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Cover: A gravid praying mantis just before she laid her ootheca—digital art on procreate. © Aakanksha Komanduri.



## Mitochondrial CO1 gene haplotype diversity of Sumatran Tiger *Panthera tigris sumatrae* (Pocock, 1929) (Mammalia: Carnivora: Felidae)

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**Abstract:** Sumatran Tigers *Panthera tigris sumatrae* inhabit 12 tiger conservation landscapes that stretch across Sumatra Island. Conservation efforts for these species require robust, information-based research, including a genetic approach. This study analyzed the haplotype diversity of *P. t. sumatrae* based on the mitochondrial CO1 (Cytochrome Oxidase Subunit 1) gene. Specifically, a nucleotide guanine at position 121 was found, distinguishing *P. t. sumatrae* from other tiger subspecies. Among the 17 sequences of *P. t. sumatrae*, two haplotypes were detected: 13 individuals were in haplotype 1 (Hap\_1), and four individuals were in haplotype 2 (Hap\_2). Hap\_1 individuals predominantly originated from Riau and North Sumatra, while Hap\_2 individuals were primarily from West Sumatra. Haplotype diversity (Hd) (0.382±0.113) and nucleotide diversity (pi) (0.00038±0.00011) confirmed the low genetic diversity. Five seized samples exhibited Hap\_2, suggesting they might have originated from Riau and North Sumatra. However, this result cannot be described as current due to the significant changes in *P. t. sumatrae* habitat. Further genetic studies, such as whole-genome analysis, are needed to detect the origin and variation of *P. t. sumatrae* across all landscapes.

**Keywords:** Forest lost, genetic diversity, Illegal trade, mtDNA, PCR, population interactions, species identification, Sumatran forest, wildlife genetic, wildlife forensic.

**Bahasa:** Harimau Sumatera *Panthera tigris sumatrae* menempati 12 area lanskap *Panthera tigris* yang berada di sepanjang Pulau Sumatera. Usaha konservasi spesies ini telah banyak dilakukan dengan melakukan berbagai macam riset, termasuk dengan pendekatan genetika. Penelitian ini dilakukan untuk menganalisis diversitas haplotipe dari *P. t. sumatrae* berdasarkan gen Cytochrome Oxidase Sub Unit 1 (CO1) DNA mitokondria (mtDNA). Dua haplotip ditemukan dari total 17 sekuen sampel *P. t. sumatrae* dengan komposisi 13 individu memiliki haplotip 1 (Hap\_1) dan empat individu memiliki haplotip 2 (Hap\_2). Haplotip 1 (H\_1) cenderung terdapat pada individu-individu dari Provinsi Riau dan Provinsi Sumatera Utara. Haplotip 2 (H\_2) cenderung terdapat pada individu-individu dari Provinsi Sumatera Barat. Nilai diversitas haplotipe (0.382) dan diversitas nukleotida (pi) (0.00038) menunjukkan rendahnya variasi genetik dari semua individu yang dianalisis. Lima sampel yang berasal dari sitaan kasus perdagangan ilegal memiliki haplotip 2 yang berarti dapat diasumsikan cenderung berasal dari Riau and Sumatera Utara. Hasil ini tentu belum dapat mendeskripsikan asal sampel *P. t. sumatrae* secara akurat dikarenakan keterbatasan sampel dan habitat *P. t. sumatrae* yang luas. Selain itu perubahan fungsi habitat yang berubah secara signifikan mengharuskan perlunya dilakukan analisis DNA lengkap *P. t. sumatrae* dari individu-individu pada populasi di semua lanskap yang tersisa.

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## INTRODUCTION

The extinction of the Bali Tiger *Panthera tigris balica* and the Javan Tiger *Panthera tigris sondaica* led to the *Panthera tigris sumatrae* being the only tiger subspecies remaining in Indonesia (Seidensticker et al. 1999). The Convention on International Trade in Endangered Species of Wild Flora and Fauna (CITES) categorizes *P. t. sumatrae* in Appendix I, which means it is prohibited from being traded (Soehartono et al. 2007). *P. t. sumatrae* naturally inhabits the Sumatran forest ecosystem, exhibiting high adaptability supported by prey availability and access to water sources (Nowell & Jackson 1996; Seidensticker et al. 1999). Additionally, factors such as vegetation density and human activity play crucial roles in determining the existence of *P. t. sumatrae* (Sanderson et al. 2006; Linkie et al. 2008; Suyadi et al. 2012).

