

RESEARCH

Open Access



Genetic diversity and relationship among Indonesian local sheep breeds on Java Island based on mitochondrial cytochrome b gene sequences

Alek Ibrahim^{1,2}, Endang Baliarti^{1*} , I Gede Suparta Budisatria¹, Wayan Tunas Artama³, Rini Widayanti³, Dyah Maharani⁴, Luis Tavares⁵ and Endang Tri Margawati²

Abstract

Background The cytochrome b (Cyt b) gene is one of the most studied mitochondrial DNA (mtDNA) genes to determine sheep's genetic profile. This study aimed to determine the genetic diversity and relationships of several Indonesian local sheep populations on Java Island, Indonesia, based on the mtDNA Cyt b gene sequences. Blood samples were collected from forty-one individual sheep in seven populations of Indonesia local sheep breeds on Java Island (Priangan = 6, Garut = 6, Batur = 7, Wonosobo = 5, Javanese Thin-Tailed/JTT = 7, Javanese Fat-Tailed/JFT = 5, and Sapudi = 5). DNA extraction was performed on blood samples, and the mtDNA Cyt b gene was amplified using specific primers (Alek-CBF: 5'-CAACCCACCACTTACAA-3' and Alek-CBR: 5'-CCTTGAGTCTTAGGGAGGTT-3'). The polymerase chain reaction (PCR) products were then sequenced, and data were analyzed using the MEGA version 7.0, DNA SP version 6.0, and NTSYS-pc version 2.11 software.

Results A total of 1140 bp complete mtDNA Cyt b gene sequences in this study obtained 1134 monomorphic sites (I), six polymorphic sites (V), one segregation site (S), and five parsimony informative sites (P) with a nucleotide diversity (Pi), the average number of nucleotide differences (K), and sequence conservation (SC) were 0.00119, 1.35610, and 0.9947, respectively. There were six haplotypes consisting of two unique haplotypes and four shared haplotypes with a haplotype diversity (Hd) of 0.5415. The genetic distance within and between populations ranged from 0.0000 to 0.0016 and 0.0000 to 0.0020, respectively. Wonosobo, JFT, and Sapudi sheep have the closest relationship, and then these three breeds were close to JTT sheep, followed by Batur, Priangan, and Garut sheep. Two haplogroups have been found based on the Ovine haplogroup clustering. All Wonosobo, JTT, JFT, Sapudi sheep, and most Batur sheep were clustered into haplogroup B. In contrast, Garut sheep were mostly clustered into haplogroup A, while Priangan sheep were clustered into both haplogroups with the same percentage.

Conclusion Seven Indonesian local sheep breeds on Java Island have a close relationship clustered into two haplogroups, namely haplogroups A and B. Most Indonesian local sheep breeds on Java Island in this study were clustered into haplogroup B, except for Garut and Priangan sheep.

Keywords Genetic variation, Indonesian local sheep, Maternal origin, mtDNA Cyt b gene, Phylogenetic

*Correspondence:

Endang Baliarti
bali_arti@ugm.ac.id

Full list of author information is available at the end of the article



© The Author(s) 2023. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

Background

Domestic sheep (*Ovis aries*) have an essential role in diverse human societies as a source of food, hide, wool, income, and a role in religious and cultural activities. In Indonesia, sheep are exceedingly popular among farmers, especially smallholder farmers, because they are easy to maintain and low cost. Indonesian local sheep breeds have been developed by the community for many years, have adapted to the environment of certain regions, and have historical value in certain areas in Indonesia [1–3]. Until 2022, eleven Indonesian local sheep breeds have been recognized by the Ministry of Agriculture of the Republic of Indonesia, spread across various regions in Indonesia [4]. The Indonesian local sheep breeds' names are usually based on the source development areas of its local sheep, for example, Garut sheep from Garut Regency, Wonosobo sheep from Wonosobo Regency, Sapudi sheep from Sapudi Island, Batur sheep from Batur district-Banjarnegara Regency, Sakub sheep from Sakub hill-Brebes Regency, Palu sheep from Palu Regency, or Kisar sheep from Kisar Island. The Ministry of Agricultural of Indonesia also develops local sheep breeds such as Compass Agrinak sheep. Indonesian local sheep breeds originating from Java Island are Priangan, Garut, Sakub, Batur, and Wonosobo sheep. In addition, other local sheep thrive on this island, namely Sapudi, Javanese Fat-Tailed (JFT), and Javanese Thin-Tailed (JTT) sheep [4, 5].

Mitochondrial DNA (mtDNA) has been the most popular marker of molecular diversity in animals over the last three decades. Most mtDNA investigations focused mainly on the Cytochrome b (Cyt b) and control region [6, 7]. Cyt b is mtDNA gene that contains abundant phylogenetic information among interspecies and intraspecies. It is considered a good marker for studying the genetic diversity and phylogenetic relationships among species within the same genus and family [7]. This gene is a member of protein-coding genes with a high evolutionary rate and higher variation ratio than other functional genes [8]. The mtDNA Cyt b is widely used for genetic diversity and phylogenetic relationship determination in domestic animals, such as goats [9–12], chickens [13–15], ducks [16, 17], pigs [18], cattle [8, 19, 20], horses [14, 21], buffaloes [22, 23], and sheep [6, 24–27].

Information on the genetic diversity of native and local livestock in Indonesia is important in developing breeding and conservation strategies [8]. Indonesian local sheep, including those on Java Island, are local livestock resources that need to be studied for their genetic profile. In a previous study using mtDNA D-loop on several Indonesian local sheep breeds on Java Island, two haplogroups were found, namely haplogroups A and B [28]. In-depth studies still need to be done to determine the

genetic diversity, phylogenetic relationship, and origin of the sheep using various techniques and gene targets. This study aimed to determine the genetic diversity and relationships of several Indonesian local sheep populations on Java Island based on the mtDNA Cyt b gene sequences.

Methods

Ethical approval

This study has been authorized and approved with the Ethical Clearance Certificate of the Faculty of Veterinary Medicine Research Ethics Commission, Universitas Gadjah Mada, with approval number 002/EC-FKH/Int./2019. The approval was also obtained from the National Political and Unity of Yogyakarta Province with the approval number 074/1850/Kesbangpol/2019 and from the Agricultural Officers and the National Political and Unity Officers in each of the sample areas used.

Sample collection

This study was conducted using blood samples from forty-one individual sheep in seven populations of Indonesian local sheep breeds on Java Island, Indonesia, as presented in Fig. 1. Sheep were sampled by a purposive sampling method, namely by determining each local sheep breed's origin and development areas, and then determining the sub-districts and villages for sampling. Blood samples were taken using a 3-cc syringe through the jugular vein previously cleaned with alcohol. The blood samples were then collected in vacutainer tubes with an anticoagulant (ethylenediaminetetraacetic acid), stored in a cooler box containing an ice pack, and transported to the laboratory for further analysis. The samples were collected from April 2019 to March 2021. However, this study was conducted from October to December 2022 in the Laboratory of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Universitas Gadjah Mada.

