Scientific Research Publishing

Open Journal of Geology, 2024, 14, 247-258
https://www.scirp.org/journal/ojg
ISSN Online: 2161-7589
ISSN Print: 2161-7570

Inferring Eupolypods Divergence Time Using Bayesian Tip-Dating

Yiran Wang[1,2], Chunxiang Li[1,3*]

[1]Key Laboratory of Palaeobiology and Petroleum Stratigraphy, Nanjing Institute of Geology and Palaeontology, Chinese Academy of Sciences (NIGPAS), Nanjing, China
[2]School of Resources and Earth Sciences, China University of Mining and Technology, Xuzhou, China
[3]Department of Cenozoic Biological Evolution and Environment, NIGPAS, Nanjing, China
Email: *cxli@nigpas.ac.cn

How to cite this paper: Wang, Y.R. and Li, C.X. (2024) Inferring Eupolypods Divergence Time Using Bayesian Tip-Dating. *Open Journal of Geology*, **14**, 247-258.
https://doi.org/10.4236/ojg.2024.142013

Received: January 8, 2024
Accepted: February 24, 2024
Published: February 27, 2024

Abstract

According to the most recent Pteridophyte Phylogeny Group (PPG), eupolypods, or eupolypod ferns, are the most differentiated and diversified of all major lineages of ferns, accounting for more than half of extant fern diversity. However, the evolutionary history of eupolypods remains incompletely understood, and conflicting ideas and scenarios exist in the literature about many aspects of this history. Due to a scarce fossil record, the diversification time of eupolypods mainly inferred from molecular dating approaches. Currently, there are two molecular dating results: the diversification of eupolypods occurred either in the Late Cretaceous or as early as in the Jurassic. This study uses the Bayesian tip-dating approach for the first time to infer the diversification time for eupolypods. Our analyses support the Jurassic diversification for eupolypods. The age estimations for the diversifications of the whole clade and one of its two subclades (the eupolypods II) are both in the Jurassic, which adds to the growing body of data on a much earlier diversification of Polypodiales in the Mesozoic than previously suspected.

Keywords

Eupolypods, Mid-Cretaceous, Fossils, Bayesian Tip-Dating

1. Introduction

The PPG classification, a comprehensive phylogeny-based classification of pteridophytes (including ferns and lycophytes), was established by the Pteridophyte Phylogeny Group in 2016 [1], and this classification has been widely accepted and cited in recent years. Based on the PPG classification, eupolypods, composed of two clades: eupolypods I and eupolypods II, include nearly 6000 species—more than half of extant fern diversity [1]. However, there are two different hypotheses on the divergence time for eupolypods based on molecular dating: Cretaceous diversification or a Jurassic one. Most DNA-based divergence-time studies indicate that eupolypods diversification occurred in the Late Cretaceous [2] [3] [4] [5] [6], but more recent studies demonstrated that eupolypods originated as early as in the Jurassic [7] [8] [9]. However, none of these multiple published DNA-based divergence-time studies utilized both morphological and molecular data, much less that have integrated both types of data into a total evidence (tip-dating) approach. This tip-dating approach allows for the inclusion of morphological data in divergence-time estimates based on both extant and extinct taxa.

So far there are three different approaches to date the diversification time for an organism group. At the very beginning when calibrating a tree, the rate-dating (clock rate) method was applied; subsequently, researchers used to date the tree based on node calibrations (node-dating), and finally, researchers used total-evidence dating (TED or tip-dating), in which the tree is dated based on simultaneous analysis of fossil and recent taxa [10] [11] [12]. Compared to the first two dating methods, the tip-dating approach of phylogenetic divergence-time estimations has been revolutionized by two recent developments: 1) the approach of tip-dating allows for the incorporation of fossils as tips in the analysis, with their phylogenetic and temporal relationships to the extant taxa inferred from the data, and 2) the fossilized birth-death (FBD) class of tree models that capture the processes that produce the tree (speciation, extinction, and fossilization) and thus provide a coherent and biologically interpretable tree prior [13]. In this study, we apply an integrative tip-dating approach that combines molecular and morphological data to re-estimate divergence times for eupolypods—the most diverse of all major lineages of ferns in the light of the stratigraphic records. In this study, we will apply the new molecular dating approach to attempt to answer the question of what geological time eupolypods start to diversify from, thereby increasing our understanding of its evolutionary history.