There are 76 tiger conservation landscapes (TCLs) around the world, 12 of them being home to *P. t. sumatrae* is located on the island of Sumatra, covering approximately 88,000 km<sup>2</sup>. These TCLs encompass various areas, including Bukit Barisan Selatan, Tesso Nilo, Kerinci Seblat, Bukit Balai Rejang-Selatan, Rimbo Panti-Batang Barat, Leuser, Berbak, Sibolga Kuala Kerumutan, Bukit Rimbang Baling, Rimbo Panti-Batang Timur, and Bukit Tigapuluh (Sanderson et al. 2006). Within these 12 landscapes of *P. t. sumatrae*, there are 18 conservation areas as well as other forested regions, including protected forests and production forests (Soehartono et al. 2007). Human activities have been a significant cause of forest loss, posing a severe threat to the sustainability of *P. t. sumatrae* (Seidensticker et al. 1999; Linkie et al. 2003; Wibisono & Pusparini 2010). Between 2000 and 2010, Sumatra saw a 3% loss in its forests (Wilcove et al. 2013), and deforested areas exhibited a 20% lower occupancy rate for *P. t. sumatrae* compared to areas that remained forested (Wibisono et al. 2011). Empirical evidence has demonstrated that habitat fragmentation, habitat loss, and isolation among populations can lead to changes in genetic composition among living species (Keyghobadi 2007). Consequently, genetic studies of *P. t. sumatrae* have become increasingly important and are a key focus of research in the 21<sup>st</sup> century.

Genetic diversity within a population plays a critical role in determining a species' ability to survive and avoid extinction. Low or diminished genetic diversity can significantly reduce a population's capacity to adapt to environmental changes and succeed in reproduction (Frankham et al. 2010). Several parameters are used to assess genetic diversity, including genetic distance, haplotype diversity, and nucleotide diversity. A haplotype

refers to a group of genes in organisms inherited from the same parent. It is defined as the inheritance of a cluster of single nucleotide polymorphisms (SNPs), which are variations in a single base within DNA sequences among individuals, particularly within the CO1 gene (Frankham et al. 2010; Liang 2013). The CO1 gene is a protein-coding gene located in mitochondrial DNA (mtDNA) and does not undergo recombination because it is maternally inherited (Ladoukakis & Zouros 2017). Consequently, individuals or closely related species will exhibit a high degree of genetic similarity (Folmer et al. 1994).

Recent genetic studies within the felid family have utilized microsatellite loci, as reported by Williamson et al. (2002). They identified an ideal set of 16 microsatellite loci for population genetic analysis. Another study successfully unveiled the phylogenetic and evolutionary relationships among the six tiger subspecies worldwide. This investigation employed three genetic markers, including mitochondrial DNA spanning approximately 6,000 bp, the class II gene DRB, and microsatellites. While these markers showed low variation between subspecies, they exhibited significant distinctions in partitioning among subspecies (Luo et al. 2004). The *P. t. sumatrae*, mitochondrial DNA study was developed with discovered 7891 bp or 46.4% (Kitpipit, Linacre, and Tobe 2009). Previously, Faizah (2008) conducted a study on the mitochondrial DNA genetic markers (Cytochrome b and D-loop) of *P. t. sumatrae*. The study involved designing primers based on the mitochondrial DNA of *Felis catus*. Additionally, Kitpipit et al. (2012) reported the identification of five single nucleotide polymorphisms (SNPs) specific to *Panthera tigris*, three specific SNPs in *P. t. sumatrae*, and three specific SNPs in *P. t. tigris*, based on an approximately 15,000 bp mitochondrial DNA sequence.

The utilization of genetic markers for *P. t. sumatrae* has been extended to various applications, including the reconstruction of *P. t. sumatrae* pedigrees by targeting the D-loop region, species identification, and phylogenetic analyses through the CO1 gene (Setianingsih 2013; Ashrifurrahman et al. 2022). Additionally, Smith et al. (2018) analyzed the impact of habitat loss and fragmentation on the genetic variation of *P. t. sumatrae* using microsatellite markers. Their findings indicated that Sumatran forest deforestation did not have a significant effect on the genetic variation of *P. t. sumatrae*, mainly due to the maintenance of heterozygosity. However, it is crucial to address the deforestation rate promptly to mitigate future risks. In this study, we investigated haplotype diversity and predicted the origin of tiger body part samples traded



from three provinces on Sumatra Island. We utilized blood and hair samples from *P. t. sumatrae* with known origins. This study provides valuable information about the geographical origin of the CO1 haplotype, previously reported by Luo et al. (2004) and Xue et al. (2015), which lacked data on the sample origin.

