR. (Subfamily Dryopteridoideae, Tribe Tectarieae)
Subfamily Polybotryoideae				
Polybotrya	/	Aspidiaceae	DR. (Tribe Dryopterideae)	DR. (Subfamily Dryopteridoideae, Tribe Dryopterideae)
Cyclodium	/	Aspidiaceae	DR. (Tribe Dryopterideae, =*Stigmatopteris*)	DR. (Subfamily Dryopteridoideae, Tribe Dryopterideae)
Maxonia	/	Aspidiaceae	DR. (Tribe Dryopterideae)	DR. (Subfamily Dryopteridoideae, Tribe Dryopterideae)
Olfersia	/	Aspidiaceae	DR. (Tribe Dryopterideae, =*Polybotrya*)	DR. (Subfamily Dryopteridoideae, Tribe Dryopterideae)
Trichoneuron	Thelypteridaceae	Athyriaceae	DR. (Tribe Physematieae)	/
Polystichopsis	DR. (*Arachniodes*)	Aspidiaceae	DR. (Tribe Dryopterideae, = *Dryopteris*)	DR. (Subfamily Dryopteridoideae, Tribe Dryopterideae, =*Arachniodes*)
Stigmatopteris	/	Aspidiaceae	DR. (Tribe Dryopterideae)	DR. (Subfamily Dryopteridoideae, Tribe Dryopterideae)
Subfamily Elaphoglossoideae				
Bolbitis	Bolbitidaceae	Lomariopsidaceae	DR. (Tribe Bolbitideae)	Lomariopsidaceae
Elaphoglossum	Elaphoglossaceae	Elaphoglossaceae	DR. (Tribe Bolbitideae)	Lomariopsidaceae
Lomagramma	Lomariopsidaceae	Lomariopsidaceae	DR. (Tribe Bolbitideae)	Lomariopsidaceae
Teratophyllum	/	Lomariopsidaceae	DR. (Tribe Bolbitideae)	Lomariopsidaceae
Mickelia	/	/	/	/
Pleocnemia	Aspidiaceae	Aspidiaceae	DR. (Tribe Dryopterideae)	DR. (Subfamily Dryopteridoideae, Tribe Tectarieae)
Megalastrum	/	/	/	/
Rumohra	DR. (*Arachniodes*)	Aspidiaceae	DR. (Tribe Dryopterideae)	DR. (Subfamily Dryopteridoideae, Tribe Rumohreae)
Lastreopsis	Aspidiaceae	Aspidiaceae	DR. (Tribe Dryopterideae)	DR. (Subfamily Dryopteridoideae, Tribe Tectarieae)
Parapolystichum	Aspidiaceae (*Lastreopsis*)	Aspidiaceae (=*Lastreopsis*)	DR. (Tribe Dryopterideae, =*Lastreopsis*)	DR. (Subfamily Dryopteridoideae, Tribe Tectarieae, =*Lastreopsis*)

[a] *Aenigmopteris* Holttum and *Dryopolystichum* Copel. are not included in the current classification of Dryopteridaceae based on recent molecular systematic studies.

parameters in Geneious 8.0.2 (Darling et al., 2010). All the sequences were aligned using MAFFT v.7 (Katoh and Standley, 2013).

2.2. Data matrices and phylogenetic analyses

We implemented phylogenetic analyses using six matrices (Table S5). Firstly, we constructed two matrices consisting of all 94 complete plastid genomes by half gaps deletion (Matrix 1) or all gaps deletion (Matrix 2) with Gblocks (Talavera and Castresana, 2007). Secondly, we constructed two gene matrices with 115 gene sequences (Matrix 3), and 85 coding-gene sequences (CDS) + four rRNA genes (Matrix 4). Thirdly, the CDS sequences of 85 protein-coding genes were translated into amino acid (AA) sequences. Furthermore, three plastid regions (*rbcL*, *rps4-trnS*, and *trnL-F*) of 61 Dryopteridaceae taxa were downloaded from NCBI (Table S2), and added to Matrix 1 as Matrix 6.

All matrices were analyzed using Maximum Likelihood (ML) analysis, Maximum Parsimony (MP), and Bayesian Inference (BI).

Table 2
Concept of subfamilies and genera of the family Dryopteridaceae.

Genera	Smith et al. (2006)	Christenhusz et al. (2011)	Zhang et al. (2013a, b)	Liu et al. (2016)	PPG I (2016)	This study
Arachniodes	Dryopteridaceae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Dryopterioideae
Leptorumohra	/	Dryopterioideae	/	/	/	/
Lithostegia	Dryopteridaceae	Dryopterioideae	/	/	/	/
Cyrtomium	Dryopteridaceae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Dryopterioideae
Dryopteris	Dryopteridaceae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Dryopterioideae
Acrophorus	Dryopteridaceae	Dryopterioideae	/	/	/	/
Acrorumohra	Dryopteridaceae	Dryopterioideae	/	/	/	/
Diacalpe	/	Dryopterioideae	/	/	/	/
Dryopsis	Dryopteridaceae	Dryopterioideae	/	/	/	/
Peranema	Dryopteridaceae	Dryopterioideae	/	/	/	/
Revwattsia	Dryopteridaceae	(uncertain placement)	/	/	/	/
Stenolepia	Dryopteridaceae	(uncertain placement)	Dryopterioideae	Dryopterioideae	/	/
Phanerophlebia	Dryopteridaceae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Dryopterioideae
Polystichum	Dryopteridaceae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Dryopterioideae
Adenoderris	Dryopteridaceae	(uncertain placement)	Dryopterioideae	/	/	/
Cyrtogonellum	Dryopteridaceae	Dryopterioideae	/	/	/	/
Cyrtomidictyum	Dryopteridaceae	Dryopterioideae	/	/	/	/
Ctenitis	Dryopteridaceae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Dryopterioideae	Ctenitidoideae
Ataxipteris	Dryopteridaceae	/	/	/	/	/
Polystichopsis	Dryopteridaceae	Dryopterioideae	Dryopterioideae	Polybotryoideae	Polybotryoideae	Polystichopsidoideae
Trichoneuron	Dryopteridaceae	/	/	Polybotryoideae	Polybotryoideae	Polystichopsidoideae
Stigmatopteris	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Polybotryoideae	Polybotryoideae	Dryopterioideae
Cyclodium	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Polybotryoideae	Polybotryoideae	Polybotryoideae
Maxonia	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Polybotryoideae	Polybotryoideae	Polybotryoideae
Olfersia	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Polybotryoideae	Polybotryoideae	Polybotryoideae
Polybotrya	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Polybotryoideae	Polybotryoideae	Polybotryoideae
Pleocnemia	Tectariaceae	Tectariaceae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Pleocnemioideae
Lastreopsis	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Lastreopsidoideae
Coveniella	Dryopteridaceae	(uncertain placement)	/	/	/	/
Megalastrum	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Lastreopsidoideae
Rumohra	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Lastreopsidoideae
Parapolystichum	/	/	/	Elapholgossoideae	Elapholgossoideae	Lastreopsidoideae
Bolbitis	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae
Elaphoglossum	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae
Mickelia	/	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae
Lomagramma	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae
Teratophyllum	Dryopteridaceae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae
Arthrobotrya	/	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	Elapholgossoideae	/

"/": Not mentioned or merged in other genera.
Accepted genera in this study are shown in bold.

ML analyses were conducted using IQ-TREE v.1.6.12 (Nguyen et al., 2015) with 10,000 ultrafast bootstrap replicates. The MP analyses were implemented in PAUP* 4.0d100 (Swofford, 2002). All characteristics were weighted equally and gaps were treated as missing data. 1000 replicates were performed with 10 tree-bisection-reconnection (TBR) searches per replicate and a maximum of 100 trees held per TBR search. MP Bootstrap support for nodes was estimated with 1000 heuristic replicates and tree bisection–reconnection branch swapping. BI analyses were conducted with MrBayes 3.2.6 (Ronquist et al., 2012), using ten million generations with one tree sampled every 1000 generations; four runs with four chains were performed in parallel. The first 25% of trees were discarded as burn-in. The Markov Chain Monte Carlo (MCMC) output was examined to check for convergence and to ensure that all the effective sample size (ESS) values were above 200 in TRACER v.1.7 (Rambaut et al., 2018).

2.3. Morphological characters coding and ancestral state reconstruction

We conducted field observation of the living plants of Dryopteridaceae, examined specimens deposited in the herbaria of BM, CHS, IBSC, K, KUN, MO, P, PE, PYU, TI, and TNS, and plant pictures on the websites (*e.g.*, GBIF: https://www.gbif.org/; CVH: https://www.cvh.ac.cn/). Firstly, we made a statistical observation of the morphological features used to identify different genera of Dryopteridaceae (*e.g.*, rhizome shape, fronds morphology, shape of rachis and costae, scales, sori and indusia) in previous studies (Pichi-Sermolli, 1977; Ching, 1978; Tryon and Tryon, 1982; Kramer et al., 1990; Wu and Ching, 1991). In order to further understand the relationship between the morphology-based classifications and molecular phylogeny of Dryopteridaceae, we further checked more morphological features of lamina division, and other appendages (hairs and glands) on costa and lamina. A total of 25 morphological features were preliminarily analyzed and 13 morphological characters (Tables S3 and S4) were finally mapped on the phylogeny tree and analyzed using RASP v.4.2 (Yu et al., 2020).

2.4. Distribution and historical biogeography

The ancestral range reconstruction of Dryopteridaceae was conducted using RASP 20200510 (Yu et al., 2020) based on the phylogenetic tree generated by Matrix 6 using the dispersal extinction-cladogenesis model (DEC; Ree and Smith, 2008). We recognized four biogeographic areas: A, Eurasia; B, Americas; C, Africa and Indian Ocean Islands; D, Australasia and Tropical Asia.

2.5. Estimation of divergence times

We estimated the divergence times of major lineages of Dryopteridaceae based on Matrix 1. One secondary molecular calibration and one fossil record were applied in divergence time analyses. The secondary calibration point was derived from earlier study of divergence times of Dryopteridaceae (128.97 (112.63–145.81)

million years ago (Ma)) (Du et al., 2021). The fertile leave fossil of *Elaphoglossum miocenicum* Lóriga, A.R. Schmidt, R.C. Moran, K. Feldberg, H. Schneid. & Heinrichs (Lóriga et al., 2014) (15.97 Ma) was utilized to calibrate the crown of *Elaphoglossum*.

