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Phylogenetic analysis of *Rhizophora mucronata* in Savu Sea Marine National Park, East Nusa Tenggara, Indonesia

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Abstract. *Ihwan, Arumningtyas EL, Retnaningdyah C, Hakim L. 2024. Phylogenetic analysis of* Rhizophora mucronata *in Savu Sea Marine National Park, East Nusa Tenggara, Indonesia. Biodiversitas 25: 4498-4506.* Mangroves, a crucial coastal ecosystem, play a crucial role in marine biodiversity and shoreline protection. This study investigates the phylogenetic patterns and haplotype analysis of *Rhizophora mucronate*, a key mangrove species, in the biodiverse Savu Sea Marine National Park within the Coral Triangle. We collected 30 *R. mucronata* samples from 15 mangrove populations in the park, including Sumba Island, Sabu Island, Rote Island, and Timor Island. The Maximum Likelihood-based phylogenetic tree reconstruction revealed distinct genetic clusters, segregating populations into two primary clades: Indo-West Pacific (IWP) and Atlantic East Pacific (AEP). Populations from the Sumba, Rote, Semau, Timor, and Sabu Islands primarily aligned with the IWP clade, while some samples exhibited unexpected clustering patterns, indicating potential unique genetic lineages. Notably, populations from Hambapraing and Tesabela showed closer genetic affinity to AEP and the outgroup species, respectively. Our subsequent haplotype analysis further unveiled a complex network of sequence-dependent populations, with notable haplotypes, such as Haplotype 2, showing widespread distribution across Sabu, Sumba, Rote, and Timor, and Haplotype 13 linking populations from Singapore, China, India, USA, Kenya, and Seychelles. These findings not only underscore the complexity of genetics but also highlight the importance of considering distinctive genetic patterns to unravel evolutionary relationships, thereby providing a solid foundation for future research and conservation initiatives.

Keywords: Atlantic East Pacific, haplotype, Indo-West Pacific, maximum likelihood, *Rhizophora mucronata*

Abbreviations: AEP: Atlantic East Pacific; IWP: Indo-West Pacific; SSMNP: Savu Sea Marine National Park

INTRODUCTION

Mangroves, the distinctive coastal ecosystems, serve as crucial intersections between terrestrial and marine environments (Arifanti et al. 2022; Anu et al. 2024). They act as breeding grounds for numerous marine species (Arceo-Carranza et al. 2021), protect coastlines from erosion (Dominicis et al. 2023), and play a pivotal role in carbon sequestration (Chatting et al. 2022). *Rhizophora mucronate* Lam., a keystone mangrove species, has garnered attention for its ecological significance and adaptability to various environments (Sefton and Woodroffe 2021; Nuraeni and Kusuma 2023)

Mangroves, including *R. mucronata*, exhibit distinct genetic variations across their range due to historical and contemporary factors (Lo et al. 2014). Pollination by insects (Hermansen et al. 2014), wind, and bird (Wee et al. 2014a), along with seed dispersal via surface currents (Triest et al. 2021), facilitate both local and global dispersal. This diverse dispersal and pollination ecology supports genetic diversity and enables *R. mucronata*'s adaptation to varied habitats. The widely distributed mangrove trees in the Indo-West Pacific (IWP) region mostly were *Rhizophora* species (Yan et al. 2016). *R. mucronata* is distributed across tropical and subtropical regions, particularly in the IWP region, extending from East Africa to the Western Pacific (Ng et al. 2015; Yan et al. 2016; Triest et al. 2021). In the southern area, *R. mucronata* is distributed from Southeast Africa, while in the northern region, it extends to the Persian Gulf (Spalding et al. 2010). Natural barriers like temperature and rainfall tolerances limit its northern spread, while dispersal constraints and habitat availability pose challenges in southern regions (Osland et al. 2017).

One of the areas in IWP regions with a diversity of *R. mucronata* is Savu Sea Marine National Park (SSMNP). Situated in the heart of the Coral Triangle, SSMNP is characterized by its unique geographical location (Lusiana et al. 2023) and diverse marine habitats (Silaban et al. 2023). The intricate interplay of complex ocean current patterns, historical sea-level fluctuations, and topographic heterogeneity (Lusiana et al. 2023) are expected to have a significant effect on the distribution and genetic diversity of *R. mucronata* in this region.

