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Genetic Diversity of Various Goat Breeds in East Java Based on DNA Microsatellite Markers

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ABSTRACT

Information on genetic diversity using microsatellite markers was essential to formulate effective conservation and breeding strategies. This study aims to identify the genetic diversity and relationships between Kacang, Senduro, Peranakan Ettawa (PE), Boer, and Saanen goats in the East Java region, Indonesia, using 12 microsatellite markers. A total of 86 goat blood DNA samples, which consisted of Kacang (n=41), Senduro (n=23), Boer (n=13), PE (n=5), and Saanen (n=4), were used in this study. The DNA was extracted based on Genomic DNA Mini Kits protocols for analysis fragment in microsatellite DNA region using specific primer recommended by the ISAG/FAO. A total of 96 alleles were identified in this study. The observed heterozygosity ranged from 0.470±0.135 (Kacang) to 0.592±0.211 (PE) and the expected heterozygosity ranged from 0.589±0.251 (Saanen) to 0.762±0.084 (PE). F statistical analysis include inter-population inbreeding rate (Fis) 0.2583 (25.83%), inbreeding rate in population (Fit) 0.3238 (32.38%), and genetic differentiation (Fst) 0.0882 (8.82%). The 11 microsatellite markers were highly informative (PIC>0.5), except the INRA063 locus markers were quite informative (PIC 0.25-0.5). The research showed that Kacang, Senduro, and PE goats had a close genetic distance and formed a cluster. Kacang and Saanen goats showed a long genetic distance at 26.9%. In conclusion, the genetic relationship among goat breeds in East Java was divided into three clusters where Boer and Saanen goats formed their cluster.

Keywords: goat; genetic relationship; genetic distance; microsatellite; fragment analysis

INTRODUCTION

In Indonesia, Kacang goats spread throughout the country and were believed to be an Indonesian native goat and the genetic source of all goat breeds in Indonesia. Kacang goat has the ability to survive and reproduce in simple feeding and rearing system practices (Khalil *et al.*, 2019). During its development, the introduction of imported goats led to crossbreeding. The crossing of Kacang goats with imported goats produces a new goat breed with optimal production of meat, milk, and other products. The results of this crossbreeding produced Peranakan Ettawa (PE) goats (Kacang and Ettawa) and Senduro goats (Kacang, Ettawa, and Jawarandu) (Sumartono *et al.*, 2015; Sumatono *et al.*, 2016). In addition to PE and Senduro goats, it is also possible that Kacang goats were crossed with imported goats that entered Indonesia in the 2000s, such as Boer and Saanen goats.

The East Java region has the second-highest goat population after Central Java, with goat breeds such as Kacang PE, Senduro, Boer, and Saanen. These goat breeds can be used for livestock development in East Java that has not been evenly distributed in every region by assessing genetic diversity among breeds. The genetic structure can be determined by genetic marking using microsatellite markers.

Microsatellites are also known as simple sequence repeats or short tandem repeats found in prokaryotes and eukaryotes throughout the genome. Microsatellite refers to deoxyribonucleic acid (DNA) regions that show short sequence repeats. Microsatellite markers are used to evaluate genetic diversity and estimate genetic distances between populations of ruminant species to evaluate the genetic relationships between livestock breeds and estimate gene flow (Perez *et al.*, 2013; Phumichai *et al.*, 2015; Seo *et al.*, 2017). The evaluation of the genetic diversity of goats using microsatellites had been conducted by several countries, such as Kanniadu, Sirohi, and Osmanabadi breeds in India, Yunnan indigenous goat breeds in China, Naine de Kabylie, Arbia, Mekatia, and M'zabite breeds in Algeria, Damani and Nachi goat breeds in Pakistan, Canindé and British Alpine goat breeds in Brazil, and Ardi, Hollandi, and Shami breeds in Saudi Arabia (Dixit *et al.*, 2012; Guang-Xin *et al.*, 2019; Tefiel *et al.*, 2018; Hussain *et al.*, 2013; Câmara *et al.*, 2017; Mahmoud *et al.*, 2020).

