Life Science and Biotechnology
Volume 2, Number 2, November 2024
Pages: 69 - 73

Life Science Biotechnology

# In silico Study of Genus Marchantia using matK loci for DNA Barcoding

**Abdillah Maulana Farhan[1], Fuad Bahrul Ulum[1*]**

[1]Biology Department, Faculty of Mathematics and Natural Sciences, Jember University, East Java, Indonesia
*Correspondence Author: fuad.fmipa@.unej.ac.id

## Abstract

Marchantia is a genus of liverworts that can be found in the Asia-Europe Region. Some of the types that can be found are *Marchantia polymorpha* subsp. *montivagans*, *Marchantia polymorpha* subsp. *ruderalis*, and *Marchantia paleacea* subsp. *diptera*. All of these species have different levels of relationhip, this is due to differences in their nucleotide structure. The purpose of this study was to identify the intolerance between Marchantia species in genomic studies using the NCBI Database. The method used in this research is the collection of nucleotides on NCBI and then construction with Mega 11 software. The marker used in this study is Maturase K (*matK*), this is because the marker is very optimal in the identification of a molecular-based species. The results of this study are *Marchantia paleacea* subsp. *diptera* matK (MP 58 and MP 54) and *Marchantia polymorpha* subsp *ruderalis* (matK sequences MC-47 and MC-44) has the highest level of relationship with a score of 99 (Neighbor-Joining Tree) and 100 (Maximum Likelihood Tree) and the outgroup of this phylogenetic is *Reboulia hemisphaerica*.

**Keywords**: marchantia, *matK*, NCBI, phylogenetic

## Introduction

The genus *Marchantia* is distinguished by its complex thalloid gametophyte, asexual reproduction via multicellular gemmae, and specialized gametangiophores that elevate reproductive structures for effective spore dispersal. It comprises approximately 40 species grouped into three clades: Clade I includes globally distributed species like *M. polymorpha*, *M. berteroana*, *M. paleacea*, *M montivagans*, and *M. ruderalis* which thrives across the Holoarctic and exhibits anthropogenic spread; Clade II features *M. quadrata* and *M. romanica*, which lack gemmae cups and rely exclusively on sexual reproduction; and Clade III, concentrated in the Indo-Australian archipelago, reflects recent diversification with species adapted to moist habitats and occasional long-distance dispersal, i.e. *M. foliacea*, *M. chenopoda*, *M. inflexa*, *M. emarginata*, *M. subinetgra* and other 31 species (Bowman et al, 2022). In Indonesia a total of 13 *Marchantia* species were recorded in Global Biodiversity Information Facility (GBIF). *M. emarginata* is the most commonly recorded species with 96 occurrences, followed by *M. treubii* (16), *M. polymorpha* (15), and *M. geminata* (12). Other species like *M. paleacea* (10) and *M. miqueliana* (7) also have notable records, while several species such as *M. berteroana*, *M. papillata*, and others are recorded only a few times, i.e. *M. acaulis*, *M. pappean*, *M. solomonensis*, *M. streimannii*, *M. subgeminata* (GBIF.org, 2024).

The genus *Marchantia*, including the widely studied *M. polymorpha*, presents significant challenges in species identification due to its phenotypic plasticity and nomenclatural ambiguity. Although Bischler-Causse and Boisselier-Dubayle (1991) reclassified *M. polymorpha* into three subspecies, i.e., *subsp. polymorpha*, *subsp. montivagans*, and *subsp. Ruderalis* based on morphological traits, distinguishing these subspecies remains difficult. Phenotypic variation influenced by environmental factors and the limited scope of genetic studies, particularly beyond Europe, hinder comprehensive understanding of its genetic structure (Shimamura, 2016). Traditional taxonomy relies on morphological characteristics, requiring extensive expertise and careful analysis. However, this approach faces challenges, especially when dealing with abundant specimens, complicated genera, or sub-optimal samples, which can make species identification lengthy, expensive, and sometimes unreliable. DNA barcoding, introduced by Paul Hebert in 2003, has emerged as an efficient complementary tool, using standardized DNA fragments like multiple plastid and nrDNA regions for plants. On the other hand, for recently diverged or rapidly radiating taxa, the species discrimination required hight cost next-generation sequencing (NGS) for complex taxonomic groups (Chen et al, 2022). DNA barcoding, with its precision and reliability, provides a valuable alternative for resolving taxonomic ambiguities by utilizing molecular markers such as *rbc*L and *matK*, for accurate species identification and supports

