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DNA sexing of Sumatran Tiger (*Panthera tigris sumatrae*) based on amelogenin genes

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Abstract

Nowadays, forensic and wildlife research, especially the Sumatran tiger needs further research with a molecular approach. Molecular approaches are needed for forensic and wildlife research including for sex identity. This study used the amelogenin gene as a marker for identification based on previous studies of Felidae species. The sample used consisted of 10 blood samples from Sumatran tigers of known sex were collected by the Dharmasraya Sumatran Tiger Rehabilitation Center (PRHSD), one hair sample, and two bone samples whose sex was unknown were collected from Natural Resources Conservation Center West Sumatra (BKSDA). The PCR results of the amelogenin gene of the Sumatran tiger confirmed the sex of 10 samples of known sex's Sumatran tiger (Four male samples and six female samples), and Three samples of unknown sex were identified as females. Male was characterized by the electrophoresis appearance of two bands, while in female's only one band, with PCR product sizes of at least 190 bp for AMELY and at least 210 bp for AMELX.

Keywords: Amelogenin; Critically Endangered; DNA Sexing; *Panthera Tigris Sumatrae*

1. Introduction

The Sumatran tiger (*Panthera tigris sumatrae*) is a *Panthera tigris* subspecies that still exists in Indonesia. The extinction of two other tiger subspecies, *Panthera tigris sondaica* and *Panthera tigris balica* as well as the deterioration of the Sumatran tiger population are inextricably linked to human exploitation. Poaching and the illegal trafficking in Sumatran tiger parts are two of these activities [1,2]. According to TRAFFIC 2007th data, there were 23 illegal marketplaces on the island of Sumatra, eight of which dealt Sumatran tiger body parts [3].

Sumatran tiger samples found at sites of illegal trade and poaching often include pieces of nails, flesh, skin, hair, and other biological materials. As a result, the identification process is hampered. Because the sample was not in good condition, it was difficult to identify it using conventional methods in aged and degraded materials, methods focused on morphology and immunology have limitations [4]. Therefore a DNA-based identification is needed that can provide information on the species and sex of the Sumatran tiger.

Information about the Sumatran tiger's sex in a population is one of the most important things. The sex composition of the population is one of the parameters in ecological studies [5]. Sex information is effective as a basis for evaluating population structure and for determining appropriate conservation management for endangered species [6]. Sex identification data will provide statistical and evolutionary information about identified sample to determine the best conservation management decision [7].

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DNA-based approach for identifying the sex of Sumatran tiger samples is currently being developed. Amplification of the Y-specific SRY locus and amplification of the amelogenin gene [8,9,10] are the most extensively used DNA-based sex identification tests in mammals [11,12,13,14]. However, the SRY gene test becomes a problem when used in non-invasive samples and the possibility of false negatives arising due to amplification of samples with low DNA quality [15]. To avoidingerrors, sex identification using the SRY locus marker must be supplemented by other genes [16].

The amelogenin gene produces the amelogenin protein, which aids in the formation of tooth enamel structure. The amelogenin gene is found on both the X and Y chromosomes in mammals [11]. Sex identification using the amelogenin gene has been reported in Felidae species (lynx, bobcat, domestic cat, and puma) [12], as well as the bengal tiger, which produce PCR products with sizes of 214 bp and 194 bp in males and 214 bp in females [17]. When compared to the X chromosome (AMELX), the Y chromosome (AMELY) has a 20 bp deletion. The lack of DNA-based identification information based on the amelogenin gene in Sumatran tigers, especially in forensic samples, is the basis for this research. The results of amplification of the amelogenin gene using primers reported by [12] can be a marker in identifying the sex of the Sumatran tiger.

2. Material and methods

2.1. Sample sources

The samples used in this study consisted of 10 blood samples from Sumatran tigers that had sex data collected by the Dharmasraya Sumatran Tiger Rehabilitation Center (PRHSD), one hair sample and two bone samples that had'n sex data, collected by the Natural Resources Conservation Center West Sumatra BKSDA.