Conservation in the context of Savu Sea Marine National Park is paramount (Paulus et al. 2023). As a designated protected area, the park is a haven for biodiversity. However, it is not immune to the challenges of climate change, habitat destruction (Ceccarelli et al. 2022), and anthropogenic disturbances (Paulus et al. 2023). Assessing the genetic diversity and structure of *R. mucronata* in this specific ecosystem is fundamental for implementing effective conservation strategies (Ihwan et al. 2019; Ihwan et al. 2020). By understanding the phylogeographic patterns and potential gene flow among populations within the park, researchers, and conservationists can make informed decisions regarding restoration efforts, sustainable management, and the preservation of this vital coastal habitat.

R. mucronata provides significant economic and ecological benefits, such as acting as a protection barrier against storms, hurricanes, and tsunamis, preventing coast erosion, and supplying natural products (charcoal, wild honey, timber, food, and medicinal elements) (Batool et al. 2014). Coastal communities previously explored the economic benefits of the products and services provided by mangrove forests (Mantiquilla et al. 2021). However, genetic diversity information for this species still needs to be improved, and genetic connectivity among its widely distributed populations has yet to be revealed. Therefore, this research article initiates a comprehensive genetic investigation of *R. mucronata* populations in the Savu Sea Marine National Park, combining molecular techniques with historical data and ecological insights. This study focuses on the genetic diversity and structure of *R. mucronata* populations in SSMNP, using molecular markers to identify unique genetic lineages and examine phylogeographic patterns. Such genetic insights are essential for designing effective conservation strategies that preserve genetic variability and adaptability, ultimately supporting restoration projects, promoting genetic resilience, and enhancing biodiversity in SSMNP. This will provide input for restoration projects, promote genetic resilience, and support biodiversity in the Savu Sea Marine National Park, East Nusa Tenggara, Indonesia. These findings will guide evidence-based management practices to mitigate the impacts of climate change and human activity on mangrove forests. Additionally, this study provides a baseline for future genetic research and monitoring efforts in the region.

MATERIALS AND METHODS

Research sampling site

This research was conducted in the Savu Sea Marine National Park, East Nusa Tenggara, Indonesia. A total of 30 *R. mucronata* were collected in this study. For the outgroup, 2 *R. apiculata* samples from fifteen mangrove populations in the SSMNP, along with *Bruguiera parviflora* (from Tesabela), were collected. These populations include locations on Sumba Island (4 locations: Kambuomang, Lumbukore, Warambadi, and Hambapraing), Sabu Island (2 locations: Lederaga and Mebba), Rote Island (4 locations: Oelaba, Papela, Baudale, and Oeseli), Timor Island (5 locations: Pariti, Paradiso, Tesabela, Salupu and Semau), all in East Nusa Tenggara (Figure 1).

