

Genetic diversity of the endemic Durian Kura-kura (*Durio testudinarius* Becc.) using morphology and SSR markers

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Research Article

Keywords: Genetic resources, microsatellites, polymorphism

Posted Date: December 21st, 2023

DOI: <https://doi.org/10.21203/rs.3.rs-3741516/v1>

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Additional Declarations: No competing interests reported.

Abstract

Durio testudinarius, commonly known as Durian kura-kura, represents a wild durian species within the Malvaceae family, exclusive to Borneo. Understanding the genetic diversity of this species is crucial for effective genetic resource management. In this study, we conducted a comprehensive analysis of the genetic diversity of *D. testudinarius* across five populations in West Kalimantan, namely Sambas, Landak, Sanggau, Sekadau, and Ketapang. Our approach integrated both morphological and molecular markers, incorporating 18 vegetative morphological characters and 20 microsatellite loci. Morphological analysis revealed variations among *D. testudinarius* accessions, encompassing traits such as tree height, leaf blade dimensions, trunk surface features, and various leaf characteristics. On the molecular level, all 20 microsatellite loci exhibited polymorphism, with allele frequencies ranging from 0.025 to 0.975. These loci collectively comprised 84 alleles across 20 accessions, averaging 4.2 alleles per locus. The observed heterozygosity and expected heterozygosity values were 0.263 and 0.268, respectively. Polymorphic information content values ranged from 0.049 to 0.869, with an average of 0.454. Ward-based cluster analysis using morphological, SSR, and combined markers generated a dendrogram, indicating that the Landak, Sekadau, and Sanggau populations tended to cluster together, while the Sambas and Ketapang populations exhibited a tendency to form distinct groups. This study provides valuable insights into the genetic diversity of *Durio testudinarius*, facilitating informed conservation and management strategies for this unique wild durian species in Borneo.

Introduction

Durio testudinarius, commonly referred to as Durian kura-kura, is a native durian species (*Durio*) belonging to the Malvaceae family and is exclusive to Borneo. It is distributed across Brunei Darussalam, Indonesia (Kalimantan), and Malaysia (Sabah and Sarawak) (POWO 2023). *D. testudinarius* has been seen in many places in Indonesia, such as Central Kalimantan (East Kotawaringin, Seruyan, and Pangkalan Bun Regencies), West Kalimantan (Sintang, Sekadau, Sambas, Landak, Kapuas Hulu, and Ketapang Regencies), and North Kalimantan (Tana Tidung Regency) (GBIF secretariat 2019). This species thrives in lowland, humid tropical environments at elevations of up to 700 meters above sea level, preferring fertile and well-drained soil conditions (Fern et al. 2014).

Durian Kura-kura exhibits distinct features that distinguish it from other species of durian cousins, including the inflorescences and fruits that appear at the lower part of the stem (Kostermans 1958). Unlike the widely grown durian species *D. zibethinus*, *D. testudinarius* has not undergone extensive exploitation or cultivation by the community. The plant is frequently observed to thrive in domestic gardens or yards as a supplementary crop, devoid of any particular upkeep or cultivation methodologies. The unique properties of *D. testudinarius* make it a rich genetic resource that may be used to produce and enhance excellent durian qualities through plant breeding initiatives (Uji 2005).

The characterization and analysis of genetic diversity play a crucial role in the management of genetic resources. Germplasm characterization involves a systematic description of accessions based on qualitative traits inherited with high heritage value, such as growth habit, plant height, and flower color. These traits serve as representatives of the population of accessions, using a list of descriptors (characters) and documented information (Jaramilo and Baena 2002). It can be difficult to distinguish between types because morphological or phenotypic characteristics frequently depend on the environment, especially during the juvenile stage before flowers or fruits have formed. Therefore, an additional approach unaffected by the environment, such as DNA (genomic) markers, is necessary to validate morphological characters. Molecular markers like microsatellites and simple sequence repeats (SSRs) can be used in a number of genetic analysis methods that are used with polymerase chain reaction (PCR) to describe genetic resources.

Microsatellites, which are sometimes called simple sequence repeats (SSRs), are short DNA sequences that have a core motif repeated twice in a row, are very variable, and are found in a lot of different organisms' genomes (Ellegren 2004). SSR markers are widely utilized in various scientific investigations, including studies on genetic diversity, paternity examination, population analysis, identification of genetic features, and forensic research (Kumar 2018). The objective of this study is to analyze the genetic diversity of the *D. testudinarius* population based on morphology and specific SSR markers. Using these markers, the study aims to give data and information about the diversity of *D. testudinarius*. This will help with the management and growth of durian genetic resources.

Materials and Methods

Plant materials

Twenty *D. testudinarius* plant samples were gathered from five places in West Kalimantan: Sambas, Landak, Sanggau, Sekadau, and Ketapang districts (see Fig. 1 and Table 1) for morphology analysis. Furthermore, for the SSR analysis, 20 plant samples were selected from the same five locations. The samples used in this research consisted of mature plants that had reached the generative phase within each population.

Morphology marker

The parameters for morphology characters in this study were based on the guide to Durian descriptors (Bioversity 2007), with minor modifications. The following characteristics were observed: tree height (m), trunk height (m), trunk circumference (cm), trunk surface, trunk growth habit, leaf upper and lower surface color, arrangement of leaves, leaf attitude, leaf blade length and width (cm), leaf blade shape, leaf apex shape, leaf base shape, and leaf blade margin. Additionally, the glossiness of the leaf's upper and lower surfaces was also taken into consideration.

