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RESEARCH ARTICLE
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Characteristics of Ternate Local Chickens Based on Single Nucleotide Polymorphism (SNP) on D-loop Mitochondrial DNA

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ABSTRACT

Local chickens excel in environmental adaptation, disease resistance, and are important for the household economy. This study aimed to identify the genetic characteristics and polymorphisms of the First Generation (G1) of local chickens using SNP analysis in the D-loop mtDNA region. A total of 20 G1 chickens were used as the initial population, with 7 selected at random for analysis. A 3mL blood sample was taken from each chicken for DNA amplification using PCR with a universal D-loop *Gallus gallus* primer, resulting in a 526bp fragment. The sequencing results showed 420nt, which was analyzed for SNPs and compared with the chicken sequence of *Gallus gallus* in GenBank (AB007725.1). The analysis identified six polymorphic sites: 33% at bases 0-100, 50% at 101-200, and 17% at 201-300. G1 individuals with feather color variations showed higher levels of polymorphism. The SNP found has the potential to be a genetic marker to differentiate G1 individuals. The novelty of this study is the identification of high-productivity local Ternate chicken clumps based on D-loop DNA as well as quantitative and qualitative phenotypic characters. These findings have the potential to be applied in similar regions to increase productivity and food security, as well as support the conservation and genetic improvement of local poultry resources in Indonesia. In conclusion, SNP analysis in the mtDNA D-loop region proves effective for evaluating genetic variability in G1 chickens. The findings support the use of SNP markers for breeding programs, genetic conservation, and future development of local chicken resources.

Keywords: Ayam Lokal, Ternate, SNP, DNA Mitokondria D-loop.

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INTRODUCTION

Knowing the genetic characterization of local Ternate chickens is important to maintain sustainability and increase the productivity of livestock genetic resources, especially local chickens in North Maluku. These local chickens have advantages in environmental adaptation and disease resistance, making them an important commodity for the household economy (Fatmona et al., 2024a; Fatmona et al., 2024b). Even so, efforts to improve the productivity and genetic quality of local chickens in this area are faced with the constraints of a lack of genetic data, because previous studies only focused on morphological aspects, and very little molecular research. Previous research stated that genetic data is necessary to increase local chicken productivity, as previous research mainly focused on the morphology (Wintari et al., 2019).

Differences in morphology and performance between local chicken individuals are thought to stem from genetic variation within and between populations (KammongKun & PromKet, 2024; Sithole et al., 2025; Temoa et al., 2025; Zhi et al., 2025). However, the difficulty of ascertaining the exact genetic origins or pedigree of local chickens is a challenge in breeding programs. Phenotype-based analyses are

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limited in distinguishing homozygous and heterozygous individuals and are not accurate enough to identify genetic mutations (Lee et al., 2022; Lindner et al., 2024; Sun et al., 2024).

To address this, a molecular approach based on Single nucleotide polymorphism (SNP) D-loop mitochondrial DNA (mtDNA) is considered to be highly relevant and accurate for identifying genetic mutations (Alcalá et al., 2024; Masila et al., 2024; Sciuto et al., 2024; Siziba & Willows-Munro, 2024) Mitochondrial DNA D-loop SNPs (mtDNA) are effective genetic markers in identifying genetic variation between individuals, tracing ancestry, and estimating the proximity of genetic relationships (González et al., 2024; Sari et al., 2024) MtDNA has maternally inherited haploid scharacteristics without recombination (Cao et al., 2024; Fatmona et al., 2024c; Feng et al., 2024; Hachad et al., 2024; Ma et al., 2024; Zhao et al., 2024) so that mitochondrial DNA D-loop SNPs make it a reliable genetic analysis tool to identify genetic variations in local chicken populations that are phenotypically quite diverse.

Local Ternate chickens are known to have diverse phenotype variations, but no genetic studies have been able to document the number and characteristics of specific clumps. To this end, efforts to cross local chickens, including Elba and Bangkok chickens, have been made as a first step towards the formation of distinctive local chicken clumps. However, the productivity level of local chickens is still low, for example in terms of egg production which is still far behind purebred chickens (Fatmona and Sjafani, 2020; Manyelo et al., 2020; Tan et al., 2024) This productivity improvement requires improving genetic quality through targeted selection and crossbreeding (Assan et al., 2024; Mokoena et al., 2024; Gowda et al., 2025).

Using SNPs in the D-loop mtDNA region, the study was able to identify the specific genetic traits of local Ternate chickens and provide a foundation for the formation of genetic markers that can support selection and breeding. In addition, the results of this study can enrich the genetic literature on local Indonesian chickens and provide methods that can be applied for the conservation and development of local chicken populations more optimally.

The novelty of this study was the genetic polymorphism analysis of First Generation (G1) local chickens using SNPs on the D-loop mtDNA region, which revealed six polymorphic sites and the relationship between feather color variation and higher levels of polymorphism. These findings demonstrate the potential of SNPs as genetic markers to differentiate individuals in populations, providing new insights for genetic characterization and local chicken breeding. The purpose of the study was to determine the genetic characteristics and polymorphisms of the First Generation (G1) using SNP analysis in the D-loop mtDNA region.

MATERIALS & METHODS

Time and Location of the Study

The research was conducted over a six-month period, from April to September 2022. Blood samples were collected from the first-generation (G1) local Ternate chickens reared at Maquaponik Farm, Gamayou Hamlet, Ternate City District, North Maluku Province, Indonesia.

Research Methods

Blood samples from seven first-generation (G1) Elba chickens and Ternate chickens were used as DNA material in this study. The samples were transported to the Laboratory of Genetics and Molecular Biology, Faculty of Animal Science, IPB University, and sequence analyses to determine the precise nucleotide order were performed at the International Livestock Research Institute (ILRI), Nairobi, Kenya.

A whole-blood sample (1mL) was collected from the brachialis vein of a G1 chicken using a 1mL syringe. The sample was transferred into a 3mL microfuge tube containing an anticoagulant (EDTA) mixed with 95% ethanol at a 1:1 ratio, and subsequently stored at 4°C until further analysis.

Molecular genetic analysis of the mtDNA D-loop gene was performed using a PCR kit (Promega). A pair of forward and reverse primers was used to amplify the mtDNA D-loop region. The primers were designed using the NCBI online primer-design tool, based on the gene sequence of the Ternate chicken G1 mtDNA D-loop deposited in GenBank (accession no. KF446986.1; size: 600 bp). Table 1 presents the primer sequences used in this study.

The universal primer sequences used were 0.2pmol/µL each of D-loop Gallus F (forward: 5′-CAT AGA CAG CTC CAA ACC AC-3′) and D-loop Gallus R (reverse: 5′-ACT AGG ATA GGA CGC AAC GC-3′). PCR amplification was carried out under the following conditions: initial denaturation at 94°C for 5min; 30 cycles of denaturation at 94°C for 45sec, annealing at 60°C for 45sec, and extension at 72°C for 90sec; followed by a final extension at 72°C for 10min. PCR products were verified by electrophoresis on a 2% agarose gel.

The primer sequences of the Gallus D-loop gene used in this study are presented in Table 1.

Table 1: Primary sequence of D-loop Gallus genes used in the study

Gen	Sekuens Primer (5′- 3′)	Access code Gen Bank	Size (bp)
D-Loop Gallus	F:5″ CAT AGA CAG CTC CAA ACC AC3″ R:5″ ACT AGG ATA GGA CGC AAC GC3″	AB007725.1	626

DNA Extraction and Purification

DNA extraction and purification were carried out using a modified phenol–chloroform method (Peck et al., 2022). DNA was extracted from 20µL of blood samples placed in a 1.5mL tube. The sample was mixed with 1000µL of 0.2% NaCl and centrifuged at 8000 rpm for 5min, after which the supernatant was discarded. Subsequently, 350µL of 1× STE (sodium–tris–EDTA), 40µL of 10% SDS, and 10µL of proteinase K (5 mg mL^{-1}) were added, and the mixture was incubated at 55°C for 2hr with gentle agitation. Following incubation, 400µL of phenol, 400µL of chloroform:isoamyl alcohol (24:1), and 40µL of 5M NaCl were added and mixed at room temperature for 1hr. The mixture was then centrifuged at 12,000 rpm for 5min, and the clear supernatant (400–164µL) was transferred into a new 1.5mL tube. Subsequently, 800µL of absolute ethanol and 40µL of 5M NaCl were added for DNA precipitation.

