HAYATI
Journal of Biosciences

Vol. 31 No. 2, March 2024 392-403
DOI:10.4308/hjb.31.2.392-403
ISSN: 1978-3019
EISSN: 2086-4094

Genetic Variability of the Long-Tailed Macaque (*Macaca fascicularis*) Populations in Urban Habitat in Padang City, West Sumatra, Indonesia

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ARTICLE INFO

Article history:
Received September 27, 2022
Received in revised form September 19, 2023
Accepted November 18, 2023

KEYWORDS:
fragmentation,
genetic variation,
Macaca fascicularis,
urban habitat

ABSTRACT

The long-tailed macaque (*Macaca fascicularis*) is a primate species recognized for its exceptional ability to adapt to urban habitat. However, urban anthropogenic activities contribute to the fragmentation of macaque natural habitat, affecting genetic variation among distinct populations. Therefore, this study aimed to assess the genetic variability of *M. fascicularis* populations in Padang City, West Sumatra, Indonesia. A total of 70 fecal samples from the wild long-tailed macaques in Gunung Padang (GPD), Gunung Meru (GMR), and Gunung Pangilun (GPG) were collected using a non-invasive method. Conventional PCR amplification and Sanger sequencing were conducted to examine a 1,200-bp mitochondrial DNA (mtDNA) fragment in the D-loop region. The analysis of genetic variation showed that only two haplotypes were present in the three populations. Both GPD and GMR shared the same haplotype (H1), while the GPG population had a distinct haplotype (H2). No intrapopulation variation was observed, and haplotype differences were found in ten nucleotide sites with transition substitution mutations. These results showed limited genetic variation among populations of the long-tailed macaque in Padang, thereby providing valuable insights for stakeholders when formulating genetic conservation policies.

1. Introduction

Macaca fascicularis, commonly known as the long-tailed macaque, crab-eating macaque, or cynomolgus macaque, is an Asian primate species belonging to the family Cercopithecidae (Roos *et al.* 2014 . This primate species comprises ten subspecies, which have few visible morphological differences. A total of seven subspecies from these subspecies, including *M. f. atriceps, M. f. condorensis, M. f. umbrosa, M. f. fusca, M. f. karimondjawae, M. f. tua*, and *M. f. lasiae*, have localized distributions on small islands. Meanwhile, the remaining three subspecies, consisting of *M. f. fascicularis, M. f. philippinensis, M. f. aurea*, have relatively large distributions. *M. f. fascicularis*, the common long-tailed macaque, has a wide geographic range in Southeast Asia, including continental and insular regions (Fooden 1995). The long-tailed macaque is categorized as endangered based on the latest update of The IUCN Red List of Threatened Species. Furthermore, this primate species is listed under Appendix II of the Convention on International Trade in Endangered Species (CITES). Populations have experienced a concerning decline of approximately 40% over the last three generations, spanning roughly 42 years, (Hansen *et al.* 2022).

Padang City, located in West Sumatra, Indonesia, represents the urban area where human activities have led to the fragmentation of macaque habitat. Within this city, three distinct urban long-tailed macaque populations exist in large social groups, primarily residing in Gunung Padang (GPD), Gunung Meru (GMR), and Gunung Pangilun (GPG). These populations inhabit separate hilly urban forests, each with varying levels of fragmentation. For instance, GPG in the city center has become completely isolated. Habitat fragmentation, whether natural

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or anthropogenic, resulting in population isolation, has consequences for these populations' genetic structure, leading to reduced genetic variation (Keyghobadi 2007; Radespiel and Bruford 2014). Populations confined to limited habitat sizes are more vulnerable to issues such as inbreeding depression, genetic drift, and restricted gene flow, all contributing to diminished genetic variation, (Schlaepfer *et al.* 2018).

Mitochondrial DNA (mtDNA) is exclusively maternally inherited and comprised of 16,600 base pairs encoding 37 genes (Gustafsson *et al.* 2016). It offers a valuable tool for genetic analysis. Sequencing the control region or complete genomic sequences of mtDNA is a widely used method for analyzing various biological samples, even those with limited DNA content (Amorim *et al.* 2019). The control region or displacement loop (D-loop) is a noncoding portion of the genome showing significant variation, containing two hypervariable regions (HVI and HVII) (Weedn *et al.* 2018; Linacre 2019). Therefore, sequences from the mtDNA D-loop region provide invaluable data for assessing genetic variation within populations and across the geographical range of the species, (Zhong *et al.* 2013).

Previous reviews regarding population genetics of the long-tailed macaque in Padang have only been carried out on GMR using protein markers (Kawamoto *et al.* 1984; Perwitasari-Farajallah *et al.* 2001). The comprehensive genetic data for both GPD and GPG still needs to be present, indicating the necessity to conduct further investigations using DNA markers, which can subsequently serve as a database for the *M. fascicularis* in western Sumatra. In addition to elucidating the genetic variation of particular populations, genetic analysis can be used in guiding conservation initiatives (Farias *et al.* 2015). This database of DNA sequences may be adopted in diverse domains, including biomedical and biogeographical investigations (Yao *et al.* 2017; Weinbauer and Mecklenburg 2022).