Table 1
Sampling location information in five populations of the research

Population	Geographical location	Elevation (m above sea level)
Sambas Botanical Gardens, Sambas District (Sbs)	01°16'21,9"N 109°29'12,5"E	34
Mandor, Landak District (Ldk)	00°14'54,4"N 109°18'59,4"E	28
Tayan Hulu, Sanggau District (Sau)	00°15'16,8"N 110°17'22,5"E	29
Nanga Taman, Sekadau District (Skd)	00°22'28,2"S 110°51'03,2"E	166
Gunung Palung National Park, Ketapang District (Ktp)	01°13'S 110°7'E	200

SSR markers

A total of 20 pairs of primers and 4 fluorescence-labeled primers (6-FAM, VIC, NED, and PET) as tails in the forward primer sequence were utilized in this study. In a previous study by Magandhi et al. (2021), microsatellite sequences derived from the whole genome data of *D. testudinarius* served as the basis for these SSR primers. We divided the SSR primers into multiplex PCR groups, each consisting of the four fluorescence-labeled SSR primers. Detailed information about the primers can be found in Table 2.

DNA extraction and amplification

A modified CTAB (cetyltrimethylammonium bromide) (Doyle and Doyle 1987) was used to get genomic DNA from leaf tissue. Tris-EDTA (TE) buffer was used as the solvent. The quantity and quality of genomic DNA were assessed using the Multiskan SkyHigh Microplate Spectrophotometer. The PCR amplification was conducted using an Agilent SureCycler 8800, and the Type-IT PCR kit (Qiagen) was used in a 10 mL reaction mixture. The reaction mixture consisted of approximately 20 ng of genomic DNA, 5 mL of 2x Type-IT Multiplex PCR Master Mix, 0.2 mM of each reverse primer, 0.1 mM of each forward primer, and 0.1 mM of each fluorescence-labeled primer. The PCR conditions were as follows: initial denaturation at 95°C for 3 minutes, followed by 32 denaturation cycles at 95°C for 30 seconds, annealing at 60°C for 1 minute and 30 seconds, extension at 72°C for 30 seconds, and a final extension at 60°C for 30 minutes. Allele number and size were determined using the capillary electrophoresis method and manually analyzed using GeneMapper Software 5 (Applied Biosystem).

Table 2
The twenty of SSR primer used in this study

Group	Primer Number	Locus ID	Primer Sequences (5' – 3')	SSR Motif	Ta (°C)
M1	P4	dt37193	F: FAM-TCCTTGAAGCTTCGGATGGT	(TA)10	60
			R: TGGAGAGAGGTAGGCTGACA		60
	P10	dt19746	F: VIC-AGGAAGTTCTCGCCGGATTG	(TTA)7	60
			R: ACCCAACCACACCATCCATC		60
	P2	dt18725	F: NED-TCGACAACCTGGAGCTTTACA	(AG)10	60
			R: TGTGGGCGATGATGAGAGTC		60
	P20	dt126809	F: PET-TGGTGGAAATTTGTCATGGGATCA	(ATTT)5	60
			R: AGGATGCCAGTCAACACCTG		60
M2	P16	dt5254	F: FAM-AGGTGTGGAAGTTTGGACCG	(TTTCA)5	60
			R: CCAGATACCAAGACCAGCGG		60
	P1	dt24564	F: VIC-GTGGTCTTCTCTGCTGCC	(TC)11	60
			R: TGATCGGGCTTTTCAGGTTCT		60
	P9	dt17599	F: NED-ATCCGCTGCTGTTCATCCAA	(AAG)7	60
			R: TAAGAGGGTGTGCGAGGGTGG		60
	P14	dt55948	F: PET-TGACCAACCATGTGATATGCTCA	(CATA)6	60
			R: GCACGCATACACAAAGATCCA		60
M3	P17	dt17930	F: FAM-ACAAGCATGCATGGACCACA	(ACAG)6	60
			R: ACAGCTGTGCCTCTCAACAG		60
	P5	dt41585	F: VIC-TTCCGCCATATCTCCATCATCA	(AG)10	60
			R: ACGATAAACTTGGAGCGTGTTT		60
	P3	dt2717	F: NED-AGCCTTTTCTTACCTCCGCT	(CT)10	60
			R: CTAACACCCCTCCACTCCGC		60
	P18	dt17247	F: PET-CACCAAAGGCCTACTCTTGCT	(TCTTT)5	60
			R: TGCGCCATGAACCACCTATT		60
M4	P15	dt30243	F: FAM-TGTGGAGGAAAGGACAACGT	(TTAA)6	60
			R: GAGAGCAAAGACCCAACCCA		60
	P6	dt2294	F: VIC-AATCTGCCGTTTGTCCAGCA	(AAT)8	60
			R: GCCCAGATGCGATTTAAGGC		60
	P8	dt33709	F: NED-TGGTGTGACGGAGAGGAT	(GAC)8	60
			R: GCAAGGCTTTTCTTCCGCAT		60
	P12	dt88788	F: PET-TCTGCGACCAAAACAAAGTGAC	(AAAT)6	60
			R: TGTAGCATCCAATGAGAAAGCAG		60
M5	P7	dt33464	F: FAM-GCCTTGATTATGCAACGTCA	(ATA)8	60
			R: TGGAAACCCAACTACACTGACT		60
	P11	dt24697	F: VIC-ATGTGCCAGCTAAGCCTGTT	(TTA)7	60
			R: TCCCCAAAGTTGCATCATACAGT		60
	P13	dt12934	F: NED-CGTTCTCGTTTCTCATTTACAGC	(TGAT)6	60
			R: AACCCATCACCCATGCCTTT		60
	P19	dt18696	F: PET-GGAGCAGATGACGAGTAACCC	(ATAAA)5	60
			R: ACCGAACTGTATGCTTTGCTTT		60
Ta annealing temperature					

