BIODIVERSITAS
Volume 24, Number 11, November 2023
Pages: 6256-6263

ISSN: 1412-033X
E-ISSN: 2085-4722
DOI: 10.13057/biodiv/d241148

DNA barcoding of Burgo chicken from Bengkulu, Indonesia, based on the cytochrome oxidase gene sub unit I mitochondria DNA

AHMAT FAKHRI UTAMA[2], JARULIS[1,2,♥], SIPRIYADI[1,2], IZUL MIFTAKHUL JANNAH[2]

[1]Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu. Jl. W.R. Supratman, Kandang Limun, Kota Bengkulu 38371, Bengkulu, Indonesia

[2]Graduate Program of Biology, Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Bengkulu. Jl. W.R. Supratman, Kandang Limun, Kota Bengkulu 38371, Bengkulu, Indonesia. Tel.: +62-736-20919, ♥email: jarulis@unib.ac.id

Manuscript received: 26 October 2023. Revision accepted: 27 November 2023.

Abstract. *Utama AF, Jarulis, Sipriyadi, Jannah IM. 2023. DNA barcoding of Burgo chicken from Bengkulu, Indonesia, based on the cytochrome oxidase gene sub unit I mitochondria DNA. Biodiversitas 24: 6268-6275.* The Burgo chicken provides a genetic source of one crossbreed chicken in Bengkulu Province. However, genetic information on Burgo chickens is not yet available, while the population of this chicken continues to decline. Research on DNA barcoding using the cytochrome oxidase subunit I mtDNA gene was conducted to obtain DNA barcodes and the relationship between Burgo chickens and other chicken species based on the COI mtDNA gene. Blood samples were obtained from Burgo chickens belonging to the Bengkulu Burgo chicken hobbyist community. Collected blood was isolated following the dneasy® blood and tissue kit protocol based on the spin-column protocol procedure, Qiagen. Amplification of genomic DNA using Polymerase Chain Reaction. The product was electrophoresed on a 1.2% agarose gel and visualized under UV light using Gel Documentation System, Axygen. Samples with bright DNA bands proceeded to the sequencing process. Sequencing results were analyzed using MEGA 11.0 software. The results showed that the target length of the Burgo chicken band was in accordance with the primer design used (752pb). SNPs were obtained at 10 specific sites in Burgo chickens and had a species barcode at site 746. The intraspecies genetic distance was 0.6%, interspecies 1.2%, and with the outgroup 14.5%, there were 12 haplotypes from all samples (n=15). Based on the result, we can conclude that the Burgo chicken is closely related to the red jungle fowl compared to other chicken species.

Keywords: Barcode, genetic study, PCR, Phasianidae

INTRODUCTION

Poultry animals like *Gallus* spp. are a source of animal protein and are usually found as pets by Indonesians. There are several chicken breeds, including local chickens, broiler chickens (layer and broilers), or ornamental chickens, that are used as a symbol of the social strata of their keepers. One of the endemic ornamental chickens in Bengkulu Province is the Burgo chicken, which can be found in several districts, such as Lebong District, Rejang Lebong District, Kepahiang District, Central Bengkulu District, North Bengkulu District, and Bengkulu City (Putranto et al. 2017). Burgo chickens are very popular with the people of Bengkulu because they have a beautiful crowing sound. As a food source, Burgo chickens produce eggs and meat, while germplasm Burgo chickens are a genetic source (Setianto et al. 2013).

Burgo chicken is the result of a crossbreed between domestic chicken and red junglefowl, in which red junglefowl is partridge that becomes the ancestor for each chicken in the world (Putranto et al. 2012). The domestication process was to begin with occur in Indochina and the southeast of Tiongkok (Wang et al. 2020). The impact of breeding appeared specific to all, which is a cruel generation of isolation that happens continuously and has a solid impact on the domestication of chicken and partridge (Gering et al. 2015; Wu et al. 2020). In any case, variety from the ancestor population has given an advantage to the domestication population (Barbato et al. 2017). However, there is still no information about the genetic Burgo chicken, which shows the relationship between Burgo chicken and other chicken species.