Microsatellite research in Indonesia has been widely carried out over the last ten years on Buffalo (Saputra *et al.*, 2020), Bali cattle (Septian & Sumantri, 2015; Puja *et al.*, 2018; Agung *et al.*, 2019), Indonesian native cattle (Sutarno *et al.*, 2015), Simmental cross cattle (Agung *et al.*, 2016), Gembrong goat (Sulabda *et al.*, 2012), Sheep (Jakaria *et al.*, 2012), Chicken (Ashari *et al.*, 2015; Saputra *et al.*, 2021), and Indonesia local duck (Maharani *et al.*, 2017; Hariyono *et al.*, 2019). All these studies were conducted to determine genetic diversity. Research on the genetic diversity of goats in East Java has been carried out by Pakpahan *et al*. (2015) using samples of the East Java Kacang goats compared to goats in Sumatra, Central Java, Maluku, Sulawesi, and Bali. However, no one has carried out the genetic characterization of goats specifically located in the East Java region. Based on these statements, this study aims to evaluate genetic characterization using microsatellite markers to determine genetic diversity between the breeds of Kacang, PE, Senduro, Boer, and Saanen goats in East Java, Indonesia.

MATERIALS AND METHODS

Collection of Blood and DNA Samples

All procedures related to animal use in this study were approved by the Animal Care and Use Committee of Brawijaya University under regulation number 021-KEP-UB-2021 (Ethical Clearance). A total of 86 goat blood samples, which consisted of Kacang (n=41) from Sawohan Village, Sidoarjo, Senduro (n=23) from Senduro Subdistrict, Lumajang, Boer (n=13) from Sumbersekar Field Laboratory of Animal Science Faculty, Universitas Brawijaya, PE (n=5) from Agro Techno Park, Universitas Brawijaya, and Saanen (n=4) from the Center for Training Ranch (BBPP) Batu Malang, were used in this study. Blood was collected from the jugular vein of the goat and placed into an EDTA k3 blood collection tube. The DNA of goat blood was extracted using Genomic DNA Mini Kits (Geneaid, Taiwan) following the protocol for DNA isolation.

Primer and DNA Amplification

A total of 12 caprine microsatellite markers (specific for goat) recommended by the International Society of Animal Genetics/Food and Agriculture Organization (ISAG/FAO, 2011) were used in the PCR process (BIO RAD T100, Singapore). The microsatellite markers consisted of MAF065, INRA023, SRCRSP9, OarAE54, OarFCB20, McM527, ILSTS087, INRA063, SPS113, OarFCB48, INRABERN172, and ILSTS011 (Table 1). The PCR reaction (30 µL) contained the template DNA (50–100 ng/µL), primers (10 pM/µL), 1 × Taq DNA polymerase, and nuclease-free water (Promega, USA).

Table 1. Sequences, dyes, and primer attachment temperatures used for amplification of microsatellite fragments recommended by ISAG/FAO (2011)

Marker	Chromosome	Primer sequences (5'>3') Forward (F) Reverse (R)	Primer attachment temperatures (°C)	Length of DNA base (Bp)	Label
MAF065	OAR15	F"AAAGGCCAGAGTATGCAATTAGGAG" R"CCACTCCTCCTGAGAATATAACATG"	58	116-158	FAM
INRA023	BTA3	F"GAGTAGAGCTACAAGATAAACTTC" R"TAACTACAGGGTGTTAGATGAACT"	58	196-215	FAM
SRCRSP9	CH112	F"AGAGGATCTGGAAATGGAATC" R"GCACTCTTTTCAGCCCTAATG"	58	99-135	HEX
OarAE54	OAR25	F"TACTAAAGAAACATGAAGCTCCCA" R"GGAAACATTTATTCTTATTCCTCAGTG"	58	115-138	HEX
OarFCB20	OAR2	F"GGAAAACCCCCATATATACCTATAC" R"AAATGTGTTTAAGATTCCATACATGTG"	58	93-112	FAM
McM527	OAR5	F"GTCCATTGCCTCAAATCAATTC" R"AAACCACTTGACTACTCCCCAA"	58	165-187	FAM
ILSTS087	BTA6	F"AGCAGACATGATGACTCAGC" R"CTGCCTCTTTTCTTGAGAG"	58	135-155	FAM
INRA063	CH118	F"GACCACAAAGGGATTTGCACAAGC" R"AAACCACAGAAATGCTTGGAAG"	58	164-186	FAM
SPS113	BTA10	F"CCTCCACACAGGCTTCTCTGACTT" R"CCTAACTTGCTTGAGTTATTGCCC"	58	134-158	HEX
OarFCB48	OAR17	F"GAGTTAGTACAAGGATGACAAGAGGCAC" R"GACTCTAGAGGATCGCAAAGAACCAG"	58	149-173	HEX
INRABERN172	BTA26	F"CCACTTCCCTGTATCCTCCT" R"GGTGCTCCCATTGTGTAGAC"	58	234-256	HEX
ILSTS011	BTA14	F"GCTTGCTACATGGAAAGTGC" R"CTAAAATGCAGAGCCCTACC"	58	250-300	HEX