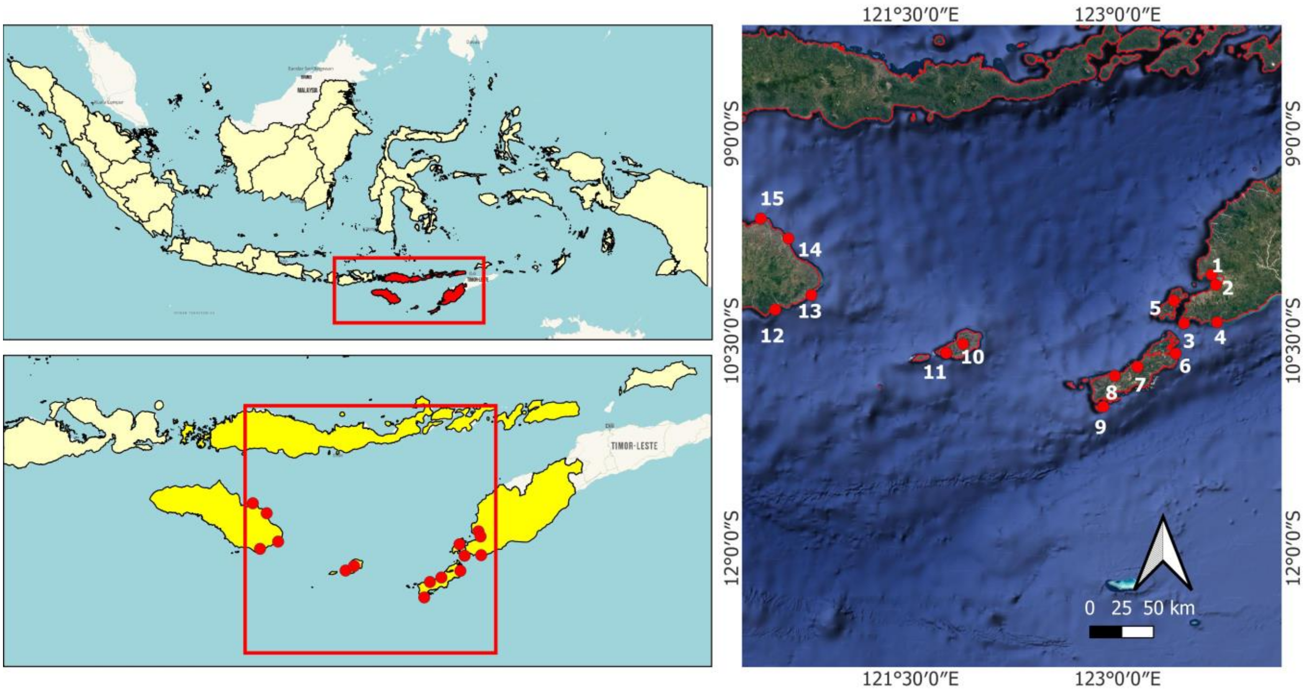

Figure 1. Study site in Savu Sea Marine National Park, East Nusa Tenggara, Indonesia: 1. Pariti, 2. Paradiso, 3. Tesabela, 4. Salupu, 5. Semau, 6. Papela, 7. Baudale, 8.Oelaba, 9. Oeseli, 10. Mebba, 11. Lederaga, 12. Kambuomang, 13. Warambadi, 14. Lumbukore, 15. Hambapraing

Field sampling and laboratory preparation

Fresh leaves from two different individual trees were collected from each population. For transport, the leaves were stored in separate bags with silica gel to maintain their freshness and prevent degradation during transit (Till et al. 2015). The samples were labeled, indicating the location, collection date, and specific tree identity. Upon arrival at the laboratory, the leaves were rigorously cleaned and sorted to exclude any contaminants or damaged specimens. Subsequently, the genetic material extraction process was initiated, following established protocols to isolate high-quality DNA from the mangrove leaf samples.

DNA isolation and amplification

DNA extraction from the silica gel-dried leaves was conducted utilizing the TIANGEN DNA Secure PlantKit according to the manufacturer's protocol. The quality of extracted DNA was assessed by separating it on a 1% agarose gel. The extracted DNA was then amplified using a PCR machine with a 30 µL reaction mixture containing 15 µL of PCR Master Mix Nexpro, 3 µL of DNA template (100 ng/µL), 6 µL of water, and 3 µL of primers (10 pmol each for the forward and reverse primers). Specifically, *rbcl1* and *rbcl2*, two chloroplast genes, were targeted for PCR amplification. These genes were chosen due to their highly conserved nature within the *R. mucronata* species (Sahu et al. 2016). The primers used for PCR amplification were *rbcl1* (`TGT CAC CAA AAA CAG AGA CT`) and *rbcl2* (`TTC CAT ACT TCA CAA GCA GC`).

The amplification process included a pre-denaturation step at 95°C for 2 minutes, followed by 35 cycles consisting of denaturation at 95°C for 30 seconds, annealing at 50°C for 30 seconds, and extension at 72°C for 120 seconds. A post-extension step was then performed at 72ºC for 7 minutes. The PCR products were subjected to Sanger sequencing, and 1st BASE Laboratories Sdn Bhd provided the sequencing services.

Data analysis

The sequencing results were assessed using sequence scanner software. The obtained sequence data underwent BLAST analysis (Basic Local Alignment Search Tool) (https://blast.ncbi.nlm.nih.gov/Blast.cgi) to evaluate their similarity. The sequence data was saved in fasta format and aligned using Clustal W within the Mega 6 software.

MEGA 6.0 was used to construct the phylogenetic tree, employing a Tamura Nei model through the Maximum Likelihood (ML) method, with a Bootstrap value set at 1000 for robustness. The alignment results were further analyzed for haplotype distribution using DnaSP version 6.0 software. The generated haplotype distribution map was visualized using Network application version 10.2 (Paradis 2018).

RESULTS AND DISCUSSION

Phylogenetic analysis

Maximum Likelihood-based phylogenetic tree reconstruction of *R. mucronata rbcL* genes revealed distinct genetic relationships among samples from various locations, clustering into two primary clades: IWP (Indo-West Pacific) and AEP (Atlantic East Pacific) (Figure 2). The outgroup, consisting of *R. apiculata* and *B. parviflora* (both from Tesabela), provided a comparative framework. The IWP *R. mucronata* population, including Sumba, Rote, Semau, Timor, and Sabu islands, exhibited distinct genetic patterns. Notably, the bootstrap analysis consistently supported the robustness of the phylogenetic tree, with a bootstrap value of 64 for *R. mucronata* across all examined areas.