Molecular techniques

The DNA was extracted based on the manufacturer's standard protocol using PureLink™ Genomic DNA Mini Kits (Invitrogen). The mtDNA Cyt b gene was amplified directly from the genomic DNA by polymerase chain reaction (PCR). The primers were designed using the Primer3 online version 4.1.0 program (<http://primer3.Ut.ee/>) [29] based on the data from the mitochondrial genome of *Ovis aries* (GenBank accession number: AF010406.1). The mtDNA Cyt b primer sequences were Alek-CBF: 5'-CAACCCACCCTTACAA-3' and Alek-CBR: 5'-CCTTGAGTCTTAGGGAGGTT-3', generated 1409 bp of the PCR product [5]. The PCR reaction consisted of 2 µL of DNA template, 25 µL of KAPA2G Fast

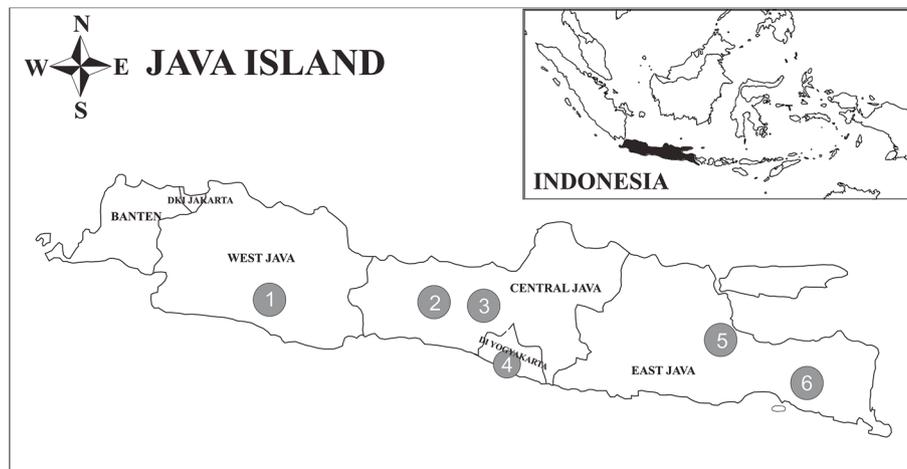


Fig. 1 Sampling sites Indonesian local sheep breeds on Java Island, Indonesia: 1. Garut Regency (Garut sheep and Priangan sheep); 2. Banjarnegara Regency (Batur sheep); 3. Wonosobo Regency (Wonosobo sheep); 4. Bantul Regency (Javanese Thin-Tailed sheep); 5. Pasuruan Regency (Javanese Fat-Tailed sheep); 6. Jember Regency (Sapudi sheep)

Ready Mix + Dye (Kapa Biosystems Ltd.), 2 μ L of forward primer, 2 μ L of reverse primer, and 19 μ L of ddH₂O. The PCR amplification was conducted using Cleaver[®] GTC96S (Cleaver Scientific Ltd.) according to the program: 5 min of pre-denaturation at 94 °C, followed by 35 cycles, each consisting of denaturation at 94 °C for 30 s, primers annealing at 55 °C for 40 s, extension at 72 °C for 90 s, then ending with a final extension at 72 °C for 8 min, and storage at 4 °C. The PCR product was visualized using 1.5% agarose gel, and electrophoresis was run at 80 mV for 45 min. The result of amplification could be seen on the ultraviolet illuminator. The purified PCR products were sequenced by 1st BASE-Asia, Malaysia. A request was made to perform a gel extraction at the ordering stage. An example of PCR product visualization results is presented in Fig. 2.

Data analyses

The primers used in this study can amplify 1409 bp of PCR products and flank complete sequences in the targeted mtDNA Cyt b gene (1140 bp). The sequence products were analyzed using the Molecular Evolutionary Genetics Analysis (MEGA) version 7.0 software [30]. The reference of mtDNA Cyt b gene sequences of wild and domestic sheep of known haplogroup (HapG) types [31] was downloaded from GenBank (<https://www.ncbi.nlm.nih.gov>) and used as comparators of Indonesian local sheep breeds on Java Island to determine the genetic relationship and assume their origin (Table 1). The mtDNA Cyt b sequences were aligned using Clustal W [32] in the MEGA version 7.0 software. The genetic variation analysis was obtained based on the nucleotide differences of the mtDNA Cyt b sequences. The genetic

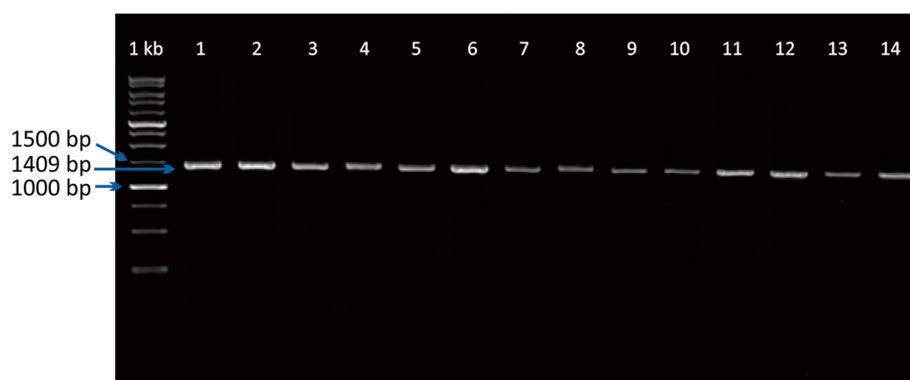


Fig. 2 Visualization of PCR product on the targeted of mtDNA Cyt b on Wonosobo sheep (1–4), Batur sheep (5–7), Javanese Thin-Tailed sheep (8–11), and Garut sheep (12–14)

Table 1 References of domestic and wild sheep

Group membership	GenBank accession number
<i>Ovis aries</i>	
Haplogroup A	HM236174, HM236175
Haplogroup B	HM236176, HM236177
Haplogroup C	HM236178, HM236179
Haplogroup D	HM236180, HM236181
Haplogroup E	HM236182, HM236183
<i>Ovis musimon</i>	HM236184
<i>Ovis vignei</i>	HM236187
<i>Ovis ammon</i>	HM236188
<i>Ovis orientalis</i>	KF312238
<i>Ovis canadensis</i>	MH094035
<i>Ovis nivicola</i>	MH779626
<i>Ovis dalli</i>	MH779627

distance analysis was obtained with the Kimura-2-parameter methods [33]. The phylogenetic tree (UPGMA) was constructed with the 1000 × bootstrap [34] and Kimura-2-parameter method [33]. The genetic diversity and haplotype diversity values were analyzed using DNA Sequence Polymorphism (DNA SP) version 6.0 software (Universitat de Barcelona, Spain) [35]. The phylogenetic tree between Indonesian local sheep breeds was constructed using NTSYS-pc version 2.11 software [20] based on the genetic distance values between breeds.

Results

Sequence variation and genetic diversity

The 1140 bp of complete mtDNA Cyt b gene sequence resulted based on the alignments of the PCR product sequences and the reference sequences. The average percentage of the nucleotide composition of the Indonesian local sheep breeds' mtDNA Cyt b gene sequences is presented in Table 2. Wonosobo, JTT, JFT, and Sapudi sheep have the same percentage of thymine composition (27.02%), and

Table 2 The average percentage (%) of nucleotide composition in Indonesian local sheep breeds based on mtDNA Cyt b gene sequences

Breed	T(U)	C	A	G	C + G
Priangan	27.11	28.51	31.45	12.94	41.45
Garut	27.15	28.46	31.46	12.92	41.39
Batur	27.09	28.52	31.42	12.97	41.49
Wonosobo	27.02	28.60	31.40	12.98	41.58
JTT	27.02	28.56	31.40	13.02	41.58
JFT	27.02	28.60	31.40	12.98	41.58
Sapudi	27.02	28.60	31.40	12.98	41.58
Overall	27.06	28.55	31.42	12.97	41.52

T thymine, C cytosine, A adenine, G guanine, U uracil

the higher was found in Garut sheep (27.15%). Wonosobo, JFT, and Sapudi sheep have the same percentage of cytosine composition (28.60%), and the lower was found in Garut sheep (28.46%). The higher percentage of adenine composition was found in Garut sheep (31.46%), and the lower was found in Wonosobo, JTT, JFT, and Sapudi sheep with the same percentage (31.40%). In addition, a higher percentage of guanine composition was found in JTT sheep (13.02%), while the lower was found in Garut sheep (12.92%).