The estimation of divergence times was carried out using Bayesian methods with BEAST v.2.6.3 (Bouckaert et al., 2014), using the GTR + G + I nucleotide substitution model, birth-death tree prior, and lognormal uncorrelated relaxed clock model. The stem of Dryopteridaceae was assigned as 128.97 Ma with a uniform prior and 112.63–145.81 Ma as the minimum–maximum age constraints. The fossil age of 15.97 Ma was incorporated as an "offset" in a lognormal distribution, with a "Mean" value of 3.0 relative to the fossil age and a "Standard deviation" of 1.0. One run consisting of two billion generations with sampling every 10,000 generations was conducted. Convergence was achieved within one billion generations, with Effective Sample Size (ESS) values exceeding 200 for all parameters. The initial 200 million generations were discarded as burn-in, and the subsequent 80,000 trees were used to construct the maximum clade credibility (MCC) tree employing TreeAnnotator v.2.6.3 (utilizing mean node heights) (Bouckaert et al., 2014).

3. Results

3.1. Characteristics of plastomes in Dryopteridaceae

A total of 53 complete plastome sequences of Dryopteridaceae were newly generated in this study (Table S1). The size of the plastomes ranged from 147,271 bp [*Arachniodes hainanensis* (Ching) Ching] to 166,133bp [*Elaphoglossum yoshinagae* (Yatabe) Makino]. The GC content of each genus was similar, with the differences of less than 1% (Table S1). The GC content of novel plastomes ranged from 40.0% [*Elaphoglossum yunnanense* (Baker) C. Chr.] to 43.5% [*Arachniodes speciosa* (D. Don) Ching] (Table S1).

The plastome collinearity analysis showed that all Dryopteridaceae plastomes were highly conserved in terms of gene content and gene order, and all had a common subregion without gene inversion (Fig. S1). The inverted repeat (IR) boundary of some *Ctenitis* species [*Ctenitis decurrenti-pinnata* (Ching) Tardieu & C. Chr., *C. rhodolepis* (C.B. Clarke) Ching, and *C. sinii* (Ching) Ohwi] expanded in the LSC direction to include *ndh*B gene. The IR boundary of six genera (*Polybotrya*, *Cyclodium*, *Maxonia*, *Olfersia*, *Trichoneuron*, and *Polystichopsis*) slightly contracted, and while no contraction was found in *Stigmatopteris*.

3.2. Molecular phylogeny

Different analytical strategies were employed to minimize systematic errors, such as removing gaps, using the coding sequences (CDS) or AA sequences, and using different tree inference methods (Table S5). The phylogenetic trees constructed using the whole chloroplast genome (deleting half or all gaps) (Matrices 1, 2, 6) had much higher support values than those constructed with only coding genes or AA (Matrices 3–5) (Fig. S2). The clades within Dryopteridaceae recovered from Matrices 1–5 (Fig. S2) were identical to the 24 clades based on Matrix 6 (Fig. 2). These 24 clades were further grouped into seven larger major clades (A–G). Most of the relationships among the major clades and clades were consistent with those reported in the previous studies (Liu et al., 2016), but were fully or strongly supported (Bayesian inference posterior probabilities [BIPP] = 1.00, maximum likelihood ML ultrafast bootstrap support values [UFBS] > 95%, & maximum parsimony bootstrap support [MPBS] > 95%).

Stigmatopteris was strongly supported as sister to the aggregate of the other six clades of the major clade A (BIPP = 1.00, UFBS = 100%, & MPBS = 97%, Fig. 2). *Arachniodes bella* (C. Chr.) Ching was found fully supported as sister to the polystichoid ferns (including *Cyrtomium* C. Presl, *Phanerophlebia* C. Presl, and *Polystichum*). All analyses resolved Polybotryoideae sensu Liu et al. (2016) into two major clades (B and C) and the *Stigmatopteris* clade. *Cyclodium*, *Maxonia*, *Olfersia*, and *Polybotrya* nested within the major clades B, and while *Polystichopsis* and *Trichoneuron* nested within the major clade C. *Ctenitis* was strongly supported as sister to the major clades B and C (BIPP = 1.00, UFBS = 100%, & MPBS = 97%) (Fig. 2). Elaphoglossoideae sensu Liu et al. (2016) were resolved into three major clades (E–G). The major clade E including five genera (*Bolbitis*, *Elaphoglossum*, *Mickelia*, *Lomagramma*, and *Teratophyllum*) was fully supported as sister to *Pleocnemia* (the major clade F). Together, they formed the sister group of the major clade G, which consisted of four genera (*Lastreopsis*, *Megalastrum* Holttum, *Parapolystichum*, and *Rumohra* Raddi). Two species of *Arthrobotrya, A. articulata* (Fée) J. Sm. and *A. wilkesiana* (Brack.) Copel., were nested within *Teratophyllum*.

3.3. Morphological character evolution

Of the 13 morphological characters (Tables S3 and S4), seven (habit, rhizome shape, frond morphology, rachis-costae architecture, appendages (including hairs, bristle and seta) on stipe base and lamina, and soral arrangement) can be used as diagnostic characters for distinguishing major clades and clades within Dryopteridaceae (Figs. 3–5). Hemi-epiphytic or epiphytic long-creeping rhizome, dimorphic fronds, and acrostichoid sori were exclusively found in the major clades B and E. The hairs from the stipe base to the lamina were synapomorphies unique of the major clade C, which distinguished it from the major clade B. Distinctive ctenitoid hairs occurred only in *Ctenitis* (the major clade D), and while yellow cylindric glands were observed only in the major clade F.

Different rachis-costae architectures were observed in major clades (Fig. 6 and Table 3). There was V-grooved rachises and costae adaxially (referred to as the dryopteridoid type; Fig. 6B) in the major clades A, B, and C. Nearly rounded or flat adaxial leaf axes (referred to as the pleocnemioid type; Fig. 6D) were found in the major clade D and F. A widened groove with a "U"-shaped bottom (known as the lastreopsid type; Fig. 6C) was observed in the major clade G. Another distinctive feature of rachis-costae architecture, the lower stipe had a typical groove (Fig. 6A1), then transformed to rounded to convex, and eventually developed into adaxial ribs on the upper rachis and costae (Fig. 6A2) (referred to as the bolbitidoid type), found exclusively in the major clade E. Other characters, such as lamina division, arrangement of pinnules above suprabasal pinnae, scales, hairs, and glands on costa and lamina, presence or absence of rachis buds, and indusial shape, showed high homoplasy or irregularity.

3.4. Historical biogeography

The ancestral range reconstruction showed that the most probable ancestral area of Dryopteridaceae was Americas (Figs. 3 and 5). Americas had the largest number of genera (17/24) and species in Dryopteridaceae, followed by Eurasia (12), Africa (9), and Australasia (9). The major clade A had a global distribution and the rest were mainly distributed in tropical to subtropical regions. *Arachniodes bella* was endemic to Africa (Madagascar). In addition to the major clade B, long-distance dispersal events across continents and oceans occurred throughout the evolutionary history of major clades A–G.

3.5. Estimation of divergence times

The results of the BEAST analysis revealed that all major clades of Dryopteridaceae have originated in the Cretaceous. The initial

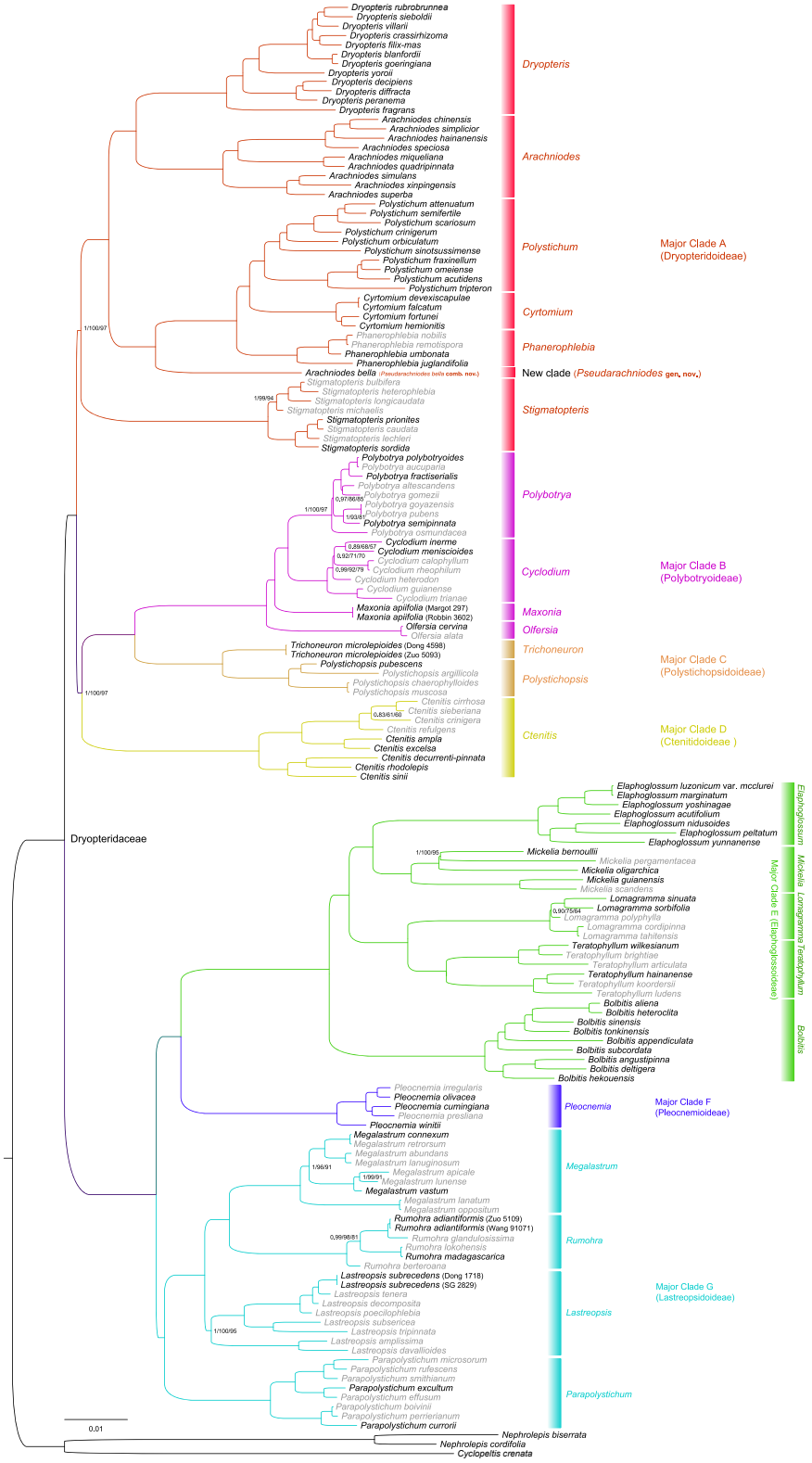

Fig. 2. Phylogeny framework of majority consensus tree of Dryopteridaceae inferred using Maximum Likelihood by IQ-TREE with GTR + R6 model based on the Matrix 6. Numbers of branch are the Bayesian confidence values (BIPP), ML bootstrap support values (UFBS), and MP bootstrap support values (MPBS) in BIPP/UFBS/MPBS order and full support values (1.00/100%/100%) are not marked.

