HAYATI
Journal of Biosciences

Vol. 33 No. 6, November 2026 1637-1647
DOI:10.4308/hjb.33.6.1637-1647
ISSN: 1978-3019
EISSN: 2086-4094

Research Article

Check for updates

OPEN ACCESS

Complete Mitochondrial Genome Characterization and Phylogenetics of Ongole Grade (PO) Cattle

Dalilah Ramadhani[1], Anik Budhi Dharmayanthi[2], Ronny Rachman Noor[3], Jakaria Jakaria[3*]

[1]*Graduate School of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia*
[2]*Research Center for Biosystematics and Evolution, National Research and Innovation Agency, Bogor 16911, Indonesia*
[3]*Department of Animal Production and Technology, Faculty of Animal Science, IPB University, Bogor 16680, Indonesia*

ARTICLE INFO

Article history:
Received May 28, 2026
Received in revised form October 12, 2026
Accepted October 21, 2026
Available Online November 5, 2026

KEYWORDS:
mitochondrial DNA,
Peranakan Ongole cattle

Copyright (c) 2026 @author(s).

ABSTRACT

The Peranakan Ongole (PO) cattle are a native Indonesian breed and a vital component of the country's livestock genetic heritage. The scarcity of mitochondrial genome data for this breed underscores the need for further research to inform future breeding programs. This study examined the mitochondrial genome of Ongole grade (PO) cattle and constructed a phylogenetic tree using both complete and partial sequences. The DNA extraction and PCR processes were conducted at the BRIN Genomics Building in Cibinong, Bogor, West Java. In addition, sequencing of the PCR products was performed at PT Genomik Solidaritas Indonesia (GSI) in South Jakarta. We analyzed blood samples from five PO bulls at the Singosari Cattle Breeding Center (BBIB), Malang, East Java, Indonesia. DNA was extracted using the Quick-DNA HMW Magbead Kit (Zymo Research, USA), and two primer pairs yielded fragments of 9,064 and 9,222 bp based on the Bos javanicus CERZA reference. Sequencing was performed using Oxford Nanopore technology. The phylogenetic analysis included full-length and specific mitochondrial regions: D-loop, Cyt-b, COX1, ND5, and 16S rRNA. The complete mitochondrial genome was 16,562 base pairs long and encoded 13 protein-coding genes, 22 tRNA genes, and the D-Loop control region. Phylogenetic trees constructed from both full and partial genomes showed consistent relationships, indicating that PO cattle are closely related to Bos javanicus CERZA (JN632606.1) and bali cattle (PQ130485.1). The study suggested that partial mitochondrial DNA markers could produce phylogenetic trees comparable to those from complete genomes. However, bootstrap support was lower with partial data, highlighting the greater resolution provided by full mitochondrial sequences.

1. Introduction

Peranakan Ongole (PO) cattle are one of Indonesia's local beef cattle breeds, found throughout most of the country, and were designated as such by the Minister of Agriculture through Decision No. 2907/Kpts/OT.140/6/2011 (BSN 2020). PO cattle result from crossbreeding Javanese cattle with Ongole cattle, which originated in India and were developed on the island of Sumba around 1930 through a grading-up process, in which Ongole cattle are domesticated members of the zebu group (humped cattle), specifically the Bos indicus subspecies. Meanwhile, Javanese cattle are believed to descend from wild Javanese cattle (*Bos javanicus*) crossed with zebu cattle (*Bos indicus*) (Hartatik 2019).

PO cattle are a local breed widely raised in Indonesia. In Central Java in particular, PO cattle make up approximately 51.93% of the existing cattle population (Hartati *et al.* 2009). However, PO cattle are currently experiencing declining productivity and genetic quality due to uncontrolled crossbreeding, necessitating efforts to preserve them as a genetic resource. Uncontrolled crossbreeding and limited

* Corresponding Author
E-mail Address: jakaria@apps.ipb.ac.id

genetic information, including livestock data, may threaten the genetic purity of local cattle. Therefore, comprehensive genetic information is essential to support sustainable breeding and conservation strategies. Efforts to increase livestock productivity through breeding and crossbreeding must be carried out in a planned manner and be accompanied by conservation strategies to preserve the genetic diversity and purity of local cattle for future generations.

To prevent the loss of genetic resources in local cattle, comprehensive genetic information is needed to support breeding programs and conservation efforts. To date, genetic information on PO cattle remains very limited; in fact, the origins and kinship relationships of PO cattle are not yet well understood. Molecular approaches using DNA analysis are now widely used to trace cattle origins and understand their phylogenetic relationships. Studies of phylogenetic relationships are often conducted using mitochondrial DNA (mtDNA) because it has a higher copy number per cell than nuclear DNA (Magee *et al.* 2014). Mitochondrial DNA (mtDNA) analysis is widely used to evaluate genetic diversity both within and between species, trace maternal lineages, and reveal evolutionary relationships between species (Bradley *et al.* 1996; Anwar *et al.* 2025). Mitochondrial DNA markers are inherited exclusively from the mother and do not recombine (Ferreira and Rodriguez 2024; Jakaria *et al.* 2025).

Molecular characterization of cattle genetic resources can be conducted using various genetic markers, including autosomal and Y-chromosomal markers, mitochondrial D-loop sequences, and whole-genome sequencing approaches (Weldenegodguad *et al.* 2019; Ajmone-Marsan *et al.* 2023). These approaches provide complementary information because nuclear and Y-chromosomal markers characterize broader genomic and paternal variation, whereas mtDNA specifically reflects maternal lineages. Complete mitochondrial genome sequencing can provide greater phylogenetic resolution than individual mitochondrial regions because it incorporates variation across the entire mitochondrial genome (Morón-López *et al.* 2022; Anwar *et al.* 2025). Consequently, complete mitogenome data may provide more detailed information for characterizing mitochondrial sequences and examining maternal phylogenetic relationships than analyses based on individual partial markers.

