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# Phylogenomic Analysis Reconstructed the Order Matoniales from Paleopolyploidy Veil

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**Abstract:** Phylogenetic conflicts limit our understanding of the evolution of terrestrial life under multiple whole genome duplication events, and the phylogeny of early terrestrial plants remains full of controversy. Although much incongruence has been solved with so-called robust topology based on single or lower copy genes, the evolutionary mechanisms behind phylogenetic conflicts such as polyploidization remain poorly understood. Here, through decreasing the effects of polyploidization and increasing the samples of species, which represent all four orders and eight families that comprise early leptosporangiate ferns, we have reconstructed a robust phylogenetic tree and network with 1125 1-to-1 orthologs based on both coalescent and concatenation methods. Our data consistently suggest that Matoniales, as a monophyletic lineage including Matoniaceae and Dipteridaceae, should be redefined as an ordinal rank. Furthermore, we have identified and located at least 11 whole-genome duplication events within the evolutionary history of four leptosporangiates lineages, and associated polyploidization with higher speciation rates and mass extinction events. We hypothesize that paleopolyploidization may have enabled leptosporangiate ferns to survive during mass extinction events at the end Permian period and then flourish throughout the Mesozoic era, which is supported by extensive fossil records. Our results highlight how ancient polyploidy can result in rapid species radiation, thus causing phylogenetic conflicts yet allowing plants to survive and thrive during mass extinction events.

**Keywords:** ancient polyploidization; ancient radiation; reticulate evolution; mass extinction; ferns; phylogeny; 1-to-1 orthologous genes

## 1. Introduction

The tree of life has been one of the biggest challenges to biologists since Darwin; phylogenetic tree reconstruction vastly improves our understanding of systematic taxonomy and the evolution of terrestrial life [1,2], which includes prokaryotes [3] and eukaryotes [4] and sub-classifications such as animals [5], insects [6], fungi [7], and plants [8–10]. However, with continuing phylogenetic research, more and more discordances come to us [11], even in deep phylogenetic relationships. In general, new studies include only a brief description of the robustness of their results with high bootstrap values and/or posterior probability and

report differences with other studies; however, the evolutionary mechanisms underlying these phylogenetic conflicts are rarely discussed.

Polyploidization, including autopolyploidization, such as whole-genome duplication (WGD), and allopolyploidization, such as hybrid polyploidization, is an important evolutionary force and a driver of plant speciation [12,13]. WGD and reticulate evolution, which are prevalent in vascular plants, especially in ferns [10,14–16], are considered as the major factors affecting the stability and reliability of the phylogenetic relationship [17,18]. Therefore, ancient polyploidy events may underlie some phylogenetic conflicts, especially those in deep phylogenetic relationships; however, this hypothesis currently lacks veritable evidence. The remaining challenge is how to reconstruct the clear phylogenetic relationship under multiple WGDs.

Previous studies have remarkably improved our understanding of the evolutionary history of ferns [19–25]. Nevertheless, phylogenetic conflicts remained in the leptosporangiate ferns, which contained seven orders, i.e., Osmundales, Hymenophyllales, Gleicheniales, Schizaeales, Salviniales, Cyatheales, and Polypodiales [20]. To date, there are at least six phylogenetic topologies between Hymenophyllales and Gleicheniales constructed by previous studies (Figure 1, Table S1). In general, Gleicheniales is considered to be a monophyletic lineage that includes Dipteridaceae, Matoniaceae, and Gleicheniaceae; this lineage is a sister group of the remaining leptosporangiate ferns except for Osmundales and Hymenophyllales (Figure 1a) [23–28]. Interestingly, Pryer et al., (2004) proposed a sistership between gleichenioid and filmy ferns (Figure 1c), which was supported by molecular, morphological, and fossil evidence. However, these studies mostly relied on a few plastid genes or those combined with nuclear genes and/or morphological traits, which may not be sufficient to resolve deep phylogenetic relationships [2].
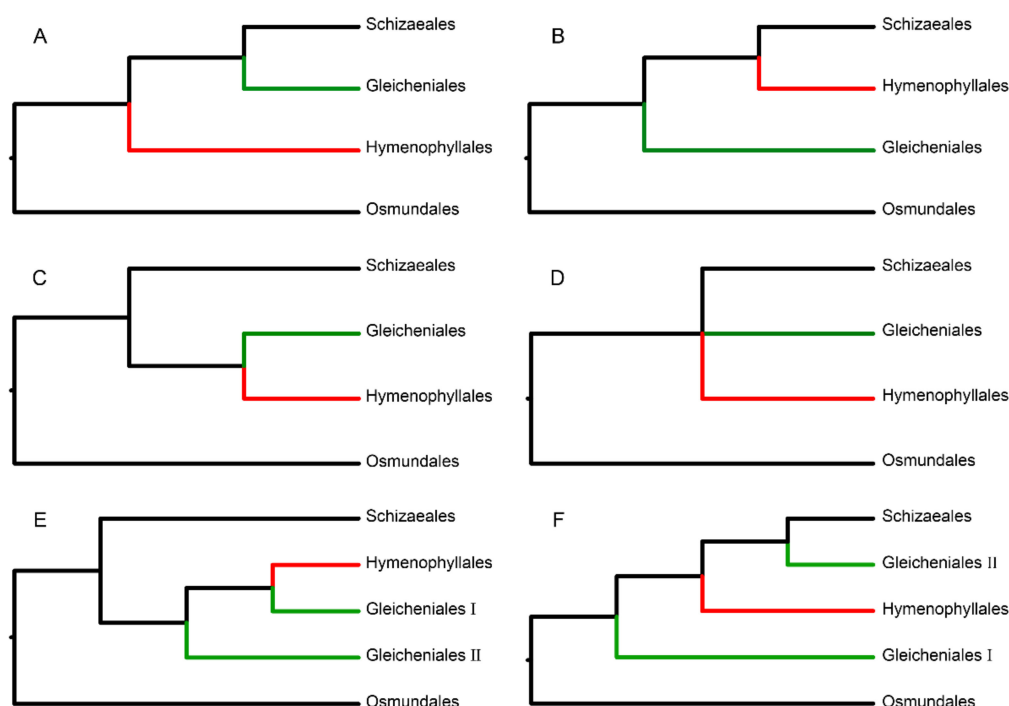