2. Analysis Methods

2.1. Sampling Taxa Set and Assembling Dataset

So far there are some studies on divergence times reported to date the diversification time for eupolypods, after reviewing the main and influential studies of fern phylochrological analyses (**Figure 1**, **Table 1**), we found only the analyses of Regalado *et al.* [8] focused on relationships among eupolypods lineages. To evaluate the possible relationships between fossil *Holttumopteris burmensis* Regalado *et al.* [8] and extant eupolypods lineages, Regalado *et al.* [8] mapped character states of the fossil *H. burmensis* on a reconstructed comprehensive, time-calibrated phylogeny of eupolypod ferns. Therefore, we used those two datasets of Regalado *et al.* [8], both morphological character matrix and DNA sequence matrix can be downloaded from the online resource of Regalado *et al.*

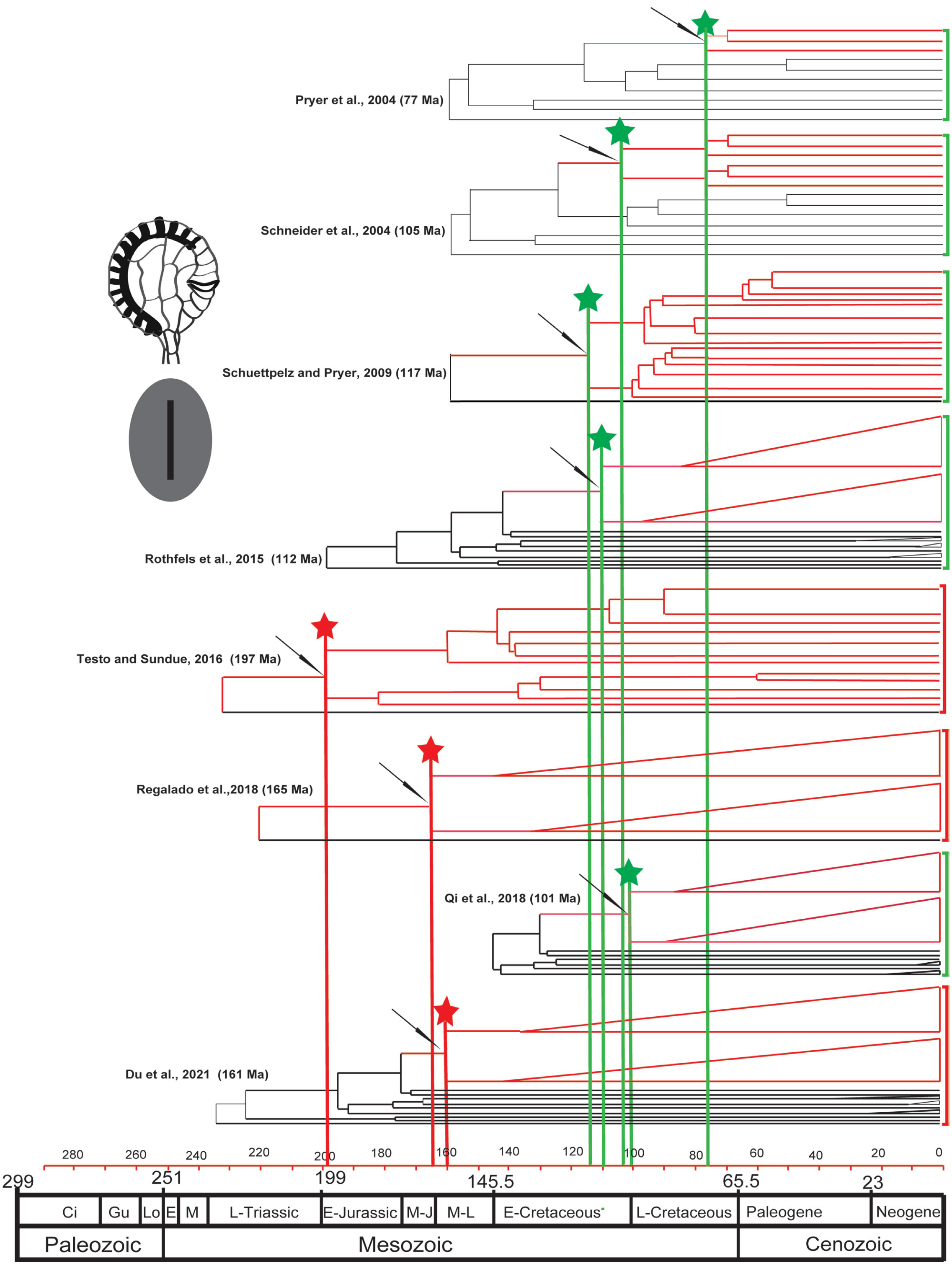


Figure 1. The divergence time estimates for eupolypod ferns (clades in red lines) are simplified from previous main studies for fern phylochrological analyses (Table 1). Mean ages in brackets after references are shown on the right side. Black arrows at nodes indicate the clades of eupolypod ferns where the mean ages are shown. Stars with lines in red indicated that the diversifications of eupolypods occurred in the Cretaceous, and green stars and lines indicated that the diversifications of eupolypods dated back in the Jurassic. Two main apomorphic characters of eupolypod ferns (sporangia with vertical annulus interrupted by the sporangium stalk and monolete spores) are shown on the right side too.

Table 1. Summary of fern phylochrological analyses in previous studies and this study.