This study aims to analyze the genetic variability of *M. fascicularis* in urban habitat within Padang City, focusing on mtDNA D-loop region sequences. To accomplish this, a total of 70 fecal samples were collected from the three populations in this city. The aim is to assess genetic diversity among GPD, GPG, and GMR and explore the relationships that exist among them. Previous reviews on the long-tailed macaque populations have successfully identified D-loop haplotypes across multiple geographic regions, spanning mainland Southeast Asia, Mauritius, Peninsula Malaysia, most Indonesian archipelago, and even the Philippines. However, the D-loop haplotype data for the Sumatra island population is limited, with available data primarily focusing on North and South Sumatra (Blancher *et al.* 2008; Liedigk *et al.* 2015; Yao *et al.* 2017). Therefore, this study provides crucial preliminary data for the western Sumatra populations. Furthermore, mtDNA D-loop data of the long-tailed macaque in Padang will be compared with available populations in GenBank to elucidate phylogenetic relationships.

2. Materials and Methods

2.1. Sample Collection

The sampling sites consisted of three locations characterized by varying degrees of fragmentation. The GPG population inhabited a fragmented habitat, entirely isolated due to the presence of housing and buildings surrounding it. In contrast, macaques in GPD resided in a semi-fragmented habitat, affording them various opportunities for migration. GMR population occupied an integrated habitat directly connected to the forest (Figure 1). All these three locations were hilly areas at altitudes ranging from 90 to 130 meters above sea level. Fecal samples of the wild *M. fascicularis* were noninvasively collected from December 2020 to February 2021. Ethical approval for this study was obtained from the Ethics Committee of LPPKM IPB University, under reference number 187-2020 IPB.

Before sample collection, group identification was conducted based on studies regarding population size at these three locations (Ilham *et al.* 2016). Group identification incorporated counting the number of macaques and troops in each population, a process performed 20, 15, and 20 times for GMR, GPD, and GPG, respectively. We followed the group of long-tailed macaques, and only fresh samples were selectively collected. 70 fecal specimens were collected during this study, and it was impossible to attribute specific individuals to each sample (as shown in Table 1). Each stool fecal sample was distinguished by its freshness, size, shape, and color to prevent resampling of the same individual. Feces found at intervals less than 1.5 m apart were not collected, following established protocols (Hayaishi and Kawamoto 2006). The swab method was used for DNA extraction to collect epithelial cells from the intestinal wall found on the

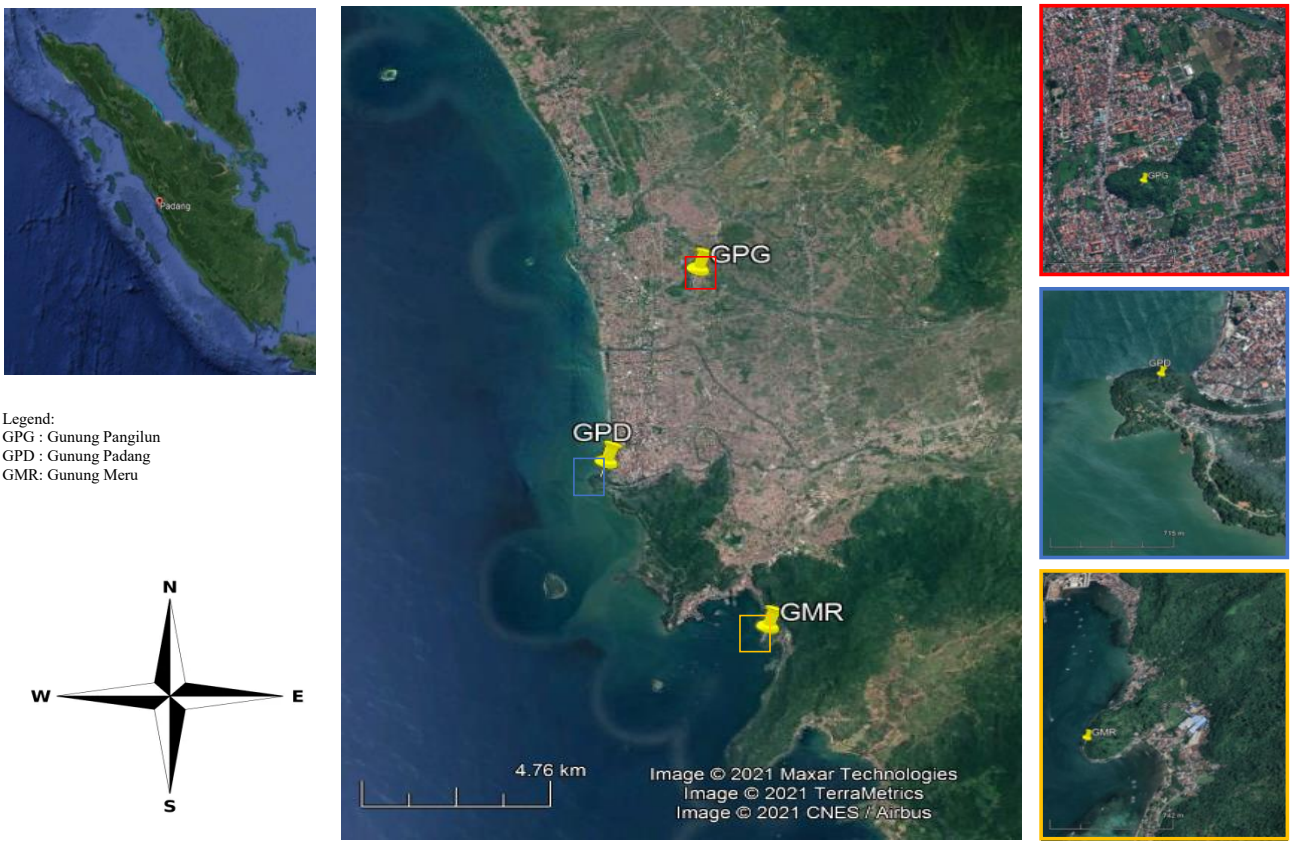

Figure 1. Map of sampling location of *M. fascicularis* in urban area in Padang City, West Sumatra, Indonesia. Gunung Pangilun (0°54'57.42"S and 100°21'59.40"E); Gunung Padang (0°57'53.32"S and 100°20'58.84"E); Gunung Meru (1°0'28.46"S and 100°23'9.91"E). Source: Google Earth, 2021

