

Molecular Insights into Pteridophytes Biosystematics

Article review

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Abstract—Plant classification is not only studied with morphological evidence but is associated with studies using molecular phylogeny approaches. Molecular phylogenetic studies can also be used to analyze plant biogeography. **Materials and methods:** Information on the Molecular Insights into Pteridophyte Phylogeny and Biosystematics of Pteridophytes was extracted from scientific journals (local, regional, and international) and other scientific databases. The publications identified totaled 342 articles; 325 were excluded because they did not meet the eligibility criteria, and 17 articles met the inclusion criteria. The results of reviews on national and international scientific publications over the past ten years found as many as 46 markers of nuclear DNA and plastid DNA used in several genera of Pteridophytes worldwide. The results of the study based on the phylogenetic approach concluded that four genera of pteridophytes are included in the monophyletic group consisting of the genus *Diplazium*, *Polypodium*, *Hymenosplenium*, and *Tectaria*. In contrast, one other genus is included in the non-monophyletic group, namely *Athyrium*, because *Athyrium skinneri* and *Athyrium alpestre* join the *Anisocampium* clade and *Cornopteris* belongs to the *Athyrium* clade.

Keywords— markers, nuclear DNA, plastid DNA, Classifications

I. INTRODUCTION

Molecular phylogenetic approaches have changed the paradigm in classification and understanding of the evolutionary history of Pteridophytes. The morphological evidence previously relied upon remains relevant, but the integration of molecular phylogenetic methods has opened the door to deeper insights into the position of species in the classification of Pteridophytes [1]. The findings from this study highlight that more than relying on morphological evidence alone is needed to understand the evolutionary relationships among Pteridophytes fully. By incorporating molecular data into the analysis, we can better understand the genetic diversity and phylogenetic relationships underlying the evolution of fern species. Molecular data provide invaluable additional insights, allowing us to identify relationships that may not be apparent through morphological characteristics alone. As such, this approach enriches the classification of Pteridophytes and helps to unravel their evolutionary history more accurately [2].

Pteridophytes using molecular phylogenetic approaches has opened the door to developing a new understanding of these species' evolution and geographic distribution [3]. The findings from this research are not only about classification but also contribute significantly to our understanding of how Pteridophytes have evolved and adapted to their environment through time. Molecular phylogenetic methods allow us to look deeper into natural history, exposing the journeys and adaptations of Pteridophytes in a way that is impossible to achieve through traditional fossil records. As such, these results enrich our knowledge of natural history and shift the paradigm in the study of fern evolution.

Through molecular analysis, this approach has successfully moved the focus away from the traditional fossil record towards a deeper understanding of the evolutionary dynamics of Pteridophytes. This is a revolution in research and a driver for further progress in viewing and interpreting the evolutionary journey of living things [4]. Thus, the importance of molecular