The sample was centrifuged at 12,000rpm for 5min, and the supernatant was discarded. The residue was washed with 800µL of 70% ethanol, centrifuged again at 12,000 rpm for 5 min, and the supernatant was removed. The pellet was then air-dried at room temperature for 2–3hr until the ethanol evaporated completely. After drying, the pellet was resuspended in 100µL of TE buffer (Tris–EDTA) to dissolve the DNA. The extracted DNA was stored at -20°C until use.

Amplification of MtDNA D-Loop genes by PCR Technique

The extracted total DNA served as the template for PCR amplification. Each 50µL PCR reaction contained 100–300ng of DNA, 25µL of 2× Green Master Mix (Promega), 0.6µL (25pmol) of each forward and reverse primer, and nuclease-free water to a final volume of 50µL. DNA amplification was performed in an Applied Biosystems 9700 thermal cycler. The CO1 gene was amplified under the following conditions: initial denaturation at 95°C for 5min; 35 cycles of denaturation at 95°C for 10sec, annealing at 60°C for 20sec, and extension at 72°C for 5min 30sec; followed by a final extension at 72°C for 5min.

Electrophoresis of the Gene Locus in the mtDNA D-loop Region

Electrophoresis was performed on a 2% agarose gel prepared in 0.5× TBE buffer and stained with PeqGreen. The gel was run in an EX Mupid Electrophoresis System at 100V for 45min. Following electrophoresis, DNA bands were visualized under a UV transilluminator at 300nm. A 100bp DNA ladder was used as a molecular weight marker to estimate fragment sizes.

Gene Sequencing in the mtDNA D-loop Regions

Samples were transported to the Genetics and Molecular Laboratory, Faculty of Animal Science, IPB University. Sequencing analysis of the mtDNA D-loop region to determine the nucleotide sequence was carried out at the International Livestock Research Institute (ILRI), Nairobi, Kenya. PCR products containing mtDNA D-loop alleles were sequenced using the Sanger sequencing method.

Sequencing results were compared with GenBank references to analyze SNP variations in the D-loop region. Amplified PCR products were sequenced to identify nucleotide sequences using the GenBank reference of *Gallus gallus* (accession no. AB007725.1). Sequencing data were visualized as electropherograms, in which each nucleotide base was represented by a distinct peak color: adenine (A) in gray, guanine (G) in purple, cytosine (C) in blue, and thymine (T) in red.

Observed Variables

Variables observed at the D-loop mtDNA G1 gene locus of Ternate Chickens include

1. Nucleotide composition.
2. Similarities and differences in nucleotides.
3. Transition substitution and nucleotide transformation.
4. Insertion and degradation of nucleotides.
5. Number of haplotypes.
6. Genetic similarities and distance.

Data Analysis

A partial sequence of the mitochondrial DNA D-loop region was used for analysis in this study. Sequence data were analyzed using several software tools. Chromas version 1.45 was employed for viewing and editing the sequence results, while forward and reverse sequences were aligned using BioEdit version 7.0.1. Nucleotide frequency analysis was performed with the Maximum Likelihood model implemented in MEGA version 7 and BioEdit (Thakur et al., 2025).

Polymorphic site analysis, nucleotide diversity, haplotype diversity, and Fu & Li's (1993) statistical tests were performed using DnaSP version 4.0 (Aiffa et al., 2025). Fu's (1997) statistic, based on the method of Fu and Li (1993), was calculated from DNA sequence data. This statistic is typically indicated by a highly negative value and serves as a sensitive indicator of demographic population expansion, often used to infer inbreeding events.

The phylogenetic tree was constructed using the neighbor-joining (NJ) method, with genetic distances estimated under the Kimura 2-parameter (K2P) model. Pairwise distance analysis and tree construction were performed in MEGA version 3.1 (Niu et al., 2025).

RESULTS

The compatibility between the lengths of the experimental fragments and the theoretical predictions for the D-loop region demonstrates the success of amplification in this study. Electrophoresis results using 1–2% agarose gel showed clear bands corresponding to fragment sizes of approximately 1 kb, indicating high amplicon quality. This suggests that the experimental design was effective in amplifying the desired mitochondrial DNA target from Ternate local chickens. The clear and consistent amplification confirms the successful characterization of single-nucleotide polymorphisms (SNPs) in the D-loop region of mitochondrial DNA.

Amplicon Size Produced

The mitochondrial DNA D-loop fragment of Ternate local chickens amplified using the M1 and HV2R primers measured approximately 1kb in size, consistent with theoretical expectations. Based on calculations, the M1 primer positioned at 15,978 bp and HV2R at 409bp yielded a fragment of 1,002bp, as follows: 16,569 – (15,978 + 1) + (409 + 1). This alignment between the experimental outcome and theoretical prediction demonstrates the successful application of the amplification strategy for genetic characterization of Ternate local chickens. Gel electrophoresis using 2% agarose revealed distinct and sharp DNA bands at the 1kb position, confirming that the resulting amplicons were of high quality and specific to the target region. No non-specific bands or degraded fragments were observed, indicating that PCR conditions were well-optimized and free from contamination. These results reinforce the reliability and reproducibility of the method for consistently amplifying the mitochondrial D-loop region.

Results of Electrophoresis Visualization

Visualization of the amplification products using 2% agarose gel electrophoresis showed distinct bands at the 1kb position for both Ternate local chicken samples, namely ASMA-KIMFST-UINSGD and YHN-KIMFST-UINSGD. The observed bands corresponded to the expected fragment size, indicating that the amplification and DNA fragmentation processes proceeded as intended. These findings support the reliability of the PCR amplification of the mitochondrial D-loop region targeted in this study. As shown in Fig. 1, the electrophoresis results of PCR products using the *Gallus gallus* D-loop marker revealed clear bands at the anticipated size, confirming the integrity and specificity of the amplification products. The consistency of the band sizes across samples further supports the reproducibility of the amplification procedure.

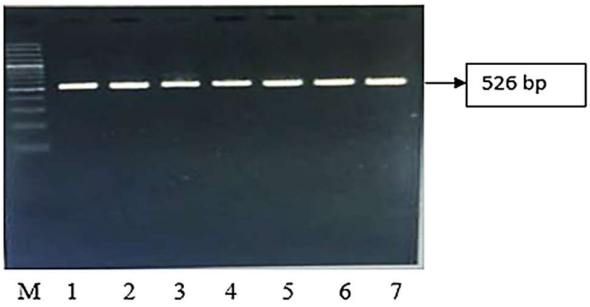

Fig. 1: Electrophoresis results of PCR products amplified with the D-loop marker of *Gallus gallus*. Note: M = 1000 bp DNA marker; lanes 1–7 = samples; bp = base pairs.

