UTTAR PRADESH JOURNAL OF ZOOLOGY

43(23): 58-65, 2022
ISSN: 0256-971X (P)

GENETIC VARIATION AND PHYLOGENETIC RELATIONSHIPS BASED ON TESTIS DETERMINING REGION-Y (SRY) GENE IN THE LAKOR GOAT BREED

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AUTHORS' CONTRIBUTIONS

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.56557/UPJOZ/2022/v43i233257

Editor(s):
(1) Dr. Ana Cláudia Correia Coelho, University of Trás-os-Montes and Alto Douro, Portugal.
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Received: 18 September 2022
Accepted: 21 November 2022
Published: 14 December 2022

Original Research Article

ABSTRACT

Lakor goat is an Indonesian indigenous goat breeds that successfully survive and is adaptive in the ecology of Lakor island in Southwest Maluku with high temperatures and curah limited rainfall. Genetic variations in Y-chromosome enable researchers to identify paternal lineages, which are informative for introgressions and migrations. This study aims to characterize genetic variation and phylogenetic relationships of Lakor goats based on male-specific region markers, i.e., sex-determining region-Y (SRY) gene. The genomes of 84 follicle samples of male goats (bucks) collected from Lakor island, were analyzed. The amplified product for SRY was 850 bp in encoding region (exon-1). Genetic variation and paternal phylogenetic relationship were established following sequencing using MEGA version X.0 software. The results of Y-chromosome alignments of the entire sequences identified two polymorphic nucleotides between individual goats within the population. Two polymorphism nucleotides, i.e. site 362 (A-G), and found in LK3, LK7, LK11, and LK13 samples, while site

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410 (C-T) was found in LK3, LK7, LK11, LK13, and LK24 samples. The two mutations are categorized as substitution (*transition type*) and this is in the exon-1 area. This study concluded that genetic variation is very low in the Lakor goat breed and phylogenetic analysis grouping is a single clade or monophyletic group.

Keywords: Genetic variation; SRY gene; Lakor goat; phylogenetics.

1. INTRODUCTION

The genus *Capra* is one of the first domesticated ruminants [1]. Three wild species, i.e bezoar (*C. aegagrus*), markhor (*C. falconeri*), and ibex goats (*C. ibex*), have been considered ancestors of domestic goats (*C. hircus*) [2]. Local goat breeds have adapted to agro-ecological conditions among other different climates, diseases, local feed, and give granting animal farming sustainability in marginal and challenging areas in developed and developing countries [3]. Moreover, *Capra hircus* are the source of meat, milk, and skin and contribute to maintaining livelihood in the harshest areas of small islands, including the Southwest Maluku Regency, especially in Lakor Island [4,5]. The local genetic resources, including *Capra* members, are threatened by several factors, including indiscriminate crossbreeding with cosmopolitan breeds and uncontrolled intermixing [6]. Therefore, the molecular characterization of available genetic resources and information on diversity and phylogenetic structure from Lakor goat based on SYR region is desirable also in this species.

Mammalian sex determination is dependent on the action of a testis-determining factor encoded by the SRY gene, which consists of a single exon. The SRY gene, which is found on The Y-chromosome is in charge of determining sex and encodes a protein in mammals [7]. The similarity in the promoter of the SRY gene between different animal species, such as goats and cattle, demonstrates this [8]. The similarity was also observed in species from different families, indicating a similar sex-determining gene function for the male sex determinant [9]. The SRY gene consists of 204 amino acids and has a centrally located region known as the high mobility group (HMG) box, which demonstrates a high degree of sequence conservation between species. Most studies have focused on SRY High mobility group (HMG) box, a protein that acts as a transcription factor by binding to and bending DNA strands [9]. According to the in vitro results, the HMG box has the ability to bind specific DNA sequences (AACAAT) and bend the DNA up to 90^{o} [10].

SRY gene expression is influenced by the 5' and 3' ends of the UTR. The TATA box is the main component that makes up the 5'-end of the UTR [11]. The most important parts of the 5' UTR for SRY gene regulation are the CAAT box, TATA box, SRY-binding site, and Sp1-binding site [8]. The presence of SNPs on the promoter region, which includes the 5' UTR and the TATA box, indicates a difference in transcription activity between genotypes formed by the SNPs [12,13]. This feature of the SRY-HMG box region making it an ideal target for the development of DNA-based therapies tests for sex determination [14].

