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Full Length Article

Revealing the Genetic Structure of Indonesia Moluccan Dipterocarps Species, *Rubroshorea selanica*

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Abstract

Among the species within the Dipterocarpaceae family, *Rubroshorea selanica* (Valmont) P.S. Ashton & J. Heck. is a rare instance of the *Rubroshorea* species that spans its distribution eastward across the Wallace Line. *R. selanica* is grouped into the genus of *Rubroshorea* and is also known as *Red Meranti* for its timber color. The species is exclusively located within the central region of the Moluccas, encompassing Buru Island, Sula Island, Obi Island and Seram Island. Our research involved sequencing two non-coding regions of chloroplast DNA and three nuclear genes to analyze the genetic variation and structuring of this species. Nucleotide diversity (π) in the intergenic spacers of chloroplasts within populations showed no variation (0) for Seram population to the highest of 0.00044 for Buru population, with an overall value of 0.00041 for the combined population. Diversity of nucleotides in non-coding regions (π_{sil}) within the nuclear gene exhibited variability across the three examined loci, ranging from the highest of 0.02034 for *GBSSI* to the lowest of 0.00450 for *Gap*C. We observed significant population structuring for the chloroplast DNA regions (F_{ST} = 0.702) and two out of the three nuclear gene loci studied (F_{ST} = 0.369 and 0.415 for *GBSSI* and *Pgi*C), suggesting that every population is unique and, as a result, carries its own importance. as a conservation unit. © 2024 Friends Science Publishers

Keywords: cpDNA; Dipterocarpaceae; Moluccas; Nuclear genes; *Rubroshorea selanica*

Introduction

The Indo-Australian Archipelago (IAA), alternatively referred to as Malesia or the Malay Archipelago, stands as one of the world's most intricate tropical regions in terms of geographical complexity. Comprising over 20,000 islands spanning the equatorial region in Southeast Asia, it encompasses Brunei, East Timor, Indonesia, Malaysia, New Guinea, the Philippines, Singapore, and Thailand (Lohman *et al.* 2011). Myers *et al*. (2000) identifies four biodiversity hotspots in this region: namely, Indo-Burma, Philippines, Sundaland, and Wallacea. Furthermore, the region is one with gradual physical transitions, typified by a decline in rainfall from west to east (Oldeman 1980). This archipelago is also divided into two biogeographic regions that correspond to the areas east and west of the Wallace Line, respectively. Wallacea is the area where the Philippine Sea, Indo-Australian and Eurasian Plates collide and is the most complex part of Southeast Asia, an unusual region of high faunal and floral endemism and the center of maximum biodiversity in the archipelago for many plants and animals. While the lowland forests of the west IAA may be dominated by members of the family Dipterocarpaceae, few species in the genera *Anisoptera, Dipterocarpus, Hopea, Vatica* and *Shorea* grow east of the Wallace Line. This pattern of distribution could raise concerns for the study of the dipterocarp's species growing in Wallacea.

Dipterocarpaceae consists of more than 470 species of tropical canopy and sub-canopy trees that grow in Asian tropical forests (Ashton 1982). Members of the family are highly valued for their timber. In global markets, dipterocarps are believed to have played a substantial role, accounting for over 50% of the total hardwood timber trade (FAO 2007). Among the dipterocarp family, *Rubroshorea* are one of the largest genera consist of 71 species (https://powo.science.kew.org/), for which vernacular names like Meranti are usually used in the context of the timber trade. This genus was recently dissected from the tribe *Shoreae* (Ashton and Heckenhauer 2022). *Rubroshorea selanica* Blume has been designated as a critically endangered species in the IUCN Red List of Threatened Species (www.iucnredlist.org). This species is known Red Meranti (Ashton and Heckenhauer 2022) and is the only species in the group whose distribution extends through to east of the Wallace Line. *R. selanica* exclusively thrives within the central region of the Moluccas, encompassing

Buru Island, Sula Island, Obi Island, Seram Island and Ambon Island. It tends to grow in clusters at the local level and holds prevalence in semi-evergreen lowland forests, typically found on well-drained, nutrient-rich soils and sometimes on limestone substrates (Newman *et al.* 1998). Furthermore, *R. selanica* plays a crucial role as a timber source. Its rapid growth, capable of reaching a diameter of 50 cm at breast height in under 30 years, makes it a promising candidate for plantation and revegetation initiatives (Subiakto 2001). Despite extensive research focusing on silviculture and widespread planting for experimental and reforestation purposes, there is currently no available study focuses on morphological differences among different islands of origin, physiological characteristics, biochemical traits well as genetic information pertaining to the species.