2.2. DNA Extraction

DNA from the total of 13 Sumatran tiger sample were isolated following the GeneAll Exgene Genomic DNA micro kit protocol. The result of DNA isolation were then electrophoresed using 1,2% agarose gel.

2.3. Polymerase Chain Reaction (PCR)

Table 1 List of Sumatran tiger Samples

No	Samples	Sex	Sample Code
1	Blood	Male	01
2	Blood	Female	02
3	Blood	Male	03
4	Blood	Female	04
5	Blood	Male	05
6	Blood	Female	06
7	Blood	Male	07
8	Blood	Female	08
9	Blood	Female	09
10	Blood	Female	10
11	Hair	Female	11
12	Bone	-	12
13	Bone	-	13

DNA amplification of the target DNA of the Sumatran tiger amelogenin gene was carried out using the SENSOQUEST PCR machine using a forward 5' CGAGGTAATTTTTCTGTTTACT 3' primer and a reverse 5' GAAACTGAGTCAGAGAGGC 3' primer [12]. The PCR reaction mixture consisted of Supermix Bioline 12.5 µl, Nuclease freewater 3.5 µl, forward primer 3 µl, reverse primer 3 µl, and DNA isolation results 5 µl, bringing the total volume to 27 µl. The mixture is put

into the machine with the conditions that predenaturation is carried out at 95 °C for 15 minutes, then denaturation is carried out at 94 °C for 30 seconds, and after that it is annealed at 51.8 °C for 60 seconds and extended at 72 °C for 60 seconds. In the last cycle, the final extension was carried out at 72 °C for 10 minutes. The amplification process with this PCR machine runs for 45 cycles. The PCR products were electrophoresed using a 3% agarose gel and visualized under ultraviolet light. Data analysis was carried out descriptively based on the DNA bands found by electrophoresis. The appearance of two bands that resulted in the sample being male and the appearance of one band from the sample being of that sex [12].

3. Results and discussion

The amelogenin gene target was successfully amplified in thirteen Sumatran tiger samples. Ten samples of Sumatran tiger blood with known sex yielded positive results that matched current data. Four male samples (sample codes 01, 03, 05, 08) exhibited double bands, while six female samples (sample codes 02, 04, 06, 07, 09, and 10) produced single bands when amelogenin gene amplification was visualized (Figure 1). This is in line with the findings of [12,18] who found that male samples produced two bands on gel electrophoresis while female samples produced only one.