Several studies have found polymorphisms in the SRY gene in ruminants i.e SRY gene polymorphism in the coding region has previously been identified in Madura cattle [15], Friesian Holstein cattle, and Sahiwal cattle [16], Bali cattle [17], and Buffalo [18]. Based on the inherited mutation (polymorphism) similarity, the polymorphism revealed specific allele types that can be used as an effective genetic marker to detect crossbreeding between livestock [18,19]. The coding region of the SRY gene on Lakor goats has never been studied before. Thus this study becomes important to further understand the coding region of the SRY gene of its contribution to Lakor goat as the flagship species in archipelagic areas.

2. MATERIALS AND METHODS

2.1 Sample Collection and DNA Extraction

This study does not require ethical approval because using non-invasive samples (hair follicles). A total of 84 sample bucks from Lakor island in Southwest Maluku Regency (Fig. 1) were collected. Hair follicles from goat tails were collected and stored in envelopes to keep dry. Bucks collection is carried out in 4 villages i.e Ketty Letpey (N=20 from 4 fams), Werwawan (N=22 from 5 farms), Yamluli (N=21 from 7 farms), and Letoda (N=21 from 5 farms). Currently, the number of bucks from Lakor goat has decreased significantly because it is the best seller compared to doe. All samples were delivered back to the animal Biotechnology Laboratory in the Indonesian Institute of Sciences, Bogor, Indonesia, for DNA extraction. DNA was extracted using a DNA isolation kit (gSYNC™ DNA Extraction Kit, Geneaid). The DNA extraction protocol is followed by manufacturer instructions and stored at -20°C until further analysis.

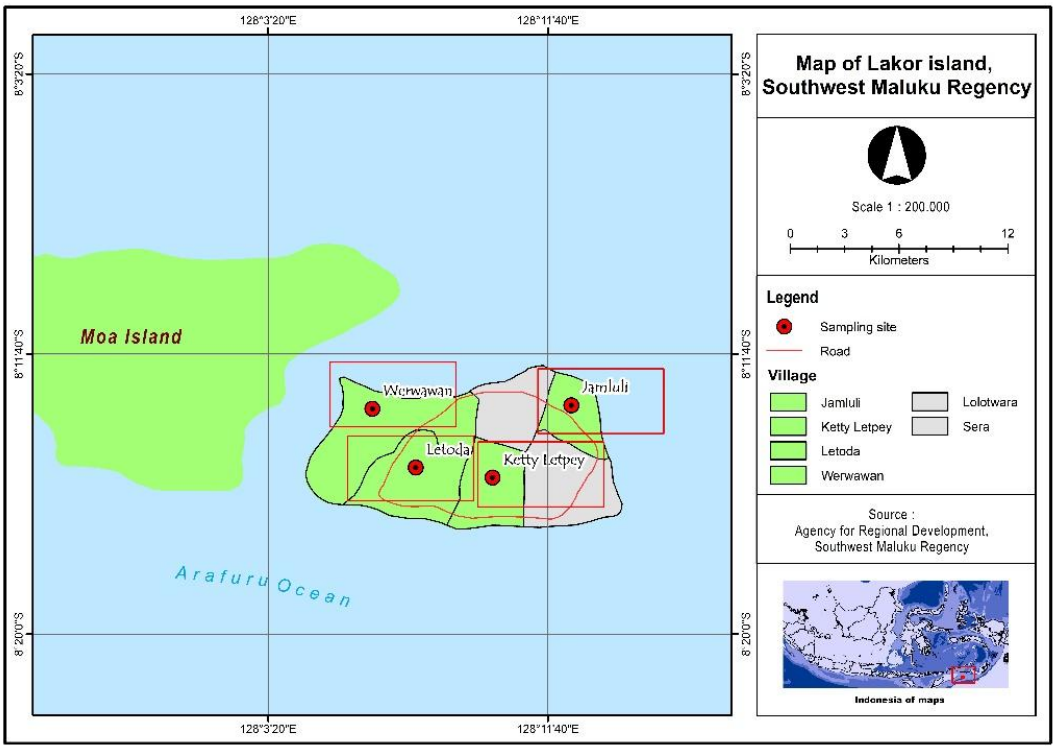

Fig. 1. Sampling sites in Lakor Island [Source: Agency for Regional Development, Southwest Maluku Regency, Indonesia]

Fig. 2. Phenotype profile of Lakor goat

Fig. 3. Location of a targeted fragment of SRY gene

Table 1. The accession number of the SRY gene used in this study (Source: NCBI Database)

