BIOTROPIA ISSN: 0215-6334 | e-ISSN: 1907-770X
The Southeast Asian Journal of Tropical Biology
Vol. 33 No. 2, 2026: 169 - 180
DOI: 10.11598/btb.2026.33.2.2715

Research Article

Check for updates

MICROSATELLITE-BASED GENETIC VARIATION IN *Rhizophora apiculata* AND *Rhizophora mucronata* FROM EAST KALIMANTAN

Adristi Shafa Widyasari[1], Abdul Razaq Chasani[1*], Istiana Prihatini[2], Tri Atmoko[3], Mukhlisi[3], Burhanuddin Adman[3], and Bina Swasta Sitepu[4]

[1]Laboratory of Plant Systematics, Faculty of Biology, Universitas Gadjah Mada, Yogyakarta 55281, Indonesia

[2]National Research and Innovation Agency, Applied Botany Research Center, Cibinong 16911, Indonesia

[3]National Research and Innovation Agency, Biota System Research Center, Cibinong 16911, Indonesia

[4]National Research and Innovation Agency, Ecology Research Center, Cibinong 16911, Indonesia

ARTICLE HIGLIGHTS

- Microsatellite markers were used to study population genetic variation in Rhizophora
- Molecular systematic analysis showed moderate genetic diversity with low heterozygosity
- Population genetic structure was dominated by within-population variation (AMOVA)
- Phenetic analyses revealed weak clustering among Rhizophora populations
- Genetic patterns supported connectivity within Rhizoporaceae populations in East Kalimantan

Article Information

Received : 30 September 2025
Revised : 30 December 2025
Accepted : 24 February 2026
Published online : 17 March 2026

***Corresponding author, e-mail:**
ar.chasani@ugm.ac.id

OPEN ACCESS
Copyright (c) 2025@author(s).

ABSTRACT

Mangroves of the family Rhizophoraceae are pantropical, with the genus *Rhizophora* forming a major component of tropical mangrove ecosystems. *Rhizophora apiculata* and *Rhizophora mucronata* are widely distributed in the Paser region, Balikpapan Bay, and the Mahakam Delta of East Kalimantan, Indonesia. This study examined genetic diversity and population structure of both *Rhizophora* species using simple sequence repeat (SSR) markers. The results showed moderate genetic diversity but relatively low heterozygosity in both species. Most genetic variation occurred within populations, as indicated by AMOVA, while *R. apiculata* exhibited lower genetic differentiation among populations and lacked private alleles, and *R. mucronata* showed higher population differentiation and possessed private alleles, suggesting localized genetic variation. Cluster and principal coordinate analyses revealed extensive overlap among populations for both species, indicating weak population structure and ongoing gene flow across the study area. Overall, the findings suggest that populations of *R. apiculata* and *R. mucronata* in East Kalimantan function as genetically connected units rather than as strongly differentiated populations. These results highlight the importance of maintaining habitat connectivity and protecting genetically unique populations to support the long-term resilience and sustainable management of mangrove genetic resources in East Kalimantan.

Keywords: microsatellite, molecular systematic, phenetic, population genetic, Rhizoporaceae.

INTRODUCTION

Mangrove ecosystems provide substantial ecological, economic, and social benefits, including support for coastal livelihoods and climate change mitigation through high carbon storage capacity. Despite their relatively low global species richness (28 genera and approximately 70 species), Indonesia supports extensive and diverse mangrove forests covering about 3.3 million ha across the archipelago (Yahya *et al.* 2014; Arifanti *et al.* 2022). However, mangroves are experiencing rapid deforestation and degradation, with an estimated 10 – 33% of their area converted or degraded in recent decades, highlighting the urgent need for effective conservation and management strategies (Richards & Friess 2016; Arifanti *et al.* 2022).

Mangrove areas in Indonesia categorized as being in critical condition total approximately 476,192 ha within forest areas and 161,432 ha outside forest areas. Previous study study related to mangrove area indicated that oil palm plantations and aquaculture are the main causes of mangrove loss in Indonesia (Richards & Friess 2016). Based Land Cover Map data, mangrove land cover in Indonesia decreased by 567,845 ha between 1990 and 2019. The area of primary and secondary mangrove land cover was to 2,483 ha in 2020, increased to 2,729 ha in 2021, and declined to 2,640 ha in 2022 (BPS 2023). Outside forest areas, 32,662 ha of mangroves are classified as critical and not suitable for rehabilitation, whereas 6,305 ha of mangroves are classified as critical but suitable for rehabilitation (BPS 2023).

Indonesian government established the Peat and Mangrove Restoration Agency (BRGM) under Presidential Regulation No. 120/2020 to reduce mangrove degradation, mandating the rehabilitation of approximately 600,000 ha of mangrove areas across nine provinces, including East Kalimantan by 2024 (Nurhati & Mardiyarso 2022). East Kalimantan Province plays a significant role in mangrove forest conservation efforts in Indonesia, being the second-largest province after Papua in terms of mangrove forest area. However, data on primary mangrove deforestation in East Kalimantan and North Kalimantan provinces in 2017 showed deforestation of 5,142 ha, the highest compared to other provinces (Salminah & Alviya 2019).

Advances in research, technology transfer, and information systems are essential for effective mangrove management in Indonesia, where rehabilitation efforts have not yet matched the rate of mangrove loss. Molecular studies, particularly those addressing the genetic diversity of mangrove species, remain limited but are increasingly important for supporting sustainable conservation and restoration strategies (Arifanti *et al.* 2022). In this context, genetic diversity data of *Rhizophora* populations in East Kalimantan provide a scientific basis for conservation planning, especially in response to environmental pressures associated with the development of the Nusantara Capital City (IKN).

This study investigated the genetic diversity and relationships of *Rhizophora apiculata* and *Rhizophora mucronata* in East Kalimantan using SSR markers. The objectives were assess genetic diversity within and among populations, and to determine phenetic kinships between the two species. The study hypothesized that both species exhibit high genetic diversity (polymorphism > 50%), that *R. apiculata* has higher diversity than *R. mucronata*, and that phenetic relationships correspond to the geographic origins of populations.

