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Genetic Variations and the Origin of Native Indonesian Goat Breeds Based on mtDNA D-Loop Sequences

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ABSTRACT

Indonesia has several breeds of goats as the source of genetic. This study aimed to characterize genetic of Indonesian goats. The phylogenetic relationship among and genetic variability within eight native Indonesian goats were analyzed using mtDNA D-loop region sequences. The results show that the length of complete sequences mtDNA D-loop in Indonesian goats are 1212 bp. There are 68 polymorphic sites and 21 different haplotypes. Haplotype diversity and nucleotide diversity based on mtDNA D-loop in eight breeds of Indonesian goats are 0.5000-1.000 and 0.248-1.1595%, respectively. The value of genetic distance between groups of native Indonesian goats ranged from 0-0.011. Indonesia goat genetic distance value against wild goat *Capra aegagrus* (AB004082) is in the range 0.0038-0.045 while against *Capra falconeri* (AB044306) is 0.087-0.095. The results indicate that the genetic diversity of Indonesian goats is not abundant. Indonesian goats originated from one maternal. Maternal origin of Indonesian goats based on mtDNA D-loop is lineage B.

Key words: Indonesian goat, mtDNA D-loop, genetic diversity, phylogeny relationship, haplotype

INTRODUCTION

Indonesia is a country with a very high biodiversity “Mega Biodiversity”. Goats are one of Indonesian germplasm that need to be developed and preserved. Goats have a high enough potential in Indonesia, as producers of meat, milk, fur and leather. From the side ethnobiological, goats are also used for religious ceremonies for Muslims and cultural ceremonies (Budisatria, 2008). Domesticated goat (*Capra hircus*) is a very adaptable animal and geographically widely spread in various continents (Chenyambuga, 2002; Peacock, 2005; Aziz, 2010).

Many authors have indicated that all the different species or any sub-genus has contributed to the domestication of goats (Epstein and Mason, 1971). Bezoar (*Capra aegagrus*) allegedly as a direct ancestor of domestic goats and markhor (*Capra falconeri*) also predicted to have contributed to some goat breeds in Central Asia (Mason, 1981; Tu, 1989; Xie, 1985; Yu, 1991). Mason (1984) stated that the species markhor have affected some breeds of goats. The most of breed goat in Indonesia is Kacang goat, since almost all Indonesian islands can be found that goat. Nevertheless, native Indonesian goats have some differences in phenotype in some areas and has been characterized into six sub-populations (Batubara, 2011).

Mitochondria DNA (mtDNA) is a genetic marker that is very important because it is passed down the maternal without recombination and has a high mutation rate. According to Tapio and

Grigaliunaite (2003) that mitochondria comprising coding regions 12S and 16S rRNA, 22 tRNA and 13 protein subunits respiratory chain enzyme complexes, also has a non-coding nucleotide sequences called displacement loop area (D-loop). The uniqueness of the D-loop region is to have the highest levels of polymorphism in mtDNA. The D-loop region is highly variable and the rate of evolution has five times faster than other regions in the area of mitochondria. Many studies based on analysis of the D-loop region to determine phylogenetic and evolutionary (Chen *et al.*, 2005; Joshi *et al.*, 2004; Bowling *et al.*, 2000).

The origin of domesticated livestock was allegedly derived from wild animals. It can be revealed by analyzing the sequences of mtDNA D-loop. Liu *et al.* (2006) examined 128 goats isolate China, which is divided into nine different breeds using mtDNA D-loop sequences complete 1213 bp. The result revealed that the goats in China come from lineage A and Joshi *et al.* (2004) conducted a study on 363 goats isolate India is divided into 10 different types of breeds by using mtDNA D-loop 457 bp. The result showed that Indian goat came from three lineages, namely A, B and C. Indonesia has some of the original goats that need to be done to determine the characterization of genetic variation and relationships are the goats that exist around the world. The purpose of this study was conducted to analyze the mtDNA D-loop sequences are complete of eight native Indonesian goats.

MATERIALS AND METHODS

Sample collection: This study used blood or tissue and hair follicle samples from 26 individuals belonging to eight sub-population of native Indonesian goats is listed in Table 1. The samples of goat with purposive sampling method, first determine the district native goat production centers and then the specified districts and villages. This study was conducted from March, to November, 2014 (Fig. 1).