However, upon examining the tree, it is evident that two samples from each island do not cluster together, raising questions about potential alternative distribution mechanisms. Analysis of the branches shows that *R. mucronata* from Hambapraing 2, Paradiso 2, Papela 2, Semau 2, and Oeseli 1 form a cohesive sub-clade. This sub-clade is distinctly separated but not loosely associated with other major IWP species. These results suggest that this group represents the earliest diverging lineages among IWP species.

An unexpected outcome is observed for *R. mucronata* from Hambapraing 1 and Tesabela 1, which cluster as sister taxa for AEP and the outgroup, respectively, compared with other IWP species. After this discovery, three IWP-originated accessions from Kenya, China, and Singapore exhibit a closer genetic affinity to *R. mucronata* species from AEP.

Haplotype analysis

Haplotype analysis was conducted to investigate the genetic diversity and population structure of *R. mucronata* species, revealing some intriguing findings. Notably, there was no significant correlation between phylogenetic construction and the geographical origin of the samples. Haplotype 2, with the highest frequency, was found in populations from Sabu, Sumba, Rote, Timor, and Semau (Figure 3). Of particular interest, five haplotypes of *R. mucronata* species from Rote were clustered closely together. With only a single base mutation, this haplotype was adjacent to the second largest haplotype, haplotype 13, which includes accessions from Singapore, China, India, USA, Singapore, Kenya, and Seychelles. Three sequences previously classified as part of the outgroup clade from Timor exhibited variations in the number of mutations and, therefore, were positioned at a considerable distance from Haplotype 2. However, several median vectors (indicated by a small white circle) remain among the haplotypes, suggesting the presence of bridging haplotypes that could be either extant or extinct unsampled sequences (Kong et al. 2016).

Discussion

Phylogenetic tree

Phylogenetic tree reconstruction reveals intriguing insights into the genetic relationships within *R. mucronata* populations, highlighting the complex interplay of evolutionary dynamics and potential ecological determinants. The clear segregation of samples into two primary clades, IWP (Indo-West Pacific) and AEP (Atlantic East Pacific), provides a foundational framework for understanding the genetic architecture of *R. mucronata* populations.

The lack of clustering of two samples from each island within the phylogenetic tree suggests the need to explore alternative distribution mechanisms. This non-congruence challenges conventional expectations, indicating that genetic affinities within islands are not solely determined by geographical proximity. The cohesive sub-clade formed by specific populations, including Hambapraing 2, Paradiso 2, Papela 2, Semau 2, and Oeseli 1, highlights a distinct evolutionary trajectory within the IWP clade. This observation suggests these populations represent early diverging lineages within the broader IWP species, providing deeper insights into the evolutionary history of *R. mucronata*.

Maximum Likelihood-based phylogenetic tree reconstruction consistently yielded bootstrap values 64 for *R. mucronata* across all examined areas. This bootstrap value indicates a moderate level of consensus regarding the genetic relationships among *R. mucronata* samples from diverse locations. The stability of the phylogenetic tree topology, which refers to the arrangement of branches and nodes in the tree, as reflected by the uniform bootstrap value, enhances the credibility of the results and supports the reliability of the delineated genetic relationships.