MATERIALS AND METHODS

Specimen Collection

Vegetative leaf specimens of *R. apiculata* and *R. mucronata* were collected in 2022 using exploratory sampling methods in East Kalimantan. Sampling locations included the Mahakam Delta (Kutai Kartanegara), Paser, and Balikpapan Bay which were selected based on the abundance of mangrove vegetation (Fig. 1).

Fresh leaf specimens were stored in envelopes and placed in plastic clips containing silica gel. Specimens were kept in dry conditions for 2 years before conducting DNA extraction in 2024.

Molecular Characterization

All tools that used were sterilized by autoclaving before use. DNA extraction followed the protocol of the Geneaid Genomic DNA Mini Kit (Plant), with minor modifications. DNA amplification was performed using four primers (Table 1).

The PCR reaction, modified from Yahya *et al.* (2014), consisted of 2.75 µL ddH_2O, 6.25 µL MyTaq Redmix HS (Bioline), 0.5 µL forward primer, 0.5 µL reverse primer, 0.2 µL BSA, and 2.5 µL DNA template diluted at a 1:9 ratio, totalling 12.5 µL. PCR was conducted using a Bio-Rad T100 Thermal Cycler. Amplification utilized primers Rm111, Rm121, RapT09, and RapT17 (Azman *et al.* 2020). PCR cycling conditions were adapted from Islam *et al.* (2004) and Rosero-Gallindo *et al.* (2002) in Faradilla *et al.* (2022), with the following modifications: Pre-PCR at 4 °C for 3 seconds, initial denaturation at 94 °C for 2 minutes, followed by 35 cycles at 94 °C for 30 seconds, annealing at 55 °C for 30 seconds, extension at 72 °C for 1 minute, and a final infinite hold at 4 °C.

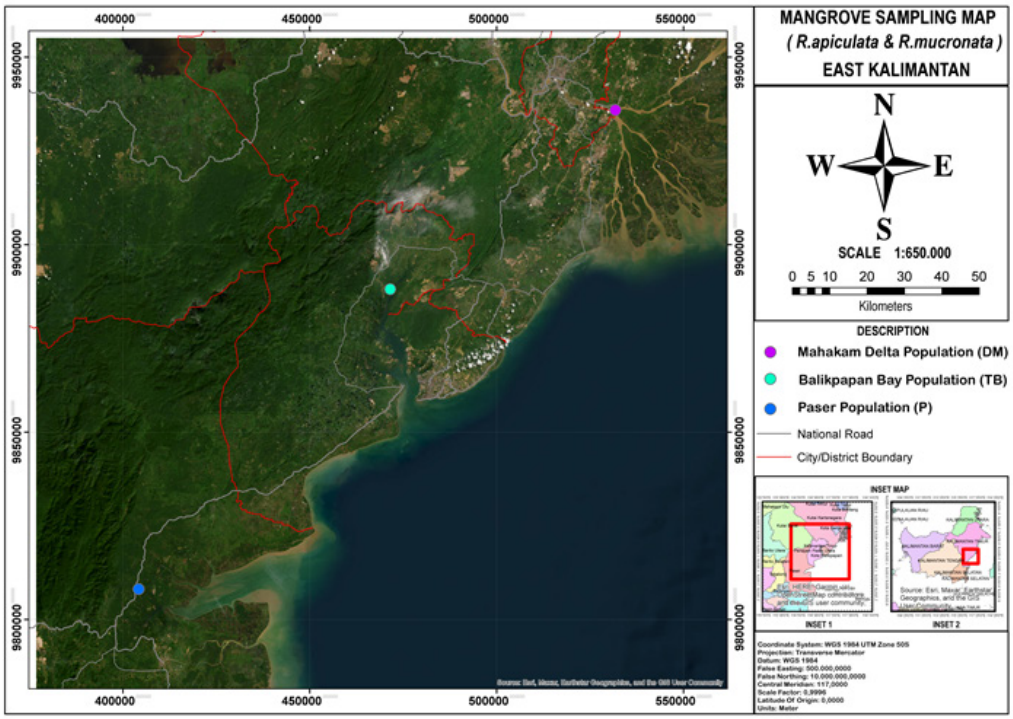


Figure 1 Map of sampling locations

Table 1 Primer Sequenc

No.	Locus	Primer sequence	Repeat motif	Accession number
1.	RM111	F: AACCGTTACTCG CGTATGCT R: CATTGCCTCCATTCCATT	(TC)13	AB721976
2.	RM121	F: TGGCCTATAGAGAAAGCGGA R: CCTTCAATCCCAAACAGC	(ATC)12	AB721982
3.	RapT09	F: AGGCTCGGGATTTTCCTTCG R: CTGGGAAGCATGGGGTAGTG	(TC)16	MT364426
4.	RapT17	F: GTGGGAGCCTGCAAAGTTTG R: GCGCGTTGAGCTCAGTTTAG	(GA)18	MT364428

DNA Electrophoresis

Electrophoresis was conducted using agarose 1.5% and TBE 1x with 100 volt and 400 Ampere for 75 minutes. The results were visualized by Gel Documentation (Biorad).

SSR Data Analysis

Microsatellite data were evaluated based on the number and size of alleles in base pairs (bp), and the allele data were manually recorded in Excel (XLS) format. These data were used to analyze genetic variation using GenAlex 6.5 software. Parameters such as expected heterozygosity, number of alleles, effective number of alleles, genetic distance, PCoA, polymorphism, and AMOVA were calculated.

SSR allele identification was based on manual evaluation, in which only high-quality, reproducible peaks were scored. Stutter bands and poorly defined peaks were excluded, and ambiguous signals were coded as missing data to ensure scoring accuracy.

Phenetic analysis was performed using a dendrogram. Dendrograms were constructed based on allele size and presence (1) or absence (0) using MVSP software. Genetic similarity was assessed using the Jaccard coefficient to generate a genetic distance matrix, which was then used to construct a dendrogram using the UPGMA method.

RESULTS AND DISCUSSION

DNA Extraction Results

A total of 63 samples were successfully extracted, consisting of 36 samples of *R. apiculata* and 27 samples of *R. mucronata*. Eight DNA samples per population were used, except for the *R. apiculata* population from the Mahakam Delta, for which only six samples were obtained due to suboptimal sample quality; as the available materials consisted of dried leaves. DNA quality assessments were performed on all samples, but only those reported in this study were successfully extracted and produced clearly detectable DNA bands.