D-loop mtDNA Sequence Analysis of G1 Chickens, Local Chickens and Elba Chickens: DNA Samples and Sequencing

The analysis of D-loop gene sequencing in first-generation (G1) chickens revealed significant genetic variation compared to the *Gallus gallus* reference sequence (AB007725.1), as illustrated in Fig. 2. A total of 52 mutation points in the form of transversions were identified, indicating nucleotide base changes between purines and pyrimidines. In addition, 52 polymorphic sites were detected, reflecting the presence of genetic diversity among the G1 chicken samples. These polymorphic sites serve as important indicators in understanding the extent of genetic variation occurring within the population. Despite the presence of genetic variation, the majority of the sequences exhibited high levels of conservation, as demonstrated by the identification of 566 monomorphic sites. These monomorphic sites represent genome regions that remain stable and unaltered, typically associated with essential biological functions. Notably, no new haplotypes were identified beyond the previously characterized haplotypes (A–L), suggesting that genetic diversity within the G1 chicken population remains limited. Furthermore, no nucleotide insertions or deletions were observed, indicating structural stability within this mitochondrial D-loop region. The sequencing results of the first-generation chickens based on haplotypes A–L and the *Gallus gallus* reference (AB007725.1) are presented in Fig. 2.

This study successfully identified genetic diversity among First-Generation (G1) chickens reared at Maquaponic Farm, Ternate. Analysis of the mitochondrial HV1 D-loop region (420 base pairs) from seven DNA samples—comprising both local chickens and Elba chickens—revealed the presence of seven distinct haplotypes and six polymorphic sites. These findings demonstrate significant within-population genetic variation. All samples were collected from Maquaponic Farm, located in West Makassar Village, Ternate City, representing a localized gene pool of chickens under semi-intensive rearing conditions.

Haplotype Identification

Analysis of seven mitochondrial DNA sequences from First-Generation (G1) chickens at Maquaponic Farm identified seven distinct haplotypes: A, B, C, D, E, F and G. Each individual possessed a unique sequence type, indicating a high level of haplotype diversity among both local chickens and Elba chickens. These findings demonstrate considerable genetic variation within the small sample set, reflecting diverse maternal lineages in the analyzed population.

Polymorphic Sites

Further analysis of the mitochondrial D-loop sequences in seven G1 chicken samples revealed six polymorphic sites at positions 49, 74, 110, 130, 131, and 214. At each of these loci, only two nucleotide variants were detected. In total, 25 polymorphic sites were identified across the entire D-loop fragment, indicating a moderate level of sequence variability. Alignment with the GenBank reference sequence (accession number AB007725.1) confirmed consistent nucleotide differences across the seven haplotypes (A–G). Notably, haplotypes B, C, F, and G differed at positions 49, 74, and 110, while haplotypes A, D, and E showed variation at positions 130 and 131. The distribution of polymorphisms is summarized in Table 2.

Haplotype Frequencies of DNA D-loop Sequences

The haplotype frequency analysis of mitochondrial DNA D-loop sequences in First-Generation (G1) chickens revealed seven distinct haplotypes, designated A through G. Haplotype frequencies ranged from 0.004 to 0.01, indicating a notable level of genetic variation within the analyzed population. Among them, haplotypes B, C, F, and G exhibited the highest frequency (0.01), whereas haplotypes A and E were the least frequent (0.004). Haplotype D showed an intermediate frequency of 0.006. The complete distribution and frequency values of each haplotype are presented in Table 3.

Table 2: Nucleotide polymorphisms were analyzed in the D-loop segment of mitochondrial DNA, and aligned with the GenBank reference (code/access number AB007725.1) in the G1 chicken population

Haplotipe	Sequence number of mitochondrial DNA control regions					
	49	74	110	130	131	214
Reff	G	C	G	T	T	C
A				C	C	
B	A	T	A	C	C	T
C	A	T	A	C	C	T
D			A	C	C	
E				C	C	
F	A	T	A	C	C	T
G	A	T	A	C	C	T

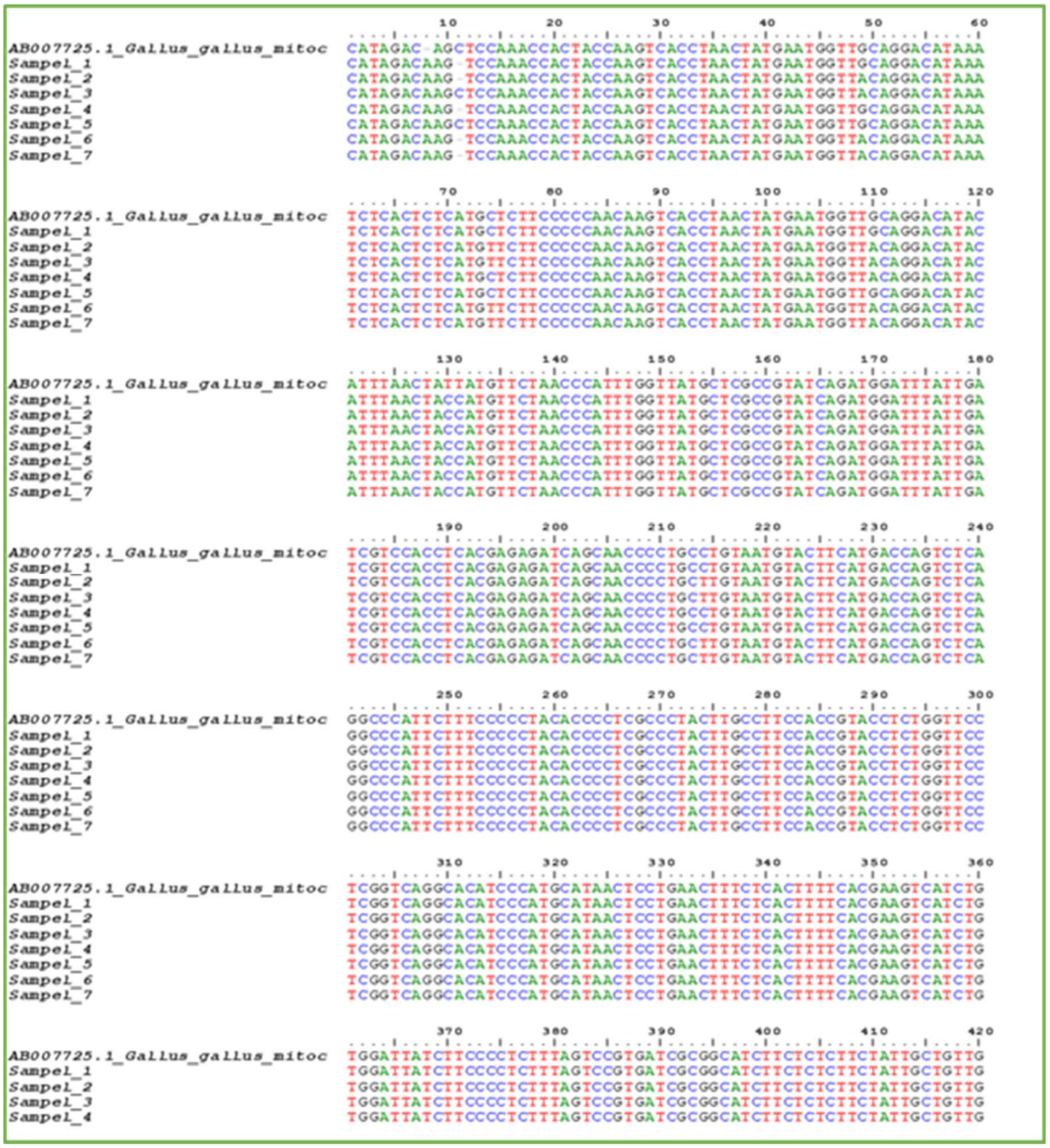

Fig. 2: Results of the sequencing of First Generation (G1) chickens based on A-L and *Gallus gallus* haplotypes (AB007725.1).

Table 3: Haplotype frequency of the D-loop sequence of chicken mitochondrial DNA G1

Haplotipe	DNA G1 (Locus)
A	0.004
B	0.01
C	0.01
D	0.006
E	0.004
F	0.01
G	0.01

Distribution of Mitochondrial DNA D-loop Fragment Sequence Polymorphic Sites

The distribution of polymorphic sites along the mitochondrial DNA D-loop fragment revealed a non-uniform pattern. The highest concentration of polymorphic sites was observed in the 101–200bp region, with an average of approximately 3.5 sites. In contrast, the 0–100bp and 201–300bp segments showed lower levels of polymorphism, with the 201–300bp region presenting the fewest polymorphic sites, averaging about 1.5 sites. These findings indicate the presence of region-specific variability along the D-loop fragment. The complete distribution pattern of polymorphic sites across the D-loop sequence is illustrated in Fig. 3.