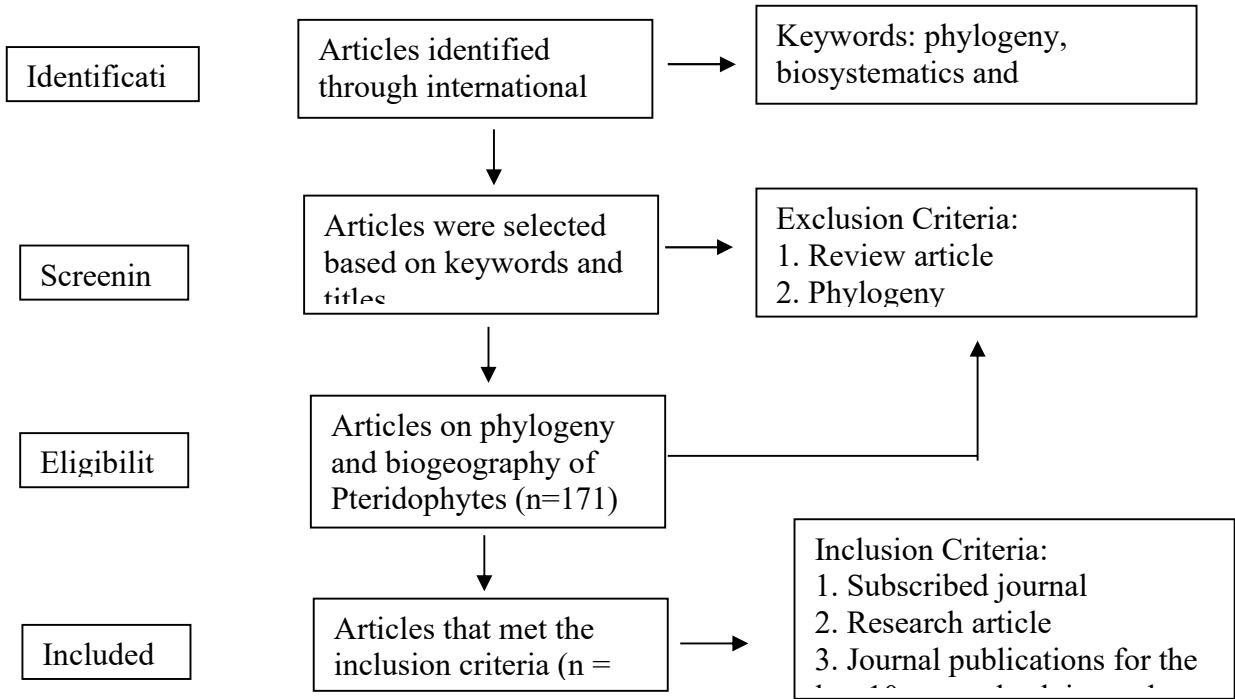
phylogenetic approaches in the context of fern biogeography not only broadens the horizons of classification but also opens a new page in evolution and adaptation research.

The molecular phylogenetic approach does not simply change the paradigm of fern classification; this approach opens a wide door to new opportunities to investigate and understand more deeply the evolutionary history and biogeography of Pteridophytes [5]. This paper will provide an overview of the development of phylogenetic studies in Pteridophytes over the past ten years based on a review of scientific article searches in international journals. The molecular phylogenetic approach not only shifts the paradigm of fern classification but also opens a wide door to new opportunities to investigate and understand Pteridophytes' evolutionary history and biogeography more deeply. This article aims to provide a comprehensive overview of the development of phylogenetic studies in Pteridophytes over the past ten years based on a search of scientific articles in international journals. By collecting and analyzing references from various articles, this study identifies recent trends and developments in molecular phylogenetic approaches to Pteridophytes. By summarizing the significant contributions of various studies, this article is expected to contribute to the global understanding of the evolution and biogeography of Pteridophytes and provide directions for further research in this field.

II. MATERIALS AND METHODS

The method used in preparing this review article involved collecting references from recent articles within ten years related to the phylogeny and biogeography of Pteridophytes. The process of searching for references was carried out through the Science Direct database using keywords such as "phylogeny," "biogeography," and "Pteridophytes." The selection of this database aims to ensure that the references collected are verified and relevant scientific sources. The search for articles was thorough and selective, with explicit inclusion and exclusion criteria applied. The articles selected had to discuss the phylogeny of Pteridophytes to ensure relevance to the research focus. In addition, journal selection was also considered by including articles from open-access and subscribed journals to ensure the quality and accessibility of information.

The analysis of the articles was done in a descriptive manner, which included summarizing and interpreting the information in the selected articles. This descriptive approach allows the reader to understand the essence of each article included in the review. As such, these review articles are not only a collection of information but also present a more in-depth understanding of recent developments in the phylogeny and biogeography of Pteridophytes. This methodological approach, which involves a thorough search, selection based on specific criteria, and descriptive analysis, is expected to provide a solid foundation for readers to understand the crucial contributions of recent articles related to the phylogeny and biogeography of Pteridophytes. Thus, this review article is a valuable source of information for researchers and practitioners in fern science.