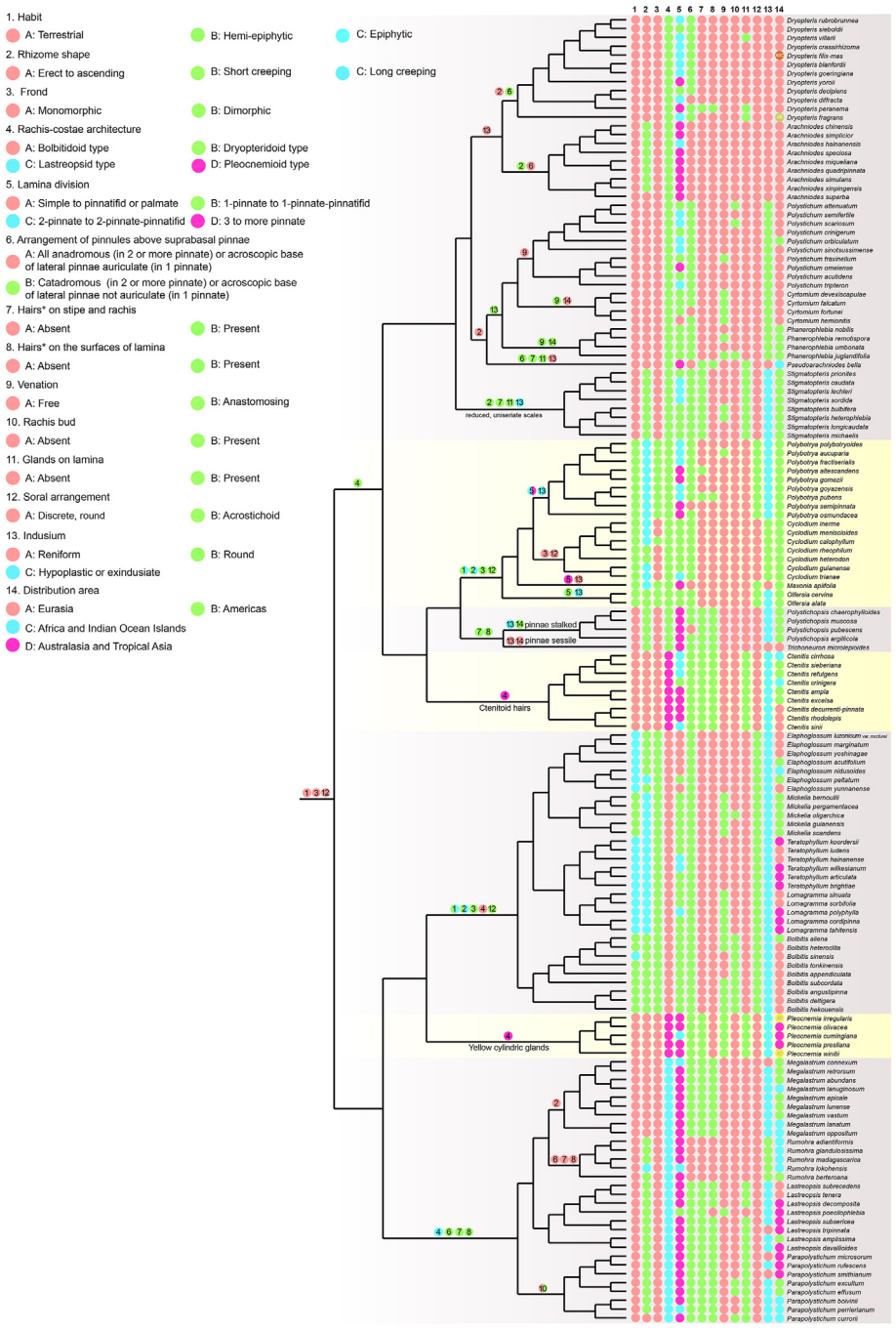

Fig. 3. Ancestral state reconstruction of morphological characters (No. 1–13) and distribution areas (No. 14) in Dryopteridaceae. The ancestral states of the genera/clades were indicated numerically above the node, and the unique autapomorphies were also marked near the node.

diversifications of Dryopteridaceae occurred approximately 104.68 (85.15–126.8) Ma, giving rise to two lineages (major clades A–D, E–G). The stem of major clade A was estimated to have emerged around 98.88 (80.7–120.24) Ma during the Cretaceous (Fig. 7, node 1). The divergence between the major clade D and the major clades B–C occurred at 96.66 (78.8–117.57) Ma (Fig. 7, node 2), and then major clades B and C diverged at 73.43 (54.21–94.33) Ma (Fig. 7, node 3). The divergences between major clade G and the major clades E and F, as well as between major clade E and major clade F, occurred at 87.52 (69.4–108.2) Ma (Fig. 7, node 4) and 81.77 (64.54–104.4) Ma (Fig. 7, node 5), respectively.

4. Discussion

4.1. Characteristics of plastomes and major evolutionary lineages in Dryopteridaceae

We reconstructed a solid and robust phylogenetic backbone of Dryopteridaceae using plastomes, effectively resolving the phylogenetic relationships among seven major clades and all 24 clades, and addressing the insufficient support of some generic relationships in previous study (Liu et al., 2016; Fig. 8). The relationships among all 24 clades and seven major

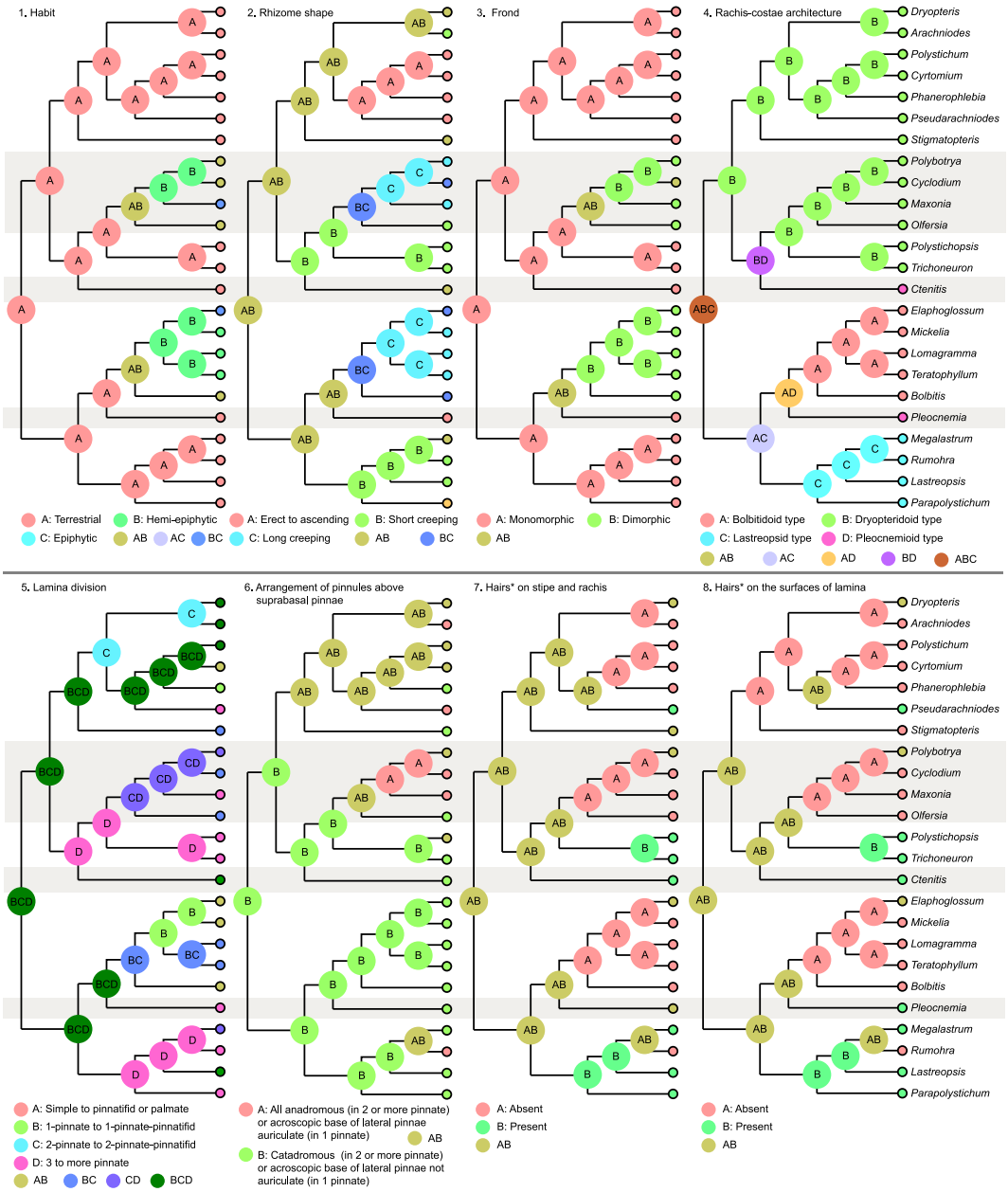

Fig. 4. The framework of inferred ancestral states for eight characters (No. 1–8). Asterisk (*): including bristle and seta.

clades of Dryopteridaceae were fully or strongly supported (Fig. 2).

The phylogenetic relationships of two uncertain subclades *Ctenitis* and *Stigmatopteris* in previous study (Liu et al., 2016) were completely resolved and supported. *Ctenitis* was resolved as sister to all other genera of Polybotryoideae sensu Liu et al. (2016) excluding *Stigmatopteris*. A small range of IR boundary expansion was detected in *Ctenitis*, and while a contraction was found in six genera of Polybotryoideae sensu Liu et al. (2016) (Fig. 8). The major clade A included most genera (except *Ctenitis*) of Dryopteridoideae sensu Liu et al. (2016), with *Stigmatopteris* being resolved as their first-diverging lineage. No IR boundary expansion or contraction was observed in the major clade A (Fig. 8). Furthermore, *Stigmatopteris* shares similar morphological characteristics with species with 1-pinnate-lamina in this major clade. *Arachniodes bella* was found in a separate clade within major clade A, distinct from other species of *Arachniodes*, indicating that it represents a distinct lineage.