Estimation of the Genetic Distance Matrix of 7 Haplotypes from the G1 Chicken Population

Table 4 presents the genetic distance values between seven haplotypes (A–G) from the G1 chicken population and the reference sequence of *Gallus gallus* (GenBank Accession: AB007725.1), as determined using mitochondrial D-loop sequences. The highest genetic divergence was observed between haplotype B and Gallus gallus, with a distance of 0.02255, while the closest relationship was recorded between haplotype A and Gallus gallus, with a value of 0.01540. Intra-population analysis showed minimal genetic divergence among certain haplotypes, such as

haplotypes C and F, which shared a distance of 0.00000, indicating identical sequences in the targeted D-loop fragment. Conversely, the highest genetic distance within the G1 population was recorded between haplotypes B and D at 0.00222, reflecting low but existing variability among some individuals.

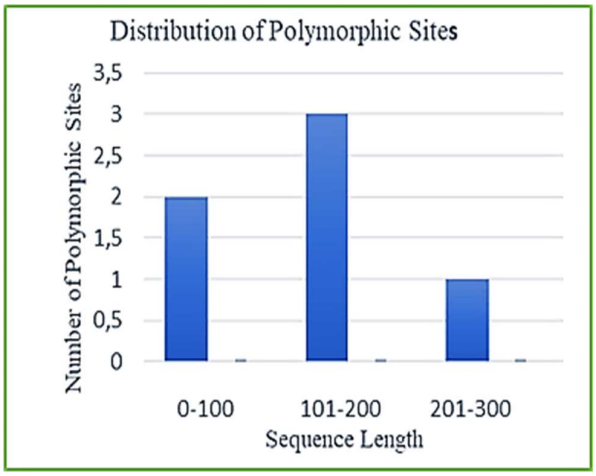

Fig. 3: Distribution of polymorphic sites along the mitochondrial DNA D-loop fragment sequence.

Phylogeny Tree

The analysis of the mitochondrial DNA D-loop region in First Generation (G1) Ternate chickens revealed significant genetic variability, as reflected in the distribution of polymorphic sites, genetic distance matrix, and phylogenetic clustering. The polymorphic site distribution (Fig. 3) demonstrated an irregular pattern, with the highest concentration observed between 101–200bp (~3.5 sites), followed by lower densities in the 0–100bp and 201–300bp regions. Table 3 presents the genetic distance values between the seven identified haplotypes (A–G) in the G1 chicken population and the reference *Gallus gallus* (AB007725.1). The maximum genetic distance (0.02255) occurred between haplotype B and Gallus gallus, while the minimum (0.01540) was between haplotype A and Gallus gallus. Within the G1 population, the lowest genetic distance was observed between haplotypes C and F (0.00000), suggesting close genetic affinity. The phylogenetic reconstruction (Fig. 4) based on haplotypes A–L and the *Gallus gallus* reference revealed five distinct clusters. Four clusters comprised G1 haplotypes, while *Gallus gallus* formed a separate, single cluster, indicating evolutionary divergence.

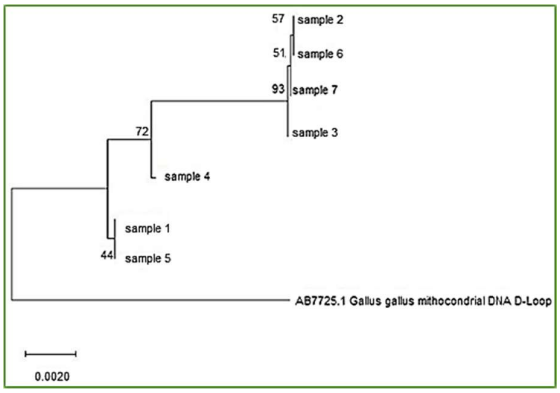

Fig. 4: Reconstruction of the First Generation (G1) chicken phylogeny tree based on the A-L haplotype and *Gallus gallus* (AB007725.1) as the out group, made using the Neighbor-joining method based on Mega software version 3.1.

DISCUSSION

The successful amplification of the D-loop region indicates that the primers and PCR conditions used in this study were well optimized. The D-loop, being a highly variable regulatory region, is widely used in phylogenetic studies to infer genetic relationships among populations and species (Cao et al., 2024; Chen et al., 2024; Fast et al., 2024). In this study, the fragment lengths produced by PCR matched the expected sizes based on theoretical predictions, reinforcing the validity of the amplification strategy. This outcome not only supports the reliability of the molecular approach used but also enables further downstream analyses such as the identification of genetic variation and phylogenetic reconstruction. As noted by Wu et al. (2024), successful D-loop amplification provides a solid foundation for understanding genetic diversity and kinship among populations. The accuracy of the amplification process, as evidenced by the gel electrophoresis results, further confirms the suitability of the methodology for analyzing SNP-based genetic characteristics in Ternate local chickens.

The concordance between the amplified fragment size and theoretical prediction reflects the accuracy of primer positioning and the appropriateness of the PCR conditions applied to target the D-loop region. The successful design of M1 and HV2R primers, along with standardized PCR protocols, ensured the amplification of the desired mitochondrial DNA fragment. These findings are consistent with those of Martínez and Menéndez-Arias (2025) and Cardona-Ramírez et al. (2025), who emphasized the significance of precise primer selection in molecular genetic

Table 4: Estimation of genetic distance matrix between seven G1 chicken haplotypes and *Gallus gallus* (AB007725.1), based on mitochondrial D-loop sequences using MEGA version 3.1

	A	B	C	D	E	F	G
AB007725.1_Gallus_gallus_mitochondrial_DNA_D-loop							
A	0.01540						
B	0.02255	0.00772					
C	0.02232	0.00748	0.00024				
D	0.01705	0.00222	0.00587	0.00563			
E	0.01540	0.00001	0.00771	0.00748	0.00221		
F	0.02255	0.00772	0.00000	0.00024	0.00587	0.00771	
G	0.02244	0.00760	0.00012	0.00012	0.00575	0.00760	0.00012

studies. The successful amplification provides a strong foundation for subsequent analyses such as single-nucleotide polymorphism (SNP) identification, genetic diversity estimation, and phylogenetic reconstruction among local chicken populations. Accurate genetic data derived from the mitochondrial D-loop region is crucial for informing conservation and selective breeding strategies. This is particularly important in the context of preserving local genetic resources to support food security and sustainable livestock development in tropical island regions.

The concordance between the observed amplicon sizes and the theoretical fragment size validates the effectiveness of the PCR method applied in this study, including the primer design and the amplification protocol (Cardona-Ramírez et al., 2025; Martínez & Menendez, 2025). These results demonstrate that both the quality of the primers and the PCR conditions were optimal for amplifying the mitochondrial D-loop region in Ternate local chickens (Chen et al., 2024). Successful amplification of this region provides a crucial foundation for subsequent molecular analyses, particularly the identification of single-nucleotide polymorphisms (SNPs) (Wu et al., 2024). SNP analysis plays an essential role in assessing genetic variation within and among populations (Cao et al., 2024). Therefore, the reliable amplification of the D-loop fragment enhances the potential to uncover meaningful genetic markers that can be used to inform conservation strategies and improve selective breeding programs for Ternate local chicken populations.