Study	Phylegenetic depth	Eupolypods/ Ferns sampled	Characters used	Dating methods	Eupolypods/Ferns Fossils used
Du *et al.* [9]	Polypodiales	162/214	Plastid 84 protein-coding genes and four rRNA genes,76 448 bp	penalized likelihood (treePL), Bayesian inference (BEAST), three root age constraints	node calibrations 6/14
Qi *et al.* [6]	Vascular plants: ferns, lycophytes, seed plants	70/129	935, 501, 348, 267 and 146 nuclear gene sets from transcriptomes	penalized likelihood (treePL)	node calibrations 4/17
Rothfels *et al.* [5]	Ferns, seed plants	31/73	25 nuclear loci 35 877 bp from transcriptomes	Bayesian methods (MrBayes version 3.2.2)	second node calibrations, 2/12
Regalado *et al.* [8]	Eupolypods	199/203	Three plastid genes (*rbcL*, *atpA*, and *atpB*; 3826 bp)	Rating dating, *i.e.*, using the standard substitution rate for plastid DNA	No calibrations 0/0
Testo and Sundue [7]	Ferns, lycophytes	2468/3973	Six chloroplast markers (*atpB*, *rbcL*, *rps4* gene, *rps4-trnS* IGS, *trnL* gene, *trnL-trnF* IGS)	Penalized likelihood (treePL)	node calibrations 7/26
Schuettpelz and Pryer [4]	Leptosporangiate ferns	242/400	Three plastid genes (*rbcL*, *atpA*, and *atpB*; >4000 bp)	Penalized likelihood in r8s version 1.71	node calibrations 5/24
Pryer *et al.* [2]	Vascular plants: ferns, lycophytes, seed plants	6/51	Four genes (plastid *rbcL*, *atpB*, *rps4*, and nuclear 18S rDNA)	Penalized likelihood in r8s version 1.60	node calibrations 1/21
Schneider *et al.* [3]	Ferns, seed plants	19/42	Two plastid genes (*rbcL*, *rps4*)	Penalized likelihood in r8s version 1.60	node calibrations 2/14
This study	Eupolypods	214/218	Three plastid genes (*rbcL*, *atpA*, and *atpB*; 3841 bp)	Bayesian Inference (MrBayes version 3.2.7a)	Tip calibrations 9/9

[8]. We added nine fossil taxa and six extant taxa to that combined dataset. Fossils included in this study and their information are shown in **Table 2**. The six additional extant species are from Desmophlebiaceae (one taxon), grammitid ferns (two taxa), and *Drynaria* (three taxa) that were not included in the dataset of Regalado *et al.* [8]. Their DNA sequences were downloaded from GenBank, and their GenBank accession numbers are followed by the species name and are shown in the chronogram of eupolypod ferns (**Figure 2**). For the data matrix of morphological characters, four more characters are added to the dataset of Regalado *et al.* [8], which includes annulus cell number of a sporangium, vascular bundle number of a petiole, spore types based on [14], and frond dimorphism. Subsequently, we combine DNA sequence data with morphological character data from 9 fossil and 209 extant taxa (2003 taxa of eupolypods and four taxa of basic polypods as outgroup members) to simultaneously infer the phylogeny and re-estimate divergence times for eupolypod ferns by a total-evidence (tip-dating) approach. We produced a matrix of 15 morphological characters coded for a

Table 2. Fossils included in this study and their information.

Fossil taxa	Selected references	Geological age and locality	Fossil ages (Ma) and prior assignments
Athyrium cretaceum Chen et Meng	Chen *et al.* [18], Deng and Chen [19], Li *et al.* [20]	Neocomian (Hauterivian-Barremian), Lower Cretaceous, Liaoning, northeastern China	Uniform (100, 145.00)
Davallia walkeri Conran, U. Kaulfuss, Bannister, Mildenhall et D. E. Lee	Conran *et al.* [21]	Early Miocene, Foulden Maar diatomite deposit, Otago, New Zealand	Uniform (20.44, 23.03)
Drynaria dimorpha J. Y. Wu et B. N. Sun	Wu *et al.* [22]	Mangbang Formation, upper Pliocene, Yunnan Province, China	Uniform (2.58, 3.60)
Holttumopteris burmensis L. Regalado, H. Schneid., M. Krings et Heinrichs	Regalado *et al.* [8]	late Albian to earliest Cenomanian, Lower Cretaceous, Kachin State, northern Myanmar	Fixed (100.50)
Onoclea sensibilis L.	Pigg and Rothwell [23], Rothwell and Stockey [24]	Paleocene, Paskapoo Formation, central Alberta, Canada	Fixed (55.80)
Protodrynaria takhtajanii Vikulin et Bobrov	Vikulin and Bobrov [25]	Paleogene flora of Tim in Russia	Fixed (33.90)
Thelypteris sp. Aline M. Homes *et al.*	Homes *et al.* [26]	late Eocene Pikopiko Fossil Forest, southern New Zealand	Fixed (34.40)
Woodwardia changchangensis Naugolnykh et Song	Song *et al.* [27]	middle Eocene of the Changchang Basin, Hainan Island, South China	Uniform (33.90, 56.00)
Woodwardia virginica (L.) J. E. Smith	Pigg and Rothwell [23]	middle Miocene Yakima Canyon flora of central Washington State, USA	Fixed (15.60)

sample of 218 fern species, the taxa were chosen to represent the phylogenetic diversity of the family level following Regalado *et al.* [8]. We also used sequences of three plastid genes (*rbcL*, *atpA*, and *atpB*; 3841 aligned positions).