Table 1. The population size of *Macaca fascicularis* at each of the three sites and the number of fecal samples collected in this study. The three populations consist of GPD = Gunung Padang; GM = Gunung Meru; GPG = Gunung Pangilun

Population	Population size		The number of samples collected in this study
	Ilham *et al.* (2016)	This study	
GMR			
Troop A	36	16	7
Troop B	28	55	9
Troop C	68	59	10
Total	132	130	26
GPD			
Troop X	15	59	20
GPG			
Troop G	10	29	11
Troop P	15	18	7
Troop S	-	17	6
Total	25	64	24

fecal surface. Catton swabs were dipped into tubes containing 2 ml of 96% ethanol. These samples were initially stored in a cool box in the field before being transferred to a -20°C storage environment (Yao *et al.* 2013). Laboratory analysis was conducted at the Primate Research Center Biotechnology Laboratory (PSSP) within LPPM IPB University.

2.2. Laboratory Methods

DNA extraction was performed using the QiaAmp™ DNA Extraction Stool and Blood mini kit (QIAGEN Inc., Hilden, Germany), following the manufacturer's protocol. Subsequently, the extracted DNA was quantified by adopting a NanoDrop™ One (Thermo Scientific) to determine its concentration. A 1,200 bp fragment of mtDNA D-loop was amplified through the polymerase chain reaction (PCR) method. This amplification comprised the use of a primer pair LqqF

(5′-TCCTAGGGCAATCAGAAAGAAAG-3′) (Li and Zhang 2004) and Saru-5R (5′-GGCCAGGACCAAGCCTATTT-3′) (Hayasaka *et al.* 1991). For PCR amplification, 1 µL of each forward and reverse primer [10 pmol/µL], 12.5 µL of MyTaq™ Red Mix Bioline, 5 µL of DNA template, and 5.5 µL of nuclease-free water were mixed. A negative control lacking a DNA template was included to detect contamination in each PCR reaction.

PCR was executed following thermocycling parameters adapted from (Hayaishi and Kawamoto 2006) with various modifications. The cycling conditions began with an initial denaturation step lasting 3 minutes at 94°C, followed by 40 cycles of denaturation for 30 seconds at 94°C, annealing for 30 seconds at 58°C, and extension for 30 seconds at 72°C. The last cycle was completed with a final extension stage for 7 minutes at 72°C. To visualize PCR products, they were electrophoresed on a 1.8 % agarose gel, which was subsequently stained with 10 µL of SYBR Safe (Invitrogen) and immersed in 1% Tris Acetic EDTA (TAE). The DNA fragments within the gel were visualized under ultraviolet light through the UV Gel Doc 2000 (Biorad) and analyzed using Quantity One software. PCR products featuring clear, targeted DNA fragments were then forwarded to 1st BASE Laboratories Sdh Bhd (Malaysia) for sequencing.

2.3. Sequence Analysis

Based on the chromatogram data, the nucleotide sequences were subjected to manual proofreading and editing. Sequence consistency was subsequently performed using ClustalW within MEGA7 (Kumar *et al.* 2016). To validate the obtained DNA sequences, GenBank BLASTn was used to identify similarities with reference sequences. Polymorphic sites and haplotype numbers were determined using DnaSP 6 (Rozas *et al.* 2017). This study also comprised a comparative analysis of the data with D-loop sequences from the long-tailed macaque populations available in GenBank. To calculate the average genetic distance between various populations, the p-distance model in MEGA7 was adopted. The STRUCTURE 2.3.4 program was used to estimate the probability of individual genetic cluster membership (Pritchard *et al.* 2010). Markov Chain Monte Carlo (MCMC) simulations were conducted for 1 million replicates, with a burn-in period 25,000. To identify the optimal cluster model, Delta K probability log plots from Structure analysis data were analyzed through the STRUCTURE HARVESTER (Earl and vonHoldt 2012). A phylogenetic tree was constructed based on the neighbor-joining (NJ) method with a bootstrap value 1000 using MEGA7 (Kumar *et al.* 2016). The haplotype network of *M. fascicularis* was reconstructed using PopART (Leigh and Bryant 2015) based on the median-joining algorithm (Bandelt *et al.* 1999). Comparative analysis of genetic variation was performed by comparing D-loop sequences of *M. fascicularis* in Padang with the available populations in GenBank (Table 2). This comparative analysis solely focused on the common subspecies *Macaca fascicularis fascicularis* (*M. f. fascicularis*), excluding all other subspecies that were allopatric on various small islands.