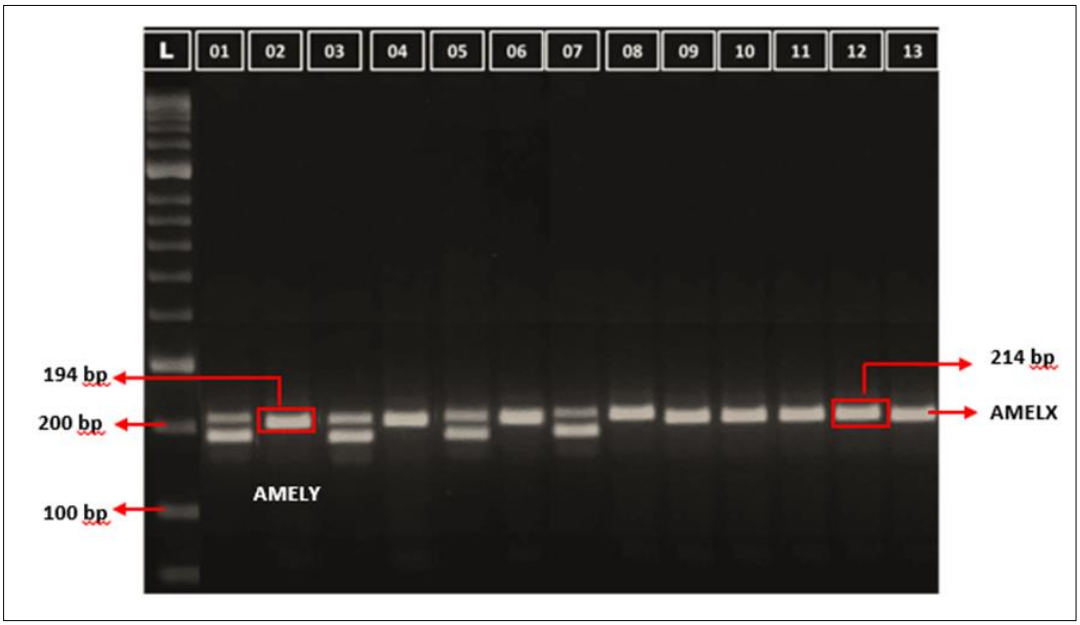

Figure 1 Electrophoresis of amelogenin gene amplification in Sumatran tigers using 3% agarose gel. L = Ladder 100 bp (base pair). Sample code 01,03,05,07: Male Sumatran tiger, Sample code 02,04,06,08,09,10,11,12,13: Female Sumatran tiger

One hair sample (sample code 11) and two bone samples (sample code 12, 13) of unknown sex were tested and found to create a single band on gel electrophoresis, indicating that all three samples were female (Figure 1). This is consistent with [19], who found that a similarity test based on the amelogenin gene in male individuals produced the same band on the amelogenin gene on the X chromosome and one band on the amelogenin gene on the Y chromosome with differences in the amount of the deletion. While the sample generates a single band with the amelogenin gene on two X chromosomes, the size of the resulting DNA band is unaffected. In male samples, several visualizations of the amplification findings displayed three DNA bands, but this had no effect on the sex analysis results [20]. The development of these three DNA bands is thought to be caused by poor amplification of poor materials, particularly stool samples, according to the majority of experts [21, 22].

The amelogenin gene amplification in Sumatran tigers yielded a PCR product with a size of at least 190 bp on the Y chromosome and at least 210 bp on the X chromosome (Figure 1). The amplification of the Sumatran tiger amelogenin gene on the Y chromosome contains a 20 bp deletion, according to the size of the PCR result. This is consistent with [12], who reported on sex identification in domestic cats (*Felis catus*), bobcats (*Lynx rufus*), and Puma sp. Using the amelogenin gene; [23] in bobcats (*Felis chaus*), Asian golden cat (*Pardofelis temminckii*), tiger (*Panthera tigris*), and mangrove cat/wild cat (*Priona viverrinus*), and [17] who reported on sex identification in Bengal tigers (*Panthera tigris tigris*) using the amelogenin gene. Differences in the amelogenin gene become indicators for sex identification. Because

the amelogenin gene has a conservative structure and can detect the X and Y chromosomes due to its nucleotide base sequence in AMELX and AMELY, it is recommended as a sex marker [24].

The recent study was amplified using a primer set that identifies the amelogenin gene area on both the X and Y chromosomes at the same time [25]. That possibility, because the amelogenin genes on the X and Y chromosomes have nearly identical base arrangements [11]. Based on the amplification results, the amelogenin gene is the most appropriate for sex identification testing by PCR among the XY homologous genes [11, 25]. However using of a pair of primers to amplify these two gene areas has a disadvantage, specifically the low primer sensitivity [25]. In the event that the Y fails, the visualization will only detect the amelogenin gene on the X chromosome if the Y chromosomal fragment amplification procedure fails. This indicates that the sample will be classified as female [23]. The primer developed by [12] is a generic primer that can be utilized in a variety of Felidae species. As a result, the results of DNA band electrophoresis did not reveal a definite band size. To improve the accuracy of the amplification results, a more specific main design and the use of more than one donor are proposed.

4. Conclusion

According to the findings of this research, the amelogenin gene can be used as a sex marker for the Sumatran tiger with the size of the PCR product from amplification of the AMELY gene at least 190 bp and the size of the PCR product resulting from amplification of the AMELX gene at least 210 bp. Nucleotide base sequencing from the amplification results can be used to estimate the size of the PCR product.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no interest in the conflict between the authors of this piece of research work. The authors agreed and assigned in hand to all matter arise to this piece of research work.

Statement of ethical approval

The present research work does not contain any experiment performed on animals/humans' subjects by any of the authors.

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