Note: Bp= base pair.

The PCR conditions included the following: initial denaturation for 5 minutes at 95 °C, then continued with 35 cycles, denaturation of 95 °C for 10 seconds, temperature primer attachment (58 °C) for 20 seconds, and elongation (amplification) at 72 °C for 30 seconds, with a final temperature of elongation at 72 °C for 5 minutes. The PCR product was visualized using 1% agarose gel and 0.5 × Tris-borate-EDTA buffer in 100 V electrophoresis (Mupid-exU, Japan) for 30 minutes. Then, the sample was stained using NAD (Nucleic Acid Diamond) and 0.5 × Tris-borate-EDTA buffer mixture for 30 minutes, and the product was visualized using GelDoc (Glite 965 GW, Taiwan). Microsatellite fragment analysis was conducted at First Base Laboratory, Selangor, Malaysia.

Data Analysis

Microsatellite polymorphisms and genetic diversity. Allele size data were generated from the analysis fragment and converted using the Convert version 1.31 for further analysis. The converted data were analyzed using Cervus version 3.0.7 to generate frequency/number of alleles, observed heterozygosity (HO), expected heterozygosity (HE), Hardy–Weinberg (HW) equilibrium, and polymorphism information content (PIC) values. Genetic differentiation (F_{ST}), the rate of inbreeding between populations (F_{IS}), and the rate of inbreeding in populations (F_{IT}) were analyzed using Genepop version 4.7.5. Polymorphism Information Content was calculated using the formula of Botstein *et al.,* (1980):

$$PIC = 1 - (\sum_{i=1}^{n-1} pi^2) - \sum_{i=1}^{n} \sum_{j=i+1} 2pi^2pj^2$$

where n was number of alleles, p_i was allele frequency in population i, and p_j was allele frequency in population j.

Genetic structure and relationships. Genetic structures for microsatellite data for each breed of goat were analyzed using POPTREEW (POPTREEW website version) (Takezaki *et al.,* 2014) to generate genetic distance using Nei's standard genetic distance (D_{ST}) method and the reconstruction of phylogeny trees between breeds. Arlequin version 3.5 was also used to generate the value of population pairwise F_{ST}. Principal coordinate analysis (PCoA) was analyzed using GenAlEx 6.51 b2 version to determine the genetic relationship between livestock breeds.

RESULTS

Microsatellite Polymorphisms and Genetic Diversity

Indicators of genetic diversity are summarized in Table 2 and Table 3. A total of 96 alleles were identified from 5 goat breeds. The number of alleles per locus ranged from 5 (INRA063) to 10 (MAF065 and ILSTS087), with an average 8 alleles per locus. Expected heterozygosity (HE) was higher than observed heterozygosity (HO) in all populations. HE ranged from 0.589±0.251 (Saanen) to 0.762±0.084 (PE), whereas the mean HO ranged from 0.470±0.315 (Kacang) to 0.592±0.211 (PE) (Table 2).