The presence of 52 transversion mutations and 52 polymorphic sites in the mitochondrial D-loop region of G1 chickens highlights notable genetic differentiation when compared to the *Gallus gallus* reference sequence (Husien et al., 2024; Chiara et al., 2025). These polymorphic sites provide strong evidence of genetic variation within the population, which may result from domestication history, genetic drift, or adaptation processes. Polymorphic regions in mitochondrial DNA are often linked to evolutionary changes that influence population structure and genetic identity. On the other hand, the observation of 566 monomorphic sites across samples indicates a high degree of genetic conservation, particularly in functionally critical regions of the D-loop, as similarly reported in other avian mitochondrial studies (Madhushan et al., 2025). The absence of new haplotypes beyond A–L suggests that the genetic pool of G1 chickens may have a narrow maternal lineage or reflect a founder effect. Additionally, the lack of base insertions or deletions implies that the D-loop segment is structurally stable, further supporting its utility in phylogenetic and population studies. These findings underscore the dual nature of mitochondrial markers: while variable enough to detect population-level differences, they also retain conserved regions critical for mitochondrial function.

The detection of seven haplotypes and six polymorphic sites within a relatively small sample size reflects a high degree of genetic variation in G1 chickens. This degree of variability suggests the potential utility of these polymorphic sites as genetic markers in future studies on local chicken populations in Indonesia. Such markers could support initiatives in genetic conservation and breeding program development. The observed genetic variation may result from multiple factors, including environmental influences, domestication practices, and founder effects at Maquaponic Farm. Environmental conditions often exert selective pressures on local populations, favoring traits that enhance survival and reproductive success. Over time, such pressures can promote adaptation and diversification, as proposed by Lovász et al. (2024), potentially leading to microevolutionary processes or even incipient speciation. Targeting the HV1 region of the mitochondrial D-loop was a strategic choice, as it is well-known for its high mutation rate and utility in detecting recent evolutionary changes. As supported by prior studies (Chen et al., 2025; Elzain et al., 2025; Geletu et al., 2025), this region is particularly informative for inferring genetic structure, phylogeography, and domestication patterns. The current results provide an important foundation for future phylogenetic analyses and the formulation of conservation strategies for indigenous chicken genetic resources in the Maluku region.

The identification of seven unique haplotypes in seven individuals suggests a remarkably high degree of genetic differentiation, which is uncommon in small, localized populations. This haplotypic diversity reflects distinct genetic backgrounds among chickens at Maquaponic Farm and implies the presence of strong genetic variability, likely shaped by domestication processes and possible historical gene flow. High haplotype diversity is a valuable indicator of evolutionary potential and adaptability, as it enhances the likelihood of population resilience under environmental pressures. As noted by Hou et al. (2024), such genetic signatures are instrumental for understanding evolutionary origins, domestication pathways, and population structure. These findings reinforce the need for integrative conservation strategies to preserve the genetic resources of local chickens, particularly in island ecosystems like Ternate where genetic drift and isolation may influence population dynamics.

The detection of 25 polymorphic sites and six consistent mutation points in a small dataset suggests that the mitochondrial D-loop region harbors significant genetic variation within the G1 chicken population. Despite the limited sample size, the presence of seven distinct haplotypes underscores the genetic richness of both local and Elba chickens maintained at Maquaponic Farm. When compared to a larger-scale study involving 434 individuals from 15 Indonesian native chicken breeds, which identified 49 polymorphic sites and 69 haplotypes (Zein & Sulandari, 2008), the current study reveals comparable diversity on a per-sample basis. This suggests that the G1 population analyzed may represent a genetically diverse subset, with high haplotype variability relative to its sample size. Such diversity in nucleotide polymorphisms, particularly in the hypervariable D-loop region, aligns with previous findings on the utility of this marker for tracking maternal lineages, domestication history, and intraspecific variation (Mutum et al., 2025; Silalahi et al., 2025). These results provide valuable insight for future studies focused on the genetic conservation and phylogenetic mapping of local chicken populations in the region.

The relatively even distribution of haplotypes among G1 chickens suggests a moderate to high level of mitochondrial genetic diversity, despite the limited sample size. The absence of a dominant haplotype—evident from the equal frequency values of haplotypes B, C, F, and G—indicates contributions from multiple maternal lineages to the genetic structure of this population. This pattern aligns with findings by Reischak et al. (2023), who noted that chicken mitochondrial haplotypes often exhibit dispersed frequencies, reflecting a complex demographic history. The presence of low-frequency haplotypes, such as A and E, may imply the retention of ancestral or rare genetic variants, which are crucial for understanding the evolutionary history and selective pressures shaping these chickens. As emphasized by Sánchez-Molano et al. (2016), rare haplotypes can carry key information about historical domestication and environmental adaptation. Furthermore, these results highlight the potential influence of domestication, artificial selection, and local ecological conditions on genetic diversity, consistent with previous reports by Gibson (2022), Guo et al. (2016), and Aminisarteshnizi et al. (2024). Thus, preserving both major and minor haplotypes should be considered a critical component in the conservation and management of indigenous chicken genetic resources, especially in insular regions such as Ternate, where environmental challenges and genetic erosion may threaten long-term population viability.

The observed concentration of polymorphic sites within the 101–200bp region suggests a higher degree of nucleotide variability in the central portion of the mitochondrial D-loop. This pattern is consistent with the known biological role of this segment in the initiation of mitochondrial DNA replication and transcription, which renders it more susceptible to mutations due to reduced selective constraints (Duraisamy et al., 2019). Conversely, the low number of polymorphic sites in the 201–300bp segment may indicate the existence of functionally conserved elements or strong purifying selection acting to maintain genomic integrity in this region. Such heterogeneity in the distribution of variation has also been reported in other avian species, where the D-loop region tends to harbor mutation hotspots in areas less involved in essential regulatory functions (Masila et al., 2024). These findings are valuable for the design of targeted molecular markers, as well as for advancing evolutionary studies and genetic conservation programs focusing on indigenous chicken populations. Understanding region-specific mutation dynamics within the D-loop enhances the efficiency of molecular diagnostics and contributes to the preservation of genetic diversity.

The observed genetic distance values between G1 chicken haplotypes and the *Gallus gallus* reference sequence suggest a moderate degree of genetic divergence, with the highest value (0.02255) indicating that certain individuals may have undergone distinct domestication histories or introgression events. These results align with findings from previous studies, which reported that local chicken populations often diverge genetically from commercial or wild strains due to regional selection, drift, and breeding practices (Asadollahpour Nanaei et al., 2022).

The low genetic distances between several haplotypes within the G1 population—particularly the complete sequence similarity between haplotypes C and F—point to possible inbreeding or a narrow genetic base. Such patterns are commonly found in semi-isolated or unmanaged breeding systems, where offspring derive from a limited number of ancestors. Nevertheless, the presence of modest genetic divergence among other haplotype pairs (e.g., B and D) suggests the retention of valuable genetic variation, which is critical for long-term breeding, adaptability, and conservation strategies (Schleimer et al., 2024). These insights emphasize the importance of genetic monitoring in local chicken populations and inform future programs aimed at maintaining diversity and improving productivity through controlled breeding interventions.

The uneven distribution of polymorphic sites, particularly the concentration within the 101–200bp segment, highlights the central D-loop region's higher mutation frequency. This region is biologically important due to its role in the initiation of replication and transcription of mtDNA, thus often acting as a mutational hotspot (Duraisamy et al., 2019). Conversely, the fewer polymorphisms in the 201–300bp segment may suggest a region under stronger selective constraints or functional conservation. Genetic distance analysis supports the notion of divergence between local G1 chickens and the commercial *Gallus gallus* strain. Although G1 chickens remain genetically related to Gallus gallus, the relatively high interspecific distances indicate the influence of separate domestication events or gene flow from diverse ancestral sources (Asadollahpour Nanaei et al., 2022).