Table 2. D-loop sequences of *M. fascicularis* and outgroup species obtained from GenBank

Species	GenBank ID	Population
Macaca fascicularis	MF893986.1[a]	Peninsula Malaysia
Macaca fascicularis	KM851018.1[b]	Peninsula Malaysia
Macaca fascicularis	KM851017.1[b]	Peninsula Malaysia
Macaca fascicularis	KM850999.1[b]	North Sumatra
Macaca fascicularis	KM850998.1[b]	North Sumatra
Macaca fascicularis	KM851035.1[b]	North Sumatra
Macaca fascicularis	KM851022.1[b]	North Sumatra
Macaca fascicularis	KM851011.1[b]	North Sumatra
Macaca fascicularis	KM851010.1[b]	North Sumatra
Macaca fascicularis	MF893951.1[a]	South Sumatra
Macaca fascicularis	MF893953.1[a]	South Sumatra
Macaca fascicularis	MF893952.1[a]	South Sumatra
Macaca fascicularis	MF893929.1[a]	Borneo
Macaca fascicularis	MF893928.1[a]	Borneo
Macaca fascicularis	MF893930.1[a]	Borneo
Macaca fascicularis	KM851006.1[b]	Borneo
Macaca fascicularis	KM851007.1[b]	Borneo
Macaca fascicularis	KM851005.1[b]	Borneo
Macaca fascicularis	MF893900.1[a]	Java
Macaca fascicularis	MF893899.1[a]	Java

Table 2. Continued

Species	GenBank ID	Population
Macaca fascicularis	MF893898.1[a]	Java
Macaca fascicularis	MF893897.1[a]	Java
Macaca fascicularis	MF893896.1[a]	Java
Macaca mulatta	NC_005943.1[c]	-
Macaca fuscata	NC_025513.1[d]	-
Macaca nigra	NC_026120.1[e]	-
Macaca cyclopis	NC_027449.1[f]	-

[a](Yao *et al.* 2017); [b](Liedigk *et al.* 2015); [c](Gokey *et al.* 2004); [d](Wang *et al.* 2014); [e](Du *et al.* 2014); [f](Huang *et al.* 2015)

Table 3. Extraction, amplification, and sequencing success. The number of *Macaca fascicularis* fecal samples collected (N) from three populations in urban habitats in Padang City. GPD = Gunung Padang; GM = Gunung Meru; GPG = Gunung Pangilun

Population	N	Successful extraction	Successful amplification	Succesful sequenced	% Sequenced
GMR	26	26	26	15	58
GPD	20	20	20	15	75
GPG	24	24	23	16	67
Total	70	70	69	46	67

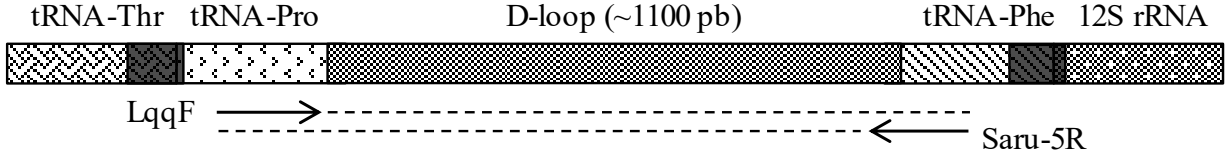

Figure 2. Sketch of the position of the LqqF and Saru-5R primer attachments at the amplification stage

3. Results

The mtDNA D-loop sequences of *M. fascicularis* were deposited in GenBank under accession numbers ON244516-ON244561. This study successfully sequenced the D-loop sequences of 1,100 base pairs for approximately 67% of the obtained genetic samples (Table 3). All samples showed sequence conformity with M. fascicularis available in GenBank (KP272058.1; KP272050.1). When subjecting the sequence to similarity searches using GenBank BLASTn, most sequences had a 95.5% matched identity and 99% query cover. The obtained D-loop sequences ranged from 1,156-1,193 bp, with the fragment of the long-tailed macaque defined as 1,095 bp based on reference sequences in Genbank (NC_012670.1). The collected sequence comprised the D-loop fragment and the flanking gene, specifically tRNA-Pro and tRNA-Phe, which served as primer attachment sites (Figure 2). For data analysis purposes, only the D-loop sequences were used.

Polymorphic analysis of the 1,092 bp D-loop sequence showed the presence of ten parsimony sites (Table 4), yielding two haplotypes. GPD and GMR populations shared the same haplotype (H1), while GPG had a distinct haplotype (H2). Polymorphic sites primarily resulted from substitution transitions, with a dominance of changes between pyrimidine bases (C↔T) over purine bases (A↔C). A significant nucleotide variation was observed within the Hypervariable I (HVI) region of the D-loop, specifically in the first 530 bp.

Concerning intrapopulation diversity analysis, no variation was observed in each population (h = 0). The nucleotide diversity among GPD, GMR, and GPG populations was significantly low (π = 0.004), and the haplotype diversity (Hd) was 0.463. Genetic distance analysis showed no difference between GPD and GMR populations (p-distance = 0; Fst = 0). In contrast, the genetic distance between GPG and the other two populations had a precise value of 0.009. The First value between GPG and the remaining two populations was 1.00, indicating a substantial differentiation between these populations.

Genetic admixture analysis showed the highest peak of Delta K at K = 2 (as shown in Figure 3A). This result indicated that the genetic structure of the long-tailed macaque populations in Padang consistently

supported the formation of two genetic clusters. The GPD and GMR populations expressed genetic identity, while GPG had a clear genetic separation. This showed there was no genetic interchange between the two clusters, as evident from the absence of individual migrants. Furthermore, Bayesian clustering yielded a bar plot pattern indicating the presence of two clusters (Figure 3B).