One source of information on the genetic characteristics of living creatures is mitochondrial DNA (mtDNA). Mitochondrial DNA has a high DNA copy number, making it suitable for analysis with a limited amount of DNA or easily degraded DNA (Ni'mah et al. 2016). MtDNA has 2 strands, namely the Heavy (H) strand, which is rich in guanine, and the Light (L), which is rich in cytosine. MtDNA is gene-dense DNA and with almost no introns, measuring 16,569 bp form 37 genes. Mitochondrial DNA base sequence comparisons have been used in population genetics and phylogeny studies in the medical field for disease tracking (Bajpai and Tewari 2010).

Putranto et al. (2012) stated that the Burgo chicken is an Indonesian genetic resource originating from a cross between the red jungle fowl (*Gallus gallus* Linnaeus 1758)) and the native chicken (*Gallus gallus* subsp. *domesticus* Linnaeus 1758)). The lack of public attention to this species of chicken has resulted in the threat of losing one of the germplasm sources. The decline in population is one of the factors that threaten the existence of Burgo chickens from Bengkulu. The decline in the Burgo chicken population is due to land conversion, resulting in habitat

fragmentation and poaching for sale. In addition, the habitat condition of the red jungle fowl is getting narrower, and the high level of hunting causes the red jungle fowl's breeding to be hampered, which can affect the existence of the Burgo chicken as a derivative of the red jungle fowl (Zahradden et al. 2005).

The DNA barcoding technique is designed to carry out fast and accurate identification based on the nucleotide base sequence of short marker genes. The use of DNA barcoding has the advantage of identifying species with a high level of accuracy compared to morphological observations (Madduppa et al. 2017). In addition to using the DNA barcoding technique, other supporting data needed are genetic character data, Single Nucleotide Polymorphism (SNP), genetic distance and phylogeny. These data are needed to explain determine the position of the chicken Burgo clade in one family (Jarulis et al. 2022). COI is a rapidly evolving mitochondrial genetic marker that is widely used to examine relationships between populations and closely related species (Kononov et al. 2016; Cock et al. 2017; Otim et al. 2018). This research was conducted to determine the DNA barcode and the relationship between Burgo chickens and other chicken species based on the COI gene in mitochondria.

MATERIALS AND METHODS

Sample collection and preservation

The 15 Burgo chickens were collected (Table 1) from the Bengkulu Burgo chicken community. The 0.5-1.0 mL of blood was taken through the pectoral vein using a 1.0 ml syringe. The blood was added into an Ethylene Diamine Tetraacetic Acid (EDTA) tube for preservation and kept in a freezer at -20°C. This research has followed the research protocol that has been approved by the LPPM code of ethics of Universitas Bengkulu with No. 15/KER-LPPM/EC/2023.

Procedures

DNA isolation

DNA total isolation was carried out using the Dneasy® Blood and Tissue Kit cat no. 69504 (50) according to the Qiagen's Spin-Column Protocol. The quality of the isolated DNA was observed on a 1.2% agarose gel using electrophoresis, then kept in a freezer at -20°C.

DNA amplification and sequencing

Replication of DNA target in the COI gene was carried out by amplification process using the polymerase chain reaction (PCR) technique. The COI gene sequence of Gallus gallus (KY039421) was used to design the primer. Primers were designed using the Primer3 Programme which is available online. The length of the COI sequence used is 1550 bp with a target product size of 752 bp, where a pair of primers are used, namely BRCO1F (5'AATGTAATCGTCACAGCCCATG-3') and BRCO1R (5'GTAAAGTAGGCTCGGGTGTCTA-3').

The PCR amplification reaction mixture (total 50 µL) consisted of 19 µL ddH2O, 25 µL Go Taq Green, 1.5 µL forward primer, 1.5 µL reverse primer, and 3 µL template DNA. The PCR machine condition during amplification as follows: predenaturation temperature 95ºC for 2 minutes, denaturation 94ºC for 1 minute, annealing 55ºC for 45 seconds, elongation 72ºC for a minute, past elongation 72ºC for 5 minutes, and cooling 4ºC for 10 minutes. The number of cycles of the denaturation-elongation stage was 30 times. The DNA (3.0 µL) product from PCR was visualized on a 1.2% agarose gel using electrophoresis and the results were photographed with a UV transilluminator (λ=302 nm). The PCR products were sent to Genetika Science Indonesia for sequencing.