The low intraspecific distances, particularly the identity between haplotypes C and F, suggest recent common ancestry or limited breeding diversity within certain lineages. The phylogenetic tree structure reinforces this interpretation, revealing clear separation between the *Gallus gallus* reference and Ternate chickens, with the latter forming multiple evolutionary clusters. This finding aligns with previous studies on native poultry populations, which report high haplotype diversity due to long-term adaptation and localized breeding (Schleimer et al., 2024; Yaemkong et al., 2024). Such diversity is critical for future genetic conservation and breeding programs aimed at enhancing productivity while preserving adaptability to local environments (Díaz-Matus et al., 2024).

Conclusion

This study successfully identified the distribution of polymorphic sites in the mitochondrial DNA D-Loop sequence of the G1 chicken population. In detail, it was found that in the base sequence between 0-100 there were two polymorphic sites (33%), in 101-200 there were three polymorphic sites (50%), in 201-300 there was one polymorphic site (17%), while in the ranges 301-400 and 401-500 there were no polymorphic sites (0%). This percentage was calculated from a total of six polymorphic sites found in G1 chickens. The sequence results showed that the genetic variability in nucleotide sequences 101-200 was the highest, with three polymorphic sites (50%). These

findings suggest that the mitochondrial DNA D-Loop segments may be a good indicator for describing genetic diversity in G1 chicken populations. This high variability in this particular region has the potential to provide valuable information for further studies of the evolution and breeding of local chickens, as well as for more effective genetic conservation strategies. The results of this study can be used as a reference to understand the genetic dynamics of G1 chickens and their contribution to the genetic diversity of poultry in Indonesia.

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Data Availability: The molecular DNA D-loop data of Ternate local chickens used in this study are available from the corresponding author upon reasonable request.

Ethics Statement: The blood sampling of the First Generation (G1) of the Ternate Local Chicken was carried out in accordance with the Indonesian National Law No. 18/2009, concerning "Livestock and Health". This sampling procedure has also received ethical approval from the North Maluku Provincial Agriculture and Food Security Office, with permit number No. 040-602 of 2022, and is carried out by competent experts to ensure that animal welfare is maintained during the research process.

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REFERENCES

Aiffa, N.O.R., Abu, W., Syafiq, M., Mohd, A., Aimi, N., Jaafar, M.A.T., Bokhari, F., Saperi, S., & Ariffin, A. (2025). Genetic diversity and population genetics of tropical shads (*Tenualosa toli*) from Sarawak, Malaysia. *Journal of Sustainability Science and Mannagement,* 20(1), 78–100. https://doi.org/http://doi.org/10.46754/jssm.2025.01.005.

Alcalá, S., Villarino, L., Ruiz-Cañas, L., Couceiro, J.R., Martínez-Calvo, M., Palencia-Campos, A., Navarro, D., Cabezas-Sainz, P., Rodriguez-Arabaolaza, I., Cordero-Barreal, A., Trilla-Fuertes, L., Rubiolo, J.A., Batres-Ramos, S., Vallespinos, M., González-Páramos, C., Rodríguez, J., Gámez-Pozo, A., Vara, J.Á.F., Fernández, S.F., & Sainz, B. (2024). Targeting cancer stem cell OXPHOS with tailored ruthenium complexes as a new anti-cancer strategy. *Journal of Experimental and Clinical Cancer Research*, 43(1), 1–27. https://doi.org/10.1186/s13046-023-02931-7.

Aminisarteshnizi, M., Moyo, N.A.G., & Raphalo, M.E. (2024). Genetic and haplotype diversity of redbreast tilapia (Coptodon rendalli) based on cytochrome oxidase subunit I and D-loop. *Journal of King Saud University - Science*, 36(11), 103585. https://doi.org/10.1016/j.jksus.2024.103585.

Asadollahpour Nanaei, H., Kharrati-Koopaee, H., & Esmailizadeh, A. (2022). Genetic diversity and signatures of selection for heat tolerance and immune response in Iranian native chickens. *BMC Genomics*, 23(1), 1–13. https://doi.org/10.1186/s12864-022-08434-7.

Assan, N., Muteyo, E., Masama, E., Mafigu, T., & Mujati, T. (2024). Crossbreeding and its implication for small-scale animal agriculture in Africa: Outcomes, both positive and negative, and future prospects. *Advances in Modern Agriculture*, 5(2), 2362. https://doi.org/10.54517/ama.v5i2.2362.

Cao, L., Chen, P., Hou, X., Ma, J., Yang, N., Lu, Y., & Huang, H. (2024). rDNA and mtDNA analysis for the identification of genetic characters in the hybrid grouper derived from hybridization of Cromileptes altivelis (female) × Epinephelus lanceolatus (male). *BMC Genomic Data*, 25(1), 1–12. https://doi.org/10.1186/s12863-023-01188-5.

Cardona-Ramírez, C., Enríquez-Valencia, C.E., Méndez-Callejas, G., Barreto, G.M., Tafur-Gómez, G.A., & Sanjuanelo-Corredor, D.W. (2025). An integrated methodological framework for the validation and verification of clinical testing by qRT-PCR. *Heliyon*, 11(1). https://doi.org/10.1016/j.heliyon.2024.e41088.

Chen, F., Lu, P., Liang, D., Wu, Y., Jiang, Z., Huang, W., & Gao, L. (2024). Phylogenetic analysis based on the complete mitochondrial genome of Discogobio brachyphysallidos (Cypriniformes: Cyprinidae) suggests the need for taxonomic revision at the genus level. *Mitochondrial DNA Part B: Resources*, 9(1), 200–203. https://doi.org/10.1080/23802359.2024.2306882.

Chen, Y., Chen, L., Lun, A. T., Baldoni, P. L., & Smyth, G. K. (2025). edgeR v4: powerful differential analysis of sequencing data with expanded functionality and improved support for small counts and larger datasets. *Nucleic Acids Research*, 53(2), gkaf018. https://doi.org/10.1093/nar/gkaf018

Chen, Y., Ma, G., Gai, Y., Yang, Q., Liu, X., de Avila, J.M., Mao, S., Zhu, M.J., & Du, M. (2024). AMPK Suppression due to obesity drives oocyte mtDNA heteroplasmy via ATF5-POLG Axis. *Advanced Science*, 11(20), 1–14. https://doi.org/10.1002/advs.202307480

Chiara, L., Paula, B.D., Maria, A., Azeredo, L.D., & Torres, T.T. (2025). Demographic and historical processes influencing Cochliomyia

hominivorax (Diptera: Calliphoridae) population structure across South America. *Parasites & Vectors*, 1–14. https://doi.org/10.1186/s13071-024-06622-w.

Díaz-Matus de la Parra, M., Inostroza, K., Alcalde, J.A., Larama, G., & Bravo, S. (2024). Characterization of the genetic diversity, structure, and admixture of 7 Chilean chicken breeds. *Poultry Science*, 103(2). https://doi.org/10.1016/j.psj.2023.103238.

Duraisamy, A.J., Radhakrishnan, R., Seyoum, B., Abrams, G.W., & Kowluru, R.A. (2019). Epigenetic modifications in peripheral blood as potential noninvasive biomarker of diabetic retinopathy. *Translational Vision Science and Technology*, 8(6). https://doi.org/10.1167/tvst.8.6.43.

Elzain, I.A., Idris, A.B., Karim, A.A., Ahmed, N.M., Elzaki, S.G., Yılmaz, S., Hassan, M.A., & Abdalla, H.S. (2025). Analysis of DNA cox1 barcoding revealed novel haplotype in Schistosoma haematobium isolated from Western Sudan. *Scientific Reports*, 15(1), 2062. https://doi.org/10.1038/s41598-025-85986-0.

Fast, K.M., Larrimore, J.D., Alley, Z.D., & Sandel, M.W. (2024). Complete mitochondrial genome of the Starhead Topminnow Fundulus dispar (Cyprinodontiformes: Fundulidae). *Mitochondrial DNA Part B: Resources*, 9(3), 342–346. https://doi.org/10.1080/23802359.2024.2327564.