SSR Fragment Length

The SSR fragment lengths obtained in this study varied depending on the primer used (Fig.2).

For *R. apiculata*, primer Rm111 produced fragments of 140 – 150 bp, Rm121 of 170 – 180 bp, RapT09 of 170 – 180 bp, and RapT17 of 270 – 280 bp. For *R. mucronata*, Rm111 resulted in 140 – 150 bp, Rm121 in 170 – 185 bp, RapT09 in 170 – 180 bp, and RapT17 in 185 – 280 bp. These results are consistent with the findings of studies conducted by Azman *et al.* (2020), who reported that the RapT09 primer has a fragment length of ~170 bp and RapT17 ~281 bp, while Rm111 is ~149 bp and Rm121 ~179 bp (Azman *et al.* 2020; Shinmura *et al.* 2012). This consistency confirms the reliability and cross-study comparability of the SSR markers used. Moreover, variation in fragment length among individuals reflects differences in the number of repeat motifs, which is a fundamental characteristic of SSR polymorphism and underpins their effectiveness in assessing genetic diversity and population structure. Therefore, the observed SSR fragment length variation supports the suitability of these markers for evaluating genetic diversity in *Rhizophora* populations in East Kalimantan.

Genetic Distance and Principal Coordinates (PCoA)

Genetic distance is a measure of genetic difference between populations or species, typically calculated using allele frequency data across multiple loci (Kanaka *et al.* 2023). The genetic distances among *R. apiculata* and *R. mucronata* samples are further discussed in the phenogram analysis.

Principal Coordinate Analysis (PCoA) is a multivariate technique that represents genetic distances in a low-dimensional Euclidean space using eigenvalue equations, which is also referred to as classical scaling or metric multidimensional scaling. The PCoA results for *R. apiculata* are shown in Figure 3. The total variation explained was 91.78%, with 56.65% on coordinate 1 and 35.13% on coordinate 2. The clustering pattern shows individuals from different populations overlapping at similar coordinates. This overlap indicates weak genetic structuring and low genetic differentiation, suggesting high genetic similarity and potential gene flow among populations.

The PCoA results for *R. mucronata* species are presented in Figure 4. The total variation in PCoA of *R. mucronata* species was 98.29%, with 54.23% on coordinate 1 and 44.07% on coordinate 2. The PCoA plot shows extensive overlap among individuals from different populations, indicating weak genetic structuring and low genetic differentiation.

Individuals from the same population formed more than one cluster but remained closely grouped, as observed among Paser samples (Rm1–Rm8). This pattern suggests high genetic similarity across populations, likely due to ongoing gene flow or recent common ancestry, and implies that *R. mucronata* populations function as a genetically connected unit, highlighting the importance of maintaining habitat connectivity for conservation.

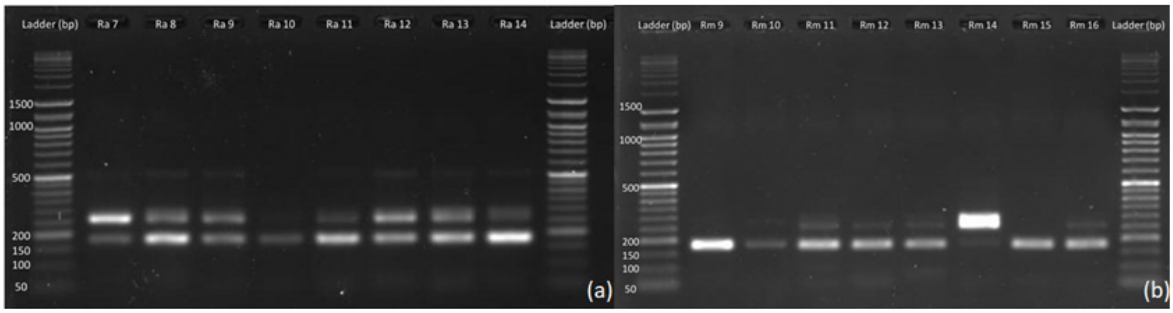


Figure 2 Gel documentation RapT17

Notes: (a) *R. apiculata*;, (b) *R. mucronata*

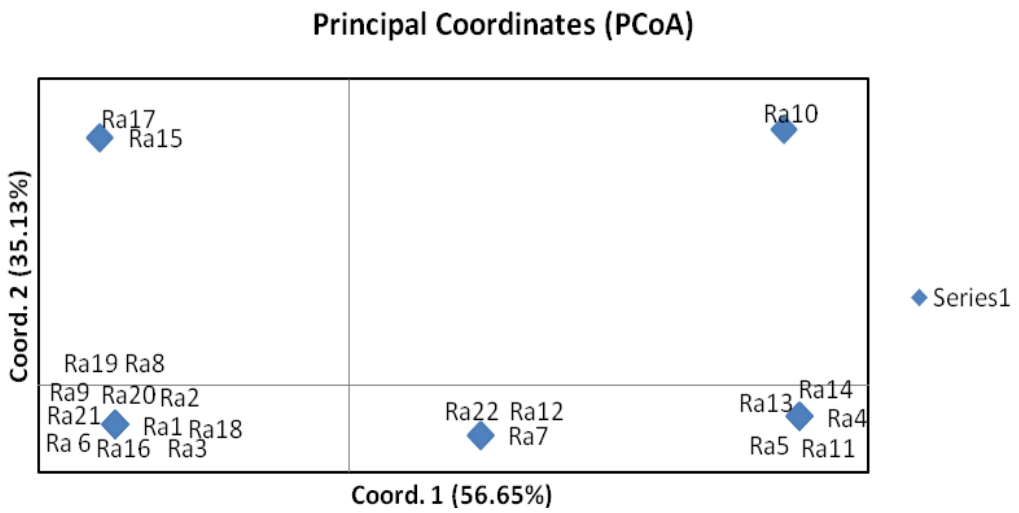


Figure 3 Principal Coordinates Analysis from *Rhizophora apiculata* at Mahakam Delta Population (Ra1-Ra6), Paser Population (Ra7-Ra14), and Balikpapan Bay Population (Ra15-Ra22)