6-FAM: GCCTCCCTCGCGCCA; VIC: GCCTTGCCAGCCCGC; NED: CAGGACCAGGCTACCGTG; PET: CGGAGAGCCGAGAGGTG

Data analysis

The morphological character data were organized and tabulated in the form of binary data with scores 1 and 0, or as nominal or interval (numeric) data using Microsoft® Excel 2016. Means were compared using the Fisher's Least Significant Difference (LSD) test at a significance level of 5%. Principal component analysis (PCA) and other statistical analyses were performed by Minitab 16 statistical software (Minitab Inc., State College, PA, USA). Cluster analysis for morphological characters was carried out using the Ward clustering method based on the coefficient of dissimilarity (Gower 1971).

The size and number of allele data from each SSR locus across samples were tabulated and organized according to codominance data using Microsoft® Excel 2016. Parameters of genetic diversity were estimated, including the observed heterozygosity (H_o), estimated heterozygosity (H_e), and polymorphic information content (PIC) for co-dominant markers, as described by Botstein et al. (1980). These parameters were calculated using GenAlEx 6.5 software (Peakall and Smouse 2012). Subsequently, a phylogenetic analysis was conducted to assess the genetic relationships between accessions. The resulting genetic relationships were visualized using a dendrogram constructed using the Ward clustering method based on the coefficient of dissimilarity (Gower 1971). The dendrogram for cluster analysis, which included both morphological and SSR markers, was performed using the PBSTAT-CL 2.1 program.

Result and Discussion

Genetic Diversity based on Morphological Characters

Among the five populations tested, six out of the 18 morphological character parameters exhibited consistent results across all accessions. These populations shared common characteristics, including straight stems, dark green and coppery brown colors on the upper and under leaf surfaces, alternate leaf arrangements, semi-erect leaf attitudes, and a glossy upper leaf surface. However, twelve other parameters exhibited variations both among different populations and within accessions of each population. These variations included quantitative characters like tree height and leaf blade length, which displayed significant differences at the 5% level, leaf blade width, and the ratio of length to width of the leaf blade, which displayed significant differences at the 1% level. Although characters of trunk height and trunk circumference revealed no significant difference by Fisher's LSD test. Other characters exhibited variations among populations, including trunk surface, leaf blade shape, leaf apex and base shape, leaf blade margin, and leaf lower surface glossiness. The tabulation of morphological characters for the five populations tested is available in Table 3. The Ketapang group documented the tallest trees, averaging 20.3 m in height, while the Sekadau group had the shortest trees, with an average height of 11.2 m. Regarding leaf measurements, the Sambas group exhibited the largest leaf length and width among the five populations, averaging 21.7 cm and 7.5 cm, respectively. Conversely, the Sekadau group had the smallest average leaf length and width, measuring 14.7 cm and 4.0 cm, respectively. Morphological features encompass both quantitative and qualitative aspects. Environmental factors, such as location disparities, treatment strategies, and unique microclimates, appear to have a significant impact on variations in quantitative characteristics, such as tree height and trunk circumference. In contrast, qualitative traits continue to be largely unaffected by environmental factors, suggesting that genetic factors are the main determinants of these features (Syukur et al. 2012).

Table 3
Morphological characters of *D. testudinarius* in five population on West Kalimantan

Character	Population					F-value
	Sambas	Landak	Sanggau	Sekadau	Ketapang	
Tree height (m)	19.1 ± 4.6a	11.3 ± 2.0b	15.7 ± 7.1ab	11.2 ± 4.2b	20.3 ± 1.5a	3.88*
Trunk height (m)	8.9 ± 5.4	7.3 ± 1.6	8.3 ± 5.2	3.6 ± 1.9	7.4 ± 1.5	1.31ns
Trunk circumference (cm)	87.1 ± 34.6	51.3 ± 15.8	51.3 ± 31.7	76.9 ± 49.1	88.1 ± 60.5	0.80ns
Trunk surface	Rough or very rough	Very rough	Rough	Rough or very rough	Rough	
Trunk growth habit	Straight	Straight	Straight	Straight	Straight	
Leaf upper surface color	Dark green	Dark green	Dark green	Dark green	Dark green	
Leaf lower surface color	Coppery brown	Coppery brown	Coppery brown	Coppery brown	Coppery brown	
Arrangement of leaves	Alternate	Alternate	Alternate	Alternate	Alternate	
Leaf attitude	Semi-erect	Semi-erect	Semi-erect	Semi-erect	Semi-erect	
Leaf blade length (cm)	21.7 ± 1.4a	16.5 ± 2.7b	18.8 ± 3.6ab	14.7 ± 4.8b	15.8 ± 0.8b	3.32*
Leaf blade width (cm)	7.5 ± 0.5a	4.3 ± 0.3cd	6.3 ± 0.6b	4.0 ± 1.4d	5.2 ± 0.2cd	16.03**
Ratio of length/width of leaf blade	2.9 ± 0.02b	3.9 ± 0.49a	3.0 ± 0.32b	3.7 ± 0.39a	3.1 ± 0.11b	8.22**
Leaf blade shape	Oblong,	Linear-oblong	Oblong or elliptic	Oblong or linear-oblong	Oblong	
Leaf apex shape	Acuminate	Long acuminate	Acuminate or long acuminate	Long acuminate	Acuminate	
Leaf base shape	Round	Acute	Acute	Acute	Acute	
Leaf blade margin	Entire	Undulate	Entire or undulate	Entire or undulate	Entire	
Leaf upper surface glossiness	Glossy	Glossy	Glossy	Glossy	Glossy	
Leaf lower surface glossiness	Not glossy or glossy	Not glossy	Glossy	Not glossy or glossy	Glossy	