The study area of the Moluccas consists of over 1,000 islands, varying in size from small, inhabited atolls to the largest island of Seram. The northern and central islands have tropical forests and volcanoes and receive rain throughout the year. Such a climate has allowed forest areas to thrive in the northern and central Moluccas. In contrast, the southeast islands are made up of mangrove swamps and tidal salt marshes and are characterized by long dry seasons. A range of unique animal species has long been known in the Moluccas, reflecting the area's transitional position between Asian and Australian faunal realms (Barlow *et al.* 2003). The Moluccas are a geological composite of different ages and processes. The islands of Buru and Seram (Central part) are composed of Australasian continent basement. Buru is known to be very simple in formation and young in age, while the North Moluccas (Halmahera and other surrounding islands) are dominated by oceanic and island-arc terrains. Although much of the North Moluccas is arc-derived, continental basement rocks have also been documented there, on Bacan and Obi (Hall *et al.* 1995). The Banggai and Sula islands have been described as satellite islands of Sulawesi (Michaux 2010), while the southeast islands in the Moluccas, known as the Banda arc, were resulted from the convergence of a volcanic arc with the Australian continental margin (Spakman and Hall 2010).

Among all major tropical forest ecosystems, Southeast Asian rainforests are witnessing the most significant rates of forest cover depletion and alteration (Achard *et al.* 2002; Mayaux *et al.* 2005). The diminishing forest cover is directly contributing to the endangerment and localized extinction of numerous plant and animal species, including those of significant ecological and economic importance within the Dipterocarpaceae family (Sodhi *et al.* 2010). According to extinction models, if the ongoing rates of habitat alteration persist, it is projected that as much as 42% of the plant and animal species in Southeast Asia could face extinction by the year 2100 (Sodhi *et al.* 2004). More severe consequences could happen in Wallacea, as it consists of thousands of islands, and islands are more fragile to habitat loss and modification (Cox and Moore 2010). Population genetic investigations in Wallacea, similar to research in conservation biology within this region, have fallen behind their counterparts in other global regions. Our study is the first investigation of genetic variation and population genetic structure in the only Wallacean *Rubroshorea* species endemic to the Moluccas, *R. selanica*. This study is aimed at estimating the extent of genetic variation and population differentiation among populations of *R. selanica*.

Materials and Methods

Plant materials

We gathered leaf samples from mature *R. selanica* trees in four distinct populations, covering their native range in four islands of Moluccas, the eastern part of Indonesian archipelago (Fig. 1). These samples were obtained from trees with diameters exceeding 30 cm at breast height, with a minimum distance of 50 m between sampled individuals. We sampled two localities in the Sula Islands (Waibau and Mangole), two localities in Seram (Hualoy and Manusela), two localities in Buru (Waitabi and Waspait) and one locality in Obi. We could not get samples from Ambon due to rapid development on the island, which has left no remaining lowland forests containing *R. selanica*. In total, 42 individuals of *R. selanica* were analyzed in this study: ten individuals were sampled from Seram, ten from Obi, nine from Sula, and thirteen from Buru. Species identification was performed in the field, based on leaf morphological characteristics that were known to both indigenous local people and local forest rangers. The leaves were also compared to trees planted abundantly in several plots of experimental forest land owned by The Forest Research and Development Centre under the Indonesian Ministry of Forestry. In this investigation, we referred to the existing literature, which confirms the exclusive occurrence of a single species of *red meranti*, *R. selanica*, across the four main islands under study. There is no supporting evidence from other publications or surveys suggesting the presence of other *Rubroshorea* species or red meranti clusters in these specified study areas (Ashton 1982; Newman *et al.* 1998; https://powo.science.kew.org/; https://www.ipni.org/). As a result, the probability of misidentification during the identification process is considered to be low.