Table 1: Source of the Indonesian goat samples

Populations	Abbreviations	Province of origin	Islands
Samosir goat	SAM	North Sumatera	Sumatera
Muara goat	MUR	North Sumatera	Sumatera
Peranakan Etawah goat	PE	D.I. Yogyakarta	Java
Jawarandu goat	JAW	D.I. Yogyakarta	Java
Kacang goat	KAC	East Java	Java
Lakor goat	LAK	Maluku	Maluku
Gembrong goat	GEM	Bali	Bali
Marica goat	MAR	South Sulawesi	Sulawesi



Fig. 1: Sampling sites native Indonesian goats

Table 2: Reference individuals of the 6 domestic goat haplogroups

Haplogroups	Geographic origin (country)	Accession numbers	References
A	India	AY155721	Joshi <i>et al.</i> (2004)
A	Italy	EF618134	Naderi <i>et al.</i> (2007)
B	Laos	AB044303	Mannen <i>et al.</i> (2001)
B	Mongolia	AJ317833	Luikart <i>et al.</i> (2001)
C	Spain	EF618413	Naderi <i>et al.</i> (2007)
C	India	AY155708	Joshi <i>et al.</i> (2004)
D	China	DQ188893	Liu <i>et al.</i> (2006)
D	India	AY155952	Joshi <i>et al.</i> (2004)
F	Sicily	DQ241349	Sardina <i>et al.</i> (2006)
F	Sicily	DQ241351	Sardina <i>et al.</i> (2006)
G	Iran	EF618084	Naderi <i>et al.</i> (2007)
G	Turkey	EF618535	Naderi <i>et al.</i> (2007)

Molecular techniques: There were 3 kinds of samples obtained which are blood, hair follicle and muscle tissue. Blood samples were isolated and purified using Genomic DNA Mini KIT (*Geneaid*) while samples from the hair follicle and skin tissue were isolated using the gSYNC™ DNA Mini Kit (*Geneaid*). The primer was designed using Primer 3 online programs with the 4.0 version ([http://primer3. UT. ee/](http://primer3.ut.ee/)) based on data from a mitochondria genome sequence of *Capra hircus* (KF952601). The mtDNA D-loop region primer sequences were as follows, SPF: 5'-GCCAATC TCCCTAAGACTCA-3' and SPR: 5'-CATCTAGGCATTTTCAGTGC-3'. The PCR conditions were: The DNA template 3 µL, Kapa ready mix 25 µL, each forward primer and reverse 1 µL and ddH₂O in a total of 50 µL. The PCR amplification were conducted using a Infinigen Thermal Cycler according to program, initial denaturation at 94°C for 6 min, followed by 35 cycles, each consisting of 30 sec denaturation at 94°C, 45 sec primers annealing at 48°C, 90 sec elongation at 72°C, then a final 5 min elongation at 72°C and storage at 4°C. The PCR product was visualized using 1% gel agarose (5 µL PCR products added with 2 µL loading dye). Electrophoresis was run on 100 mV condition for 60 min and the amplification result could be seen on UV light. The purified PCR products were sequenced directly by 1st BASE Sequencing INT Malaysia.

Data analysis: Length of amplification product D-loop region sequence is 1212 bp for each individual and analyzed using the MEGA version 6.0 software (Tamura *et al.*, 2013). D-loop region sequences native Indonesian goats aligned along with the D-loop sequences of *Capra hircus* from GenBank by using Clustal W in the MEGA program. Analysis of genetic variation seen by nucleotide sequence differences D-loop region. Genetic distances were analyzed by the method of Kimura with two parameters (Kumar *et al.*, 2001). Genetic diversity and haplotype diversity analyzed by DNASP5 software. Phylogenetic tree (Neighbor-joining tree) is constructed based on mtDNA D-loop sequences by bootstrap test 1000x repetition (Felsenstein, 1985) and Kimura 2-parameter method (Kimura, 1980). Statistical analysis was performed by using software DNASP5.0.

In addition, to provide more complete information about Indonesian goats origin, then some mtDNA D-loop sequences of wild and domesticated goat downloaded from GenBank and used as a comparison native Indonesian goat to look at the genetic distance and assumed their origin. The access code is listed in Table 2.

