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An overview of the diversity of Asian Elephants in Indonesia in Terms of Their Genetics

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Abstract. There are two species diversity of Asian elephants in Indonesia, namely: Sumatran elephant (*Elephas maximus sumatranus*) and Borneo elephant (*Elephas maximus borneensis*). This literature study aims to identify and analyze the diversity of Asian elephants in Indonesia, namely: Sumatran elephants (*Elephas maximus sumatranus*) and Borneo elephants (*Elephas maximus borneensis*) as seen from their genetics (Microsatellites). This elephant is listed in the red list book of the International Union for Conservation of Nature (IUCN) from precarious to critically endangered. Meanwhile, the Borneo Elephant (*Elephas maximus borneensis*) is included in the IUCN red list with the endangered category. The Amely gene of the Sumatran elephant's Y chromosome is used to trace offspring paternally. Meanwhile, Borneo elephants have low genetic diversity due to the low average number of alleles and heterezygotes and genetic research on Borneo elephants is also still small. For this reason, it is necessary to make further conservation efforts to maintain the Borneo elephant species that have unique characteristics. This Borneo elephant is a unique species, because it is smaller or dwarf in size than other elephants, with straight tusks and has a long tail.

Keyword: *Elephas maximus sumatranus, Elephas maximus borneensis, IUCN, Gen Amely, Heterezigot*

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1 Introduction

Indonesia is a country that has a lot of biodiversity or also known as megadiversity. Indonesia has a diversity of species, one of which is elephants. Elephants are mammals that live in small groups that are impregnated by adult female elephants. Meanwhile, most young male elephants live in separate herds from adult females. In Indonesia the Asian elephant (*Elephas maximus*) is found only in Sumatra (*Elephas maximus sumatranus*) and eastern Borneo (*Elephas maximus bornensis*) [1].

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Sumatran elephants have distinctive characteristics, namely: 1). Have a weight of about 3-5 tons and a height of about 2-3 meters; 2). Lighter skin color compared to other Asian elephants, and on its ears there is often depigmentation such as reddish-white spots; 3). Only male elephants have long tusks, while in female elephants if they have short tusks: 4). Sumatran elephants have two protrusions on the top of their heads; 5). It has 5 nails on the front foot and 4 nails on the balakang foot. This elephant population is spread across 7 provinces, namely: Nangroe Aceh Darussalam, North Sumatra, Riau, Jambi, Bengkulu, South Sumatra and Lampung [2].

Sumatran elephant (*Elephas maximus sumatranus*) is a protected animal based on Government Regulation No. 7 of 1999. This elephant is listed in the red list book of the International Union for Conservation of Nature (IUCN) from precarious to critically endangered [3]. Since 1990 Asian elephants have been included in the Appendix I group category in Indonesia based on the Convention on International Trade of Endangered Species (CITES) or often referred to as (Conservation of International Trade in Animals and Plants) which is a list of lists on the protection of all threatened plant and wildlife species from all forms of trade [4].

These Borneo elephants are a unique species, as they are smaller in size or dwarf than other elephants, with straight tusks and have a long tail. This elephant is also called a Dwarf Elephant or Dwarf Elephant. This elephant in English is referred to as the Bornean Pygmi Elephant. The distribution of Borneo elephants covers the watersheds of the Kinabatangan River, (Sabah, Malaysia) and Nunukan (North Kalimantan, Indonesia). The habitat of this elephant is: forests in Tulin Onsui district, Nunukan Regency, North Kalimantan. People in the elephant habitat area often refer to this wildlife as "grandmother" which means as a sign of community respect for the wildlife [5].

The Borneo elephant (*Elephas maximus borneensis*) is a sub-species of the Asian elephant, with morphological and behavioral differences. Currently, the Borneo Elephants are estimated to have 2,000 left in the wild, and are only found in the northern part of the island of Borneo. This Borneo elephant is already endangered according to the International Union for Conservation of Nature (IUCN) because it is caused by rampant human activities that cause shrinkage and disruption of its habitat [6]. Until now, how these elephants evolved with their body shapes that are very different from other species and their distribus that are very separate from other species is still a mystery. So based on the description above, it is necessary to conduct further research related to the diversity of Asian elephants in Indonesia in terms of genetics through literature study research or field research. Which can provide benefits in the form of wider information to the community about this elephant and indirectly instill in the community to maintain the survival of this species.

2 Research Purposes

This study aims to identify and analyze the diversity of Asian elephants in Indonesia, namely: Sumatran elephants (*Elephas maximus sumatranus*) and Borneo elephants (*Elephas maximus borneensis*) in terms of their genetics (Microsatellites).

3 Methods

The research methods used in this study are: literature studies. The literature study method is a series of activities related to the method of collecting library data, reading and recording, and managing research materials [7].

