Phylogenetical Relationships between Kejobong Goat and Other Goats Based on Mt-DNA D-loop Sequence Analysis

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ABSTRACT

The aim of this study was to investigate phylogenetical relationships between Kejobong goat and Kacang goat as well as Etawah Grade goat using genetic diversity, haplotype, and genetic distance analysis based on D-loop sequences. A total of 76 blood samples belonged to three local goats, namely Kacang goat (KC), Etawah Grade goat (EG), and Kejobong goat (KJ). The DNA genome was extracted based on the manufacturer's standard protocol using gSYNC DNA Mini Kit (Geneaid Biotech Ltd.) for sequence analysis control region (D-loop) in mitochondrial DNA (mtDNA) using specific primer. The results revealed that there were a total of 36 nucleotide substitutions, those were 1 indel (insertion or deletion), 34 haplotypes with Pi = 0.00253±0.00029 and Hd = 0.925±0.018 in three local goats, while intra-breed in this study showed Pi value of EG, KC, and KJ were 0.00452±0.00077, 0.00214±0.00028 and 0.00143±0.00018, respectively and Hd value were 0.985±0.025, 0.926±0.032, and 0.855±0.044, respectively. Genetic distances between KJ and KC, KJ and EG, and between KC and EG were 0.0018; 0.0034, and 0.0036, respectively. The highest NHap was observed in KC (17), followed by EG (15) and KJ (11); and all three local goats were in the same cluster in phylogeny tree. In conclusion, genetically, Kejobong goat is a crossbred of Kacang goat and Etawah Grade goat and has closer genetic relationships to Kacang goat than to Etawah Grade goat.

Keywords: genetic diversity, haplotype, genetic distance, d-loop, local goat

INTRODUCTION

Goat (*Capra hircus*) is one of the earliest domesticated farm animals and it plays an important role in agricultural, economic, and cultural sides for all human civilizations (Joshi *et al.,* 2004). The domestication of goats in the region spread throughout the world along terrestrial and maritime routes of migration and commerce (Piras *et al.,* 2012). Goats most likely descend from the wild Bezoar (*Capra aegagrus*) (Naderi *et al.*, 2007; Naderi *et al.*, 2008) and also one of the most important livestock species in the world for providing good animal production such as meat, milk, and fiber even under harsh environmental condition (Zeuner, 1963; Mason, 1984; Lin *et al.,* 2013). As one of the tropical countries with high level of biological diversity (biodiversity) in the world, Indonesia has many breeds of local goat. Many of them were Kacang goat (KC), Etawah Grade goat (EG), and Kejobong goat (KJ). KC and EG are the most commonly found in Indonesia (Batubara *et al.,* 2011; Lestari *et al.,* 2017). While KJ only can be found in Central Java, Indonesia at exactly in Purbalingga District. KJ has been recently recognized as "rumpun" by decree No 301/Kpts/SR.120/5/2017 (Ministry of Agriculture, 2017).

It was assumed that KJ was a crossbred of KC and EG goat that experienced phenotypic selection from generation to generation by the local farmers leading to black hair color (Kurnianto *et al.,* 2012; Kurnianto *et al.,* 2013). Studies about KJ have been done in resource characterization (Sodiq, 2009), genotype and phenotype characteristics (Kurnianto *et al.,* 2012), morphology (Kurnianto *et al.,* 2013; Suryani *et al.,* 2013), sperm characteristic (Wibowo *et al.*, 2013), hair color (Permatasari *et al.,* 2014), productivity, and hair color patterns (Setiaji *et al.,* 2013), characteristics of mtDNA (Jiyanto *et al.,* 2014; Harlistyo *et al.,* 2014) and prolification (Febriana *et al.,* 2017). However, study about phylogenetical relationship information of KJ with KC and EG is still lack.

