

Research Article



Genetic Diversity and Phylogenetic Analysis of Bligon Goats Using Partial Mitochondrial Cytochrome B Gene

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Abstract | Indonesia has some local breeds of goats, including the Bligon goat, a crossbreed between the Peranakan Etawah and the Kacang goat. Though it is economically valuable, genetic studies of Bligon goats remain scarce. This study analyzed genetic diversity and the phylogenetic relationship of Bligon goats using mitochondrial Cytochrome b (*Cyt b*) gene sequences. Nine blood samples of Bligon goats from Kulon Progo, Yogyakarta, were collected, and DNA was extracted using the Geneaid gSYNC™ DNA Extraction Kit. PCR amplification of an 837 bp fragment of *Cyt b* was performed, and agarose gel electrophoresis and Sanger sequencing were then performed. Sequence data were generated using MEGA software, and genetic distances were estimated using the Kimura 2-parameter model, whereas a phylogenetic tree was constructed using the Neighbor-Joining method. Results revealed low genetic diversity in Bligon goats, with six polymorphic sites in 837 bp. Genetic distance calculation (0.000–0.011) also revealed close genetic similarity, demonstrating a well-conserved lineage. Phylogenetic analysis clustered Bligon goats with the Gembrong breed, suggesting a shared ancestry of Indonesian goats. Unlike domestic *Capra hircus* references, Bligon goats had a distinct genetic signature while differing from wild *Capra* species. These findings highlight the need to conserve Bligon goats as a genetic resource. The study underscores the need for more genetic research to enable sustainable breeding programs and livestock biodiversity conservation in Indonesia.

Keywords | *Cyt b* gene, Phylogenetics, Polymerase chain reaction (PCR), Bligon goat, Genetic diversity

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INTRODUCTION

Indonesia possesses a range of native goat breeds with distinct morphological and genetic characteristics shaped by adaptation to different environmental conditions and production systems. The goats form an integral part of the

agricultural economy in Indonesia, serving as a source of income, meat, and milk for smallholder farmers (Ilham *et al.*, 2023). Goat farming is prevalent in rural areas, providing most households economic stability and food security. Goat consumption is primarily influenced by cultural and religious beliefs, particularly around activities like Eid al-

Adha, when livestock demand increases significantly (Sujarwanta *et al.*, 2024). Among the many indigenous breeds, the Bligon goat is of particular interest. It is a crossbreed between Peranakan Etawah and Kacang goats, prevalent throughout Java. Despite its importance in farming systems, there has not been extensive work on the genetic diversity of Bligon goats, and it remains an area to study further.

Genetic diversity is an important element of goat breeding and conservation because it influences the adaptability of a breed to the environment, disease resistance, and productivity. Mitochondrial DNA (mtDNA) is a valuable tool in assessing genetic relationships between goat populations and in other domestic animals. Studies have indicated that mtDNA studies provides valuable information on genetic diversity, phylogenetic relatedness, and maternal lineage tracing that is important in the explanation of the evolutionary history of goat breeds (Nguluma, 2021; Nguluma *et al.*, 2021; Silva *et al.*, 2020). Indonesian local goats have been revealed in previous mtDNA studies to possess restricted maternal genetic diversity, suggesting a common ancestral origin and potential historical interbreeding (Lestari *et al.*, 2018; Pakpahan *et al.*, 2015). This finding underscores the importance of further genetic studies in determining whether native breeds, like Bligon goats, maintain specific genetic identities or have significant genetic similarities with other local breeds.

One of the most significant genetic markers used in goat phylogenetic studies is the cytochrome b (*Cyt b*) gene, which has been widely used to assess genetic diversity and evolutionary distances among various goat breeds. Research suggests that *Cyt b* analysis can illustrate close genetic relationships among indigenous goat populations from various regions of Asia, suggesting historical patterns of domestication and differentiation among breeds (Chowdhury *et al.*, 2019). As most earlier studies have focused on other Indonesian goat breeds, comprehensive genetic work has not been conducted on Bligon goats. Therefore this study analyzed genetic diversity and the phylogenetic relationship of Bligon goats using mitochondrial Cytochrome b (*Cyt b*) gene sequences. Identifying their genetic makeup through mtDNA and *Cyt b* analysis will be critical to evaluating their genetic distinctiveness, conserving them, and improving breeding programs. Improved knowledge of the genetic composition of Bligon goats will be valuable to the sustainable development of native goat breeds in Indonesia, supporting biodiversity conservation and local rural agriculture development.