F statistical analysis estimated inter-population inbreeding rate (Fis), inbreeding rate in population (Fit), and genetic differentiation (Fst). F_{IS}, F_{IT}, and F_{ST} values were significantly different from zero, with values ranged from 0.0538 (SRCRSP9) to 0.4974 (OarFCB20), 0.1263 (SRCRSP9) to 0.5252 (OarFCB20), and 0.0069 (INRA063) to 0.1655 (SPS113), respectively. Their average values were 0.258, 0.324, and 0.088, respectively. The loci PIC value ranged from 0.451 (INRA063) to 0.788 (SRCRSP9), which indicated that the microsatellite markers were moderate (0.25–0.5) to highly (>0.5) informative (Table 3).

Genetic Structure and Relationships

The result of genetic distance analysis using Nei's standard genetic distance (D_{ST}) shows that the genetic distance ranged from 0.037 to 0.757, which was the closest distance (0.037) between Senduro and PE and the farthest distance (0.757) between Boer and Saanen (Table 4). Figure 1 shows the phylogenetic tree reconstruction, which describes the relationship between goats in East Java, using the unweighted pair-group method with arithmetic mean using Nei's genetic distance (Dst-corrected). The phylogeny tree showed that there are three clusters: the clusters that contained the blood of Kacang goats consisted of four breeds, where Kacang goats were separated, followed by Senduro and PE goats in one cluster: Boer goats; Saanen goats formed their own cluster, which showed a long genetic distance with Kacang goat at 26.9%.

The results of the analysis using PCoA show that the distribution of Kacang goats was centered on the right side of the graph, the distribution of Senduro goats were spread almost throughout the graph, indicating the origin of the Senduro goats used in the study,

Table 2. Number of alleles (Na), observed heterozygosity (Ho), and expected (He), and polymorphism information content in five goat breeds observed

Breed	N	Na±SD	Ho±SD	He±SD	PIC
A	41	5.67±1.67	0.470±0.135	0.636±0.134	0.585
B	23	5.75±1.36	0.479±0.178	0.679±0.109	0.620
C	13	5.00±1.35	0.546±0.199	0.702±0.103	0.629
D	5	4.17±0.72	0.592±0.211	0.762±0.084	0.634
E	4	3.00±1.04	0.542±0.334	0.589±0.251	0.460

Note: A=Kacang goat; B=Senduro goat; C=Boer goat; D=Peranakan Ettawa goat; E=Saanen goat; N=number of sample; Na=Number of alleles; Ho=observed heterozygosity; He=expected heterozygosity; PIC=polymorphism information content; SD=standart deviation.

Table 3. Statistics (Fis, Fit, Fst) between five goat breeds

Loci	Size (bp)	Na	Ho	He	PIC	F_{IS}	F_{IT}	F_{ST}	HW
MAF065	116-146	10	0.547	0.762	0.724	0.252	0.298	0.061	NS
INRA023	196-212	9	0.531	0.806	0.780	0.324	0.351	0.041	NS
SRCRSP9	116-134	8	0.733	0.817	0.788	0.054	0.126	0.077	NS
OarAE54	116-138	9	0.628	0.728	0.698	0.131	0.142	0.013	NS
OarFCB20	96-106	6	0.349	0.720	0.662	0.497	0.525	0.055	***
McM527	154-170	8	0.558	0.722	0.681	0.155	0.261	0.125	NS
ILSTS087	135-153	10	0.462	0.749	0.706	0.327	0.408	0.120	***
INRA063	172-180	5	0.430	0.492	0.451	0.123	0.129	0.007	NS
SPS113	135-155	8	0.547	0.762	0.723	0.188	0.322	0.166	*
OarFCB48	150-168	8	0.360	0.666	0.631	0.391	0.488	0.159	***
INRABERN172	233-245	7	0.302	0.575	0.535	0.436	0.493	0.101	***
ILSTS011	267-285	8	0.488	0.712	0.667	0.255	0.340	0.114	***
Mean	-	8	-	-	-	0.258	0.324	0.088	-
SD	-	1.5	-	-	-	0.137	0.141	0.053	-

Note: Bp= base pair; Na= number of alleles; Ho= observed heterozygosity; He= expected heterozygosity; PIC= polymorphism information content; FIS= Rate of inbreeding between populations; FIT= Rate of inbreeding in populations; FST= Genetic differentiation; HW= Hardy-Weinberg; NS= not significant; SD= standard deviation; *** p<0.001.