The phylogenetical relationship can be discovered through gene sequence analysis by knowing their genetic diversity, haplotype, and genetic distance (Liu *et al.,* 2013). On the other hand, information about genetic diversity and haplotype is important to support conservation and breeding program. Analysis of genetic diversity can provide information about domestication events, relationship among breeds, the loss of within-breed genetic diversity, and breed structure. They are also important to establish conservation priorities (Toro

et al., 2009). In recent years, studies on characterizing genetic diversity in goat breeds from some different countries and regions have been conducted by researchers. Among the studies are such as in Chinese goat (Wang *et al.*, 2008), Sardinian goat (Piras *et al.*, 2012), Arabian goat (Al-Araimi *et al.*, 2017), East Asian goat (Lin *et al.*, 2013), Moroccan goat (Benjelloun *et al.*, 2011), Egyptian goat (Othman & Mahfouz, 2016; Ahmed *et al.*, 2017), African goat (Kibegwa *et al.*, 2016; Githui *et al.*, 2016; Awotunde *et al.*, 2015; Royo *et al.*, 2009), American goat (Amills *et al.*, 2009), Anatolian goat (Akis *et al.*, 2014), Pakistani goat (Naqvi *et al.*, 2017), and genetic diversity information in animal farm has been reviewed recently by Groeneveld *et al.* (2010).

Previous researchers had been largely applied mitochondrial DNA (mtDNA) polymorphism, especially the displacement loop (D-loop) or the control region to understand evolution and to identify maternal lineage of modern domestic goats. D-loop has represented the most informative genomic element to study diversity in both closely related species and individuals within species, mainly because this gene is maternal inheritance without recombination and has high mutation rate (Upholt & David, 1977). Many studies based on the D-loop analysis had been largely used to understand genetic diversity, haplotype diversity, genetic distance, and phylogenetical relationships (Chen *et al.*, 2005; Joshi *et al.*, 2004; Luikart *et al.*, 2001; Sultana *et al.*, 2003). Study of D-Loop in animal farm in Indonesia have been done in goats (Batubara *et al.*, 2011; Harlistyo *et al.*, 2014; Pakpahan *et al.*, 2015), duck (Purwantini *et al.*, 2013; Purwantini & Ismoyowati, 2014; Susanti *et al.*, 2017ª; Susanti *et al.*, 2017ᵇ), chicken (Sulandari *et al.*, 2008; Zein & Sulandari, 2008; Zein & Sulandari, 2009; Sulandari & Zein, 2009) and cattle (Abdullah *et al.*, 2012; Sari *et al.*, 2016). Thus, based on the reason above, this study was aimed to investigate the phylogenetical relationships between Kejobong goat, Kacang goat, and Etawah goat based on genetic diversity, haplotype, and genetic distance analysis using D-loop sequences.

MATERIALS AND METHODS

Sample Collection

A total of 76 blood samples belonged to three breeds of Indonesian local goat were collected from Purbalingga regency, Kendal regency, and Grobogan regency, Central Java Province (Figure 1) (Table 1). Purposive sampling was used to determine the samples. Samples were determined based on some criteria. Firstly, the samples were based on research location that had the largest population and as the development area of each breed; secondly, the samples were 1-2 years old goats; thirdly, all the samples on each breed had no genetic relationship one another. Phenotype appearance of each breed that taken as sample refers to Ministry of Agriculture decree number 2840/Kpts/LB.430/8/2012 (KC: Small and short body posture; white, black, brown hair color or a combination of all three; small head with a straight nose profile, sword-shaped horn, and hanging ears); 695/Kpts/PD.410/2/2013 (EG : Long and tall body

posture; white, black, brown hair color combination and longer in the neck and hips, convex nose profile; curved backwards horn; long and dropping ear); 301/Kpts/SR.120/5/2017 (KJ : Bigger body posture than KC but smaller than EG; black hair color or black with a white stripe, slightly convex nose profile, curved backwards horn; hanging outward and not folding ear)

Blood was taken using 3 cc syringe through *jugular venous* that was cleaned with alcohol before. The blood was then collected in vacutainers tubes with anticoagulant (EDTA), and then was stored in a cool box containing ice gel and transported to the laboratory for analysis. The DNA genome were extracted based on the manufacturer's standard protocol using gSYNC DNA mini kit (Geneaid Biotech Ltd.) for sequence analysis control region (D-loop) in mitochondrial DNA (mtDNA).