MATERIALS AND METHOD

ETHICAL APPROVAL

The use of goats in this study was conducted following ethical guidelines reviewed and approved by the Ethical

Clearance Commission of the Veterinary Medicine Faculty, Universitas Gadjah Mada (UGM). This approval was validated through the Ethical Clearance Statement No: 086/EC-FKH/Int./2022, issued on October 15th, 2022.

SAMPLE COLLECTION

Nine blood samples were collected from Banjarsari Village, Kulon Progo Regency, and Yogyakarta goat farms. Blood samples were collected from the jugular vein and immediately stored in EDTA tubes to prevent coagulation. The samples were then preserved at -20°C to maintain DNA integrity for further analysis.

DNA ISOLATION

DNA extraction was performed using the Geneaid gSYNC™ DNA Extraction Kit Quick Protocol (Geneaid Biotech, Taiwan). The isolation procedure involved several steps, including cell lysis, DNA binding, washing, and elution, to ensure the extraction of high-quality DNA.

POLYMERASE CHAIN REACTION (PCR)

The extracted DNA was amplified using specific primers: forward primer (AHCB-F: 5'-ATT-GACCTCCCAACCCCATC-3') and reverse primer (AHCB-R: 5'-TGTGTGGAGGAAGGGTACAA-3'), producing a PCR product of 837 bp. The amplification was carried out in a 50 µl reaction mixture consisting of 25 µl of 2x MyTaq HS Red Mix (Meridian Bioscience, US), 20 µl of nuclease-free water, 3 µl of DNA template, and 1 µl of each primer. PCR was performed using a Cleaver GTC96S thermal cycler (Cleaver Scientific Ltd, England).

The PCR conditions included an initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 55°C for 40 seconds, and extension at 72°C for 1 minute. The amplification process concluded with a final extension step at 72°C for 10 minutes to ensure complete elongation of the DNA fragments.

AGAROSE GEL ELECTROPHORESIS AND SEQUENCING

The PCR products were analyzed using 1.5% agarose gel electrophoresis to confirm successful amplification. Electrophoresis was conducted at 100V for 40 minutes, and the DNA bands were visualized using a UV transilluminator. The amplified DNA from all nine samples was then subjected to Sanger sequencing, which was performed at the Integrated Research and Testing Laboratory (LPPT), Universitas Gadjah Mada.

DATA ANALYSIS

The sequencing results were analyzed using Molecular Evolutionary Genetic Analysis (MEGA) software version 11.0.13. Raw sequence chromatograms were initially viewed and manually edited to ensure base calling accuracy

and to trim low-quality ends. Consensus sequences were assembled by aligning forward and reverse reads. Sequence alignment was performed using the Clustal W algorithm integrated in MEGA with default parameters. The aligned sequences were further inspected to ensure proper alignment and to correct for possible misalignments in hyper-variable regions. Haplotype analysis was conducted using MEGA by identifying variable sites and unique sequence patterns within the aligned data. Polymorphic sites were calculated based on the aligned sequences. Genetic distances between sequences were calculated using the Pairwise distance method under the Kimura 2-parameter (K2P) model, which corrects for multiple substitutions at the same site and accounts for transition/transversion bias. A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method implemented in MEGA, also employing the K2P model. Statistical support for the tree topology was assessed using a bootstrap analysis with 1000 replicates. Reference sequences from domestic and wild goats were retrieved from the National Center for Biotechnology Information (NCBI) GenBank database and included in the analysis to infer evolutionary relationships and validate the phylogenetic structure of the sample sequences.

RESULTS AND DISCUSSION

AMPLIFICATION VISUALIZATION

The PCR amplicons were subsequently analyzed using 1.5% agarose gel electrophoresis and visualized using a UV transilluminator, as shown in [Figure 1](#). The DNA bands were distinct and well separated in wells 1–9, indicating a good-quality DNA concentration for further research ([Arslan et al., 2021](#)). This confirms that the PCR amplification successfully produced an 837 bp DNA fragment. Based on these results, samples were analyzed further using Sanger sequencing for detailed genetic analysis.