The mtDNA Cyt b gene sequences were aligned in polymorphism sites (variable sites), as presented in Fig. 3. It is shown that the complete mtDNA Cyt b sequence (1140 bp) in the individual sample was only found in six different nucleotide sites between observed samples, namely at the 189th, 309th, 495th, 792nd, 828th, and 982nd sites. The genetic diversity parameters of the mtDNA Cyt b gene sequence of seven Indonesian local sheep breeds on Java Island are presented in Table 3. This table shows that from the forty-one samples used with the 1140 bp of complete mtDNA Cyt b sequence was found 1334 monomorphic sites (I), six variable sites (V), one segregation site (S), and five parsimony informative sites (P) with the nucleotide diversity (Pi), the average number of pairwise differences (K), and sequence conservation (SC) were 0.00119, 1.35610, and 0.9947, respectively. Not found the insertion and deletion in the sequence analysis.

The haplotype diversity parameters of seven Indonesian local sheep breeds based on mtDNA Cyt b gene sequences were presented in Table 4. It is shown that based on analysis of the sample used was found six

Sample	Breed	Sites					
		1	3	4	7	8	9
PRG1	Priangan	C	C	G	T	C	C
PRG2	Priangan	.	T	A	.	T	.
PRG3	Priangan	.	T	A	.	T	.
PRG4	Priangan
PRG5	Priangan	.	T	A	.	T	.
PRG6	Priangan
GRT1	Garut
GRT2	Garut	T	.
GRT3	Garut	.	T	A	.	T	.
GRT4	Garut	.	T	A	.	T	.
GRT5	Garut	.	T	A	.	T	.
GRT6	Garut	T	.	A	.	T	.
BTR1	Batur	.	T	A	.	T	.
BTR2	Batur
BTR3	Batur	.	.	.	T	T	.
BTR4	Batur	.	.	.	T	T	.
BTR5	Batur
BTR6	Batur
BTR7	Batur
WSB1	Wonosobo
WSB2	Wonosobo
WSB3	Wonosobo	C	C	G	T	C	C
WSB4	Wonosobo
WSB5	Wonosobo
JTT1	JTT	.	.	.	G	T	.
JTT2	JTT	.	.	.	G	T	.
JTT3	JTT	.	.	.	G	T	.
JTT4	JTT
JTT5	JTT
JTT6	JTT
JTT7	JTT
JFT1	JFT
JFT2	JFT
JFT3	JFT
JFT4	JFT
JFT5	JFT
SPD1	Sapudi
SPD2	Sapudi
SPD3	Sapudi
SPD4	Sapudi
SPD5	Sapudi

Fig. 3 Variable sites position of mtDNA Cyt b gene sequences in Indonesian local sheep breeds

Table 3 Nucleotide diversity in Indonesian local sheep breeds based on mtDNA Cyt b gene sequences

Breed	<i>n</i>	<i>I</i>	<i>V</i>	<i>S</i>	<i>P</i>	Indel	SC	Pi	K
Priangan	6	1137	3	0	3	0	0.9974	0.00158	1.80000
Garut	6	1136	4	2	2	0	0.9965	0.00158	1.80000
Batur	7	1136	4	2	2	0	0.9965	0.00142	1.61905
Wonosobo	5	1140	0	0	0	0	1.0000	0.00000	0.00000
JTT	7	1138	2	0	2	0	0.9983	0.00100	1.14286
JFT	5	1140	0	0	0	0	1.0000	0.00000	0.00000
Sapudi	5	1140	0	0	0	0	1.0000	0.00000	0.00000
Overall	41	1134	6	1	5	0	0.9947	0.00119	1.35610

n number of samples, *I* number of monomorphic sites (invariable sites), *V* number of polymorphism sites (variable sites), *S* number of segregation sites (singleton sites), *P* parsimony-informative sites, *Indel* insertion-deletion, *SC* sequence conservation, *Pi* nucleotide diversity, *K* average number of pairwise differences

Table 4 Haplotype diversity in Indonesian local sheep breeds based on mtDNA Cyt b gene sequences

Breed	<i>n</i>	nHap	Hd	nHapG A	nHapG B
Priangan	6	2	0.600	3	3
Garut	6	4	0.800	4	2
Batur	7	3	0.667	1	6
Wonosobo	5	1	0.000	0	5
JTT	7	2	0.571	0	7
JFT	5	1	0.000	0	5
Sapudi	5	1	0.000	0	5
Overall	41	6	0.541	8	33

n numbers of samples, *nHap* numbers of haplotype, *Hd* haplotype diversity, *nHapG A* numbers of haplogroup A, *nHapG B* numbers of haplogroup B

haplotypes (Hap) consisting of two unique haplotypes (Hap-3 and Hap-4) and four shared haplotypes (Hap-1, Hap-2, Hap-5, and Hap-6) with the haplotype diversity (*Hd*) value was 0.5415. The distribution of each haplotype is presented in Table 5. It is shown that haplotype 1 (Hap-1) has the most haplotype members, they were 27 members consisting of three Priangan sheep, one Garut sheep, four Batur sheep, five Wonosobo sheep, four JTT

sheep, five JFT sheep, and five Sapudi sheep members. In haplotype 2 (Hap-2), seven sample members consisted of three Priangan sheep, three Garut sheep, and one Batur sheep. In addition, haplotype 3 (Hap-3) and haplotype 4 (Hap-4) each have one sample member, while Hap-5 and Hap-6 have two and three sample members, respectively.

This study's alignment of amino acids was conducted by comparing the sample sequences to the *Ovis aries* sequences from various haplogroups and genera (Table 1). Alignment of 1140 bp complete mtDNA Cyt b sequence in this study was found 380 amino acids. There were 11 different amino acids from several amino acids obtained compared to the references, but there was no difference in amino acids between Indonesian local sheep breeds in this study (Fig. 4).

Genetic distance of Indonesian local sheep breeds and *Ovis aries* haplogroups

The genetic distance between seven Indonesian local sheep breeds on Java Island and the sheep reference in the study is presented in Fig. 5. It is shown that genetic distance within the population (0.0000–0.0016) and between populations (0.0000–0.0020) in this study were categorized to close distance. The farthest distance

Table 5 Haplotypes distributions in Indonesian local sheep breeds based on mtDNA Cyt b gene sequences

Haplotypes	Breed							Overall	
	PRG	GRT	BTR	WSB	JTT	JFT	SPD	<i>n</i>	%
Hap-1	3	1	4	5	4	5	5	27	68.85
Hap-2	3	3	1	–	–	–	–	7	17.07
Hap-3	–	1	–	–	–	–	–	1	2.44
Hap-4	–	1	–	–	–	–	–	1	2.44
Hap-5	–	–	2	–	–	–	–	2	4.88
Hap-6	–	–	–	–	3	–	–	3	7.32
Total	6	6	7	5	7	5	5	41	100.00

Hap haplotype, *n* number of samples, *PRG* Priangan sheep, *GRT* Garut sheep, *BTR* Batur sheep, *WSB* Wonosobo sheep, *JTT* Javanese Thin-Tailed sheep, *JFT* Javanese Fat Tailed sheep, *SPD* Sapudi sheep