Research using mtDNA markers has been widely conducted to reveal genetic diversity. However, most studies still rely on partial mtDNA sequences such as the D-loop (Jakaria *et al.* 2019), the cytB gene (Hartatik *et al.* 2019), the 12S rRNA gene (Suselowati *et al.* 2023), and the COI gene (Utami *et al.* 2023). Therefore, analyses based on partial mitochondrial markers provide only a limited picture of mitochondrial sequence variation in PO cattle. This limitation can be overcome by performing complete mitochondrial genome sequencing, which provides a broader picture of mitochondrial sequence variation and has the potential to improve phylogenetic resolution. One sequencing technology that is increasingly used in genomic studies is Oxford Nanopore Technology (ONT), a third-generation sequencing technology, especially in resolving complex or long repetitive regions found in the mitochondrial genome (Anwar *et al.* 2025). This study aimed to characterize the mitochondrial genome of PO cattle and examine their maternal phylogenetic relationships using complete and selected partial mtDNA sequences.

2. Materials and Methods

2.1. Ethical Clearance

This study was conducted after approval from the Ethics Committee on Animal Care and Use of the National Research and Innovation Agency (BRIN) (approval number: 071/KE.02/SK/03/2024).

2.2. Methods

2.2.1. Animals, DNA Extraction, and Sequencing

The Molecular Genetics Laboratory at the Faculty of Animal Science, IPB, collected blood samples from PO bulls at BBIB Singosari, Malang, East Java, in September 2024. Blood samples of 5–8 Ml were collected from the jugular vein into Vacutainer tubes containing K3EDTA. We assigned the samples identification codes PO2, PO3, PO4, and PO5. Table 1 presents each animal's identity, based on its livestock name and corresponding sample code. Total DNA extraction was performed using the Quick-DNA HMW Magbead Kit (Zymo Research, USA) according to the manufacturer's instructions. DNA quantity and quality were tested using the Qubit 4.0 Fluorometer (Thermo Fisher Scientific, USA) and the NanoDrop One Spectrophotometer (Thermo Fisher Scientific, USA).

We obtained the mitochondrial genome through amplicon-based targeted sequencing using the Oxford Nanopore Technologies (ONT) platform. Two fragments, each approximately 9 kb in size, were amplified using GoTaq Long PCR Master Mix (Promega) with two pairs of primers: the first forward primer 5'-

Table 1. Identity of the individual PO cattle samples from BBIB Singosari used in the study

Sample code	Bull code	Name	Origin	Birth date	Sample collection month
PO2	21334	Akbar	Indonesia	20/07/2013	December 2024
PO3	21431	Alindo	Indonesia	10/10/2014	December 2024
PO4	21532	Delta	Indonesia	12/12/2015	December 2024
PO5	21740	BJJ. Wick	Indonesia	15/01/2017	December 2024

ATGAGTTGGTAGTTTCGGTTGG- 3' and the first reverse primer 5'- AGTTGATTGTTGGGGGTAGAGC-3', as well as the second forward primer 5'-ACCAAACAGAACGCCTAAACG-3' and the second reverse primer 5'- GCTATGAAGAAGAGGGCAAATG-3' (Anwar *et al.* 2025). We tagged the amplicons using the Native Barcoding Kit V14 (SQK-NBD114.96), incorporated them during library preparation, and sequenced them on the R10.4.1 flow cell on the GridION (ONT, Oxford, UK).

Sequencing results were processed using high-accuracy basecalling with Guppy Base-calling was performed with the high-accuracy model, applying a minimum quality score of Q9. The resulting data were saved in FASTQ format, which was then demultiplexed to separate reads by sample barcodes. The demultiplexing process used the minKNOW core v23.04.8 software. We then trimmed adapters using Porechop v0.2.4 and filtered reads with NanoFilt v2.3.0 (De Coster *et al.* 2018) to retain only those with a minimum average quality score of Q10 (Dhorne-Pollet *et al.* 2020). The quality of read data was assessed using NanoPlot v1.42.0. The alignment results were mapped against two reference genomes, namely *Bos javanicus* bali cattle (PQ130485.1) and Bos indicus Ongole cattle (OQ886063.1), using Minimap2 v2.28 with the "map-ont" parameter, and the results were output in Binary Alignment Map (BAM) format (Li 2018).

The consensus sequence was generated using iVar v1.4.2 by applying a minimum quality score threshold of Q20, a minimum base frequency threshold of 0.6, a minimum indel frequency threshold of 0.8, and a minimum coverage depth of 30× (Grubaugh *et al.* 2019). The steps from trimming to consensus sequence assembly were performed using Galaxy Europe (https://usegalaxy.eu/). We manually curated, validated, and aligned the final assembly, particularly in the D-loop region, using Sanger sequencing data from the same PO cattle sample.

2.3. Data Analysis

The consensus assembly was annotated using MITOS2 (Donath *et al.* 2019). The organizational structure of the PO cattle mitogenome was described based on the annotation results, which included the location and size of fragments, as well as the start and stop codons in protein-coding genes (PCGs), rRNA, tRNA, and control regions (CRs), using Geneious Prime v2025.0.3, while the anticodons were analyzed using the MITOS2 bioinformatics web tool. We visualized tRNA structures, including the anticodons obtained from the MITOS2 annotation results, as well as their secondary structures, using the Forna tool (Kerpedjiev *et al.* 2015).

We aligned sequence data from the samples and reference sequences using MAFFT. Phylogenetic trees were reconstructed using the Neighbor-Joining (NJ) method with MEGA v11.0.13. Reference cattle species obtained from NCBI were Bos indicus (access ID NC_005971.1), Bos indicus (access ID AY126697.1), *Bos taurus* (access ID NC_006853.1), *Bos primigenius* (access ID NC_013996.1), and *Bos javanicus* (access ID PQ130485. 1, *Bos javanicus* (access ID JN632606.1), *Bos frontalis* (access ID NC_036020.1), and *Bos gaurus* (access ID NC_024818.1). *Syncerus caffer* (NC_020617.1) was used as the outgroup. The final mitogenome sequence "RefPO" was submitted to GenBank via the BankIT tool in accordance with the available instructions.

3. Results

3.1. Structure and Characteristics of the Whole Genome mtDNA

The mitochondrial genome obtained is 16,562 base pairs (bp) long, consisting of 13 protein-coding genes (PCGs), 22 tRNAs, 2 rRNAs, and 1 control region (D-loop); its sequence has been submitted to NCBI (GenBank: PZ533979). A visualization of the PO cattle mitochondrial genome based on its circular structure, annotated genes, and the assembly of the complete mitochondrial genome is presented in Figure 1.