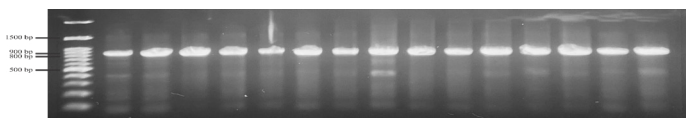


Figure 1: Agarose gel electrophoresis of PCR-amplified *Cyt b* gene fragments from Bligon goat DNA samples. The image shows distinct DNA bands at the expected size of 837 bp.

SEQUENCE ANALYSIS

The alignment analysis of an 837 bp *Cyt b* gene fragment of *Capra hircus* produced interesting data on genetic variation in Bligon goats and their phylogenetic relationships to other domestic and feral goat populations. The results showed that 831 of the 837 nucleotide positions were monomorphic, indicating significant conservation in the *Cyt b* gene. These conserved regions point to strict functional limitations, as mutations within these areas could

interfere with important mitochondrial processes involved in cellular respiration.

However, six positions (309, 366, 495, 501, 594, and 643) were discovered to be polymorphic, pointing towards the genetic diversity of the goat samples under investigation. Five sites (366, 495, 501, 594, and 643) were parsimony-informative, as these positions had nucleotide substitutions shared by at least two different sequences. These informative sites play a crucial role in phylogenetic analysis and help to determine the evolutionary relationships among populations. One site (309) was a singleton, i.e., it contained a single nucleotide difference found in only one sequence, indicating a new or recent mutation in this patient. The nucleotide substitutions detected were mainly transitions (A↔G and C↔T), typical of mitochondrial DNA, as they are less likely to impact protein structure significantly. This is consistent with known mutation patterns in mitochondrial genes, which always contain more transitions than transversion mutations ([Zou and Zhang, 2021](#)).

Comparing Bligon goats with other Indonesian local breeds, such as Gembrong, established a high genetic similarity, which aligns with Bligon goats being classified as members of the *Capra hircus* species. Despite the similarity, polymorphic sites show some genetic diversity within the population that would be of potential value in future breeding and conservation programs. The results highlight the genetic homogeneity of the Bligon goat but also indicate that genetic heterogeneity remains, which can result in breed differentiation or environmental adaptation ([Rahmawati et al., 2022](#)). These findings highlight the importance of mitochondrial DNA, particularly the *Cyt b* gene, in describing genetic affinities and diversity in Indonesian local goat breeds. The description of parsimony-informative and singleton sites sheds light on the richness of genetic variety within the Bligon goat population. Further studies with additional mitochondrial markers and broader sampling can augment our understanding of Indonesian goat breeds' genetic makeup and evolutionary process. The precise locations of the polymorphic sites and the corresponding changes in nucleotides are presented in [Table 1](#), giving a clear representation of the genetic variability encountered in this study.

The alignment result of the partial *Cyt b* gene at position 215 amino acid, as shown in [Table 2](#), indicates genetic variation between goat populations. The control *Capra hircus* (AB004075.1) possesses a different amino acid compared to the Gembrong goat, Bligon goats (KB 1–9), and the control genome (NC_005044.2:1), which all possess the same amino acid at this position. Notably, the wild relatives *Capra ibex*, *Capra falconeri*, and *Capra aegagrus* retain the ancestral state, suggesting significant evolutionary conservation. Because *Cyt b* is a critical protein in mitochondri-

al electron transport and cellular respiration, variation at functionally significant positions like amino acid 215 could influence metabolic efficiency and adaptation to local environmental conditions. The similarity between the amino acid changes of Gembrong and Bligon goats indicates recent common descent or selective pressures that might have acted on their genetic makeup (Pakpahan *et al.*, 2015).

Table 1: Multiple sequence alignment of *Cyt b* gene sequences from Bligon goat samples (KB 1–9) and reference *Capra* species.

Site	309	366	495	501	594	643
<i>Capra hircus</i> AB004075.1	T	A	A	G	C	A
<i>Capra hircus</i> NC 005044.2:1	C	G	.	A	T	G
Etawah	.	G	.	A	.	.
Gembrong	C	G	.	A	T	G
KB 1	C	G	.	A	T	G
KB 2	C	G	.	A	T	G
KB 3	C	G	G	A	T	G
KB 4	C	G	.	A	T	G
KB 5	C	G	.	A	T	G
KB 6	C	G	.	A	T	G
KB 7	C	G	.	A	T	G
KB 8	C	G	.	A	T	G
KB 9	C	G	.	A	T	G
<i>Capra ibex</i>	.	.	.	A	.	G
<i>Capra falconeri</i>	.	.	.	A	.	.
<i>Capra aegagrus</i>	.	G	.	A	T	.