Sample	Breed	Sites									Sample	Breed	Sites												
		1	1	1	2	2	2	3	3	3			1	1	1	2	2	2	3	3	3				
		2	2	5	5	3	4	9	0	0			7	2	2	5	5	3	4	9	0	0	7		
		2	3	9	6	9	8	0	5	2	4	1			2	3	9	6	9	8	0	5	2	4	1
PRG1	Priangan	I	A	I	I	N	I	M	I	V	I	I	JTT6	JTT	I	A	I	I	N	I	M	I	V	I	I
PRG2	Priangan	JTT7	JTT
PRG3	Priangan	JFT1	JFT
PRG4	Priangan	JFT2	JFT
PRG5	Priangan	JFT3	JFT
PRG6	Priangan	JFT4	JFT
GRT1	Garut	JFT5	JFT
GRT2	Garut	SPD1	Sapudi
GRT3	Garut	SPD2	Sapudi
GRT4	Garut	SPD3	Sapudi
GRT5	Garut	SPD4	Sapudi
GRT6	Garut	SPD5	Sapudi
BTR1	Batur	HM236174.1	<i>Ovis aries</i> - Merino	Haplogroup A
BTR2	Batur	HM236175.1	<i>Ovis aries</i> - Romney	Haplogroup A
BTR3	Batur	HM236176.1	<i>Ovis aries</i> - Karakas	Haplogroup B
BTR4	Batur	HM236177.1	<i>Ovis aries</i> - Karakas	Haplogroup B
BTR5	Batur	HM236178.1	<i>Ovis aries</i> - Karakas	Haplogroup C	.	.	.	S
BTR6	Batur	HM236179.1	<i>Ovis aries</i> - Morkaraman	Haplogroup C	.	.	.	S
BTR7	Batur	HM236180.1	<i>Ovis aries</i> - Morkaraman	Haplogroup D	.	.	.	V
WSB1	Wonosobo	HM236181.1	<i>Ovis aries</i> - Morkaraman	Haplogroup D	.	.	.	V
WSB2	Wonosobo	HM236182.1	<i>Ovis aries</i> - Awassi	Haplogroup E	.	.	.	S
WSB3	Wonosobo	HM236183.1	<i>Ovis aries</i> - Tuj	Haplogroup E	.	.	.	S
WSB4	Wonosobo	HM236184.1	<i>Ovis musimon</i>	<i>Ovis musimon</i>
WSB5	Wonosobo	HM236187.1	<i>Ovis vignei</i>	<i>Ovis vignei</i>
JTT1	JTT	HM236188.1	<i>Ovis ammon</i>	<i>Ovis ammon</i>	.	T	.	.	T	I	V	I	.	
JTT2	JTT	KF312238.2	<i>Ovis orientalis</i>	<i>Ovis orientalis</i>	.	.	.	S
JTT3	JTT	MH094035.1	<i>Ovis canadensis</i>	<i>Ovis canadensis</i>	T	.	M	.	V	I	.	.	M	.
JTT4	JTT	MH779626.1	<i>Ovis nivicola</i>	<i>Ovis nivicola</i>	T	.	.	.	V	I	.	.	M	V
JTT5	JTT	MH779627.1	<i>Ovis dalli</i>	<i>Ovis dalli</i>	T	T	.	.	V	I	.	.	M	.

Fig. 4 Amino acid diversity of mtDNA Cyt b gene in Indonesian local sheep breeds

Breed	1	2	3	4	5	6	7	8	9	10	11	12
1. Priangan	0.0016											
2. Garut	0.0015	0.0016										
3. Batur	0.0016	0.0019	0.0014									
4. Wonosobo	0.0013	0.0019	0.0009	0.0000								
5. Javanese Thin Tailed	0.0017	0.0020	0.0013	0.0008	0.0010							
6. Javanese Fat Tailed	0.0013	0.0019	0.0009	0.0000	0.0008	0.0000						
7. Sapudi	0.0013	0.0019	0.0009	0.0000	0.0008	0.0000	0.0000					
8. <i>Ovis aries</i> - HapG A	0.0013	0.0010	0.0023	0.0026	0.0026	0.0026	0.0026	0.0000				
9. <i>Ovis aries</i> - HapG B	0.0013	0.0013	0.0010	0.0009	0.0009	0.0009	0.0009	0.0018	0.0000			
10. <i>Ovis aries</i> - HapG C	0.0093	0.0090	0.0103	0.0106	0.0106	0.0106	0.0106	0.0080	0.0097	0.0000		
11. <i>Ovis aries</i> - HapG D	0.0084	0.0087	0.0092	0.0088	0.0096	0.0088	0.0088	0.0080	0.0097	0.0142	0.0000	
12. <i>Ovis aries</i> - HapG E	0.0071	0.0068	0.0080	0.0084	0.0084	0.0084	0.0084	0.0057	0.0075	0.0031	0.0120	0.0008

Fig. 5 Genetic distance within (bold font) and between (standard font) breeds of Indonesian local sheep breeds based on mtDNA Cyt b gene sequences

value within the population was found in Priangan and Garut sheep with the same value (0.0016), while the closest distance value was found in Wonosobo, JFT, and Sapudi sheep (0.0000). In addition, the farthest distance value between the population was found between Garut and JTT sheep (0.0020), while the closest distance value was found between Wonosobo and JFT sheep, Wonosobo and Sapudi sheep, and JFT and Sapudi sheep with the same values (0.0000).

Phylogenetic analysis

The phylogenetic tree of Indonesian local sheep breeds and the sheep breeds referenced in this study are presented in Fig. 6. The phylogenetic analysis resulted that Indonesian local sheep breeds on Java Island were clustered in two haplogroups, namely haplogroup A (HapG A) and haplogroup B (HapG B). Haplogroup A consists of haplotypes 2 and 4, while haplogroup B consists of haplotypes 1, 3, 5, and 6. In this study, the phylogenetic tree