## MATERIALS AND METHODS

Five seized samples (PTS 1, PTS 3, PTS 5, PTS 6, and PTS 8) of *P. t. sumatrae* from illegal trading were collected from the West Sumatra Natural Resources

Conservation Agency (BKSDA). These samples consisted of various body parts, including hairs and bones, from *P. t. sumatrae* that had been confiscated from illegal traffickers arrested by authorities on Sumatra Island. In addition, we obtained nine blood samples (PTS 9, PTS 10, PTS 11, PTS 12, PTS 14, PTS 15, PTS 16, PTS 19, and PTS 20) and one hair sample (PTS 4) from the Dharmasraya Sumatran Tiger Rehabilitation Center (PR-HSD), as shown in Figure 1. The blood samples were collected from *P. t. sumatrae* individuals that had been evacuated from conflicts with humans in recent years in three provinces (West Sumatra, East Sumatra/Riau, North Sumatra), except for PTS 11, whose origin was unknown. Then,

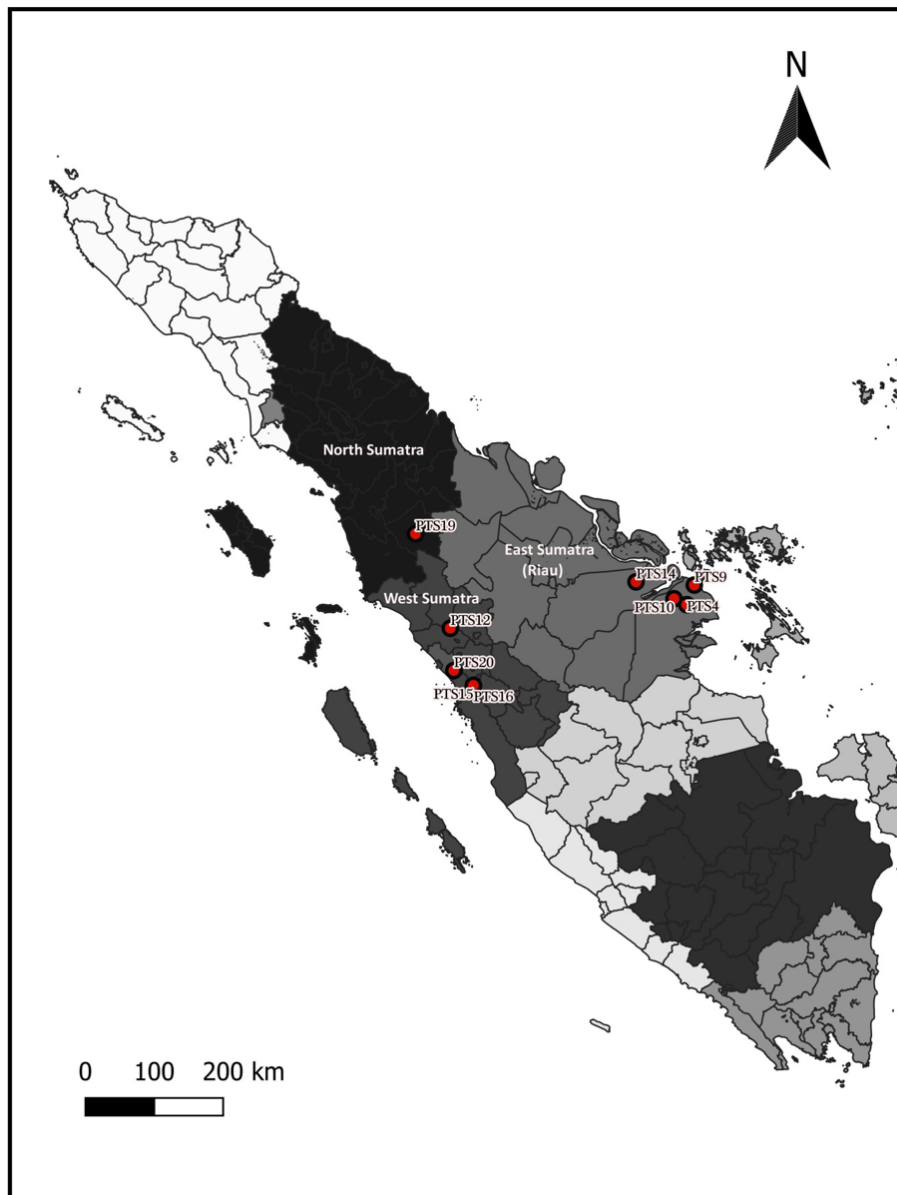


Figure 1. Geographic locations of *Panthera tigris sumatrae* samples used for molecular genetic analyses.

the tigers will be rehabilitated to be released back into their habitat. All collected samples were placed into 1.5 ml microtubes, appropriately labeled, photographed, and stored at room temperature. For validation and comparison with previous studies, we utilized sequence data (mtDNA) of *P. t. sumatrae* as assessed by (Kitpipit et al. 2012).