Table 2: Amino acid variation at position 215 of the *Cyt b* protein among Bligon goat samples (KB 1–9) and reference *Capra* species.

Site	215
<i>Capra hircus</i> AB004075.1	T
<i>Capra hircus</i> NC 005044.2:1	A
Etawah	.
Gembrong	A
KB 1	A
KB 2	A
KB 3	A
KB 4	A
KB 5	A
KB 6	A
KB 7	A
KB 8	A
KB 9	A
<i>Capra ibex</i>	A
<i>Capra falconeri</i>	.
<i>Capra aegagrus</i>	.

GENETIC DISTANCE

The genetic distance of Bligon goats (KB 1–9), as shown in Table 3, reveals that this breed has a highly conserved genetic structure, emphasizing its status as a well-preserved Indonesian local goat (Lestari *et al.*, 2018; Pakpahan *et al.*, 2015). The genetic distances of the Bligon goat samples range from 0.000–0.011, which signifies minimal variation within the population. Several instances of zero genetic distance among individuals (e.g., KB 1 and KB 2, KB 4 and KB 8) indicate close genetic similarity, supporting that Bligon goats have been intensively conserved with an unchanged genetic line through generations. Such stability shows that Bligon goats have not undergone strong genetic drift or crossbreeding with other breeds, re-establishing that they are a distinct local genetic resource. The close genetic relationship of Bligon goats to the Gembrong goat, the only available Indonesian native goat breed in the current study, also warrants the conservation of their genetic characteristics. Bligon goats, at a genetic distance of 0.006, are genetically closer to Gembrong than any other domestic or wild goat breeds, suggesting a common origin among Indonesia’s native goat breeds. Their genetic proximity to Etawah goats (0.003–0.006) also suggests some historical genetic exchange, although Bligon goats remain distinct in their genetic constitution.

Compared to *Capra hircus* reference sequences (AB004075.1, NC 005044.2:1), Bligon goats share genetic distances of 0.003 to 0.009, once again confirming their placement in the domestic goat lineage but with a unique genetic identity of their own. On the other hand, the genetic distance between Bligon goats and wild goat species such as *Capra ibex*, *Capra falconeri*, and *Capra aegagrus* is significantly greater (ranging from 0.041 to 0.065). This illustrates a clear evolutionary divergence of Bligon goats from wild forms, which further attests that they are a fully domesticated and conserved breed.

The genetic similarity among Bligon goats underscores the significance of Bligon goats in Indonesia’s agricultural and cultural heritage. Unlike commercial breeds of goats that can be under selective breeding for specific traits, Bligon goats have been conserved as a local gene pool, protecting the originality of their traits. Their survival is likely due to traditional breeding practices focusing on conserving indigenous traits suited to local environmental conditions rather than artificial selection for commercial traits. The uniformity of their genetic structure suggests that Bligon goats have been a long-standing element of Indonesia’s livestock diversity and constitute a valuable genetic resource for future breeding and sustainable livestock development.

PHYLOGENETIC ANALYSIS

The phylogenetic tree obtained from mitochondrial sequences provides an overall view of the genetic

Table 3: A pairwise genetic distance matrix is based on *Cyt b* sequences among Bligon goat samples (KB 1–9) and references to *Capra* species.