Different letters within the same row indicate statistically significant differences (P 0.05) according to Fisher's least significant difference test. A double asterisk (**) indicates significance at the 1% level, a single asterisk (*) indicates significance at the 5% level, and "ns" indicates no significant difference.

Principal component analysis (PCA) was conducted on twelve morphological characters that exhibited variation among the test accessions. These characters included tree height, trunk height, trunk circumference, trunk surface, leaf blade length, leaf blade width, ratio of length/width of leaf blade, leaf blade shape, leaf apex shape, leaf base shape, leaf blade margin, and leaf lower surface glossiness. The PCA resulted in the first and second components explaining variances of 39.6% and 16.7%, respectively. The bi-plot provides insight into the relationship among morphological characters within the first two components. In the bi-plot, tree height, trunk height, trunk circumference, leaf blade shape, leaf apex shape, leaf blade margin, leaf blade width, and ratio of length/width of leaf blade exhibit similar strong loadings on principal component 1. On the other hand, trunk surface, leaf blade length, leaf base shape, and leaf lower surface glossiness demonstrate similar strong loadings on principal component 2. Based on the distances between individuals within each origin population, dashed circles served as that population's representation. The Sambas group was distinguished by characters of tree height, trunk height and circumference, and leaf blade length and width, while the Ketapang group exhibited high values for leaf lower surface glossiness. The Sekadau group differentiated by ratio of length/width of leaf blade, leaf blade shape, leaf apex shape, and leaf base shape, while Landak members grouped based on ratio of length/width of leaf blade, leaf apex shape, and leaf blade margin. The Sanggau group spreaded near the center of the bi-plot pivot point, indicating a relatively neutral tendency towards specific characters, but had notable values for leaf lower surface glossiness, trunk height and circumference, and leaf blade width and shape.

Allelic Diversity and Connections Within and Between *D. testudinarius* populations

A total of 20 loci were examined in 20 accessions of *D. testudinarius* from five populations in West Kalimantan. All loci exhibited polymorphism, showing varying numbers and frequencies of alleles, except for locus P15, which showed only one allele (Table 4). The number of alleles per locus varied between 1 and 12, while the allele frequencies ranged from 0.025 to 0.975. Notably, locus P4 exhibited the highest number of alleles, totaling 12. The alleles of P4 ranged in size from 172 to 200 bp, with the 182 bp allele being the most frequent at 0.25, while the 172 and 198 bp alleles were the least frequent at 0.025. Overall, the distance between the closest alleles was 2 bp, while the distance between the farthest alleles was 14 bp.

Table 4
Allele sizes and frequencies of 20 microsatellites loci analyzed in 20 accessions of *D. testudinarius*

Locus	Allele Size and Frequency											
P1	176	181	183	189	197	200	205					
	0.117	0.175	0.200	0.200	0.025	0.158	0.125					
P2	250	252	254	256	264							
	0.200	0.500	0.200	0.075	0.025							
P3	186	188	190	192								
	0.650	0.300	0.025	0.025								
P4	172	174	176	178	182	184	186	188	190	194	198	200
	0.025	0.075	0.100	0.150	0.250	0.025	0.100	0.025	0.050	0.075	0.025	0.100
P5	231	241										
	0.975	0.025										
P6	254	261	263									
	0.025	0.075	0.900									
P7	223	229	232	235	238	241	244	246				
	0.050	0.100	0.050	0.100	0.350	0.150	0.175	0.025				
P8	219	225	228	231	237	239	242					
	0.325	0.050	0.075	0.250	0.125	0.075	0.100					
P9	219	222										
	0.950	0.050										
P10	216	219										
	0.500	0.500										
P11	243	246	249	252								
	0.075	0.725	0.075	0.125								
P12	188	192										
	0.900	0.100										
P13	294	299	303									
	0.350	0.425	0.025									
P14	307	313	315	317	319	329						
	0.025	0.367	0.075	0.408	0.050	0.075						
P15	241											
	0.800											
P16	249	259	265	270	275	280						
	0.100	0.425	0.100	0.325	0.025	0.025						
P17	197	211	215	219								
	0.425	0.425	0.075	0.075								
P18	230	241										
	0.025	0.975										
P19	246	256										
	0.025	0.975										
P20	233	241										
	0.975	0.025										

Allele frequencies are listed under the allele sizes

The variability in sample numbers and characteristics may lead to diverse allele counts, even when utilizing the same primer (Sugiantari et al. 2015). Polymorphisms within SSRs are a result of variations in the number of motif repetitions due to polymerase strand slippage during DNA replication or imperfections in recombination (Hosseinzadeh-Colagar et al. 2016; Vieira et al. 2016). Generally, a high number of alleles at a specific genetic locus serves as an indicator of substantial polymorphism. Polymorphism denotes the presence of multiple genetic variants (alleles) within a population (Chesnokov and Artemyeva 2015). The greater the number of alleles identified at a particular locus, the higher the level of polymorphism in that region. In this study, locus P4 was indicated to have a greater degree of polymorphism compared to other loci due to the largest number of alleles.

