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Identification of Mangrove using DNA Barcoding and Vegetation Analysis at the Sarawet Mangrove Ecotourism Site, North Minahasa Regency

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Abstract. This study aims to identify the diversity of mangrove species in the Mangrove Ecotourism area of Sarawet Village, Likupang, North Minahasa Regency, using a molecular taxonomy approach based on DNA barcoding and an ecological approach through vegetation analysis. Vegetation data collection was conducted descriptively and qualitatively using transect and plot methods. Molecular analysis included DNA extraction from mangrove leaf samples, amplification of the matK and rbcL genes using Polymerase Chain Reaction (PCR), followed by sequencing and nucleotide base comparison using the Basic Local Alignment Search Tool (BLAST). The morphological vegetation analysis found six mangrove species at the research site, namely *Rhizophora mucronata*, *Rhizophora apiculata*, Bruguiera sp., *Sonneratia alba*, *Aegiceras corniculatum*, and *Ceriops tagal*. Meanwhile, the molecular identification results of DNA barcoding showed that the matK gene sequence in the SRA sample had a 99.98% similarity with *Sonneratia alba*, and the rbcL gene sequence in the SRC sample had a 100% similarity with *Aegiceras corniculatum*. The combination of molecular identification and vegetation analysis provides accurate data on mangrove species composition, which is highly essential as a foundation for monitoring, sustainable management, and conservation of the mangrove ecotourism area as a buffer zone.

Key words: Sarawet, DNA barcoding, mangrove, vegetation.

INTRODUCTION

Mangroves are one of the coastal buffer ecosystems, found in tropical and subtropical regions (Tomlinson, 2016). Mangrove vegetation has a strong ability to grow in seawater with high salinity. Mangroves also possess unique characteristics compared to other plants; these include their distinctive formation, which extends in a neat arrangement from the mainland to the shoreline, and their rich diversity of flora, fauna and habitats (Matatuta et al., 2019 ; Darwati et al., 2022) In environments with surface air circulation and standing water that result in constant sediment exchange and replenishment, mangroves can grow and reproduce to their full potential on various substrates, including sand, sandy soil, clay, mud, and stony soil (Rambu et al. 2019)

The plant diversity of an ecosystem can be determined through vegetation analysis. The biodiversity of an ecosystem can be determined through vegetation analysis. Mangrove vegetation analysis is essential for understanding the structure and composition of the ecosystem (Ruruh dan Ernikawati, 2021; Sakti et al., 2024). Changes in the structure and composition of vegetation within an ecosystem can be monitored through periodic vegetation analysis. Therefore, monitoring vegetation within an ecosystem is crucial, as changes in vegetation can affect ecosystem stability, resilience, productivity, trophic structure, and the movement of ecosystem components (Sarnubi et al., 2020)

It is hoped that this study on the identification and vegetation of mangroves can serve as a guide for further research and provide information to local authorities for the formulation of comprehensive regulations in mangrove ecotourism areas. As mangrove ecotourism areas function as buffer zones for protected forests overseen by the UPTD KPH Unit V, information regarding this mangrove vegetation is of great importance.

Mangroves are one of the coastal buffer ecosystems. In addition to morphological and ecological approaches, organism identification has now evolved towards molecular taxonomy. Organism identification in mangrove ecosystems has now evolved towards molecular taxonomy (Afifah, 2025). DNA barcoding is a technique for taxonomic identification using one or more standard DNA regions that are universally present in the target lineage, and which possess sufficient genetic sequence variation to distinguish species and identify individuals with precision (Ariyanti et al., 2021; Yustina et al., 2025).

Molecular analysis, including techniques such as Polymerase Chain Reaction (PCR), requires DNA of sufficient quality. DNA extracted from plant cells often still contains a number of contaminants, such as polysaccharides, proteins and secondary metabolites (polyphenols, tannins and alkaloids), which can affect the purity of the DNA (Abdel-Latif dan Osman, 2017). The presence of these contaminants can inhibit the activity of polymerase and restriction enzymes (Seth et al., 2018). Therefore, this study combines DNA barcoding analysis using the matK and rbcL genes with vegetation analysis to comprehensively identify and map mangrove species.

METHODS

The research was conducted from April to September 2025. The study was carried out at the Sarawet Likupang mangrove ecotourism site in North Minahasa Regency, during the day at low tide. Identification of mangrove with mangrove identification catalog. Molecular identification via DNA barcoding was performed using mangrove leaves. Other materials included MyTaq HS Red Mix (Bioline), Genomic DNA Mini Kit (Geneaid), rbcL primer, matK primer, agarose powder, 1x TBE buffer, 1 kb DNA ladder, and micropipette tips. Total DNA was extracted from leaf samples to serve as a template for the PCR reaction. The success of the reaction was detected using agarose gel electrophoresis. PCR products successfully amplifying the rbcL and matK genes were sent for sequencing. Sequencing chromatograms were edited using Geneious v5.6 software (Drummond et al., 2012), followed by a BLAST (Basic Local Alignment Search Tool) search to compare them with DNA sequences in GenBank, and identification via BOLD (Barcode of Life Database). The method used was qualitative descriptive research. Data collection involved measuring mangrove species in Sarawet Likupang Village.