Leaves morphological characters

In conjunction with the genetic study that constitutes the focal point of this research, we conducted leaf dimension measurements. This experiment serves as an initial assessment to broadly examine whether there are discernible morphological differences among leaves in relation to the genetic diversity and structure that may be derived from the genetic study results. The investigation of morphological disparities among leaves were conducted to the available leaves' samples collected from the field used for the genetic

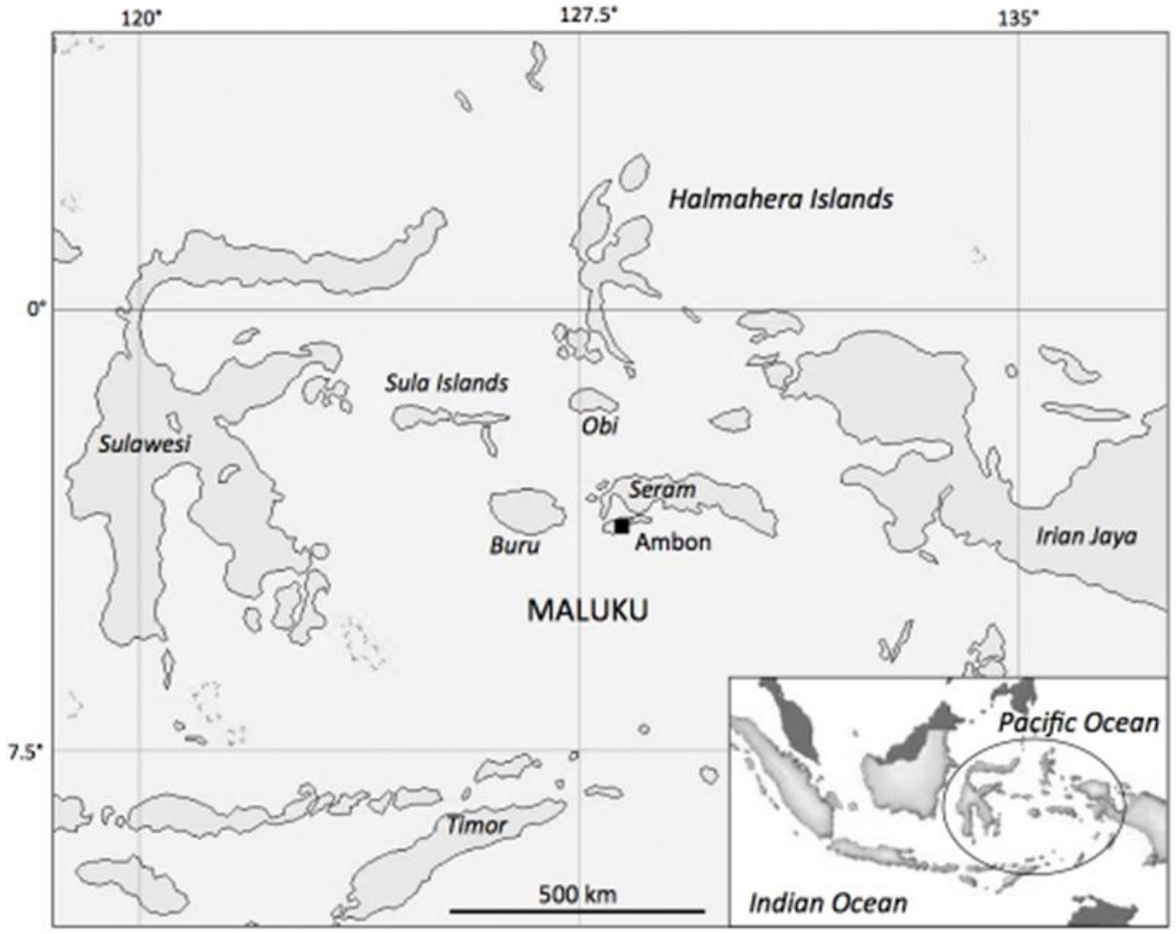

Fig. 1: Locations of the sampled populations for the genetic diversity and population structure analyses of *Rubroshorea selanica*. Circled area in the map at the bottom right is enlarged

study and also herbarium collection. We measured variables of leaf length, leaf width, ratio of the leaf length and leaf width, and the quantification of secondary veins. The scope of measurement extended beyond the leaves exclusively utilized in this study, incorporating specimens from the *R. selanica* stand planted in Bogor, Island of Java.