3.2. Composition of AT-Skew and GC-Skew

The total length of the protein-coding genes (PCGs) is 11,403 bp, or 68.85% of the total

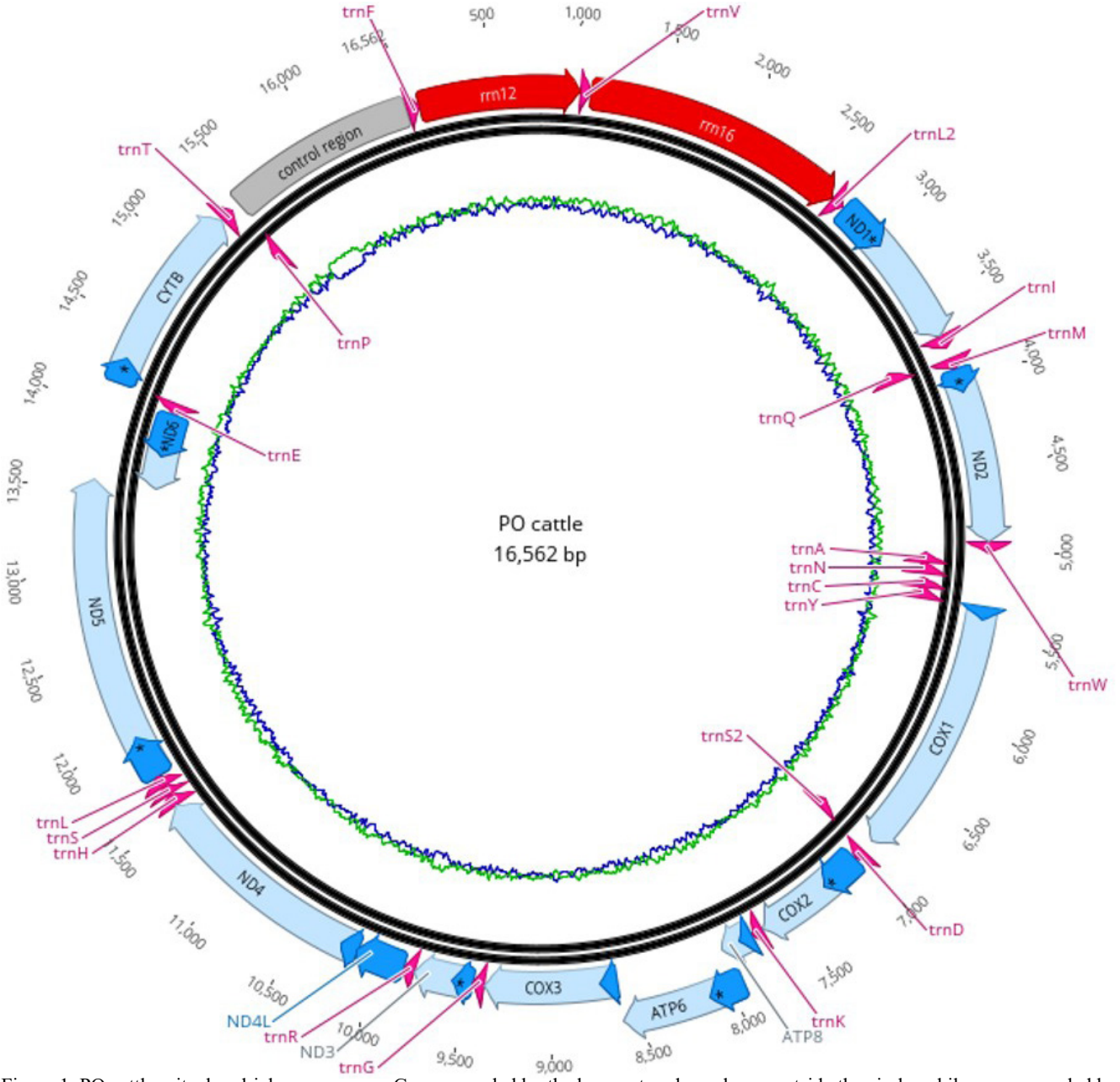


Figure 1. PO cattle mitochondrial genome map. Genes encoded by the heavy strand are shown outside the circle, while genes encoded by the light strand are shown inside the circle

mitochondrial genome of PO cattle. The protein-coding genes include 7 NADH dehydrogenase subunits (ND1–ND6 and ND4L), 3 cytochrome oxidase subunits (COX1–COX3), 2 ATP synthase subunits (ATP6 and ATP8), and 1 cytochrome b gene (CYTB). The nucleotide composition and AT-skewness and GC-skewness values for each protein-coding gene (PCG) were analyzed to describe the base composition characteristics of the PO cattle mitochondrial genome (Table 2).

3.3. Secondary Structure of tRNA

The tRNA structures were obtained from MITOS2 annotations, and their secondary structures were visualized using the Forna tool (https://rna.tbi.univie.ac.at/forna/). The lengths of the tRNA genes in the PO

Table 2. Nucleotide composition of the bovine PO mitochondrial genome, as well as AT-skewness and GC-skewness of 13 protein-coding genes (PCGs)

Regions	size (bp)	A(%)	T(%)	G(%)	C(%)	A + T(%)	G + C(%)	AT-skew	GC-skew
nad1	956	32.74	27.20	11.92	28.14	59.94	40.06	0.092	-0.405
nad2	1,042	36.47	27.06	8.93	27.54	63.53	36.47	0.148	-0.510
cox1	1,545	28.35	29.58	16.76	25.31	57.93	42.07	-0.021	-0.203
cox2	684	33.63	27.19	15.35	23.83	60.82	39.18	0.106	-0.216
atp8	201	41.29	26.37	6.47	25.87	67.66	32.34	0.221	-0.600
atp6	681	33.33	28.63	11.31	26.73	61.96	38.04	0.076	-0.405
cox3	784	27.04	28.70	14.54	29.72	55.74	44.26	-0.030	-0.343
nad3	346	31.21	28.03	12.14	28.61	59.24	40.75	0.054	-0.404
nad4l	297	31.99	30.98	11.78	25.25	62.97	37.03	0.016	-0.364
nad4	1,378	32.00	28.01	11.39	28.59	60.01	39.98	0.066	-0.430
nad5	1,821	32.62	27.73	10.71	28.94	60.35	39.65	0.081	-0.460
nad6	528	21.21	41.48	29.36	7.95	62.69	37.31	-0.323	0.574
cytb	1,140	31.67	26.14	13.33	28.86	57.81	42.19	0.096	-0.368

cattle mitochondrial genome vary, ranging from 60 bp (trnS1) to 75 bp (trnL2). The 22 tRNA genes in the complete mitochondrial genome of PO cattle exhibit the classic cloverleaf secondary structure. The tRNA structure plays a crucial role in tRNA function during mitochondrial protein translation. The tRNA structures are shown in Figure 2.

