

Biosystematics Of Athyrium And Biogeography Of Deparia (Athyriaceae) Based On Molecular Study Review

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Abstract—Athyriaceae is a family of ferns consisting of five genera, two of which are *Athyrium* and *Deparia*. *Athyrium* is a genus of ferns, and until now, the classification of this species is still a matter of debate among researchers through morphological and molecular approaches. Likewise, biogeography studies on the genus *Deparia* (Athyriaceae) are still a mystery to researchers. Thus, this paper will review molecular phylogenetic studies on *Athyrium* and *Deparia* biogeography based on a review of previously published literature. A systematic literature review (SLR) is used in this paper.

Keywords—*Athyrium*, Biogeography, *Deparia*, Molecular

I. INTRODUCTION

The Athyriaceae family is a fern plant consisting of several genera including *Athyrium*, *Deparia*, *Lunathyrium*, *Dryoathyrium*, and *Parathyrium*. *Athyrium* has the largest number of species in the Athyriaceae family (Wang et al, 2013) [1]. About 160-220 species are known. There are 200 species of the genus *Athyrium*, most of which are distributed in Southeast Asia to the Himalayas (Krame & Kato, 1990) [2]. The *Athyrium* genus group is characterized by two lines on the petiole, the base of the petiole is wide and has large air holes (pneumatophores), and there are grooves that extend from the rachises to the costae and the shape of the sorus is like a J (Sundue and Rothfels, 2014) [3]. The very common stature of Athyroid ferns has been the subject of debate since it was published by Roth (1800). In Japan, 20 species of *Athyrium* are known [4]. The proposed infrageneric classification of *Athyrium* is generally based on morphology [5]. [6] introduced only two species groups (*A. filix-femina* group and *A. puncticaule* group) based on the presence or absence of cartilaginous cells on the leaf margins. [7] proposed the first formal infrageneric classification by introducing two subgenera (*Athyrium* and *Echinoathyrium*), five sections (*Filix-femina*, *Polystichoides*, *Niponica*, *Echinoathyrium*, and *Strigoathyrium*), and ten series (*Filix-femina*, *Exindusiata*, *Macrocarpa*, *Fimbriata*, *Niponica*, *Biserrulata*, *Mackinnoniana*, *Epiraches*, *Strigillosa*, and *Pectinata*). [8] modified [7] and introduced two series into the *Athyrium wallichianum* and *A. dissitifolium* groups. Due to differences in the classification of *Athyrium* based on morphology, a literature search was conducted on molecular studies on the genus *Athyrium*, biogeographic history, species variation, and current classification.

In order to see the distribution of the genus in Athyriaceae, a biogeography study was also carried out in this case research on the genus *Deparia*. *Deparia* consists of 60-70 species, which are found in tropical, subtropical, and temperate forests. It is also found in Africa and the Indonesian islands of the western Indian Ocean (mostly in Madagascar), Australia, Northern New

Zealand, northeastern North America, the Hawaiian Islands, and various South Pacific Islands. Sect. *Deparia* is endemic to the Pacific Ocean Islands, including the Bonin Islands and the Hawaiian Islands. The biogeographic history of *Deparia* has received attention due to its ability to spread over long distances [9]. Phylogenetic biogeography is the only feasible approach to understanding the mechanisms underlying the distribution of the genus *Deparia*. In addition, polyploidy in this genus is also of particular interest, whether polyploidy correlates with dispersal distance or range expansion in the genus *Deparia* so further research is still needed.