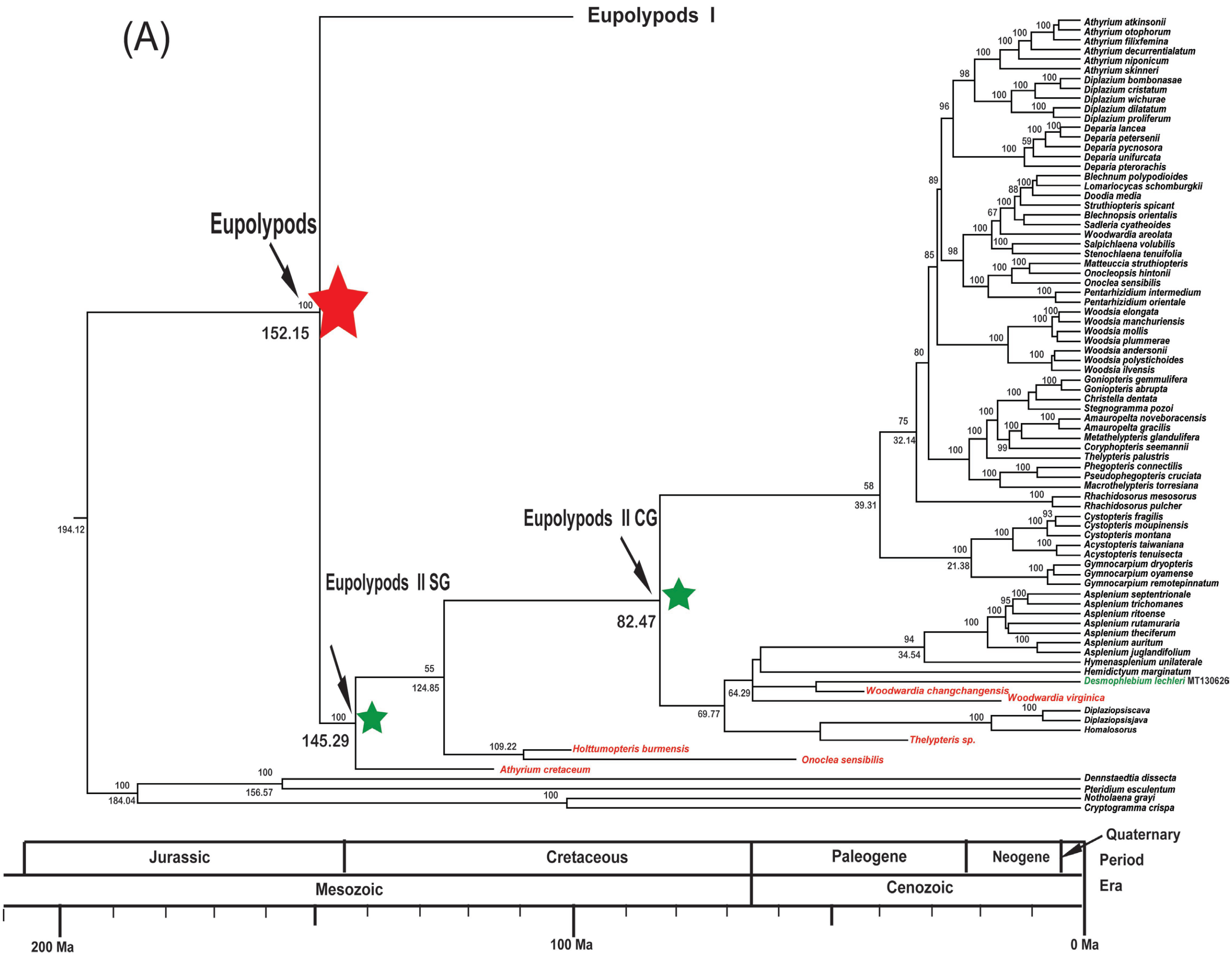
2.2. Set Calibration Priors for Bayesian Tip-Dating Analyses

Bayesian tip-dating analysis using the fossilized birth-death process (FBD) was previously conducted only for one fern lineage, the Marattiales [13]. They fitted a variety of complex, hierarchical Bayesian models that varied in assumptions regarding how morphological characters evolve, how evolutionary rates vary across the tree, and whether rate shifts are coincident with geologic intervals [13]. May *et al.* [13] used stepping-stone analysis [15] to estimate marginal likelihoods for each model to determine the best-fit model. We applied the same best-fit model configuration as determined by [13] to our updated character data matrix. Our tip dating analyses were performed in Mrbayes 3.2.7a [11] [16] following the manuals downloaded from http://mrbayes.net. The FBD model was used as the tree prior, the ages of fossil terminals were provided as uniform or fixed priors with bounds equal to the limits of the estimated ages of their deposits (**Table 2**). We used a "diversity" setting in the sampling strategy since we strived to include as many eupolypods terminals as possible, and set the sample probability prior to 0.0178; this was done because we included 214 terminals, while the diversity of the eupolypods lineage is currently of about 6000 species and we expected it contains about that same number of undescribed species, so, 214 (eupolypods taxa this study) divided by (6000 + 6000, the total diversity of the eupolypods) is equal to 0.0178. Our analyses were run for 20 million generations, sampling every 5000 generations. Bayesian posterior probabilities (PP) were calculated for the majority rule (>50%) consensus tree of all sampled trees after discarding the first 25% as burn-in. Visualized trees and all the nodes were checked in FigTree v.1.4 [17].