Polymerase Chain Reaction and Sequencing

The Control region mtDNA were amplified directly from the genomic DNA by *Polymerase Chain Reaction* (PCR). The primers were used SPF (5'-GCCAATCTCCCTAAGACTCA-3') and SPR (5'-CATCTAGGCATTTTCAGTGC-3') (Pakpahan *et al.*, 2015) that generated 1342 bp of PCR product. The PCR reaction consisted of 3 µL DNA template, 25 µL KAPA2G Fast Ready Mix + Dye (Kapa Biosystems Ltd.), 1 µL forward primer, 1 µL reverse primer, and 20 µL ddH2O. PCR used Infinigen Thermal Cycler with condition : pre-denaturation at 94°C for 5 min, followed by 35 cycles, each consisting of denaturation at 94°C for 30 sec, primers annealing at 50°C for 45 sec, elongation at 72°C for 90 sec, then final elongation at 72°C for 5 min. PCR product was stored at 4°C and 1% Agarose gel

Table 1. Number of sample and sample origin

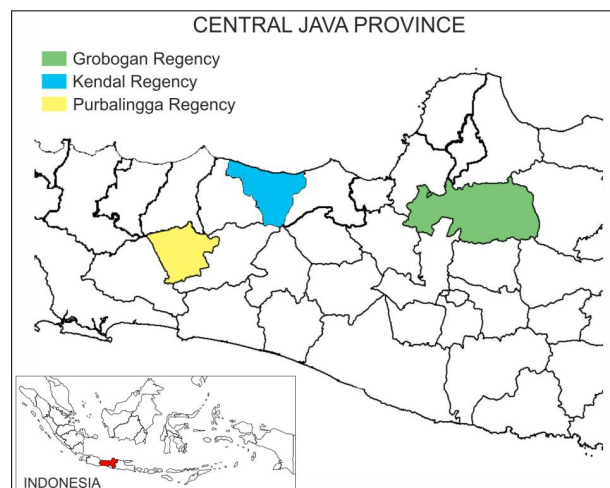| Breed	n	Origin
Kacang (KC)	30	Grobogan Regency
Kejobong (KJ)	29	Purbalingga Regency
Etawah Grade (EG)	17	Kendal Regency

Note: n= number of sample

Figure 1. Sampling location

was used to visualize it. Electrophoresis was performed at 100 V for 20 min. The result of amplification could be seen on UV light and sequenced by 1st Base-Asia, Malaysia.

Data Analyses

Genetic diversity and genetic distance. A total of 1191 bp of 76 mtDNA D-loop sequences were used to identify genetic diversity and genetic distance. Data were analyzed using Molecular Evolutionary Genetics Analysis (MEGA) version 6.0 (Tamura *et al.*, 2013). Alignment of sequences was achieved using the Clustal W program (Thompson *et al.*, 1994). Genetic distance among breed was calculated by Kimura 2-parameter model (Kimura, (1980). The number of nucleotide variable sites (V), singleton site (S), parsimony site (P), nucleotide diversity (Pi), and haplotype diversity (Hd) were calculated using DNA Sequence Polymorphism (DnaSP) version 5.1 (Librado & Rozas, 2009).

Haplotype analysis and phylogenetical relationships. Number of haplotypes (NHap) and type of haplotype were calculated using DnaSP version 5.1 (Librado & Rozas, 2009). Phylogeny tree was constructed by Neighbor-Joining (NJ) using MEGA version 6.0 (Tamura *et al.*, 2013) based on seventy-six sequences from this study and forty sequences of other goat breeds in the world that originated from GenBank as comparator. Phylogeny tree used to demonstrate genetic relationship of KJ, KC, and EG and their haplogroups. The forty sequences that originated from GenBank were consisted of fifteen from lineage A (EF618134; AB004081; AB004082; AY155721; AJ317563; AJ317593; AJ317633; AJ317663; AJ317693; AJ317733; AJ317763; AJ317793; AJ317801; AJ317815; AJ317825), nine from lineage B (AB044303; AJ317826-33), nine from lineage C (EF618413; AY155708; AJ317834-40), two from lineage D (DQ188893; AY155952), three from lineage F (DQ241349; DQ241351; NC020623), and two from lineage G (EF618084; EF618535) as out-group using the Kimura 2-parameter model with 1000 bootstrap replications (Saitou & Nei, 1987; Felsenstein, 1985; Kimura, 1980).