Laboratory methods start with DNA isolation for each sample using GeneAll Exgene Genomic DNA micro. Each type of sample used different protocols according to the kit guide. Then, pairs of primers used to amplify CO1 gene segments were performed using forward primers PTSF 5 'AGTTACTGCCCATGCCTTTG 3' and reverse primers PTRS 5 'CAGGCCTAGGAAATGCTGAG 3' (Ashrifurrahman et al. 2022). The primers would amplify 1129 bp of the Sumatran tiger CO1 gene sequences. Finally, PCR reactions were performed using 25 µl reaction volume containing 11 µl supermix of bioline, 9 µl nuclease freewater, 1 µl forward primer, 1 µl reverse primer, 3 µl DNA isolate. PCR machine was set to start from predenaturation at 96°C for 1 minute to ensure complete denaturation, then denaturation was carried out at 96°C for 30 seconds, annealing at 50°C for 30 seconds, and elongation at 72°C for 1.5 minutes. The last cycle at 72°C for 3 minutes, this PCR process runs for 40 cycles.

The amplification product was sent to Firstbase Company in Malaysia to be purified and sequencing reaction. The sequencing process used Applied Biosystems highest capacity-based genetic analyzer platforms and used the BigDye® Terminator v3.1 cycle sequencing kit chemistry. The forward and reverse DNA sequences were then combined using the DNA STAR (Burland 2000). The *P. t. sumatrae* sequences were then aligned using the Clustal X version 1.8. Polymorphism sequence analysis was carried out using DNA sequence polymorphism 5.10. To analyze the changes in the nucleotide base (haplotype), calculating the haplotype diversity and nucleotide diversity (Rozas 2009). MEGA (Molecular Evolutionary Genetics Analysis) version 7 was used for nucleotide base differences analysis (Kumar et al. 2016). The AMOVA (Analysis of Molecular Variance) and FST (Population-based gene flow measures) analysis was calculated with Arlequin 3.5.2.2 (Excoffier et al. 2010).

Furthermore, the various haplotypes identified in the genetic variation analysis were visually represented on a map using QGIS 3.6. The known coordinates of *P. t. sumatrae* were inputted into the QGIS software. Each *P. t. sumatrae* specimen was labeled based on the specific haplotype type that had been determined in the

analysis of haplotype diversity. This mapping approach provided a clear visual representation of the distribution of haplotypes among *P. t. sumatrae* populations.