No	GenBank Acc No	Breeds/Name of isolate
1	MF741782.1	*Capra hircus* haplotype Y2A_Y2C SRY (SRY)
2	MF741781.1	*Capra hircus* haplotype Y1C SRY (SRY)
3	MF741780.1	*Capra hircus* haplotype Y1B SRY (SRY)
4	MF741779.1	*Capra hircus* haplotype Y1A2 SRY (SRY)
5	EU581862.1	*Capra hircus* sex-determining region Y (SRY)
6	MF741778.1	*Capra hircus* haplotype Y1A1 SRY (SRY)
7	AF026566.1	*Ovis aries* SRY gene promoter region

2.2 Primer Design and Amplification

The set primer was designed based on GenBank NCBI NW_017189563.1. The primer sequences utilized for the SRY gene amplification were SRY-F: 5′- AGGTATTGAGGGGAGGTATT-3′ and SRY-R: 3′-AATTGAGATAAAGCGTGCCT-5′ with predicted melting temperatures of 54.53°C for forward and 55,42°C for reverse. Then, this study explored of that location to know the genetic variation and paternal phylogenetic in Lakor goat breed based on SRY gene (Fig. 3). Amplification of *SRY* region in the Lakor goat population using the PCR method.

2.3 Sequencing and Analysis

The sequencing of the purified PCR products was done precisely by 1st base sequencing INT (Singapore) with Sanger method. Nucleotides in each amplicon *SRY* gene were aligned by BioEdit version 7.0 to identify nucleotide variation, and the electropherograms were checked one by one [20].

2.4 Phylogenetic Tree Construction

The phylogenetic tree was analyzed by MEGA X version 10.2.6 [21]. The raw sequence data were trimmed to get full of the SRY gene, exon-1 region. A 850 bp sequence as a basis for both analyses. Seven complete sequences of the SRY gene from several goat breeds (from the NCBI Database) were collected and built for phylogenetic tree construction (Table 1). The phylogenetic tree was constructed by Neighbor-joining (NJ), and maximum likelihood (ML) methods [21], 1.000 bootstraps, and the Kimura 2-parameter models.

3. RESULTS AND DISCUSSION

A total of 84 DNA sample bucks were isolated and used as a template for SRY and gene amplification by PCR. After alignment, two polymorphism sites were found i.e 362 (A-G), and 410 (C-T) between individual goats within the Lakor goat population based on SRY gene analyzed (Table 2). Several genetic studies on Lakor goats have reported that inbreeding depression is thought to have occurred, causing a decline in the genetic performance of this breed [4,5,22]. Volkandari et al. [4], reported that only one genotype (TT) was found and monomorphic based on *the POU1F1|PstI* gene of the Lakor goat. Based on the GH gene analysis showed that polymorphism was found with two variants of genotypes (AA and AB) and two alleles (A and B), besides AB genotype was dominant in all populations (93.7%), and concluded that the populations were in a state of disequilibrium [22].

The result showed that two polymorphism nucleotides, i.e site 362 (A-G), and found in LK3, LK7, LK11, and LK13 samples, while site 410 (C-T) found in LK3, LK7, LK11, LK13, and LK24 samples. The two mutation points are categorized as substitution (*transition type*) and this happens in the exon-1 area and not in the intron area. These results showed differences with the ZFY gene because 2 polymorphic nucleotides were found in the intron region [23]. Field facts show that polymorphic nucleotides are only found in samples from the villages (origin sites) of Ketty-Letpey and Werwawan. If within a population found low intra-individual genetic diversity (0-3 nucleotides) showed that the population has suffered from inbreeding depression [24]. Rumanta et al. [5] stated that the four nucleotide mutations in the mitochondrial COI gene are assumed to result from genetic compression caused by adaptation to a dry tropical environment.

The maintenance system Lakor goats are generally done conventionally by releasing the goat in an unattended pasture on feed quality and mating control. This will cause a decrease in livestock performance with low body weight growth and a high chance of inbreeding. Genetic facts show that inbreeding has a great chance of causing a decrease in the quality of goat breeds and, if not handled thoughtfully, will impact population decline and extinction. The recent studies stated that there is a strong suspicion of inbreeding depression in the Lakor goat population, and has reached a maximum point with a deficient level of genetic variation and is predicted to undergo a process of genetic erosion and has an impact on population profile characteristics of Lakor goat [4,5,22].