The overall level of polymorphism can be quantitatively assessed using two separate measures: heterozygosity and PIC value (Shete et al. 2000). Heterozygosity refers to the probability of an individual having different alleles at a specific marker site, influenced by the number of alleles present and their respective frequencies within the population. The range of values varies from zero, representing the complete absence of heterozygosity, to 1.0, indicating a substantial number of alleles with equal frequencies (Serrote et al. 2020). The twenty loci comprised a total of 84 alleles across 20 accessions from the five populations, with an average of 4.2 alleles per locus (Table 5). The Sekadau population had the highest number of alleles, with a total of 57 and an average of 2.85 alleles per locus. On the other hand, the Ketapang population had the lowest number of alleles, with a total of 29 and an average of 1.45 alleles per locus. The average values of H_o and H_e across loci were 0.263 and 0.268, respectively. Among the populations studied, the Sekadau population exhibited the highest averages of H_o and H_e , namely 0.363 and 0.453, respectively. These results suggest that the Sekadau population exhibits the highest genetic diversity among the other four populations. Nevertheless, the Sekadau population demonstrates a smaller H_o value compared to the H_e value ($H_o < H_e$), indicating a tendency for a higher prevalence of homozygous alleles relative to heterozygous alleles. Conversely, the Sanggau population displayed the lowest average H_o , which was 0.188, indicating the lowest genetic diversity among the populations studied.

The PIC value of a marker reflects its capability to identify polymorphisms among individuals within a population. Botstein et al. (1980) categorized markers with PIC values exceeding 0.5 as highly informative, those with values between 0.25 and 0.50 as moderately informative, and those below 0.25 as less informative. The result of this study showed that the PIC values across loci ranged from 0.049 to 0.869, with an average of 0.454. According to Botstein's criteria, loci P1, P2, P4, P7, P8, P13, P14, P16, and P17 were considered highly informative markers, loci P3, P10, and P10 were considered moderately informative markers, and the remaining loci were considered less informative markers.

According to molecular variance analysis, within-population variance accounts for a slightly higher percentage, specifically 54%, compared to between-population variance, which comprises 46%. (Fig. 4). This result differs from the findings documented by Handayani (2016) for other *Durio* relatives. Handayani observed that the genetic variation in Lai (*D. kutejensis*) was significantly attributed to variation within populations, which reached 88%, while the remaining 12% was attributed to variation across populations. The small variation within the population of *D. testudinarius* is likely associated with constrained pollination, resulting from the positioning of the *D. testudinarius* inflorescences at the stem's base. The location of inflorescences results in a greater abundance of insect pollinators transporting pollen, as opposed to birds or bats. However, Nyffeler and Baum (2000) argued that birds and bats are the main pollinators for *D. testudinarius*. The positioning of these inflorescences or fruits is also believed to restrict seed distribution, leading to less genetic diversity within the population in comparison to other *Durio* relatives that produce fruits on branches. Additional observations are necessary to verify this suspicion regarding the process of pollination and seed dispersal in *D. testudinarius*.

Table 5
Genetic diversity of 20 microsatellites loci in five population of *D. testudinarius*

Locus	Sambas (n = 4)			Landak (n = 4)			Sanggau (n = 4)			Sekadau (n = 4)			Ketapang (n = 4)			All Population (n = 20)	
	Na	Ho	He	Na	Ho	He	Na	Ho	He	Na	Ho	He	Na	Ho	He	Na	Ho
P1	5	0.500	0.750	3	0.250	0.531	3	0.250	0.656	3	0.250	0.531	3	0.333	0.611	7	0.317
P2	2	0.250	0.219	1	0	0	2	0.500	0.375	4	0.750	0.563	1	0	0	5	0.300
P3	1	0	0	1	0	0	2	0.250	0.219	3	0.750	0.594	1	0	0	4	0.200
P4	2	0	0.375	2	0	0.5	3	0.250	0.656	6	0.750	0.781	1	0	0	12	0.200
P5	1	0	0	1	0	0	1	0	0	2	0.250	0.219	1	0	0	2	0.050
P6	1	0	0	2	0.250	0.219	1	0	0	3	0.250	0.531	1	0	0	3	0.100
P7	4	1.000	0.719	2	0.250	0.219	3	0.500	0.531	4	0.250	0.656	3	0.750	0.531	8	0.550
P8	2	0.250	0.469	3	0.500	0.594	1	0	0	3	0.250	0.531	3	1.000	0.594	7	0.400
P9	1	0	0	1	0	0	1	0	0	2	0	0.375	1	0	0	2	0
P10	1	0	0	2	0.500	0.375	1	0	0	2	0.500	0.375	1	0	0	2	0.200
P11	2	0.500	0.375	2	0.750	0.469	2	0.250	0.469	2	0.250	0.469	1	0	0	4	0.350
P12	1	0	0	1	0	0	1	0	0	2	0	0.375	2	0.500	0.375	2	0.100
P13	2	0.250	0.219	2	0.250	0.219	1	0	0	3	0.750	0.594	0	0	0	3	0.250
P14	2	0.500	0.375	2	0.667	0.444	2	0.250	0.219	5	0.500	0.750	3	0.750	0.656	6	0.533
P15	1	0	0	1	0	0	1	0	0	1	0	0	0	0	0	1	0
P16	4	0.750	0.656	3	0.750	0.531	3	0.500	0.594	5	0.250	0.781	2	0.500	0.375	6	0.550
P17	3	1.000	0.594	3	0.750	0.656	2	0.750	0.469	2	1.000	0.500	2	1.000	0.500	4	0.900
P18	1	0	0	1	0	0	1	0	0	2	0.250	0.219	1	0	0	2	0.050
P19	1	0	0	1	0	0	1	0	0	2	0.250	0.219	1	0	0	2	0.050
P20	1	0	0	1	0	0	2	0.250	0.219	1	0	0	1	0	0	2	0.050
Total across loci	38	5.000	4.751	35	4.917	4.757	34	3.750	4.407	57	7.25	9.063	29	4.833	3.642	84	5.100
Average across loci	1.90	0.250	0.238	1.75	0.246	0.238	1.70	0.188	0.220	2.85	0.363	0.453	1.45	0.242	0.182	4.20	0.255