**Figure 1.** Six topologies of the phylogenetic relationship in the early leptosporangiate ferns. The corresponding references are shown in Table S1. The red branches indicate Hymenophyllales, and the green branches indicate Gleicheniales. Gleicheniales I indicates Gleicheniaceae, and Gleicheniales II indicates Dipteridaceae and Matoniaceae.

Following the development of next-generation sequencing and phylogenomic analysis, Shen et al., (2018) and Qi et al., (2018) reconstructed the framework of fern phylogeny based on massive transcriptomic datasets with 69 and 129 samples, respectively. This revealed

that gleichenioid ferns are not a monophyletic group and that Gleicheniaceae is a sister group of Hymenophyllaceae (Figure 1e), which means that Dipteridaceae might be another order of Dipteridales [29]. However, Matoniaceae, belonging to Matoniales [30] and considered as the sister lineage of Dipteridaceae [25,28], was not included in these two studies. Taxon sampling, which is considered as a common pitfall in phylogenetics [31,32], plays an important role in phylogenetic inference accuracy [5,33–35], and thus it is expected that a degree of the sampling within a study can be compromised by systematic errors [36]. Here, in order to increase taxa coverage, we analyzed the transcriptome data of 31 ferns, including 21 newly sequenced species. These data represent all of the four orders (Osmundales, Hymenophyllales, Gleicheniales, and Schizaeales) and eight families (Osmundaceae, Hymenophyllaceae, Matoniaceae, Dipteridaceae, Gleicheniaceae, Lygodiaceae, Schizaeaceae, and Anemiaceae) that comprise early leptosporangiate ferns.

It has been proposed that ferns underwent multiple cycles of polyploidization accompanied by subsequent diploidization but without apparent chromosome loss [37]. Moreover, evidence of repeated WGD during the diversification of leptosporangiate ferns was reported [38,39]. Due to the evolutionary significance of polyploidization and its effect on the phylogenetic relationship [13,18], we hypothesized that ancient polyploidization may have occurred in the early leptosporangiate ferns. This may have enabled ferns to survive and flourish during the Mesozoic era, but it has hampered the reconstruction of a clear and stable phylogenetic relationship to date.

In this study, we aimed to perform different analytical strategies to decrease the effect of polyploidization on phylogenomic analysis by 1-to-1 ortholog genes and to reconstruct robust phylogenetic relationships within early leptosporangiate ferns using sufficiently representative taxa. In addition, to improve our understanding of the impact of polyploidization in fern evolution, we investigated whether WGD and reticulate evolution underlie the ambiguous evolutionary history of early leptosporangiate ferns.

## 2. Results

### 2.1. Assessment of RNA-Seq Data

New transcriptomic data were generated from 21 fern species (Table S2, RS200-RS305). After filtering, approximately 1354.3 million total reads (about 200 Gb) were obtained. The average of Q30 and N50 were 94% and 1741 bp, respectively. In order to assess the assembly completeness of our transcriptomic data, we employed the core set of orthologs that are ultra-conserved in embryophyte species, which contained 1440 BUSCOs. Following a BLAST against our transcriptomic data, the gene coverage of all samples used in this study was more than 60%, except for *Hymenophyllum digitatum* (RS202, 57%) and *Callistopteris apiifolia* (RS208, 56%), and at least 808 conservative orthologs were matched (Figure 2).