	<i>Capra hircus</i> AB004075.1	<i>Capra hircus</i> NC 005044.2:1	<i>Etawah</i> Gem- brong	KB 1	KB 2	KB 3	KB 4	KB 5	KB 6	KB 7	KB 8	KB 9	<i>Capra</i> <i>ibex</i>	<i>Capra</i> <i>falconeri</i>	<i>Capra</i> <i>aegagrus</i>
<i>Capra hircus</i> AB004075.1															
<i>Capra hircus</i> NC 005044.2:1	0.009														
<i>Etawah</i>	0.003	0.006													
<i>Gembrong</i>	0.009	0.000	0.006												
KB 1	0.009	0.000	0.006	0.000											
KB 2	0.009	0.000	0.006	0.000	0.000										
KB 3	0.011	0.002	0.008	0.002	0.002	0.002									
KB 4	0.009	0.000	0.006	0.000	0.000	0.002	0.002								
KB 5	0.009	0.000	0.006	0.000	0.000	0.002	0.000	0.000							
KB 6	0.009	0.000	0.006	0.000	0.000	0.002	0.000	0.000	0.000						
KB 7	0.009	0.000	0.006	0.000	0.000	0.002	0.000	0.000	0.000	0.000					
KB 8	0.009	0.000	0.006	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000				
KB 9	0.009	0.000	0.006	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000			
<i>Capra ibex</i>	0.063	0.063	0.063	0.063	0.063	0.065	0.063	0.063	0.063	0.063	0.063	0.063	0.063		
<i>Capra falconeri</i>	0.041	0.048	0.041	0.048	0.048	0.049	0.048	0.048	0.048	0.048	0.048	0.048	0.048	0.051	
<i>Capra aegagrus</i>	0.006	0.003	0.003	0.003	0.003	0.005	0.003	0.003	0.003	0.003	0.003	0.003	0.003	0.063	0.044

relationships between Bligon goats (KB 1–9) and various reference *Capra* species, including domestic and wild goats. The tree shows a highly supported pattern of clustering supporting the classification of Bligon goats as a conserved Indonesian goat breed. The samples of Bligon goats form an extremely well-supported monophyletic group, as attested by the 100% bootstrap value, demonstrating the existence of very high genetic homogeneity. Their clumping together near very little genetic difference indicates that Bligon goats possess a well-conserved genetic lineage. This clade is nested within the broader *Capra hircus* clade and is genetically closely related to the Gembrong goat (MK234884.1), another indigenous Indonesian breed. The genetic closeness of Bligon and Gembrong goats suggests a shared ancestral origin or limited genetic introgression from foreign breeds, further highlighting their characterization as locally adapted breeds.

In addition to clustering closely with the Gembrong goat, the Bligon goats cluster near the domestic goat reference genome (NC 005044.2:1) and Etawah breed (KY305183.1). However, they remain genetically distinct from *Capra aegagrus* (NC 028161.1), the wild ancestor of domestic goats that indicates their domestication status, and they have no substantial introgression from the wild (Amills *et al.*, 2017; Colli *et al.*, 2015; Dong *et al.*, 2015). The phylogenetic tree distinctly separates domestic goats from wild species, with Bligon and Gembrong goats firmly in the *Capra hircus* branch, while wild goats such as *Capra ibex* (AF217256.1) and *Capra falconeri* (AB044309.1) perch on different, more divergent branches. The significant genetic distance from Bligon goats to such wild types further confirms their character as a stable domesticated breed with long-term continuity of genetics.

The robust bootstrap values over the whole tree confirm the credibility of these phylogenetic associations, demonstrating that Bligon goats have been within the *Capra hircus* gene pool without substantial outside genetic contributions. In contrast to numerous contemporary commercial breeds having experienced extensive crossbreeding and selection for particular productive characteristics (Ribeiro *et al.*, 2018), Bligon goats demonstrate genetic stability, presumably because of conventional breeding practices that seek to maintain local gene resources. The clumping pattern shows that Bligon goats have been maintained as a distinct genetic population, contributing to the genetic diversity of Indonesia's livestock populations. The phylogenetic analysis reveals the importance of Bligon goats as a genetic asset, and conservation is required to ensure the sustainability and conservation of this indigenous breed. Their genetic stability, as indicated by their distinct clustering and close relationship with other Indonesian breeds, also bears witness to their being a conserved local breed of great cultural and agricultural importance.

The phylogenetic tree in Figure 2 visually illustrates these relationships, illustrating the genetic proximity of Bligon goats to domestic goat reference sequences and their divergence from wild goats.