4.2. Morphological evolution in Dryopteridaceae

Although Dryopteridaceae were previously classified into three subfamilies (Liu et al., 2016), no morphological synapomorphies were found to define each subfamily. However, each of the major clade identified here is morphologically definable (Fig. 8). The rachis-costae architecture remains consistent in fresh plants of different major clades and can be served as an identifying feature of Dryopteridaceae, although distortion occurred in dried specimens, which can lead to potential confusion.

Genera in major clade A are predominantly terrestrial, characterized by the dryopteridoid rachis and costae, monomorphic fronds,

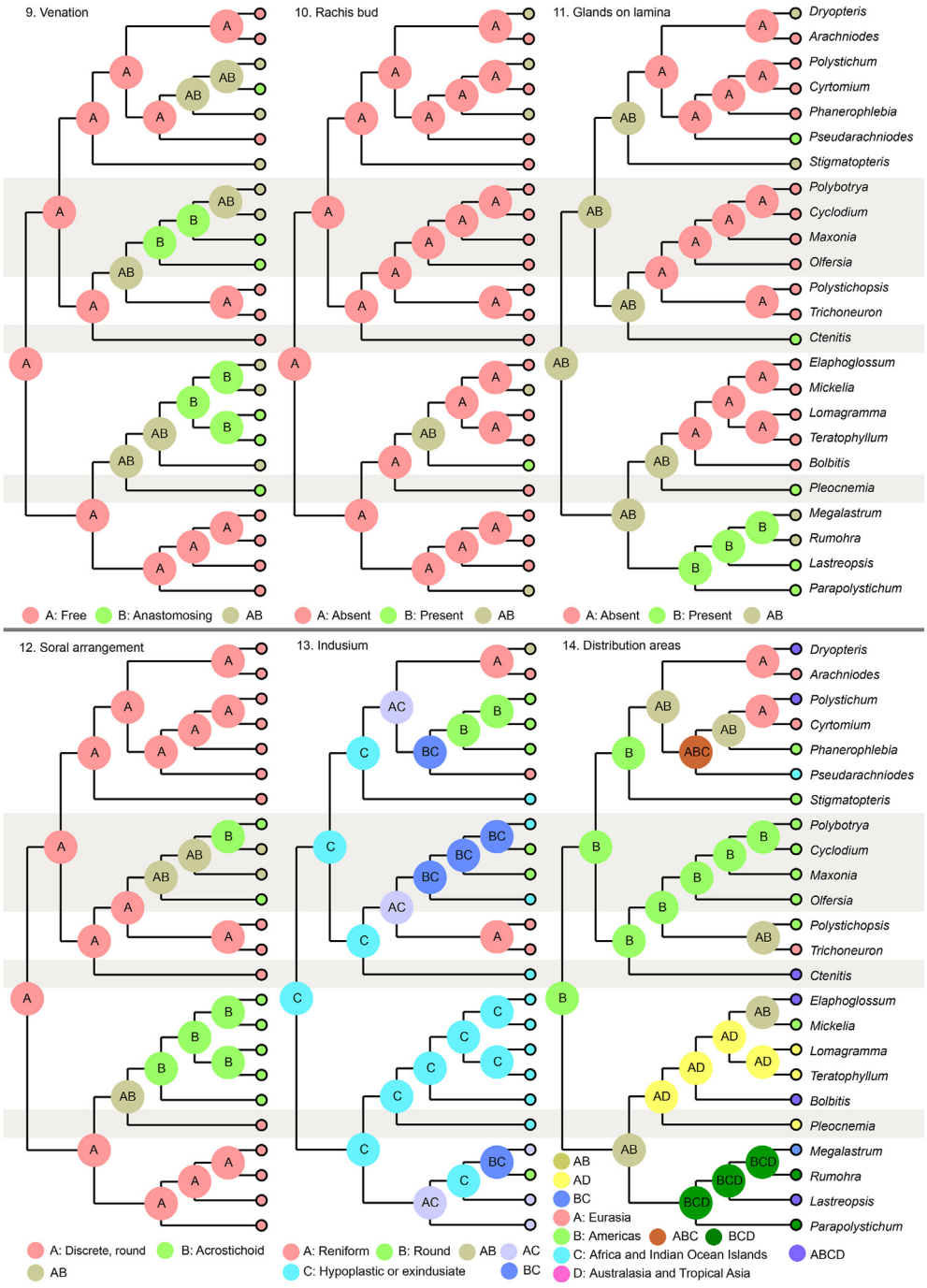

Fig. 5. The framework of inferred ancestral states for five characters (No. 9–13) and distribution areas (No. 14).

and rounded sori (Fig. 8) although a few species of some genera have occasional hairs (including bristles and setae), uniseriate scales, and glands on stipe, rachis, and lamina, anastomosing venation, rachis bud, and exindusiate sori. *Stigmatopteris* and *Arachniodes bella* were included in the major clade A based on well-supported relationships and shared morphological characteristics (Fig. 8). *Ctenitis* (major clade D) has ctenitoid hairs and pleocnemioid rachis-costae type, and was identified as the sister to major clades B and C, necessitating its reclassification outside of Dryopteridoideae.

Both major clades B and C also have dryopteridoid rachis-costae type. However, most genera and species within the major clade B are climbing or hemi-epiphytic, with dimorphic fronds, while species of the major clade C are terrestrial, with monomorphic fronds (Fig. 8). Among the two genera of major clade C, *Polystichopsis* had been merged in *Arachniodes* (Kramer et al., 1990; Wu and Ching, 1991) or *Dryopteris* (Tryon and Tryon, 1982), until recent molecular evidence supported its recognition as an independent genus (Schuettpelz and Pryer, 2007; Prado and Moran, 2015). Morphologically, *Polystichopsis* has pubescent stipe and lamina, which are distinctly from *Arachniodes* and *Dryopteris*, as well as from genera of the major clade B. Prior to the acquisition of molecular sequences, although *Trichoneuron* was placed in Athyriaceae or Thelypteridaceae, it was considered distinctive among all ferns by Ching (1965b) and Pichi-Sermolli (1977). In fact, this Asia-

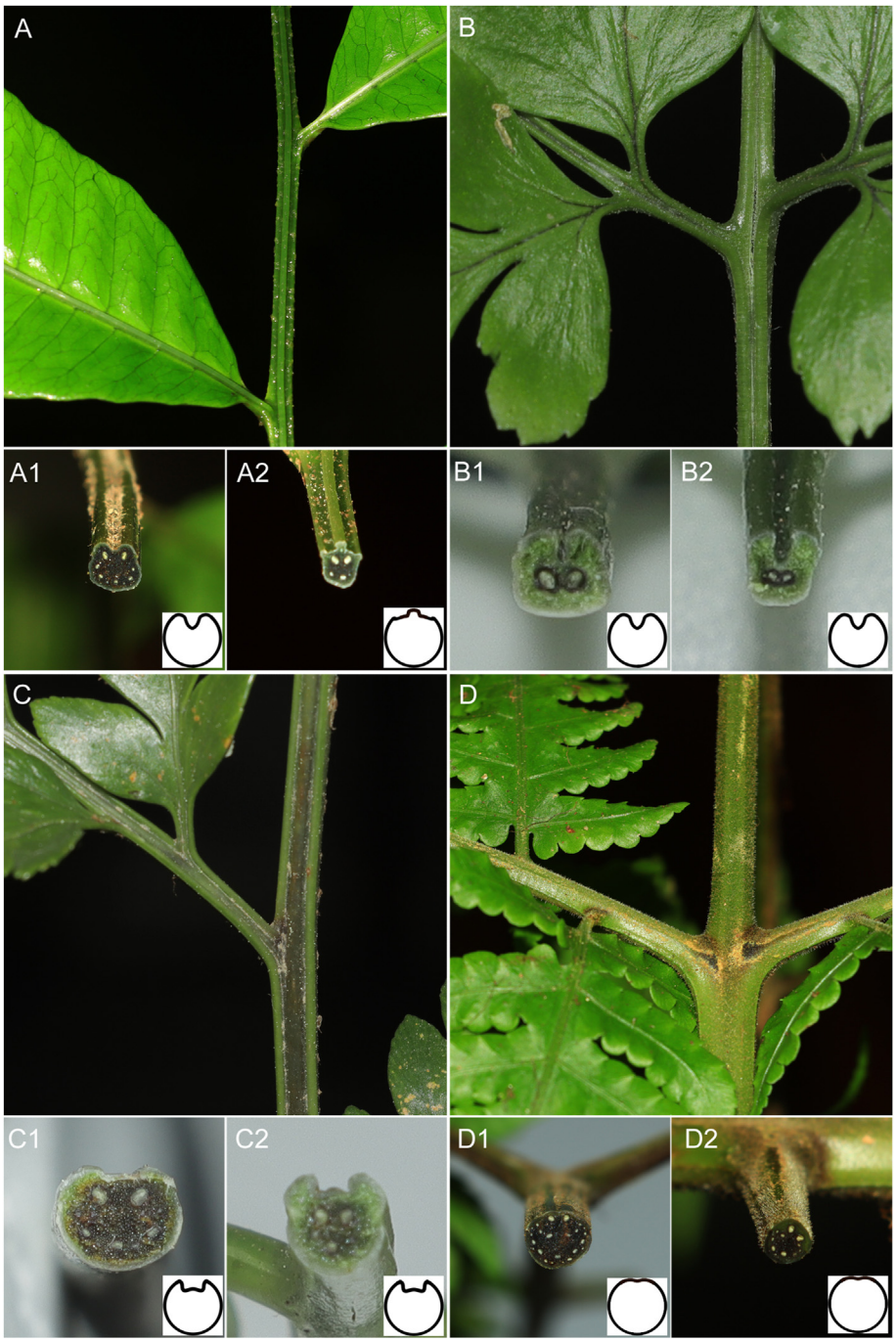

Fig. 6. The shape of stipe (A1, B1, C1, & D1), rachis (in simple to 1-pinnate leaves, A2) and pinna rachis (in 2- to more pinnate leaves, B2, C2, & D2) in cross-section and adaxially. A, Bolbitidoid type [*Bolbitis deltigera* (Bedd.) C. Chr.]; B, Dryopteridoid type [*Dryopteris sparsa* (D. Don) Kuntze]; C, Lastreopsid type [*Rumohra adiantiformis* (G. Forst.) Ching]; D, Pleocnemioid type (*Pleocnemia winitii* Holttum).

endemic genus shares many morphological similarities with *Polystichopsis*, such as terrestrial habitat, monomorphic frond, dryopteridoid rachis-costae type, hairs on stipe and lamina, and rounded sori, but *Trichoneuron* is distinguished by its sessile or subsessile pinnae.