## RESULTS

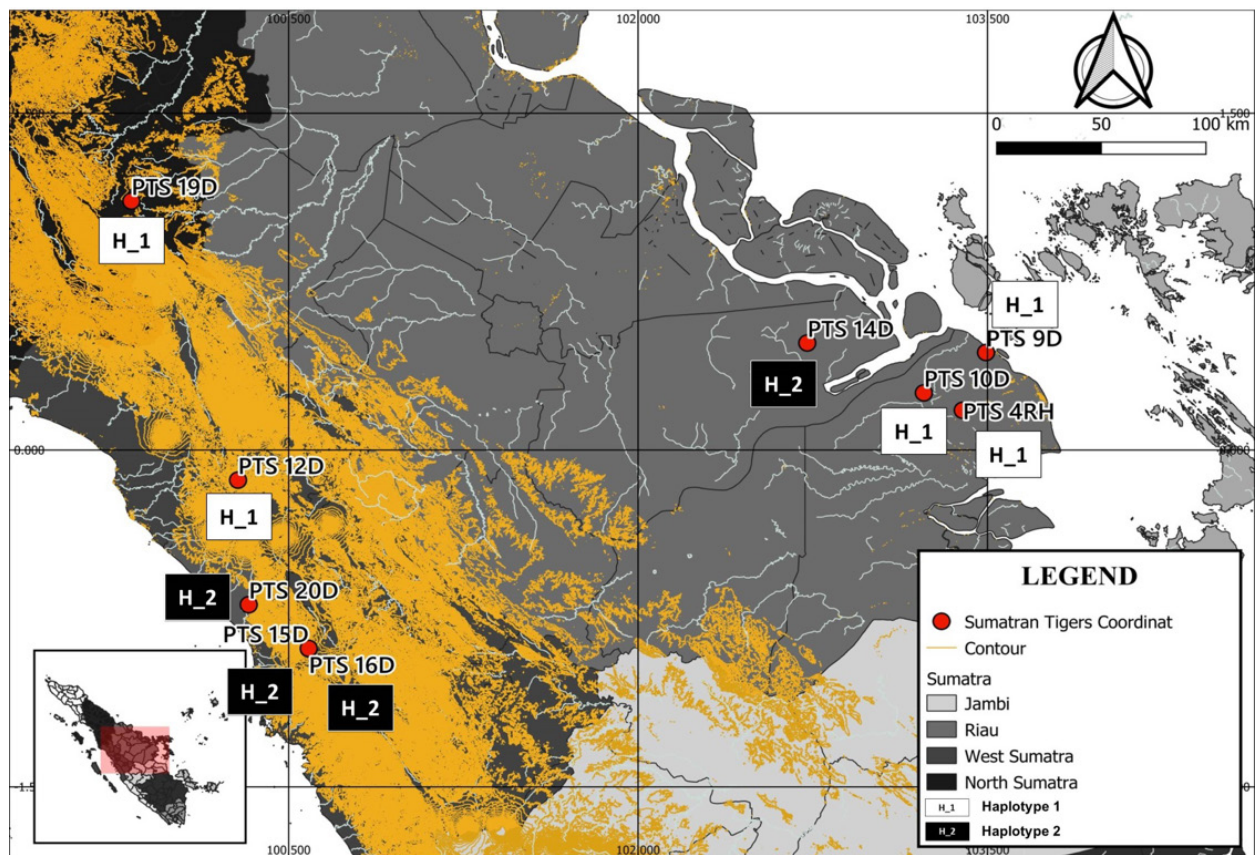
In total, 17 samples of *P. t. sumatrae* were sequenced for a 999 bp segment of the mtDNA CO1 gene. The analysis revealed the presence of two distinct mtDNA haplotypes: haplotype 1 (Hap\_1), consisting of 13 individuals, and haplotype 2 (Hap\_2), which was found in four individuals. Hap\_1 included PTS 4, PTS 9, PTS 10, PTS 11, PTS 12, PTS 19, PTS 1, PTS 3, PTS 5, PTS 6, and PTS 8, while Hap\_2 was identified in PTS 14, PTS 15, PTS 16, and PTS 20. The accuracy of these haplotypes was verified through a thorough examination of the electropherograms obtained during the sequencing process. The analysis was conducted using the MEGA 7.0 program, and DNA-to-protein translation was applied for amino acid translation. It's worth noting that the sequence variability observed in other research studies corroborates the mutations found at these specific sites. Additionally, to ensure the accuracy, several amino acid sites were carefully examined and corrected, with the best frame selected from multiple frames generated by the DNA-to-Protein Translation program. Confirmation was also obtained from NCBI data with accession number AEJ88608.1. Lastly, as part of the DNA-to-amino acid translation process, the initial two nucleotide bases (TT) and the final nucleotide base (A) were removed for consistency and accuracy.

The amino acids were obtained from the translation of the 996 nucleotide bases of *P. tigris* sequences (332 amino acids) with eight various sites (Table 2). Notably, all variations observed in the amino acids were synonymous mutations and transitional mutations. The substitutions detected in the nucleotide base sequences analyzed served to differentiate between tiger subspecies. Specifically, based on subspecies-specific nucleotides, *P. t. amoyensis* is characterized by guanine at position 17, adenine at 121 and 302, and thymine at 422. On the other hand, *P. t. altaica* and *P. t. corbetti* share the same specific nucleotides: adenine at 121, cytosine at 825, and thymine at 920. *P. t. corbetti* does not have a specific site for this study. Of particular significance is the discovery of a specific nucleotide, guanine at position 121, which serves as a distinctive marker distinguishing *P. t. sumatrae* from other tiger subspecies (Table 3).

The genetic variation in the *P. t. sumatrae* population

**Table 1.** Haplotype variation, haplotype diversity value (Hd) and nucleotide diversity (Pi) in the *Panthera tigris sumatrae* sequences.