RESULTS

Total DNA extraction from plant samples (SRA, SRB, SRC) was carried out using the Tissue Genomic DNA Mini Kit (Geneaid). Polymerase Chain Reaction (PCR) amplification was carried out in a total volume of 40 µl, comprising 20 µl MyTaq HS Red Mix 2x (Bioline), 1.5 µl each of forward and reverse primers (10 µM), 15 µl ddH_2O, and 2 µl DNA template. Amplification of the matK gene in SRA and SRB samples used the matK-1Rf and matK-3Fr primers. Meanwhile, amplification of the rbcL gene in SRC samples used the rbcLaF and rbcLaR primers. The PCR machine conditions were set with an initial denaturation step at 95°C for 3 minutes, followed by 35 cycles of denaturation at 95°C for 20 seconds, primer annealing at 50°C for 30 seconds, extension at 72°C for 30 seconds, and concluded with a final extension at 72°C for 1 minute.

Examination of the PCR products using 0.8% agarose gel electrophoresis confirmed successful amplification, as evidenced by the presence of a single band of approximately 600 bp for the rbcL gene (located between the 500 bp and 750 bp standard markers), and a band of approximately 900–950 bp for the matK gene (located between the 750 bp and 1000 bp

markers). This indicates that the rbcL and matK primers bind effectively to their respective loci on the target chloroplast DNA. The SRA sample showed a DNA size of 600 bp from the PCR process, while the SRC showed a band size of 950 bp (**Figure 1**). The success of this PCR also confirms that the previous DNA extraction process produced a sufficient concentration and purity of DNA to proceed to the sequencing stage. The quality of the DNA from the sequencing results can be considered good, as evidenced by the chromatogram readings showing high, non-overlapping peaks.

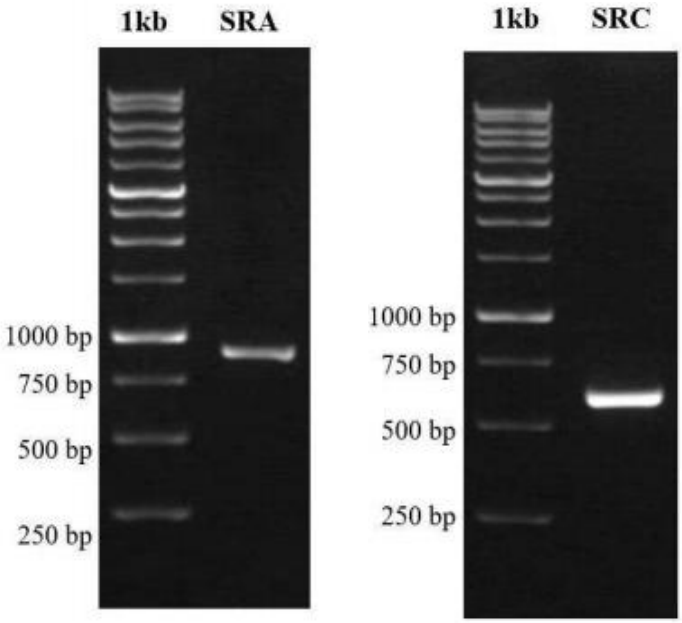

Figure 1. Visualization results of PCR products of SRA and SRC samples

The results of DNA sequence alignment using the Basic Local Alignment Search Tool (BLAST) indicate that the matK gene sequence in the SRA sample exhibits the closest similarity, at 99.98%, with the species *Sonneratia. alba (***Table 1)**. Furthermore, the results of molecular identification based on the rbcL gene sequence in the SRC sample showed an absolute similarity of 100% with the species *Aegiceras corniculatum* (**Table 1)**.

Table 1. Results of analysis using BLAST

No.	Sample Code	Blast Results	Per. Ident	Accesion
1.	SRA	*Sonneratia alba*	99.98%	KU984625.1
2.	SRC	*Aegiceras corniculatum*	100%	ON758270.1

In addition to molecular identification, morphological identification during vegetation analysis at the study site directly identified five mangrove species in the field, namely:

1. *Rhizophora mucronata*: This species is characterised by broad, elliptical, green leaves with pointed tips, and small spots on the underside of the leaves. The flowers *of R. mucronata* have four white, hairy petals, each 7 mm long. Morphologically, the fruit also appears longer and larger when compared to *R. apiculata.*
2. *R. apiculata*: This tree typically reaches a height of 2.3–5.8 metres, with a trunk diameter of up to 48 cm. Its bark is brown to greyish in colour. The main characteristic of its adaptive morphology lies in its root system, which includes aerial roots and prop roots.
3. *Bruguiera* sp.: This plant is characterised by rough-textured bark ranging in colour from light to dark grey, as well as a root system that extends above the substrate. The

leaves are lanceolate in shape, dark green on the upper surface and light green on the lower surface.

