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SHORT COMMUNICATION: IDENTIFICATION AND CHARACTERISATION OF COI GENE IN FEMALE SUMATRAN ELEPHANT (*Elephas maximus sumatranus*) IN ELEPHANT TRAINING CENTRE, WAY KAMBAS NATIONAL PARK

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Abstract *:*

Sumatran Elephant is a subspecies of endemic Asian elephants on the island of Sumatra and is included in the Red List of the International Union for Conservation of Nature (IUCN) with critically endangered status. The building of the Elephant Training Centre (ETC) in Way Kambas National Park (WKNP) is one of the conservation efforts of Sumatran elephants. Small and closed population size lead to an increased risk of inbreeding that triggers reduction in genetic variation and viability and increases the risk of extinction. The phylogenetic pattern of Sumatran elephants in Indonesia has shown a low population genetic diversity. Genetic diversity information is indispensable to support the direction of decision making in Sumatran elephant conservation policy. The DNA isolation of Sumatran elephants in ETC, WKNP has performed as a first step to trace its genetic variation. The advanced step of DNA isolation is the use of Cytochrome Oxidase subunit I (COI) gene for identification of genetic characteristics in Sumatran elephants. The COI gene is one of the genes on the mitochondrial genome and in molecular studies it is used as a genetic marker to study genetic characteristics between species and individuals. Identification and characterisation are done by sequencing process and data analysis in the form of electroforegram using Molecular Evolution Genetics Analysis (MEGA) software version 6.0. to see the genetic diversity of the female Sumatran elephant population in ETC, WKNP. Based on the results of the analysis it is indicated that the genetic distance of 24 individual female Sumatran elephant from PLG, TNWK is 0.000 with a homology value of 100%, strengthened by the construction of phylogenetic tree. The absence of genetic distance indicates a close genetic relationship, so it can be concluded all individual female Sumatran elephants in the PLG, TNWK is derived from one population group.

Keywords: Sumatran elephant, COI gene, genetic distance, homology, phylogenetic

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1. Introduction

Sumatran elephant is a subspecies of Asian elephants endemic to the island of Sumatra. It is also included in the Red List of the International Union for Conservation of Nature (IUCN) with critically endangered status. Sumatran elephants characterized as a land mammal with a grouping pattern led by adult females (matrilineal) [1] [2]. One of the conservation efforts site for Sumatran elephants is in ETC, WKNP which is part of the area of the natural habitat of Sumatran elephants. The presence of ETC plays an important role in conservation efforts, such as handling Sumatran elephants that are affected by habitat fragmentation, wild hunting, and human-wildlife conflict with national park buffer village communities. The population size of Sumatran elephants in ETC, WKNP in 2016 shows a total of 66 individuals with 36 male elephants, and 30 female elephants [2]. Currently with the birth of two elephants, the total number of Sumatran elephants increases to 68 individuals [3].

Sumatran elephants as critically endangered animals depend on their ability to maintain their survival rate, while in closed populations such as ETC increases the chances of inbreeding, which is caused by the absence of supporting data describing the genetic diversity of the elephant population within it. The genetic diversity of Sumatran elephants in the population affects its ability to adapt to changing environmental conditions. Reducted genetic diversity increases the potential for extinction. Sumatran elephant as an umbrella species with various challenges for its survival; including low genetic diversity should be saved from such threats. Information on the genetic diversity of the Sumatran elephant population in ETC, WKNP is needed in determining the policy direction, management and strategy of Sumatran elephant conservation efforts. The molecular genetic analysis approach is done by sequencing tests to analyse genetic diversity at the population level. COI gene as one of genetic markers is used to study genetic characteristics of female Sumatran elephants in ETC, WKNP.

2. Materials and Methods

2.1 Materials

The tools used in this research are 0.2 µl micro tubes, Laminar Air Flow (LAF), vortex, Micropipette, Microtip, Veriti Thermal Cycler, agarose gel electrophoresis, Digital Documents (Digi doc), Canon cameras, QI Aquick column, Spin column, centrifuge, Microtube, System sequencer type 3130 Applied Biosystem. The material used is the blood DNA extracts of female Sumatran elephant,and a master mix consisting of MyTaq™ HS Red Mix, nuclease-free water as well as primary forward and reverse cytochrome oxidase subunit I (COI) gene, Tris-Acetate-EDTA (TAE) buffer solution, 100 bp SYBR safe marker.

2.2 Methods

Process of PCR started with the making of Master mix by homogenizing MyTaqTM HS Red Mix and primary reverse and forward cytochrome oxidase subunit I (COI) gene Cat No. 10336022 Invitrogen (table 1) and nuclease free water (NFW). A total of 30 µl of the master mix is homogenized with 5 µl of DNA extraction results.

Table 1. COI primary sequence for Sumatran elephants

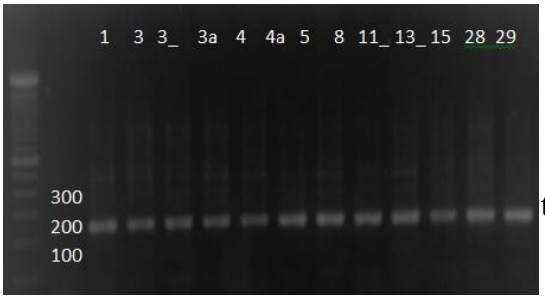
Primary	Sequence
Forward	5'GTGTCATTGTCACAGCACAC '3
Reverse	5'CTGCCAGAGGAGGATATCG '3

The results of the PCR further processed with agarose gel electrophoresis and visualized by Digi Doc. The result then purified to obtain DNA fragments. The process with sequencer takes up as many as 25 cycles with predenaturation, denaturation, forging and extension stages. Data analysis of the sequencing results were analysed using Molecular Evolution Genetics Analysis (MEGA) software version 6.0 [4]. To obtain phylogenetic tree from the COI gene sequencing result of 24 female Sumatran elephants in ETC, WKNP.