3. Results

3.1. Tip-dating Analyses

Tip-dating analyses of the total-evidence dataset (combined 3841 molecular characters and 15 morphological characters) resulted in a total of 214 eupolypod taxa falling into two separate clades: eupolypod I with 126 taxa and eupolypod II with 78 taxa (**Figure 2**). The age estimation for the diversification of eupolypods is in Jurassic, mean 152.15 Ma, with a 95% highest posterior density (HPD) interval of 128.76 - 178.80 Ma, the diversification of eupolypod II also occurred in



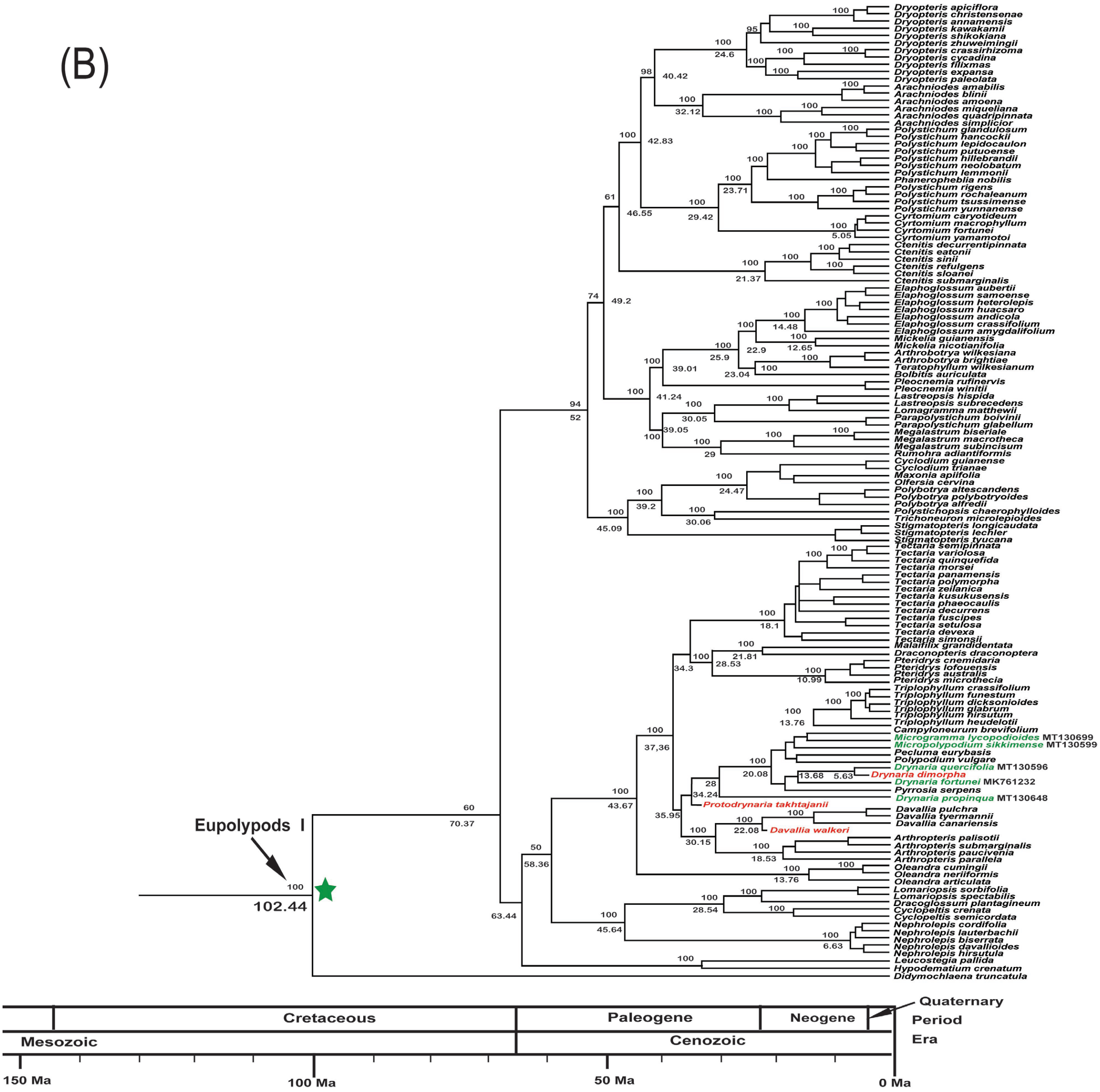


Figure 2. The chronogram of eupolypod ferns using tip-dating approach with the Fossilized Birth-Death model in a Bayesian framework. Nine fossil taxa are in red, six species in green with a Genbank accession number are the extra taxa added to the dataset [8]. Numbers above the branches indicate posterior probability (pp%, only shown over 50), numbers below the branches indicate the mean age (Ma, only shown for the main clades), and the nodes where the main lineages of eupolypods diversified are marked with a star. SG, stem group; CG, crown group. (A) the Eupolypods II part, (B) the Eupolypods I part.