Table 4. Population pair-wise Fst (bottom diagonal) and Nei's standard genetic distance (top diagonal)

Goat breeds	Kacang	Senduro	Boer	Peranakan Ettawa	Saanen
Kacang	***	0.16	0.233	0.14	0.751
Senduro	0.055	***	0.183	0.037	0.627
Boer	0.083	0.075	***	0.182	0.757
Peranakan Ettawa	0.077	0.037	0.07	***	0.275
Saanen	0.269	0.228	0.261	0.113	***

Note: Fst= Genetic differentiation.

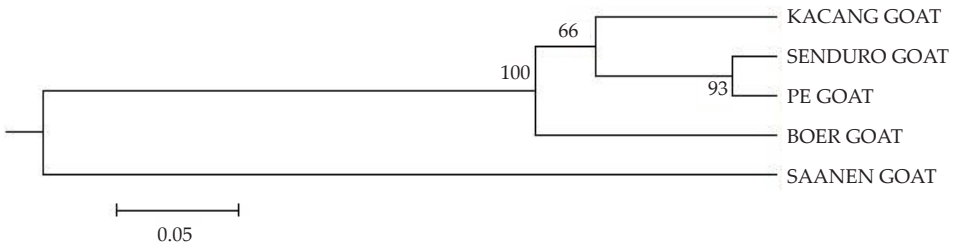

Figure 1. The reconstruction of the UPGMA phylogeny tree of Kacang, Senduro, PE (Peranakan Ettawa), Boer, and Saanen goat breeds with Nei genetic distance

besides the genetics of Kacang, Ettawa, and Jawarandu goats. They were also crossed with another goat. Boer goats were centered on the bottom left of the graph, PE goats were centered on the top left of the graph, and Saanen goats were centered on the bottom left of the graph without any contact with other goats (Figure 2). PCoA (Figure 3) revealed three clusters: Kacang, Boer, and Saanen. Senduro and PE goats were included in the Kacang. Meanwhile, Boer and Saanen were separated from their own cluster.

DISCUSSION

In this research, all microsatellite locus were polymorphic, with the average number of alleles was 8±1.5. These results were lower than the other research in Turkish and Albanian goat populations with the number of alleles of 14.55 (Bulut *et al.*, 2016) and 11.03 (Hoda *et al.*, 2011), respectively. Allele frequencies at all loci showed that Kacang, Senduro, and Boer goats had more diverse allele sizes than PE and Saanen goats. This could be due to the small number of samples used for analysis on the Saanen and PE goat breeds. The low frequency of goat DNA samples used causes a small number of alleles to be obtained so that the use of genetic markers for diversity studies is less effective (Kim *et al.*, 2001).

The expected heterozygosity value was higher than the observed heterozygosity value in all populations studied, indicating that almost all loci showed a positive deviation from the HW equilibrium (Dixit *et al.*, 2012). In addition, there was a decrease in the heterozygosity value (Ho < He), which indicated inbreeding and endogamy degree or mating in groups due to an intensive selection process (Pan & Jinzeng,

