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PATERNAL PHYLOGENETIC RELATIONSHIPS BASED ON ZINC FINGER PROTEIN-Y (ZFY) GENE IN THE LAKOR GOAT BREED

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. Authors RMK, PK, MM and MR conceived the idea. Authors RMK, MR and SDV wrote the first draft of the manuscript. Authors MM, PK and MR were involved in the design and experimental work. Authors RMK, MR, MM, SDV and PK were involved in the analysis. All authors read and approved the final manuscript.

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ABSTRACT

This study aims to trace the kinship of the Lakor goat breed based on Paternal Linked using the Zinc Finger Protein-Y (ZFY) region on the Y-chromosome. This study began with non-invasive sampling (hair follicles) from 64 bucks (male individuals), followed by DNA extraction. The extracted DNA molecules are used as a template for amplification of the ZFY target region, with PCR and sequencing techniques. The PCR product of each target gene is sequenced to determine its nucleotide sequence. The molecular data of the target gene resulting from sequencing and DNA sequences obtained from the international database are aligned with the Clustal W program software present in MEGA version X. Phylogenetic analysis shows that the Lakor goat breed as monophyly above 90% bootstrap homology, and two haplotypes i.e *C. aegagrus* haplotype zI ZFY and the

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other in *C. hircus* haplotype Y1 ZFY. These haplotypes differ only in two nucleotides in our alignment, which is site 321 (C-T), in the haplotype *C. aegagrus* haplotype zI ZFY, and site 512 (G-A) in *C. hircus* haplotype Y1 ZFY. Phylogenetic analysis indicated that *C. aegagrus* (bezoar) is the ancestor of the native goats in Indonesia, including the Lakor goats.

Keywords: Phylogentic; gene ZFY; Y-chromosome; Lakor goat.

1. INTRODUCTION

Goats are one of the important livestock commodities in Indonesia. Goats are known as livestock producing meat, milk, and skin in addition to religious events and traditional ceremonies. This makes goats have great potential to be developed in the livestock industry. There are many goat breeds that have adapted to the environment and geography of the Indonesian territory i.e Marica, Benggala, Ettawah, Kosta, Kacang, Gembrong, Muara, Samosir, [1,2] and Lakor goats [3,4]. Lakor goat are native goat from Southwest Maluku Regency, precisely on the island of Lakor and have been designated as a family of local Indonesian goats with limited distribution based on the Decree of the Minister of Agriculture No. 2912 / Kpts / OT.140 / 6/2011. According to data [5], the population of Lakor goats in 2020 reached 45,109 heads.

The diversity of nucleotides in certain loci in the chromosomes of an animal species, has a close relationship with the survival activity of that species in natural populations when facing environmental changes [6]. The study of the genetic diversity of Lakor goats using paternal DNA (Y-chromosomes) in the SRY region [7] and ZFY [8] plays a role in increasing the comprehensive understanding of these livestock in natural habitats so that appropriate breeding actions can be designed so as to enrich the concept of livestock breeding in Indonesia.

The development of molecular biology techniques and the disclosure of the DNA arrangement of organisms caused many molecular studies to be carried out in breeding efforts. Numerous studies on various mammalian species prove that the paternal hierarchy for the Y-chromosome at the ZFY locus can provide an overview of the diversity of core DNA in a comprehensive manner with the ultimate goal of understanding the characteristics of the decline in inheritance traits based on paternal lineage [1,8]. In most Eutherian species, the ZFY gene is found as a single copy on the X and Y chromosomes [8], and is included in the coding region.

It is estimated that until now there are still many local Indonesian goat nations that have not been characterized and some may be scarce or the population is very limited. Besides has not been explored its characteristics, potential and genetic diversity to be used as a source of improving the genetic quality of local goats endemic in Indonesia. Therefore, this study aims to examine the paternal relationship of phylogenetics in the Lakor Goat Breed, based on the ZFY gene on the Y-chromosome. It is hoped that genetic information based on this molecular study will be very helpful for designing policies for the development and improvement of the genetic quality of Lakor goats as an endemic species, as well as through tracking the status of pure strains resulting from unguided crosses with other local goat clumps.