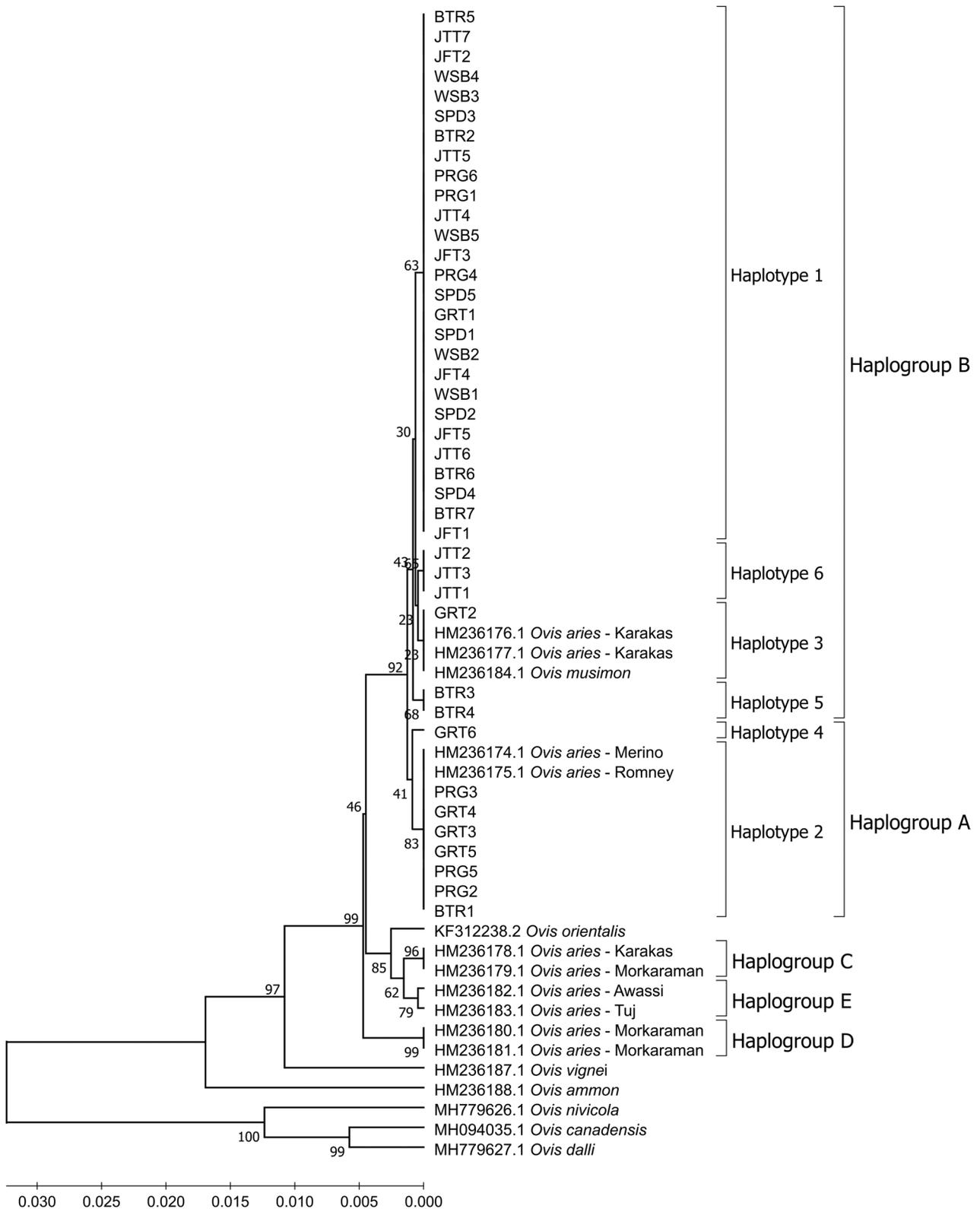


Fig. 6 Phylogenetic tree of Indonesian local sheep breeds and the other sheep in various genera and haplogroups based on mtDNA Cyt b sequences. PRG: Priangan sheep, GRT: Garut sheep, BTR: Batur sheep, WSB: Wonosobo sheep, JTT: Javanese Thin-Tailed sheep, JFT: Javanese Fat Tailed sheep, and SPD: Sapudi sheep

between Indonesian local sheep breeds on Java Island was constructed based on the genetic distance between them, as presented in Fig. 7.

Discussion

The present results show that the nucleotide base composition of the mtDNA Cyt b gene on all observed Indonesian local sheep has almost the same percentage. Overall, nucleotide base composition from the highest to lower, namely adenine (31.42%), cytosine (28.55%), thymine/uracil (27.06%), and guanine (12.97%). The Wonosobo, JTT, JFT, and Sapudi sheep have the same percentage of G + C (41.58%) and have a higher value than the other sheep observed, while the lower value was the Garut sheep (41.39%). Overall, the G + C percentage (41.52%) in observed Indonesian local sheep breeds was lower than the A + T percentage (58.48%). The nucleotide composition in this study was similar to published studies in goats [36], chicken [37], and cattle [7] that the G + C nucleotides composition in these animals was lower than A + T nucleotides composition.

Our results show that nucleotide diversity within and between breeds was only found in Priangan, Garut, Batur, and JTT sheep. The Wonosobo, JFT, and Sapudi sheep have the same mtDNA Cyt b gene sequence. This figure also shows that Priangan and Garut sheep were the sheep breeds with the highest nucleotide variation. No unique nucleotide base sequence was found in the different sheep breeds observed in this study. It indicates that no specific nucleotide base in the mtDNA Cyt b gene sequence can be used to characterize Indonesian local sheep breeds on Java Island in this study.

Furthermore, the results show that Priangan and Garut sheep have a higher nucleotide diversity/Pi (0.00158) and the average number of pairwise differences/K (1.8000) than other observed sheep breeds. Batur sheep had slightly higher nucleotide diversity than JTT sheep. In the same number of samples (7 samples), Batur sheep had slightly more variable sites than JTT sheep, so the values of Pi (0.00142 vs 0.00100) and K (1.61905 vs 1.4286)

were also higher, and lower sequence conservation (SC) values (99.65% vs 99.83%). Wonosobo, JFT, and Sapudi sheep were monomorphic, or there was no difference in nucleotide sequences in the mtDNA Cyt b gene. The low Pi value was also found in nineteen local sheep breeds in Xinjiang (0.00052–0.00665) [38] and six Egyptian sheep breeds (0.00087–0.00595) [6]. The low average pairwise differences (K) value were also found in Rahmani sheep (0.982), Saidi sheep (1.735), and Fallahi sheep (2.066) [6]. Based on within breeds, the higher haplotype diversity (Hd) was found in Garut sheep (0.800) from six samples used, resulting in four haplotypes, while the lower value was found in Wonosobo, JFT, and Sapudi sheep (0.000), resulting in the same haplotype in each breed (Tables 4 and 5). The Hd value in the Xinjiang local sheep [38], Mongolian native sheep [39], and Egyptian sheep [6] breeds were 0.464–0.893, 0.800–0.960, and 0.643–0.871, respectively.

The present results show that in 41 samples of observed Indonesian local sheep no difference in amino acid, both within and between sheep breeds. In this study, the nucleotide base changes occurred on the third codon, except for the C > T nucleotide base change that occurred in the first codon of the 982nd site (Fig. 3). Differences in nucleotide sequences do not change the amino acid structures of the mtDNA Cyt b gene in observed Indonesian local sheep, and the amino acid structure remains the same in all observed Indonesian local sheep breeds. It indicates that the mtDNA Cyt b gene was conserved relatively and that most nucleotide mutations do not change the amino acid structure [26]. This study's absence of amino acid diversity indicates that the mtDNA Cyt b gene cannot be used as a unique marker to characterize the observed Indonesian local sheep breeds on Java Island. Nucleotide mutation can be occurred by transition mutation and transversion mutation. Nucleotide mutation in the protein-encoding genes can be resulting synonymous amino acids (silent substitution) and non-synonymous amino acids. Most non-synonymous amino acids are due to the substitution of nucleotides in the first and second

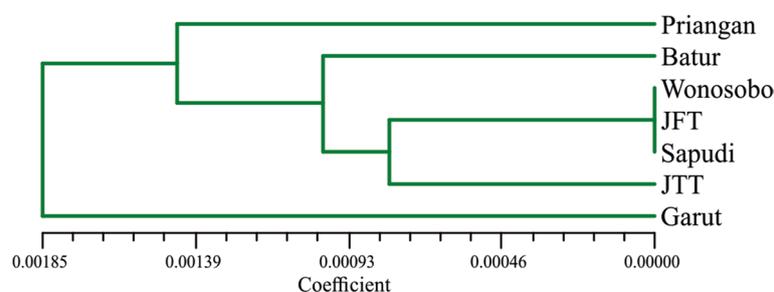


Fig. 7 Phylogenetic tree of observed Indonesian local sheep breeds based on genetic distance values of mtDNA Cyt b gene sequences

codons [11]. This study has no difference in amino acid structure due to the observed sheep breeds clustered into *Ovis aries* of haplogroup A and/or haplogroup B (Fig. 4). These haplogroups have the same amino acid structure in the mtDNA Cyt b gene. The difference in structure occurred when compared to *Ovis aries* of haplogroups C, D, and E in the 156th site (I>V) and 159th site (N>S). The mtDNA Cyt b gene sequence of Indonesian local sheep breeds in this study also has the same amino acid structure as *Ovis musimon* and *Ovis vignei* (Fig. 4).