Loci studied

We conducted PCR amplification of two cpDNA intergenic spacers, *trn*T-*trn*L and *trn*L-*trn*F, using the universal primers outlined in Taberlet *et al*. (1991). Partial sequencing of three nuclear genes—*Gap*C, *Pgi*C and *GBSSI*—was carried out. *Gap*C amplification and sequencing employed primers developed by Iwanaga *et al*. (2012), while we developed primers for *GBSSI* and *Pgi*C, utilizing DNA sequences from species within the *Shorea* genus available in GenBank, as detailed by Kamiya *et al*. (2005).

DNA isolation, amplification and sequencing

We employed a modified CTAB method as described by Murray and Thompson (1980) to extract genomic DNA from adult leaves. For each individual, we carried out nested PCR to amplify partial regions of the three nuclear genes of interest under the following conditions: 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s and extension at 72°C for 7 min. In practice, the number of cycles for nested PCR ranged from 30 to 35, depending on the efficiency of amplification.

For the amplification of the cpDNA intergenic spacers *trn*T-*trn*L and *trn*L-*trn*F, we employed a touchdown PCR program: 2 min at 94°C; 4 cycles of 45 s at 94°C, followed by 45 s at 59°C (decreasing by 1°C each cycle) and then 2.5 min at 68°C; 26 cycles of 45 s at 94°C, 45 s at 55°C and 2.5 min at 68°C; and finally, a 30-min extension at 68°C.

Before sequencing, we purified the PCR products by employing rAPid Alkaline PhosphataseTM (Roche Diagnostics, Tokyo, Japan) and exonuclease I (Exo I; New England Biolabs, Ipswich, Massachusetts, USA). Subsequently, we performed direct sequencing for both strands using an ABI Prism 3100 automatic sequencer (Applied Biosystems, Bedford, MA, USA).

Data analysis

We visually inspected DNA sequences and used the ATGC program (Cosmo Bio, Tokyo, Japan) to assemble their forward and reverse sequences. To evaluate the levels of nucleotide polymorphism, we calculated haplotype diversity (Hd) (Nei 1987) and nucleotide diversity (π) (Nei 1987) for each of the loci under investigation. Additionally, we used Tajima's D (Taberlet *et al.* 1991) to evaluate deviations from selective neutrality and underlying assumptions, including a stable population size without migration. To conduct this assessment, we generated 95% confidence intervals for Tajima's D statistics for each locus by performing 10,000 replicates of coalescent simulations following the standard neutral model (Hudson 1990). To determine the phylogenic position of *R. selanica* with other species within the other previously *Shorea* genus, we aligned our sequences with those made previously available for the *trn*L-*trn*F cpDNA region (Tsumura *et al.* 2011) and *PgiC* nuclear gene (Kamiya *et al.* 2005) by using BioEdit and constructing the tree using ClustalX.

We employed ARLEQUIN v. 3.5 (Excoffier *et al.* 2007) to investigate population structure. Genetic differentiation was assessed using analysis of molecular variance (AMOVA). Additionally, we computed pairwise F_{ST} values to analyze population structuring between all possible population pairs.

All the sequence data was deposited in DDBJ database (Accession no. AB775786-95 for cp DNA, AB777347-395 for *GapC*, AB777523-575 for *GBSSI* and we are submitting for *PgiC* and will attach as soon as they are available).

Results

Differences on leaves dimension

Through measurements of several leaf dimensions across different populations, variation within and among populations have been identified (Table 1). The analysis of leaf length, width, leaf ratio and the number of secondary veins has highlighted distinct variations among the studied groups. These observed differences underscore the need for further investigation, specifically through this study, to confirm and better understand of the genetic diversity residing within and among population.