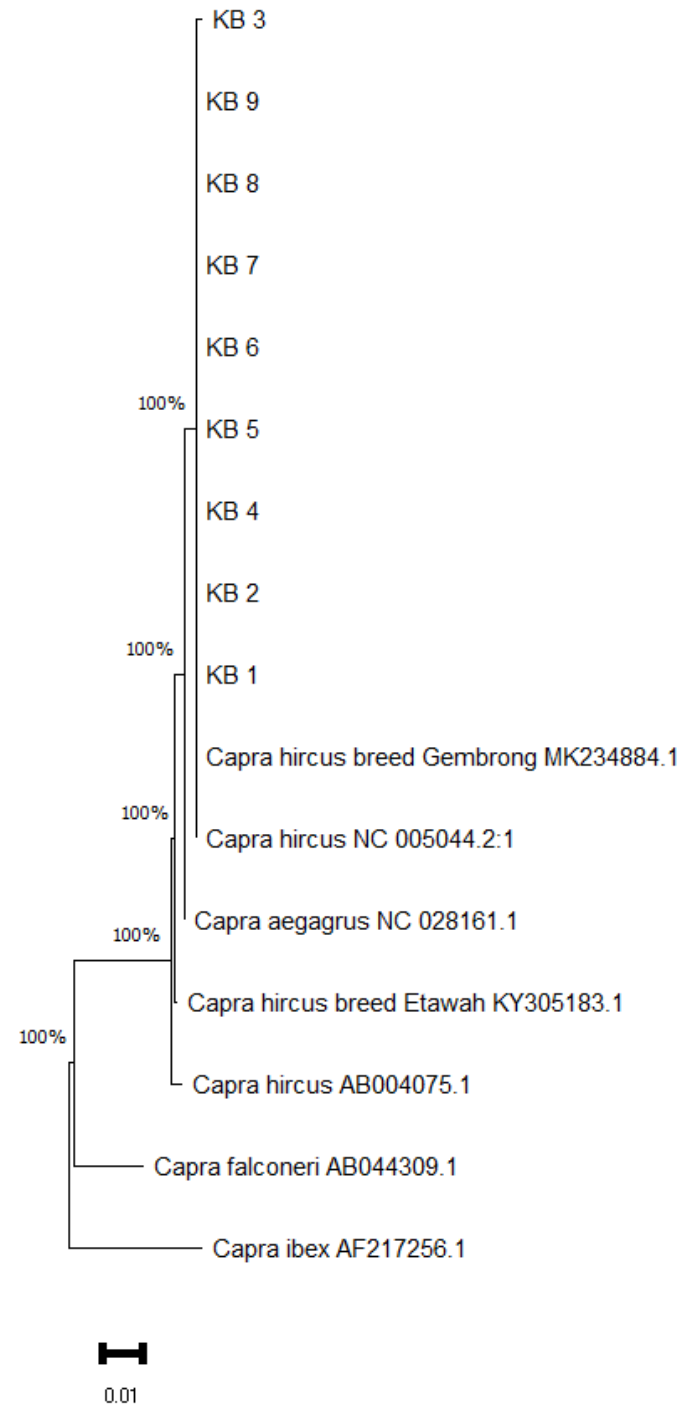


Figure 2: The phylogenetic tree based on Cyt b sequences illustrates the genetic relationships among Bligon goat samples (KB 1–9) and reference *Capra* species.

The genetic analysis of Bligon goats (KB 1–9) confirms their strong genetic stability and close relationship with the Gembrong goat, reinforcing their status as a well-preserved Indonesian breed. The 837 bp *Cyt b* gene sequencing revealed minimal genetic variation, with only a few polymorphic sites indicating limited diversity. Genetic distance analysis showed high similarity within the population and clear separation from wild goat species, confirming their domesticated status. Phylogenetic analysis further supported their distinct clustering within the *Capra hircus* lineage. These findings highlight the importance of Bligon goats as a valuable genetic resource, emphasizing the need for conservation to maintain their genetic integrity and contribution to Indonesia's livestock diversity.

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NOVELTY STATEMENTS

This study is the first to assess the genetic diversity and phylogenetic relationships of Bligon goats, a crossbred Indonesian breed, using mitochondrial *Cytochrome b* gene sequences. Despite their hybrid origin, Bligon goats exhibit low genetic divergence from domestic *Capra hircus* references and are distinctly separated from wild goat lineages. These findings highlight the genetic stability of Bligon goats and underscore their importance as a conserved local genetic resource, valuable for sustainable breeding strategies and biodiversity preservation in Indonesia.

AUTHOR'S CONTRIBUTIONS

Regita Cahyani Pantoro, Krisna Noli Andrian, and Reski Amalia contributed to conceptualization, investigation, sample collection, data analysis and interpretation, and original draft preparation. Medania Purwaningrum was involved in conceptualization, investigation, sample collection, reviewing, and editing. Yuriati and Triatmojo were involved in conceptualization, investigation, and sample collection. Aris Haryanto contributed to reviewing, editing, and funding acquisition. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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