2. MATERIALS AND METHODS

2.1 Sample Collection and Location

This study does not require ethical approval because using non-invasive samples (hair follicles). A total of 64 samples male goats (bucks) were collected. Hair follicles from goat tails were collected and stored in envelopes to keep dry. Bucks sampling is carried out in four villages (Fig. 1) i.e Ketty Letpey (n=17 from 2 farms), Werwawan (n=15 from 1 farms), Yamluli (n=16 from 3 farms), and Letoda (n=16 from 2 farms).

2.2 DNA Extraction and Primer Design

DNA was extracted using DNA Extraction Kit (gSYNC TM DNA Extraction Kit, Geneaid). The DNA extraction protocol followed by manufacture instructions. DNA template were stored at -20^{o}C before future analysis. Primer of ZFY gene were designed using Primer BLAST (Available:https://www.ncbi.nlm.nih.gov/tools/primer-blast/). The primer sequences utilized for the ZFY gene amplification were ZFY-F: 5′-AGGCACAGTCACAGAGTAGC-3′ and ZFY-R: 3′-AAATCGCCACCTTTTGGCAG-5′ (Fig. 2) with predicted melting temperatures of 54.53°C for forward and 55,42°C for reverse.

2.3 Amplification of ZFY Gene using PCR Methods

Genome amplification of 64 bucks from Lakor island resulted in excellent bands, with a length of

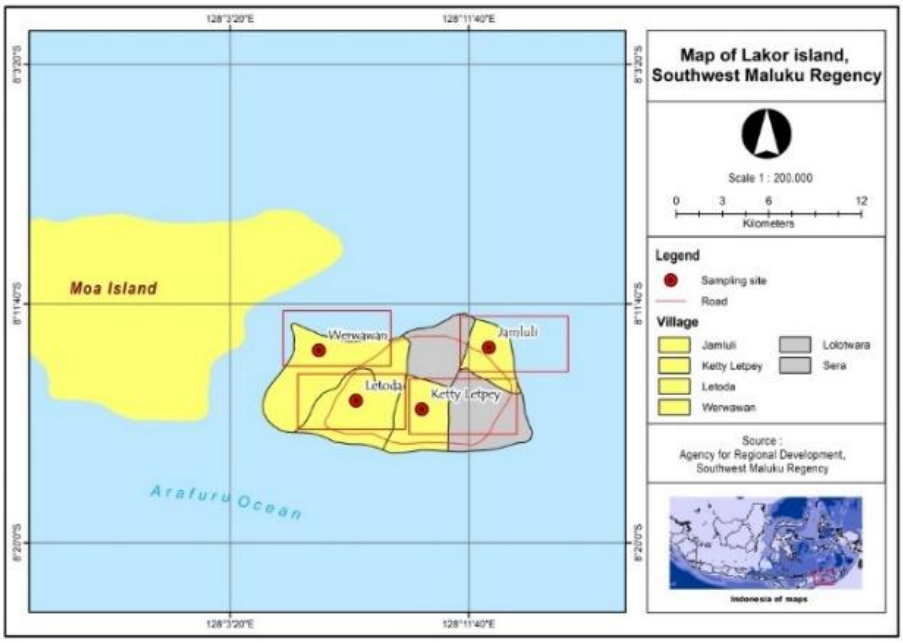

Fig. 1. Sampling sites in Lakor Island
[Source: Agency for Regional Development, Southwest Maluku Regency, Indonesia]