Species assignment and nucleotide polymorphism

In a phylogenetic analysis, we found a total of 15 individuals harboring distinct haplotypes for the *PgiC* gene that fell into the *Shorea* White Meranti group (tree not shown). Those atypical *PgiC* sequences came from five individuals in the Buru population and all ten individuals in Obi. This result was quite incongruent with the morphological identification of the individuals, and observed nucleotide substitution patterns in the *trn*L-*trn*F region (Tsumura *et al.* 2011), which provided specific diagnostic DNA markers for the Red Meranti group. One possible reason for this incongruence would be the occurrence of intraspecific hybridization. Although intraspecific hybridization has been observed in several Dipterocarp species (Ishiyama *et al.* 2003, 2008; Kamiya *et al.* 2011), hybridization among different taxonomical sections or even genera has not been reported. This might lead to species misidentification, and there is a chance we may have collected unknown Dipterocarps species growing in the Moluccas since the region lacks intensive research and comprehensive records on native Dipterocarps. In light of these conditions, we have excluded the fifteen individuals having atypical sequences in all genetic analyses to avoid overestimation of genetic diversity and genetic structuring. Our analyses were based only on the individuals that showed *Shorea* Red Meranti sequences in both their cpDNA and nuclear *PgiC* gene sequences.

The alignment lengths for the chloroplast intergenic spacers *trn*T-*trn*L and *trn*L-*trn*F were 1961 bp, while the nuclear genes *Pgi*C, *Gap*C, and *GBSSI* had lengths of 794, 667 and 668 bp, respectively, resulting in a total of 4090 bp. The haplotype diversity within the combined population (H_d) was 0.615 for cpDNA, while it spanned from 0.709 to 0.904 for nuclear genes. In the case of chloroplast intergenic spacers, population nucleotide diversities ranged from definitely no variation in Seram (0) to slightly 0.00044 in Buru, with the overall value 0.00041 for the pooled population. Nuclear regions exhibited variability for their silent site nucleotide diversity (π_{sil}) among the three investigated loci, with *GBSSI* showed the highest (0.0203) to *Gap*C as the lowest (0.0045). For non-synonymous sites (π_{nonsyn}), this measure ranged from 0.00035 (PgiC) to 0.00456 (GBSSI) (refer to Table 2 and 3).

Population structure

The level of genetic differentiation between populations was estimated from both cpDNA and nuclear gene regions. Significant and considerably high population differentiation (F_{ST}) was found among populations, with values of 0.702 for cpDNA (Table 2) and 0.415 and 0.369 for *PgiC* and *GBSSI*, respectively (Table 3). Only for the *GapC* nuclear gene region was no significant population differentiation found (F_{ST} value = 0.046). When examining pairwise F_{ST} values among the sampled populations, we observed significant population structuring in both the cpDNA and nuclear gene datasets, except for the pair of Seram and Sula islands for *GapC* (Table 4).

Discussion

The species *R. selanica* is characterized by oblong or ovate leaves measuring between 9 – 18 cm × 3 – 7 cm, exhibits leaf venation patterns with 19 – 23 pairs of secondary (Newman *et al.* 1998). Measurements

Table 1: Measurements of several morphological characters *in Rubroshorea selanica* leaves from five different origins

No.	Origin	Leaves length		Leaves width		Leaves ratio	Number of secondary veins	
		Range (cm)	Average (cm)	Range (cm)	Average (cm)		Range	Average
1.	Buru Island	7,0 - 17,5	11,3	4,0 - 7,5	5,3	1,6 - 2,4	9 - 24	17
2.	Obi Island	15,1 - 27,1	20,3	4,5 - 11,5	7,7	2,1 - 3,4	13 - 23	18
3.	Java - planted	8,0 - 18,3	12,8	4,0 - 7,7	4,9	2,2 - 2,6	15 - 23	18
4.	Sula Island	9,5 - 10,5	10	3,0 - 5,0	4	2,1 - 3,2	18 - 20	18
5.	Seram Island	14,0 - 22,0	18,7	5,1 - 9,0	6,9	2,2 - 3,3	16 - 19	18

Table 2: Estimates of nucleotide variation, neutrality tests, and population differentiation of *Rubroshorea selanica* as inferred from chloroplast intergenic spacer *trn*T-*trn*L and *trn*L-*trn*F sequences

Population	N	*Hd*	*S*	*k*	π	Tajima's *D*	F_{ST}
Buru	8	0.714	2	0.857	0.00044	0.41421	
Seram	10	0.000	0	0.000	0.00000	n.a.	
Sula	9	0.556	1	0.556	0.00029	1.40117	
Total	27	0.615	3	0.798	0.00041	0.06046	0.70222***