### 2.2. Evolutionary History of the Early Leptosporangiate Ferns

After careful screening using the ortholog-derived pipeline (Figure S1) described in Shen et al., (2018), 1125 1-to-1 orthologs consisting of 878,952 nucleotides, each of which represented at least 16 species, were identified and used in subsequent analyses. We used both coalescent- and concatenation-based methods to infer the phylogenetic relationships of early leptosporangiate ferns (Figure 3). All species trees indicated that Gleicheniaceae is the sister group of Hymenophyllaceae and that sistership also exists between Dipteridaceae and Matoniaceae; however, Gleicheniales, which included Gleicheniaceae, Dipteridaceae, and Matoniaceae, was not a monophyletic group (Figures 3 and 4). As shown by both coalescent and concatenation analyses based on the DNA dataset, these four families are a monophyletic lineage with high confidence (Figures 3 and 4); however, analyses using the other datasets (1 and 2 codons and Protein datasets) showed that these four families are a paraphyletic group with low BS values (Figure 3).
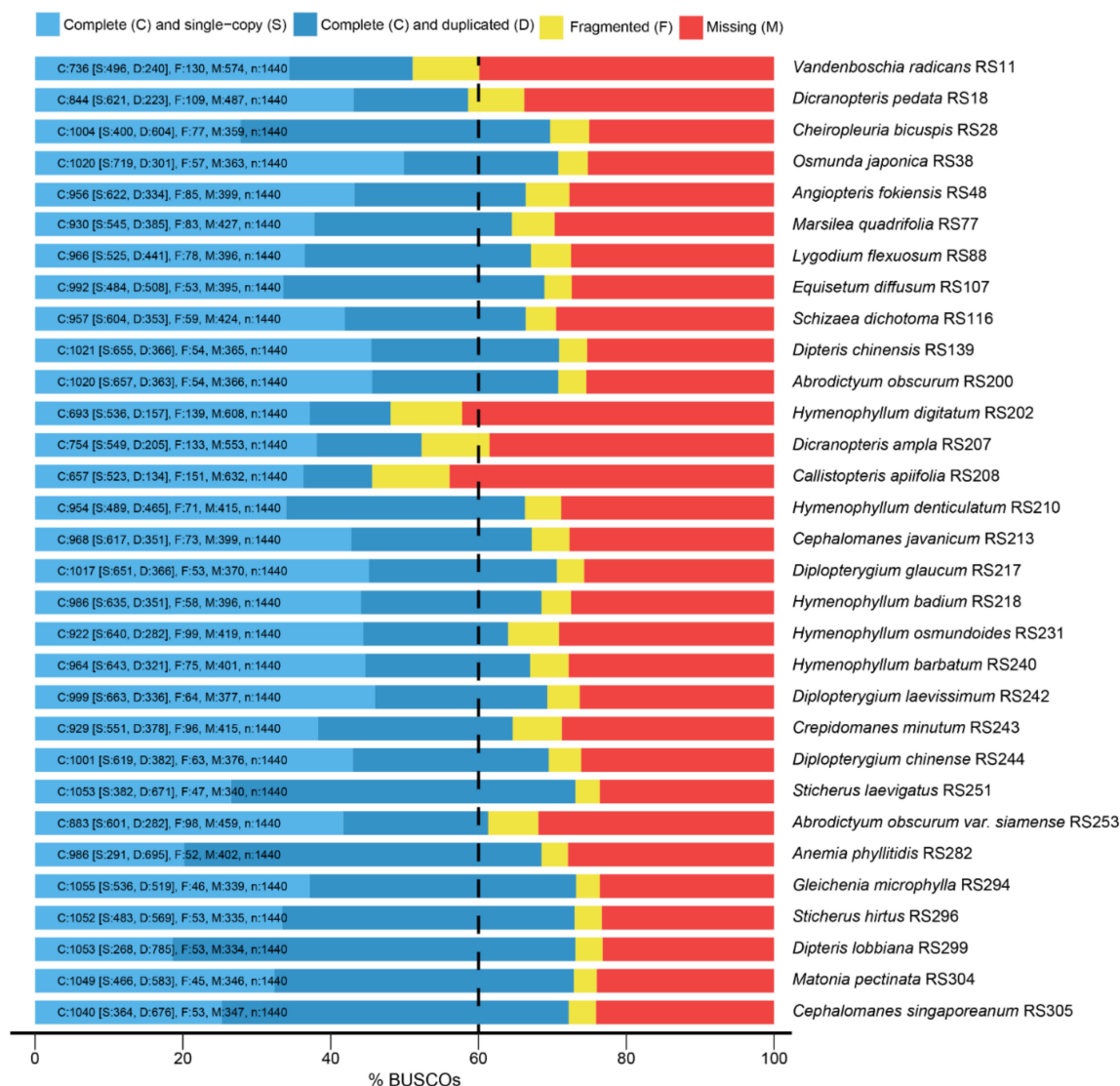
**Figure 2.** The assessment of transcriptome assembly completeness by Basic Universal Single Copy Orthologs (BUSCOs) analysis. Except for *Hymenophyllum digitatum* (RS202) and *Callistopteris apiifolia* (RS208), transcriptome assemblies of the sampled species were complete and orthologs shared more than 60% sequence identity.