Genetic Relationship between Accessions

We used cluster analysis with the Ward method to look at the genetic relationships between the 20 *D. testudinarius* accessions in this study. The results were shown in a dendrogram (Fig. 4). The analysis, employing morphological markers, SSR, and a combination of both, revealed distinctive patterns. When considering morphological markers alone, all accessions were classified into two groups. Group 1 included accessions from Sekadau, Landak, and Sanggau, excluding accession 4 from Sanggau (Sau4), while Group 2 encompassed all accessions from Ketapang, Sambas, and Sau4. Conversely, SSR markers yielded a dendrogram grouping the 20 accessions from five populations into two primary groups. Group 1 exclusively consisted of members from Ketapang, while Group 2 further divided into two sub-groups: group 2a incorporated accessions from Sekadau, Landak, Sanggau, and one member from Sambas (Sbs4), and group 2b included the remaining members from Sambas. The combination of morphological and SSR markers in the dendrogram divided all accessions into three main groups. Group 1 was subsequently divided into two sub-groups: group 1a encompassed all members from Sanggau and one member from Sekadau (Skd3), and group 1b included all members from Landak and the remaining three members from Sekadau. Meanwhile, Group 2 comprised all members from Ketapang, and the final group, Group 3, included all members from Sambas. Overall, cluster analysis consistently showed a pattern of grouping based on morphological markers, SSR, or a mix of the two. This showed that the Landak, Sekadau, and Sanggau populations tended to group together. This pattern highlighted the close kinship among accessions from these three populations. Conversely, the Sambas and Ketapang populations exhibited a tendency to form distinct groups, despite their close genetic proximity.

Conclusion

Variations were observed among *D. testudinarius* accessions based on morphological characters, encompassing quantitative traits such as tree height, leaf blade length, leaf blade width, and the ratio of leaf blade length to width, as well as qualitative traits such as trunk surface, leaf blade shape, leaf apex and base shape, leaf blade margin, and leaf lower surface glossiness. All loci exhibited polymorphism, except for locus P15, with the number of alleles per locus ranging from 1 to 12 and allele frequencies spanning from 0.025 to 0.975. These loci collectively constituted 82 alleles across 20 accessions from the five

populations, averaging 4.2 alleles per locus. The observed heterozygosity and expected heterozygosity displayed average values of 0.263 and 0.268, respectively. Polymorphic information content values across loci ranged from 0.049 to 0.869, with an average of 0.454. Molecular variance analysis indicated that within-population variance accounted for a slightly higher percentage, specifically 54%, compared to between-population variance, which comprised 46%. The dendrogram, generated through Ward-based cluster analysis using morphological, SSR, and combined markers, revealed that the Landak, Sekadau, and Sanggau populations tended to cluster together. In contrast, the Sambas and Ketapang populations exhibited a tendency to form distinct groups.

Declarations

Author Contribution

The research was planned and designed by SO and DM. The study experiments were conducted by MM and DM. The data was examined by DM. MM drafted the manuscript. The manuscript was reviewed and edited by SO, DM, YW and SU. The manuscript was finalized by MM. The work was reviewed and approved by all of the authors.

Acknowledgment

This research was funded by the Ministry of Research and Technology/National Research and Innovation Agency of the Republic of Indonesia through the Doctoral Dissertation Research (PDD) grant scheme for the 2021 fiscal year, contract number 1/E1/KP.PTNBH/2021. And appreciation to the National Research and Innovation Agency (BRIN) for supporting graduate study through the *Degree by Research* scheme of the 2019 year.