II. MOLECULAR TAXONOMY OF ATHYRIUM

2.1. Comparison of Phylogenetic Trees of Athyrium Species Using Different DNA Genomes

Some phylogenetic studies of thyroid ferns were carried out using molecular data [10], no comprehensive phylogenetic studies at the species level that focus on this group have been done, instead only at the regional level (Japanese *Athyrium* by [4]. Phylogenetic studies of *Athyrium* based on molecular analysis using 8 chloroplast DNA genomes (Five Plastid DNA: *atpA*, *atpB*, *matK*, *rbcL*, *rps4*, and three non-coding regions: *rps4-trnS* intergenic spacer (IGS), *trnL-F* IGS and *rpl32-trnP* IGS) and genomic DNA in *rbcL* and *trnL* genes are expected to solve the problem of phylogenetic relationships and grouping and species position in the genus *Athyrium*. Biosystematics studies based on molecular approaches in *Athyrium* also aim to determine the phylogenetic relationship between species in the genus *Athyrium* using a combination of several molecular DNA genome with a combination of plastid DNA such as *atpA*, *atpB*, *matK*, *rbcL*, *rps4*, and three non-coding regions namely *rps4-trnS* intergenic spacer (IGS), *trnL-F* IGS and *rpl32-trnP* IGS and genomic DNA in the *rbcL* and *trnL* genes to be able to solve the problem of phylogenetic relationships and grouping and species position in the *Athyrium* genus.

Phylogenetic analysis methods with MP, MI, and BI produce a combination of data that can display two parsimony trees. The results of phylogenetic analysis based on molecular data using 8 chloroplast DNA obtained nucleotide chain length data 7925 of 109 taxa with constant character 4104 and parsimony informative character 2798 with CI value 0.4322 and RI 0.6495. Of the 8 DNA markers used, the highest percentage value of constant characters is owned by *rbcL* while the lowest is owned by *matK* and *rpl32-trnP* IGS. Based on character informative parsimony data, the highest value is owned by *rpl32-trnP* IGS and has very high saving properties while *rbcL* has the lowest value.