Based on the high-confidence species tree, we determined divergence times in the early leptosporangiate ferns based on a Bayesian relaxed clock model. Our results showed that the leptosporangiate ferns originated in the Carboniferous period about 334 Mya [95% HPD = (261–401 Mya)], and the origins of Gleicheniales and Hymenophyllales were dated to almost the same periods of late Permian and early Triassic (Figures 4 and S2). Following this, early leptosporangiate ferns diversified throughout nearly the entire Mesozoic era. Subsequently, we estimated the species-specific diversification rates based on the reconstructed phylogenetic tree with time estimation. This showed that speciation rates were varied both among tree branches and through time. The highest diversification rate (about 2 Myr$^{-1}$) was calculated in the common ancestor of Hymenophylaceae and Gleicheniaceae, and, generally, speciation rates were more than 0.5 Myr$^{-1}$ in the deep branches of the early leptosporangiate ferns (Figure 4).
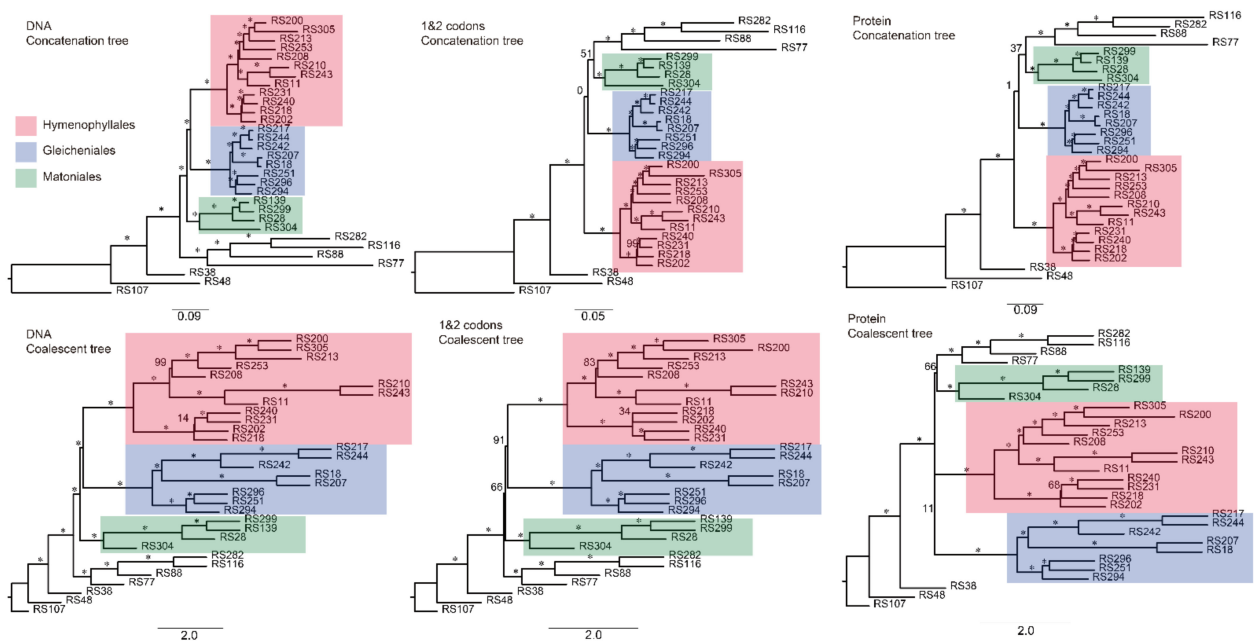
**Figure 3.** Six phylogenetic trees of different strategies. The number on the branch is the bootstrap value, and the asterisk (*) indicates a bootstrap support (BS) value of 100. The DNA concatenation tree, the DNA coalescent tree, and the 1 and 2 codons coalescent tree are consistent with high confidence (almost all of BS = 100), whereas the other trees are incongruent with low BS values.
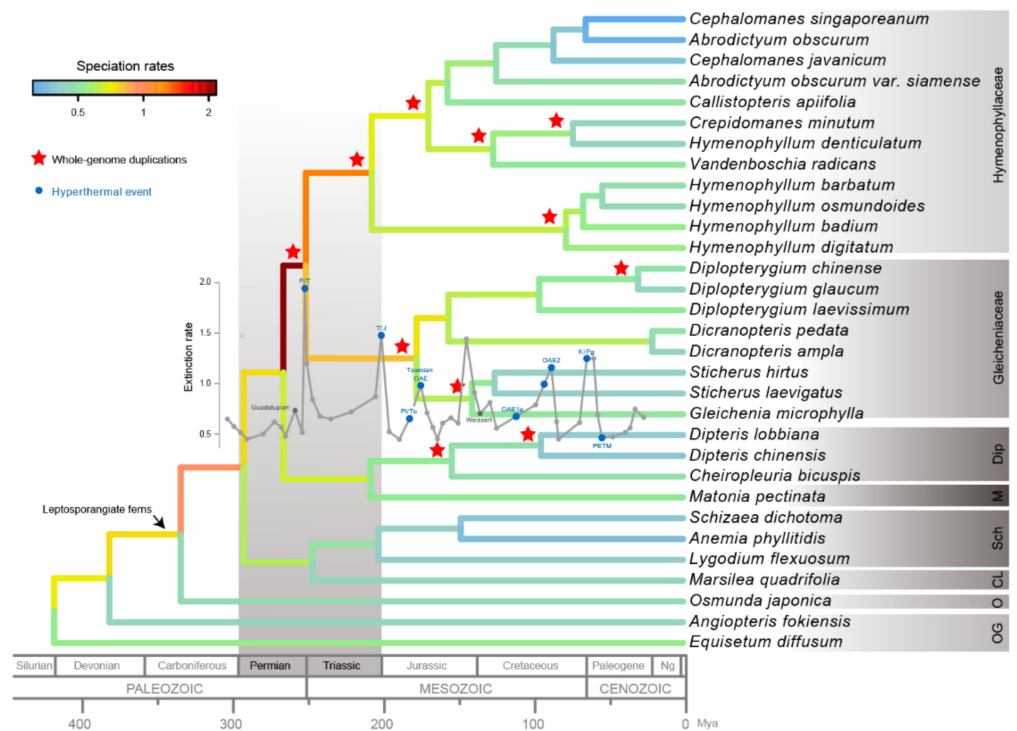