Elaphoglossoideae sensu Liu et al. (2016) were clustered into three major clades (Figs. 2 and S2), which is generally in agreement with previous studies (Labiak et al., 2014; Liu et al., 2016), but with strong support for the relationships among the three major clades. In morphology-based classification, Kramer et al. (1990) placed Elaphoglossoideae within Lomariopsidaceae and the latter two groups (the *Lastreopsis* and allies, and *Pleocnemia*) in the tribes Tectarieae and Rumohreae (*Rumohra*) of Dryopteridaceae. The major clade E encompassed all genera of Bolbitidaceae sensu Tryon and Tryon (1982) and Elaphoglossoideae sensu Pichi-Sermolli (1977) and sensu Ching (1978), as well as *Lomagramma* of Lomariopsidaceae sensu Pichi-Sermolli (1977) and sensu Ching (1978). All sampled species of the major clade E exhibit the bolbitidoid rachis-costae type—ribbed adaxially on upper rachis and costae. The rachis-costae architecture in the major clade F is the lastreopsid type, and that in the major clade G is the pleocnemioid type (Figs. 6 and 8). In addition to

Table 3
Comparison of the types of rachis-costae architecture of Labiak et al. (2014) and this study.

Labiak et al. (2014)		This study	
Types	Identification characteristic	Types	Identification characteristic
Dryopteridoid type	/	Dryopteridoid type	A V-shaped groove on rachises and pinnae rachis adaxially.
Bolbitidoid type	/	Bolbitidoid type	The lower stipe was usually grooved, or occasionally rounded, then turned convex, and finally ribbed adaxially on the upper rachis and costae.
Pleocnemia type	/	Pleocnemioid type	The leaf axes adaxially were nearly round, prominulous or flat.
Lastreopsid type	A thickened marginal ridge of the lamina that is confluent with the sulcus walls of the rachis, and dense pubescence present within the sulcus.	Lastreopsid type	The groove was widened and the bottom was U-shaped (sometimes shallowly).
Megalastrum type	The lamina is decurrent onto the rachis as a narrow wing that is not thickened, and the rachis is not or only hardly sulcate.		

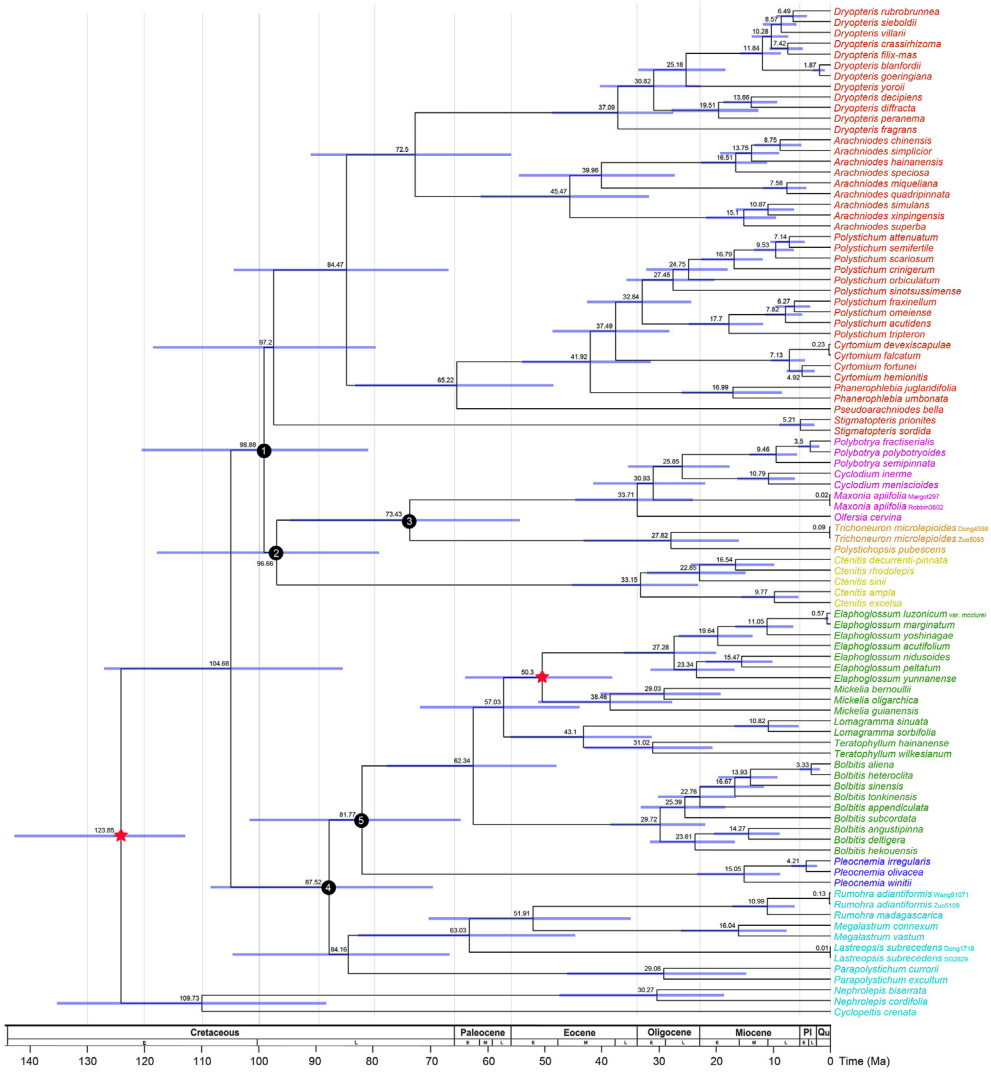

Fig. 7. Time tree for Dryopteridaceae estimated using the 94-taxon dataset. Node bars indicate the 95% HPD, numbers on the bars indicate the mean age (Ma). The calibrated nodes are indicated by red stars, and key nodes are indicated by numbers in circles. Geological timescale and subdivisions: E, Early; L, Late; M, Middle; Pl, Pleistocene; Qu, Quaternary.

differences in rachis-costae architecture, the habit, rhizome shape, frond morphology, soral arrangement, and venation can also be used to distinguish the major clade E, F, and G (Fig. 8). The species of the major clade E are epiphytic, hemi-epiphytic or climbing, mostly with creeping rhizome, dimorphic fronds, and acrostichoid sori, and while those of major clades F and G are terrestrial plants with erect to short-creeping rhizome, monomorphic fronds, and rounded sori. The pleocnemioid rachis-

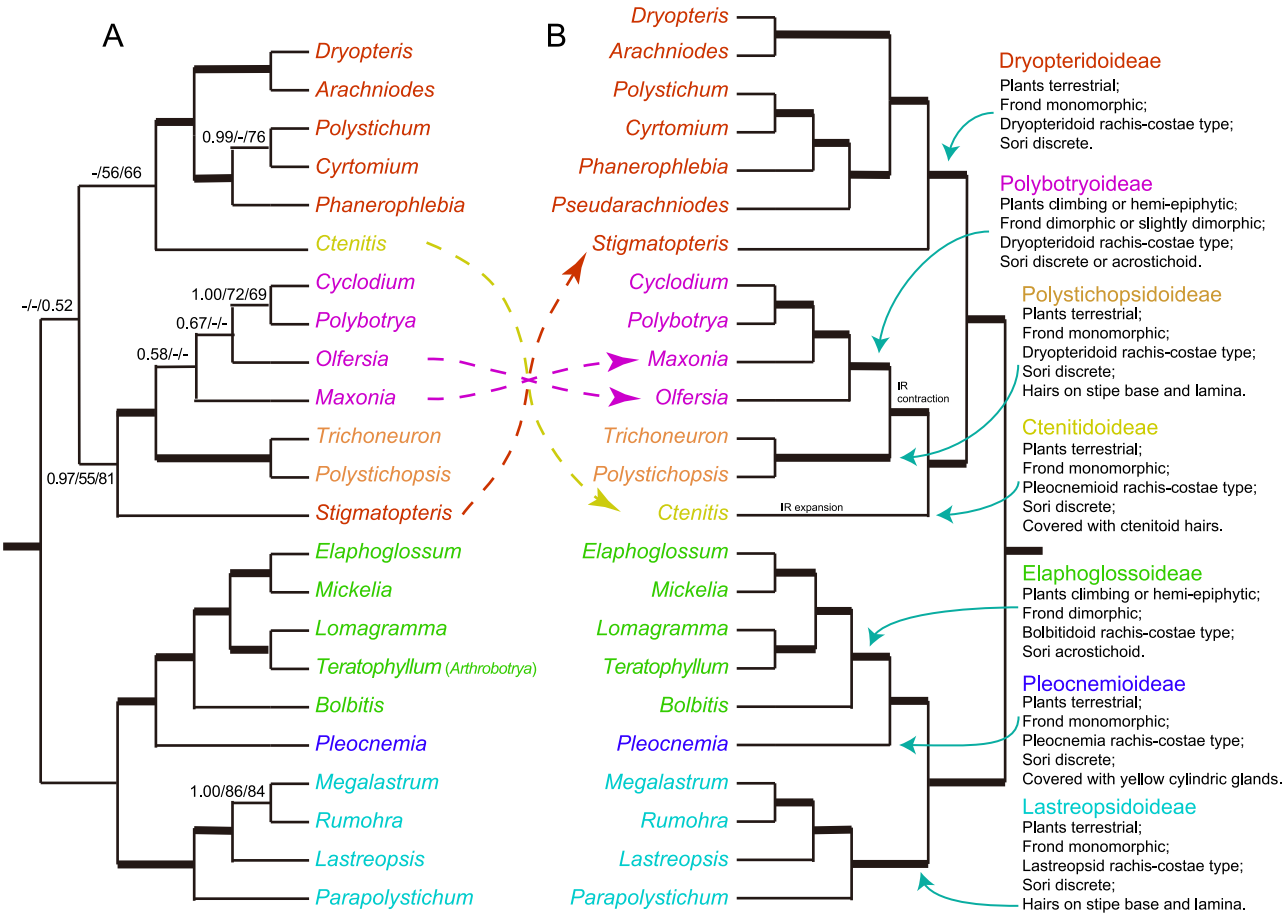

Fig. 8. Schematic trees of Dryopteridaceae at generic level showing differences between Liu et al. (2016) (A) and this study (B). The identification characteristics of subfamilies are marked on the tree (B). Support values of BIPP/UFBS/MPBS were indicated above or below each branch. Thickened branch: the relationship was strongly supported (BIPP > 0.95, MPBS > 90%, UFBS > 90%). A dash '-': BIPP < 0.90, MPBS < 50%, UFBS < 50%.

costae architecture, combined with the yellow cylindric glands, distinguishes *Pleocnemia* as a distinct clade from all other genera of Dryopteridaceae.