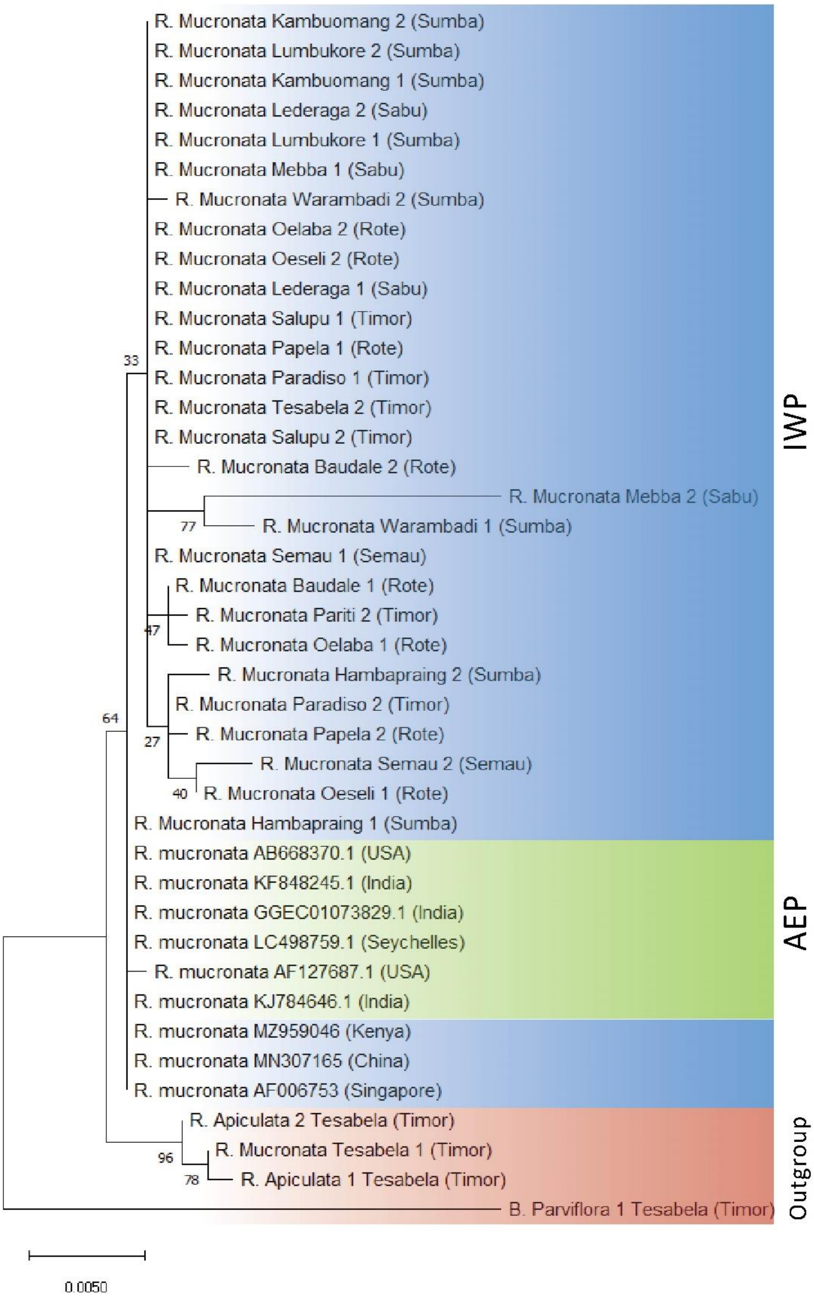

Figure 2. Genetic relatedness among the *R. mucronata* population in Savu Sea Marine National Park. The numbers above the branches represent bootstrap support values based on 1000 replicates. The words in brackets indicate the sample extraction site. Abbreviations are as follows: AEP (Atlantic East Pacific) and IWP (Indo-West Pacific)