deeper insights into genetic diversity for studies in Megabiodiversity countries with a high species number (Nguyen, 2020).

Indonesia is renowned for its extraordinarily rich genetic and biodiversity; however, its DNA barcode records in global databases are notably underrepresented. Developing a national DNA barcode library and advancing DNA barcoding initiatives are essential steps to safeguard its genetic diversity and strengthen biodiversity conservation and management efforts. Since its introduction in 2003, DNA barcoding has provided a robust and standardized method for species identification, offering high repeatability and stability across diverse developmental stages and even in processed or degraded specimens. This molecular approach addresses the shortcomings of morphology-based methods, enabling more accurate taxonomic resolution (Priyono et al,. 2023). In plants, the chloroplast gene regions *rbcL* and *matK* have emerged as the core DNA barcoding loci due to their universality and relatively high species-level resolution. However, the effectiveness of these markers varies across taxonomic groups, with some studies highlighting differential resolution capabilities (Su'udi et al, 2023). While DNA barcoding has been extensively applied to vascular plants, its application to *Marchantia* in Indonesia remains underexplored. The *matK* gene markers is frequently used because of the existence, effectiveness, and sustainability of the mutation rate of these two genes which are found in almost all plants (Rohimah et al., 2018; Suudi, 2023). This in silico study aimed to evaluate the species resolution capability of the *matK* locus for the genus *Marchantia* using National Center for Biotechnology Information (NCBI) data, contributing to the identification of effective barcoding markers for species identification, conservation, and biodiversity research in Indonesia.

## Materials and Methods

### Collection Nucleotide from NCBI

The nucleotide data of *Marchantia* genus in FASTA fromat were downloaded from NCBI. We focused only on the genus of Marchantia specimens with mat-K sequence. The species were *Marchantia polymorpha* subsp. *polymorpha*, *Marchantia polymorpha* subsp. *montivagans*, *Marchantia polymorpha* subsp. *ruderalis*, *Marchantia paleacea* subsp. *diptera*, and the outgroup in this study was *Reboulia hemisphaerica*. The listed species were evaluated with BLAST to determine the Query cover value, E value, per ident, and country of Origin of the *Marchantia* sequences (Altschul and Pop, 2017). BLAST was performed using Fasta sequences from one of the species namely, *Marchantia polymorpha* subsp. *ruderalis* with Accesion Number KX451208.

### Phylogenetic Tree Construction

Phylogenetic trees between the species were constructed with Mega 11 software based on Neighbour-Joining Tree and Likelihood Maximum with 1000 Bootstrap, Kimura 2-parameter substitution model (Tamura et al, 2021). Neighbour-Joining Tree was a faster analysis to generate a tree as a starting point and the more complex methods, such as Maximum Likelihood provided a higher accuracy in examining complex evolutionary relationships. *Reboulia hemisphaerica* was used as an outgroup To assess the species resolution of a given barcode locus, a species was deemed successfully resolved if conspecific individuals formed a single monophyletic branch in the phylogenetic tree with strong bootstrap support. Conversely, if conspecific individuals were divided across paraphyletic branches, it was classified as a failure in species identification (Sikdar et al, 2016).

## Results and Discussion

Using the keyword "species+matK" to search for sequences in the NCBI GenBank database, a total of 14 matK region DNA sequences were identified (Table 1). The sequence lengths varied significantly, ranging from 805 bp to 1,113 bp.