4 Result and Discussion

A. Sumatran Elephant *(Elephas maximus sumatranus)*

Microsatellite analysis of Sumatran elephant feces samples (*Elephas maximus sumatranus*) in Tesso Nilo National Park, Riau. Based on the analysis's findings, it was recognized that all of the microsatellite loci employed in this study were in the Hardy-Weinberg equilibrium. The Hardy-Weinberg test results on Genepop on the Web Version 4.2, which demonstrated a P-Value value of >0.05 on all microsatellite loci used, serve as proof of this. The locus Emu10, which included four alleles and had observed heterozygosity values of 0.795 and heterozygosity expectations of 0.728, had the greatest heterozygosity value. The locus Emu04, which included two alleles and had observed heterozygosity values as high as 0.110 and expected heterozygosity as low as 0.104, had the lowest heterozygosity value [8].

The individual kinship level of Sumatran elephants (*Elephas maximus sumtranus*) in Taman Safari Indonesia II is based on the analysis of mitochondrial DNA loop displacement genes through the Polymerase Chain Reaction (PCR) method. Identities against the Asian Elephant d-loop gene (*Elephas maximus*) for the Sumatran elephants Subaru and Nebi were determined to be 99% and 98%, respectively. Subaru Sumatran elephants and Sumatran Nebi elephants have a genetic distance of zero percent, according to the reconstruction of phylogenetic trees using MEGA software, the Neighbor Joining 1000x bootstrap method, and pairwise distance. The genetic gap between the two Sumatran elephants and the Asian elephant reference is 1.4%, and the distance between the African elephant reference is 5%. The results of this study indicate that the Sumatran elephants Subaru and Nebi have a genetic similarity rate of 100% based on the D-loop gene, demonstrating their close genetic relationship [9].

The result of the Asian elephant Amely gene sequence (*Elephas maximus*) from Genbank with access code AY823325. Genetic variation of Sumatran elephants maternal using D-loop performed with mitochondrial DNA showed low genetic diversity results, where there was one dominant haplotype reaching 91.4% of 105 individuals from five Sumatran elephant conservation sites, where the genetic distance in the population ranged from 0.0-0.0003 and the genetic distance between populations was 0.0-0.0022. The intraspecies variability of the Amely gene is relatively homogeneous and paternally Sumatran elephants and Asian elephants have the same haplotype, while interspecific variations can form 3 clades where the position of Sumatran elephants is in the Asian elephant clade [10].

The phylogenetic analysis of Sumatran elephants. Using the MEGA 11 tool, phylogenetic analysis was performed based on the genetic distance between individual elephants. Elephants in PLG showed modest nucleotide variations, but compared to outgroups, they underwent considerable modifications, according to alignment data. Delesion, insertion, and replacement mutations are the three current types of mutations. Based on measurements of genetic distances and nucleotide sequence equations of the D-loop mitochondrial region, reconstruction of phylogenetic trees revealed that Sumatran elephants in PLG Padang Sugihan have a close relationship between individuals and are grouped in the same subclaster with an average similarity between individuals of 100% [11].

Genetic analysis of Sumatran elephants in Seblat Natural Ecotourism Park based on partial of mitochondrial cytochrome b-gene. The results of DNA quantification showed that the genomic DNA extracted from 11 captive elephants was in high concentrations. It ranges from 491.7 to 596 ng/Ml. Analysis of the Basic Local Alignment Search Tool (BLAST) showed that all elephants were closely related to *Elephas maximus*. Based on the alignment of the samples with two subspecies of the Asian elephant, *E. maximus* and *E. maximus indicus*, all samples are closely related to *E. maximus borneensis* [12].

Molecular study on mt-DNA COX2 gene of Sumatran elephant (*Elephas maximus sumatranus*). More than 20 nucleotide changes were found when samples from Mammuthus primigenius and African Elephants were compared in this study. These discrepancies can be linked to differences in the two species' environmental conditions. The COX2 nucleotide-based phylogenetic trees demonstrate that all samples belong to the same group. As a result, the COX2 gene in Sumatran elephants has an extremely low mutation rate. Less than 0.25% of the DNA in other subspecies of elephas maximus and sumatran elephas maximus differs [13].

Identification and Characterisation Of COI Gene In Female Sumatran Elephant (*Elephas maximus sumatranus*) In Elephant Training Centre, Way Kambas National Park. In this study, a molecular genetic analysis approach was carried out with sequencing tests to analyze genetic diversity at the population level. Based on the results of sequential data analysis, it is known that the genetic distance of 24 female Sumatran elephant individuals in TNWK is 0.000 with a homology value of 100%, strengthened by the construction of phylogenetic trees. The absence of genetic distance indicates the existence of close kinship relationships between female individuals in the population in the TNWK DLL, so the entire population comes from one population group. Sumatran elephant conservation efforts in the future will require genetic information as a way to identify each individual Sumatran elephant [14].