N, number of individuals analyzed per population; *Hd*, haplotype diversity; S, number of segregating sites; *k*, number of nucleotide differences; π, nucleotide diversity; F_{ST}, population differentiation; n.a., not available; *** $P < 0.0001$

Table 3: Assessments of nucleotide diversity in *Rubroshorea selanica* based on three nuclear genes

Region	Population	N	*Hd*	π*non-syn*	π*sil*	Tajima's *D*	F_{ST}
GapC	Buru	16	0.500	0.00000	0.00206	1.68710	
	Seram	20	0.747	0.00271	0.00910	-0.38485	
	Sula	18	0.712	0.00144	0.00868	-0.77240	
	Total	54	0.709	0.00158	0.00450	-0.51160	0.04565
PgiC	Buru	16	0.733	0.00113	0.01106	1.17542	
	Seram	20	0.516	0.00000	0.00488	-0.33177	
	Sula	18	0.765	0.00000	0.01113	2.16458*	
	Total	54	0.825	0.00035	0.01327	0.58653	0.41482***
GBSSI	Buru	16	0.733	0.00000	0.00765	-1.15152	
	Seram	20	0.974	0.01030	0.02721	0.59395	
	Sula	18	0.850	0.00045	0.01184	0.60105	
	Total	54	0.904	0.00456	0.02034	-0.21472	0.36930***

N, number of traces/sequences studied each population; *Hd*, the diversity of haplotype; $\pi_{non\text{-}syn}$, nucleotide diversity in non-synonymous sites; π_{sil}, nucleotide diversity in synonymous and non-coding regions; F_{ST}, genetic differentiation among population; *** $P < 0.0001$

Table 4: Pairwise F_{ST} for cpDNA and nuclear genes in Moluccan *Rubroshorea selanica* population

Regions	Population					
		Buru		Seram		Sula	
cpDNA	Buru	0.0000		0.90740	***	0.46222	***
	Seram	0.97406	***	0.0000		0.39394	**
GapC	Buru	0.0000		0.11683	*	0.10107	*
	Seram	0.11683	*	0.0000		-0.03656	
PgiC	Buru	0.0000		0.56040	***	0.39664	***
	Seram	0.56040	***	0.0000		0.18132	**
GBSSI	Buru	0.0000		0.46290	***	0.40130	***
	Seram	0.46290	***	0.0000		0.23655	***

***$P < 0.0001$; **$P < 0.01$

conducted across five distinct populations revealed substantial variations in both leaf dimensions and the number of secondary veins. Leaves originating from Obi Island and Seram Island exhibited larger length and width dimensions compared to other population leaves as well as the reference measurements. The length-to-width ratio of these leaves significantly influenced their oblong shape, distinctly visible in the leaves from Obi and Seram. While the average number of secondary veins per leaf remained relatively consistent across populations, notable variations in the count were identified, particularly in the Buru population. This suggests the presence of individuals in the Buru population with a significantly higher number of secondary veins than their counterparts in the same island. The observed variations in leaf size parameters among the five populations prompt further investigation, and a population genetic study of *R. selanica* represents a pertinent endeavor to elucidate the diversity and genetic structure of this Moluccan-endemic species.

Outcrossing and strong self-incompatibility traits are prominent features of Dipterocarps species (Appanah and Chan 1982; Sakai *et al.* 1999; Lee *et al.* 2000; Nagamitsu *et al.* 2001; Obayashi *et al.* 2002).