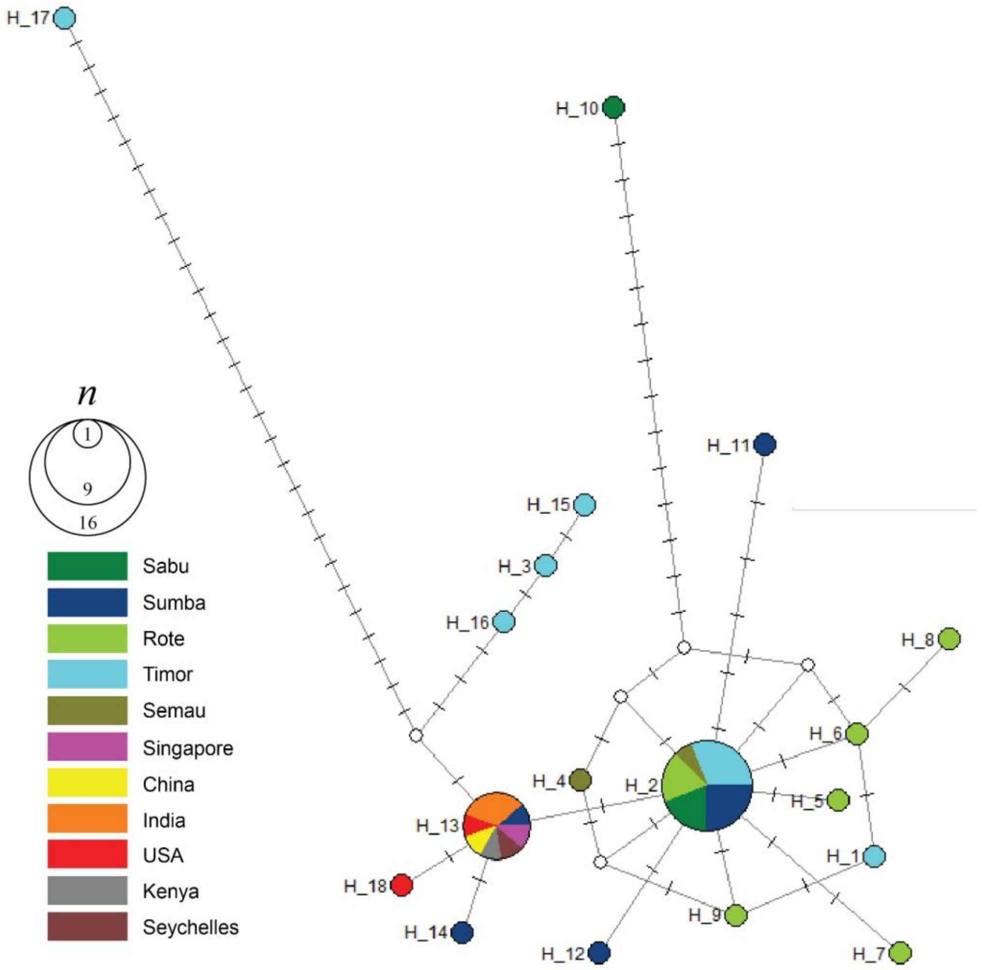

Figure 3. Haplotype network diagram

It is crucial to understand that bootstrap value primarily assesses the precision rather than the accuracy of the phylogenetic tree. This distinction is key to interpreting the results correctly and staying engaged with the topic. A high bootstrap value at a node indicates strong support for the branch but does not guarantee its correctness; the node could still be positioned inaccurately. This becomes particularly evident when the tree-building method introduces errors in deducing the relationships within a dataset. Bootstrapping may produce a robust result, but this does not necessarily ensure that the result is accurate (Russo and Selvatti 2018; Simon 2022).

An unexpected clustering pattern is observed for *R. mucronata* from Hambapraing 1 and Tesabela 1, which aligns as sister taxa for AEP and the outgroup, respectively, rather than with other IWP species. This divergence from expected clustering highlights the complexity of genetic affinities and prompts further investigation into the factors contributing to this unusual pattern. Subsequent analysis reveals that three IWP-originated accessions from Kenya, China, and Singapore exhibit a closer genetic affinity to *R. mucronata* species from AEP, challenging traditional assumptions about genetic isolation between geographically distant populations.

The distinctive genetic variability observed within Indonesian *R. mucronata* populations reveals a dynamic interplay between environmental constraints and anthropogenic influences. Factors such as weather, soil, and water characteristics, including salinity, temperature, pH, tidal flooding, and nutrient availability, significantly contribute to the observed genetic patterns (Feng et al. 2023; Chenani et al. 2023; Lira and Granado 2023). Additionally, a previous study indicated that the SSMNP is threatened by pollution, overfishing, and climate change, which could significantly impact the region's livelihoods and environment (Paulus et al. 2023). The complex relationship between the genetic makeup of these populations and their ecological context emphasizes the need for a more holistic understanding of the delicate balance between evolutionary forces and environmental factors (Rellstab et al. 2015).

During data collection, the authors did not directly observe any obvious destructive activities by coastal communities affecting the mangrove population/ecosystem. However, previous reports from the Directorate of Conservation of Areas and Fish Species of the Directorate General of Marine, Coastal, and Small Islands of the Ministry of Marine Affairs and Fisheries of Indonesia have documented the impact of fishermen's activities on mangrove ecosystems. The growing number of fishermen in coastal waters is intensifying pressure on marine resources, exacerbated by harmful fishing practices. This threatens the sustainability of shallow marine ecosystems such as mangroves and coral reefs, with damage averaging 70% (Paulus et al. 2023). Anthropogenic activities emerge as influential factors contributing to habitat loss, changes in natural environments, and genetic diversity (Almeida-Rocha et al. 2020; Exposito-Alonso et al. 2022). These impacts on genetic structure affect ecological functions and biodiversity composition (Canty et al. 2022).

While the phylogenetic tree provides valuable insights, it is essential to recognize the potential limitations associated with bootstrap values. The relatively low bootstrap values observed in certain branches may indicate a weak consensus in branching formations, especially when relying on analyses based on a single gene. This cautionary note aligns with the observations by Lemoine et al. (2018) and highlights the importance of interpreting phylogenetic relationships cautiously, especially when relying on single-gene analyses.