3.4. Reconstruction of Phylogenetic and Genetic Distance

The phylogenetic tree of PO cattle and various comparative breeds obtained from GenBank was constructed using full-length and partial mitochondrial sequences, including the D-loop, cytochrome b, COX1, ND5, and 16S rRNA. This tree illustrates the evolutionary relationships among cattle breeds based on these mitochondrial sequences. The analysis resulted in several clusters, as shown in Figure 3. Both the full and partial sequence-based trees consistently depict PO cattle's relationships with other breeds: PO2–PO5 form a single cluster that is closely related to *Bos javanicus* CERZA (JN632606.1) and Bali cattle (PQ130485.1), with a 99% bootstrap support. This grouping confirms that PO cattle at BBIB Singosari, Malang, share significant maternal genetic similarity with *Bos javanicus*.

A smaller genetic distance value signifies a closer genetic relationship between PO cattle and the reference cattle breed. Table 3 shows the genetic distance calculations between PO cattle and reference sequences from GenBank. These results show that PO4 has a very small genetic distance of 0.000 from *Bos javanicus* CERZA (JN632606.1). Meanwhile, PO2, PO3, and PO5 exhibit genetic distances from *Bos javanicus* CERZA (JN632606.1) ranging from 0.001 to 0.002. The genetic distance between PO cattle and the outgroup species, *Syncerus caffer*, is 0.135. A higher genetic distance from the outgroup indicates a more distant phylogenetic relationship. Genetic distances close to zero indicate low nucleotide-sequence variation between PO cattle individuals and the reference sequences within their group. The consistency between the phylogenetic results and these genetic distances supports two maternal lineages in the analyzed samples.

4. Discussion

The mitochondrial genome consists of circular, double-stranded DNA molecules in most eukaryotes. These strands are distinguished by their nucleotide composition: one is rich in guanine (H-strand) and the other is rich in cytosine (L-strand) (Chinnery *et al.* 2013). Most genes (28 out of 37) are located on the heavy strand (H-strand), while the light strand (L-strand) encodes eight tRNA genes (trnQ, trnA, trnN, trnC, trnY, trnS2, trnE, and trnP) and one protein-coding gene (ND6). The protein-coding genes include seven NADH dehydrogenase subunits (ND1–ND6 and ND4L), three cytochrome oxidase subunits (COX1–COX3), two ATP synthase subunits (ATP6 and ATP8), and one cytochrome b gene (CYTB).

The mitochondrial genome of PO cattle is longer than the Bovinae average (16.3–16.4 kb) (Arbizu *et al.* 2022; Anwar *et al.* 2025). Differences in mtDNA genome length in PO cattle are thought to result from variation in D-loop size, as the D-loop is a non-coding region within mtDNA. These variations generally result from D-loop mutations. According to Ishida *et al.* (1994),

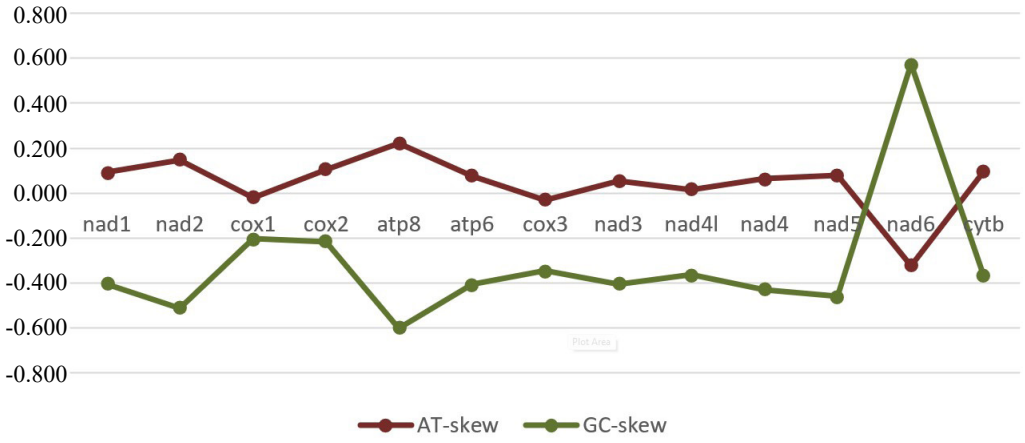


Figure 2. AT-skewness and GC-skewness plots for 13 protein-coding genes (PCGs)