Fatmona, S., Hoda, A., & Utami, S. (2024a). Phenotype character of the first generation (G1) results of crossing elba chicken with ternate village chicken to improve the quality of local chicken. *Advances in Animal and Veterinary Sciences*, 12(8), 1410–1421. https://doi.org/http://dx.doi.org/10.17582/journal.aavs/2024/12.8.1410.1421.

Fatmona, S., & Sjafani, N. (2020). Phenotype diversity of free-range chickens (*Gallus gallus* domesticus) in Ternate City. *Cannarium* 18(1), 30–43.https://doi.org/10.33387/cannarium.v18i1.2163.

Fatmona, S., Utami, S., & Gunawan, (2024b). Reproduction performance as a basis for selecting female balinese cattle in traditional breeding in east Halmahera regency. *Advances in Animal and Veterinary Sciences*, 12(7), 1214-1222. https://dx.doi.org/10.17582/journal.aavs/2024/12.7.1214.1222.

Fatmona, S., Sjafani, N., Sulasmi, Utami, S., Wahyuni, S., Sahil, J., & Keintjem, J.R.M. (2024c). Genetic distance and kinship relationship of Walik Kembang Sula bird (Ptilinopus melanosphila) based on mtDNA CO1 in North Maluku, Indonesia. *International Journal of Veterinary Science*, 14(3), 548-556. https://doi.org/10.47278/journal.ijvs/2024.264.

Feng, Z., Peng, F., Xie, F., Liu, Y., Zhang, H., Ma, J., Xing, J., & Guo, X. (2024). Comparison of capture-based mtDNA sequencing performance between MGI and illumina sequencing platforms in various sample types. *BMC Genomics*, 25(1), 1–10. https://doi.org/10.1186/s12864-023-09938-6.

Fu, Y.-X. (1997). Statistical tests of neutrality of mutations against population growth, hitchhiking and background selection. *Genetics, 147*(2), 915–925. https://doi.org/10.1093/genetics/147.2.915

Fu, Y.-X., & Li, W.-H. (1993). Statistical tests of neutrality of mutations. *Genetics, 133*(3), 693–709. https://doi.org/10.1093/genetics/133.3.693

Geletu, T.T., Tang, S., & Zhao, J. (2025). Genetic diversity and differentiation of cultured Nile tilapia populations from Ethiopia revealed by ddRAD-seq: implications for better hatchery management. *Aquatic Living Resources*, 38. https://doi.org/10.1051/alr/2024018

Gibson, A.K. (2022). Genetic diversity and disease: The past, present, and future of an old idea. *Evolution*, 76, 20–36. https://doi.org/10.1111/evo.14395.

González, S., Repetto, L., Gutiérrez, V., Olivera, M.E., Botto, C.C., Leone, Y., Merino, M. L., Braga, F.G., Duarte, J.M.B., Maldonado, J.E., & Cosse, M. (2024). Revisiting the conservation genetics of Pampas deer (Ozotoceros bezoarticus). *Therya*, 15(1), 5–16. https://doi.org/10.12933/therya-24-5379.

Gowda, K.B., Jerry, D.R., & Zenger, K.R. (2025). Genetic improvement of farmed insect species: Programmes, progress, and prospects. *Journal of Insects as Food and Feed*, 4588. https://doi.org/10.1163/23524588-00001387.

Guo, Z., Zhao, S., Fan, H., Du, Y., Zhao, Y., & Wang, G. (2016). Identification of sequence polymorphisms in the D-Loop region of mitochondrial DNA as a risk factor for colon cancer. *Mitochondrial DNA Part A: DNA Mapping, Sequencing, and Analysis*, 27(6), 4244–4245. https://doi.org/10.3109/19401736.2014.1003920.

Hachad, M., Burnet, J.B., Sylvestre, É., Duy, S.V., Villemur, R., Sauvé, S., Prévost, M., Qiu, J.Y., Pang, X., & Dorner, S. (2024). β-D-glucuronidase activity triggered monitoring of fecal contamination using microbial and chemical source tracking markers at drinking water intakes. *Water Research*, 254, 121374. https://doi.org/10.1016/j.watres.2024.121374.

Hou, Y., Gan, J., Fan, Z., Sun, L., Garg, V., Wang, Y., Li, S., Bao, P., Cao, B., Varshney, R.K., & Zhao, H. (2024). Haplotype-based pangenomes reveal genetic variations and climate adaptations in moso bamboo populations. *Nature Communications,* 15(1), 8085. https://doi.org/10.1038/s41467-024-52376-5.

Husien, H.M., Saleh, A.A., Hassanine, N.N.A.M., Rashad,A.M.A., Sharaby, M.A., Mohamed, A.Z., Abdelhalim, H., Hafez, E.E., Essa, M.O.A., Adam, S.Y., Chen, N., & Wang, M. (2024). The evolution and role of molecular tools in measuring diversity and genomic selection in livestock populations (Traditional and Up-to-Date Insights): A comprehensive exploration. *Veterinary Sciences*, 11(12), 627. https://doi.org/10.3390/vetsci11120627.

KammongKun, J., & PromKet, D. (2024). Growth performance and morphology traits associated with neuropeptide Y (NPY) genes expression in native chickens. *Advances in Animal and Veterinary Sciences*, 12(11), 2263-2274. https://doi.org/https://dx.doi.org/10.17582/journal.aavs/2024/12.11.2263.2274.

Lee, K.P., Anthony, N.B., Orlowski, S.K., & Rhoads, D.D. (2022). SNP-based breeding for broiler resistance to ascites and evaluation of correlated production traits. *Hereditas*, 159(1), 1–15. https://doi.org/10.1186/s41065-022-00228-x.

Lindner, M., Verhagen, I., Mateman, A.C., van Oers, K., Laine, V.N., & Visser, M.E. (2024). Genetic and epigenetic differentiation in response to genomic selection for avian lay date. *Evolutionary Applications*, 17(7), 1–17. https://doi.org/10.1111/eva.13703.

Lovász, L., Sommer-Trembo, C., Barth, J.M.I., Scasta, J., Grancharova-Hill, R., Lemoine, R., Kerekes, V., Merckling, L., Svenning, J., & Fages, A. (2024). Rewilded horses in European nature conservation-a genetics, ethics and welfare perspective. *Biological Reviews*, 100, 407–427. https://doi.org/10.1111/brv.13146.

Ma, Z., Huang, W., Xu, J., Qiu, J., Liu, Y., Ye, M., & Fan, S. (2024). Analysis of deafness susceptibility gene of neonates in northern Guangdong, China. *Scientific Reports*, 14(1), 1–5. https://doi.org/10.1038/s41598-023-49530-2.

Madhushan, A., Weerasingha, D.B., Ilyukhin, E., Taylor, P.W.J., Ratnayake, A.S., Liu, J.K., & Maharachchikumbura, S.S.N. (2025). From Natural Hosts to Agricultural Threats: The Evolutionary Journey of Phytopathogenic Fungi. *Journal of Fungi*, 11(1), 25. https://doi.org/10.3390/jof11010025.

Manyelo, T.G., Selaledi, L., Hassan, Z.M., & Mabelebele, M. (2020). Local chicken breeds of Africa: Their description, uses and conservation methods. *Animals*, 10(12), 2257. https://doi.org/10.3390/ani10122257.

Martínez, J., & Menéndez-arias, L. (2025). Next-Generation Sequencing Methods to Determine the Accuracy of Retroviral Reverse Transcriptases: Advantages and Limitations. *Viruses*, *17*(2), 173. https://doi.org/10.3390/v17020173.

Masila, E.M., Ogada, S.O., Ogali, I.N., Kennedy, G.M., Too, E.K., & Ommeh, C.S. (2024). Mitochondrial DNA D-loop polymorphisms among the galla goats reveals multiple maternal origins with implication on the functional diversity of the HSP70 gene. *Genetics Research*, 2024, 5564596. https://doi.org/10.1155/2024/5564596.