Unresolved clades within phylogenetic trees can often be attributed to the inherent limitations of using short sequence lengths. Recent studies on mangrove species have shown that by extending genetic sequence lengths and incorporating additional genomic datasets, researchers can significantly enhance phylogenetic resolution and clarity, thereby resolving previously ambiguous evolutionary relationships. Such methodological refinements are imperative for enhancing the reliability and robustness of phylogenetic reconstructions, providing a more accurate depiction of the evolutionary relationships within *R. mucronata* populations (Giri 2023; Miraki et al. 2023; Hamilton and Presotto 2024).

Ultimately, the comprehensive exploration of the genetic landscape of *R. mucronata* populations provides a foundational understanding of their evolutionary dynamics and ecological influences. The unexpected clustering patterns challenge preconceived notions about genetic affinities, highlighting the need for a nuanced examination of the factors shaping population genetics. The complex interplay between environmental constraints, anthropogenic activities, and the observed genetic variability underscores the complexity of factors influencing the genetic diversity of *R. mucronata*. Continued research efforts, incorporating larger datasets and refined methodologies, are crucial for unraveling the evolutionary relationships within this ecologically significant species.

Haplotype analysis

Haplotype analysis has revealed a complex network of sequence-dependent populations within the genetic landscape of *R. mucronata* species. This method facilitated a detailed examination of the variations and relationships among haplotypes, shedding light on the genetic diversity present in populations across different geographical locations.

In examining the variability of sequences within clades, contrary to expectations, no statistically significant correlation was found between the construction of the phylogenetic tree and the geographical origin of the sampled *R. mucronata* populations. This finding suggests that factors beyond mere geographical proximity contribute to the observed genetic variations, pointing to potential evolutionary influences and ecological dynamics shaping the genetic makeup of these populations. Other factors, such as colonization time, extent of gene flow, dispersal, and colonization success, may have more profound effects on the current patterns of population structure (Berbel-Filho et al. 2020).

In alignment with our findings, comprehensive studies on mangrove genetic diversity have revealed similar patterns of population structuring of *R. apiculata* and *R. mucronata* in Southeast Asia (Wee et al. 2014a; Yahya et al. 2014; Ng et al. 2015). Additionally, ecological theories regarding the influence of tidal patterns on mangrove ecosystems suggest that the observed genetic variations may result from the complex interplay of ecological factors shaping *R. mucronata* populations (Wee et al. 2014b; Wee et al. 2014a; Yahya et al. 2014; Yan et al. 2016).

Haplotype 2 stands out due to its high frequency and wide distribution across diverse regions. This haplotype includes populations from Sabu, Sumba, Rote, Timor, and Semau, indicating a shared genetic signature among these geographically distinct areas. Further investigation into the specific genetic markers defining Haplotype 2 may provide valuable insights into the mechanisms driving its prevalence and dispersal.

Despite its relatively small size, the island of Rote exhibited notable genetic diversity, with five distinct haplotypes found in close proximity. The clustering of haplotypes raises questions about localized factors influencing genetic differentiation and adaptation within this population.

Analysis of genetic proximity and relationships among different haplotypes reveals the presence of a distinctive Haplotype 13. This haplotype, characterized by its unique genetic composition, includes accessions from diverse locations such as Singapore, China, India, the USA, Singapore, Kenya, and Seychelles. The presence of these geographically distant locations within the same haplotype suggests the potential for shared evolutionary pressures or historical genetic exchanges among these populations.

Introducing the concept of inbreeding adds another layer to this discussion. According to a study conducted by Inomata et al. (2009), investigating *R. apiculata*, *Rhizophora mucronate*, and *Rhizophora stylosa* revealed positive FI5 (inbreeding coefficient) estimates were observed in the nuclear DNA data. These estimates suggest a scarcity of heterozygotes within subpopulations, potentially due to inbreeding or the Wahlund effect (Inomata et al. 2009). Biparental inbreeding influences the significant genetic variation observed among the samples (Mori et al. 2015). Local populations that are involved in the dispersal of the species play a crucial role in mitigating biparental inbreeding effects (Duminil et al. 2016). Earlier studies on *R. apiculata* and *R. mucronata* have similarly reported a deficit of heterozygotes, which is consistent with these findings.

Similarly, two studies on AEP *Rhizophora* species utilizing microsatellite markers reported analogous outcomes.