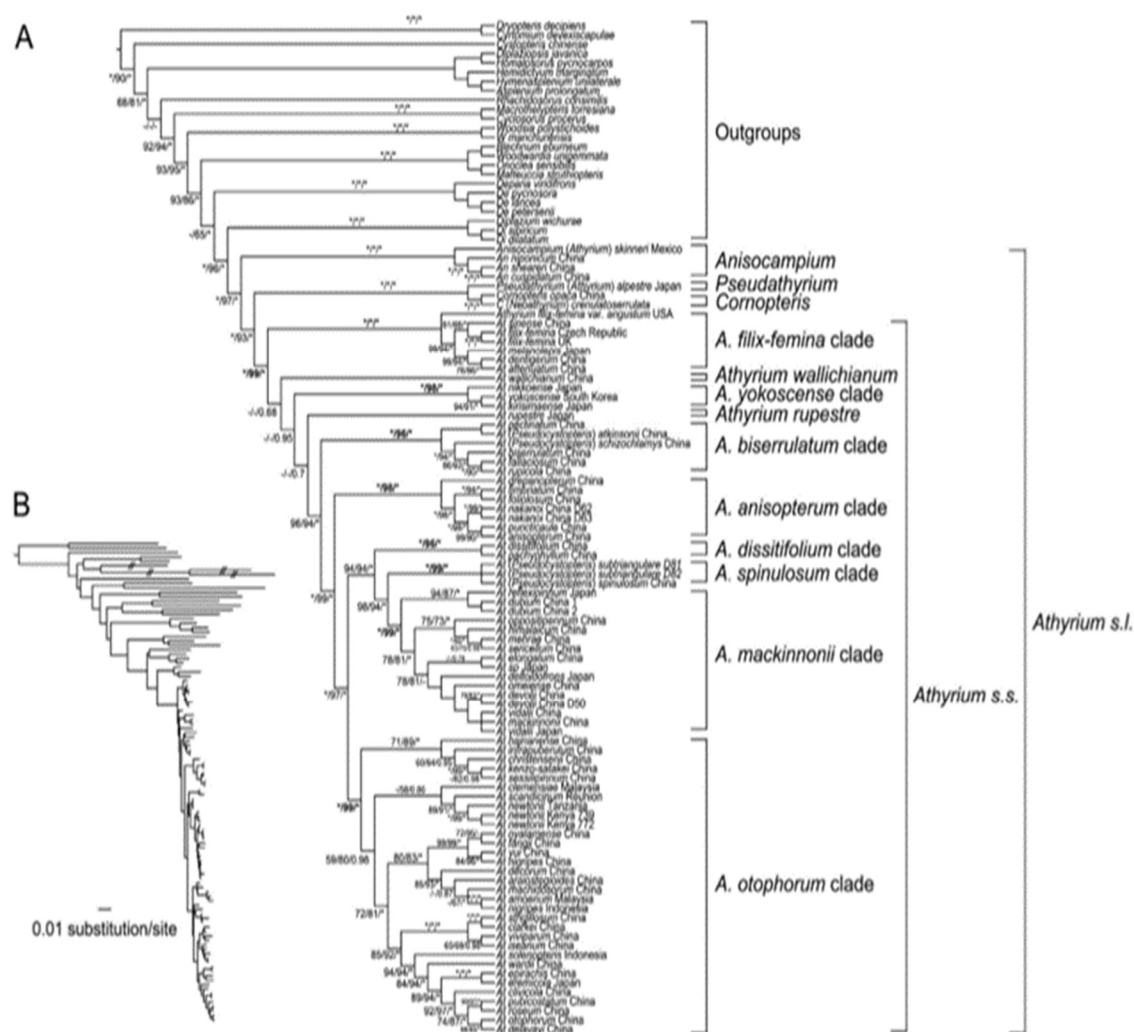


Figure 1. Relationship-based on phylogenetic studies on *Athyrium* and its relatives.

Based on the phylogenetic tree using bayesian analysis and the combination of molecular data, *Athyrium*, *Anisocampium*, *Pseudoathyrium*, and *Cornopteris* are monophyletic groups as shown in Figure 1. *Athyrium skinneri* belongs to the *Anisocampium* group while *A. alpestre* belongs to the *Pseudathyrium* group. Four species of *Pseudocystopteris* are not monophyletic because they are found in different clades. Based on MP, ML, and BI analysis with a combination of molecular data, a phylogenetic tree with ten clades supports the grouping of *Athyrium* species. The clade is 1. *A. felix femina* and all supporting species, 2. *A. wallichianum* which is a single species, 3. *A. yokoscense* with several adjacent species, 4. *A. rupestre* which is a single species, 5. *A. biserrulatum* which is a combination of previous species that have been placed in *Athyrium* and *Pseudocystopteris*, 6. *A. anisopterum* with other species in one group, 7. *A. distitifolium* with the same species morphology as *A. Pachyphyllum*, 8. *A. spinulosum* which consists of two species formed by testing is part of *Pseudocystopteris*, 9. *A. mackinnonii* where the species formed is tested as a section of *Echinoathyrium* or section *mackinnoniana*, 10. *A. otophorum* several species formed are placed in sect. *Otophora* in *Athyrium*.