One thing to note is that our morphological statistics and evaluations are based on the vast majority of species within each genus, but there may be extreme variations within genera, as observed in cases such as *Dryopteris cochleata* (D. Don) C. Chr. with dimorphic fronds, *Polybotrya semipinnata* Fée with catadromous pinnules, and *Elaphoglossum piloselloides* (C. Presl) T. Moore with hairs on the stipe, rachis, and lamina.

4.3. Historical spatiotemporal dynamics in Dryopteridaceae

The origin of Dryopteridaceae dates back to the early Cretaceous (Du et al., 2021), when the global climate was shifting towards a new climatic condition characterized by increased rainfall and temperatures (Chaboureau et al., 2014). Ancestral area analysis suggests that this family likely originated in Americas. The early diversification of major clades of Dryopteridaceae was influenced by global climate changes, primarily through dispersal and vicariance events. The stems of all major clades emerged successively in the late Cretaceous (98.88–73.43 Ma) (Fig. 7). Although the precipitation rate decreased slightly between 95 Ma and 70 Ma, it maintained high in the Late Cretaceous (Chaboureau et al., 2014). The emergence of new bioclimatic continents set the stage for plant ecological expansion and may eventually lead to successful ecological radiation and plant diversification (Chaboureau et al., 2014).

In the early Cenozoic period, significant transformations of tropical forests occurred (Azuma et al., 2001; Fritsch et al., 2015; Bansal et al., 2022; Lim et al., 2022) and subtropical evergreen broadleaved forests emerged (Yu et al., 2017; Qin et al., 2023), the ancestors of the major clades then spread to Africa, Australia, Pacific islands, and Asia, forming more genera during the Paleocene and the Eocene. As global temperatures increased in the late Paleocene and early Eocene and forest compositions evolved, the major clades B and E of Dryopteridaceae began to occupy different ecological niches, which may have driven the differentiation of hemi-epiphytic and epiphytic genera to adapt to the new environment. As global temperatures dropped in the late Cenozoic, some genera (*e.g.*, *Dryopteris* and *Polystichum*) experienced explosive growth as they adapted to the new temperate ecological niche.

4.4. The systematic relationships of four enigmatic taxa

Due to insufficient samples and sequence data in previous studies, some genera of Dryopteridaceae were found to be not monophyletic (Lu et al., 2019), and the phylogenetic placement of some genera remained to be resolved (Liu et al., 2016). Our results address these questions well and provide solid evidence for resolving the systematic relationships of four enigmatic taxa.

4.4.1. Ctenitis

Ctenitis is a unique genus characterized by ctenitoid hairs on the rachis, pinna rachis and prominent costae on pinnules, and has long been treated as a member of Tectariaceae (or "Aspidiaceae") (Ching, 1978; Kramer et al., 1990; Wu and Ching, 1991). Previous studies based on plastid sequences weakly supported *Ctenitis* as the sister clade to Dryopteridoideae (Liu et al., 2016), suggesting its tentative placement in Dryopteridoideae (Liu et al., 2016; PPG I, 2016). Our study strongly supported *Ctenitis* as the sister group to six genera of Polybotryoideae sensu Liu et al. (2016) (the major clades B and C) in the present study. Furthermore, the synteny of the plastomes also distinguishes *Ctenitis* from other genera within Dryopteridaceae. The rachis-costae architecture of *Ctenitis* is the pleocnemioid type, and its erect to ascending rhizome is distinct from that of Polybotryoideae sensu Liu et al. (2016) (Figs. 6 and 8). Therefore, we defined *Ctenitis* as an independent major clade within Dryopteridaceae.

4.4.2. Stigmatopteris

Based on molecular phylogenetic and morphological evidence, we thoroughly settled the systematic location of *Stigmatopteris*, and suggested that it was removed from the subfamily Polybotryoideae and placed in the subfamily Dryopteridoideae (Fig. 8). The relationship of *Stigmatopteris* had remained poorly resolved in previous studies (Labiak et al., 2014; Liu et al., 2016; Moran and Labiak, 2016; Lu et al., 2019), and it was tentatively placed in subfamily Polybotryoideae (Liu et al., 2016; PPG I, 2016). In our study, *Stigmatopteris* is strongly supported as the sister group to all other clades of the major clade A. Morphologically, *Stigmatopteris* shares some morphological characteristics with other members of the major clade A in terms of monomorphic fronds and discrete round sori.

4.4.3. Arachniodes bella

Arachniodes bella (Ching, 1962) was originally treated as a member of *Dryopteris* in the early 20th century (Bonaparte, 1925), or placed in *Polystichopsis* (Humbert, 1958; Tardieu-Blot, 1958). Morphologically, *Arachniodes bella* shares similarities with *Arachniodes* (especially *A. superba* Fraser-Jenk.) in terms of lamina division, anadromously arranged frond segments, and reniform indusia, and also similar to most species of *Dryopteris* in terms of erect to ascending rhizomes. Most notably, this species has setiform hairs and glands on the stipe, rachis, lamina and indusium (Fig. 9), which distinguishes it not only from *Arachniodes* and *Dryopteris* (Table 4), but also from most genera within Dryopteridoideae (except for *Stigmatopteris*, which has reduced uniseriate hair-like scales). Based on the comprehensive evidence of morphology, molecular phylogeny, and special distribution area, we suggested that *Arachniodes bella* should be treated as a new genus within Dryopteridaceae in the hereafter taxonomic treatment.

4.4.4. Arthrobotrya

The classification of *Arthrobotrya* has always been a controversial topic. It was originally separated from *Polybotrya* (Smith, 1875) based on the presence of articulate pinnae and pinnules, and included only two species, *Arthrobotrya articulata* and *A. wilkesiana* (PPG I, 2016). Holttum (1938) noticed the similarity between *Arthrobotrya* and *Teratophyllum*, and merged *Arthrobotrya* in *Teratophyllum*. He (Holttum, 1938) further divided *Teratophyllum* into *T.* sect. "*Euteratophyllum*" (=*T.* sect. *Teratophyllum*; thin rhizome, biseriate leaves, and pinnate acrophylla) and *T.* sect. *Polyseriatae* (thicker rhizome, polyseriate leaves, and bipinnate acrophylla), and included *Arthrobotrya* in *T.* sect. *Polyseriatae* with three species (*T. articulatum* (Hook.) Mett. ex Kuhn, *T. brightiae* (F. Muell.) Holttum, and *T. wilkesianum* (Brack.) Holttum). PPG I (2016) treated

Fig. 9. Specimens and photograph of *Pseudarachniodes bella*. A. Holotype (http://coldb.mnhn.fr/catalognumber/mnhn/p/p00483193), B. Isotype (http://coldb.mnhn.fr/catalognumber/mnhn/p/p00483194), C. Photograph of *G. Rouhan* et al., *253* (http://coldb.mnhn.fr/catalognumber/mnhn/p/p00749177) (Photo by G. Rouhan/MNHN). D. Abaxial surface of rachis, showing setiform hairs (MNHN-P00483194), E. Abaxial surface of lamina, showing setiform and glandular hairs (MNHN-P00483194), F. Glandular indusia (MNHN-P00749186).

Table 4
Comparisons of morphological characters and distribution area in *Pseudarachniodes* and related genera.

	Pseudarachniodes	*Arachniodes*	*Dryopteris*	*Polystichopsis*
Rhizome	**Erect**	Long creeping, short creeping, and ascending, rarely erect	Erect or ascending, rarely creeping	Long to short creeping, and ascending
The arrangement of frond segments	**Anadromously**	Anadromously	Mostly catadromously	Mostly anadromously
Hairs	**Setiform, glandular**	Not hairy, rarely with hairlike and twisted scales	Not hairy or occasionally hairy	Whitish pilose hairs
Glands	**Present on rachises, laminae, and indusia**	Absent	Absent or present occasionally on laminae and/or rarely on indusia	Absent
Distribution area	**Madagascar**	Tropical and subtropical regions	Worldwide	Tropical and subtropical regions of New Word

Arthrobotrya as a separate genus following Moran et al. (2010a). Our finding indicates that *T.* sect. *Polyseriatae* is monophyletic.

The inclusion of *Teratophyllum brightiae* with intermediate morphological traits in *Arthrobotrya* will lead to instability of the key morphological characteristics distinguishing *Teratophyllum* from *Arthrobotrya* (thin/thicker rhizome, biseriate/polyseriate leaves; pinnate/bipinnate acrophylla). Holttum (1966) also reported that these differences were variable between young and adult plants. Moran et al. (2010a) noted that the type of soral paraphysis differs between *Teratophyllum* and *Arthrobotrya* (branched vs. simple). However, this characteristic has not been thoroughly tested in all species. Furthermore, *Teratophyllum* and *Arthrobotrya* share similar habits and overlapping distribution areas. Therefore, we support treating *Arthrobotrya* as a synonym for *Teratophyllum*.

4.5. Infra-familial classification of Dryopteridaceae

The present results underscore the necessity of redefining all major clades by integrating both molecular phylogeny and morphological data. Many families and genera of ferns with distinctive morphological traits were included within Dryopteridaceae according to current and previous phylogenetic studies (Moran et al., 2010a; Labiak et al., 2014; Moran and Labiak, 2015; Liu et al., 2016). Examples include Peranemataceae (Ching, 1978), Bolbitidaceae (Ching, 1978), Elaphoglossaceae (Pichi-Sermolli, 1977; Ching, 1978), and *Lomagramma* of Lomariopsidaceae (Pichi-Sermolli, 1977; Ching, 1978), as well as two genera (*Ctenitis* and *Pleocnemia*) of Tectariaceae (as "Aspidiaceae" [Pichi-Sermolli, 1977; Ching, 1978]). The three-subfamily classification of Dryopteridaceae was based primarily on molecular phylogenetic relationships of three plastid coding regions, but the relationships between the major clades were not strongly supported (Liu et al., 2016). Each subfamily is difficult to define morphologically. Without morphological evidence, it is very challenging to define each subfamily based solely on phylogenetic relationships. By integrating current phylogenetic results (Fig. 2) with morphological data (Fig. 3), we have two possible taxonomic options: either dividing Dryopteridaceae into seven subfamilies or eliminating all subfamilies. Dryopteridaceae currently encompass about 20% of the fern species, whose member genera were previously assigned to different families (Table 1) displaying distinct, even unique morphological characteristics. Therefore, based on the results of morphological and phylogenetic studies, we propose hereafter in the taxonomic treatment a new classification that divides Dryopteridaceae into seven subfamilies, including four new ones (Fig. 8).