3. Results and Discussion

The DNA quality test of the amplification results of the blood samples carried out on 24 individual with a dissolved agarose gel electrophoresis. The DNA samples used are the extraction results of female Sumatran elephant blood sample that has been amplified using PCR. Visualisation of the results of agarose gel electrophoresis is done by using digital document (Digi doc). The results obtained from the visualization of agarose gel using a Digi doc in the form of DNA ribbon luminescence are visible in 13 samples.

Agarose gel electrophoresis test result for 13 DNA samples for individuals Pepi with isolation number (IN) 1, Dita (IN 3), Sulli (IN 3_), Rahmi (IN 3a), Wulan (IN 4), Poniyem (IN 4a), Gunturia (IN 5), Mega (IN 8), Lingling (IN 11_), Karmila (IN 13_), Kartijah (IN 15), Arni (IN 28), and Dona (IN 29) after PCR, showed good DNA ribbon except in sample named Pepi (IN 1) (Figure 1).



the sample Agarose gel electrophoresis test result for 12 DNA samples for individualsBunga (IN 27), Riska (IN 30), Meli (IN 32), Alma (IN 34), Yulia (IN 8a), Amalia (IN 14_), Yeti (IN 24), Mela (IN 28_), Heli (IN 36), Pleno (IN 12) Pepi (IN 1) dan Queen (IN 1_) after PCR showed good DNA ribbon except in samples identified asBunga (IN 27), Riska (IN 30), Meli (IN 32), Alma (IN 34) (Figure 2).

Figure 2.Qualitative DNA test results of the sample extracts (12 samples)

The next step was to re-extract the 4 DNA samples;Bunga (IN 27) Riska (IN 30), Meli (IN 32) dan Alma (IN 34) which then showed good results (Figure 3).

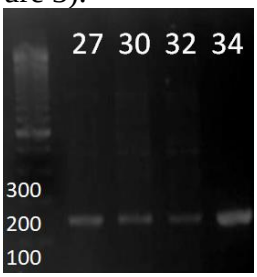


Figure 3. Qualitative DNA test results of the re-extracted sample extracts (4 samples)

After testing the quality of DNA extraction results, the samples were proceeded to sequencing and data analysis process of the electroforegram using the Molecular Evolution Genetics Analysis (MEGA) software version 6.0 [5]. to read the genetic variations of Sumatran elephants in ETC, WKNP based on its nucleic acid to determine its genetic distance and homology [6]. Information on the genetic diversity of the Sumatran elephant population in ETC, WKNP is needed in determining the policy direction, management and strategy of Sumatran elephant conservation efforts. The molecular genetic analysis approach is done by sequencing tests to analyse genetic diversity in the population level. The COI gene as one of the genetic markers is used to study genetic characteristics of female Sumatran in ETC, WKNP. Based on the results of the sequential data analysis, it is known that the genetic distance from the 24 individual female Sumatran elephants in ETC, WKNP is 0.000 with a homology value of 100%, strengthened by the construction of phylogenetic tree.

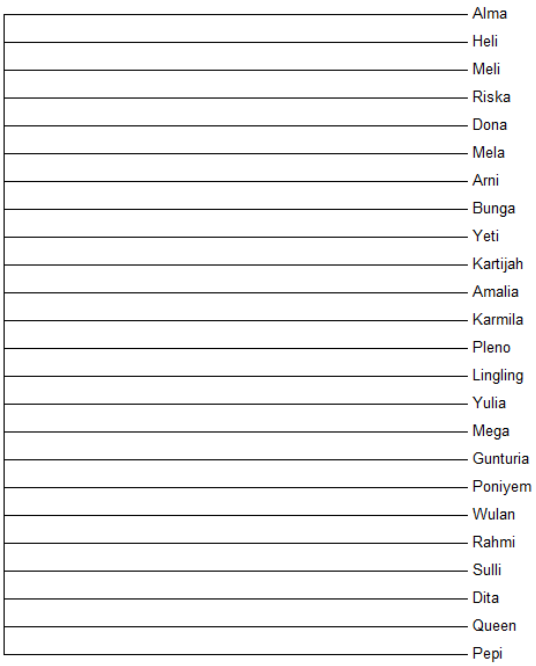


Figure 4. Constructed phylogenetic tree of female Sumatran elephants in ETC, WKNP

The absence of genetic distance as shown in Figure 4 indicates a close kinship relationship between the female individuals in the population in in ETC, WKNP, so that the entire population was derived from one population group. The future of Sumatran elephant conservation effort will need genetic information as a way to identify each of the Sumatran elephant individual. The data results of this research of the can be

used as a supporting reference to the decision making in policy direction of Sumatran elephant conservation effort in captivity, for example to determine whether or not translocation of elephants from and to ETC as an attempt to reduce the probability of inbreeding is needed.

4. Conclusion

Genetic distance from 24 individual female Sumatran elephants in ETC, WKNP is 0.000 with the homology value of 100%, supported by the construction of phylogenetic tree. The absence of genetic distance indicates a close genetic relationship, so it can be concluded that all individual female Sumatran elephants in ETC, WKNP is derived from one population group.

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