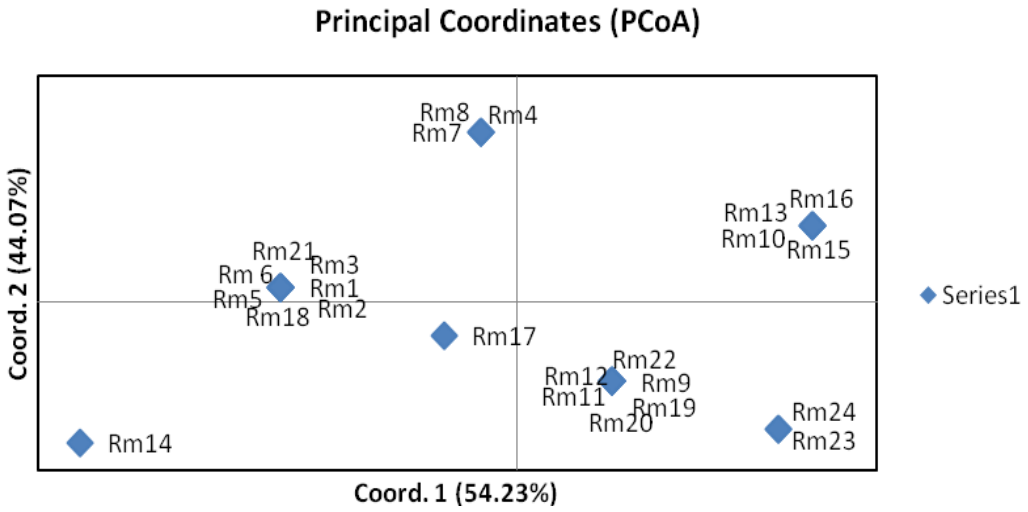


Figure 4 Principal Coordinate Analysis from *Rhizophora mucronata* at Paser Population (Rm1-Rm8), Mahakam Delta Population (Rm9-Rm16), and Balikpapan Bay Population (Rm17-Rm24)

Allele Frequency, Heterozygosity, F-statistics and Polymorphism

Allele frequencies for the Paser, Balikpapan Bay, and Mahakam Delta populations are shown in Table 2. Differences in allele presence and frequency among populations, such as the absence of allele 140 at the Rm111 locus in Paser and the dominance of allele 150, indicate variation in allelic composition across sites.

High-frequency alleles observed at specific loci, including allele 170 at Rm121 in Mahakam Delta and alleles 270 and 280 at RapT17 in Balikpapan Bay and Paser, suggest unequal allele distribution among populations. However, the absence of population-specific fixed alleles and the presence of shared high-frequency alleles imply limited genetic differentiation and possible gene flow among populations.

The highest allele frequencies in the Paser population were observed at locus Rm121 and RapT09, where allele 170 reached a frequency of 1.00. In the Balikpapan Bay population, the highest allele frequency was detected at locus Rm121 for allele 180 (0.875), while in the Mahakam Delta population, allele 150 at locus Rm111 showed a frequency of 1.00.

The occurrence of high-frequency and fixed alleles suggests dominance of specific alleles within populations, which may reflect limited allelic variation at certain loci. However, the presence of shared alleles among populations indicates genetic similarity and potential gene flow. All allele frequencies fell within the expected range of 0 to 1, and the sum of allele frequencies equalled 1 at each locus, confirming the reliability of the genetic data (Aoki & Fukasawa 2024).

Differences in allele patterns among the Paser, Balikpapan Bay, and Mahakam Delta populations are presented in Table 3. The Paser population had a lower number of different alleles (Na) than the other two populations, indicating slightly lower genetic variation. Similar effective numbers of alleles (Ne) across populations suggest that alleles are distributed in a comparable manner, with no strong genetic separation.

The absence of private alleles and the presence of shared alleles among populations indicate gene flow and genetic connectivity. Heterozygosity values were low to moderate, with Balikpapan Bay showing the highest value, suggesting relatively higher genetic diversity at this site.

Table 2 Allele frequencies in *R. apiculata* and *R. mucronata* population

Locus	Allele	Paser	Balikpapan Bay	Mahakam Delta
R. apiculata				
Rm111	140	0.000	0.125	0.250
	150	1.000	0.875	0.750
Rm121	170	0.667	0.375	0.938
	180	0.333	0.625	0.063
RapT09	170	0.750	0.714	0.813
	180	0.250	0.286	0.188
RapT17	270	0.400	0.750	0.500
	280	0.600	0.250	0.500
R. mucronata				
Rm111	140	0.000	0.125	0.250
	150	1.000	0.875	0.750
Rm121	170	0.667	0.375	0.938
	180	0.333	0.625	0.063
RapT09	170	0.750	0.714	0.813
	180	0.250	0.286	0.188
RapT17	270	0.400	0.750	0.500
	280	0.600	0.250	0.500

Table 3 Allele patterns and heterozygosity of *R. apiculata* population

Population	N	N	Na	Na Freq. ≥ 5%	Ne	I	Ho	He	F
		(mean ± SE)							
Paser	21	5.250 ± 0.479	1.750 ± 0.250	1.750 ± 0.250	1.581 ± 0.205	0.468 ± 0.158	0.225 ± 0.131	0.325 ± 0.110	0.278 ± 0.337
Balikpapan Bay	31	7.750 ± 0.250	2.000 ± 0	2.000 ± 0	1.613 ± 0.126	0.550 ± 0.061	0.259 ± 0.102	0.368 ± 0.053	0.358 ± 0.275
Mahakam Delta	32	7.750 ± 0.250	2.000 ± 0	2.000 ± 0	1.543 ± 0.181	0.493 ± 0.097	0.313 ± 0.231	0.324 ± 0.080	0.131 ± 0.436

Heterozygosity, F-statistics, and Polymorphism in Populations of *R. apiculata* Species

The sample sizes used in this study (Paser: N = 21; Balikpapan Bay: N = 31; Mahakam Delta: N = 32) are comparable to those applied in previous SSR-based studies of *Rhizophora* and are sufficient to characterize major patterns of genetic diversity (Yahya *et al.* 2014). Although larger sample sizes are often employed in SNP-based analyses (Ruang-areerate *et al.* 2023), SSR markers generally require fewer individuals to provide reliable estimates of genetic variation.