RESULTS

Variations and haplotypes based on mtDNA D-loop complete sequences of Indonesian goats. All Indonesian goats have a nucleotide length 1212 bp while, *C. aegagrus* (AB004082) and

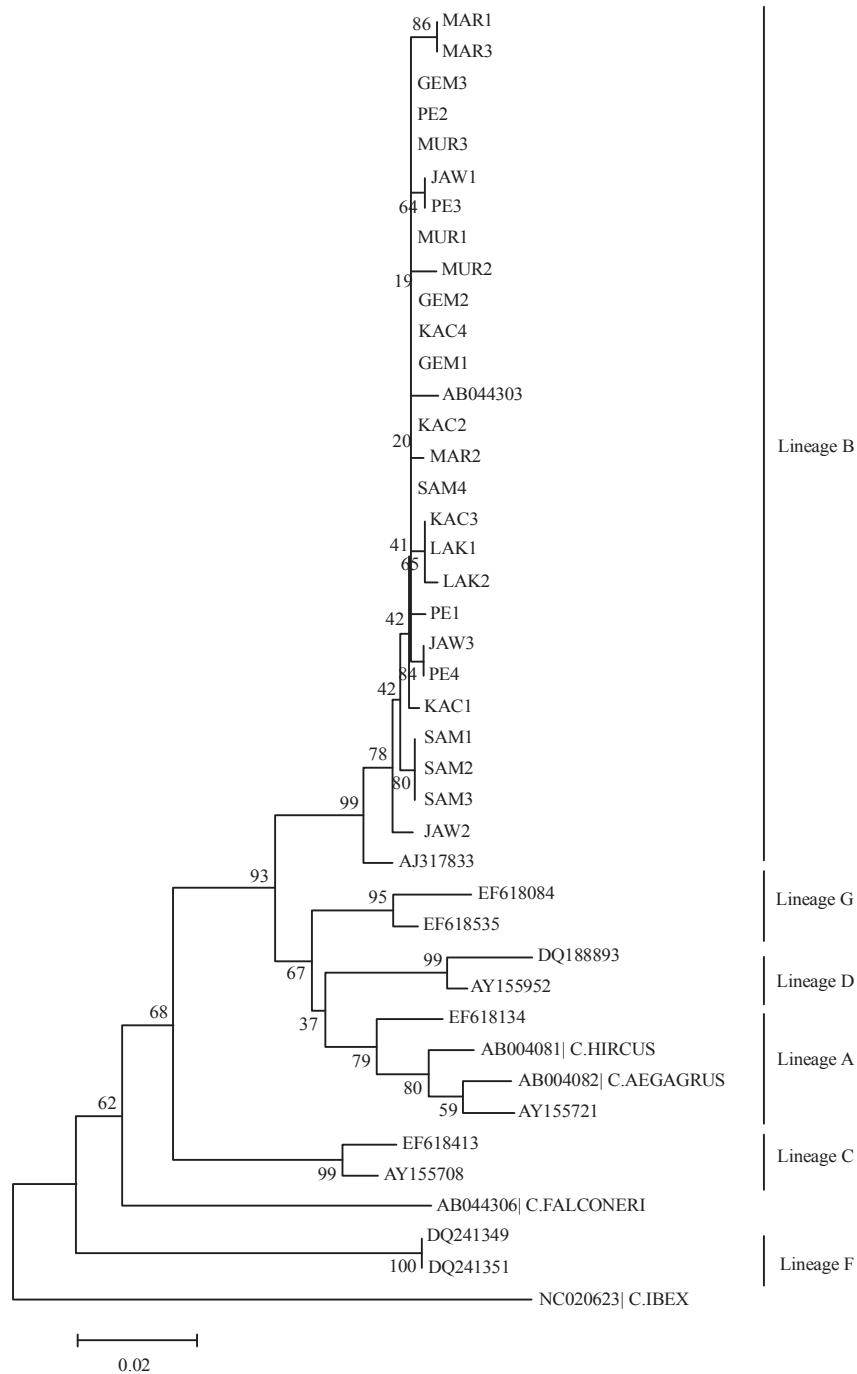


Fig. 3: Phylogenetic tree constructed from the neighbor-joining method using the Kimura 2-parameter model and assumes $\alpha = 0.29$. The number at the branch node indicates the percentage occurrence in 1000 bootstrap replications. The bar scale indicates the genetic distance among mtDNA D-loop haplotypes

and AB044306) and some *Capra hircus* which have different haplogroups (lineage A, B, C, D, F and G). The whole goat from Indonesia into the lineage B (Fig. 3). The 26 individuals as representatives