	Haplotypes	Samples	Origin	Haplotype diversity (Hd)	Nucleotide diversity (Pi)
1	Hap_1	PTS_1	Unknown	0.382±0,113	0.00038±0,00011
2		PTS_3	Unknown		
3		PTS_4	Riau		
4		PTS_5	Unknown		
5		PTS_6	Unknown		
6		PTS_8	Unknown		
7		PTS_9	Riau		
8		PTS_10	Riau		
9		PTS_11	Unknown		
10		PTS_12	West Sumatra		
11		PTS_19	North Sumatra		
12		JF357969_1_Pt.sumatrae	Unknown		
13		JF357970_1_Pt.sumatrae	Unknown		
14	Hap_2	PTS_14	Riau	0.382±0,113	0.00038±0,00011
15		PTS_15	West Sumatra		
16		PTS_16	West Sumatra		
17		PTS_20	West Sumatra		



**Figure 2.** Haplotypes distribution of *Panthera tigris sumatrae*.

**Table 2.** Variations in the amino acid of *Panthera tigris* cytochrome oxidase subunit 1 gene.

	Species	G (Glycine)	G (Glycine)	L (Leucine)	A (Alanine)	L (Leucine)	L (Leucine)	I (Isoleucine)
		GGA	GGG	CTG	GCC	TTA	TTG	ATC
		15/5	120/40	300/100	420/140	823/275	840/280	918/306
1	PTS 1	-	-	-	-	-	-	-
2	PTS 3	-	-	-	-	-	-	-
3	PTS 4	-	-	-	-	-	-	-
4	PTS 5	-	-	-	-	-	-	-
5	PTS 6	-	-	-	-	-	-	-
6	PTS 8	-	-	-	-	-	-	-
7	PTS 9	-	-	-	-	-	-	-
8	PTS 10	-	-	-	-	-	-	-
9	PTS 11	-	-	-	-	-	-	-
10	PTS 12	-	-	-	-	-	-	-
11	PTS 14	-	-	-	-	-	TTA	-
12	PTS 15	-	-	-	-	-	TTA	-
13	PTS 16	-	-	-	-	-	TTA	-
14	PTS 19	-	-	-	-	-	-	-
15	PTS 20	-	-	-	-	-	TTA	-
16	<i>P. t. amoyensis_HM589215 1</i>	GGG	GGA	CTA	GCT	-	-	-
17	<i>P. t. sumatrae_JF357969 1</i>	-	-	-	-	-	-	-
18	<i>P. t. sumatrae_JF357970 1</i>	-	-	-	-	-	-	-
19	<i>P. t. corbetti_JF357972 1</i>	-	GGA	-	-	CTA	-	ATT
20	<i>P. t. altaica_KF297576 1</i>	-	GGA	-	-	CTA	-	ATT
21	<i>P. t. altaica_MH124080 1</i>	-	GGA	-	-	CTA	-	-
22	<i>P. t. altaica_MH124110</i>	-	GGA	-	-	CTA	-	ATT
23	<i>P. t. altaica_MN624080 1</i>	-	GGA	-	-	CTA	-	ATT

was characterized by two distinct haplotypes: Haplotype 1 (H\_1) and Haplotype 2 (H\_2), each distinguished by a single nucleotide site. Haplotype 1 (H\_1) is characterized by the guanine nucleotide base at position 842, while Haplotype 2 (H\_2) features an adenine base at the same position (Table 3). This result aligns with findings by Luo et al. (2004), who reported one nucleotide base variation in eight *P. t. sumatrae* samples based on a 409 bp segment of the CO1 gene sequence. Five of these individuals had guanine at position 7382 bp, while the remaining three had adenine bases. Furthermore, Xue et al. (2015) examined five *P. t. sumatrae* museum samples using the same primers as Luo et al. (2004), with four samples having guanine bases and one sample featuring adenine bases in the same order as previously reported. Despite the reporting of haplotypes, the geographical origin of these haplotype samples within *P. t. sumatrae* has not been previously documented. A map illustrating the distribution of these haplotypes is presented in

Figure 2.

Haplotype diversity throughout the population of *P. t. sumatrae* in this study was low at  $0.382 \pm 0,113$  (Table 1). Haplotype diversity values range from 0 to 1, with values exceeding 0.5 indicating high haplotype diversity, while values below 0.5 suggest low diversity (Curry et al. 2015). The nucleotide diversity ( $\pi$ ) value of the 17 sequences was  $0.00038 \pm 0,00011$ . From several previous reports, other tiger subspecies also have low mtDNA nucleotide diversity, including *P. t. tigris*  $0.000355 \pm 0.000256$ , *P. t. jacksoni*  $0.00118 \pm 0.000670$ , respectively.