B. Borneo Elephant *(Elephas maximus borneensis*)

DNA Analysis Indicates That Asian Elephants Are Native to Borneo and Are Therefore a High Priority for Conservation. Elephants from Borneo have a murky history. According to two opposing theories, they are either native, dating to the Pleistocene, or introduced, descended from elephants brought to Europe in the 16th and 17th centuries. They have either been categorized taxonomically as a distinct subspecies or as belonging to the Indian or Sumatran subspecies [15].

Elephants are only found in Borneo's extreme northeast, where their distribution is restricted to around 5% of the entire island (Figure 1). The distinct b-haplotype BD was fixed in the Borneo population. With a few slight changes to the terminal branches, the maximum parsimony, neighbour joining, and maximum likelihood methods of phylogenetic studies produced similar tree topologies. Bornean and other 'Sundaland'-specific haplotypes (Borneo: BD; peninsular Malaysia: BQ, BV; Sumatra: BS, BU, BT, BR) occupied basal positions in all trees' b-clade phylogenies (Figure 2) and were deduced from internal nodes in a parsimony network of haplotypes (Figure 3) [15].

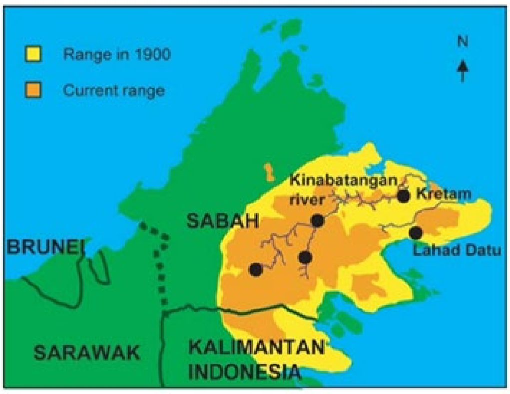

Figure 1. Asian Elephant Range and Sampling Locations in Borneo Solid lines demarcate country borders and the dotted line the boundary between the Malaysian states of Sabah and Sarawak. Black dots indicate areas of sample collection.

The distinct b-haplotype BD was fixed in the Borneo population. Maximum parsimony, neighbor-joining, and maximum likelihood phylogenetic analysis approaches all produced similar tree topologies, with some slight terminal branch rearrangements. Bornean and other 'Sundaland'-specific haplotypes, such as those found in Borneo (BD), Peninsular Malaysia (BQ, BV), and Sumatra (BS, BU, BT, BR), occupied basal positions in all trees' b-clade phylogenies. These haplotypes were obtained from internal nodes in a parsimony network (Figure 3) [15].

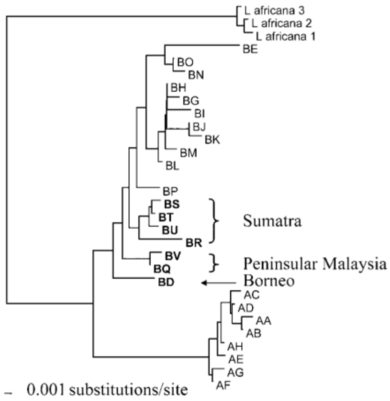

Figure 2. A Neighbour-Joining Phylogram of Asian Elephant Haplotypes Rooted with an African Elephant Out-GroupSunda Region haplotypes are in bold.

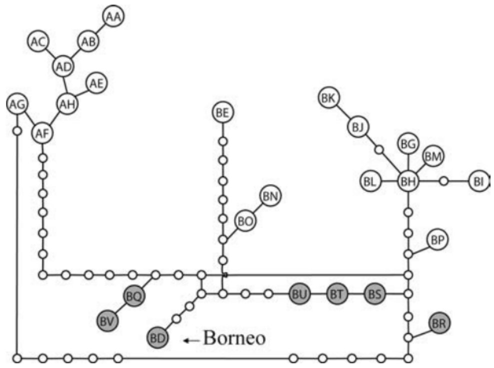

Figure 3. Network of Asian Elephant Haplotypes Based on Statistical Parsimony Grey circles with letters denote haplotypes unique to the Sunda region (BD: Borneo; BQ, BV: peninsular Malaysia; BR, BS, BT, BU: Sumatra). White circles with letters denote haplotypes found in mainland Asia (excluding peninsular Malaysia) and Sri Lanka. The small open circles denote hypothetical haplotypes. Haplotypes beginning with the letters A and B belong to the two clades a and b, respectively.