Table 3: Estimates of evolutionary divergence over sequence pairs between groups

Populations	CH	SAM	MUR	PE	JAW	KAC	LAK	GEM	MAR	CA	CF
CH		0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.007	0.003	0.016
SAM	0.035		0.002	0.002	0.002	0.003	0.002	0.002	0.002	0.007	0.014
MUR	0.035	0.003		0.000	0.000	0.002	0.001	0.001	0.001	0.007	0.013
PE	0.035	0.003	0.000		0.000	0.002	0.001	0.001	0.001	0.007	0.013
JAW	0.035	0.003	0.000	0.000		0.002	0.001	0.001	0.001	0.007	0.013
KAC	0.041	0.008	0.005	0.005	0.005		0.002	0.002	0.003	0.008	0.014
LAK	0.037	0.005	0.002	0.002	0.002	0.007		0.001	0.002	0.007	0.013
GEM	0.039	0.006	0.003	0.003	0.003	0.008	0.004		0.002	0.007	0.013
MAR	0.042	0.009	0.006	0.006	0.006	0.011	0.008	0.008		0.007	0.014
CA	0.008	0.038	0.038	0.038	0.038	0.044	0.041	0.042	0.045		0.017
CF	0.108	0.089	0.087	0.087	0.087	0.095	0.090	0.089	0.095	0.112	

CH: *C. hircus*, CA: *C. aegagrus*, CF: *C. falconeri*, SAM: Samosir goat, MUR: Muara goat, PE: Peranakan etawah goat, JAW: Jawarandu goat, KAC: Kacang goat, AK: Gembrong goat, MAR: Marica goat, number of base substitutions per site from averaging over all sequence pairs between groups are shown. Standard error estimate(s) are shown above the diagonal. Analyses were conducted using the Kimura 2-parameter model. The rate variation among sites was modeled with a gamma distribution (shape parameter = 0.29)

Table 4: Maximum likelihood estimate of substitution matrix

Parameters	A	T/U	C	G
A	-	4.09	3.74	9.18
T/U	4.51	-	19.79	1.99
C	4.51	21.62	-	1.99
G	20.75	4.09	3.74	-

Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in normal

from Indonesian goats provides evidence that comes from one origin ancestor (maternal origin). Eight sub-population of native Indonesian breeds is specific to the area of livestock development.

Genetic distance goat population based on mtDNA D-loop in Indonesian goats: The value of genetic distance between groups of native Indonesian goats ranged from 0-0.011 (Table 3). The highest genetic distance between populations goat Indonesia is MAR with KAC. Some populations have a very close genetic distance that PE: MUR; JAW: MUR; JAW: PE. Indonesia goat population has sufficient genetic distance away with *Capra hircus* (AB004081) is 0.035-0.042. Indonesia goat genetic distance value against wild goat *Capra aegagrus* (AB004082) is in the range 0.0038-0.045 while, against *Capra falconeri* (AB044306) is 0.087-0.095.

Each entry is the probability of substitution (r) from one base (row) to another base (column). Substitution pattern and rates were estimated under the Tamura *et al.* (2013) model. Rates of different transitional substitutions are shown in bold and those of transversional substitutions are shown in normal (Table 4). Relative values of instantaneous r should be considered when evaluating them. For simplicity, sum of r values is made equal to 100, the nucleotide frequencies are A = 31.46, T/U = 28.52, C = 26.11% and G = 13.91%. For estimating ML values, a tree topology was automatically computed. The maximum Log likelihood for this computation was 2096.124. The analysis involved 26 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data were eliminated. There were a total of 1212 bp positions in the final dataset. Evolutionary analyses were conducted in MEGA6.

Population genetic structure of mtDNA D-loop in Indonesian goat: Parameter of population genetic structure of mtDNA D-loop in eight sub population Indonesian goats shown in Table 5. The haplotype diversity and nucleotide diversity of mtDNA D-loop in eight Indonesian native goats were 0.500-1.000 and 0.248-1.1595%, respectively. The two indices reflecting population genetic diversity revealed abundant haplotype type and genetic diversity in eight Indonesian native goat breeds.

Table 5: Genetic diversity of mtDNA D-loop in Indonesian native goat

Breeds	Sample size	Number of haplotypes	Haplotype diversity	Nucleotide diversity
Samosir goat	4	2	0.500±0.265	0.00248±0.00131
Muara goat	3	2	0.667±0.314	0.00110±0.00052
PE goat	4	4	1.000±0.177	0.00124±0.00030
Jawarandu goat	3	3	1.000±0.272	0.00440±0.00132
Kacang goat	4	4	1.000±0.177	0.01595± 0.00422
Lakor goat	2	2	1.000±0.500	0.00248±0.00124
Gembrong goat	3	3	1.000±0.272	0.00440±0.00122
Marica goat	3	3	1.000±0.272	0.00935±0.00312