The nucleotide diversity within the surveyed chloroplast intergenic spacers of sampled *R. selanica* populations showed lower value than the nucleotide diversity of the cpDNA in common *Rubroshorea* species such as *R. curtisii* (π_{sil} = 0.00155) located in the Malay Peninsula and Borneo (Kamiya *et al.* 2012). Nucleotide diversity for the *Pgi*C locus is similar to that of *R. acuminata* (0.0112) but higher than that of widespread *R. parvifolia* (0.0064) and *R. curtisii* (0,005) (Ishiyama *et al.* 2008). Conversely, in the *Gap*C locus, the nucleotide diversity of silent site for *R. selanica* was resembles those values observed in other common *Rubroshorea* species such as *R. curtisii*, *R. leprosula*, *R. parvifolia* and *R. acuminata* (Ishiyama *et al.* 2003, 2008), but surpassed the diversity observed in *Anthoshorea javanica*, an endemic *Shorea* species in western coast of Sumatra and West Java (Rachmat *et al.* 2012). For the GBSSI locus, the nucleotide diversity of silent sites in *R. selanica* (0.0234) was approximately twice as high as in *R. parvifolia* (0.0113) (Iwanaga *et al.* 2012).

Typically, endemic species and those with limited geographic distributions tend to possess lower genetic variation compared to widely distributed species (Hamrick *et al.* 1992). *R. selanica* displayed lower genetic diversity only in the analyzed cpDNA region and did not exhibit a decrease in genetic diversity relative to other *Shorea* species in the nuclear gene regions. Although this is not the typical observation, Gitzendanner and Soltis (2000) have demonstrated that endangered species can sometimes display levels of diversity as high as or even higher than their more common counterparts. Within the Dipterocarpaceae family, the rare species *Richetia blumutensis* exhibits greater genetic diversity than other widely distributed species, including the scattered species *R. dasyphylla* (Cao *et al.* 2009). Other research has also demonstrated that specific rare and geographically restricted species maintain genetic variation levels akin to closely related, widely dispersed and frequently encountered congeners (Young *et al.* 1996; Gitzendanner and Soltis 2000).

Considering the distribution of the endemic *R. selanica* is limited to only some islands in the Moluccas, its level of genetic differentiation is particularly interesting.

The significant variation identified within the chloroplast genome of the studied species is likely the result of extended separation and isolation throughout its evolutionary history. *Shorea* species' seeds are known to disperse through wind and gravity, with dispersal distances reaching up to 500 meters. However, in forested conditions, more than 50% of mature seeds typically were found within the distance of 50 meters from their parent trees (Chan and Appanah 1980; Takeuchi *et al.* 2004), indicating restricted dispersion despite the lightweight seeds and long-winged fruits characteristic of the species (Fukue *et al.* 2007). Geographical disjunction within the species' distribution has likely led to the development of distinct lineages on isolated islands due to the cumulative effects of mutation and genetic drift. The continual absence of land bridges between these islands since their emergence (Jong 1998) has further reinforced the isolation process by restricting genetic exchange among populations.

Furthermore, strong genetic structuring was evident in two of the three studied nuclear loci (F_{ST} = 0.415 and 0.369 for *Pgi*C and *GBSSI* respectively). In both tropical and temperate zone trees, it is generally believed that gene flow through pollen is more extensive than seeds (Hamilton and Miller 2002; Petit *et al.* 2005). Nonetheless, species within the *Shorea* genus primarily rely on small and less mobile pollinators, such as beetles (Chrysomelidae and Curculionidae, Coleoptera) and thrips (Thrips and Megalurothrips, Thysanoptera), which have limited active migration capabilities (Appanah and Chan 1981; Sakai *et al.* 1999). Given that these populations have their origin in distinct islands, the population structuring revealed through nuclear loci could reasonably be attributed to restricted pollen flow.

Indeed, genetically structured populations are not uncommon among *Shorea* and *Rubroshorea* species and limited gene flow has been observed in several dipterocarp species in various regions. Studies in Malaysian Borneo, for instance, have shown significant fine-scale genetic structure in *Richetia xanthophylla* and *Parashorea tomentella*, partly due to constraints on both seed and pollen dispersal (Kettle *et al.* 2011). Significantly high genetic differentiation across adult populations of *Veteriopsis seychellarum*, an endemic dipterocarp species in the Seychelles, has also been observed (F_{ST} = 0.30), a trend even stronger for juveniles of the species (F_{ST} = 0.42) (Finger *et al.* 2012). Population differentiation (in terms of F_{ST}) in *R. leprosula* within the same island was found to be 0.099, but became larger (0.302) when estimated between Sumatra and Kalimantan (Cao *et al.* 2009). Kamiya *et al.* (2012) found a F_{ST} value of 0.519 between the Malay Peninsula and Borneo populations of *R. curtisii*, while Iwanaga *et al.* (2012) showed the level of population differentiation between *R. parvifolia* populations of either peninsular Malaysia or Sumatra and Borneo to be > 0.25.