**DISCUSSION**

The low levels of haplotype ( $0.382 \pm 0,113$ ) and nucleotide diversity ( $0.00038 \pm 0,00011$ ) were found in *P. t. sumatrae* from this research. Recent reports on Felidae mtDNA diversity show comparable values. For example,



Table 3. Specific nucleotide bases in *Panthera tigris* sequences.

No	Sample	Site							
		Luo et al. 2004	6479	6583	6764	7130	7287	7304	7382
		mtDNA (NC_010642.1)	6543	6647	6828	7194	7351	7368	7446
		CO1 (NC_010642.1)	263	367	548	668	1071	1088	1166
		CO1 in this study	17	121	302	422	825	842	920
1	PTS 1		A	G	G	C	T	G	C
2	PTS 3		.	.	.	.	.	.	.
3	PTS 4		.	.	.	.	.	.	.
4	PTS 5		.	.	.	.	.	.	.
5	PTS 6		.	.	.	.	.	.	.
6	PTS 8		.	.	.	.	.	.	.
7	PTS 9		.	.	.	.	.	.	.
8	PTS 10		.	.	.	.	.	.	.
9	PTS 11		.	.	.	.	.	.	.
10	PTS 12		.	.	.	.	.	.	.
11	PTS 14		.	.	.	.	.	A	.
12	PTS 15		.	.	.	.	.	A	.
13	PTS 16		.	.	.	.	.	A	.
14	PTS 19		.	.	.	.	.	.	.
15	PTS 20		.	.	.	.	.	A	.
16	JF357969 1 <i>P t sumatrae</i>		.	.	.	.	.	.	.
17	JF357970 1 <i>P t sumatrae</i>		.	.	.	.	.	.	.
18	JF357972 1 <i>P t corbetti</i>		.	A	.	.	C	.	T
19	MH124110 1 <i>P t altaica</i>		.	A	.	.	C	.	T
20	MN624080 1 <i>P t altaica</i>		.	A	.	.	C	.	T
21	KF297576 1 <i>P t altaica</i>		.	A	.	.	C	.	T
22	MH124080 1 <i>P t altaica</i>		.	A	.	.	C	.	.
23	HM589215 1 <i>P t amoyensis</i>		G	A	A	T	.	.	.

*Panthera pardus saxicolor* exhibited comparably low diversity levels in haplotypes (0.247) and nucleotides (0.00078) (Farhadinia et al. 2020), *Puma concolor* mtDNA diversity (0,006) (Caragiulo et al. 2013), In the case of *Panthera tigris* in the Sundarbans, haplotype diversity was 0.50, and nucleotide diversity was 0.00266 (Aziz et al. 2022). Previous research by Luo et al. (2004), involving the analysis of several gene sequences in mtDNA (4078 bp), consistently reported low nucleotide diversity values (0.00717±0.00444). The levels of genetic variation, whether high or low, as determined by the CO1 gene play a significant role in determining the genetic relatedness between populations and taxa. Lower genetic variation indicates a closer relationship among individuals or populations of living organisms, especially in the case of tigers. This condition has implications for the geographic isolation of tigers, suggesting that they

were separated approximately 100,000 years ago (Luo et al. 2004; Xue et al. 2015).

A total 17 individuals were analyzed there were nine individuals have known wild origin. The nine individuals are spread across three provinces on the island of Sumatra. There are four individuals from West Sumatra Province (PTS 12, PTS 15, PTS 16, PTS 20), four individuals from Riau Province (PTS 14, PTS 10, PTS 4, PTS 9), and one individual from North Sumatra Province (PTS 19). These individuals can serve as representatives to determine the distribution of haplotypes forensic samples that unknown origin (PTS 1, PTS 3, PTS 5, PTS 6, PTS 8) and GeneBank data sequences (*P. t. sumatrae*\_JF357969.1 and *P. t. sumatrae*\_JF357970). All *P. t. sumatrae* forensic samples showed that conceivable from Riau and North Sumatra.

Haplotype 2 (Hap\_2) was found in all seized samples

of poaching and illegal trade in *P. t. sumatrae*, suspected from Riau and North Sumatra provinces. However, this assumption is not entirely accurate because Hap\_2 also exists in individuals from West Sumatra. This suggests the possibility that confiscated tiger samples could originate from other populations on the island of Sumatra.

For the current number of samples, there was a propensity for all districts to have the same haplotype variation, particularly Riau and West Sumatra. The haplotype distribution indicates the sharing of haplotypes by individuals from West Sumatra and Riau Provinces. Furthermore, the sharing of haplotype 1 (H\_1) from Riau and North Sumatra Provinces come to pass. The distribution of haplotypes shown in Figure 2 is not significant indicating the specific haplotypes from each province.