Based on the genetic distance value, Indonesian local sheep breeds in this study have a close relationship with domestic sheep from haplogroup A and haplogroup B (Fig. 5). Priangan sheep have the same genetic distance value between *Ovis aries* from haplogroups A and B (0.0013). The genetic distance between Garut sheep and Haplogroup A sheep (0.0010) was lower than between Garut sheep and haplogroup B sheep (0.0013). It indicates that Garut sheep are closer to domestic sheep clustered in haplogroup A. In contrast, the genetic distance in Batur, Wonosobo, JTT, JFT, and Sapudi sheep was higher between domestic sheep in haplogroup B (0.0023–0.0028) than in haplogroup A (0.0009–0.0010). It indicates that Batur, Wonosobo, JTT, JFT, and Sapudi sheep were closer to domestic sheep clustered in haplogroup B than haplogroup A. These results reflect a previous study that Indonesian local sheep breeds clustered to haplogroup A and haplogroup B. They were mostly closer to haplogroup B than haplogroup A based on mtDNA D-loop sequences [28, 40]. The genetic distance in this study was lower than the Northernmost snow sheep population (0.0020–0.0028) [41] and six Egyptian sheep breeds (0.0012–0.0053) [6].

The phylogenetic analysis reported that the previous study clustered the domestic sheep into five haplogroups, namely haplogroups A, B, C, D, and E in Asia and Europe sheep breeds [40]. Indonesian local sheep in this study were clustered into haplogroup A and haplogroup B (Fig. 6). All Wonosobo, JTT, JFT, and Sapudi sheep were clustered into haplogroup B. Most Batur sheep were also clustered into haplogroup B, except for BTR1 sheep clustered into haplogroup A. Garut sheep were clustered into haplogroup A, except for GRT1 and GRT2. In addition, Priangan sheep have the same number of members clustered into both haplogroups. Thus, most of the Indonesian local sheep breeds on Java Island in this study were clustered into haplogroup B, except for Garut and Priangan sheep. These results were consistent with previous studies using mtDNA D-loop sequences that Indonesian local sheep breeds can be clustered into two haplogroups, namely haplogroup A and haplogroup B [28, 40]. Predominantly haplogroup B than haplogroup A was also found in Altanbulag sheep [1], Romanian

Racha sheep [42], Tsigai sheep and Cikta sheep [27], but in contrast to Nepal sheep [43], Mongolian sheep [1, 39], and Tibetan sheep [44].

Our results indicate that Wonosobo, JFT, and Sapudi sheep have the closest relationship. These three sheep breeds were then close to JTT sheep, followed by Batur, Priangan, and Garut sheep. This study found that most Garut sheep were clustered into haplogroup A, while Priangan sheep have the same percentage in haplogroups A and B (Table 4 and Fig. 6). This study also found one sample of Batur sheep clustered into haplogroup A. The researchers in the previous study stated that haplogroup A in the domestic sheep is predominantly found in Asian breed types [25, 33], while haplogroup B is predominantly found in European breed types and partially in Eastern Asia breed types [40, 45–48]. This study indicates that most Indonesian local sheep breeds on Java Island originated from European breed types. It is also supported by the close relationship between Indonesian local sheep breeds and *Ovis musimon* (European Mouflon) in the phylogenetic analysis (Fig. 6). It contrasts with Meadows et al. [40] and Guangxin et al. [49] report that domestic sheep from Indonesia, Mongolia, and Tibet have a close relationship with Asian sheep or domestic sheep with haplogroup A, while domestic sheep with haplogroup B are predominantly found in European sheep. It can be an exception for domestic sheep breeds in Indonesia, considering that Indonesia is a country with a strategic area for exploring and trading with various countries. In addition, this country had previously been occupied by the Dutch government for more than 300 years [50]. During that time, various livestock was imported from outside the area to be developed in this country including sheep [51]. Thus, Indonesian local sheep breeds may have resulted from crosses with breeds of European origin [28, 40, 48, 52].

The Garut, Priangan, and Batur sheep which were partly clustered into haplogroup A could be due to the crossing with Merino sheep in these three sheep, or maybe these sheep were indeed of an Asian breed of origin [28]. According to the Ministry of Agriculture of the Republic of Indonesia, Garut sheep resulted from crossing between Merino sheep from Australia, Kaapstad sheep from Africa, and Javanese Thin-Tailed sheep (local sheep) [53]. Priangan sheep resulted from crossing between local sheep, Texel sheep, and Merino sheep [54]. Batur sheep resulted from crossing between Merino sheep and thin-tailed sheep (local sheep) [55, 56]. However, genetically it has not been clearly explained in those Ministry of Agriculture Decree. This study proved that Garut, Priangan, and Batur sheep have a close relationship with Merino

sheep from Australia (HM236174), clustered with haplogroup A.

This study shows that most Indonesian local sheep breeds on Java Island were clustered to haplogroup B, which these sheep have a close relationship with European sheep breeds, whereas Indonesia on the Asia continental. However, this study also found the sheep samples clustered to haplogroup A, in which these sheep have a close relationship with Asian sheep breeds. Considering the history and facts in this study, the Indonesian local sheep breeds that developed on Java Island were indicated as resulting from crosses between Asian sheep types and European sheep types. This finding corroborates previous studies conducted with mtDNA D-loop sequences [28]. Further research still needs to be done to corroborate these results. Research with more target genes, larger samples, wider sampling area, complex methods, analyses, and comparative data with more various breeds both from Indonesia and outside the country will assist in characterizing and exploring the origins of Indonesian sheep breeds.

Conclusion

In conclusion, based on the mtDNA Cyt b gene sequence, there was a close relationship between Indonesian local sheep breeds on Java Island. Wonosobo, JFT, and Sapudi sheep had the closest relationship, and then these three breeds were close to JTT sheep, followed by Batur, Priangan, and Garut sheep. All Wonosobo, JTT, JFT, and Sapudi sheep and most Batur sheep were clustered to haplogroup B, which has a close relationship to European breed types. In contrast, most Garut sheep were clustered to haplogroup A, which has a close relationship to Asian breed types. In addition, Priangan sheep were clustered into both haplogroups with the same proportion.

Abbreviations

A	Adenine
bp	Base pair
BTR	Batur
C	Cytosine
Cyt b	Cytochrome b
D-loop	Displacement loop
DNA	Deoxyribonucleic acid
DNA SP	DNA Sequence Polymorphism
G	Guanine
GRT	Garut
Hap	Haplotype
HapG	Haplogroup
Hd	Haplotype diversity
I	Invariable sites
Indel	Insertion-deletion
JFT	Javanese Fat Tailed
JTT	Javanese Thin Tailed
K	Average number of pairwise differences
MEGA	Molecular Evolutionary Genetics Analysis

mtDNA	Mitochondrial deoxyribonucleic acid
NTSYS	Numerical Taxonomy and Multivariate Analysis System
P	Parsimony-informative sites
PCR	Polymerase chain reaction
Pi	Nucleotide diversity
PRG	Priangan
S	Singleton sites
SC	Sequence conservation
SPD	Sapudi
T	Thymine
U	Uracil
UPGMA	Unweighted Pair Group Method with Arithmetic Mean
V	Variable sites
WSB	Wonosobo

Acknowledgements

The authors are thankful to the Direktorat Penelitian UGM dan Tim Peningkatan Reputasi UGM menuju World Class University-Kantor Jaminan Mutu UGM, for funding this study (grant no. 10922/UN1.PII/Dit-Lit/PT.01.02/2022), and to the National Research and Innovation Agency (Badan Riset dan Inovasi Nasional/BRIN) with the Post-Doctoral Program. The authors also thank Faculty of Animal Science Universitas Gadjah Mada; PT. Agro Investama, and UPT Pembibitan Ternak dan Hijauan Makanan Ternak of Jember Regency for the support and thank all farmers.