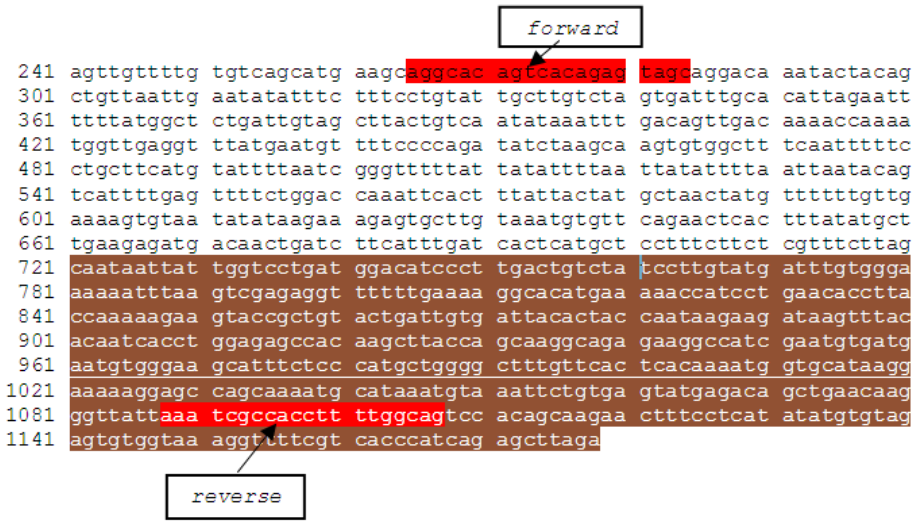

Fig. 2. Location of targeted fragment of *ZFY* gene

approximately 843 bp. Amplicons were visualized using 1,5% agarose gel electrophoresis with 1 kb DNA ladder as a molecular weight indicator. The PCR products were sequenced using forward primers and reverse primers used for the amplification process.

2.4 Statistical Analysis

The multiple alignments of ZFY gene nucleotides were analyzed using ClustalW software [9]. The sequences of ZFY gene were analyzed by MEGA software version X.0 [10]. Genetic distance was analyzed by the Kimura method with two parameters [11]. PCR products were sequenced, both forward and reverse. Sequencing results were aligned using the ClustalW program. Reverse sequences were used as comparisons of forward sequences to obtain an accurate sequence result. The phylogenetic tree was constructed based on nucleotide sequences by the neighbor-joining method with a bootstrap value 1000x. The in group and out group used as a comparison and was taken from GenBank data i.e *Capra aegagrus* haplotype zI ZFY protein (ZFY)

gene (AY082495.1), *Capra falconeri* haplotype Cf1 ZFY protein (ZFY) gene (AY082499.1), *Capra hircus* haplotype Zc1 ZFY protein (ZFY) gene (AY082500.1), *Capra hircus* haplotype Y2A_Y2B ZFY (ZFY) gene (MF741777.1), *Capra hircus* haplotype Y1 SFY (ZFY) gene (MF741776.1), *Capra cylindricornis* haplotype zA ZFY protein (ZFY) gene (AY082497.1), *Capra nubiana* haplotype CiNs20 ZFY protein (ZFY) gene (AY082504.1), *Capra ibex ibex* haplotype zB ZFY protein (ZFY) gene (AY082501.1), *Capra pyrenaica* haplotype zK ZFY protein (ZFY) gene (AY082505.1), *Capra sibirica* haplotype zE ZFY protein (ZFY) gene (AY082509.1), *Capra hircus* breed Murciana zinc finger protein Y (ZFY) gene (MF448233.1), and *Capra hircus* breed Saanen zinc finger protein Y (ZFY) gene (MF448234.1).

3. RESULTS AND DISCUSSION

3.1 Genetic Variation in ZFY Promoter

We have amplified and sequenced 843 nucleotides corresponding to a fragment of the ZFY gene promoter from 64 male of Lakor goats were analyzed based on sequences of forward and reverse *ZFY* sequences. Forward and reverse sequences were used for the editing process by observing sequence electropherogram. The polymorphic nucleotides were identified that may function as genetic markers between individual within the population. Lakor goat (LK1 ZFY) from Ketty Letpey village was a basis comparison to determine the site variables for other samples. The dot on the alignment image showed homology with LK1 ZFY sample. There were two site variables in the amplified *ZFY* gene. Based on the alignment results, it shows that there are two nucleotide that function as genetic markers i.e 321 (A-G), and 512 (G-A) (Table 1).

The data in Table 1 shows that the low diversity of nucleotides in the population is due to inbreeding depression. This data is in line with the analysis of genetic diversity in Lakor goat breed besed on COI gene mtDNA [2]. Two nucleotides, that is sites positions 321 (C-T) found in LK17, and LK23, site 512 (G-A) found in LK8, LK11, and LK20. The two mutation points are categorized as substitution (*transition type*) and this happens in