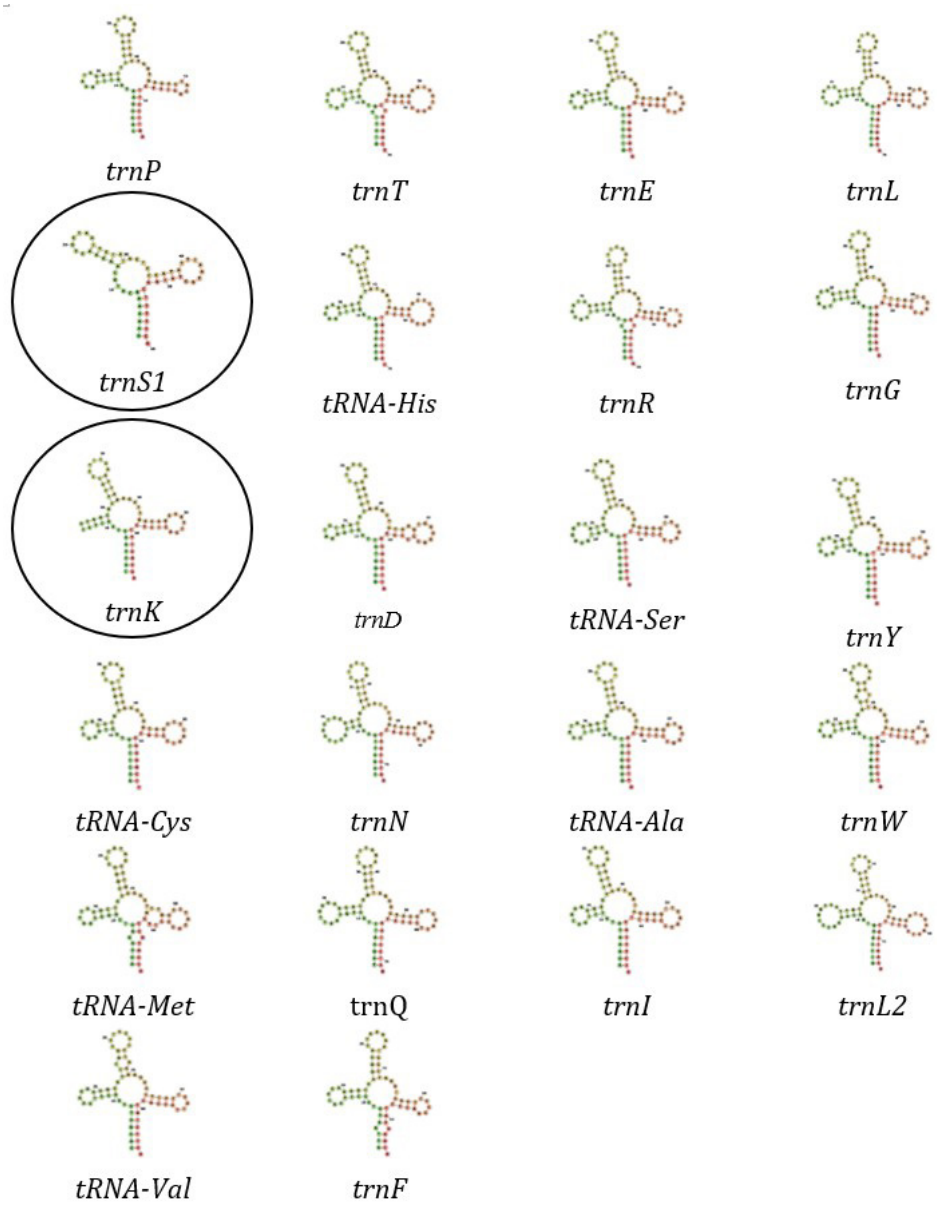


Figure 3. Secondary structures of 22 tRNA genes in PO cattle

Table 3. Genetic distance between PO cattle and comparison cattle breeds from GenBank (GB) based on the total mtDNA genome, with genetic distance values (below the diagonal) and standard deviations (above the diagonal)

Cattle breed	1	2	3	4	5	6	7	8	9	10	11	12	13
*B. frontalis*_NC036020.1		0.001	0.009	0.009	0.009	0.009	0.009	0.009	0.014	0.014	0.014	0.014	0.027
*B. gaurus*_NC024818.1	0.003		0.009	0.009	0.009	0.009	0.009	0.009	0.014	0.014	0.014	0.014	0.027
B. javanicus CERZA_JN632606.1	0.042	0.042		0.000	0.000	0.000	0.000	0.000	0.015	0.015	0.015	0.015	0.028
PO 04	0.042	0.041	0.000		0.000	0.000	0.000	0.000	0.015	0.015	0.015	0.015	0.028
Bali cattle_PQ130485.1	0.041	0.041	0.000	0.000		0.000	0.000	0.000	0.015	0.015	0.015	0.015	0.028
PO 02	0.041	0.041	0.002	0.001	0.001		0.000	0.000	0.015	0.015	0.015	0.015	0.028
PO 05	0.041	0.041	0.001	0.001	0.001	0.000		0.000	0.015	0.015	0.015	0.015	0.028
PO 03	0.041	0.041	0.002	0.002	0.001	0.000	0.000		0.015	0.015	0.015	0.015	0.028
B. indicus Nellore_AY126697.1	0.068	0.068	0.070	0.070	0.070	0.070	0.070	0.070		0.000	0.003	0.003	0.028
*B. indicus*_NC005971.1	0.068	0.068	0.070	0.070	0.070	0.070	0.070	0.070	0.000		0.003	0.003	0.028
*B. primigenius*_NC013996.1	0.068	0.068	0.071	0.071	0.071	0.071	0.071	0.071	0.015	0.015		0.001	0.028
*B. taurus*_NC006853.1	0.069	0.069	0.073	0.072	0.072	0.073	0.072	0.072	0.016	0.016	0.005		0.028
Syncerus caffer_NC020617.1	0.131	0.131	0.135	0.135	0.135	0.135	0.135	0.135	0.136	0.136	0.136	0.137	

within mtDNA fragments, the D-loop region is the most hypervariable and has a higher mutation rate than other mtDNA regions. Sari *et al.* (2016) identified four mutations in Aceh cattle, while Liu *et al.* (2006) reported 65 polymorphic sites in the mtDNA D-loop region of cattle, most of which were transition mutations, indicating high genetic variability in the mtDNA D-loop region.

The mitochondrial genome of PO cattle consists primarily of PCGs ending with the TAA stop codon, except for cytb, which ends with the AGA stop codon. Additionally, incomplete TAA stop codons were identified in certain PCGs, such as Nad1, which is missing one adenine, while Nad2, Nad3, Nad4, and Cox3 exhibit double adenine deletions, as shown in Table 1.

The nucleotide composition shows 33.6% adenine (A), 25.8% cytosine (C), 13.5% guanine (G), and 27.1% thymine/uracil (T/U). Protein-coding genes (PCGs) use ATG and ATA as start codons. The stop codons identified are TAA and AGA, and the incomplete stop codon is TA-. Anwar *et al.* (2025) also reported incomplete stop codons in Bali cattle.