In a comparative analysis comprising D-loop sequences of *M. fascicularis* from Padang and other populations available in GenBank, partial mtDNA D-loop (515 bp) was used. Genetic distance analysis showed that populations of Padang were relatively closer to those of Borneo, Java, and South Sumatra, with genetic distances ranging from 0.016 to 0.019. Despite its geographic proximity to North Sumatra, populations of Padang tended to have a higher genetic distance from others (Table 5).

The reconstructed neighbor-joining phylogenetic tree (Figure 4) showcased two distinct clades representing the long-tailed macaque populations. Padang, Java, Borneo, and South Sumatra populations formed a monophyletic clade, while North Sumatra and Peninsula Malaysia populations were grouped into separate clades. This grouping was supported by the result of network analysis, corroborated by the pattern observed in the phylogenetic tree (Figure 5). Haplotype network analysis showed 19 haplotypes from six populations of *M. Fascicularis* and was organized into two distinct haplogroups. The Macaque haplotype in Padang City was closely related to populations of Java, South Sumatra, and Borneo. In contrast, North Sumatra and Peninsula Malaysia populations were clustered together within the same haplogroup.

4. Discussion

This study offered preliminary insights into the D-loop sequence of *M. fascicularis* in Padang. Populations in this region showed a significant characteristic of low genetic variation, with only two haplotypes identified among GPD, GMR, and GPG. GPD population in the semi-fragmented area shared the same haplotype as GMR. Despite their separation approximately 7 km apart, both populations inhabited hilly regions that were nearly connected and situated

Table 5. The genetic distance (p-distance) among populations of *Macaca fascicularis* based on the mtDNA D-loop

Population	1	2	3	4	5	6
Padang	-					
Peninsula Malaysia	0.038	-				
North Sumatra	0.043	0.018	-			
South Sumatra	0.019	0.034	0.038	-		
Borneo	0.016	0.040	0.042	0.016	-	
Java	0.017	0.039	0.043	0.021	0.023	-

Table 4. Variant site of mtDNA D-loop in *Macaca fascicularis* determined in this study. The three populations consist of GPD = Gunung Padang; GM = Gunung Meru; GPG = Gunung Pangilun

Population	Haplotype	Nucleotide substitution sites									
		161	163	193	202	213	222	229	282	302	530
GMR	H1		T	G	C	A	A	T	C	T	C
GPD	H1		T	G	C	A	A	T	C	T	C
GPG	H2		C	A	T	G	G	C	T	C	T

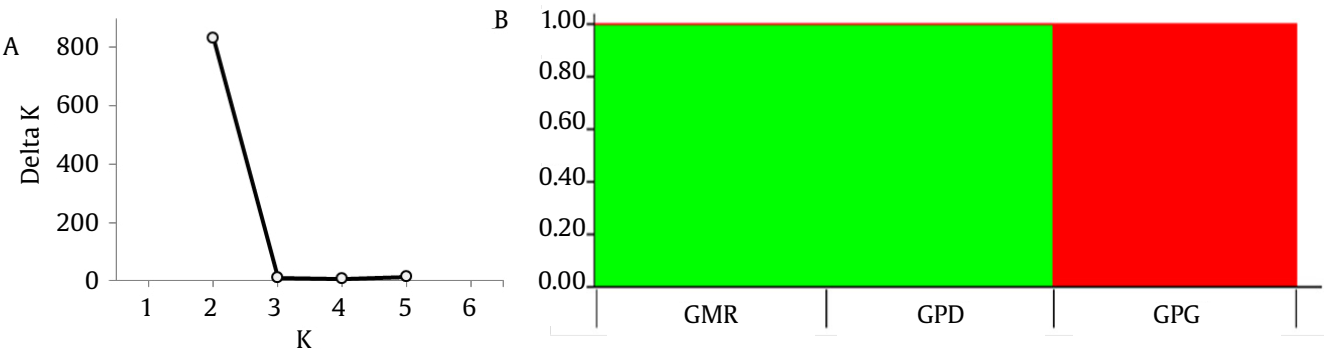

Figure 3. Population structure analysis based on mtDNA D-loop of *Macaca fascicularis* from three populations in Padang. (A) Estimated number of genetic clusters (K) with a range of 1-6, (B) STRUCTURE analysis bar plot configured two clusters