RESULTS

Genetic Diversity and Genetic Distance

A total of 1191 bp of 76 mtDNA D-loop sequences were obtained from KJ, KC, and EG. The analysis revealed a total of 36 nucleotide substitutions, those were 1 indel (insertion or deletion), 34 haplotypes with Pi = 0.00253±0.00029 and Hd = 0.925±0.018. While intra-population, diversity measures calculated for each goat breed are presented in Table 2. Analysis of mtDNA D-loop sequences identified that EG had the highest variable sites, there were 27 variable sites, consist of 16 singleton sites and 11 singleton sites. Then followed by KC that had 16 variable sites with 8 singleton sites and 8 parsimony sites. While KJ had 12 variable sites that consisted of 8 singleton sites and 4 parsimony sites. Singleton and parsimony sites positions were presented in Table 3 and each goat breed in this study had 1 indel (Table 4). Among the population, the highest Pi and Hd were observed in EG and the lowest was in KJ. Pi value of EG, KC and KJ were 0.00452±0.00077, 0.00214±0.00028, and 0.00143±0.00018 respectively, and Hd value were 0.985±0.025, 0.926±0.032, and 0.855±0.044 respectively. While the highest NHap was observed in KC (17), followed by EG (15) and KJ (11). Genetic distance analysis results among KJ with KC and EG based on the 1191 bp D-loop sequence are presented in Table 5. Results showed that genetic distance between KJ and KC was 0.0018, whereas between KJ and EG was 0.0034. While genetic distance between KC and EG was 0.0036.

Haplotype and Phylogenetical Relationship

In this study, the 36 polymorphisms observed in three goat populations classified sequences into 34 haplotypes that are shown in Table 4 and sample identity in Table 6. Point mutation in this study was observed as many as 85.71 % transition and 14.29 % transversion, and there was an indel in sites 1075. H2, H21, and H34 went through C insertion, while in other haplotypes went through deletion. Three breeds of local goats in

Table 3. Singleton and parsimony site on mtDNA D-loop in three Indonesian local goats

Breed	Singleton site position	Parsimony site position
KC	404, 535, 543, 562, 607, 632, 1110, 1189	457, 469, 483, 610, 613, 1005, 1074, 1079
KJ	24, 520, 543, 607, 632, 783, 937, 1061	457, 469, 483, 610
EG	479, 487, 543, 607, 610, 632, 636, 895, 924, 971, 1005, 1018, 1047, 1062, 1105, 1161	457, 469, 480, 482, 483, 562, 1074, 1079, 1081, 1110, 1189

Note: KC= Kacang goat; KJ= Kejobong goat; EG= Etawah Grade goat.

Tabel 2. Genetic diversity in three Indonesian local goats

Breed	n	V	S	P	Indel	NHap	Pi (±SD)	Hd (±SD)
All	76	35	-	-	1	34	0.00253 (±0.00029)	0.925 (±0.018)
KC	30	16	8	8	1	17	0.00214 (±0.00028)	0.926 (±0.032)
KJ	29	12	8	4	1	11	0.00143 (±0.00018)	0.855 (±0.044)
EG	17	27	16	11	1	15	0.00452 (±0.00077)	0.985 (±0.025)

Note: All = three breeds of Indonesian local goat; KC = Kacang goat; KJ = Kejobong goat; EG = Etawah Grade goat; n = number of sample/sequence, V = number of variabel site, S = number of singleton site, P = number of parsimony site, Indel = number of insertion or deletion site, NHap = number of haplotype, Pi = nucleotide diversity value, Hd = haplotype diversity value.