Based on Table 2, the AT-skewness values for the mitochondrial protein-coding genes of PO cattle show that 10 genes (ND1, ND2, COX2, ATP8, ATP6, ND3, ND4L, ND4, ND5, and CYTB) have positive values, indicating a predominance of the adenine (A) base over the thymine (T) base, while three genes (COX1, COX3, and ND6) have negative values, indicating a predominance of the thymine (T) base. The GC-skewness values for the mitochondrial protein-coding genes of PO cattle are generally negative, indicating a predominance of the cytosine (C) base over guanine (G), except for nad6, which has a positive GC-skew value. The AT and GC skew values of the 13 PCGs are consistent with a study on Bali cattle (Anwar *et al.* 2025), which reported unique skewness patterns in COX1, COX3, and ND6. These variations indicate nucleotide preferences and gene-specific compositional biases influenced by different mutation pressures (Min and Hickey 2007). The patterns of AT-skewness and GC-skewness values for each protein-coding gene are visualized in Figure 3.

Based on Figure 2, the secondary structures of tRNA S1 and tRNA K show abnormalities resulting from the loss of the dihydrouridine (DHU) arm. These abnormal tRNA structures are consistent with vertebrate tRNA characteristics, similar to those reported in Bali cattle (Jakaria *et al.* 2025) and Peruvian Creole cattle (Arbizu *et al.* 2022).

The total mitochondrial genome length of PO cattle ranged from 16,495 to 16,562 bp, whereas that of the comparison cattle breeds ranged from 16,338 to 16,519 bp in Table 4. These results indicate that mitochondrial genome lengths were relatively uniform across all samples, although some variations were observed among cattle breeds. The D-loop region, which serves as the mitochondrial control region and exhibits relatively high genetic variability, showed greater variation in sequence length than the other mitochondrial regions analyzed. In

Table 4. Comparison of the lengths of the full and partial mitochondrial genomes in PO cattle and several cattle breeds from GenBank

Cattle breed	Total mitochondrial genome marker (bp)	Partial mitochondrial genome marker (bp)				
		D-loop	Cyt-b	COX1	ND5	16S rRNA
PO 2	16,562	1134	1140	1545	1821	1572
PO 3	16,500	1071	1140	1545	1821	1572
PO 4	16,518	1089	1140	1545	1821	1572
PO 5	16,495	1067	1140	1545	1821	1572
B. indicus (NC005971.1)	16,341	913	1140	1545	1821	1570
B. indicus (AY126697.1)	16,341	913	1140	1545	1821	1570
B. taurus (NC006853.1)	16,338	910	1140	1545	1821	1571
B. primigenius (NC013996.1)	16,338	909	1140	1545	1821	1571
B. javanicus (PQ130485.1)	16,387	957	1140	1545	1821	1572
B. javanicus (JN632606.1)	16,519	896	1140	1545	1821	1572
B. frontalis (NC036020.1)	16,346	903	1140	1545	1821	1569
B. gaurus (NC024818.1)	16,345	723	1140	1545	1821	1569
Syncerus caffer (NC020617.1)	16,359	931	1140	1545	1821	1569

PO cattle, the D-loop length ranged from 1,067 to 1,134 bp, whereas in the comparison cattle breeds, it ranged from 723 to 957 bp. This variation indicates differences in the sequence characteristics of the mitochondrial control region among the cattle breeds analyzed. In contrast, the lengths of several mitochondrial protein-coding genes and RNA regions were relatively conserved. The Cyt-b, COX1, and ND5 genes consistently exhibited lengths of 1,140 bp, 1,545 bp, and 1,821 bp, respectively, across all samples. Meanwhile, the 16S rRNA region showed only slight variation, ranging from 1,569 to 1,572 bp.

Phylogenetic tree analysis based on the full mtDNA genome showed that PO cattle form a clade closely related to the *Bos javanicus* CERZA clade (JN632606.1) and Bali cattle (PQ130485.1), with a 99% bootstrap value using both the full and partial mitochondrial genomes (Figure 4). These results indicate that, maternally, PO cattle at BBIB Singosari, Malang, are closely related to *Bos javanicus*, suggesting that PO cattle resulting from crossbreeding with a mitochondrial genome source derive from *Bos javanicus* as their maternal ancestor. According to Hartati *et al.* (2015), this relationship can be traced to the history of the "Ongolisasi" program, which took place between 1915 and 1929, during which Sumba Ongole (SO) cattle were distributed to various regions in Indonesia, including Java. In these regions, SO cattle were crossed with local *Bos javanicus* (also known as Javanese cattle or banteng), producing offspring known as Ongole cattle or Ongole crossbreeds (PO) (Hartati *et al.* 2015).

Phylogenetic tree construction using partial mtDNA markers (D-loop, cytochrome b, COX1, ND5, and 16S rRNA) shows that the resulting topology is similar to that of the complete mitochondrial genome. However, using partial mitochondrial genome markers yields less stable phylogenetic inferences. The use of partial markers for phylogenetic analysis is widespread, particularly the D-loop marker (Sari *et al.* 2016), the cytochrome b gene (Hartatik *et al.* 2019), 16S rRNA (Misrianti *et al.* 2022), COI (Wulandari *et al.* 2019), and 12S rRNA (Suselowati *et al.* 2023). Nevertheless, using the complete mitochondrial genome provides better phylogenetic resolution.

In conclusion, the mitochondrial genome of PO cattle is 16,562 bp long and contains 13 protein-coding genes, 22 tRNA genes, and a control region (D-loop) that is similar to the sequences of *Bos javanicus* CERZA (JN632606.1) and sapi bali (PQ130485.1). The selected partial mtDNA markers generally recovered the same broad maternal grouping, although with lower and less consistent branch support than the complete mitogenome. Because the present study is based on four animals from a single breeding center and mitochondrial DNA represents maternal inheritance only, broader sampling and complementary nuclear and Y-chromosomal markers are required before conclusions can be made about the overall genetic diversity and ancestry of PO cattle.