The number of alleles per locus (Na) was low to moderate (1.75 – 2.00) and fell within the lower range reported for *R. apiculata* populations in the Greater Sunda Islands (Yahya *et al.* 2014). This pattern suggests relatively limited allelic richness in the studied populations compared with more genetically diverse regions.

Consistently, the effective number of alleles (Ne) was low and narrowly distributed among populations (1.54 – 1.61), similar to values reported in both SSR- and SNP-based studies of *R. apiculata* (Yan *et al.* 2016; Ruang-areerate *et al.* 2023). These results may reflect historical demographic constraints and the influence of genetic drift, a common feature of mangrove populations, rather than recent population fragmentation.

Heterozygosity (H) is a key indicator of genetic diversity in natural populations, reflecting the proportion of loci with different alleles at the individual level and overall genetic variability at the population level. Heterozygosity is commonly expressed as observed heterozygosity (Ho) and expected heterozygosity (He). Expected heterozygosity (He) estimates the proportion of individuals expected to be heterozygous at a randomly selected locus under Hardy–Weinberg equilibrium, whereas observed heterozygosity (Ho) represents the actual proportion of heterozygotes in the population (Kanaka *et al.* 2023).

In this study, Ho values in the Paser (0.225) and Balikpapan Bay (0.259) populations were comparable to those reported for several *R. apiculata* populations in Indonesia, including Langkat and Padang (Yahya *et al.* 2014). The higher Ho value observed in the Mahakam Delta population (0.313) was similar to values reported for Untung Jawa, Banyuwangi, Tarakan, and other Indo–West Pacific populations (Yahya *et al.* 2014; Yan *et al.* 2016). Comparable Ho values have also been reported for Malaysian populations using SSR markers (Azman *et al.* 2020), indicating that heterozygosity levels observed in this study fell within the typical range for the species.

Expected heterozygosity (He) ranged from 0.324 to 0.368 across populations and was consistent with values reported from Indonesia and Malaysia (Yahya *et al.* 2014; Azman *et al.* 2020). The generally moderate He values, together with Ho values that were slightly lower than He, may suggest limited genetic diversity and the influence of inbreeding or historical demographic processes. From a conservation perspective, these patterns indicate that although current genetic diversity is comparable to regional populations, continued habitat degradation and fragmentation could further reduce heterozygosity. Maintaining habitat integrity and connectivity among mangrove populations is, therefore, essential to conserve genetic diversity in *R. apiculata*.

The fixation index (F) value in the Paser population (0.278) was similar to those reported for the Untung Jawa (0.279) and Banyuwangi (0.229) populations, while the Mahakam Delta population showed a lower F value (0.131), comparable to that of the Tarakan population (0.134) (Yahya *et al.* 2014). In contrast, the Balikpapan Bay population exhibited the highest F value (0.358), exceeding those reported for most populations in Yahya *et al.* (2014). The fixation index (F), developed by Sewall Wright, is commonly used to assess deviations from random mating and to infer levels of inbreeding or population structure in natural populations (Kanaka *et al.* 2023). The relatively higher F value observed in the Balikpapan Bay population may indicate a greater degree of inbreeding or restricted gene flow, whereas the lower values in Mahakam Delta suggest more effective mixing of alleles. From a conservation perspective, elevated F values highlight populations that may be more vulnerable to genetic erosion and, therefore, require careful management to maintain gene flow and genetic diversity.

Polymorphism percentage observed in *R. apiculata* was 100% in the Balikpapan Bay and Mahakam Delta populations, while the Paser population showed a lower value of 75% (Table 4).

The mean value of 91.67% in the present study is slightly lower than those reported for *R. apiculata* populations in the Gulf of Thailand and the Andaman Sea, which exhibited polymorphism

levels of 99.92% and 99.05%, with a mean of 94.49% (Ruang-areerate *et al.* 2023). High levels of allelic polymorphism are generally associated with higher genetic diversity (Petolescu *et al.* 2024). The reduced percentage of polymorphism in the Paser population may indicate lower genetic variability.

The percentage of polymorphic loci observed in *R. mucronata* was 100% in the Balikpapan Bay population, 75% in the Paser population, and 50% in the Mahakam Delta population (Table 4). Overall, the mean level of polymorphism in *R. mucronata* was lower than that observed in *R. apiculata*. High levels of allelic polymorphism are generally associated with greater genetic diversity (Petolescu *et al.* 2024). The reduced polymorphism observed in the Mahakam Delta population may indicate lower genetic variability, which could reflect local environmental pressures. From a conservation perspective, populations with lower polymorphism may be more vulnerable to genetic erosion and, therefore, warrant increased management attention.

Allele Frequency and Sample Size in Populations of *R. mucronata* Species

Private alleles were detected in the Balikpapan Bay and Paser populations of *R. mucronata*, with values of 0.25 and 0.50, respectively (Table 5). The Balikpapan Bay population exhibited a private allele at locus Rm111 (allele 160) with a frequency of 0.125, while the Paser population showed two private alleles at loci Rm121 and RapT17 (allele 185), each with a frequency of 0.50.

Private alleles, defined by their exclusive occurrence in a single population, are informative indicators of population differentiation and local genetic uniqueness (Szpiech & Rosenberg 2011). The presence of private alleles in the Paser and Balikpapan Bay populations suggests some degree of localized genetic variation, which may reflect restricted gene flow or historical demographic processes. From a conservation perspective, populations possessing private alleles may warrant special attention, as they contribute uniquely to the overall genetic diversity of the species (Ribeiro *et al.* 2013).

Table 4 Polymorphism percentage of *R. apiculata* and R mucronata

Species	Population	Polymorphism (%)
R. apiculata	Paser	75.00
	Balikpapan Bay	100.00
	Mahakam Delta	100.00
	Mean	91.67
	SE	8.33
R. mucronata	Paser	75.00
	Balikpapan Bay	100.00
	Mahakam Delta	50.00
	Mean	75.00
	SE	14.43

Table 5 Private allele of *R. mucronata*

Population	Locus	Allele	Freq
Teluk Balikpapan	Rm111	160	0.125
Paser	Rm121	185	0.250
Paser	Rap T17	185	0.250

Heterozygosity, F-statistics, and Polymorphism in Populations of *R. mucronata* Species

R. mucronata populations showed an average number of alleles of 1.50 in the Mahakam Delta population and 2.25 in both the Balikpapan Bay and Paser populations (Table 6).