PE: Peranakan etawah

DISCUSSION

Goat breeds in Indonesia: Indonesia has several breeds of goats that have a difference in phenotype and has been developed in Indonesia. Samosir goat came from the Samosir island and has a specific color that has white fur all over the body and has a small body conformation. Muara goat is a result of crossbreeding Boer goat with Kacang goat on the Dutch colonial era in Indonesia. This goat has a more compact body conformation and the livestock producers of meat. The PE goats are a result of crossbreeding Etawah goat (India) with Kacang goat in ancient times. This goat has a pattern of black and white coat color is uniform and as a producer of milk. Jawarandu goat is a result of crossbreeding PE goat with Kacang goat. This breed has a body conformation similar to the PE goat. Kacang goat is a goat that spread to almost all regions of Indonesia. This breed has a small body and very adaptable to various environmental conditions. Lakor goat has a population of slightly and only in Lakor Island. This breed has light brown fur color and still maintained traditionally is released into the forest. Gembrong goat is the goat of the most unique phenotype. This breed has a white coat color and very long, so it can be used as a producer of goat hair. Marica goat has phenotype almost same with Kacang goat and goat breeds is already endangered.

Genetic diversity of mtDNA D-loop in Indonesian goats: Mitochondria DNA D-loop diversity is very high, it is likely in part the result of the mutation rate control region is high. Sequent complete of mtDNA D-loop is 1212 bp of the eight sub-population Indonesian goat showed a very close genetic distance. This provides evidence that the Indonesian goats originated from a common ancestor and all sub-populations belong to the lineage B. There is some genetic variants of mtDNA D-loop sequences are transitions, transversions, insertions and deletions. In this study using 26 individuals showed 68 polymorphic sites and 21 haplotypes.

Nucleotide diversity and haplotype diversity of mtDNA D-loop region are the important indices for assessing population polymorphism and genetic differentiation. Haplotype diversity and the nucleotide diversity of mtDNA is greater, then the polymorphism of the population will be higher. Batubara (2011) states that the nucleotide diversity mtDNA is 0.1-0.8% in six goat breeds native to Indonesia. In this study stated that the nucleotide diversity of mtDNA D-loop in eight goat breeds native to Indonesia is 0.248-1.1595%. This shows that the genetic diversity of Indonesian goats is abundant. This difference may be due to the difference in length of the amplification of mtDNA region.

The Origin of the domestic Indonesian goats: The phylogenetic tree showed that all the goats Indonesia joined in one lineage (Fig. 3). Based on previous studies, said there were some domestic goat lineage throughout the world. All domestic animals originated from wild animals. There is some wild goat which still survives to this day and is thought to be an ancestor of goat

domestication. Many authors suspect that *Capra aegagrus* and *Capra falconeri* the strongest candidates as ancestor *Capra hircus* (Mason, 1981; Tu, 1989; Xie, 1985; Yu, 1991, Kang *et al.*, 2011). But we cannot prove clearly that *C. aegagrus* and *C. falconeri* is the ancestor of goat domestication. This is due to that mtDNA has limited information and sample size used. Indonesian goat breeds have a genetic distance closer to *C. aegagrus* than *C. falconeri*. Luikart *et al.* (2001) examined 406 individuals from 88 breeds partially sequenced the mtDNA Hyper Variable Region (HVR) in distributed across the world and defined lineages A, B and C of which lineages B detected in India, Malaysia, Mongolia and Pakistan. Liu *et al.* (2006) examined 50 mtDNA HVR (481 bp) sequences of lineage B from six Asian goats, which were classified into two subclasses and considered that lineage B.

Naderi *et al.* (2007) investigated 2430 goats from different countries and shows six very different groups according to mitochondrial haplogroup are referred to as A, B, C, D, F and G. The results of the research show that the haplogroup A is at most at 53%. A haplogroup spread almost all over the world and is the first haplogroup. Haplogroup B is found throughout most of Asia and a small portion coming from Sub-Saharan Africa and goat Europe from Greece. Goats of haplogroup C is from across Asia and Europe and haplogroup D is present throughout Asia and Northern Europe. Group F goat came from Sicily and the group G is present in the Middle East and North Africa.

CONCLUSION

Sequent complete of mtDNA D-loop is 1212 bp in eight Indonesian goat breeds of the 26 individuals goat declared 68 polymorphic sites and 21 different haplotypes. Haplotype diversity and nucleotide diversity based on mtDNA D-loop in eight breeds of Indonesian goats is 0.5000-1.000 and 0.248-1.1595%, respectively. The Value of genetic distance between groups of native Indonesian goats ranged from 0-0.011. Indonesia goat genetic distance value against wild goat *Capra aegagrus* (AB004082) is in the range 0.0038-0.045 while, against with *Capra falconeri* (AB044306) is 0.087-0.095. The all Indonesian goat breeds, including to the haplogroup B (lineage B).

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