The pairwise F_{ST} values in Table 4 indicate that the populations on each island surveyed had genetically differentiated with respect to each other in terms of both their maternally inherited cpDNA and all three nuclear genes. Among a thousand islands spanning the Moluccas region, *R. selanica* has been documented exclusively on four islands—Buru, Seram, Obi and Sula—which have markedly different geological histories of emergence. It is essential to note that the continual absence of land bridges between those islands since their formation (Jong 1998) probably limited past genetic exchange, leading to a notable level of genetic variation between populations. The intricate geological history of these islands may not align with a straightforward isolation-distance pattern. For instance, the F_{ST} values between the neighboring Buru and Seram Island

were notably high amounting 0.907 for cpDNA and 0.101 – 0.560 for nuclear gene regions.

Although Seram and Buru share close stratigraphic similarities, they exhibit different structural pattern (Charlton 2000). Moreover, even though Buru and Seram are currently in close proximity, 5 million years ago, Buru was situated considerably north of Seram (Hall *et al.* 1995), making intensive contact or gene flow improbable at that time. While Buru is known as a recent island, Seram is an ancient landmass with origins dating back to the late Miocene to early Pliocene, has maintained isolation from other landmasses throughout its history (Audley-Charles 1993). This prolonged period of isolation has given rise to a diverse array of endemic species such as 16 bird species exclusive to Seram and its satellite islands.

Understanding the genetic structure, which involves the distribution of genetic diversity within and among populations, holds significant importance in devising conservation strategies for endangered species, particularly when it may not be feasible to protect every population. In cases of low population structure, the loss of a single population may have a limited impact on the overall genetic diversity of the species. However, in instances of high structure, the extinction of a single population could substantially diminish the species-wide genetic diversity. Our study has revealed that each of the island populations of *R. selanica* displays distinct genetic structures, underscoring the significance of conserving all these populations due to their genetic differentiation.

Based on the available information, two primary management and conservation strategies are warranted. The first strategy would entail an *in situ* conservation plan, defining core areas that are entirely free from disturbance. The second management approach would focus on *ex situ* conservation, aimed conserving genetic diversity from all island of distribution. For the sort term strategy, it is crucial to collect as many seeds as possible to obtain a thorough inclusiveness of the species' genetic diversity, spanning its entire distribution. Considering the important status of this species for Indonesia’s plantation program, closer and deeper study of the species should be undertaken.

Conclusion

Our study on *R. selanica* in the Moluccas region has revealed its unique genetic diversity and population structure, notably its distribution extending east of the Wallace Line. By employing a comprehensive multi-marker genetic analysis, we have underscored the significant impact of geographic isolation on genetic differentiation among populations. This knowledge carries vital conservation implications, emphasizing the importance of treating these genetically distinct populations as separate conservation units to preserve their genetic diversity and adaptability. The significance of undertaking a more in-depth study of *R. selanica* becomes evident in the quest for a more comprehensive understanding of the species' evolution and adaptation mechanisms. While our current research has shed light on its genetic diversity and population structure, delving deeper into ecological, physiological, and biochemical aspects is essential to unravel a more complete picture. A broader study encompassing various facets of the species is imperative not only for enhancing our knowledge of its biology but also for formulating more effective conservation strategies. This comprehensive approach is crucial in revealing the intricacies of *R. selanica*'s evolutionary processes and in ensuring a holistic understanding that can contribute to its sustainable conservation and management. Furthermore, our research contributes to a broader understanding of genetic dynamics in Wallacea's complex island geography, enriching the field of biogeography.

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Author Contributions

HHR, KK and KH designed the study; HHR and AH collected the samples, HHR conducted the laboratory works; HHR and AH analyzed data; HHR and AH write the first draft; HHR, AH, KK and KH edit and review the draft. All authors have approved the final version of the manuscript.

Conflict of Interest

All authors declare no conflict of interest.

Data Availability

Data presented in this study will be available on a fair request to the corresponding author.

Ethics Approval

Not applicable to this paper.

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