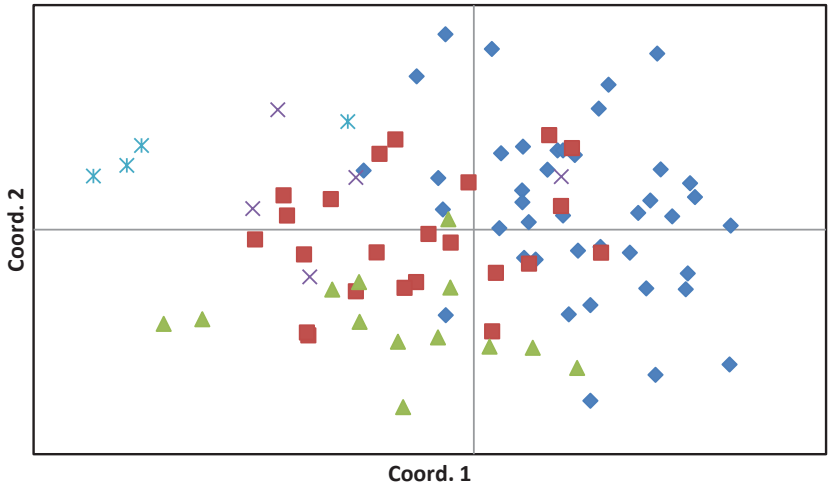

Figure 2. Principal coordinate analysis (PCoA) based on 12 microsatellite loci from 86 individual goats of 5 breeds in East Java. Kacang= ◆; Senduro= ■; Boer= ▲; PE= ×; Saanen= ✳.

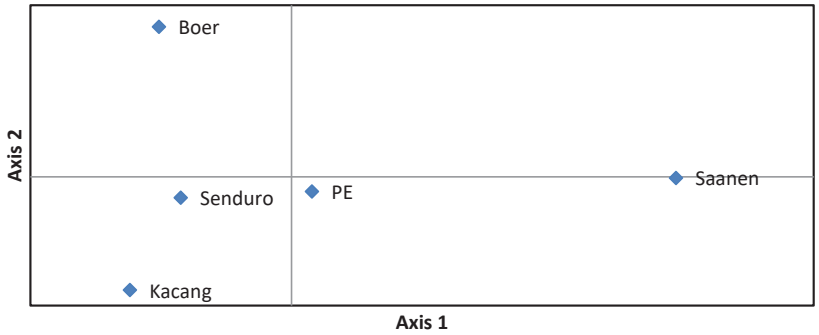

Figure 3. Principal coordinate analysis (PCoA) between goat breeds in East Java

2010). Although the He value was higher, it was lower compared to Algerian goat (Tefiel *et al.*, 2018) and Saudi Arabian goat (Mahmoud *et al.*, 2020), but higher than Ardi goat (Aljumaah *et al.*, 2012), indigenous Tsawana goat (Maletsanake *et al.*, 2015), and Egyptian goat (El-Sayed *et al.*, 2016). The low heterozygosity value in the study indicated high uniformity in the population of each goat breed. Uniformity caused by inbreeding can increase homozygosity and decrease heterozygosity. This was also evidenced by the positive values of Fis observed. A positive F_{IS} value indicated a decrease in heterozygosity in the population, meanwhile negative or close to 0 explained by negative assortative mating, balancing selection at a locus, or as an asymmetrical sex migration that produced an outbreeding effect (Parreira & Chikhi, 2015). Heterozygosity decrease can be caused by inbreeding and the Wahlund effect. The Wahlund effect refers to the reduction in heterozygosity observed due to the (cryptic) population substructure (Hoda *et al.*, 2011). The high inbreeding is caused by undirected mating, small effective population size, including the ratio of productive males and females, preferential mating behavior towards certain livestock, isolated closed populations, and random genetic drift (Thiruvenkadan *et al.*, 2013). The F_{IS} value was higher than the previous study on Ardi (Aljumaah *et al.*, 2012), four Small East African goats (Nguluma *et al.*, 2018), Saudi Arabian (Mahmoud *et al.*, 2020), Thai (Seilsuth *et al.*, 2016), and Jordan (Al-Atiyat *et al.*, 2015) goats. The F_{IT} value in this study was higher than that of the Algerian (Tefiel *et al.*, 2018), Egyptian and Saudi Arabian (Mahrous *et al.*, 2013), and Nigerian (Murital *et al.*, 2015) goats. The observed F_{ST} value was 8.82% between populations. This indicated that 8.82% of the total genetic variation was due to the differences between populations and that 91.18% was due to the differences between individuals. The obtained F_{ST} value was higher than the West African local (Missohou *et al.*, 2011), Portuguese (Bruno-de-Sousa *et al.*, 2011), and Kerala (Radhika *et al.*, 2015) goats but lower than homologous Portugal and Brazilian goat (Oliveira *et al.*, 2010).