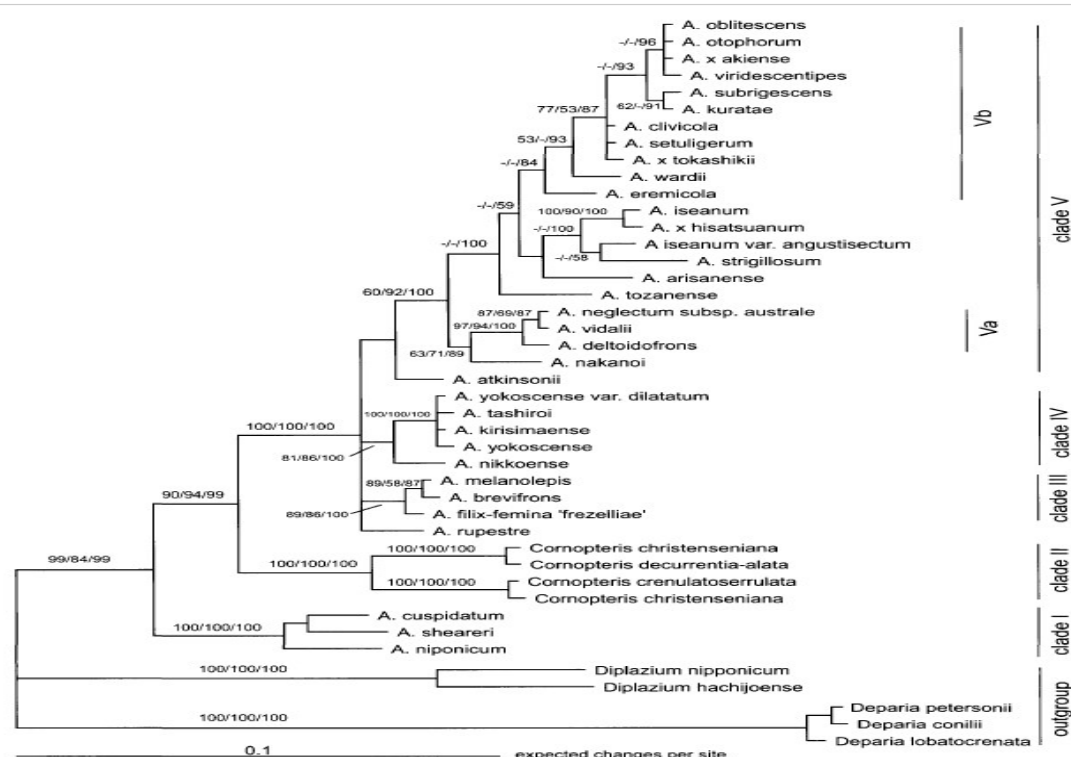


Figure 2. Phylogenetic tree based on trnL-F data using Bayesian analysis. Supported by NJ bootstrap (BS)/MP bootstrap (BS)/Bayesian Posterior Probabilities (PP) values.

Based on morphological characters, monophyly can be seen in Athyrioid ferns and 8 clades that support synapomorphism in morphological characters. This can be seen from the basic morphology of the petiole in *Athyrium* which is large with pneumatophore and the number of basic chromosomes $X = 40$. Synapomorphism is formed in *Athyrium* based on the spines found in the costa or costulla as a smooth perispora and is found in *A. mackinnonii* or *A. otophorum* (Figure 2). This study can solve the problem of generic and infrageneric relationships using molecular data. Based on MP, ML and BI data, a strong phylogenetic framework was obtained in *Athyrium* species. Phylogenetic analysis based on molecular data and data set total evidence on *Athyrium* with *Anisocampium*, *Cornopteris* and *Pseudocystopteris* as a monophyletic group with BSMP = 100%, BSML > 80% and BI pp = 1) which is in accordance with research [11]. Four *Pseudocystopteris* samples were found to belong to different clades of *Athyrium*, and two *Athyrium* species were closely related to *Anisocampium* (*A. skinneri*) or *Cornopteris* (*Athyrium alpestre*). Although current research organizes the concept by including *Anisocampium* and *Cornopteris* [12] into the genus *Athyrium*, this study still proposes the separation of these genera because these two genera have different types from the *Athyrium* type. The clade described in this study is in accordance with taxonomic and phylogenetic studies conducted [4]. New records in this study are the clades of *A. biserrulatum*, *A. yokoscense*, and *A. spinulosum*, and two independent species were found, namely *A. wallichianum* and *A. Rupestre*. Based on the results of this study, the combination of DNA data from chloroplast and nuclear DNA has proven to be an appropriate method for detecting the origin of a hybrid and solving the problem of kinship relationships in the genus *Athyrium*.

2.2. Biogeographic History of the Genus Deparia (Athyriaceae)

Ferns have a wider biogeographical area compared to seed plants because of the easier spore ability to germinate [13]. The long-distance distribution of ferns is not only based on the fossil record [14] but is often inferred from molecular phylogenetic studies [15]. Ferns account for 80% of extant species, after the breakup of Gondwana and Laurasia [16]. In this paper, we will explain the history of the biogeography of one of the genera of Athyriaceae, namely *Deparia*.

Biogeography also correlates with the discovery of new species. The high endemism in the Hawaiian Islands can be proven in