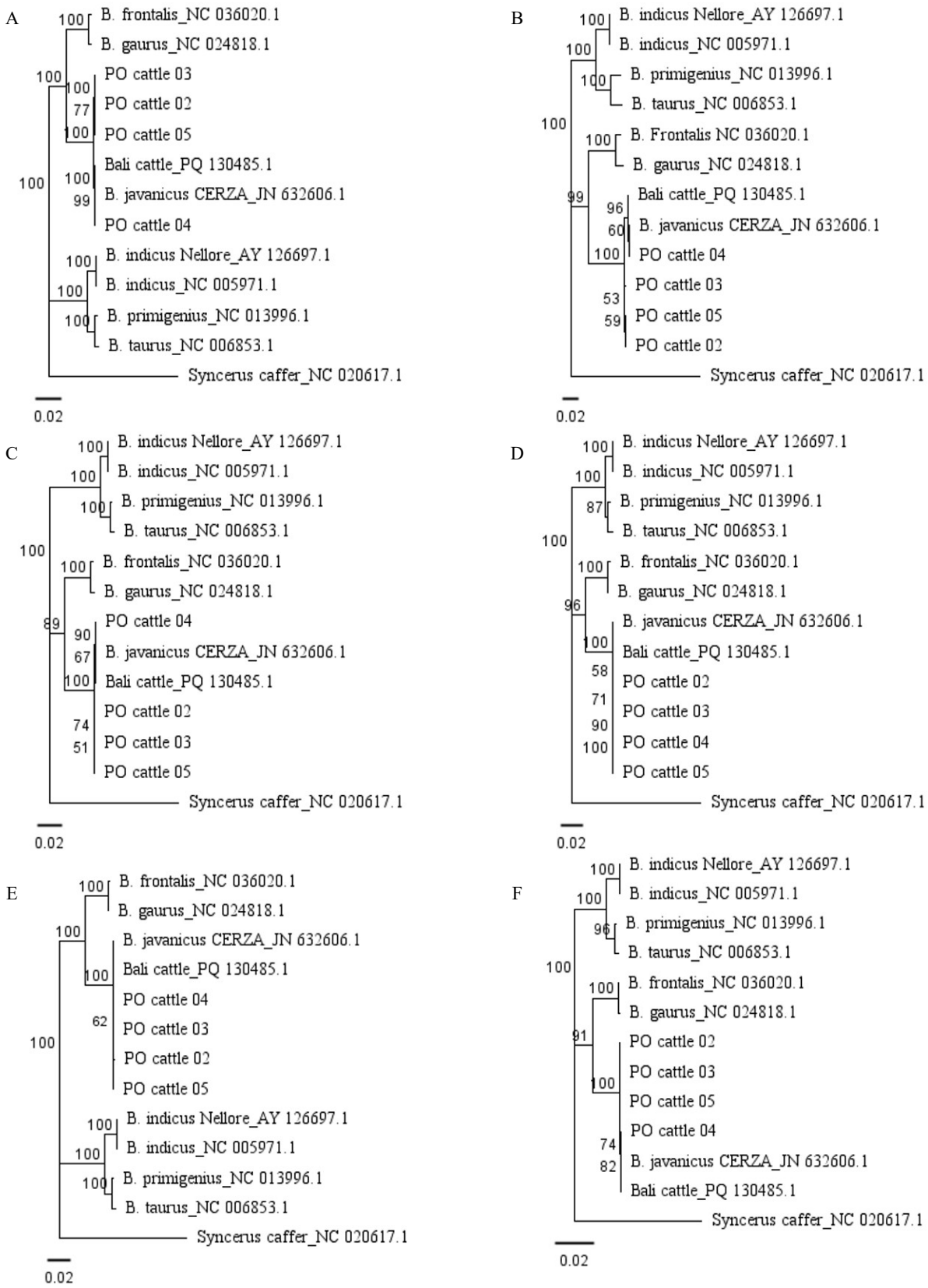


Figure 4. Phylogenetic tree of PO (A) based on the total mitochondrial DNA genome (B) based on the D-loop region of mtDNA (C) based on Cyt-b mtDNA, (D) based on COX1 mtDNA, (E) based on ND5, (F) based on 16S Rrna

Acknowledgements

The authors thank the Ministry of Higher Education, Science, and Technology for supporting their research through the Master's Thesis Research (PTM) scheme with contact number 160/C3/DT.05.00/PLBARU/2026 and number 18958/IT3.D10/PT.01.03/ P/B/2026.

References

Ajmone-Marsan, P., Colli, L., Ginja, C., Kantanen J., Lenstra, J.A., 2023. Genomic Characterization of Animal Genetic Resources. FAO Animal Production and Health Guidelines No. 32. FAO, Rome. https://doi.org/10.4060/cc3079en

Anwar, S., Shidiq, F., Noor, R.R., Semiadi, G., Dharmayanthi, A.B., Furqon, A., Khaerunnisa, I., Sutikno, S., Margawati, E.T., Jakaria, 2025. The complete mitochondrial genome assembly of bali cattle (*Bos javanicus*) using oxford nanopore long-read sequencing technology. *Gene.* 966, 149706. https://doi.org/10.1016/j.gene.2025.149706

Arbizu, C.I., Ferro-Mauricio, R.D., Chávez-Galarza, J.C., Vásquez, H.V., Maicelo, J.L., Poemape, C., Corredor, F.A., 2022. The complete mitochondrial genome of a neglected breed, the Peruvian creole cattle (*Bos taurus*), and its phylogenetic analysis. *Data.* 7, 76. https://doi.org/10.3390/data7060076

Bradley, D.G., MacHugh, D.E., Cunningham, P., Loftus, R.T., 1996. Mitochondrial diversity and the origins of african and european cattle. *PNAS.* 93, 5131. https://doi.org/10.1073/pnas.93.10.5131

[BSN] Badan Standarisasi Nasional, 2020. SNI 7651-5: *Bibit Sapi Potong-Bagian 5: Peranakan Ongole*. BSN, Jakarta.

Chinnery, Francis, P., Hudson, G., 2013. Mitochondrial genetics. *BMB.* 106, 135-159. https://doi.org/10.1093/bmb/ldt017

De Coster, W., D'Hert, S., Schultz, D.T., Cruts, M., Van Broeckhoven, C., 2018. NanoPack: visualizing and processing long-read sequencing data. *Bioinformatics.* 34, 2666-2669. https://doi.org/10.1093/bioinformatics/bty149

Dhorne-Pollet, S., Barrey, E., Pollet, N., 2020. A new method for long-read sequencing of animal mitochondrial genomes: application to the identification of equine mitochondrial DNA variants. *BMC Genomics.* 21, 785. https://doi.org/10.1186/s12864-020-07183-9

Donath, A., Jühling, F., Al-Arab, M., Bernhart, S.H., Reinhardt, F., Stadler, P.F., Middendorf, M., Bernt, M., 2019. Improved annotation of protein-coding genes boundaries in metazoan mitochondrial genomes. *NAR.* 47, 10543-10552. https://doi.org/10.1093/nar/gkz833