Subfam. 1. Dryopteridoideae (Major clade A).
Subfam. 2. Ctenitidoideae (Major clade D).
Subfam. 3. Elaphoglossoideae (Major clade E).
Subfam. 4. Lastreopsidoideae (Major clade G).
Subfam. 5. Pleocnemioideae (Major clade F).
Subfam. 6. Polybotryoideae (Major clade B).
Subfam. 7. Polystichopsidoideae (Major clade C).

5. Taxonomic treatment

Dryopteridaceae Herter, Revista Sudamer. Bot. 9: 15. 1949. —Type: *Dryopteris* Adans.

Plants evergreen or deciduous, terrestrial, epilithic, hemiepiphytic, or epiphytic. **Rhizomes** erect, ascending, creeping, or climbing, dictyostelic, scaly. **Fronds** calathiform, caespitose, or remote from one another, with pinnules anadromous at base and anadromously or catadromously arranged distally; stipe, rachises, and pinnules articulate or not, scaly, grooved, round or ribbed adaxially, hairy or not. **Lamina** monomorphic or dimorphic, 1–5-pinnate, or simple, rarely imparipinnate, scaly, glandular, hairy, or glabrous; thinly papery, papery, or leathery. **Venation** pinnate and free, or variously anastomosing to form 1 to multiple rows of areoles, with or without included veinlets. **Sori** acrostichoid or discrete, terminal, subterminal, or dorsal on veins, indusiate or exindusiate. **Indusia** orbicular or reniform or rarely ovoid, superior, lateral, or rarely inferior, sessile or rarely stalked, entire or toothed. **Spores** monolete, achlorophyllous, with prominent perispore. Chromosome number $x = 41$.

Seven subfamilies and **24 genera** with more than 2100 species: nearly cosmopolitan, but highest diversity found in temperate regions (*Dryopteris*, *Polystichum*) and tropical mountain regions (*Elaphoglossum*).

5.1. Key to subfamilies of Dryopteridaceae

1a. Rachis, pinna rachis (in 2- to more pinnate leaves), and costae (in simple to 1-pinnate leaves) adaxially grooved……**2**
1b. Rachis, pinna rachis (in 2- to more pinnate leaves), and costae (in simple to 1-pinnate leaves) adaxially not grooved, or only grooved near stipe base……**5**
2a. Rachis and pinna rachis adaxially grooved, with widened bottom (lastreopsid type)……**Lastreopsidoideae**
2b. Rachis and pinna rachis adaxially deeply grooved, without widened bottom (dryopteridoid type)……**3**
3a. Root climbers (starting on soil, then later scandent) or hemiepiphytic, rarely terrestrial; rhizomes short or long creeping, fronds dimorphic or sub-dimorphic, sori acrostichoid, rarely discrete……**Polybotryoideae**
3b. Terrestrial; rhizomes erect, ascending or short creeping, fronds monomorphic, sori discrete……**4**
4a. Rhizome erect, ascending or short creeping, if creeping, base of the stipe only scaly……**Dryopteridoideae**
4b. Rhizome short creeping, base of the stipe hairy……**Polystichopsidoideae**
5a. Sori acrostichoid; fronds dimorphic; Stipe base grooved or not, flat to slightly convex on upper stipe, then turned convex, and finally ribbed adaxially on the upper rachis and costae (bolbitidoid type)……**Elaphoglossoideae**

5b. Sori discrete; fronds monomorphic; Costae and costules adaxially slightly convex, rounded or nearly flat (pleocnemioid type)……..……**6**

6a. Veins anastomosing, with scales and yellow glands on lamina……...……**Pleocnemioideae**

6b. Veins free, with scales and multicellular ctenitoid hairs on lamina …..……...……**Ctenitidoideae**

5.2. Subfamily 1. ***Dryopteridoideae*** *B.K. Nayar, Taxon 19: 235. 1970*

—Type: *Dryopteris* Adans.

Plants terrestrial. **Rhizomes** erect, ascending or creeping, apex densely scaly. **Stipes** densely scaly on the base and the lower parts, and the upper parts clothed with smaller scales, soft hairs or glands. **Fronds** caespitose or approximate, monomorphic. **Laminae** simple to 3 to more pinnatifid, herbaceous, papery or nearly leathery; pinnules anadromous or catadromous, rachis and pinna rachis adaxially grooved. **Veins** free or anastomosing. **Sori** orbicular, dorsal or rarely terminal. **Indusia** present or not, reniform or round, superior or inferior, sessile or with a long stalk.

Genera: *Arachniodes* Blume, *Dryopteris* Adans., *Cyrtomium* C. Presl, *Phanerophlebia* C. Presl, *Polystichum* Roth, *Pseudarachniodes* Z.Y. Zuo & Rouhan, *Stigmatopteris* C. Chr.

Pseudarachniodes Z.Y. Zuo & Rouhan **gen. nov**.

Type: ***Pseudarachniodes bella*** (C. Chr.) Z.Y. Zuo & Rouhan (Baisonym: *Dryopteris bella* C. Chr.) (Fig. 9).

Plants terrestrial. **Rhizomes** erect, densely clothed with scales. **Scales** entire, narrowly lanceolate to oblong, scarious, dark brown to dark castaneous, most often contorted. **Stipes** up to 30 cm, scaly at the base, covered with sparse to dense hairs, most setiform (straight and white) mixed with some shorter glandular ones. **Laminae** usually 3- or more pinnate (sometimes 2-pinnate-pinnatifid), to *ca.* 50 × 45 cm, triangular to lanceate, herbaceous to nearly leathery, most basal pinnae symmetrical, pinnules of suprabasal pinnae anadromous, rachis and costae adaxially grooved, adaxial surfaces of leaves subglabrous, rachises, costae and abaxial surface of leaves and veins with a few linear and dark brown scales in addition to sparse to dense hairs similar to those of stipes (setiform and clear), bright castaneous and spherical glands scattered mostly on abaxial surfaces. **Veins** free. **Indusia** orbicular-reniform, glandular, deciduous.

Distribution and diversity: Currently with one species, endemic to Madagascar, *P. bella* (C. Chr.) Z.Y. Zuo & Rouhan.

Pseudarachniodes bella (C. Chr.) Z.Y. Zuo & Rouhan **comb. nov.**

Baisonym: *Dryopteris bella* C. Chr. in Bonap., Notes Pteridol. 16: 164, t.8 (1925).

Polystichopsis bella (C. Chr.) Tardieu, Amer. Fern J. 48(1): 33 (1958).

Arachniodes bella (C. Chr.) Ching, Acta Bot. Sin. 10(3): 261 (1962).

Type: Madagascar, *H. Perrier de la Bâthie 7439* (P!).

Additional examined specimens: Madagascar, *H. Humbert 18182* (P!) *& 18513* (P!); *H. Humbert* et al. *24.718* (P!), *F. Rakotondrainibe 1423* (K!, P!) *& 1705* (K!, MO!, P!); *G. Rouhan* et al. *253* (P!) *& 257* (P!).

Key to genera of subfamily Dryopteridoideae

1a. Scales with uniseriate cilia on the margins; laminae with internal punctate glands; veins ending in a conspicuous hydathode; indusia absent……...……***Stigmatopteris***

1b. Scales without uniseriate cilia on the margins; laminae with or without internal punctate glands; veins without hydathode; indusia present in most species……..……**2**

2a. Indusia (when present) reniform, rarely horseshoe-shaped, globose or subglobose; pinnae and/or pinnules lacking a basal acroscopic lobe; lobes or segments not spinulose-tipped……**3**

2b. Indusia peltate; pinnae and/or pinnules with a basal acroscopic lobe; lobes or segments spinulose-tipped……**6**

3a. Pinnules of suprabasal pinnae catadromously arranged……..……***Dryopteris***

3b. Pinnules of suprabasal pinnae anadromously arranged……...……**4**

4a. Rhizomes creeping……***Arachniodes***

4b. Rhizomes erect……...……**5**

5a. Laminar tissue with sparse to dense, setiform hairs (especially abaxially) and some small scales……..……***Pseudarachniodes***

5b. Laminar tissue glabrous or nearly so, only with small scales……...……***Dryopteris*** (sect. *Acrorumohra*)

6a. Laminae 1–3-pinnate, apices pinnatifid ……***Polystichum***

6b. Laminae imparipinnate, with an apical pinna somewhat dissected at base……**7**

7a. Veins free or with 1–3 series of marginal anastomoses……...……***Phanerophlebia***

7b. Veins anastomosing, with 2 to more rows of areoles……...……***Cyrtomium***

5.3. Subfam. 2. ***Ctenitidoideae*** *Z.Y. Zuo & Jin Mei Lu,* ***subfam. nov.***

—Type: *Ctenitis* (C. Chr.) C. Chr.

Plants terrestrial. **Rhizomes** erect to ascending, rarely short-creeping, apex densely scaly. **Fronds** clustered, monomorphic. **Stipes** scaly at base or throughout. **Laminae** 1–4-pinnate, herbaceous or papery, lanceolate to triangular, with small scales and hairs on both surfaces or rarely glabrous; costae of pinnules round, always covered with multicellular ctenitoid hairs (often reddish and jointed, twisted), pinnules catadromous, rachis and pinna rachis adaxially flat to rounded. **Veins** free. **Sori** medial or rarely submarginal. **Indusia** present or not, sometimes very small and hidden by maturing sporangia.

Genus: *Ctenitis* (C. Chr.) C. Chr.

5.4. Subfam. 3. ***Elaphoglossoideae*** *(Pic. Serm.) Crabbe, Jermy & Mickel, Fern Gaz. 11: 154, 1975*

—Type: *Elaphoglossum* Schott ex J. Sm.

Plants mostly epiphytic, occasionally hemi-epiphytic or climbing. **Rhizomes** short to long-creeping, densely scaly at apex. **Fronds** clustered or widely spaced, dimorphic. **Stipes** mostly scaly at base. **Laminae** simple, 1-pinnate, rarely 2-pinnate, rachis and costae glabrate or hairy, pinnules catadromous, lower rachis adaxially with or without grooves, gradually convex upwards, and finally adaxially ribbed on the upper rachis and costae; terminal pinna articulated or not. **Veins** free or anastomosing. **Sori** acrostichoid. **Exindusiate**.

Genera: *Bolbitis* Schott, *Elaphoglossum* Schott ex J. Sm., *Lomagramma* J. Sm., *Mickelia* R.C. Moran, Labiak & Sundue, *Teratophyllum* Mett. ex Kuhn.