the genus *Deparia*. This endemism is also found in one of the *Athyrium* species found in the Hawaiian Islands. *Athyrium* is estimated to have a total of 230 species that live in both terrestrial and epiphytes. The *Athyrium* species diversity center in the Sino-Himalayan region reported as many as 91 species originating from Southern China, the Sichuan Basin, Tibet, Yunnan Plateau and Nepal [17]. Species diversity is also related to the current classification. The classification of the genus *Athyrium* is constantly changing using a phylogenetic approach [18], [19], [3], [12]. In 2016 the Pteridophyte Phylogeny Group (PPG) published the Athyriaceae family which consists of three genera, namely *Athyrium*, *Deparia*, and *Diplazium* with an estimated number of species of 650 species. In the 2016 PPG publication, 9 endemic species were found in the Hawaiian Islands including *Athyrium microphyllum* (Sm.) Alston, *Deparia cataracticola* M. Kato, *Deparia fenzliana* (Luer.) M. Kato, *Deparia kaalaana* (Copel.) M. Kato, *Deparia marginalis* (Hillebr.) M. Kato, *Deparia prolifera* (Kaulf.) Hook. & Grev., *Diplazium arnottii* Brack., *Diplazium molokaiense* W.J. Rob., and *Diplazium sandwichianum* (C. Presl) Diels [21]. This publication also found a new species in the genus *Athyrium* in the Hawaiian Islands which is different from the one published by PPG, I, 2016. This study will be discussed further in this paper.

