International Journal of IJAS Agriculture System

INTERNATIONAL JOURNAL OF
AGRICULTURE SYSTEM (IJAS)
ISSN Online: 2580-6815 | ISSN Print: 2337-9782

VOLUME 1 ISSUE 2, DECEMBER 2013

Available online since December 10, 2013

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Genetic Relationship amongst *Marica, Kacang* Goat and *Capra* species

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Abstract: *Marica* goat is an endemic species that can be found only in South Sulawesi, Indonesia. It has been reported by the FAO as endangered species because its number of population has been significantly decreased. Domestication by local people has been done by cultivating them with *Kacang* goat that leads to an interbreeding process. Conservation of the goats must be done immediately due to its rapidly decreasing population. However, accurate identification and quantification of the goat by DNA analysis is highly important. The objective of this study is to determine the differences amongst marica, kacang and *Capra hircus, C. caucasica* and *C. falconeri* from the GenBank. The sequence of the mitochondrial DNA (mtDNA) at d-loop region of 30 *Marica* goats and five *Kacang* goats from three districts in South Sulawesi were investigated. Their nucleotide sequences were compared with the sequence of the GenBank's *Capra sp* and were analyzed using Dendogram neighbor joining tree. The results showed that there were a few nucleotide differences between some *Marica* and *Kacang* goat that were located at 20, 840 and 980bp. In addition, both nucleotides sequence have short genetic distance compared to *C. hircus*. However, comparing with other *Capra* sp, the distance was significantly far. Meanwhile, according to the dendogram, it was found that all Goats and *Capra* sp came from the same ancestral lineage. It can be concluded that *Marica* and *Kacang* goats could be very closely related with *C. hircus* but they were different from the *C. caucasica* and *C. falconeri*.

Keywords: *Marica* goat; *Kacang* goat; *Capra hircus*; South Sulawesi; mtDNA

1. Introduction

One of Indonesian's germplasm commodities are goats. Goats spread in different regions with different climates and separated in a long time. Various selections of environmental factors and treatment cause genetic changes in goat's population (Rout *et al.*, 2008).

Marica goat is a type of local goat that can be found only in South Sulawesi Province, especially in the district of Maros, Jeneponto, Soppeng and Makassar (Fitra *et al.*, 2009). The goats are genetically potential to adapt in the dry land with a very low annual

rainfall. *Marica* goats can survive in the dry season although they only eat dry grass in the rocky ground. Unfortunately, according to Food and Agriculture Organization (2000) Report, its population is very low and is categorized as endangered species.

Traditionally, local communities raise various kinds of goats in the same field and cages. It also happened on *Marica* goat that was reared together with *Kacang* goat, consequently interbreeding and genetic mixing among goat are inevitable. According to previous observation and interview with a local traditional breeder, *Marica* goats have strong characteristic even they resulted from the combinations of *Marica* and *Kacang* goats. Thus, even the goats have strong characteristic of Marica goat, it does not guarantee that they are original *Marica* goats.

Molecular genetic studies within and across breeds are essential for the population management (Hall and Bradley, 1995; Ruane, 2000; Simianer, 2005). Performing such purpose, it is believed that using mitochondria DNA (mtDNA) is simply enough than using circular DNA. Mamalian mtDNA shows several special features such as absences of intron, material inheritance, lack of recombination events and a high mutation rate (Irwin *et al.*, 1991). Furthermore, it brings enough information to figure out goat ancestries (Chen *et al.*, 2005). Complete sequences of bovine mtDNA were published by Anderson *et al.* (1982), so the mtDNA have widely used for genetic diversity and phylogenetic analysis among different cattle breeds (Bradley *et al.*, 1996; Troy *et al.*, 2001). Specifically, D-loop area at mtDNA, non coding area, has nucleotide sequence that is useful to determine phylogenetic relationship (Hou *et al.*, 2006). Meanwhile, the preservation of *Marica* goats should be done immediately considering the number of its population has declined sharply. However, before conducting conservation effort, determining the original *Marica* goat is essential. This study is therefore conducted to determine the kinship between *Marica* and *Kacang* goats and the original *Marica* from *Kacang* goats.