Cerón-Souza et al. (2010) observed a deficiency of heterozygotes in nearly all studied populations, while (Takayama et al. 2013) found this deficiency in approximately one-third of their investigated populations. However, both studies detected null alleles, which complicates the assessment of the impact of their impact on reported heterozygosity estimates. *Rhizophora* species, as noted by (Kondo et al. 1987), limited pollen dispersal, which can restrict reproductive potential and genetic connectivity, thereby compromising the resilience of these mangroves to environmental change. Additionally, self-pollination in this species may lead to the production of non-viable seeds (López et al. 2021).

Inbreeding, resulting from the reproduction of closely related individuals, increases genetic homogeneity within a population (Inomata et al. 2009). Although Haplotype 13 suggests historical gene flow among diverse populations, the phenomenon of inbreeding underscores the potential for increased genetic similarity within specific demographic groups due to familial relationships. By integrating these two perspectives, we gain a nuanced understanding of the genetic constitution and historical dynamics, elucidating both the migratory patterns of genes across geographically distinct regions and the impact of familial connections on genetic homogeneity within discrete groups.

Examining sequences initially categorized as part of the outgroup clade from Timor reveals variations in the number of mutations, thus placing the sequences significantly different from Haplotype 2. This suggests distinct evolutionary trajectories within the Timor population, adding a layer of complexity to the broader phylogenetic framework and highlighting the nuanced intrapopulation genetic variations.

Intriguingly, among the identified haplotypes, several median vectors (explained in the results) persist. These vectors, representing intermediate genetic states, suggest the existence of bridging haplotypes. Bridging haplotypes, whether extant or extinct and unsampled, are crucial in elucidating potential genetic connections and evolutionary transitions among the observed haplotypes.

Genomic implications and future directions

The genetic complexity of *R. mucronata* is revealed through phylogenetic reconstruction and haplotype analysis, highlighting distinct patterns and unique haplotypes. Despite a lack of correlation between genetic and geographic factors, understanding the genetic markers driving these variations remains elusive. Future genomic studies employ advanced techniques to uncover deeper insights into adaptive traits and responses to environmental stimuli.

Median vectors and bridging haplotypes suggest intermediate genetic states within *R. mucronata* populations, offering insights into historical dynamics and adaptive radiations. Integrating population genomics can reveal the interplay between genetic drift, gene flow, and selection. This multidisciplinary approach is crucial for understanding adaptation and evolution in *R. mucronata* amidst changing environmental conditions and anthropogenic pressures. Therefore, to conserve the genetic diversity of *R. mucronate*, it is crucial to implement strategies that protect and restore mangrove habitats, such as establishing conservation zones, promoting sustainable land-use practices, and preventing deforestation. Additionally, genetic monitoring programs should be established to track changes in genetic diversity over time and identify potential threats.

Therefore, genomic insights from phylogenetic and haplotype analyses illuminate future research on *R. mucronata*. This exploration enriches our understanding of mangrove evolution and underscores the importance of conserving the genetic diversity of this ecologically crucial species amidst environmental challenges. Future studies should aim to identify specific genetic markers associated with adaptive traits to support breeding programs focused on enhancing resilience to environmental stressors. Additionally, research should investigate the effects of climate change on genetic diversity and structure, as well as investigate gene-environment interactions to understand better how *R. mucronata* populations adapt to changing conditions. Integrating genomic data with environmental and ecological data will provide a comprehensive understanding of the evolutionary processes shaping *R. mucronata* populations.

In conclusion, this study provides valuable insights into the genetic landscape of *R. mucronata* in the SSMNP. Phylogenetic analysis revealed two primary clades: IWP (Indo-West Pacific) and AEP (Atlantic-East Pacific). The IWP population from Sumba, Rote, Semau, Timor, and Sabu islands formed a distinct sub-clade. On the other hand, Hambapraing 1 and Tesabela 1 clustered with AEP and the outgroup, indicating potential historical gene flow.

Haplotype analysis revealed Haplotype 2 as the most frequent, covering populations from Sabu, Sumba, Rote, Timor, and Semau. Notably, Rote exhibited diversity with five distinct haplotypes. Haplotype 13 included accessions from distant regions like Singapore, China, India, USA, Kenya, and Seychelles, suggesting historical genetic exchanges. These findings underscore the significance of environmental constraints, including temperature, humidity, and anthropogenic activities, as influential drivers of genetic diversity. These findings highlight the complexity of genetic diversity shaped by both environmental factors and human activities. Conservation efforts are crucial to safeguard the genetic diversity of *R. mucronata* populations. Future research should focus on identifying genetic markers with adaptive traits and understanding the impacts of climate change on genetic diversity. Integrating genomic with ecological data will enhance our understanding of the evolutionary processes shaping *R. mucronata* populations.

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