III. RESULT AND DISCUSSIONS

A search of scientific publications was conducted on 342 articles to obtain the latest information on the phylogeny and biogeography of Pteridophytes. Of these, 328 articles were excluded because they did not meet the predetermined inclusion criteria, while ten articles met the inclusion criteria and became the main focus of the study. The flow of articles per year can be visually seen in Figure 1. The articles that passed the inclusion criteria selection underwent further review. In this process, each article was carefully re-examined based on several critical criteria, such as the name of the researcher, year of publication, plant studied, phylogenetic approach applied, DNA markers used, research methodology, and results obtained. This approach was taken to ensure the accuracy and quality of the data integrated into the study and to guarantee that the analytical results obtained have a high level of confidence. The detailed review process provided a solid foundation to ensure that the selected articles made relevant and reliable contributions to our understanding of recent developments in the phylogeny and biogeography of Pteridophytes. The literature search results reflecting research progress on the phylogeny and biogeography of Pteridophytes can be found in the collected details in Table 1.

The study of phylogeny and biogeography of Pteridophytes has dramatically expanded in the last ten years. Several leading research studies have discussed geographical distribution analysis and applied phylogenetic approaches to compiling a genus's biogeographic history. This can be learned from various published genera, including *Deparia* and *Diplazium* (Athyriaceae, Eupolypod II) [6] ; [7]. This study explicitly highlights the significant role of polyploidy in providing a deeper understanding of the geographic range expansion of these Pteridophytes. In the context of the genus *Deparia*, phylogenetic reconstructions based on four cpDNA genes (*rps16-matK IGS*, *trnL-LF*, *matK*, and *rbcL*) revealed that *Deparia* originated on the Asian/East Asian continent about 27.7 million years ago. This analysis also sheds light on the restricted distribution of this genus, mainly found in East Asia, Northeast, and North America. Phylogenetic reconstructions involving four cpDNA genes in *Deparia* indicate that *Deparia* has undergone indirect speciation. However, the long-distance distribution of the genus includes regions such as Africa/Madagascar, Southeast Asia, the South Pacific Islands, Australia/New Guinea/New Zealand, and the Hawaiian Islands. A more detailed study, specifically in the context of *Deparia*'s dispersal to the Hawaiian Islands. The findings resulting from this study not only illustrate a comprehensive picture of the movement and adaptation history of *Deparia* in different regions but also provide profound insights that deepen the understanding of the evolutionary complexity and distribution of this genus on a global scale [6]. The study of the phylogeny and biogeography of *Deparia* provides a valuable contribution to our understanding of how Pteridophytes can acclimatize and thrive in various ecosystems, including in very remote regions such as the Hawaiian Islands. As such, this study is a significant addition to the scientific literature and lays the foundation for further research on the evolution and biogeography of Pteridophytes globally.

Table 1. Research progress on phylogeny and biogeography of Pteridophytes in the last ten years

No	Author	Year	Title of Article	Marker
1	Zhou and Zhang	2023	Phylogeny, character evolution, and classification of Selaginellaceae (lycophytes)	<i>rbcL</i> , <i>pgiC</i> , <i>SQD1</i>
2	Liu <i>et al.</i>	2022	Phylogenetic Relationships of Grammitid Fern Diversity of Gunung Tama Abu (Sarawak)	<i>rbcL</i>
3	Wei <i>et al.</i>	2021	Plastid phylogenomics provides novel insights into the infrafamilial relationship of Polypodiaceae	817 plastid genomes (plastomes)
4	Wei and Zhan	2020	Phylogeny of <i>Diplazium</i> (Athyriaceae) revisited: Resolving the backbone relationships based on plastid genomes and phylogenetic tree space analysis	51 plastid genomes
5	Testo et al.	2019	Phylogenetic and Morphological Analyses Support the Resurrection of <i>Dendroconche</i> and the Recognition of Two New Genera in Polypodiaceae Subfamily Microsorioideae	<i>rbcL</i> 10gene, <i>rps4</i> gene, <i>rps4-trnIIS</i> intergenic spacer, and 12 <i>trnL-trnF</i> intergenic spacer