2. Materials and Methods

1.1 Animal Sample

DNA was obtained from whole blood sample because the DNA have specific genetic characteristic and the size is smaller than nucleus DNA (Chen *et al.*, 2005). It was impossible to find a number of *Marica* goats that have the same characteristic due to the lack of its number. All *Marica* goats comprising of seven goats from Maros (TB5, TB4, S1, S3, S2, TB6 and TB8), three goats from Makassar (MK3, MK4 and MK5) and 20 goats from Jeneponto (JNP induk, JNP anak, MCJ2, MCJ3, I, Btg1, Btg2, Btg3, Btg4, TB VIII, B1, B2, B3, K1, K2, K3, Mks7, Mks8, Mks10, Mks7Jantan) were analysed. Almost all the goats were female except Mks7Jantan which was male. On the other hand, five *Kacang* goats were taken from Makassar.

1.2 DNA isolation

A total of 10 ml of blood were taken aseptically from the jugular vein of each *Marica* and *Kacang* goats and placed in vacuum glass tubes containing anticoagulant ACD (acid citrate dextrose). All samples were stored in a cooler and brought to laboratory for further analysis (Yadav and Yadav, 2008).

In this study, only mitochondria DNA was used and purified following the method of Sambrook *et al* (1989).

2.3 PCR Amplification

The primers that were used in this study were designed to amplify D-loop region (Table 1). Primer for the D-loop was designed based on data *Capra hircus* sequence (GenBank Accession number: GU295658.1). Program of Primary 3 output (http://www-genome.wi.mit.edu/cgi.bin/primr3.cgi/results_from-primer3) was used to select primers which are predicted to give good results. PCR Reaction consisted of 2.5 mM $MgCl_2$, 10 mM dNTPs, 100-300 ng template DNA, 10 *p*mol each primer and 2U *Taq Polymerase* (Bio Lab) and its buffer.

Table 1. Base sequences and its melting temperatures of primers for amplifying D-loop region of mtDNA of the goats.

PRIMER					
Target	Name	R/F	Base sequence	Number of Base	Melting Temperature (^{0}C)
D-loop	CHF	F	5' CTCACATTAAACCTGAGTGG 3'	20	59,0
D-loop	CHR	R	5' ATGCAGTTAAGTCCAGCTAC 3'	20	60,7

The thermal profile included an initial denaturation at 94°C for 2 min following denaturation at 94°C for 2 minutes, annealing at 72°C for 2 minutes and 35 cycles of elongation and extension at 72°C for 1 min, using PCR amplification Infigen. PCR products were migrated at 1.2% agarose gel migrated using 1xTBE buffer Submarine Electrophoresis (Hoefer, USA). The gel was stained with ethidium bromide and observed under UV Transluminator (λ = 300nm) exposure, and the 1000 bp of standard molecular used as a ladder.

2.4 Purification of PCR product using Phylogenetic analysis

Products of PCR amplification was purified using GFX purification kit (Amersham, USA). Alignment of sequences was obtained using the Clustal W program (Thompson *et al.*, 1994). The result was compared with DNA sequence of *Capra hircus* (GenBank Accession number: GU295658.1), *Capra hircus* breed Inner Mongolia White (GenBank: Accession number GU068049.1), *Capra falconeri* (GenBank Accesion number: FJ207525.1) and *Capra caucasica* (GenBank Accession number: JN632609.1)

3. Results and Discussion

Figure 1 shows the profile of PCR amplification products of mtDNA gene. Some of the DNA bands show a smear and even no bands at all. These might be due to the degradation process during DNA purification or the DNA chains were cut shorter during purification process. Some DNA do not form visible band that could be caused by the small amount of sample or annealing temperature. As results, only 11 band are clearly seen which are from B2F,