**Figure 4.** The evolutionary history of early leptosporangiate ferns. The bootstrap support (BS) value of each branch is 100 (not indicated in the species tree). The extinction rate was sourced from Clapham and Renne (2019). The red stars indicate the inferred whole genome duplication events. Abbreviations: K/Pg, end-Cretaceous; OAE, oceanic anoxic event; PETM, Paleocene–Eocene Thermal Maximum; Pl/To, Pliensbachian–Toarcian; P/T, end-Permian; T/J, end-Triassic; Dip, Dipteridaceae; M, Matoniaceae; Sch, Schizaeales; CL, core leptosporangiate; O, Osmundales; and OG, outgroups.

### 2.3. Paleopolyploidization and Reticulate Evolution in the Early Leptosporangiate Ferns

We applied gene-tree-species tree reconciliation methods to identify and locate WGD events in the early leptosporangiate ferns. After filtering the tandem duplications, at least 11 WGD events were identified during the evolution of early leptosporangiate ferns, especially in the ancestors of Hymenophyllaceae and Gleicheniaceae (Figures 4 and S3). Following this, we reconstructed the phylogenetic network of early leptosporangiate ferns and uncovered a complex evolutionary network with 106 splits and 954 edges. Apparently, the elusive network topology was clearly divided into nine groups with high confidence (Figure 5). Once rooted with *E. diffusum* as the outgroup, the four families (Matoniaceae, Dipteridaceae, Gleicheniaceae, and Hymenophyllaceae) were aggregated as one group. However, the relationships between these families were trifurcate rather than bifurcate due to the presence of hyper-complexed reticulate evolution (Figure S4). Moreover, reticulate evolution was also prevalent in the inner taxa of these families.
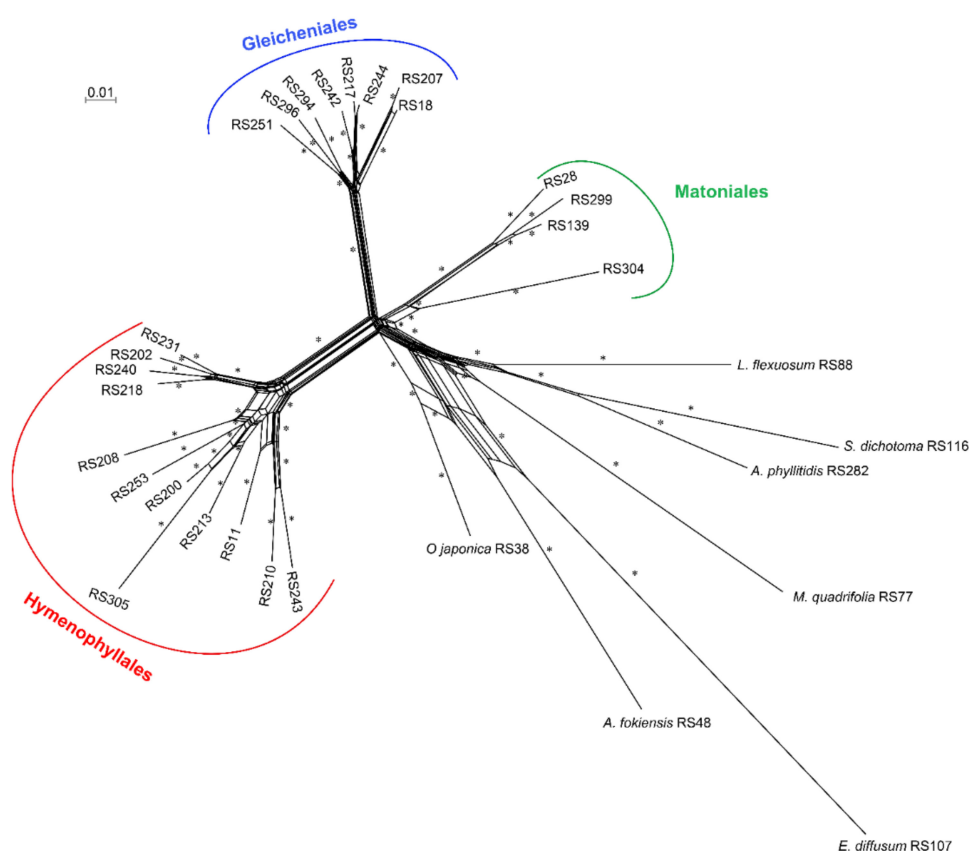


**Figure 5.** Reticulate evolution in the early leptosporangiate ferns. The asterisk (*) indicates bootstrap support (BS) values of 100.