Table 2. Genetic variation of Lakor goat based on the *SRY* gene

Samples	Origin (village)	Polymorphic	Nucleotides
		3	4
		6	1
		2	0
LK1 SRY	Ketty Letpey	A	C
LK2 SRY	Ketty Letpey	.	.
LK3 SRY	Ketty Letpey	G	T
LK4 SRY	Ketty Letpey	.	.
LK5 SRY	Ketty Letpey	.	.
LK6 SRY	Ketty Letpey	.	.
LK7 SRY	Werwawan	G	T
LK8 SRY	Werwawan	.	.
LK9 SRY	Werwawan	.	.
LK10 SRY	Werwawan	.	.
LK11 SRY	Werwawan	G	T
LK12 SRY	Werwawan	.	.
LK13 SRY	Werwawan	G	T
LK14 SRY	Yamluli	.	.
LK15 SRY	Yamluli	.	.
LK16 SRY	Yamluli	.	.
LK17 SRY	Yamluli	.	.
LK18 SRY	Yamluli	.	.
LK19 SRY	Yamluli	.	.
LK20 SRY	Letoda	.	.
LK21 SRY	Letoda	.	.
LK22 SRY	Letoda	.	.
LK23 SRY	Letoda	.	T
LK24 SRY	Letoda	.	.

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

Since a long time, ago the Leti, Moa, and Lakor islands (abbreviated as "*Lemola*") have a local wisdom i.e "*lutur* (*stone fence*)," which is circular and made of stone structures as high as 1 meter and serves to protect food crops, livestock, and the surrounding area grazing from threats. Concerning the lutur tradition, the local community of Lakor island has a conventional understanding that is still sustainable today, i.e., protecting the mixing of livestock between villages. This is done by making *lutur* (*stone fence*) around the boundary zone between villages to limit the mobilization of livestock to enter neighboring villages. Strongly suspected *lutur* to be responsible for genetic erosion in the Lakor goat population. The genetic erosion seen in the Lakor goat population is seen in the low genetic diversity population. It is caused by the high inbreeding and bottleneck population, which causes a decrease in biological fitness. All scientific findings from studies on this population must be applied to save germplasm endemic to Maluku. Thus, education is needed for stakeholders at the district level and in each village and farmers not to limit genetic mixing between villages by using *lutur*.

In addition, another fact that is also considered in the study of genetic aspects for the development of Lakor goats is the isolation of populations on small and outer islands so that there is no insertion of gene flow into the gene pool. For species inhabiting islands that have been domesticated, the loss of genetic diversity is generally due to loss of habitation, a dramatic decrease in population numbers, and the absence of male introgression from outside the breed [24,25]. Overall, the low genetic variation in the Lakor goat population is due to improper breeding mechanisms and brood management. In this study, we discovered that when population genetic variation decreases, it is critical to minimize genetic loss such that it is not significant.

The examination of the phylogenetic relationship of the Lakor goat based on SRY gene using two evolution (NJ and ML) models (Fig. 3). The consistency of these two evolutionary models showed that the SRY gene could separate the intra and inter-species levels associated with the phylogram. The phylogenetic signal based on the SRY gene showed that a Lakor goat breed is a monophyletic group or single clade as in previous COI mtDNA gene analyses

[5]. All samples used to create this phylogenetic tree were grouped into one clad with a genetic distance of 0.0%. This study is the first attempt toward a comprehensive genetic characterization of Lakor goats based on paternal phylogenetic studies. Our findings suggest that the observed level of genetic diversity is not high. This is possible because the Lakor goats are farther from the center of domestication and are in the last stage of the spread and evolution and are related to the process of colonization on a small island.

Based on the phylogenetic analysis of the SRY gene on the Y-chromosome, it was shown that evolutionarily, the Lakor goat breed had a kinship with beef goats and not dairy goats. The facts of this study support previous findings that the Lakor goat shares a kinship with *C. aegagrus* based on mtDNA analysis. Our paternal phylogenetic analysis following based on the mtDNA COI gene [5]. These findings show that *C. aegagrus* (bezoar) is the ancestor of Indonesian indigenous goats including the Lakor goat, which has evolved through adaptation in various habitats and times. The Lakor goat is a hybrid between an Etawah descendant and an Indonesian native goat known as the Kacang goat. However, the Etawah goat is genetically dominant [26], and geographical isolation has contributed to the evolution of this unique breed. Currently, several studies on the functional area of Lakor goats including Growth Hormone (GH) [22] and POU1F1 (*PstI*) gene [4] showed the same pattern as the one inferred from the SRY gene. It is suspected that the breed shows a retention of diversity in the face of population reduction. Conservation will be much more difficult when the population becomes genetically poor and practical and easy to implement when the population is genetically stable.