Table 4. Polymorphic sites on mtDNA D-loop in three Indonesian local goats

Haplotype	244	404	457	469	479	480	482	483	487	520	534	535	562	601	613	632	638	683	895	924	937	971	1015	1018	1047	1061	1062	1074	1079	1081	1105	1110	1150	1161	1189	Indel 1075
H1	A	A	A	T	A	A	A	A	T	C	G	T	G	T	T	T	C	C	C	C	C	C	A	A	C	C	A	C	T	C	A	C	A	T	G	-
H2	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
H3	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H4	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H5	.	.	.	.	.	.	.	G	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H6	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H7	.	G	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H8	.	.	.	.	.	.	.	G	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H9	.	.	.	.	.	.	.	G	.	.	.	C	.	.	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H10	.	.	C	.	.	.	.	.	.	.	.	C	.	T	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H11	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H12	.	.	.	.	.	.	.	.	.	.	.	C	A	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H13	.	.	.	.	.	.	.	.	T	.	.	C	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H14	.	.	.	.	.	.	.	.	.	C	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H15	.	.	.	.	.	.	.	G	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H16	.	.	.	.	.	.	.	.	.	.	.	C	.	T	.	.	C	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H17	.	.	.	.	.	.	.	G	.	.	.	C	.	T	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H18	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H19	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
H20	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	-
H21	G	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C
H22	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	-
H23	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	-
H24	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	T	.	C	T	.	.	-
H25	.	.	T	.	G	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	T	.	C	.	T	.	-
H26	.	.	T	.	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	.	C	.	.	.	-
H27	.	.	.	.	.	A	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	-
H28	.	.	.	.	A	A	.	G	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	.	C	C	T	.	.	-
H29	.	.	.	.	.	G	.	G	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	T	T	.	C	T	C	.	-
H30	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	-
H31	.	.	.	.	.	.	.	.	.	T	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	.	C	-
H32	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	-
H33	.	.	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	T	T	.	T	T	.	.	.	.	.	.	.	.	.	.	.	.	-
H34	.	.	C	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	.	.	.	.	C	C	C	.	.	.	.	.	.	.	.	C

Table 5. Genetic distance based on mtDNA D-loop in three Indonesian local goats

Breed	KC	KJ	EG
KC	0.0000		
KJ	0.0020	0.0000	
EG	0.0040	0.0030	0.0000

Note: KC= Kacang goat; KJ= Kejobong goat; EG= Etawah Grade goat.

Table 6. Haplotype of three breeds of Indonesian local goats based on mtDNA D-loop

Haplotype	N	Breeds (Number of sample within each breed)
H1	1	KC (1)
H2	6	KC (2), KJ (2), EG (2)
H3	10	KC (4), KJ (4), EG (2)
H4	1	KC (1)
H5	16	KC (7), KJ (9)
H6	5	KC (3), KJ (2)
H7	7	KC (1), KJ (6)
H8	1	KC (1)
H9	1	KC (1)
H10	1	KC (1)
H11	1	KC (1)
H12	1	KC (1)
H13	2	KC (2)
H14	2	KC (1), KJ (1)
H15	2	KC (1), EG (1)
H16	1	KC (1)
H17	1	KC (1)
H18	1	KJ (1)
H19	1	KJ (1)
H20	1	KJ (1)
H21	1	KJ (1)
H22	1	KJ (1)
H23	1	EG (1)
H24	1	EG (1)
H25	1	EG (1)
H26	1	EG (1)
H27	1	EG (1)
H28	1	EG (1)
H29	1	EG (1)
H30	1	EG (1)
H31	1	EG (1)
H32	1	EG (1)
H33	1	EG (1)
H34	1	EG (1)

N = number of haplotype

this study were defined into 34 haplotypes and H5 was the most common (Table 6). It contained 16 sequences from KC and KJ. Whereas H2 and H3contained from whole breeds, as many as 6 and 10 sequences respectively. A total 12 of 34 haplotypes were only found in EG (H23 until H34) and it was the largest number among the other breeds in this study. While 10 haplotypes (H1, H4, H8, H9, H10, H11, H12, H13, H16, H17) were observed in KC and 5 haplotypes (H18, H19, H20, H21, H22) were observed in KJ. The phylogeny tree showed that KJ, KC, and EG were in the same cluster and there

was no clear grouping in each breed (Figure 2). Those three goats were in the same cluster with other goat breed from Genbank in B Haplogroup.

DISCUSSION

Genetic Diversity and Genetic Distance

Based on genetic diversity analysis, the result was different from some previous researches and no one of them has indel site. Batubara *et al.* (2011) reported based on 957 bp D-loop sequence that KC had 4 haplotypes with 6 variable sites. While study conducted by Pakpahan *et al.* (2015) reported a total 1212 bp of D-loop sequence were alignment, then KC had 34 variable sites while EG had 3 variable sites and each of them had 4 haplotypes. Harlistyo *et al.* (2014) revealed based on 548 bp D-loop sequence, there were 11 variable sites in KJ which formed 7 haplotypes. These different results might be caused by the research method including analysis method, sample location, and the number of sample (Jia *et al.*, 1999; Liu *et al.*, 2006; Liu *et al.*, 2007; Zhao *et al.*, 2011)