Table 1. Nucleotides variation of Lakor goat based on the *ZFY* gene

Samples	Origin (village)	Polymorphic Nucleotides	
		3	**5**
		2	**1**
		1	**2**
LK1 ZFY	Ketty Letpey	C	G
LK2 ZFY	Ketty Letpey	.	.
LK3 ZFY	Ketty Letpey	.	.
LK4 ZFY	Ketty Letpey	.	.
LK5 ZFY	Ketty Letpey	.	.
LK6 ZFY	Ketty Letpey	.	.
LK7 ZFY	Werwawan	.	.
LK8 ZFY	Werwawan	.	A
LK9 ZFY	Werwawan	.	.
LK10 ZFY	Werwawan	.	.
LK11 ZFY	Werwawan	.	A
LK12 ZFY	Werwawan	.	.
LK13 ZFY	Werwawan	.	.
LK14 ZFY	Yamluli	.	.
LK15 ZFY	Yamluli	.	.
LK16 ZFY	Yamluli	.	.
LK17 ZFY	Yamluli	T	.
LK18 ZFY	Yamluli	.	.
LK19 ZFY	Yamluli	.	.
LK20 ZFY	Letoda	.	A
LK21 ZFY	Letoda	.	.
LK22 ZFY	Letoda	.	.
LK23 ZFY	Letoda	T	.

Description: Identification with the first sequences, and nucleotides identical is denoted by a dot