Jurassic, mean 145.29 Ma, with a 95% HPD interval of 121.05 - 165.88 Ma, while eupolypod I diversified later, which is in Cretaceous, mean 102.44 Ma, with a 95% HPD interval of 71.96 - 136.22 Ma (Figure 2).

3.2. Phylogenetic Positions of Fossils

Our tip-dating tree shows that the phylogenetic placements of fossils are mostly in accordance with their previous taxonomic allocations, among the nine fossil records, six fossils are supposed to belong to eupolypod II based on previously published morphologic descriptions and their taxonomic status, and as be predicted based on morphological studies, they all occupied the stem positions of eupolypod II lineage in our tip-dating tree (**Figure 2**). Three fossils are supposed to belong to eupolypod I are all in the lineage of eupolypod I. However, only three fossils of eupolypod I have more concrete and accurate phylogenetic placements, for an example, the fossil *Davallia walker* [21] is clustered with its extant *Davallia* relatives (**Figure 2**).

We suppose that providing a more concrete and accurate phylogenetic placement for those fossils that are dated back as old as the origin of the whole lineage is challenging, because tip-dating analyses allow the fossil age to inform about their phylogenetic hypothesis. More fossils as well as larger DNA sequence datasets for extant taxa may compensate for the limited number of morphological characters identifiable on fossils, serving as a backbone for reliably estimating the phylogeny of both extinct and extant taxa.

4. Discussions

4.1. Diversification of Eupolypods

So far, there have been eight main and influential studies of fern phylochronological (divergence time estimation) analyses related to the diversification of eupolypods, five of the eight previous studies supported a Cretaceous diversification of eupolypods, the more recent three studies supported a Jurassic diversification of eupolypods (**Table 1**), our tip-dating analyses supported the second hypothesis. This result is nevertheless important because it provided another estimation based on the recently developed tip-dating analyses that eupolypods originated before the Cretaceous, as early as the Jurassic. This result is consistent with the few divergence time estimates based on the different molecular dating methods (node-dating [7] [9] and rate-dating [8]). On the other hand, the fossil record has always been considered as crucial information as an independent source of evidence for diversification of eupolypods. Recent studies on the Myanmar amber fossils (late Albian-earliest Cenomanian, Cretaceous, c. 100. 5 Ma) have recorded remarkable fossils—both lineages of eupolypods has at least one representative fossil: eupolypod II with the fossil of Thelypteridaceae [8] and eupolypod I with fossil of Dryopteridiaceae [28] [29]. Both fossils mentioned above and newly available fossils (scales and spores assigned to eupolypods [30]) further substantiate that Polypodiales were more diverse and abundant in the mid-Cretaceous forest ecosystems of Myanmar clearly showing that eupolypods originated before the mid-Cretaceous, probably as early as the Early Jurassic [30], this is consistent with the few divergence time estimates based on other molecular dating studies [7] [8] [9] and our tip-dating analyses.

4.2. Bayesian Tip-Dating

Extinct organisms, *i.e.*, the fossils, could provide crucial information about the origin and time of origination of extant groups, however, in previous studies (**Table 1**), stratigraphic age ranges of fossils were used to calibrate molecular clock analyses only (the traditional node-dating method), morphological characters, however, were not directly incorporated. The very early rate-dating method, using the standard substitution rate of nucleic acids or amino acids, did not use the information provided by the fossils at all. The importance of morphological phylogenetics for rigorously dating the Tree of Life is now widely recognized and has been revitalized by methodological developments such as the application of tip-dating Bayesian approaches [31]. The introduction of tip calibrations allows fossil species to be included alongside their living relatives, which overcomes several drawbacks in the traditional stepwise approach to some extent, however, it also requires more knowledge about statistics and more priors need to be done when running the program. How to set the priors for the divergence times, the priors for the evolutionary rates, and the models for the character changes that are all key components in this method, probably continue to be a significant challenge for its users [16].

5. Conclusion

For the first time, this study combined morphological data from extinct and extant taxa with DNA sequence data to estimate diversification ages for eupolypods, the most diverse of all major lineages of ferns. Not only did we obtain the age estimates, but also the phylogenetic positions of fossils in one tip-dating analysis. The results further strengthen previous Jurassic diversification hypotheses for eupolypods. This is undoubtedly a successful experimentation. Hence, this study unequivocally validates fossil tip-dating as a promising tool within a phylogenetic framework. To bring more details regarding the fern evolutionary history to light, more studies using tip-dating methods are currently underway.