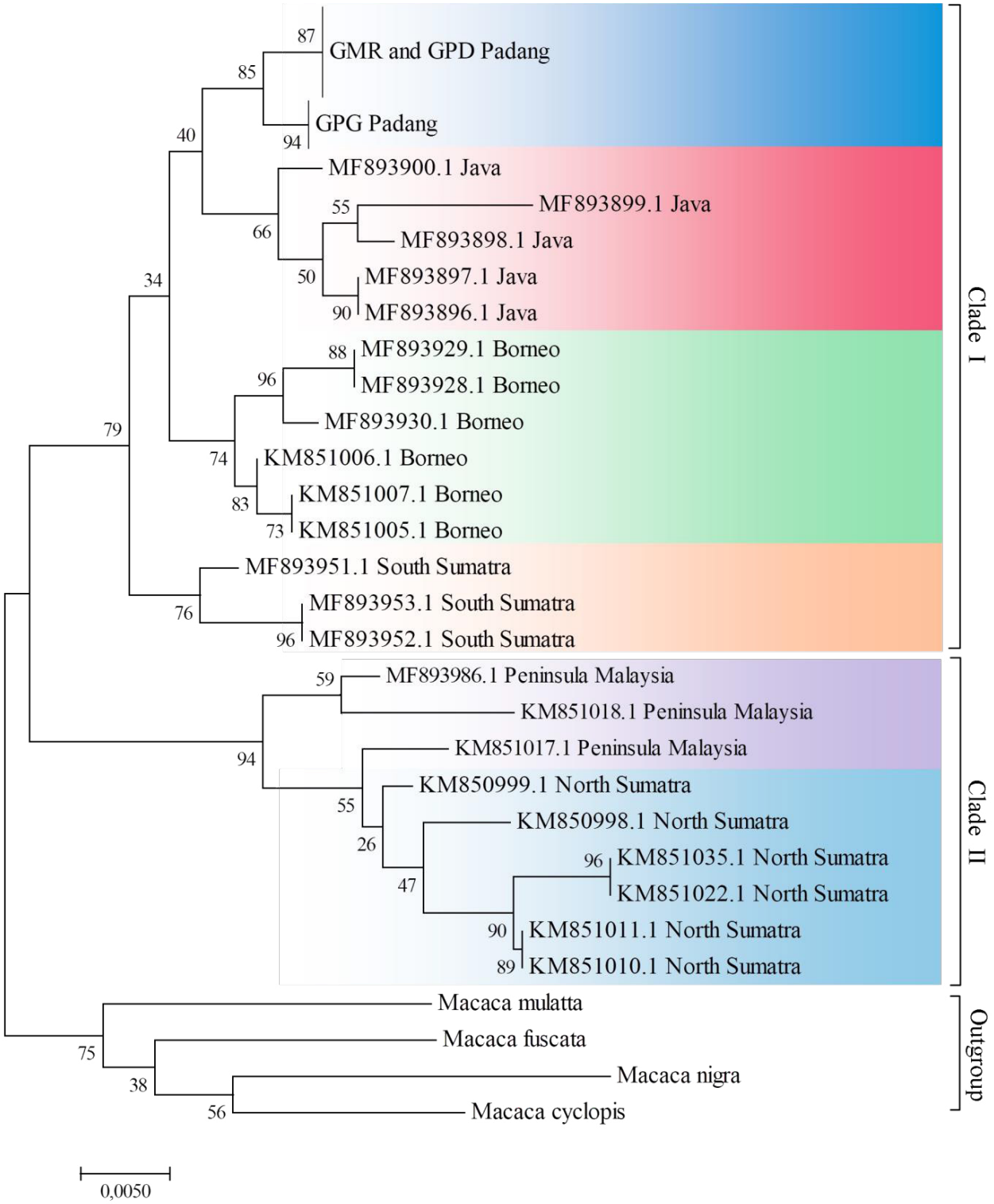

Figure 4. The neighbor-joining phylogenetic tree of *Macaca fascicularis* based on mtDNA D-loop (515 bp) was estimated using the Kimura 2-parameter method. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1,000 replicates) was shown next to the branches. The optimal tree with the sum of branch length = 0.214 was shown

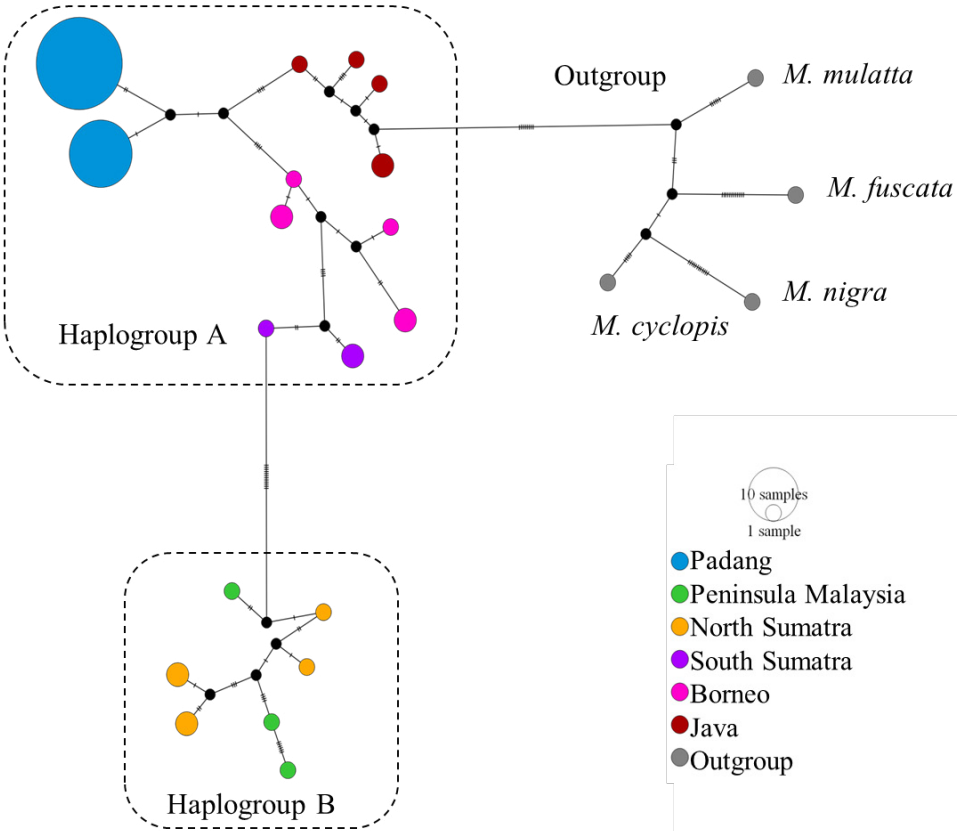

Figure 5. The Median-joining network generated by PopART illustrates the relationships of *Macaca fascicularis* in six populations, including the outgroup species. Each circle represents a haplotype with a different diameter based on the haplotype frequency