## 3. Discussion

### 3.1. Ancient Polyploidization Plays an Important Role in the Evolutionary History of Plants

Polyploidy is significant in many ways in the evolutionary process of organisms [14], and its occurrence is prevalent in vascular plants, especially in ferns [16]. Here, we identified and located WGD events based on the optimal species tree. As expected, at least 11 WGD events were identified during the evolutionary history of early leptosporangiate ferns (Figures 4 and S3). In the literature, it is widely contested how polyploidization affects species diversification [13,40,41]. On the one hand, polyploidization is considered as an evolutionary "dead end" [42,43] that results in a lower diversification rate than that of diploid relatives. There is lacking evidence for WGD events that survive in the long term, and polyploids speciate more slowly and exhibit higher rates of extinction

than their diploid progenitors [44]. On the other hand, WGD events often cause higher diversification rates but only after a delay of potentially up to several million years [45,46]. Although we have not carefully investigated the link between species diversification rates and paleopolyploidization, it seems that with the occurrence of more WGD events, there is higher species diversity and a higher diversification rate in clades (Figure 4). This partly indicates that ancient polyploidization, similar to ancient hybridization [47], is a major driver of rapid radiation [17].

In this study, we increased the species representation of early leptosporangiate ferns and used a large-scale transcriptomic dataset including 1125 1-to-1 orthologs; however, the reconstruction of a consistent species tree remained elusive (Figures 3 and 4). Potentially, these topological incongruences are the result of rapid diversification and the complicated phylogenetic network that exists among the four monophyletic lineages Hymenophyllaceae, Gleicheniaceae, Dipteridaceae, and Matoniaceae (Figures 4 and S4), which might also be the result of ancient polyploidization. In other words, the evolutionary histories of many organisms may have an inherent radiate pattern, such as trifurcate (Figure S4), owing to complicated biological processes, such as polyploidization and hybridization [48].

In addition to being a driver of rapid evolution, polyploidization might also lead to increased tolerance to a broader range of ecological and environmental conditions [49–51], leading to a better chance of survival during mass extinction events [17,52,53]. Based on our calculated divergence times, the ancestors of Matoniaceae, Dipteridaceae, Gleicheniaceae, and Hymenophyllaceae originated about 266 Ma [95% HPD = (233–351 Ma)] during the late Carboniferous and early Triassic periods (Figure 4) [54]. At that time, the most extensive mass extinction event known to date, referred to as the Permian-Triassic (P-T) event, occurred [55], whereby the eruption of the Siberian Traps exposed an enormous amount of fresh basalt and triggered $CO_2$ release, rapid global warming, and acid rain [56]. Regarded as an evolutionary bottleneck, the P-T event narrowed down the gene pool and left a small number of species to repopulate the Earth, upon which there were many empty and new niches. The early leptosporangiate ferns were among those to survive, which then flourished throughout the whole Mesozoic era, especially Dipteridaceae, Matoniaceae, and Gleicheniaceae [54,57,58]. The success of these families may be due to ancient polyploidization (Figure 4). In fact, it seems that polyploidization is strongly correlated with extinction events, in that almost every extinction event is linked to polyploidy events in the evolutionary history of life [13,15]. On the one hand, these polyploidy events may have enabled early leptosporangiate ferns to survive the extinction events. On the other hand, these extinction events may have generated massive vacant ecological niches, thus providing opportunities for heliophytes such as Dipteridaceae and gleichenioid ferns to flourish.

### 3.2. Matoniales Should Be Redefined as a New Ordinal Rank

After increasing the taxa coverage, especially in Matoniaceae, and decreasing the effect of ancient polyploidization, we reconstructed the phylogenetic relationships of the early leptosporangiate ferns with a large-scale transcriptomic dataset. Although phylogenetic conflicts persisted among the four families Hymenophyllaceae, Glecheniaceae, Dipteridaceae, and Matoniaceae following both coalescent- and concatenation-based analysis using different kinds of datasets, Matoniaceae was undoubtedly shown to be a sister group of Dipteridaceae (Figures 3 and 4), which is consistent with previous studies [28,59] and supported by our phylogenetic network (Figures 5 and S4). Furthermore, Gleicheniales was clearly a paraphyletic lineage (Figures 3 and 4), which was also reported by Shen et al., (2018) and Qi et al., (2018). In both our concatenation- and coalescent-based species trees, Matoniaceae and Dipteridaceae, as a monophyly, were clearly paraphyletic to Gleicheniaceae (Figure 4). Thus, these combined results suggest that Matoniaceae and Dipteridaceae should be redefined as a new order.

For a long time, pteridologists have recognized significant differences among the Matoniaceae, Dipteridaceae, and Gleicheniaceae [60], so that Matoniaceae and Dipteridaceae have been treated into the two orders, Matoniales [30] and Dipteridales [29], respectively.