Key to genera of subfamily Elaphoglossoideae

1a. Laminae simple (rarely divided); veins free, rarely anastomosing or connected by their tips to form a submarginal strand……..……***Elaphoglossum***

1b. Laminae pinnate; veins free or anastomosing……..……**2**

2a. Plants climbing, with differentiated bathyphylls (juvenile and lower fronds) and acrophylls (mature upper fronds); rhizomes long-creeping; paraphyses present, simple, or stellate or capitate, long-stalked, the stalk of 1 or 2 rows of cells……...…**3**

2b. Plants epiphytic or terrestrial, without differentiated bathyphylls and acrophylls; rhizome ascending or creeping; paraphyses absent, or rarely present, short-stalked, the stalk of 3 rows of cells……...…**4**

3a. Venation of sterile fronds anastomosing; laminae catadromous (or isodromic) toward apex……..……***Lomagramma***

3b. Venation of sterile fronds free; laminae often anadromous toward apex……...……***Teratophyllum***

4a. Buds usually present near the apices of pinnae or terminal segments……***Bolbitis***

4b. Buds absent or present in the “axil” of the pinna (on the base of the costae)……..…***Mickelia***

5.5. Subfam. 4. ***Lastreopsidoideae*** *Z.Y. Zuo,* ***subfam. nov.***

—Type: *Lastreopsis* Ching.

Plants terrestrial. **Rhizomes** erect, decumbent, or creeping, scaly at apex. **Stipes** scaly at base, stipe and other parts densely clothed in soft hairs. **Fronds** approximate, monomorphic. **Laminae** 3 or more pinnate, herbaceous or papery, basal pinnae largest, with hairs on both surface; pinnules anadromous or catadromous, rachis and pinna rachis with widened grooves adaxially. **Veins** free. **Sori** terminal on veins. **Indusia** present or not, sometimes very small and hidden by maturing sporangia.

Genera: *Lastreopsis* Ching, *Megalastrum* Holttum, *Parapolystichum* (Keyserl.) Ching, *Rumohra* Raddi

Notes: *Parapolysticum* and *Lastreopsis* (s.s.) are very similar morphologically, and there are no distinct morphological features that distinguish the two genera (Labiak et al., 2014). We can partially distinguish some species of *Parapolystichum* by the erect rhizome and the buds on the abaxial rachis, which are absent in *Lastreopsis*. However, these characteristics are not stable between species within *Parapolystichum*. The rachis of the lamina, pinna rachises, and costules are pubescent adaxially in *Megalastrum* (Moran and Prado, 2010). Labiak et al. (2014) summarized the rachis-costa architecture of *Megalastrum* as the Megalastrum type. We consider it as a special form of the Lastreopsid type—the bottom of the groove on rachis is conspicuous or not, and the groove is shallow.

Key to genera of subfamily Lastreopsidoideae

1a. Lamina nearly leathery, hairless; indusia peltate…***Rumohra***

1b. Lamina herbaceous to papery, with hairs abaxially; indusia reniform, hypoplastic, or exindusiate……**2**

2a. Rachis and pinna rachis slightly grooved with a narrow bottom; pinnae and segments often obtuse, toothless; basal basiscopic lobe of the distal pinnules decurrent on the costae with the distalmost veins arising from the pinna rachis (not the costule) ……***Megalastrum***

2b. Rachis and pinna rachis grooved with widened bottom; pinnae and segments acuminate, usually dentate; basal basiscopic lobe of the pinnules not decurrent, veins arise from the costule……....……**3**

3a. Rhizomes erect, ascending to creeping; upper portion of rachis usually with buds……..……***Parapolystichum***

3b. Rhizomes creeping; upper portion of rachis never with buds……...……***Lastreopsis***

5.6. Subfam. 5. ***Pleocnemioideae*** *Z.Y. Zuo,* ***subfam. nov.***

—Type: *Pleocnemia* C. Presl.

Diagnosis: **Plants** terrestrial. **Rhizomes** erect or rarely creeping, apex densely covered with linear scales. **Fronds** clustered, monomorphic. **Stipes** only scaly at base. **Laminae** 2–4-pinnate, herbaceous or papery, basal pinnae largest, with yellow cylindric glands along costules and veins abaxially; pinnules catadromous, rachis and pinna rachis rounded adaxially. **Veins** along costae anastomosing. **Sori** dorsal on free veins. **Indusia** present or not.

Genus: *Pleocnemia* C. Presl.

5.7. Subfam. 6. ***Polybotryoideae*** *Hong M. Liu & X.C. Zhang, Plant Syst. Evol. 302(3): 330 (2016)*

—Type: *Polybotrya* Humb. & Bonpl. ex Willd.

Plants climbing or hemi-epiphytic. **Rhizomes** short- to long-creeping, covered with scales. **Fronds** clustered or widely spaced, dimorphic or slightly dimorphic. **Stipes** mostly scaly at base. **Laminae** 1- to 4-pinnate, rachis and costae loosely to densely hairy (but glabrous in *Olfersia*), pinnules anadromous or catadromous, rachis, pinna rachis and costa sulcate adaxially. **Veins** free or rarely anastomosing. **Sori** acrostichoid or round. **Indusia** round, or exindusiate.

Genera: *Cyclodium* C. Presl, *Maxonia* C. Chr., *Olfersia* Raddi, *Polybotrya* Humb. & Bonpl. ex Willd.

Notes: Polybotryoideae are characterized by creeping rhizomes and usually strongly dimorphic sterile and fertile leaves. The perines tend to be finely echinulate. The sori of *Olfersia* and *Polybotrya* are exindusiate, whereas those of *Cyclodium* and *Maxonia* are indusiate, the indusia being round and peltate. *Olfersia* is atypical in this subfamily because of its glabrous laminae, conform terminal pinna (imparipinnate laminae), and vein tips connected to form a submarginal strand.

Key to genera of subfamily Polybotryoideae

1a. Sterile and fertile fronds subdimorphic; rhizomes short-creeping……..***Cyclodium***

1b. Sterile and fertile fronds strongly dimorphic; rhizomes long-creeping……...……**2**

2a. Sterile laminae imparipinnate, glabrous; **v**ein tips connected by a submarginal connecting strand……..……***Olfersia***

2b. Sterile laminae 2- to 3-pinnate-pinnatifid**;** vein tips not connected by a submarginal connecting strand……..……**3**

3a. Rhizomes with meristeles surrounded by a dark sclerenchymatous sheath; sori non-indusiate……***Polybotrya***

3b. Rhizomes with meristeles not surrounded by a dark sclerenchymatous sheath; sori and indusial round……...…***Maxonia***

5.8. Subfam. 7. ***Polystichopsidoideae*** *Z.Y. Zuo,* ***subfam. nov.***

—Type: *Polystichopsis* (J. Sm.) C. Chr.

Plants terrestrial. **Rhizomes** creeping, densely covered with linear scales. **Stipes** densely clothed in soft hairs, with scales only at base. **Fronds** approximate, monomorphic. **Laminae** 3 or more pinnate, herbaceous or papery, basal pinnae largest, with hairs on both surface; pinnules anadromous or catadromous, rachis and

pinna rachis sulcate adaxially. **Veins** free. **Sori** terminal on veins. **Indusia** present or not, sometimes very small and hidden by maturing sporangia.

Genera: *Polystichopsis* (J. Sm.) C. Chr., *Trichoneuron* Ching.

Notes: Morphologically, *Polystichopsis* and *Trichoneuron* are very similar and cannot be distinguished significantly. However, they are typically differentiated by geographical isolation. *Polystichopsis* is endemic to tropical America, whereas *Trichoneuron* only occurs in Asia.

Key to genera of subfamily Polystichopsidoideae

1a. Pinnae with distinct stalks, and pinnules anadromous or catadromous; only in the Neotropic……..……***Polystichopsis***

1b. Pinnae sessile or sub-sessile, and pinnules catadromous; only in Asia……...……***Trichoneuron***

6. Conclusions

Based on extensive field collections, the use of herbarium specimens, and better genome skimming technique, this study provides new insights into the phylogenetic relationships among different lineages of Dryopteridaceae. We reconstructed a robust topology of Dryopteridaceae worldwide and identify some morphological synapomorphies that support the lineages (subfamilies) we have identified. The robust phylogenetic backbone and the reconstructed morphological character evolution here provide an extendable framework for future studies of taxonomy, biogeography, and diversification of Dryopteridaceae.

We present a new classification of Dryopteridaceae recognizing 24 genera in seven subfamilies, of which four subfamilies and one genus are newly established. A recent classification of Selaginellaceae into seven subfamilies and 19 genera (Zhou and Zhang, 2023) is comparable to the classification of Dryopteridaceae presented here. As the fern community prepares to update the PPG classification, pteridologists need to consider how to make the classification of ferns more practical. For example, for taxonomic identification of Dryopteridaceae, Polypodiaceae, and Pteridaceae, which each contain more than 1000 species, how can we first determine the higher taxon (family or subfamily) to which a species belongs and then assign it to a lower taxon (genus), rather than identifying the genus first and then assigning it to the corresponding family. The monophyly with morphological synapomorphy is more suitable to define a "natural" familial or infra-familial group.

Data availability

Data will be made available on request.

CRediT authorship contribution statement

Zheng-Yu Zuo: Writing – original draft, Software, Methodology, Formal analysis, Data curation, Conceptualization. **Germinal Rouhan:** Writing – review & editing, Resources, Project administration. **Shi-Yong Dong:** Writing – review & editing, Resources. **Hong-Mei Liu:** Writing – review & editing, Supervision. **Xin-Yu Du:** Methodology. **Li-Bing Zhang:** Writing – review & editing, Resources, Project administration, Investigation. **Jin-Mei Lu:** Writing – original draft, Writing – review & editing, Visualization, Validation, Supervision, Resources, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare no conflict of interest.

Acknowledgements

We would like to thank the curators and staff of the CHS, KUN, MO, P, PE herbaria that offered their facilities and help in examining herbarium specimens, and thank Dr. Ran Wei (Institute of Botany, Chinese Academy of Sciences) for sample collection, Robbin C. Moran (The New York Botanical Garden) for discussions on the morphology of Dryopteridaceae, improving the manuscript, and providing photographs of several neotropical genera. We also thank the Experimental Center of Molecular Biology of the Germplasm Bank of Wild Species, Kunming Institute of Botany, Chinese Academy of Sciences for suggestion of laboratory work, and the iFlora High Performance Computing Center of the Germplasm Bank of Wild Species (iFlora HPC Center of GBOWS, KIB, CAS) for computing. Madagascar collecting permits were granted to GR by Madagascar National Parks and the "Ministère de l'Environnement, de l'Ecologie et des Forêts". The authors are also grateful for field assistance from CNRE-Madagascar and MBG-Madagascar. The MNHN gives access to the collections in the framework of the RECOLNAT National Research Infrastructure. The study was funded by the National Natural Science Foundation of China (Grant No. 31970232) and the National Key Basic Research Program of China (Grant No. 2014CB954100).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pld.2024.07.010.

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