Haplotype distribution in this study indicates that no specific grouping formed between these three provinces. The AMOVA and FST (Population-based gene flow measures) analysis was calculated with Arlequin 3.5.2.2 (Excoffier et al. 2010). FST was not calculated between areas within the North Sumatra population due to contributing only one sample that would have skewed the result. AMOVA analysis, run with each of the main areas within Riau group and West Sumatera group, resulted in an FST of 0.2. In line with Smith et al. (2015) reported on microsatellite analysis of 37 samples of *P. t. sumatrae* originating from the North, West, East and South of Sumatra. The data showed inconsistent group separations between regions using three different software. First, structure analysis shows two subgroups, Northern Riau and the island of Sumatra in general. Second, Tess's analysis shows two subgroups, namely the Southern Way Kambas group and the Sumatra Island group in general. Finally, Geneland's analysis indicates four subgroups, namely Northern Sumatra, Eastern Sumatra, mixed East-West Sumatra, and Southern Sumatra. Gene flow values from west to east are 0.20. This value indicates the existence of a migration history of *P. t. sumatrae* is quite high from the west to the east. At the same time the value of 0.06 gene flow from the main area of Sumatra to the southern region indicates the low gene flow of *P. t. sumatrae* to South Sumatra.

The mitochondrial CO1 haplotypes presented here show historic connectivity between Riau and West Sumatra. The *P. t. sumatrae* sharing haplotype in this study is due to the maternal lineage between populations in each province. Any population of *P. t. sumatrae* seems to be bordered by a mountain range from South to North Sumatra. The mountain range might not be a barrier for each population to interact. The adaptability

and roaming abilities of the *P. t. sumatrae* are among the factors that support the possibility of interaction between populations in each province. Franklin et al. (1999) reported that the territory of adult male *P. t. sumatrae* is 110 km<sup>2</sup> and for adult females around 50–70 km<sup>2</sup>. Griffiths (1994) also reported home ranges of adult male tigers of about 180 km<sup>2</sup> at altitudes ranging 100–600 m, 274 km<sup>2</sup> at altitudes of 600–1,700 m. Mitochondrial DNA CO1 genes are inherited maternally. The sharing of the haplotypes of each population from this study shows the distribution of individual females carrying specific haplotypes from the original population. The distance from the origin of the discovery of *P. t. sumatrae* in West Sumatra Province to *P. t. sumatrae* in Riau and North Sumatra Provinces is in the range of 200–400 km. Geographical facts support the possibility of interactions or encounters between populations. Priatna's (2012) research reinforces that female *P. t. sumatrae* can have a home range of 376.8 km<sup>2</sup>.

The interaction among *P. t. sumatrae* populations on the Sumatra island was estimated to have occurred tens to hundreds years ago. The anthropogenic influence was not great enough to fragment the *P. t. sumatrae* habitat. Currently, it is very unlikely that interactions and breeding between populations naturally because of the fragmentation and reduction of forest areas on the island of Sumatra. From 1985–1997, it was recorded that 61% of the total forest on the island of Sumatra had disappeared (Holmes 2002). Genetic studies of the *P. t. sumatrae* population with microsatellite markers show that the genetic structure of the *P. t. sumatrae* population is still good with preserved heterozygosity values to minimize the risk of genetic drift. However, the high rate of forest fragmentation and loss will accelerate the risk of decreasing genetic variation (Kenney et al. 2014; Smith et al. 2018).

Despite the limitations of our sample size, this study generated the first report of CO1 genetic datasets for *P. t. sumatrae* population in several origin locations. The CO1 mtDNA haplotypes exhibited here show historic connectivity, and maintain genetic connectivity within both East and West Sumatra. The Initial overview of *P. t. sumatrae* gives a basic picture of how the genetic structure (Smith et al. 2018). Furthermore, to detect the confiscated sample origin and accomplish the case of illegal trade with genetic forensic tools, ideally reveal the complete mtDNA and nuclear markers of *P. t. sumatrae* with most recent technology.

## CONCLUSION

Haplotype diversity and sharing haplotypes showed the possibility of interaction by each population in the past. Evidenced by the haplotypes distribution in several regions (West, North, East Sumatra). One variation in *P. t. sumatrae* is the important data and supports the previous studies. The results of this study can also determine the origin of unknown samples, although not too significant. Other genetic studies on the entire population of *P. t. sumatrae* with geological time observation are needed.

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