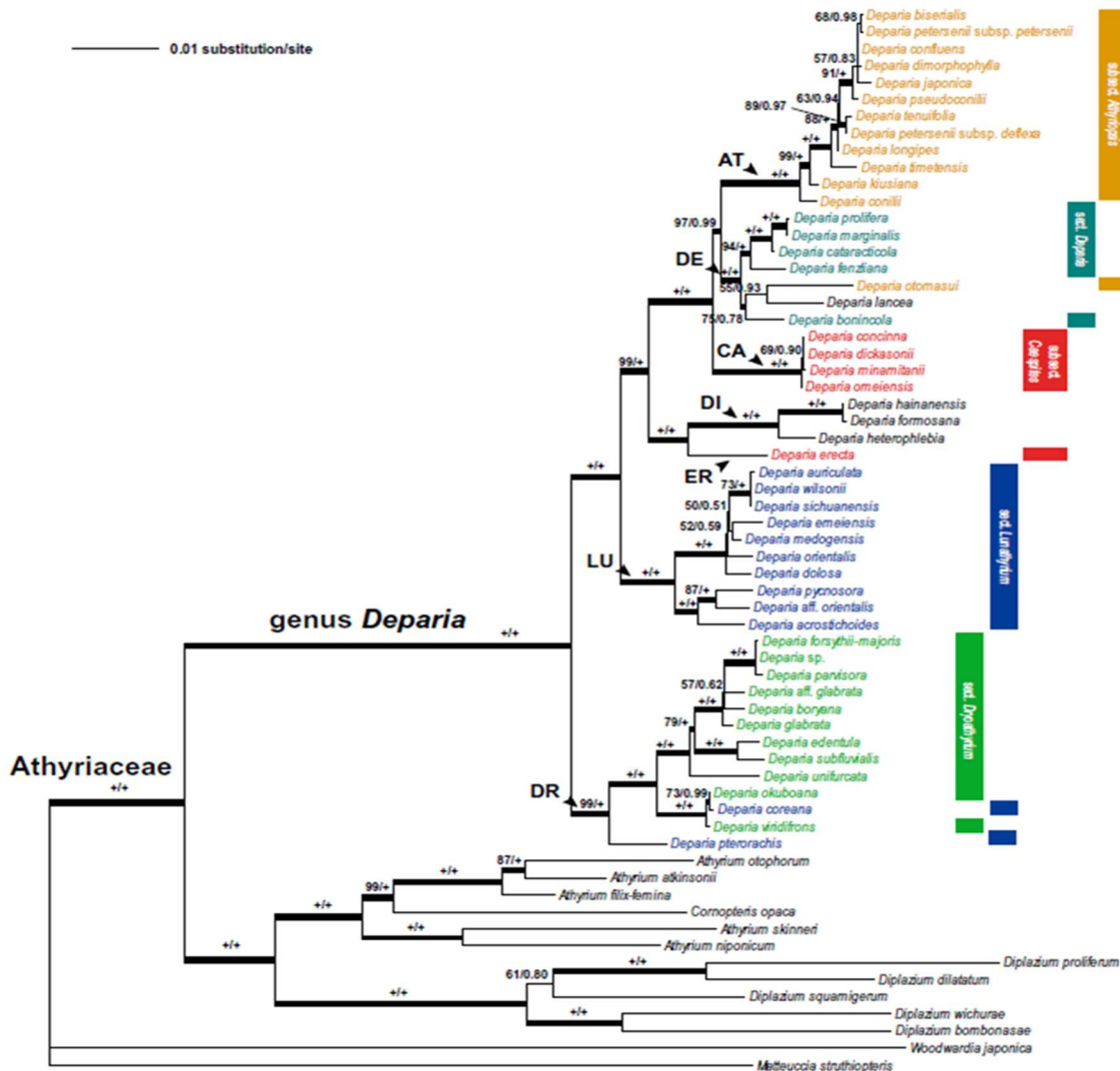


Figure 3. Maximum likelihood (ML) epigram of *Deparia* based on the rps16-matK IGS + matK + rbcL + trnL-L-F dataset.

The phylogenetic patterns and divergence times of *Deparia* based on cpDNA regions are shown in Figure 1. The phylogenetic relationships obtained are consistent with previous studies [21] but provide greater resolution of species groups, with seven highly supportive clades (ML BS > 99 and posterior Bayesian probability = 1.00; Figure 3). DR clade sect. *Dryoathyrium*, LU clade of sect. *Lunathyrium*, CA clade for subsect. *Caespites*, DE clade for sect. *Deparia*, AT clade for subsect. *Athyriopsis*, and DI clade for *Dictyodroma*. ER clade, there is only one species, *Deparia erecta*, which is considered a subsection. However, this

species is sister to Dictyodroma (i.e., DI clade), and is not related to any other subsection as a member of *Caespites* (i.e., CA clade).

A comparative study of divergence times in the genus *Deparia* was obtained from the results of a previous study (Rothfels et al., 2015), the divergence time of *Deparia* (24.7 and 21.6 Ma) estimated in this study is 26.41-28.70 Ma. Based on the biogeographic analysis inferred by using the DEC model on the *Deparia* phylogeny reveals that *Deparia* originated on the Asian/East Asian continent. Based on its dispersal history, it can be concluded that *Deparia* can spread to Africa/Madagascar, northeastern North America, Hawaiian Islands, South Pacific islands, Southeast Asia, and Australia/New Guinea/New Zealand. This long-distance dispersal is the result of a very long period of dispersal because its divergence period is thought to be much younger than the breakup of Gondwana and the separation of Laurasia. This marked divergent time is indicated by the relative DNA of tissue cores in leaves from *Deparia prolifera*, and the diploid *D. lancea*. *D. prolifera* has a genome almost twice the size of diploid *D. lancea*. This suggests that *D. prolifera* is a polyploid and tetraploid-like species. The dispersal and extinction events inferred by the DEC model are mapped on the chronogram in Figure 3.