within the coastal zone. This high genetic similarity suggested that GPD and GMR originated from a standard ancestral population. It was observed that habitat fragmentation in several locations did not appear to induce genetic changes within these two populations. City development, often accompanied by habitat degradation and fragmentation, could adversely impact the natural habitat of macaques. Fragmentation resulting from the construction of new roadways, which separated GPD and GMR populations over the past few decades, occurred significantly faster than the DNA mutation rate observed in the primate. The substitution mutation rate of mtDNA D-loop sequences in the primate was estimated to be 1.5% per million years (Ho *et al.* 2005).

GMR, located in areas directly connected to the primary forest of the Bukit Barisan mountain range, was expected to represent other populations in the forest. Theoretically, gene flow among populations occurs in unfragmented habitats, leading to genetic variation. The study results showed a low level of gene diversity within GMR. This surprising outcome could be attributed to the abundant availability of human-provided food in the GMR area. Populations had relied on human-provided sustenance, consuming human food three times more frequently than natural food sources within the forest (Ilham *et al.* 2016). Provisioning activities at GMR had become a regular practice among the local community, with specific individuals visiting the location deliberately while others made brief stops.

Food provisioning to the long-tailed macaque groups was observed to have influenced their ranging patterns, as evidenced by studies comparing home ranges among these various groups. For instance, an investigation in Baluran National Park showed that the group with access to food from humans had a home range approximately 23 times smaller (10.62 ha) compared to those without access to human-provided food (249.90 ha) (Hansen *et al.* 2020). The long-tailed macaque inhabiting the natural habitat in the West Bali National Park forest maintained a home range of 32.01 ha. Meanwhile, the Ubud population in urban Ubud population had a smaller

home range of 8.7 ha (Brotcorne 2014). Further study is necessary to explore whether provisioning might affect genetic variation in long-tailed macaques due to the changes in their behavior. In this study, no sampling was conducted on populations residing in the forest untouched by anthropogenic activities for data comparison. No investigation has been conducted regarding the home range of GMR, making it uncertain whether these populations overlapped with others in the forest, thereby facilitating gene flow between subpopulations.

Examining the genetic structure of the long-tailed macaque populations in Padang city through the STRUCTURE 2.3.4 program showed genetic similarities between GPD and GMR, suggesting their shared matrilineal lineage. The distribution of *M. fascicularis*, characterized by philopatry in females and migration in males, played a significant role in shaping the similarity of haplotypes within each population. Female philopatry indicated that mature females remained within their birth group, while adult males often migrated or dispersed from their original group (Van Noordwijk and Van Schaik 1985; De Ruiter and Geffen 1998). Female philopatry in macaques led to an apparent geographic clustering pattern based on maternally inherited mtDNA haplotypes. This was marked by homogeneity within populations and substantial diversity between different populations (Melnick and Hoelzer 1992). Female emigration incorporated splitting social groups within one matrilineal line into smaller groups, with the females often moving to adjacent home ranges (Dittus 1988; De Ruiter and Geffen 1998). This phenomenon supported the discovery of similar D-loop mtDNA haplotypes within GMR and GPD.

The discovery of a specific haplotype within GPG showed the significant differentiation between this population and the other two populations. GPG, situated in the middle of the city, had experienced complete fragmentation, effectively closing off the possibility of natural gene flow with other populations. No factual information was found about when the population started to inhabit the area. Historically, GPG was originally a hilly region densely covered with trees, which served as a bunker during the colonial era. GPG become an essential example of the genetic impact of fragmentation. This relatively small population resided within a confined home range of approximately 26 ha. During sample collection, the presence of three subgroups of *M. fascicularis* in GPG was detected, comprising an estimated total population of 64 individuals. In small populations, the risk of inbreeding depression and reduced genetic diversity was heightened as the effects of gene drift were increased, and there was every likelihood of breeding between relatives (Sarre and Georges 2009).

Habitat fragmentation driven by human activities could have a profound impact on animal populations. In the coastal areas of Madagascar, the lemur (*Eulemur cinereiceps*) experienced a decline in genetic variation and population size, showing the effects of population isolation, anthropogenic disturbances, and exposure to hurricanes (Brenneman *et al.* 2012). Similarly, habitat fragmentation, caused by both natural barriers and human activities, contributed to genetic differentiation and limited gene flow between populations of *Macaca thibetana* (Yao *et al.* 2013). On the other hand, Habitat fragmentation did not uniformly lead to a significant reduction in genetic variation. For instance, chimpanzee populations (*Pan troglodytes schweinfurthii*) residing in the Gishwati Forest Reserve, a forest fragment in western Rwanda, managed to maintain high genetic diversity. These populations served as promising candidates for conservation strategies, including connectivity with larger populations through forest corridors (Chancellor *et al.* 2012).