Authors' contributions

WTA and EB supervised and coordinated the study. IGSB, ETM, and RW designed the study and interpreted the data. AI performed the experiment, analyzed the data, and drafted the manuscript. DM, ETM, and LT took part in preparing and critical checking this manuscript. All authors read and approved the final manuscript.

Funding

The financial support to cover the research activities were obtained from the Direktorat Penelitian UGM dan Tim Peningkatan Reputasi UGM menuju World Class University—Kantor Jaminan Mutu UGM with the Post-Doctoral Program (grant no. 10922/UN1.PII/Dit-Lit/PT.01.02/2022). DNA materials used in this activity were collected from PMDSU (Pendidikan Magister menuju Doktor untuk Sarjana Unggul) project.

Availability of data and materials

All data are primary data and generated from the research, research materials belong to our laboratory (Laboratory of Biochemistry and Molecular Biology).

Declarations

Ethics approval and consent to participate

This study has been authorized and approved with the Ethical Clearance Certificate of the Faculty of Veterinary Medicine Research Ethics Commission, Universitas Gadjah Mada, with the approval number 002/EC-FKH/Int./2019.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Author details

¹Department of Animal Production, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281. ²Research Center for Applied Zoology, National Research and Innovation Agency (BRIN), Bogor, Indonesia 16911. ³Department of Biochemistry and Molecular Biology, Faculty of Veterinary Medicine, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281. ⁴Department of Animal Breeding and Reproduction, Faculty of Animal Science, Universitas Gadjah Mada, Yogyakarta, Indonesia 55281. ⁵Department of Animal Science, Faculty of Agriculture, Universidade Nacional Timor Lorosa'e, Dili, Timor-Leste.

Received: 4 January 2023 Accepted: 12 March 2023

Published online: 17 March 2023

References

- Kim YS, Tseveen K, Batsukh B, Seong J, Kong HS (2020) Origin-related study of genetic diversity and heteroplasmy of Mongolian sheep (*Ovis aries*) using mitochondrial DNA. *J Anim Reprod Biotechnol* 35:198–206
- Ibrahim A, Budisatria IGS, Widayanti R, Artama WT (2019) The impact of religious festival on roadside livestock traders in urban and peri-urban areas of Yogyakarta, Indonesia. *Vet World* 12:1408–1415
- Ibrahim A, Budisatria IGS, Widayanti R, Artama WT (2019) Consumer's preferences for sheep attributes for Eid al-Adha celebration in Yogyakarta, Indonesia. *IOP Conf Ser Earth Environ Sci* 387:012001
- Ibrahim A, Budisatria IGS, Baliarti E, Sari APZNL, Artama WT, Widayanti R, Margawati ET, Fadholly A, Atmoko BA (2022) Qualitative morphological characterization of male Indonesian local sheep breeds on Java Island, Indonesia. *Indian J Anim Res*. <https://doi.org/10.18805/ijar.bf-1555>
- Ibrahim A (2021) Profil domba lokal di Pulau Jawa ditinjau dari karakteristik fenotipik dan genetik serta peranannya di masyarakat. Dissertation, Universitas Gadjah Mada
- Othman O, Germot A, Khodary M, Petit D, Maftah A (2018) Cytochrome b diversity and phylogeny of six Egyptian sheep breeds. *Annu Res Rev Biol* 22:1–11
- Hartatik T, Hariyono DNH, Adinata Y (2019) Short communication: genetic diversity and phylogenetic analysis of two Indonesian local cattle breeds based on cytochrome b gene sequences. *Biodiversitas* 20:17–22
- Prihandini PW, Primasari A, Luthfi M, Efendy J, Pamungkas D (2020) Genetic diversity of mitochondrial DNA cytochrome b in Indonesian native and local cattle populations. *J Ilmu Ternak dan Vet* 25:39–47
- Lestari DA, Sutopo KE (2017) Amino acid diversity on the basis of cytochrome b gene in Kacang and Ettawa Grade goats. *J Indones Trop Anim Agric* 42:135–146
- Hermes TR, Frachetti MD, Voyakin D et al (2020) High mitochondrial diversity of domesticated goats persisted among Bronze and Iron Age pastoralists in the Inner Asian Mountain Corridor. *PLoS One* 15:1–21
- Lestari DA, Purbowati E, Sutopo S, Kurnianto E (2018) Amino acid sequence based on cytochrome b gene in Kejobong goat and its genetic relationships among several local goats in Asia. *Vet World* 11:1196–1202
- Pakpahan S, Artama WT, Widayanti R, Suparta IG (2016) Genetic characteristics and relationship in different goat populations of Indonesia based on cytochrome b gene sequences. *Asian J Anim Sci* 10:29–38
- Phromnoi S, Lertwatcharasarakul P, Phattanakunanan S (2022) Genetic diversity and phylogenetic analysis of Khiew-Phalee chickens (Thailand) based on mitochondrial DNA cytochrome b gene sequences. *Biodiversitas* 23:750–756
- Farag MR, El Bohi KM, Khalil SR, Alagawany M, Arain MA, Sharun K, Tiwari R, Dhama K (2020) Forensic applications of mitochondrial cytochrome b gene in the identification of domestic and wild animal species. *J Exp Biol Agric Sci* 8:1–8
- Dave AR, Chaudhary DF, Mankad PM, Koringa PG, Rank DN (2021) Genetic diversity among two native Indian chicken populations using cytochrome c oxidase subunit I and cytochrome b DNA barcodes. *Vet World* 14:1389–1397
- Henrik PD, Ismoyowati, (2018) Morphometrics and genetic diversity of Tegal, Magelang and their crossbred ducks based on Cytochrome b gene. *J Indones Trop Anim Agric* 43:9–18
- Adeola AC, Sola-Ojo FE, Opeyemi YA et al (2022) Genetic diversity and population structure of muscovy duck (*Cairina moschata*) from Nigeria. *PeerJ* 10:e13236
- Chi HNQ, Minh TT, Quan NTM et al (2021) Cytochrome b based genetic relationship of wild boars from Dak Nong Province of Vietnam. *Ho Chi Minh City Univ Education J Sci* 18:2138–2146
- Hartatik T, Maharani D, Sidadolog JHP, Fathoni A, Sumadi, (2018) Haplotype diversity of partial cytochrome b gene in Kebumen ongole grade cattle. *Trop Anim Sci J* 41:8–14
- Tarekegn GM, Ji XY, Bai X, Liu B, Zhang W, Birungi J, Djikeng A, Tesfaye K (2018) Variations in mitochondrial cytochrome b region among Ethiopian indigenous cattle populations assert *Bos taurus* maternal origin and historical dynamics. *Asian-Australasian J Anim Sci* 31:1393–1400
- Giantisis IA, Diakakis NE, Avdi M (2018) Genetic composition and evaluation of the status of a non-descript indigenous horse population from Greece, the Macedonian Pacer. *J Equine Vet Sci* 71:64–70
- Sun T, Wang S, Hanif Q, Chen N, Chen H, Lei C (2020) Genetic diversity of mitochondrial cytochrome b gene in swamp buffalo. *Anim Genet* 51:977–981
- Rusdin M, Solihin DD, Gunawan A, Talib C, Sumantri C (2020) Genetic variation of Eight Indonesian swamp-buffalo populations based on cytochrome b gene marker. *Trop Anim Sci J* 43:1–10
- Kamalakkannan R, Kumar S, Bhavana K, Prabhu VR, Machado CB, Singha HS, Sureshgopi D, Vijay V, Nagarajan M (2021) Evidence for independent domestication of sheep mtDNA lineage A in India and introduction of lineage B through Arabian sea route. *Sci Rep* 11:1–16
- Koshkina OA, Deniskova TE, Dotsev AV, Kunz E, Upadhyay M, Krebs S, Solovieva AD, Medugorac I, Zinovieva NA (2021) A study of maternal variability of Russian local sheep breeds based on analysis of cytochrome b gene polymorphism. *Agric Biol* 56:1134–1147
- Sofla SS, Seyedabadi HR, Javanrouh Aliabad A, Seyed Sharifi R (2017) Genetic diversity and molecular phylogeny of Iranian sheep based on cytochrome b gene sequences. *Iran J Appl Anim Sci* 7:283–287
- Gáspárdy A, Zenke P, Kovács E, Annus K, Posta J, Sáfár L, Maróti-Agóts Á (2022) Evaluation of maternal genetic background of two Hungarian autochthonous sheep breeds coming from different geographical directions. *Animals* 12:1–12
- Ibrahim A, Budisatria IGS, Widayanti R, Artama WT (2020) The genetic profiles and maternal origin of local sheep breeds on Java Island (Indonesia) based on complete mitochondrial DNA D-loop sequences. *Vet World* 13:2625–2634
- Kőressaar T, Lepamets M, Kaplinski L, Raime K, Andreson R, Remm M (2018) Primer3-masker: Integrating masking of template sequence with primer design software. *Bioinformatics* 34:1937–1938
- Kumar S, Stecher G, Tamura K (2016) MEGA7: Molecular Evolutionary Genetics Analysis version 7.0 for bigger datasets. *Mol Biol Evol* 33:1870–1874
- Meadows JRS, Hiendleder S, Kijas JW (2011) Haplogroup relationships between domestic and wild sheep resolved using a mitogenome panel. *Heredity* (Edinb) 106:700–706
- Thompson JD, Higgins DG, Gibson TJ (1994) CLUSTAL W: Improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res* 22:4673–4680
- Kimura M (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J Mol Evol* 16:111–120
- Felsenstein J (1985) Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* (NY) 39:783–791
- Rozas J, Ferrer-Mata A, Sanchez-DelBarrio JC, Guirao-Rico S, Librado P, Ramos-Onsins SE, Sanchez-Gracia A (2017) DnaSP v6: DNA sequence polymorphism analysis of large datasets. *Mol Biol Evol* 34:3299–3302
- Pakpahan S, Artama WT, Widayanti R, Suparta IG (2016) Molecular phylogenetic of Hutan Sumatera goat (Sumatran serow) and domestic goat (*Capra hircus*) in Indonesia based on analysis mitochondrial cytochrome b gene. *Asian J Anim Vet Adv* 11:331–340
- Adamu MJ, Suhaili Z, Umar AS, Zubair RU (2016) Genetic diversity of indigenous chicken from the East Coast of Peninsular Malaysia inferred from control region of mitochondrial DNA. *Am J Innov Res Appl Sci* 2:282–288
- Sulaiman Y, Kaleri HA, Huang X, Aniwashi J, Zhao C, Xiong Y (2013) Phylogeny of 19 indigenous sheep breeds in Xinjiang inferred from cytochrome b. *J Anim Plant Sci* 23:1268–1275
- Ganbold O, Lee S, Seo D, Paek WK, Manjula P (2019) Genetic diversity and the origin of Mongolian native sheep. *Livest Sci* 220:17–25
- Meadows JRS, Li K, Kantanen J et al (2005) Mitochondrial sequence reveals high levels of gene flow between breeds of domestic sheep from Asia and Europe. *J Hered* 96:494–501
- Dotsev AV, Kunz E, Kharzinova VR et al (2021) Mitochondrial DNA analysis clarifies taxonomic status of the Northernmost Snow sheep (*Ovis nivicola*) population. *Life* 11:1–11
- Dudu A, Ghita E, Costache M, Georgescu SE (2016) Origin and genetic diversity of Romanian Racka sheep using mitochondrial markers. *Small Rum Res* 144:276–282
- Gorkhali NA, Han JL, Ma YH (2015) Mitochondrial DNA variation in indigenous sheep (*Ovis aries*) breeds of Nepal. *Trop Agric Res* 26:632–641