4. *S. alba*.: Morphologically, it can be a small to medium-sized tree (1.7–3 metres), with characteristic vertical pneumatophores. The leaves are elongated, inversely ovate in shape, with rounded or blunt tips.
5. *Ceriops tagal*: The trunk of this species is covered in smooth, greyish-brown bark that is sometimes slightly cracked, supported by a relatively short prop root system. The leaves range in shape from round, oblong, to elliptical, with some leaf blades exhibiting edges that curve inwards.
6. *A. corniculatum* : A small, upright tree. It generally grows to a height of between 2 and 7 metres; the leaves are ovate and rounded-elliptical in shape, with blunt or rounded tips, and are green in colour; the fruit is sharply curved and tapers to a point, and is light green in colour

The identification and mapping of mangroves are vital for the sustainable management of coastal ecosystems, which provide a wide range of benefits. Ecologically (environmentally), the mangrove root system is highly effective at preventing erosion and abrasion caused by coastal waves. Mangrove forests also act as blue carbon sinks, supporting climate change mitigation efforts; they provide vital habitats for maintaining biodiversity, such as waterbirds, shrimp and crabs; and can function as filters for pollutants and sediments. From an economic perspective, this mangrove diversity is a key source of livelihood for local communities and fishermen. Mangrove forest areas also hold promising potential for the development of ecotourism, and their raw materials can be processed into high-value products such as syrup, coffee, dodol, and natural dyes for batik fabric. Furthermore, mangroves provide a supply of local resources, such as traditional medicines and materials for construction and firewood.

DISCUSSION

The success of the PCR amplification process, as indicated by the appearance of a single band on agarose gel electrophoresis (approximately 600 bp for rbcL and 900–950 bp for matK), indicates that the rbcL and matK primers have successfully bound with precision to the target loci in the chloroplast DNA. The success of this amplification is highly dependent on the initial stage, namely DNA extraction. The extraction process in this study was deemed successful in obtaining a DNA concentration and purity level adequate for use as a PCR template. This is in line with the statements by Hakim (2014) and Purnami (2009), who stated that the purity of the template DNA is a crucial factor in the smooth running of the amplification processFurthermore, the high quality of the PCR product's DNA was also confirmed by sequencing results. Based on the chromatogram (electropherogram) readings, the staining of the dominant nucleotide bases was identified as falling within the 'good' to 'very good' categories. This quality is characterised by high, non-overlapping peaks. It is these accurate sequence readings that enable molecular species identification via the Basic Local Alignment Search Tool (BLAST) with a very high similarity level, thereby definitively confirming the identity of the SRA specimen as *S. alba* (99.98%) and the SRC specimen as *A. corniculatum* (100%).

From the results of morphological vegetation identification in Sarawet Likupang Village, five main taxa comprising the mangrove formation were identified, namely *R. mucronata, R.apiculata, Bruguiera sp., S. alba., A. corniculatum* , and *C. tagal*. Mapping the composition of these mangrove species is essential as a foundation for the sustainable management of coastal ecosystems.

Ecologically, the presence of these species provides crucial environmental protection. Adaptive morphological characteristics such as aerial roots and prop roots in *Rhizophora* sp., as well as vertical breathing roots in *Sonneratia* sp., play an effective role in absorbing wave energy, thereby preventing coastal abrasion and erosion. The mangrove forests in this area also function as blue carbon sinks, actively supporting global climate change mitigation efforts. This ecosystem also functions as a natural filtration system for sediments and pollutants, helping to maintain the quality of the surrounding waters. Furthermore, the dense vegetation creates essential habitats (ecological niches) that support wildlife biodiversity, serving as shelters and feeding grounds for fish, shrimp, crabs, and various species of waterbirds.

From a socio-economic perspective, the conservation of this mangrove biodiversity is a key pillar of livelihoods for local communities and fishermen along the Likupang coast Area Research indicates that ecotourism areas hold promising potential for boosting the local economy. Furthermore, raw materials derived from mangrove trees (such as the fruit or leaves of certain species) can be processed into high-value derivative products, including syrup, dodol, coffee, and natural dyes for textiles. The utilisation of local resources also includes the empirical use of mangrove plants as ingredients in traditional medicine, whilst the wood is widely used by local communities for basic building materials and firewood. The integration of molecular and vegetation data ultimately demonstrates the importance of comprehensive monitoring and conservation of mangrove ecosystem functions.

CONCLUSION

The results of DNA barcoding research based on BLAST analysis show that the matK gene sequence of the nearest specimen is 99.98% similar to *S. alba*, and the rbcL gene is 100% similar to *A. corniculatum*. Vegetation identification in Sarawet identified 6 mangrove species, namely: *R. mucronata*, *R. apiculate*, *Bruguiera* sp, *S. alba*, *A. corniculatum*, and *C. tagal.*

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