Acknowledgements

The authors wish to thank the editors and anonymous reviewers for constructive suggestions and comments that have improved the paper. This research was supported by the Basic Frontier Scientific Research Program of the Chinese Academy of Sciences (No. ZDBS-LY-DQC021-02). The authors deeply appreciate the support.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] PPG I (2016) A Community-Derived Classification for Extant Lycophytes and Ferns. *Journal of Systematics and Evolution*, **54**, 563-603. https://doi.org/10.1111/jse.12229

[2] Pryer, K.M., Schuettpelz, E., Wolf, P.G., Schneider, H., Smith, A.R. and Cranfill, R.

(2004) Phylogeny and Evolution of Ferns (Monilophytes) with a Focus on the Early Leptosporangiate Divergences. *American Journal of Botany*, **91**, 1582-1598. https://doi.org/10.3732/ajb.91.10.1582

[3] Schneider, H., Schuettpelz, E., Pryer, K.M., Cranfill, R., Magallon, S. and Lupia, R. (2004) Ferns Diversified in the Shadow of Angiosperms. *Nature*, **428**, 553-557. https://doi.org/10.1038/nature02361

[4] Schuettpelz, E. and Pryer, K.M. (2009) Evidence for a Cenozoic Radiation of Ferns in an Angiosperm-Dominated Canopy. *Proceedings of the National Academy of Sciences, USA*, **106**, 11200-11205. https://doi.org/10.1073/pnas.0811136106

[5] Rothfels, C.J., Li, F.W., Sigel, E.M., Huiet, L., Larsson, A., Burge, D.O., Ruhsam, M., Deyholos, M., Soltis, D.E., Stewart, C.N., *et al.* (2015) The Evolutionary History of Ferns Inferred from 25 Low-Copy Nuclear Genes. *American Journal of Botany*, **102**, 1089-1107. https://doi.org/10.3732/ajb.1500089

[6] Qi, X.P., Kuo, L.Y., Guo, C., Li, H., Li, Z., Qi, J., Wang, L., Hu, Y., Xiang, J., Zhang, C.F., Guo, J., Huang, C.H. and Ma, H. (2018) A Well-Resolved Fern Nuclear Phylogeny Reveals the Evolution History of Numerous Transcription Factor Families. *Molecular Biology and Evolution*, **127**, 961-977. https://doi.org/10.1016/j.ympev.2018.06.043

[7] Testo, W. and Sundue, M. (2016) A 4000-Species Dataset Provides New Insight into the Evolution of Ferns. *Molecular Biology and Evolution*, **105**, 200-211. https://doi.org/10.1016/j.ympev.2016.09.003

[8] Regalado, L., Schmidt, A.R., Krings, M., Bechteler, J., Schneider, H. and Heinrichs, J. (2018) Fossil Evidence of Eupolypod Ferns in the Mid-Cretaceous of Myanmar. *Plant Systematics and Evolution*, **304**, 1-13. https://doi.org/10.1007/s00606-017-1439-2

[9] Du, X.Y., Lu, J.M., Zhang, L.B., Wen, J., Kuo, L.Y., Mynssen, C.M., Schneider, H. and Li, D.Z. (2021) Simultaneous Diversification of Polypodiales and Angiosperms in the Mesozoic. *Cladistics*, **37**, 518-539. https://doi.org/10.1111/cla.12457

[10] Ronquist, F., Klopfstein, S., Vilhelmsen, L., Schulmeister, S., Murray, D.L. and Rasnitsyn, A.P. (2012) A Total-Evidence Approach to Dating, Applied to the Early Radiation of Hymenoptera. *Systematic Biology*, **61**, 973-999. https://doi.org/10.1093/sysbio/sys058

[11] Ronquist, F., Teslenko, M., Mark, P., van der Ayres, D., Darling, A., Höhna, S., Larget, B.L., Liu, M.A., Suchard and Huelsenbeck, J.P. (2012) MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice across a Large Model Space. *Systematic Biology*, **61**, 539-542. https://doi.org/10.1093/sysbio/sys029

[12] Zhang, C., Stadler, T., Klopfstein, S., Heath, T.A. and Ronquist, F. (2016) Total-Evidence Dating under the Fossilized Birth-Death Process. *Systematic Biology*, **65**, 228-249. https://doi.org/10.1093/sysbio/syv080

[13] May, M.R., Contreras, D.L., Sundue, M.A., Nagalingum, N.S., Looy, C.V. and Rothfels, C.J. (2021) Inferring the Total-Evidence Timescale of Marattialean Fern Evolution in the Face of Model Sensitivity. *Systematic Biology*, **70**, 1232-1255. https://doi.org/10.1093/sysbio/syab020

[14] Wang, Q.X. (2001) Study on the Spore Morphology of Polypodiales (Filieales) from China. PhD Dissertation, Northeast Forestry University, Harbin, 291 p.