The informativeness of observed loci was measured using PIC. Microsatellites with high PIC values are useful for the study of genetic variation. In this study, the 11 microsatellite markers used had a high informative value (PIC>0.5), and one marker INRA063 had a moderate informative value of 0.451 (0.25–0.5). PIC (<0.25) has a low information value. The PIC value in studies on Chinese dairy goats using 15 microsatellites was 0.3963–0.8663 (Wang *et al.*, 2017), that of Markhoz goats were 0.653–0.793 (Asroush *et al.*, 2018), and that of Gaddi goats of Western Himalayas were 0.7148–0.909 (Singh *et al.*, 2015).

Indicators of the genetic distance of F_{ST} values and Nei's genetic distances revealed a genetic relationship among five goat populations. Estimation of Fst value

between each nation/population shows that Kacang and Saanen goats have the highest genetic differentiation followed by Boer and Saanen goats, Senduro and Saanen goats, PE and Saanen goats, and Senduro and PE goats showed the lowest genetic differentiation between each pair per population. Genetic differentiation between Kacang and Senduro goats and Senduro and PE goats showed a low value; and it indicated the high genetic similarity between goat breeds. The genetic similarity is directly proportional to the phenotypic similarity between PE and Senduro goats, including a convex facial profile, beard in male goats, short tail and slightly curved straight back, and getting higher up to the hips (Zhang *et al.,* 2009; Belay *et al.,* 2014). The similarity of the phenotypes of PE and senduro goats with Kacang goats is not apparent, because the initial cross between Kacang and Ettawa goats produced offspring whose phenotype was more similar to that of the Ettawa goat, the similarity with the Kacang goat, which was more adaptive to the environment in Indonesia.

Reconstruction of the phylogeny tree was used to describe the relationship among the five goats population in East Java. Phylogeny tree analysis indicated a high share of the gene pool between Kacang and Senduro, Kacang and PE, as well as Senduro and PE goats. The PCoA analysis result also confirmed these results by grouping Senduro and PE in the Kacang goat cluster. This result confirmed the origin of Senduro and PE goats, which have the blood of Kacang goats. Senduro goat was a cross between Kacang, Ettawa, and Jawarandu goats (Decree of the Minister of Agriculture number 1055/Kpts/SR.120/2014), and PE goat was a cross between Kacang and Ettawa goats (Decree of the Minister of Agriculture number 695/Kpts/PD.410/2013). The Boer goats used in this study showed a fairly close genetic distance to Kacang goats, but in different clusters. It can be possible that the Boer goats used have higher blood lines Boer goats than local goats from crosses. The difference between the pure Boer goat and the Boer used in the study is that the pure Boer has short hair and big and sturdy legs (Tesema *et al.,* 2018; Manirakiza *et al.,* 2020), while the Boer in this study has long hair, thin legs, and has long hair on the chest to the front legs which is not found in the pure Boer goat.

Genetically, Kacang, Senduro, and PE have a close genetic distance and form one cluster. However, Boer and Saanen goats form their clusters. The results of the study provide information on the development of the goat breed in East Java and can be used in sustainable livestock utilization in the future. The information on genetic diversity using microsatellite markers was critical to meet the demands of future breeding programs and formulate strategies for the conservation and development of goats in East Java.

CONCLUSION

The use of 12 microsatellite markers was highly informative and polymorphic in detecting the genetic diversity on five goat breeds in East Java. Preventing further loss of alleles should be considered by implementing effective breeding strategies to reduce inbreeding and increase heterozygosity. All identified alleles in this research were able to classify the genetic relationship into three clusters, i.e., cluster of Kacang goats (Kacang, Senduro, and PE), cluster of Boer goats, and cluster of Saanen goats.

CONFLICT OF INTEREST

The authors confirm that there is no conflict of interest regarding this manuscript.

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