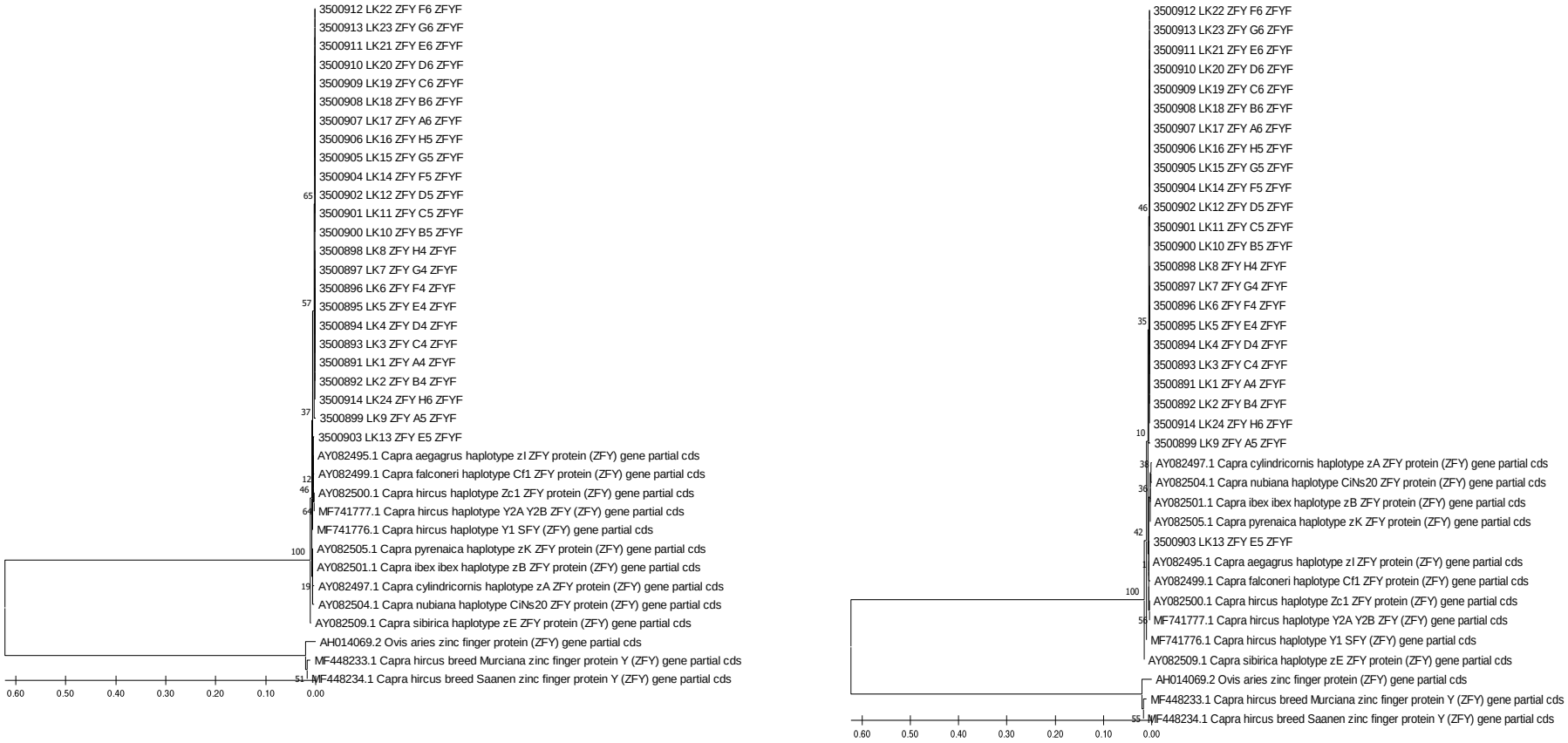

Fig. 3. Phylogram of Lakor goat based on NJ (*left*), and ML (*right*) methods

the intron area and not in the exon. The alignment resulted in two haplotypes, one in *C. aegagrus* haplotype zI ZFY and the other in *C. hircus* haplotype Y1 ZFY. These haplotypes differ only in two nucleotide in our alignment, which is site 321 (C-T), in the haplotype *C. aegagrus* haplotype zI ZFY, and site 512 (G-A) in *C. hircus* haplotype Y1 ZFY.

3.2 Evolutional History

The evolutionary history was inferred using the Neighbor-Joining (NJ) and Maximum Likelihood (ML) methods [12]. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) are shown next to the branches. The tree with the highest log likelihood (-1691.71) is shown. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. This analysis involved 37 nucleotide sequences. The total of codon and noncoding position were a total of 566 positions in the final dataset.

The phylogenetic tree based on ZFY gene indicated that the Lakor goat shares a kinship with three other goat breeds from China i.e *C. aegagrus*, *C. falconeri*, and *C. hircus* Y1 ZFY (Fig. 3). Curently, the link between evolution and taxonomic division of the family Bovidae disagreements and various classification schemes based on their phyletic relationships, exist for bovids. The results of morphological and molecular analysis have suggested that the Bovidae family ought to be taken into account a monophyletic group in which all the offspring are formed a clade by evolving from a common ancestor. In contrast, the Bovidae family paraphyletic grouping, where the majority of a common ancestor descendants excluding a few, evolutionary ancestors form a group. Descendants, who are a distinct group, have been outlined in a number of papers [13].

These two evolutionary models show that the ZFY gene has the ability to separate intra and inter species related levels with a philogram. Phylogenetic signals based on the ZFY gene indicates the Lakor goat breed as a monophyletic group with bootstrap value above 90% (Fig. 3). The domestic goat and *C. falconeri* (Markhor), a wild goat that has contributed to a number of goat species in Asia, are both descended from *C. aegagrus* (bezoar), which is also known as the bezoar [2,14]. Indonesian goats share genetics with *C. aegagrus*. This demonstrates that *C. aegagrus*, often known as the bezoar, is the ancestor of Indonesia indigenous goats [2]. Our phylogenetic tree (NJ and ML) analysis appears to reflect two separate lineages between *C. falconeri* and *C. aegagrus*.

According to Rumanta et al. [2] that *C. aegagrus* (bezoar) is the evolutionary ancestor of the Lakor goat, which has evolved over time through a process of adaptation in various habitats, as well as the local goats of Indonesia (Fig. 3). Based on the ZFY gene promoter, the NJ and ML tree topology convincingly demonstrates the historical Lakor goat strong evolutionary connection to other goat species. The several study reported that domestic goats in Asia descended primarily from a single ancestor i.e *C. aegagrus* [15,16]. Beside Chen et al. [17] reported that two species of wild goats from the Middle East *C. aegagrus* and *C. ibex* may have influenced the development of Asian domestic goats.

4. CONCLUSIONS

Phylogenetic analysis shows that the Lakor goat breed as monophyly above 90% bootstrap homology, and two haplotypes i.e *C. aegagrus* haplotype zI ZFY and the other in *C. hircus* haplotype Y1 ZFY. These haplotypes differ only in two nucleotides in our alignment, which is site 321 (C-T), in the haplotype *C. aegagrus* haplotype zI ZFY, and site 512 (G-A) in *C. hircus* haplotype Y1 ZFY. Phylogenetic analysis indicated that *C. aegagrus* (bezoar) is the ancestor of the native goats in Indonesia, including the Lakor goats.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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