The BLAST analysis results revealed that all *Marchantia* in this study exhibited a query cover value of 100%, with the exception of *Reboulia hemisphaerica*, which had a query cover value of 85%. This high query cover indicates a close genetic relationship among the Marchantia species (Rizqoni et al., 2024). The value of per ident or percentage identify obtained in this data Marchantia from Japan gets value of 100% which means that the nucleotide match between the query sequence and the subject sequence shows a similarity between nucleotides (Boratyn et al., 2013).

BLAST results revealed that all sample sequences analyzed in this study originated from the Asia-Europe region. Among the samples, *Marchantia polymorpha*, a thalloid liverwort of the genus *Marchantia*, was included in the analysis. Taxonomically, *M. polymorpha* comprises three subspecies: *M. polymorpha subsp. ruderalis*, *M. polymorpha subsp. montivagans*, and *M. polymorpha subsp. polymorpha* (Linde et al., 2020). Morphologically, these subspecies exhibit distinct differences. *M. polymorpha subsp. polymorpha* is characterized by a continuous black line along the center of the thallus, while *M. polymorpha subsp. ruderalis* has a thinner, discontinuous black line. *M.*

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*polymorpha subsp. montivagans* is the most morphologically distinct, as its thallus lacks a central black line entirely (Kwon et al., 2019; Linde et al., 2020; Zheng et al., 2022). Another species analyzed, *Marchantia paleacea*, is abundant in Asia, particularly in Japan. A notable subspecies, *M. paleacea subsp. diptera*, is distinguished by its yellowish thallus and a pattern created by prominent pores, which differentiates it from other *Marchantia* species (Shimamura, 2017). Due to database limitations for matK markers in NCBI, this study focused on *M. polymorpha subsp. ruderalis*, *M. polymorpha subsp. montivagans*, and *M. paleacea subsp. diptera*.

Table 1. BLAST Result for Genus *Marchanti*a using *matK*

| No. | Species | Accesion Number | Query Cover (%) | E Value | Per Ident (%) | Origin |
|---|---|---|---|---|---|---|
| 1. | *Marchantia polymorpha* subsp. *ruderalis*, MC-57) | KX451208 | 100 | 0.0 | 100 | Japan |
| 2. | *Marchantia polymorpha* subsp. *ruderalis*, MC-59) | KX451204 | 100 | 0.0 | 100 | Japan |
| 3. | *Marchantia polymorpha* subsp. *montivagans*, MA-38 | KX451192 | 100 | 0.0 | 100 | Iceland |
| 4. | *Marchantia polymorpha* subsp. *montivagans*, MA-39 | KX451195 | 100 | 0.0 | 100 | Iceland |
| 5. | *Marchantia polymorpha* subsp. *ruderalis*, MC-04 | KX451201 | 100 | 0.0 | 100 | Bosnia and Herzegovina |
| 6. | *Marchantia polymorpha* subsp. *ruderalis*, MC-47 | KX451200 | 100 | 0.0 | 100 | Netherlands |
| 7. | *Marchantia polymorpha* subsp. *ruderalis*, MC-29 | KX451207 | 100 | 0.0 | 100 | Poland |
| 8. | *Marchantia paleacea* subsp. *diptera*, MP-55 | KX451211 | 100 | 0.0 | 97,56 | Japan |
| 9. | *Marchantia paleacea* subsp. *diptera*, MP-54 | KX451206 | 100 | 0.0 | 97,56 | Japan |
| 10. | *Marchantia paleacea* subsp. *diptera*, MP-58 | KX451209 | 100 | 0.0 | 97,56 | Japan |
| 11. | *Marchantia paleacea* subsp. *diptera* isolate MP_18 | KJ437439 | 100 | 0.0 | 97,56 | Japan |
| 12. | *Marchantia paleacea* subsp. *diptera*, MP-53 | KX451214 | 100 | 0.0 | 97,52 | Japan |
| 13. | *Marchantia polymorpha* subsp. *montivagans*, MA-86 | KX451188 | 100 | 0.0 | 100 | Poland |
| 14. | *Marchantia polymorpha* subsp. *ruderalis*, MC-44 | KX451198 | 100 | 0.0 | 100 | Finland |
| 15. | *Reboulia hemisphaerica* | AF264671 | 85 | 0.0 | 89,55 | Ireland |