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Figures



Figure 1

Map of sampling location in five populations in West Kalimantan

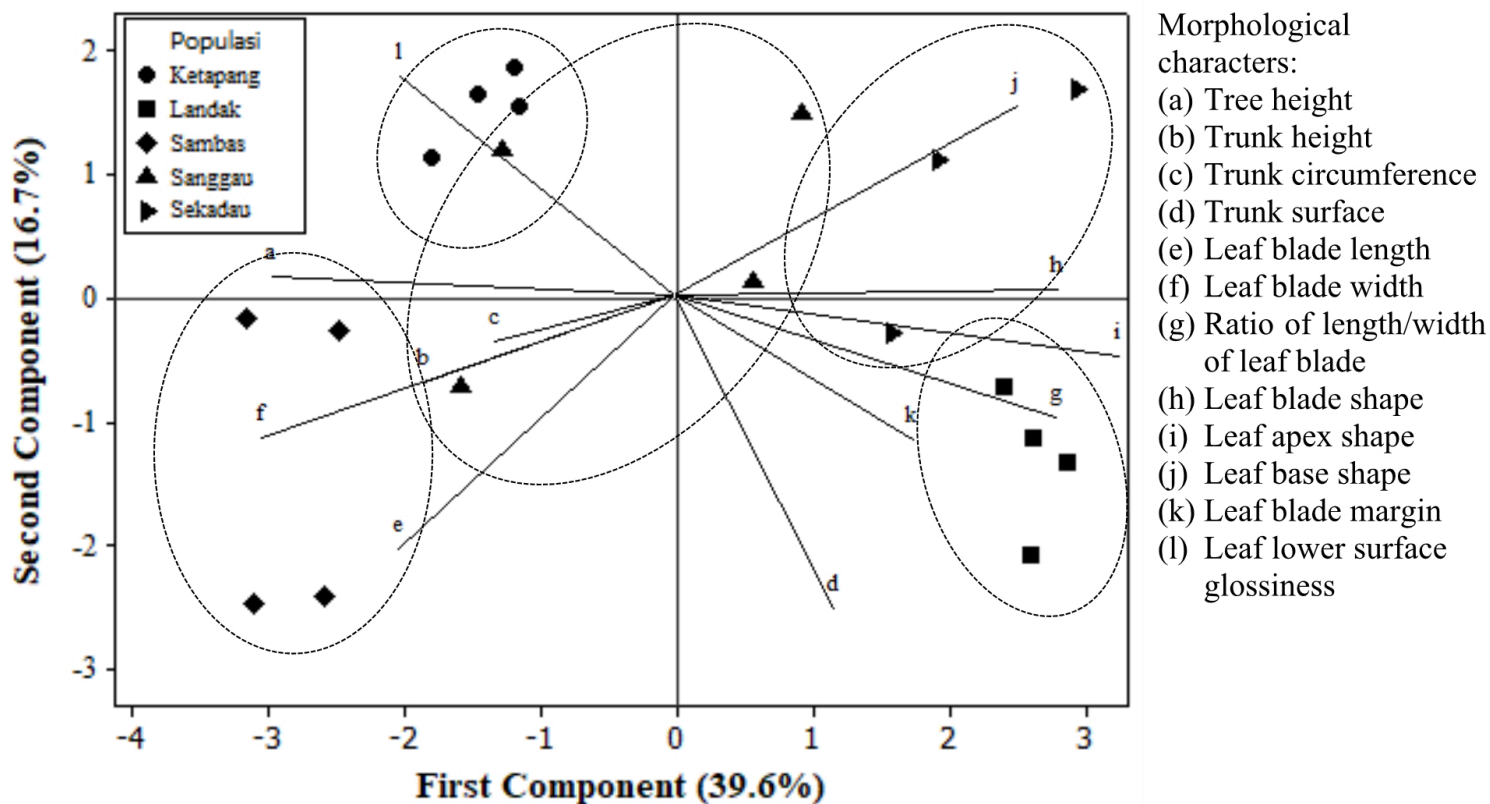


Figure 2

Principal component analysis of five *D. testudinarius* populations based on morphological characters

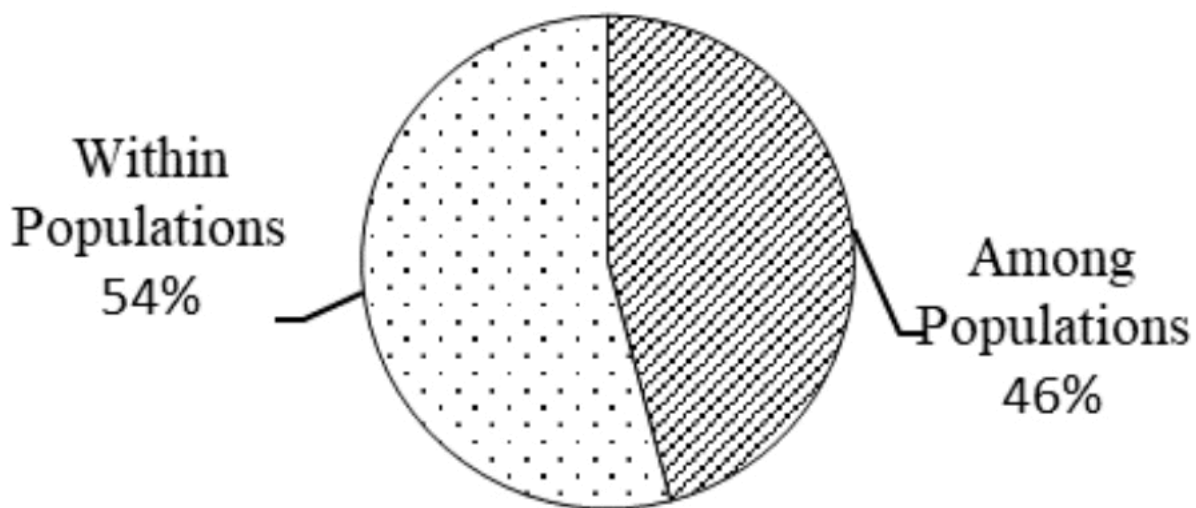


Figure 3

Percentages of molecular variance of the five populations based on 20 loci of SSR markers