[15] Xie, W., Lewis, P.O., Fan, Y., Kuo, L. and Chen, M.H. (2011) Improving Marginal Likelihood Estimation for Bayesian Phylogenetic Model Selection. *Systematic Biology*, **60**, 150-160. https://doi.org/10.1093/sysbio/syq085

[16] Zhang, C. (2021) Using Bayesian Tip-Dating Method to Estimate Divergence Times and Evolutionary Rates. *Vertebrata Palasiatica*, **4**, 333-341. (In Chinese)

[17] Rambaut, A. (2012) FigTree, Version 1.4. http://tree.bio.ed.ac.uk/software/figtree

[18] Chen, F., Deng, S.H. and Sun, K.Q. (1997) Early Cretaceous Athyrium Roth from Northeastern China. *Palaeobotanist*, **46**, 117-133. https://doi.org/10.54991/jop.1997.1356

[19] Deng, S.H. and Chen, F. (2001) The Early Cretaceous Filicopsida from Northeast China. Geological Publishing House, Beijing, 374 p.

[20] Li, C.X., Ma, J.Y., Hao, J.S. and Yang, Q. (2023) On the Systematic Position of Early Cretaceous Fern Genus “Athyrium”. *Palaeoworld*, **32**, 116-123. https://doi.org/10.1016/j.palwor.2022.07.003

[21] Conran, J.G., Kaulfuss, U., Bannister, J.M., Mildenhall, D.C. and Lee, D.E. (2010) Davallia (Polypodiales: Davalliaceae) Macrofossils from Early Miocene Otago (New Zealand) with *in Situ* Spores. *Review of Palaeobotany and Palynology*, **162**, 84-94. https://doi.org/10.1016/j.revpalbo.2010.06.001

[22] Wu, J.Y., Sun, B.N., Xie, S.P., Ding, S.T. and Wen, W.W. (2012) Dimorphic Fronds and *in Situ* Spores of Drynaria (Polypodiaceae) from the Upper Pliocene of Southwest China. *Review of Palaeobotany and Palynology*, **172**, 1-9. https://doi.org/10.1016/j.revpalbo.2012.01.007

[23] Pigg, K.B. and Rothwell, G.W. (2001) Anatomically Preserved *Woodwardia virginica* (Blechnaceae) and a New Filicalean Fern from the Middle Miocene Yakima Canyon Flora of Central Washington, USA. *American Journal of Botany*, **88**, 777-785. https://doi.org/10.2307/2657030

[24] Rothwell, G.W. and Stockey, R.A. (1991) *Onoclea sensibilis* in the Paleocene of North America, a Dramatic Example of Structural and Ecological Stasis. *Review of Palaeobotany and Palynology*, **70**, 113-124. https://doi.org/10.1016/0034-6667(91)90081-D

[25] Vikulin, S.V. and Bobrov, A.E. (1987) A New Fossil Genus Protodrynaria (Polypodiaceae) from the Paleogene Flora of Tim (the South of the Middle Russian Upland). *Botanicheskii Zhurnal*, **72**, 95-98. (In Russian)

[26] Homes, A.M., Cieraad, E., Lee, D.E., Lindqvist, J.K., Raine, J.I., Kennedy, E.M. and Conran, J.G. (2015) A Diverse Fern Flora Including Macrofossils with *in Situ* Spores from the Late Eocene of Southern New Zealand. *Review of Palaeobotany and Palynology*, **220**, 16-28. https://doi.org/10.1016/j.revpalbo.2015.04.007

[27] Song, H.Z., Naugolnykh, S.V., Wu, X.K., Liu, X.Y. and Jin, J.H. (2022) Fertile Woodwardia from the Middle Eocene of South China and Its Implications for Palaeogeography and Palaeoclimate. *Plant Diversity*, **44**, 565-576. https://doi.org/10.1016/j.pld.2021.09.003

[28] Poinar, G.O. and Buckley, R. (2008) *Cretacifilix fungiformis* gen. and sp. nov., an Eupolypod Fern (Polypodiales) in Early Cretaceous Burmese Amber. *Journal of the Botanical Research Institute of Texas*, **2**, 1175-1182.

[29] Regalado, L., Schneider, H., Mülle, P. and Schmidt, A.R. (2023) Character Evolution of Modern Eupolypods Supports the Assignment of the Fossil Fern *Cretacifilix fungiformis* to Dryopteridaceae. *Frontiers in Ecology and Evolution*, **11**, Article ID: 1162577. https://doi.org/10.3389/fevo.2023.1162577

[30] Li, C. and Zhang, L. (2019) Diversification of Eupolypods in Mid-Cretaceous—Evidenced by Myanmar Amber Forest. *Open Journal of Geology*, **9**, 726-730. https://doi.org/10.4236/ojg.2019.910086

[31] Dalmassoa, A., Peláez-Campomanesb, P. and López-Antoñanzas R. (2022) Relative Performance of Bayesian Morphological Clock and Parsimony Methods for Phylogenetic Reconstructions: Insights from the Case of Myomiminae and Dryomyinae Glirid Rodents. *Cladistics*, **38**, 702-710. https://doi.org/10.1111/cla.12516