The mean allele frequency values corresponded to the observed average number of alleles. The effective number of alleles was highest in the Paser population (2.159), followed by Balikpapan Bay (1.918) and Mahakam Delta (1.421). Similarly, the Shannon diversity index was highest in the Paser population (0.704), intermediate in Balikpapan Bay (0.680), and lowest in Mahakam Delta (0.325).

These patterns suggest higher genetic diversity in the Paser and Balikpapan Bay populations compared to the Mahakam Delta population. The lower diversity observed in Mahakam Delta may indicate reduced allelic richness, highlighting the importance of maintaining genetic diversity in this population for conservation purposes.

In this study, the highest He value was observed in the Balikpapan Bay population (0.449), followed by the Paser population (0.443), while the Mahakam Delta population showed a lower He value (0.325). Observed heterozygosity values were lower than expected across all populations, with Ho values of 0.299 in Balikpapan Bay, 0.219 in Paser, and 0.167 in Mahakam Delta. These Ho values are lower than those reported for *R. mucronata* populations in Tarakan and Sulawesi (Yan *et al.* 2016), suggesting reduced levels of realized heterozygosity in the present study. The consistent pattern of Ho < He may indicate a deficit of heterozygotes, potentially reflecting inbreeding, population substructure, or historical demographic effects.

The fixation index analysis further supports this pattern, with a mean FST value of 0.273, indicating moderate genetic differentiation among populations (Table 7).

The mean FIS (0.489) and FIT (0.572) values were positive, suggesting an excess of homozygotes within populations and across loci, although negative values were observed at the RapT17 locus. Positive FIS values are commonly interpreted as evidence of inbreeding or limited genetic diversity (Kanaka *et al.* 2023; Yahya *et al.* 2014). From a conservation perspective, these results suggest that *R. mucronata* populations in the study area may be experiencing reduced genetic variability, emphasizing the importance of maintaining habitat connectivity and gene flow to mitigate further genetic erosion.

Table 6 Allelic pattern and heterozigosity of *R. mucronata* population

Population	N	Na	Na Freq. ≥ 5%	Ne	I	Ho	He	No. Priv. Alleles
	(mean±SE)							
Mahakam Delta	7.500 ± 0.500	1.500 ± 0.289	1.500 ± 0.289	1.421 ± 0.243	0.325 ± 0.147	0.167± 0.167	0.228 ± 0.132	0 ± 0
Balikpapan Bay	7.750± 0.250	2.250 ± 0.250	2.250 ± 0.250	1.918 ± 0.243	0.680 ± 0.122	0.299± 0.173	0.449 ± 0.080	0.250 ± 0.250
Paser	7.000± 1.000	2.250 ± 0.479	2.250 ± 0.479	2.159 ± 0.452	0.704 ± 0.254	0.219± 0.180	0.443 ± 0.154	0.500 ± 0.289

Table 7 Fstat value of *R. mucronata*

	Rm111	Rm121	RapT09	Rap T17	Mean	SE
FIS	1.000	0.856	0.350	-0.250	0.489	0.283
FIT	1.000	0.927	0.545	-0.183	0.572	0.271
FST	0.244	0.495	0.300	0.054	0.273	0.091
NM	0.773	0.255	0.584	4.395	1.502	0.970

Analysis of Molecular Variance (AMOVA)

The AMOVA results showed that most molecular variation of R. apiculate occurred within populations (83%), while variation among populations was relatively low (17%) (Table 8).

This pattern indicates weak genetic differentiation among populations and suggests substantial genetic similarity. Similar results have been reported in previous studies of *Rhizophora*, including Yahya *et al.* (2014), who found 67% of variation within populations and 33% among populations, as well as Ruang-areerate *et al.* (2023) and Azman *et al.* (2020), who reported higher within-population variation than among-population variation. The predominance of within-population genetic variation is commonly interpreted as evidence of ongoing gene flow or recent common ancestry among populations. From a conservation perspective, this pattern suggests that maintaining connectivity among populations is important to preserve genetic diversity and prevent further population differentiation.

In regard to *R. mucronata*, the AMOVA results showing that a greater proportion of molecular variation was found within populations (66%) than among populations (34%), indicated moderate genetic differentiation among populations. This pattern suggests that while populations are not completely isolated, some level of population structuring is present. This result differs from the findings of Wee *et al.* (2015), who reported higher among-population variation relative to within-population variation, indicating stronger genetic differentiation in their study. Such differences may reflect variation in geographic scale, sampling design, or historical and environmental factors influencing population connectivity. From a conservation perspective, the presence of substantial within-population variation highlights the importance of preserving local populations, while the moderate among-population differentiation suggests that maintaining gene flow among populations remains important to sustain overall genetic diversity.

Phenetic Relationship Analysis

The phenogram for *R. apiculata* was divided into two large clusters (Fig. 5). The first cluster with samples Ra5_DM to Ra4_DM and the second cluster with samples Ra2_DM to Ra1_DM. The phenogram showed that the samples have shared alleles so their position in the phenogram were dispersed. This clustering is consistent with the PCoA results. The phenogram for *R. mucronata* was also divided into two large clusters with a similarity of 0.169 (Fig. 6). The first cluster with samples Rm16_DM to Rm4_P and the second cluster with samples Rm24_TB to Rm1_P. This grouping is consistent with the grouping results that appear in PCoA.