44. Liu J, Ding X, Zeng Y et al (2016) Genetic diversity and phylogenetic evolution of Tibetan sheep based on mtDNA D-Loop sequences. *PLoS One* 11:e0159308
45. Mereu P, Pirastu M, Barbato M, Satta V, Hadjisterkotis E, Manca L, Naitana S, Leoni GG (2019) Identification of an ancestral haplotype in the mitochondrial phylogeny of the ovine haplogroup B. *PeerJ* 7:e7895
46. Hiendleder S, Kaue B, Wassmuth R, Janke A (2002) Molecular analysis of wild and domestic sheep questions current nomenclature and provides evidence for domestication from two different subspecies. *Proc R Soc B Biol Sci* 269:893–904
47. Guo J, Du LXL-X, Ma Y-H, Guan W-J, Li H-B, Zhao Q-J, Li X, Rao A-Q, Rao S-Q (2005) A novel maternal lineage revealed in sheep (*Ovis aries*). *Anim Genet* 36:331–336
48. Tapio M, Marzanov N, Ozerov M, Činkulov M, Gonzarenko G, Kiselyova T, Murawski M, Viinalass H, Kantanen J (2006) Sheep mitochondrial DNA variation in European, Caucasian, and Central Asian areas. *Mol Biol Evol* 23:1776–1783
49. Guangxin E, Yong-Ju Z, Ri-su N et al (2017) Meta-analysis evidence of maternal lineages in Chinese Tibetan sheep using mtDNA D-loop panel. *Mitochondrial DNA Part A* 28:579–583
50. Vickers A (2005) *A History of Modern Indonesia*. Cambridge University Press, London
51. Directorate General of Livestock Services (2003) National report on animal genetic resources Indonesia, a strategic policy document. Ministry of Agriculture of the Republic of Indonesia, Jakarta
52. Baihaqi ZA, Widiyono I, Nurcahyo W (2019) Prevalence of gastrointestinal worms in Wonosobo and thin-tailed sheep on the slope of Mount Sumbing, Central Java, Indonesia. *Vet World* 12:1866–1871
53. Ministry of Agriculture (2011) Keputusan Menteri Pertanian Nomor 2914/Kpts/OT.140/6/2011 Tentang Penetapan Rumpun Domba Garut. Ministry of Agriculture of the Republic of Indonesia, Jakarta
54. Ministry of Agriculture (2017) Keputusan Menteri Pertanian Republik Indonesia Nomor 300/Kpts/SR.120/5/2017 Tentang Penetapan Rumpun Domba Priangan. Ministry of Agriculture of the Republic of Indonesia, Jakarta
55. Ministry of Agriculture (2011) Keputusan Menteri Pertanian Nomor 2916/Kpts/OT.140/6/2011 Tentang Penetapan Rumpun Domba Batur. Ministry of Agriculture of the Republic of Indonesia, Jakarta
56. Ibrahim A, Artama WT, Budisatria IGS, Yuniawan R, Atmoko BA, Widayanti R (2021) Regression model analysis for prediction of body weight from body measurements in female Batur sheep of Banjarnegara District, Indonesia. *Biodiversitas* 22:2723–2730

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.