Mokoena, K., Mbazima, V., & Tyasi, T.L. (2024). A literature review on effect of chicken crossbreeding on egg production traits. *World's Poultry Science Journal*, 81 (1), 151–167. https://doi.org/10.1080/00439339.2024.2443184.

Mutum, R.S., Das, A., Ghosh, S.K., & Wangkheimayum, V.D. (2025). The origins of "Kaunayen" game fowls of Manipur, India: Insights from mitochondrial D-loop sequence analysis. *Poultry Science*, 104(1), 104667. https://doi.org/10.1016/j.psj.2024.104667.

Niu, M., Fu, D., Wang, H., Liu, Y., Du, X., Zhao, Q., & Wei, J. (2025). Microsatellite and mitochondrial COI provide novel insights into the population genetic structure of white prunicola scale (*Pseudaulacaspis prunicola*) in China. *Ecology and Evolution*, 15(1), e70865. https://doi.org/10.1002/ece3.70865.

Peck, C., Jackobs, F., & Smith, E. (2022). The PortaLyzer, a DIY tool that allows environmental DNA extraction in the field. *HardwareX*, 12, e00373. https://doi.org/10.1016/j.ohx.2022.e00373.

Reischak, D., Rivetti, A. V., Jr, Otaka, J.N.P., Domingues, C.S., Freitas, T.L., Cardoso, F.G., Montesino, L.O., da Silva, A.L.S., Malta, F., Amgarten, D., Goés-Neto, A., de Oliveira, A.F., & Camargos, M.F. (2023). First report and genetic characterization of the highly pathogenic avian influenza A(H5N1) virus in Cabot's tern (*Thalasseus acuflavidus*), Brazil. *Veterinary and Animal Science*, 22, 100319. https://doi.org/10.1016/j.vas.2023.100319.

Sánchez-Molano, E., Tsiokos, D., Chatziplis, D., Jorjani, H., Degano, L., Diaz, C., Rossoni, A., Schwarzenbacher, H., Seefried, F., Varona, L., Vicario, D., Nicolazzi, E.L., & Banos, G. (2016). A practical approach to detect ancestral haplotypes in livestock populations. *BMC Genetics*, 17(1), 1–11. https://doi.org/10.1186/s12863-016-0405-2.

Sari, R.M., Saepuloh, U., Rizaldi, & Perwitasari-Farajallah, D. (2024). Genetic

Variability of the Long-Tailed Macaque (Macaca fascicularis) Populations in Urban Habitat in Padang City, West Sumatra, Indonesia. *HAYATI Journal of Biosciences*, 31(2), 392–403. https://doi.org/10.4308/hjb.31.2.392-403.

Schleimer, A., Cantú-Salazar, L., Luttringer, A., & Frantz, A.C. (2024). Spatial prioritisation for crested newt conservation in Luxembourg: Insights from population genetics and species distribution modelling. *Ecological Solutions and Evidence*, 5(1), e12310. https://doi.org/10.1002/2688-8319.12310.

Sciuto, S., Colussi, S., Esposito, G., Meletiadis, A., Prearo, M., Pizzul, E., Acutis, P.L., Gozlan, R.E., & Pastorino, P. (2024). Buccal swabs for long-term DNA storage in conservation genetics of fish: One-and-a-half-year analysis timeframe. *Global Ecology and Conservation*, 51, e02867. https://doi.org/10.1016/j.gecco.2024.e02867.

Silalahi, P., Sitorus, T.F., & Putra, W.P.B. (2025). Genetic characterization of sumatran mirah chicken based on mitochondrial D-loop region sequence. *HAYATI Journal of Biosciences*, 32(1), 196–202. https://doi.org/10.4308/hjb.32.1.196-202.

Sithole, S.M., Nephawe, K.A., Mpofu, T.J., Mtileni, B., Mphaphathi, M.L., & Ngcobo, J.N. (2025). Extinction status, challenges, and conservation approaches of South African indigenous and village chickens: A systematic review. *Agriculture*, *15*(2), 216. https://doi.org/10.3390/agriculture15020216.

Siziba, V.I., & Willows-Munro, S. (2024). An assessment of South African small mammal barcode sequence libraries: Implications for future carnivore diet analyses by DNA. *African Journal of Ecology*, 62(1), e13233. https://doi.org/10.1111/aje.13233.

Sun, S., Wei, L., Chen, Z., Chai, Y., Wang, S., & Sun, R. (2024). Nondestructive estimation method of live chicken leg weight based on deep learning. *Poultry Science*, 103(4), 103477. https://doi.org/10.1016/j.psj.2024.103477.

Tan, X., Zhang, J., Dong, J., Huang, M., Li, Q., Wang, H., Bai, L., Cui, M., Zhou, Z., Yang, S., & Wang, D. (2024). Whole-genome variants dataset of 209 local chickens from China. *Scientific Data*, 11(1), 169. https://doi.org/10.1038/s41597-024-02995-w.

Temoa, C.W., Massawa, J., Djiotsa, F.D., Manjely, Y., & Meutchieye, F. (2025). Morphological characteristics of local chicken populations (*Gallus gallus* domesticus) in farmed basins of the Far North Region, Cameroon. *Open Journal of Animal Sciences*, 15(1), 51–68. https://doi.org/10.4236/ojas.2025.151004.

Thakur, K., Sharma, D., Sharma, A., Sharma, A.K., Mahajan, D., Brar, B., Kumari, H., Kumar, S., Bala, M., Kumar, S., & Kumar, R. (2025). In silico analysis of mitochondrial DNA genes: implication for conservation of Tor putitora (Hamilton, 1822). *Scientific Reports*, 15(1), 106. https://doi.org/10.1038/s41598-024-83669-w.

Wintari, M., Pharmawati, M., & Wiratmini, N. (2019). Genetic diversity and relationships of local chickens based on RAPD Marker. *Metamorfosa: Journal of Biological Sciences, 6*(2), 230-236. https://doi:10.24843/metamorfosa.2019.v06.i02.p13.

Wu, J.P., Wu, J.L., Chen, Y.B., Xu, W.H., Xie, W.Q., Tang, Z.Q., Ding, G.H., & Ma, L. (2024). The complete mitochondrial genome of the Baishanzu horned toad Boulenophrys baishanzuensis (Anura: Megophryidae). *Mitochondrial DNA Part B: Resources*, 9(1), 209–213. https://doi.org/10.1080/23802359.2024.2307995.

Yaemkong, S., Phromnoi, S., Mingchai, C., & Jongjitvimol, T. (2024). Characterization of phenotypic variation in indigenous chicken populations in lower northern thailand to improve chicken breeding. *International Journal of Zoology*, 2024(1), 9985076. https://doi.org/10.1155/2024/9985076.

Zein, M.S.A., & Sulandari, S. (2008). Genetic diversity of Lombok chickens based on D-loop mitochondrial DNA sequences. *Jurnal Ilmu Ternak dan Veteriner*, 13(4), 308–314. https://doi.org/10.14334/jitv.v13i4.575.

Zhao, Y., Hou, Y., Zhao, X., Liufu, T., Yu, M., Zhang, W., Xie, Z., Zhang, V.W., Yuan, Y., & Wang, Z. (2024). The clinical, myopathological, and genetic analysis of 155 Chinese mitochondrial ophthalmoplegia patients with mitochondrial DNA single large deletions. *Molecular Genetics and Genomic Medicine*, 12(1), e2328. https://doi.org/10.1002/mgg3.2328.

Zhi, Y., Geng, W., Li, S., Chen, X., & Kamal, M. (2025). Advanced molecular system for accurate identification of chicken genetic resources. *Computers and Electronics in Agriculture*, 231, 109989. https://doi.org/10.1016/j.compag.2025.109989.