**Phylogenetic of Genus Marchantia**

The phylogenetic tree construction of the study based on Neighbor-Joining Tree and Maximum Likelihood Tree are presented in Figure 1. The resolution capacity of a DNA barcode refers to its effectiveness in distinguishing and identifying species based on the interspecific variations in DNA sequences. A species is regarded as successfully resolved when its individuals form a distinct monophyletic branch in a phylogenetic tree, demonstrating clear genetic separation from other species (Sikdar et al., 2018).

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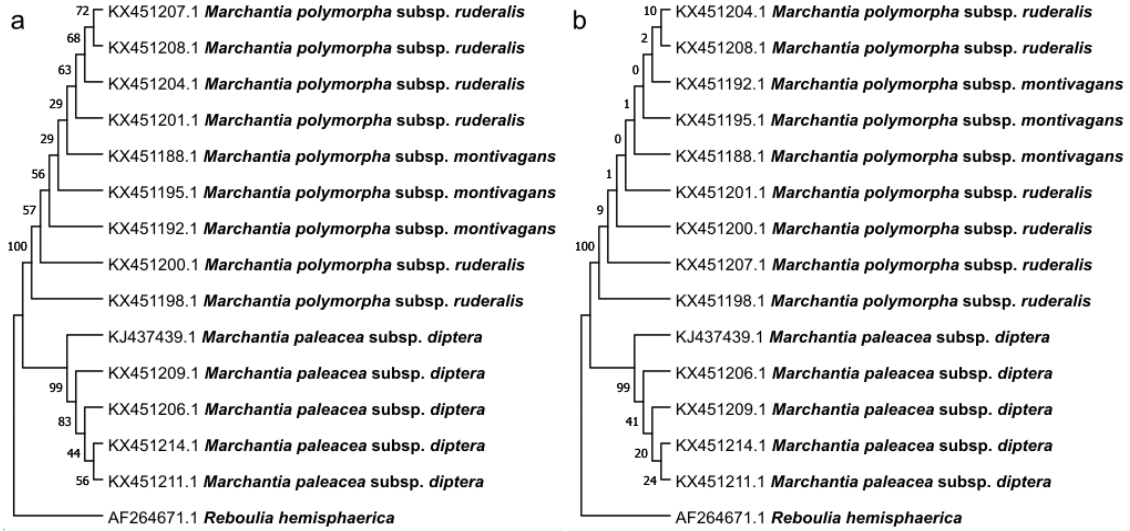

Figure 1. Phylogenetic tree of *Marchantia* based on *matK* sequences: a: Neighbour-Joining Tree; b: Maximum Likelihood

The study revealed that *Marchantia paleacea subsp. diptera* (matK sequences MP 58 and MP 54) exhibited the highest level of genetic similarity, likely due to both samples originating from Japan, resulting in closely matching nucleotide sequences. This relationship was supported by a score of 99 on the Neighbor-Joining Tree and 100 on the Maximum Likelihood Tree. The outgroup in the phylogenetic tree was identified as *Reboulia hemisphaerica*, as it does not belong to the genus *Marchantia* but is classified within the same order, Marchantiales (Luthfiah et al., 2021).

Furthermore, the *Marchantia* genus distributed across Asia and Europe demonstrated nucleotide structure similarities, with a query cover value of 100%. The phylogenetic analysis confirmed that *M. paleacea subsp. diptera* (matK sequences MP 58 and MP 54) and *Marchantia polymorpha* subsp *ruderalis* (matK sequences MC-47 and MC-44) had the closest genetic relationship, consistent with their shared geographic origin and subspecies classification. The phylogenetic tree construction further validated these findings, with *Reboulia hemisphaerica* serving as the designated outgroup.

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