Genetic diversity (Pi) value in this study was in low category while haplotype diversity (Hd) value was in high category. According to Nei (1987), genetic diversity (Pi) value ranged from 0.01 to 0.001 and defined into 3 categories i.e., high category ranged from 0.008 to 0.01, medium category ranged from 0.005 to 0.007, and low category ranged from 0.001 to 0.004. Meanwhile, haplotype diversity value (Hd) in range ≥0<0.5 was included in low category, while Hd value in range >0,5≤1 was included in high category. The higher haplotype diversity, the higher genetic diversity will be and vice versa. Haplotype diversity and nucleotide diversity of mtDNA are two important indices for assessing population polymorphism and genetic differentiation (Pereira *et al.*, 2005; Liu *et al.*, 2007; Zhao *et al.*, 2011). Liu *et al.* (2006) and Liu *et al.* (2007) revealed that the bigger the value of haplotype diversity and nucleotide diversity of mtDNA, the higher the population polymorphism. Low genetic diversity indicates the presence of inbreeding. This condition is caused by the random animal mating (uncontrolled mating) which is often conducted by tranditional farmers. Inbreeding will lead to a depression of life and reproduction (Charlesworth & Willis, 2009). The potency of inbreeding will continue in the event of random mating among animals that already has extremely close genetic distance. This condition may lead to the depression of biological fitness (inbreeding depression) such as homozygous individuals, mutation defects from bottlenecks effects, recessive alleles, and imbalances of gene flow (Wright et al., 2008; Pekkala *et al.*, 2014). Based on genetic distance analysis indicated that KJ was closer with KC than EG genetically. This result was in parallel to the report of Suryani *et al.* (2013) that KJ had a closer relationship with KC than EG based on cranium measurement analysis. Yet another research reported that morphologically, KJ was in the same group with EG outside of KC group (Kurnianto *et al.*, 2013).
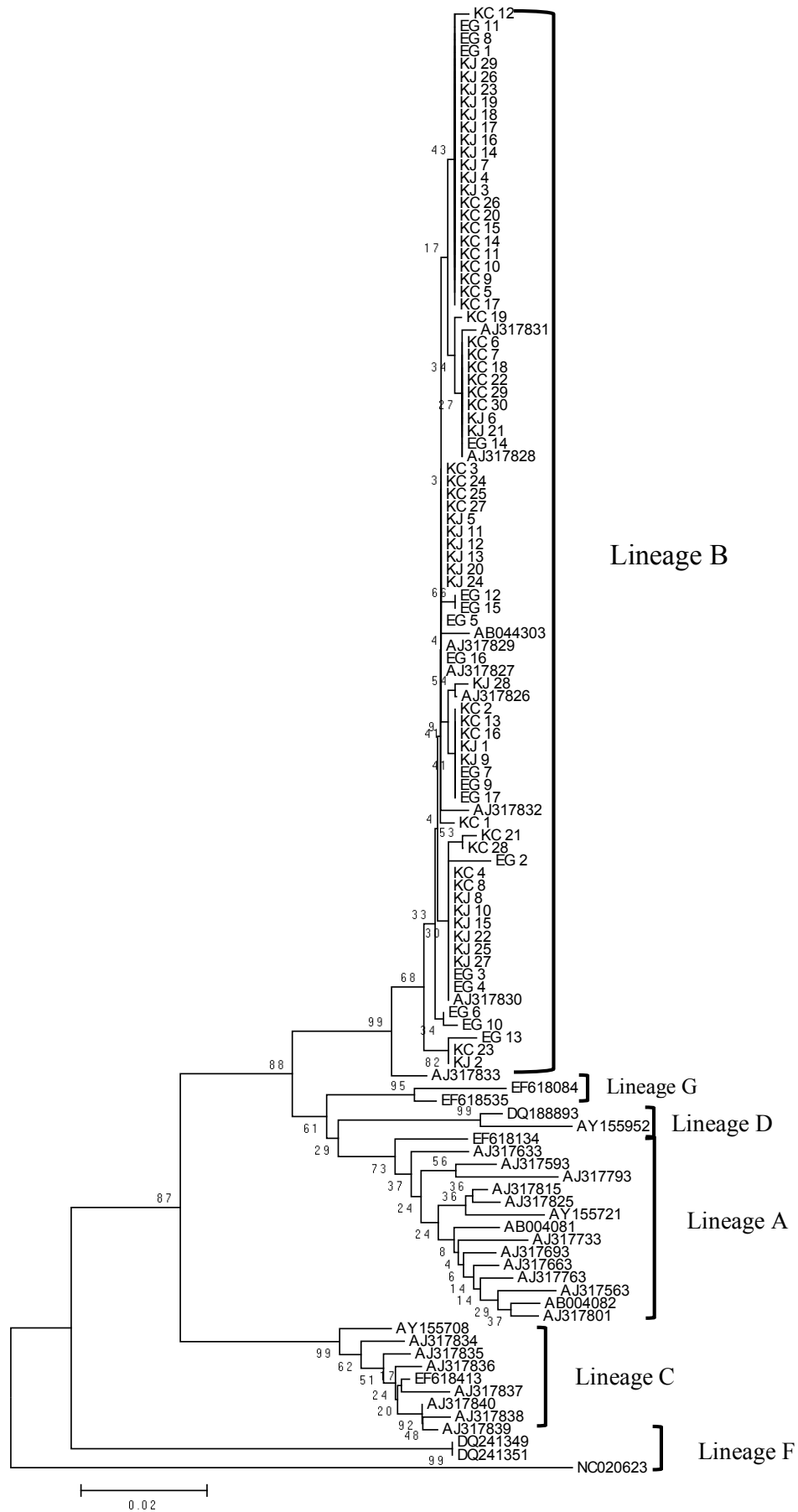
Figure 2. Phylogeny tree of three Indonesian local goats compared to other goats from different lineages