6	Bouret <i>et al.</i>	2018	First insights on the biogeographical history of <i>Phlegmariurus</i> (Lycopodiaceae), with a focus on Madagascar	<i>rbcL</i> , <i>trnH-psbA</i> dan <i>trnL+trnL-trnF</i>
7	Wei <i>et al.</i>	2018	A total evidence phylogeny of the lady fern genera <i>Athyrium</i> Roth (Athyriaceae) with a new infrageneric classification.	<i>atpA</i> , <i>atpB</i> , <i>matK</i> , <i>rbcL</i> , <i>rps4</i> dan tiga non-coding <i>rps4-trnS</i> intergenic spacer (IGS), <i>trnL-F</i> IGS dan <i>rpl32-trnP</i> IGS
8	Xu <i>et al.</i>	2018	A global plastid phylogeny uncovers extensive cryptic speciation in the fern genera <i>Hymenasplenium</i> (Aspleniaceae).	<i>atpB</i> , <i>rbcL</i> , <i>rps4</i> , <i>rps4-trnS</i> , <i>trnL intron</i> dan <i>trnL-F</i>
9	Vicent <i>et al.</i>	2017	Phylogenetics and biogeography of <i>Lomaridium</i> (Blechnaceae)	<i>trnL</i> gen dan <i>trnL-trnF</i> intergenic spacer, <i>rbcL</i> dan <i>rps4-trnS</i> intergenic spacer
10	Zhang <i>et al.</i>	2017	A global phylogeny of the fern genera <i>Tectaria</i> (Tectariaceae: Polypodiales) based on plastid and nuclear markers identifies major evolutionary lineages and suggests repeated evolution of free venation from anastomosing venation.	<i>atpA</i> , <i>matK</i> , <i>rbcL</i> , <i>rps4</i> , <i>rps4-trnS</i> intergenic spacer, <i>rps16-matK</i> intergenic spacer, <i>trnL intron</i> dan <i>trnL-F</i> intergenic spacer
11	Hannequien <i>et al.</i>	2017	Global phylogeny and biogeography of the fern genera <i>Ctenitis</i> (Dryopteridaceae), with a focus on the Indian Ocean region.	<i>rbcL</i> , <i>trnL-F</i> dan <i>psbA-trnH</i>
12	Kuo <i>et al.</i>	2016	Historical biogeography of the fern genera <i>Deparia</i> (Athyriaceae) and its relation with polyploidy.	<i>rps16-matK</i> IGS, <i>trnL-LF</i> , <i>matK</i> dan <i>rbcL</i>
13	Sundue <i>et al.</i>	2015	Morphological innovation, ecological opportunity and the radiation of a major vascular epiphyte lineage.	<i>atpB</i> , <i>rbcL</i> , <i>trnL-trnF</i> , <i>rps4-trnS</i> dan <i>trnG-trnR</i>
14	Ding <i>et al.</i>	2014	Phylogeny and character evolution of the fern genera <i>Tectaria</i> (Tectariaceae) in the old world inferred from chloroplast DNA sequences.	<i>atpB</i> , <i>ndhF</i> plus <i>ndhF-trnL</i> , <i>rbcL</i> , <i>rps16-matK</i> plus <i>matK</i> dan <i>trnL-F</i>

Phylogeny and biogeographic history can also be studied in the genus *Phlegmariurus* (Lycopodiaceae). A study of the *Phlegmariurus* involving the analysis of three regions of plastid DNA (*rbcL*, *trnH-psbA*, and *trnL+trnL-trnF*) in 250 *Phlegmariurus* species from Madagascar and surrounding islands, including the western Indian Ocean showed the existence of widespread species such as *Phlegmariurus phlegmaria*, *P. squarrosus*, and *P. verticillatus*. Analysis of fossil calibration results indicates that *Phlegmariurus* originated in the late Carboniferous period and diversified in the early Eocene period. However, biogeographic analyses highlight the uncertainty regarding the biogeographic origin of *Phlegmariurus*, with the belief that the genus diversified in the Neotropics and Australasia [8].