Table 8 AMOVA of *R. apiculata* and *R. mucronata*

Species	Source	Df	SS	MS	Est. Var.	%
R. apiculata	Among Pops	2	307.197	153.598	12.586	17%
	Within Pops	19	1179.167	62.061	62.061	83%
	Total	**21**	**1486.364**		**74.648**	**100%**
R. mucronata	Among Pops	2	916.667	458.333	46.205	34%
	Within Pops	21	1862.500	88.690	88.690	66%
	Total	**23**	**2779.167**		**134.896**	**100%**

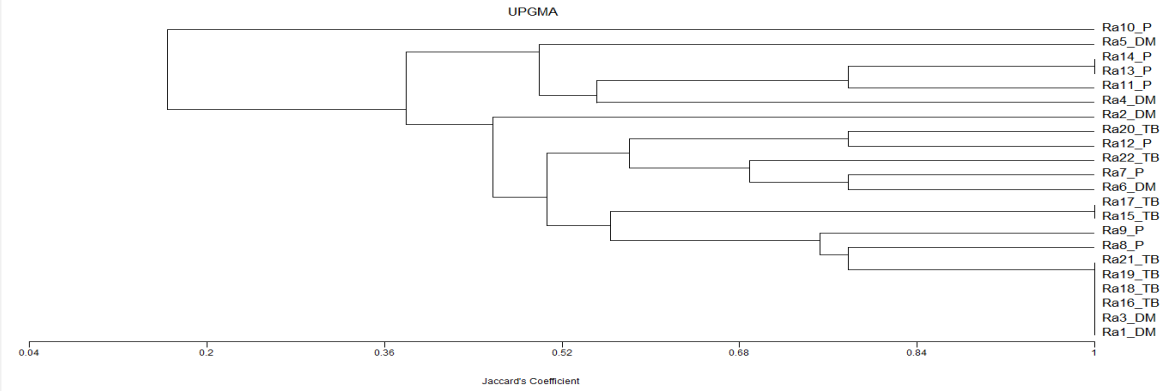


Figure 5 Phenogram for *R. apiculata*

Notes: TB = Balikpapan Bay; DM = Mahakam Delta; P = Paser.

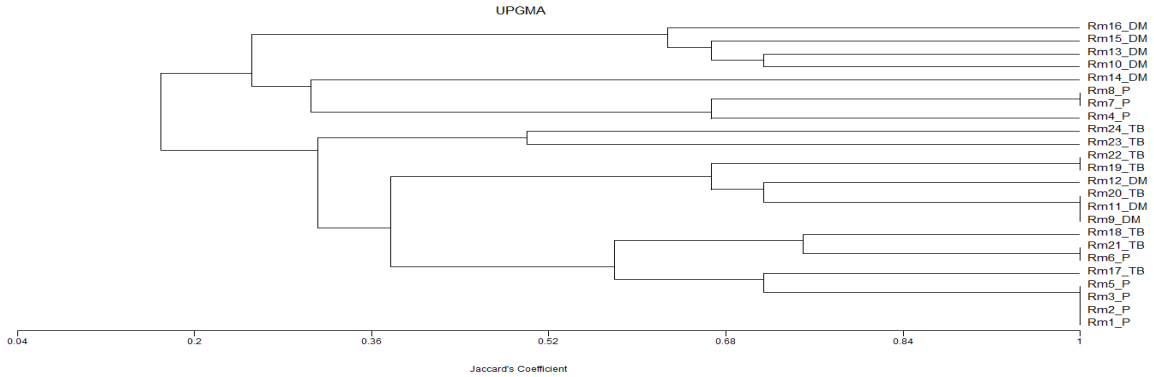


Figure 6 Phenogram for *R. mucronata*

Notes: TB = Balikpapan Bay; DM = Mahakam Delta; P = Paser.

Phenetics is a taxonomic approach that classifies organisms based on overall similarity, commonly visualized using phenograms that represent phenetic relationships among taxa. Phenograms do not infer evolutionary history but illustrate similarity based on clustering patterns. The use of UPGMA enables the application of phenetic analyses in molecular studies because the method is compatible with quantitative molecular data, allowing morphological characters to be replaced by molecular markers without altering the analytical framework. Consequently, phenetic trees are widely applied in molecular research to visualize genetic similarity and variation among populations or species and to support species classification based on genetic data. Such analyses contribute to evaluating genetic structure and relationships among taxa (Kranke 2024).

Genetic diversity in mangrove populations reflects their adaptive capacity to environmental change and demographic processes and is therefore a key consideration in conservation and restoration planning, particularly for maintaining gene flow, preventing inbreeding, and selecting appropriate reproductive material (Canty *et al.* 2022). Although advances in phylogeny and phylogeography have expanded understanding of mangrove genetic structure, their application in conservation practice remains limited. However, integrating genetic and genomic approaches can enhance mangrove management and long-term population resilience (Hoffmann & Sgró 2011; Wee *et al.* 2019).

In this study, AMOVA results indicating higher genetic variation within populations than among populations, together with low heterozygosity values, underscore important conservation implications for *R. apiculata* and *R. mucronata* in East Kalimantan. These patterns suggest that individual populations harbor unique genetic variation that should be conserved in situ, particularly in Paser, the Mahakam Delta, and Balikpapan Bay, and support the use of locally sourced propagules and stakeholder involvement in restoration efforts.

CONCLUSION

SSR analysis revealed moderate genetic variation but generally low heterozygosity in *Rhizophora apiculata* and *R. mucronata* populations from East Kalimantan. Most genetic variation occurred within populations, consistent with AMOVA results, suggesting that population are largely genetically connected. *R. mucronata* exhibited higher population differentiation and the presence of private alleles, indicating population-specific genetic variation. Population clustering was not clearly associated with geographic origin, suggesting historical or ongoing gene flow. These findings emphasize the importance of conserving multiple mangrove populations, particularly those with unique alleles and maintaining habitat connectivity to preserve genetic diversity and support long term resilience. Effective conservation strategies should prioritize the protection of existing populations, systematic genetic documentation, and strengthened local management of mangrove forest in East Kalimantan.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the support of BRIN (PEE scheme No. 950/II/HK.01.00/4/2022) for funding sample collection, as well as LPDP-BRIN (RIIM No. 82/II.7/HK/2022) and the Faculty of Biology, Universitas Gadjah Mada, for providing research supplies.

REFERENCES

Aoki S, Fukasawa K. 2024. Kernel density estimation of allele frequency including undetected alleles. Peer J 12(4):1-15. DOI: 10.7717/peerj.17248.

Arifanti VB, Sidik F, Mulyanto B, Susilowati A, Wahyuni T, Subarno, Novita N. 2022. Challenges and strategies for sustainable mangrove management in Indonesia: A Review. Forests 13(5):1–18. DOI: 10.3390/f13050695.