Soon after, Mationaceae and Dipteridaceae were merged into Gleicheniales along with Gleicheniaceae, based on monophyletic evidence [28,59,61] and some morphological traits such as root steles with 3–5 protoxylem poles [62] and antheridia with 6–12 narrow, twisted, or curved cells in walls [63], which usually are called gleichids because of common traits of forking branches. In fact, these traits are very common among early leptosporangiate ferns, such as *Schizaea dichotoma* (Schizaeaceae), *Lygodium digitatum* (Lygodiaceae), *Regnellidium diphyllum* (Marsileaceae), and *Gonocormus minutus* (Hymenophyllaceae), and therefore do not provide the classification significance for high order rank. Furthermore, this previous classification is challenged by both our results and those of previous phylogenomic studies [19,22], which supported that Matoniaceae and Dipteridaceae are a monophyletic group but are paraphyletic with Gleicheniaceae, so they should not be merged into the order Gleicheniales. Morphologically, the species in these two families share a number of common morphological characters, such as a creeping rhizome, dichotomous or fan-like split leaves (Figure 6), and flattened sporangia with slightly oblique or sub-erected annulus [64]; they are more or less similar in their spore morphologies (sub-triangular or elliptisoidal spore shape with trilete marks or monolete) [65], and they are mainly distributed in tropical regions in Asia, extending to China and Japan [60,66,67]. Moreover, the sister group of Gleicheniales and Hymenophyllales shared some common sporangia located on the elongated receptacles, with equatorial transverse-oblique annulus and not interrupted by very short stalk, although they look very different in habit [59,60,64].



**Figure 6.** The habit of species from Matoniaceae and Dipteridaceae. (**A**): *Matonia pectinata*; (**B**): *Dipteris lobbiana*; (**C**): *Dipteris chinensis*; and (**D**): *Cheiropleuria bicuspis*.

Based on this robust evidence from phylogenomics and morphology, we propose to redefine a general ordinal rank of Matoniales from Dipteridales Doweld (2001) and Matoniales Reveal (1993) based on the International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) [68]. This order should be composed of extant Dipteridaceae (including *Cheiropleuria* and *Dipteris*) and Matoniaceae (*Matonia* and *Phanerosorus*), with a total of 15 species [20,63], which flourished with widespread global distribution throughout the Mesozoic era together with their related group of Gleicheniaceae [54,69–71]. Certainly, the redefinition of order Matoniales will provide new insights regarding the diversification and prosperity of fossil genera of Matoniaceae and Dipteridaceae, such as *Clathropteris*, *Dictyophyllum*, *Hausmannia*, *Goeppeetella*, *Camptopteris*, *Thaumatopteris*, *Phlebopteris*, *Matonidium*, *Weichselia,* and so on [57,69,72–75].

An order is a higher taxonomic rank in biological classification, and most of the accepted orders in extant land plants are long-established [20,76]. Only a few new orders of land organisms, such as Mantophasmatodea [6,77] and Hypopterygiales [9], have been

recently published and accepted based on phylogenomic evidence. In the extant lycopods and ferns, more than 40 valid orders were reported, but only 14 of these are accepted in PPG I [20,63]. However, more careful studies may be required to verify the treatments of these synonym names in the future [78]. In this study, as an example, we have reconstructed the new order Matoniales from the synonym names with phylogenomic analysis, which was opposed to previous treatments.

## 4. Materials and Methods

### 4.1. Taxon Sampling

Taxon sampling bias was considered as an important factor affecting the phylogenetic relationship [36]. According to PPG I, gleichenioid ferns include approximately 172 species in 10 genera in the three families Dipteridaceae, Matoniaceae, and Gleicheniaceae [20]. In this study, we collected samples of 12 species of gleichenioid ferns in 7 genera representing the 3 families, in particular Matoniaceae, and 12 species of filmy ferns in seven genera. For other early leptosporangiate ferns, we used published data, including those for Osmunda japonica (Osmundaceae), *Lygodium flexuosum* (Lygodiaceae), *Schizaea dichotoma* (Schizaeaceae), and *Anemia phyllitidis* (Anemiaceae), the latter of which was a newly sequenced species. In addition, *Equisetum diffusum* (Equisetaceae) and *Angiopteris fokiensis* (Marattiaceae) as the outgroups and *Marsilea quadrifolia* (Marsilieaceae) as a proxy of core leptosporangiate ferns were used in this study. All samples were collected with permission from the natural reserves of Shanghai Chenshan Botanical Garden in China, and the voucher specimens are kept in the Shanghai Chenshan Herbarium (CSH). The information of materials is shown in Table S2.

### 4.2. Data Acquisition and Quality Assessment

We performed RNA extraction, sequencing, and transcriptome assembly as per in our previous study [19]. In order to assess the gene coverage of each assembly, we performed Basic Universal Single Copy Orthologs (BUSCOs) analysis using a core set of orthologs conserved in Embryophyta species (Embryophyta odb9) [79]. The description of transcriptome data was shown in Table S2.