Molecular phylogenetic analysis has also been published on several genera of Pteridophytes, including *Dryopteris*, *Polystichum*, and *Ctenitis* (Dryopteridaceae). The analysis shows that the genus *Dryopteris* emerged about 42 million years ago. *Dryopteris* species are not monophyletic because indirect events dominated their evolutionary history in North America, with long-distance dispersal occurring in species in Central America and Southern North America. This phylogenetic analysis is supported by studies using one coding protein (*rbcL*) and six noncoding chloroplast genomes (*psbA-trnH*, *trnP-petG*, *rps4-trnS*, *trnL-F*, *trnG-trnR*, and *rbcL-accD*). In the genus, *Polystichum* (Dryopteridaceae), phylogenetic analysis using *rbcL*, *rps4-trnS*, and *trnL-F* plastid DNA showed that *Polystichum* forms a monophyletic clustered genus, supported by morphological and distribution data. *Polystichum* revealed two monophyletic clades in two subgenera, *P. subg. Polystichum* and *P. subg. Haplopolystichum* [9]. Phylogeny and biogeography studies on the genus *Ctenitis* (Dryopteridaceae) globally using chloroplast

genomic DNA sequences of *rbcL*, *trnL-F*, and *psbA-trnH* intergenic spacers showed that *Ctenitis* is of neotropical origin [10]. This research presents a significant contribution to the scientific literature, forming the basis for further understanding the evolution and distribution of Pteridophytes globally.

Phylogeny analysis was also conducted on the genus *Hymenasplenium* (Aspleniaceae), using six plastid markers (*atpB*, *rbcL*, *rps4*, *rps4-trnS*, *trnL intron*, and *trnL-F*). This study indicated that *Hymenasplenium* is a monophyletic genus [11]. Meanwhile, in a molecular phylogeny study on the genus *Tectaria* (Tectariaceae), based on eight plastid DNA markers (*atpA*, *matK*, *rbcL*, *rps4*, *rps4-trnS* intergenic spacer, *rps16-matK* intergenic spacer, *trnL intron*, and *trnL-F* intergenic spacer), plus one nuclear marker (*pgiC*), showed that *Tectaria* also clustered monophyletically [12]. Similarly, phylogenetic and evolutionary studies of the genus *Tectaria* (Tectariaceae), based on chloroplast DNA sequences with five plastid DNA sequences (*atpB*, *ndhF* plus *ndhF-trnL*, *rbcL*, *rps16-matK* plus *matK*, and *trnL-F*), confirmed that *Tectaria* forms a monophyletic group [13]. However, in the genus *Athyrium*, analysis using five plastid DNAs (*atpA*, *atpB*, *matK*, *rbcL*, *rps4*) and three non-coding *rps4-trnS* intergenic spacers (IGS), *trnL-F* IGS, and *rpl32-trnP* IGS, revealed that *Athyrium* is not monophyletic. The results suggest that *A. skinneri* and *A. alpestre* belong to the *Anisocampium* and *Cornopteris* clades, respectively, and ultimately to the genus *Athyrium* [14].

Phylogeny and biogeography studies on the genus *Phlegmariurus* (Lycopodiaceae) were conducted based on three plastid DNA regions (*rbcL*, *trnH-psbA*, and *trnL+trnL-trnF*) in 250 species in Madagascar and surrounding islands, including the western Indian Ocean. The results show that some species have broad distributions, such as *Phlegmariurus phlegmaria*, *P. squarrosus*, and *P. verticillatus*. Biogeographic analysis and fossil calibration revealed that *Phlegmariurus* originated in the late Carboniferous Period and underwent significant divergence during the early Eocene. However, the biogeographic origin of *Phlegmariurus* remains uncertain, with predictions that the genus diversified in the Neotropics and Australasia [8]. This study underscores the complexity of the distribution and evolutionary history of the *Phlegmariurus* genus, highlighting the importance of combining molecular analyses and fossil data in understanding the evolutionary of Pteridophytes.

Phylogenetic and biogeographic history studies were also conducted on species in the genus *Lomaridium* (Blechnaceae), which account for about 85% of genetic variation. Analysis using three plastid markers (*trnL gene* and *trnL-trnF* intergenic spacer, *rbcL*, and *rps4-trnS* intergenic spacer) revealed that the ancestry of *Lomaridium* can be traced back to the Paleocene epoch. Its native range is thought to include Australia, the Central Tropics, and South America. Dispersal patterns suggest a long-distance dispersal event, with *Lomaridium* diversification involving various regions, such as the Caribbean (*L. binervatum*), several islands in the Pacific (*L. schottii*), Africa, and Madagascar. The biogeographic analysis highlights the extensive long-distance dispersal events in *Lomaridium* [15], providing a deep understanding of this genus's evolutionary history and worldwide distribution.