Azman A, Ng KKS, Ng CH, Lee CT, Tnah LH, Zakaria NF, Lee SL. 2020. Low genetic diversity indicating the threatened status of *Rhizophora apiculata* (*Rhizophora*ceae) in Malaysia: Declined evolution meets habitat destruction. Sci Rep 10(1):1–12. DOI: 10.1038/s41598-020-76092-4.

[BPS] Badan Pusat Statistik. 2023. Luas penutupan lahan Indonesia di dalam dan di luar kawasan hutan tahun 2014–2022 menurut kelas (ribu ha) [Land cover area of Indonesia inside and outside forest zones in 2014–2022 by class (thousand ha)]. Jakarta (ID): Badan Pusat Statistik; [Internet] [cited 2024 Mar 20]. Available from: https://www.bps.go.id/id/statistics-table/1/MjA4NCMx/luas-penutupan-lahan-indonesia-di-dalam-dan-di-luar-kawasan-hutan-tahun-2014-2022-menurut-kelas--ribu-ha-.html

Canty SWJ, Kennedy JP, Fox G, Matterson K, González VL, Núñez-Vallecillo ML, Rowntree JK. 2022. Mangrove diversity is more than fringe deep. Sci Rep 12(1):1–10. DOI: 10.1038/s41598-022-05847-y.

Faradilla FA, Prihatini I, Suranto, Susilowati A. 2022. Genetic variation of Austropuccinia psidii in some species of Myrtaceae as host plants in Java, Indonesia based on Simple Sequence Repeats (SSR) Markers. Biodiversitas 23(1):256–263. DOI: 10.13057/biodiv/d230131.

Hoffmann AA, SgróCM. 2011. Climate change and evolutionary adaptation. Nature 470(7335):479–485. DOI: 10.1038/nature09670.

Islam MS, Lian C, Kameyama N, Wus B. Hogetsu T. 2004. Development of microsatellite markers in *Rhizophora* stylosa using a Dual- Suppression-Polymerase Chain Reaction Technique. Mol Ecol Notes 4:110-112. DOI: 10.1046/j.1471-8286.2003.00585.x.

Kanaka KK, Sukhija N, Goli RC, Singh S, Ganguly I, Dixit SP, Malik AA. 2023. On the concepts and measures of diversity in the genomics era. Curr Plant Biol 33:100278. DOI: 10.1016/j.cpb.2023.100278.

Kranke N. 2024. How phenograms and cladograms became molecular phylogenetic trees. J Hist Biol 57:423–443. DOI: 10.1007/s10739-024-09782-8.

Nurhati IS, Murdiyarso D. 2022. Strategi nasional pengelolaan ekosistem mangrove [National strategy for mangrove ecosystem management]. [Internet] [Accessed 2024 Mar 15]. Available from: https://www.cifor.org/knowledge/publication/879.

Petolescu C, Sarac I, Popescu S, Tenche-Constantinescu AM, Petrescu I, Camen D, Onisan E. 2024. Assessment of genetic diversity in alfalfa using DNA polymorphism analysis and statistical tools. Plants 13(20). DOI: 10.3390/plants13202853.

Ribeiro DO, Vinson CC, Nascimento DSS, Mehlig U, Menezes MPM, Sampaio I, Silva MB. 2013. Isolation of microsatellite markers for the red mangrove, *Rhizophora* mangle (*Rhizophora*ceae). Appl Plant Sci 1(9):9–11. DOI: 10.3732/apps.1300003.

Richards DR, Friess DA. 2016. Rates and drivers of mangrove deforestation in Southeast Asia, 2000-2012. Proceedings of the National Academy of Sciences of the United States of America 113(2):344–349. DOI: 10.1073/pnas.1510272113.

Rosero-Galindo C, Gaitan-Solis E, Cárdenas-Henao H, Tohme J, Toro-Perea N. 2002. Polymorphic microsatellites in a mangrove species, *Rhizophora* mangle L. (*Rhizophora*ceae). Mol Ecol Notes 3(1):220–222. DOI: 10.1046/j.1471-8286.

Ruang-areerate P, Naktang C, Khanbo S, Yundaeng C, U-thoomporn S, Kongkachana W, Pootakham W. 2023. Assessment of the genetic diversity and population structure of *Rhizophora mucronata* along coastal areas in Thailand. Biology 12(3):1–14. DOI: 10.3390/biology12030484.

Salminah M, Alviya I. 2019. Efektivitas kebijakan pengelolaan mangrove [Effectiveness of mangrove management policy]. Jurnal Analisis Kebijakan Kehutanan 16(1):11–29.

Shinmura Y, Wee AK, Takayama K, Meenakshisundaram SH, Asakawa T, Onrizal, …, Kajita T. 2012. Isolation and characterization of 14 microsatellite markers for *Rhizophora mucronata* (*Rhizophora*ceae) and their population use in range-wide population studies. Conserv Genet Res 4(4):951–954. DOI: 10.1007/s12686-012-9681-y.

Szpiech ZA, Rosenberg NA. 2011. On the size distribution of private microsatellite alleles. Theor Popul Biol 80(2):100–113. DOI: 10.1016/j.tpb.2011.03.006.

Wee AKS, Mori GM, Lira CF, Núñez-Farfán J, Takayama K, Faulks L, Kajita T. 2019. The integration and application of genomic information in mangrove conservation. Conserv Biol 33(1):206–209. DOI: 10.1111/cobi.13140.

Wee AKS, Takayama K, Chua JL, Asakawa T, Meenakshisundaram SH, Onrizal, Kajita T. 2015. Genetic differentiation and phylogeography of partially sympatric species complex *Rhizophora mucronata* Lam. and R. stylosa Griff. using SSR markers. BMC Evol Biol 15:57. DOI: 10.1186/s12862-015-0331-3.

Yahya AF, Hyun JO, Lee JH, Kim YY, Lee KM, Hong KN, Kim SC. 2014. Genetic variation and population genetic structure of *Rhizophora apiculata* (*Rhizophora*ceae) in the greater Sunda Islands, Indonesia using microsatellite markers. J Plant Res 127(2):287–297. DOI: 10.1007/s10265-013-0613-z.

Yan YB, Duke NC, Sun M. 2016. Comparative analysis of the pattern of population genetic diversity in three Indo-West Pacific *Rhizophora* mangrove species. Front Plant Sci 7 (1434):1–17. DOI: 10.3389/fpls.2016.01434.