### 4.3. Phylogenetic Tree and Network Analysis

In order to decrease the effect of paralogs on phylogenetic analysis [80], 1-to-1 orthologs prediction (also illustrated in Figure S1) was in compliance with Shen et al., (2018), but we restricted at least 16 (51.6%) species in each 1-to-1 orthologs matrix, which could decrease the effect of polyploidization on phylogenomic reconstruction [81]. We performed the phylogenomic analysis based on both the coalescent and concatenation methods using three kinds of datasets, including DNA, protein, and especially the first two codons, because saturated third codon positions might radically influence the reconstruction of phylogenetic trees under multiple WGDs [82,83]. In coalescent-based analysis, each gene tree was constructed by RAxML v8.2.4 [84] with the PROTCAT JTTF and GTR model for protein and DNA sequences, respectively; 100 random replicates were performed to calculate bootstrap probabilities. The coalescent-based species tree was constructed by ASTRAL v4.10.4 [85] with 100 random replicates of multilocus bootstrapping [86]. In concatenation-based analysis, a multiple-gene supermatrix was constructed by concatenating the 1-to-1 orthologs matrix, and we created the maximum likelihood (ML) tree by RAxML v8.2.4, with the GTR + $\Gamma$4 + I model for the DNA and codon matrices and the JTTF model for the corresponding protein matrix. Following this, tree visualization was performed using FigTree v1.4.3 (http://tree.bio.ed.ac.uk/software/figtree/, accessed on 16 January 2021). Due to numerous complicated evolutionary processes, a phylogenetic network is required in order to graphically represent a reticulate evolutionary history [48]. In this study, we reconstructed the evolutionary network of the early leptosporangiate ferns with SplitsTree4 v4.15.1 [87]. In order to assess the bootstrap support (BS), we performed 1000 random

replicates using the Neighbor-Net methods [88] with the Kimura 2-parameter model, and *E. diffusum* was used as the outgroup to infer a rooted phylogenetic network.

### 4.4. Divergence Time Estimation

To date divergence times, we used the concatenated alignment of orthologs and calibrated with the ages of two fossils—*Senftenbergia plumosa* [89]: 354 Ma, and *Hopetedia praetermissa* [90]: 228 Ma—as the minimum age of leptosporangiate ferns and Hymenophyllaceae, respectively, and a maximum age constraint of 450 Ma for monilophytes in a Bayesian relaxed clock method using MCMCTree in PAML v4.9i [91] based on the same robust phylogenetic tree constructed by coalescent and concatenation methods with the DNA and codon matrices.

### 4.5. Polyploidization Inference and Localization

Due to the many limitations of Ks plot analysis [92], especially that it cannot detect more ancient WGD events [93–95], which may be prevalent in ferns, we employed a gene-tree sorting and counting algorithm, namely, Multi-tAxon Paleopolyploidy Search (MAPS) [96], to locate WGD events with full gene family dataset, including all paralogs. This algorithm uses a given species tree to filter for subtrees within complex gene trees consistent with relationships at each node in the species tree. WGDs will produce a large burst of shared duplications across taxa and gene trees. We filtered gene trees with less than 16 (51.6%) taxa and defined a WGD as a threshold duplication percentage of more than 10% [15] because lower percentages might result from tandem duplications.

### 4.6. Species Diversification Rates

Understanding how diversification rates vary through time and across species groups is vital to understanding the emergence of the current biodiversity on Earth [97]. However, variation in speciation and extinction rates may exist both across lineages and through time [98]. Here, we used a Bayesian model-based approach, namely, ClaDS of R script (https://github.com/OdileMaliet/ClaDS, accessed on 20 May 2021) [97], to quantify the lineage-specific speciation rates on the basis of our phylogenetic tree with time estimation. The extinction rate data was sourced from Clapham and Renne (2019) with the authors' permissions.

## 5. Conclusions

To decrease the effect on phylogenetic reconstruction from the WGDs, we have reconstructed the evolutionary history of early leptosporangiate ferns through representative species sampling and analysis of large-scale transcriptomic data by 1-to-1 orthologs. Following careful phylogenomic analysis, we found that the phylogenetic relationships of early leptosporangiate ferns are not bifurcate but rather have a trifurcate pattern due to rapid radiate evolution caused by ancient polyploidization. We propose that Matoniales, which include Dipteridaceae and Matoniaceae, should be redefined as a new ordinal rank based on powerful evidence from 1-to-1 orthologs trees, as with the first two codons. Furthermore, our study found that paleopolyploidization played an important role in the evolutionary history of early leptosporangiate ferns, potentially enabling the survival of these ferns during the P-T mass extinction event and promoting speciation throughout the Mesozoic era.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/plants11121529/s1, Figure S1: The pipeline of 1-to-1 orthologs prediction; Figure S2: The timetree of early leptosporangiate ferns; Figure S3: The inference of WGD events in early leptosporangiate ferns; Figure S4: The rooted reticulate evolution in the early leptosporangiate ferns; Table S1: Summary of main studies of basal leptosporangiate ferns; Table S2: The information of samples and transcriptome data used in this study.

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