Ferreira, T., Rodriguez, S., 2024. Mitochondrial DNA: inherent complexities relevant to genetic analyses. *Genes.* 15, 617. https://doi.org/10.3390/genes15050617

Grubaugh, N.D., Gangavarapu, K., Quick, J., Matteson, N.L., De Jesus, J.G., Main, B.J., Tan, A.L., Paul, L.M., Brackney, D.E., Grewal, S., Gurfield, N., Van Rompay, K.K.A., Isern, S., Michael, S.F., Coffey, L.L., Loman, N.J., Andersen, K.G., 2019. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. *Genome Biol.* 20, 8. https://doi.org/10.1186/s13059-018-1618-7

Hartati, Sumadi, Hartatik, T., 2009. Identifikasi karakteristik genetik sapi peranakan ongole di peternakan rakyat. *Bul. Anim. Sci.* 33, 64-73. https://doi.org/10.21059/buletinpeternak.v33i2.118

Hartati, Utsunomiya, Y.T., Sonstegard, T.S., Garcia, J.F., Jakaria, Muladno, 2015. Evidence of *Bos javanicus* × *Bos indicus* hybridization and major QTLs for birth weight in Indonesian peranakan ongole cattle. *BMC Genetics.* 16, 75. https://doi.org/10.1186/s12863-015-0229-5

Hartatik, T., Hariyono, D.N.H., Adinata, Y., 2019. Genetic diversity and phylogenetic analysis of two Indonesian local cattle breeds based on cytochrome b gene sequences. *Biodiv.* 20, 17-22. https://doi.org/10.13057/biodiv/d200103

Hartatik, T., 2019. *Analisis Genetik Ternak Lokal*. UGM Press, Yogyakarta.

Ishida, N., Hasegawa, T., Takeda, K., Sakagami, M., Onishi, A., Inumaru, S., Komatsu, M., Mukoyama, H., 1994. Polymorphic sequence in the d-loop region of equine mitochondrial DNA. *Anim. Gen.* 25, 215-221. https://doi.org/10.1111/j.1365-2052.1994.tb00196.x

Jakaria, J., Musyaddad, T., Rahayu, S., Muladno, M., Sumantri, C., 2019. Diversity of D-loop mitochondrial DNA (mtDNA) sequence in bali and sumba ongole cattle breeds. *JITAA.* 44, 335-345. https://doi.org/10.14710/jitaa.44.4.335-345

Jakaria, J., Dharmayanthi, A.B., Ulum, M.F., Anwar, S., Noor, R.R., 2025. Complete mitochondrial genome sequence of bali cattle (*Bos javanicus*). *Biodiv.* 26, 1545-1552. https://doi.org/10.13057/biodiv/d260406

Kerpedjiev, P., Hammer, S., Hofacker, I.L., 2015. Forna (force-directed RNA): simple and effective online RNA secondary structure diagrams. *Bioinformatics.* 31, 3377-3379. https://doi.org/10.1093/bioinformatics/btv372

Li, H., 2018. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics.* 34, 3094-3100. https://doi.org/10.1093/bioinformatics/bty191

Liu, R.Y., Xia, X.L., Lei, C.Z., Zhang, M.Z., Chen, H., Yang, G.S., 2006. Genetic diversity of mitochondrial DNA D-loop sequences in cattle breeds in Guizhou. *Yi Chuan Hereditas.* 28, 279-284.

Magee, D.A., MacHugh, D.E., Edwards, C.J., 2014. Interrogation of modern and ancient genomes reveals the complex domestic history of cattle. *Anim. Front.* 4, 7-22. https://doi.org/10.2527/af.2014-0017

Min, X.J., Hickey, D.A,, 2007. DNA asymmetric strand bias effects the amino acid composition of mitochondrial proteins. *DNA Res.* 14, 201-206. https://doi.org/10.1093/dnares/dsm019

Misrianti, R., Wijaya, S.H., Sumantri, C., Jakaria, J., 2022. Investigating genetic diversity of Indonesian native cattle breeds using mitochondrial DNA 16S rRNA gene. *Turk J Vet Anim Sci.* 46, 457-464. https://doi.org/10.55730/1300-0128.4216

Morón-López, J., Vergara, K., Sato, M., Gajardo, G., Ueki, S., 2022. Intraspecies variation of the mitochondrial genome: An evaluation for phylogenetic approaches based on the conventional choices of genes and segments on mitogenome. *PLOS ONE.* 17, e0273330. https://doi.org/10.1371/journal.pone.0273330

Sari, E.M., Jianlin, H., Noor, R.R., Sumantri, C., Margawati, E.T., 2016. Phylogenetic analysis of aceh cattle breed of Indonesia through mitochondrial D-Loop region. *JGEB.* 14, 227-231. https://doi.org/10.1016/j.jgeb.2015.12.001

Suselowati, T., Noor, R.R., Jakaria, J., 2023. Haplotype and p hylogenetic diversity using mitochondrial 12S rRNA gene marker in bali cattle. *JITAA.* 48, 169-181. https://doi.org/10.14710/jitaa.48.3.169-181

Utami, S., Jamil, A., Nidom, R.V., Natsir, M., Prayitno, H., Prakoso, D., Ansori, A.N.M., Indrasari, S., Nidom, C.A., 2023. Genetic diversity of crossbred cattle using cytochrome oxidase subunit I (COI) gene in South Sulawesi, Indonesia. *Indian J. Anim. Res.* 58, 28-34. https://doi.org/10.18805/IJAR.BF-1600

Weldenegodguad, M., Popov, R., Pokharel, K., Ammosov, I., Ming, Y., Ivanova, Z., Kantanen, J., 2019. Whole-genome sequencing of three native cattle breeds originating from the northernmost catlle farming regions. *Front. Genet.* 9, 728. https://doi.org/10.3389/fgene.2018.00728

Wulandari, A., Nurgiartiningsih, V.M.A., Kuswati, Susilorini, T.E., Agung, P.P., 2019. Filogeni beberapa sapi lokal Indonesia menggunakan DNA mitokondria COI (Cytochrome Oxidase Subunit I). *JITRO.* 6, 278-282. https://doi.org/10.33772/jitro.v6i2.6165