Phylogeny was also studied in the fern genus *Grammitid* (Polypodiaceae), analysis using five plastid DNA genes (*atpB*, *rbcL*, *trnL-trnF*, *rps4-trnS*, and *trnG-trnR* intergenic spacer) confirmed that grammitids form a monophyletic group with ancestors estimated to have originated in the Neotropical period about 31.4 million years ago [16] ; [17] ; [18] . This finding underscores the continuity of grammatic evolution and dispersal in the context of a long history in the Neotropics.

Furthermore, phylogeny analysis on the genus *Hymenasplenium* (Aspleniaceae) using six plastid markers (*atpB*, *rbcL*, *rps4*, *rps4-trnS*, *trnL intron*, and *trnL-F*) revealed that *Hymenasplenium* is a monophyletic genus [11], providing a solid understanding of the evolutionary relationships among species in this genus. Similarly, a molecular phylogeny on the genus *Tectaria* (Tectariaceae) with eight plastid DNA markers and one nuclear marker showed that *Tectaria* is a monophyletic group [12]. Further studies on the phylogeny and evolution of the genus *Tectaria*, based on the chloroplast DNA sequences of five plastid DNA sequences, confirmed this monophyletic group [13]. These findings support our understanding of the phylogenetic structure and evolutionary history of the genus *Tectaria* in a global context.

Molecular phylogenetic studies on two species of grammatid group ferns collected at Gunung Tama Abu in Sarawak yielded exciting findings. In this study, *Acrosorus streptophyllus* forms a clade that is sister to *A. friderici-et-pauli*. By adding *Acrosorus* samples from one to two, the phylogenetic hypothesis assembled supports the monophyly of this genus for the first time based on DNA sequence data. A new Bornean accession from Borneo, *Scleroglossum pyxidium*, was shown to be a sister to the clade of *S. sulcatum* accessions. Meanwhile, *Prosaptia alata* samples formed a clade with other accessions of this species [19]. The importance of this study lies not only in its phylogenetic discoveries but also in its implications for the current

understanding of the diversity and relationships among species in this group. In particular, adding data from Borneo and further understanding of phylogenetic relationships may pave the way for further research and conservation of the species.

Meanwhile, the latest study of *Selaginella* phylogeny also achieved significant milestones. Using data from six plastid samples and nuclear loci, including new samples, the study covered 684 accessions representing approximately 300 species. This significant increase, by 74% from the previous most extensive sampling, allowed for a more comprehensive phylogeny to be constructed. The evolution of 10 morphological characters was explored in this new phylogenetic context. The main findings involved the separation of *Selaginella* s.l. into seven subfamilies and 19 genera, with nine new genera diagnosable as significant clades. Publication of this study's results is expected to contribute significantly to understanding *Selaginella* phylogeny, facilitate communication among researchers, stimulate further research, and support plant conservation efforts [20].

IV. CONCLUSIONS

In the last decade, research focused on Pteridophytes has made significant progress by analyzing nuclear and plastid DNA markers. 46 DNA markers have been identified and applied to several genera of Pteridophytes worldwide. The phylogenetic approach adopted in this study has revealed intriguing information on the evolutionary relationships among the genera. From the results of this study, it was found that four fern genera, namely *Diplazium*, *Polypodium*, *Hymenosplenium*, and *Tectaria*, form a monophyletic group. This finding suggests a close evolutionary relationship between the four genera. However, an equally exciting finding relates to the genus *Athyrium*. Unlike the previous four genera, *Athyrium* belongs to a non-monophyletic group. Phylogenetic analysis demonstrated that *Athyrium skinneri* and *Athyrium alpestre* simultaneously belong to the *Anisocampium* and *Cornopteris* clades, which are part of the *Athyrium* clade. Understanding the phylogenetic relationships between genera can form a more detailed picture of how Pteridophytes evolved. These findings provide profound insights that can form the basis for further classification and research into the divergence and evolution of Pteridophytes.

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