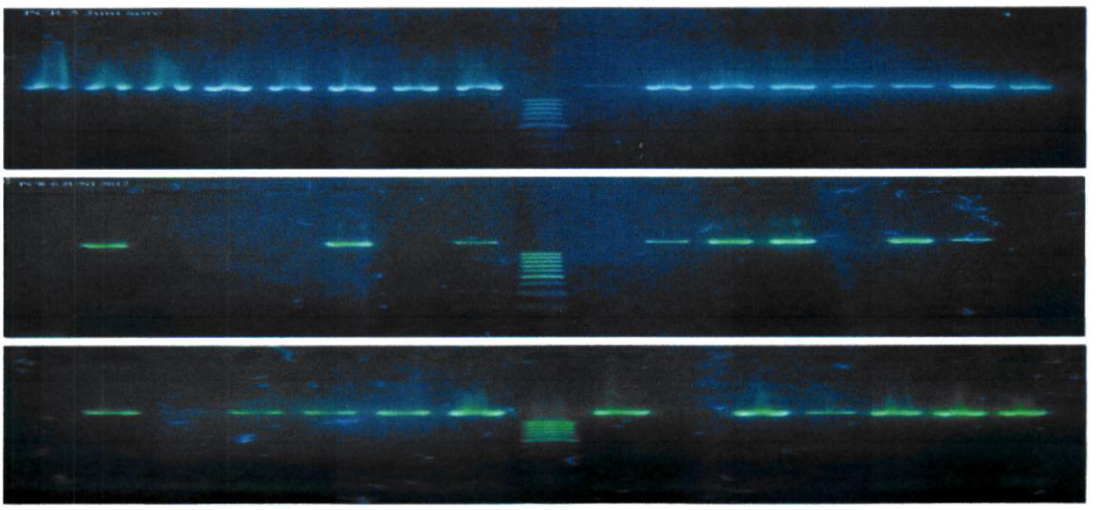

Figure 1. The results of PCR amplification of D-loop region of mtDNA of marica and kacang goats (Ladder : 1000 bp).

Table 2. Sequences comparison of the nucleotides polymorphisms of D-Lopp DNA region.

#C_hircus	AATCCTACGA TCAATTCCCA ACAAACTAGG AGGAGTCCTA	[40]
#B_2_F	....	[40]
#BTG_4_F	....	[40]
#I_Betina_F	....	[40]
#K_3_F	....	[40]
#JNP_induk_F	.... C	[40]
#Kacang_1_F	....	[40]
#MK_3_F	....	[40]
#TB_7_F	....	[40]
#TB_8_o_F	....	[40]
#S3_F	....	[40]

BTG4F, I-Betina-F, K3-F, JNP Induk_F, S3_F, Mk 3_F, TB_7_F and TB_8_o_F for marica goats, kacang_1_F for kacang goat.

Table 2 illustrated some pertinent information about nucleotides sequence in the range 40bp. Almost all *Marica* and *Kacang* goat showed the same nucleotide sequences as *C. hircus* except for the parent JNP which is *Marica* goat sample from Jeneponto. This goat has nucleotide cytosine at 20bp while others have nucleotide adenine.

In addition, there are similar trend occurred in the range 960 and 1,000bp (Table 3) excluding TB_7_F and *Kacang* _1_F, respectively. Sample TB_F_7 which is *Marica* goat from Jeneponto has nucleotide cytosine while the others *Marica, Kacang* and *C. hircus* goats have thymine. On the other hand, at position 980bp, *Kacang*_1_F (*Kacang* goat) has guanine while others goat have adenine. Meanwhile, in the region of 840bp, two *Marica* goats (S3-F and MK_3F) show a difference where at 807bp, both of them have adenine nucleotide, while the others have guanine.

Table 4 described the differences of nucleotide sequence between the researched goats and the goat from GenBank which are *C. causica*, *C .falconeri* and *C. hircus* inner Mongolia white cashmire. It can be clearly seen that the genetic distance have no difference between *Marica* goat and

Table 3. Sequences comparison of the nucleotides polymorphisms of D-loop region of mtDNA, range 840 bp, 960 bp and 1,000 bp.

#C_hircus	ATTTTATGAT CTACTTCACG TGTACGTACA TAATATTAAT	[840]
#B_2_F	....	[840]
#BTG_4_F	....	[840]
#I_Betina_F	....	[840]
#K_3_F	....	[840]
#JNP_induk_F	....	[840]
#Kacang_1_F	....	[840]
#MK_3_F	.....A..	[840]
#TB_7_F	....	[840]
#TB_8_o_F	....	[840]
#S3_F	.....A..	[840]
#C_hircus	ATAAAGACAT AATATGTATA TCGTACATTA AACGATCTCC	[960]
#B_2_F	....	[960]
#BTG_4_F	....	[960]
#I_Betina_F	....	[960]
#K_3_F	....	[960]
#JNP_induk_F	....	[960]
#Kacang_1_F	....	[960]
#MK_3_F	....	[960]
#TB_7_F	.C.....	[960]
#TB_8_o_F	....	[960]
#S3_F	....	[960]
#C_hircus	CCCATGCATA TAAGCACGTA CAATGTCCTT ATTAGCAGTA	[1000]
#B_2_F	....	[1000]
#BTG_4_F	....	[1000]
#I_Betina_F	....	[1000]
#K_3_F	....	[1000]
#JNP_induk_F	....	[1000]
#Kacang_1_F	....G	[1000]
#MK_3_F	....	[1000]
#TB_7_F	....	[1000]
#TB_8_o_F	....	[1000]
#S3_F	....	[1000]

C. hircus. This trend is similar to *Kacang* goat and *C. hircus*. This condition can be explained by showing the dendogram Neighbor Joining Tree (Bootstrapping 1000 replications) as seen in Figure 2.