The results of this study did not find a haplotype (SNP) as reported by Vidal et al. [27]. There are 2 possibilities that caused no haplotype to be found in this study, i.e SNP positions discovered by Vidal et al. [27] i.e (*SRY*-2971T > A, *SRY*-3098G > A, *SRY*-1876A > C , is outside the amplified area (mRNA). The haplotypes found by Vidal et al. [27] were obtained based on the analysis of 4 regions i.e (SRY, AMELY, ZFY, and DDX3Y). Besides, the breeds used in the Vidal et al. [27] as many as thirty-one populations from nine countries, while our study only analyzed the SRY region with one breed i.e the Lakor goat. Study Underhill and Kivisild [28] stated that differentiation of paternal lineages via analysis of Y-chromosome variation adds significantly to what can be inferred from mitochondrial DNA and autosomal variation. Thus paternal lineages can be traced both within and between populations.

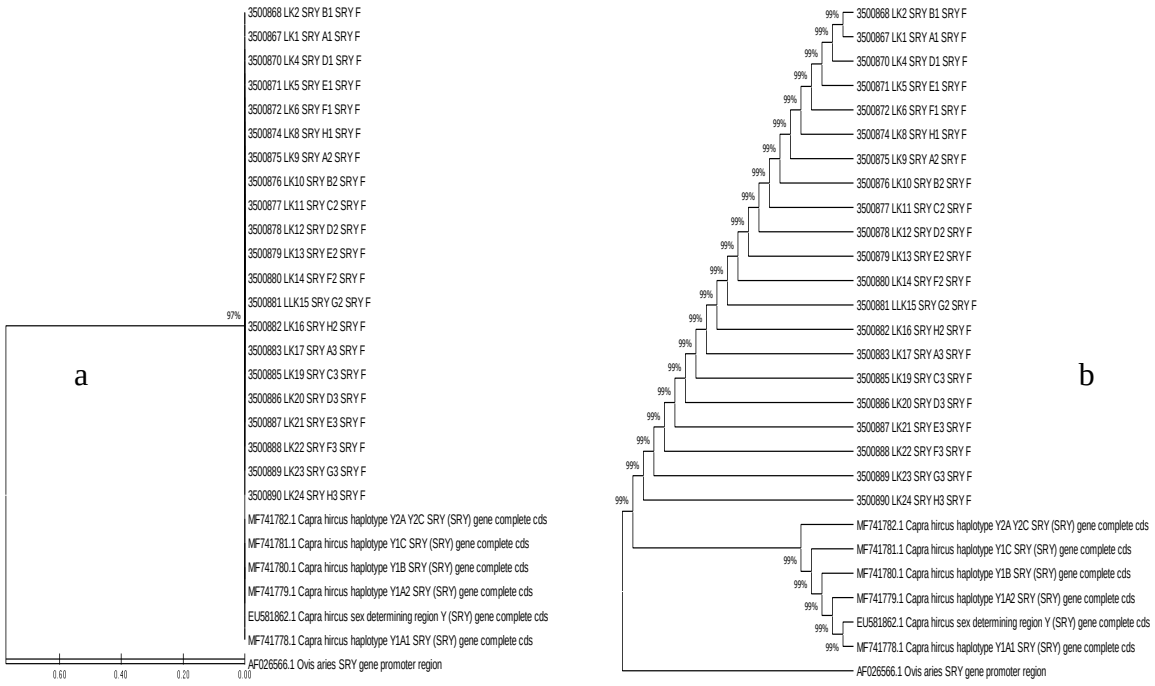

Fig. 4. Phylogram of Lakor goat based on SRY gene; NJ (a) and ML (b) Methods

4. CONCLUSIONS

Genetic diversity is very low in the Lakor goat breed based on the SRY gene. The phylogenetic study revealed that the Lakor goat is a single clade or monophyletic group. The result showed that two polymorphism nucleotides, i.e site 362 (A-G), and found in LK3, LK7, LK11, and LK13 samples, while site 410 (C-T) was found in LK3, LK7, LK11, LK13, and LK24 samples. Two polymorphism nucleotides are categorized as substitution (*transition type*) and this is in the exon-1 area.

ACKNOWLEDGEMENTS

We acknowledge the authorities of the Agriculture and Livestock Office, Southwest Maluku Regency, Animal molecular laboratory, Indonesian Institute of Science for availing us of all the necessary help we needed at the time of this study. The authors would like to thank Barnabas Giartua and Albertus Sairudy for helping collected samples in Lakor Island. The authors declare that they are received funding source or grant from the Universitas Terbuka, Indonesia, through the Institute for Research and Community Service (Grant number: 2663/UN31.LPPM/PM/2020) to support this study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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