In addition, 15 Borneo elephants were genotyped for five polymorphic autosomal microsatellite loci and their results were compared to 136 five-locus genotypes of Asian elephants from nine range states. Five unlinked, neutral-selection loci showed simple Mendelian inheritance in all populations tested for Hardy-Weinberg equilibrium and linkage disequilibrium. The total number of alleles per locus across Asian elephant populations ranged from 2.0 (EMX-2) to 11.0 (LafMS03) (x, SE 4.60, 1.51); the average number of alleles across loci, per population (excluding Borneo), ranged from 2.0 (Sumatra) to 3.6 (Sri Lanka) (x, SE 14 2.93, 0.155); the observed heterozygosity H0 across all populations (excluding Borneo), ranged Comparatively, all of the following indices showed that the Borneo population had very little genetic diversity:

the proportion of polymorphic loci was 0.4; the number of alleles per locus was 1-2 (x, SE 14 1.40, 0.219); the gene diversity was 0-0.13 (x, SE 14 0.04, 0.024); and the heterozygosity H0 was 0-0.07 (x , SE 0.01, 0.013). Comparatively, all of the following indices showed that the Borneo population had very little genetic diversity: the proportion of polymorphic loci was 0.4; the number of alleles per locus was 1-2 (x, SE 14 1.40, 0.219); the gene diversity was 0-0.13 (x, SE 14 0.04, 0.024); and the heterozygosity H0 was 0-0.07 (x x, SE 0.01, 0.013). Additionally, across all populations, the average number of alleles and observed heterozygosity was larger than it was in Borneo [15].

Genetic analyses favour an ancient and natural origin of elephants on Borneo. We discover that genetic evidence supports a theory that Bornean elephant populations had a bottleneck during the last glacial period, probably as a result of Borneo's colonization, and have since slowly recovered. Overall, the evidence is in favor of Bornean elephants having naturally colonized the area at a time when large terrestrial mammals could do so from the Sunda shelf and sea levels were much lower. Our findings are crucial for understanding the rare history of elephant colonization of Borneo as well as for the long-term preservation of these animals [16].

The Bornean elephant is genetically unique from other subspecies of Asian elephants found on the mainland, making it one of the most important populations for Asian elephant conservation. The low levels of genetic diversity seen in nuclear genetic markers, such as microsatellites and single nucleotide polymorphisms for this species, are compatible with the absence of variation at the mtDNA regulatory region. Such decreased genetic variety has been attributed to either (i) the occurrence of recent founder events or (ii) the preservation of a small population size predominantly brought on by postglacial [16].

The opposing theory contends that, despite the potential introduction of domestic elephants to Borneo, the wild population was not brought there by humans and that the Bornean elephant is native to the island. This theory gained popularity when a groundbreaking study revealed the Bornean elephant's unique genetic makeup and its genetic descent from a Sundaic line. Although no samples from the extinct Javan elephant have yet been sequenced, it is interesting to note that the single mitochondrial (mtDNA) haplotype discovered in the Bornean elephant was not seen in any of the current Asian elephant populations from South and Southeast Asia. According to calculations based on mtDNA sequence divergence, the Bornean elephant haplotype and its nearest relative split off from a common ancestor roughly 300,000 years ago [16].

5 Conclution

Based on the reconstruction of the phylogenetic tree Sumatran elephants in PLG Padang Sugihan are closely related between individuals and are grouped in the same subclaster with an average similarity between individuals of 100% based on the value of genetic distances and nucleotide sequence equations of the mitochondrial D-loop region. Analysis of the diversity of male Sumatran elephants uses the Amely gene from the Y chromosome to trace offspring paternally. The individual kinship level of Sumatran elephants (*Elephas maximus sumtranus*) in Taman Safari Indonesia II is seen based on the analysis of mitochondrial DNA loop displacement genes through the Polymerase Chain Reaction (PCR) method. Gene sequence analysis using the NCBI BLAST and Bioedit programs, where the results showed that the Sumatran elephant Subaru had an ident of 99% against the Asian Elephant d-loop gene (Elephas maximus), while the Sumatran elephant Nebi had an ident of 98%. Elephants have a very limited distribution in Borneo, being restricted to approximately 5% of the island in the extreme northeast. 15 Borneo elephants were genotyped for five polymorphic autosomal microsatellite loci and their results were compared to 136 five-locus genotypes of Asian elephants from nine range states. Five unlinked, neutral-selection loci showed simple Mendelian inheritance in all populations tested for Hardy-Weinberg equilibrium and linkage disequilibrium. Across all populations, the average number of alleles and observed heterozygosity was larger than it was in Borneo. Sumatran elephants have a higher genetic diversity than their relatives as can be seen from their phylogenic tree, DNA loop displacement gene, and Amly gene. Meanwhile, Borneo elephants have low genetic diversity due to the low average number of alleles and heterezygotes and research on the genetics of Kalimantan elephants is also still small. For this reason, it is necessary to make further conservation efforts to maintain the Borneo elephant species which has unique characteristics.

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