The observed low genetic variation in the long-tailed macaque populations in Padang, as indicated by nucleotide diversity values ($\pi = 0.004$) and haplotype diversity (Hd) of 0.463, suggesting limited gene flow between populations. This pattern of low genetic variation was also found in the discovery of Alas Purwo and Baluran, East Java. Both populations of long-tailed macaques in those regions had reduced genetic diversity, with nucleotide diversity values (π) ranging from 0.0012 to 0.0025 and haplotype diversity (Hd) between 0.257 and 0.275. Meanwhile, no shared haplotypes were observed between the populations of Alas Purwo and Baluran, indicating a high degree of genetic differentiation between these two populations, (Wandia *et al.* 2015). A similar trend of low genetic diversity was evident in *Macaca thibetana huangshanensis* within the Huangshan Mountain region, characterized by low haplotype and nucleotide diversity ($Hd = 0.5586$ and $\pi = 0.0067$). In this population, analysis of 30 samples from 7 regions showed the presence of only three haplotypes, suggesting signs of inbreeding and genetic drift, contributing to the observed low genetic diversity (Li *et al.* 2008).

The comparative analysis of *M. fascicularis* in Padang with the others, as evident in the phylogenetic tree, indicated a separation among populations within Sumatra. The populations of South Sumatra and Padang, located in West Sumatra, formed in the same clade, separate from those of North Sumatra. Previous reviews on the intraspecific phylogeny of *M. fascicularis* included populations distributed across mainland Southeast Asia, the Sunda Islands, the Philippines, and Timor. This phylogenetic reconstruction consistently showed the divergence of two major clades separating the continental and insular populations (Tosi and Coke 2007; Liedigk *et al.* 2015; Yao *et al.* 2017). However, it was crucial to observe that previous investigations did not include populations in the western region of Sumatra. The D-loop mtDNA data obtained represented the initial genetic information for the Padang populations. Moreover, mtDNA analysis showed that *M. fascicularis* in southern Sumatra clustered alongside the other insular populations in Java, Kalimantan, and the Philippines (Tosi and Coke 2007). A unique grouping was expressed within populations of North Sumatra, forming a clade with the mainland (Liedigk *et al.* 2015) and the divergence time between the mainland-Sumatran and insular clades, estimated using fossil calibration with the molecular clock method, ranged from 1.2 to 2.2 Mya (Tosi and Coke 2007; Liedigk *et al.* 2015; Yao *et al.* 2017).

The phylogenetic analysis reinforced the existence of separation on Sumatra island and shed light on the distinctive structure within the North Sumatra region. This phenomenon of population division extended to other primate species, having a shared phylogeographic pattern. For instance, the Sumatran orangutans (*Pongo abelii*) were presently confined to the northern region of Sumatra, particularly around Lake Toba. The Tapanuli orangutans (*Pongo tapanuliensis*) were exclusively distributed in Batang Toru, marking the southernmost distribution limit of the extant orangutans south of Lake Toba (Nater *et al.* 2017). This geographical boundary at Lake Toba had similarly contributed to divergence patterns observed in other taxa. *Hylobates agilis* spanned from the southern region of Lake Toba to the southern tip of Sumatra, while *Hylobates lar* was restricted to the northern territories (Whittaker *et al.* 2007).

The separation of *M. fascicularis* populations in Sumatra was substantiated by distinct phylogenetic clusters observed in both mitochondrial and nuclear DNA data (Yao *et al.* 2017; Yao *et al.* 2020). This clear separation of the long-tailed macaque in the region was linked to the Toba supereruption event (Yao *et al.* 2017), which transpired approximately 73kya and comprised four major eruptions within the last 1.2 million years (Chesner *et al.* 1991). Although the lineage divergence between northern and southern Sumatran populations preceded this cataclysm (~1.88 Mya), the Toba eruption was observed to influence the survival and genetic composition of the long-tailed macaque (Yao *et al.* 2017). Another hypothesis showed the extinction of this primate species in Sumatra, followed by subsequent recolonization. The northern region of Sumatra might have been recolonized from the mainland, while recolonization in the southern part could have originated from Borneo and Bangka (Liedigk *et al.* 2015; Yao *et al.* 2017).

In conclusion, this study showed the efficacy of a practical genetic survey approach using a noninvasive sampling method in urban long-tailed macaque populations in Padang. The analysis of mtDNA D-loop sequences suggested that GMR, GPD, and GPG had a limited genetic variation. Moreover, mtDNA D-loop sequences contributed crucial genetic insights into the Padang populations, representing the first genetic record from the western region of Sumatra. The observed low genetic variation of *M. fascicularis* in the region presented a pressing challenge for conserving this endangered species. These results indicated the importance of developing a conservation strategy that focused on enhancing the connectivity between fragmented urban habitats. Achieving this connectivity necessitated the creation and safeguarding of wildlife corridors. While habitat fragmentation remained a challenging issue to prevent, the prospect of reintroduction to restore genetic diversity warranted careful consideration. Human provisioning of the wild primate species significantly influenced the natural behavior, and its potential impact on genetic variation warranted further investigation. Practical conservation efforts would require close collaboration with stakeholders to enforce regulations regarding the interaction with and feeding of wild animals.

Acknowledgements

We acknowledge the Ministry of Finance of the Republic of Indonesia for the research funding of the LPDP Scholarship program. We thank The Natural Resources Conservation Agency (BKSDA) of West Sumatra and The Primate Research Center (PSSP) IPB

for logistical permits. We thank the people of Gunung Padang, Gunung Meru, and Gunung Pangilun for their assistance and cooperation during sample collecting. Thanks to the Biotechnology Laboratory of the Primate Research Center (PSSP) staff for laboratory assistance. Gratitude to Puji Rianti and Erin Vogel for advice in writing this manuscript.

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