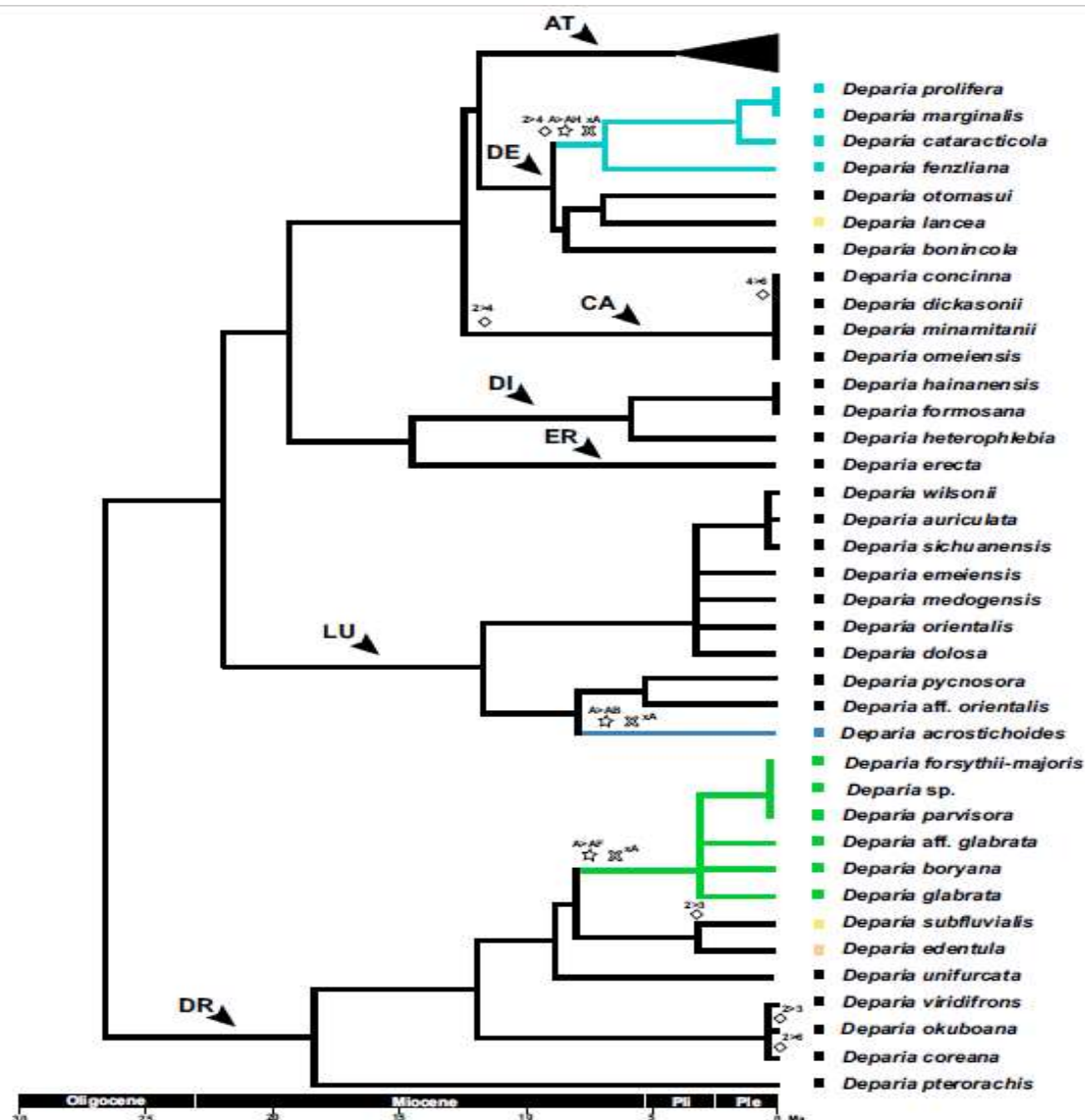


Figure 4. Chronogram of the cpDNA phylogeny of *Deparia*. Color differences in the branches indicate the distribution of ancestors.

The results of this study support *Deparia* originating from the Asian/East Asian continent around 27 Ma (20.97-34.50 Ma for

95% Highest Posterior Density) with long-distance dispersal from the Asian/East Asian continent to Africa during the Miocene to Pliocene (Figure 3). This long-distance spreading has been inferred from various ages (Le P  chon et al., 2016). Further research based on more comprehensive sampling is needed to confirm the biogeographic scenarios leading to *Deparia* divergence in Africa/Madagascar. The dispersal of *Deparia* species from the Asian/East Asian continent to northeastern North America could have occurred via the Bering land bridge, which connects the two continents (Tiffney and Manchester, 2001). This possibility is also implied by the results of the divergence time estimates, which indicate *D. acrostichoides* is an endemic plant. Thus, vicariant speciation due to land loss or past transoceanic climate change, as well as long-distance dispersal, can be easily explained using *D. acrostichoides*.

In addition to their dispersal history events, the infraspecific range expansions of *D. subfluvialis*, *D. edentula*, *D. lancea*, *D. petersenii* subsp. *deflexa*, and *D. petersenii* subsp. *Petersenii* also indicates a biogeographic increase in an area after speciation. These species expanded from the Asian/East Asian continent or Asian/East Asian continent + Southeast Asia and further to adjacent areas, including Southeast Asia, southern Pacific islands, Australia/New Guinea/New Zealand, and Africa/Madagascar. The genus *Deparia* originated in Asia/East Asia around 27.7 Ma. It was found to have spread to Africa/Madagascar, South Asia, south Pacific islands, Australia/New Guinea/New Zealand and the Hawaiian Islands. Especially for the spread to the Hawaiian Islands, it is associated with its polyploidization ability, because the level of polyploid spread is higher than diploid.

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