Haplotype Analysis and Phylogenetical Relationship

Haplotype analysis showed some of the same haplotypes among KJ, KC, and EG. This haplotype similarity could be created as an evidence that KJ was a crossbreed of KC and EG. Hence, phylogeny tree demonstrated that KJ, KC, and EG were randomly scattered in the same cluster together (Figure 2). Haplotype was formed since the presence of point mutation. Therefore, the same point mutation position in a sequence sample will lead to the same haplotype. Shared haplotypes of goat breed in this study may come from ancient haplotypes, divergence or genetic flow in the goat population (Liu *et al.* 2006; Pakpahan *et al.* 2015). This study proved that KJ, KC, and EG were belong to B haplogroup (Figure 2). It was reported by Lin *et al.* (2013) that mtDNA analysis of Eastern Asian goats revealed the predominant lineage B in Southeast Asia. Haplogroup is a group of haplotypes sharing a common ancestor. This group has been through a single-nucleotide polymorphism mutation. There are 6 number of haplogroups on domesticated goat in the world, those are A, B, C, D, F, and G lineages (Luikart *et al.*, 2001; Mannen *et al.*, 2001; Chen *et al.*, 2005; Fan *et al.*, 2007; Naderi *et al.*, 2007). Luikart *et al.* (2001) reported the most common and widely distributed lineage across all continents was lineage A and it went through a relatively ancient population expansion. Meanwhile, lineage B was found mostly in eastern and southern Asia covering China, Mongolia, Laos, Pakistan, India, and Malaysia. The observation of lineage C was together with samples from Mongolia, Switzerland, and Slovenia. Lineage D is rare. It was found only in Pakistan, India, and China. Nowadays, lineages F and G, having very few samples, were found in Pakistan, India, Spain, Italia, Southwestern Asia, Nothern and Eastern Africa. These facts indicate how complicated it is an origin of domestic goats (Sultana *et al.*, 2003; Joshi *et al.*, 2004; Azor *et al.*, 2005; Fernandez *et al.*, 2006; Sardina *et al.*, 2006; Colli *et al.*, 2015; Kigebwa *et al.*, 2016; Ahmed *et al.*, 2017).

CONCLUSION

This result of this study proved genetically that Kejobong goat is a crossbred of Kacang goat and Etawah Grade goat. Kejobong goat has closer genetic relationship with Kacang goat than Etawah Grade goat. Low genetic diversity of these three local goats suggests efforts to preserve local goat as one of animal genetic resource in Indonesia.

ACKNOWLEDGEMENT

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