It can be seen clearly that based on dendogram Neighbor Joining Tree, all *Marica* and *Kacang* goats have a far genetic distance compare to *C. falconeri* and *C. caucasica*, but their distance is closely related to species of *C. hircus*. Meanwhile, a closer study on this dendogram shows that all goats sample has similar genetic distance.

They are few substitution mutations found in *Kacang* and *Marica* goats. It might happen as a result of adaptation process to environmental condition such as the lack of feed resources and selection by breeders. In Jeneponto, for example, the dry season duration is over seven months per year.

Table 4. Matrix index of nucleotide sequence's difference between *Marica* and *Kacang* goats compare to the sequence from the GenBank's goats.

		1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	C_hircus														
2	B_2_F	0													
3	BTG_4_F	0	0												
4	I_Betina_F	0	0	0											
5	K_3_F	0	0	0	0										
6	JNP_induk_F	1	1	1	1	1									
7	Kacang_1_F	1	1	1	1	1	2								
8	MK_3_F	1	1	1	1	1	2	2							
9	TB_7_F	1	1	1	1	1	2	2	2						
10	TB_8_o_F	0	0	0	0	0	1	1	1	1					
11	S3_F	1	1	1	1	1	2	2	0	2	1				
12	Capra_caucasica	102	102	102	102	102	103	103	103	103	102	103			
13	Capra_falconeri	79	79	79	79	79	80	80	80	80	79	80	106		
14	C_hircus_Inner_ Mongolia_White_Cashmere	38	38	38	38	38	39	39	39	39	38	39	96	88	

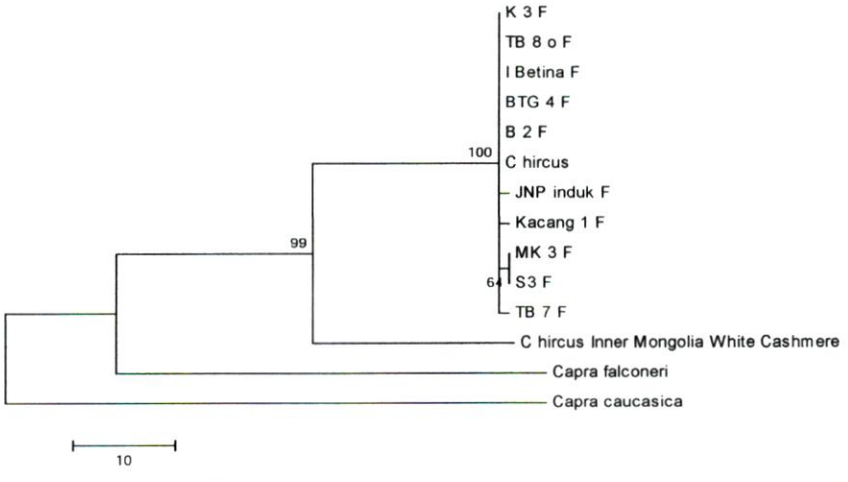

Figure 2. Dendrogram neighbor joining tree displaying the relationship between the samples and goats' particular references belonging to the *Capra* sp as determined by D-loop region of mtDNA gene analyses. The numbers at the node are bootstrap values based on 1,000 re-samplings. The bar represents the number of mutations per sequence position

Thus, the feeding grass is less available and dry. Elrod dan Stansfield (2007) explained that the total gene pool may change when phenotype characteristic is suitable with the environment. Furthermore, the genes will be inherited to the offspring. Meanwhile, a research conducted by Agha *et al.* (2008) showed that the studied Mediterranean breeds sampled from African and European populations seem to have differentiated from each other with only little genetic exchange between the geographically isolated populations. Therefore, the mutation of population is often caused by genetic drift or

selection (Nei and Kumar, 2000).

4. Conclusion

It can be conclude that even though there are some difference nucleotides in some *Marica* and *Kacang* goats, all of the nucleotides sequence alignments are similar to the nucleotide of *C. hircus* (AF533441). However, it is different from *C. caucasica* and *C. falconeri*. Future work strongly recommended to perform the same analysis on the other area of D-loop mtDNA outside the area of 40,960 and 1,000bp to figure out other similarities and dissimilarities between *Kacang* and *Marica* goats.

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