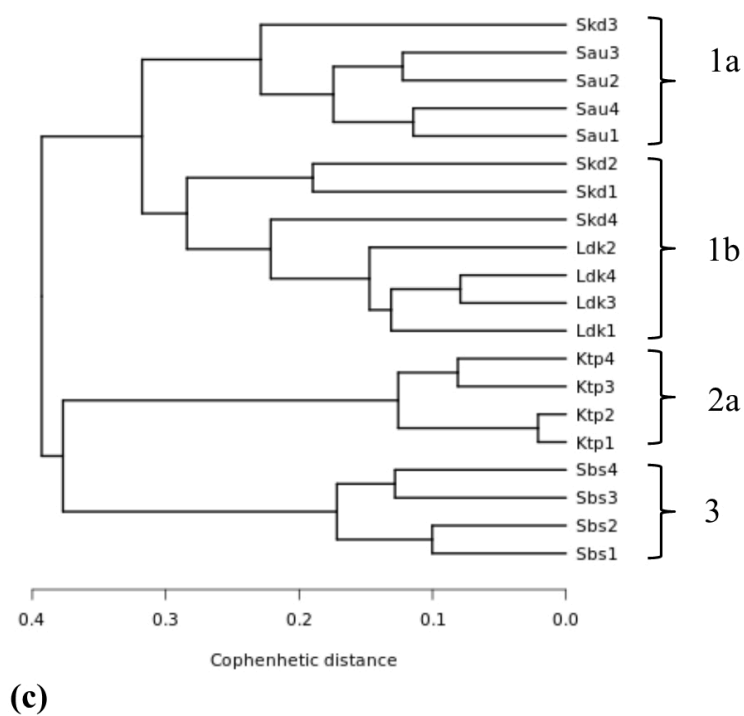
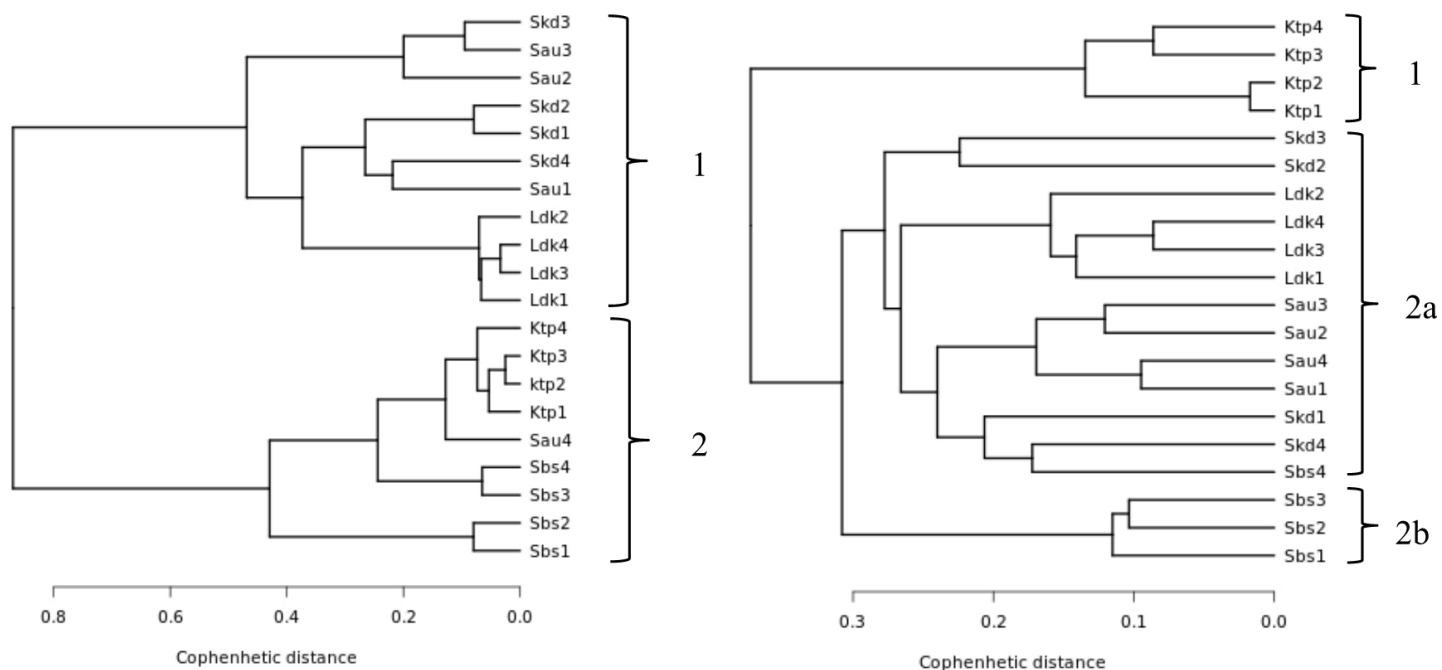


Figure 4

Dendrogram for cluster analysis of 20 *D. testudinarius* accessions using Ward clustering method based on morphological characters (a), SSR markers (b), as well as a combination of morphology and SSR markers (c)