Data analysis

The nucleotide sequences from the sequencing results in alignment used the Clustal W program Molecular Evolutionary Genetic Analysis (MEGA) 11.0 (Tamura et al. 2013). The BIOEDIT software version 7.0.9 (Hall et al. 2011), was used to edit the COI gene sequence, visualize the electrogram and nucleotide base sequence. Each individual's gene sequence was aligned with the COI gene in GenBank via the BoLD System to view the samples' similarity. Genetic distance was calculated using the Kimura 2-parameter method (Kumar et al. 2018). Phylogenetic trees were reconstructed using the Neighbor-Joining (NJ) method with 1000 replications (Tamura et al. 2013).

RESULTS AND DISCUSSION

DNA band visualization

Based on the visualization of the PCR result, we obtained a bright and clear band with a target DNA of 752 bp, followed by sequencing (Figure 1).

Single Nucleotide Polymorphism

The results of the nucleotide sequence alignment of the COI gene of Bengkulu Burgo chicken (*G. gallus*) population showed the existence of single nucleotide polymorphism (SNP) variations between the individuals studied (Table 1). This table shows that 10 specific nucleotide sites were found in the COI gene with a length of 752 bp. These specific sites are found in sequence numbers 26 -32, 43, 228 and 745.

DNA barcoding

The *COI* gene, apart from having more conserved properties compared to other genes found in mitochondrial DNA. Therefore, the *COI* gene can be used as a DNA barcode or specific marker to differentiate taxa at the species level. Based on the results of sequence alignment of *G. gallus* (Burgo) samples with data samples from *Genbank*, namely *Gallus sonneratii* Temminck 1813 (gray junglefowl)(NC007240.1), *Gallus lafayettii* R.Lesson 1831 (ceylon junglefowl) (NC007239.1), *Gallus varius* Shaw 1798*)* (green junglefowl) (NC007238.1) and *G. gallus* (KY039421.1) 26 barcode sites were obtained consisting of 25 genus barcode sites and 1 species barcode site.

Haplotype networks

Results of haplotype network analysis using PopART v1.7 software. Figure 2 shows the genetic relationships between populations of *G. gallus* and species of *Gallus* spp. Haplotype network analysis using a median-joining network found 17 haplotypes formed from the COI gene sequence of *G. gallus*.

Phylogeny

Phylogenetic tree reconstruction of 15 individuals of Bengkulu's Burgo chickens to be one group and separated from other species. Based on reconstruction using Neighbor Joining model (Figure 3). Neighbor Joining is a phylogenetic analysis method based on differences in the rate of evolution of each branch. The components in NJ analysis are operational taxonomy units (OTU) and evolutionary distance.

Table 1. SNP among individual Bengkulu's Burgo chickens based on the COI gene (752pb)

Individual	Sequence number									
	26	**27**	**28**	**29**	**30**	**31**	**32**	**43**	**228**	**745**
G. gallus (KY039421.1)	G	T	C	A	T	A	A	G	T	A
G. gallus (Burgo) 1	.	C	.	T	.	G	.	.	.	.
G. gallus (Burgo) 2	C	.	T	G	.	.	.	.	.	.
G. gallus (Burgo) 3	C	.	T	G	.	.	.	.	.	T
G. gallus (Burgo) 4	C	.	T	G	.	.	.	.	.	T
G. gallus (Burgo) 5	C	.	T	G	C	.	.	.	.	T
G. gallus (Burgo) 6	.	.	.	.	.	.	.	.	.	T
G. gallus (Burgo) 7	C	.	.	G	C	.	.	.	.	T
G. gallus (Burgo) 8	C	.	T	G	.	.	.	.	.	.
G. gallus (Burgo) 9	.	.	.	.	.	.	.	.	.	.
G. gallus (Burgo) 10	.	C	.	T	.	G	C	.	C	T
G. gallus (Burgo) 11	.	C	.	T	.	G	.	A	.	T
G. gallus (Burgo) 12	C	.	T	G	.	.	.	.	.	T
G. gallus (Burgo) 13	.	C	.	T	.	G	.	.	.	T
G. gallus (Burgo) 14	C	.	T	G	C	.	.	A	.	.
G. gallus (Burgo) 15	.	C	.	G	.	.	.	A	.	T

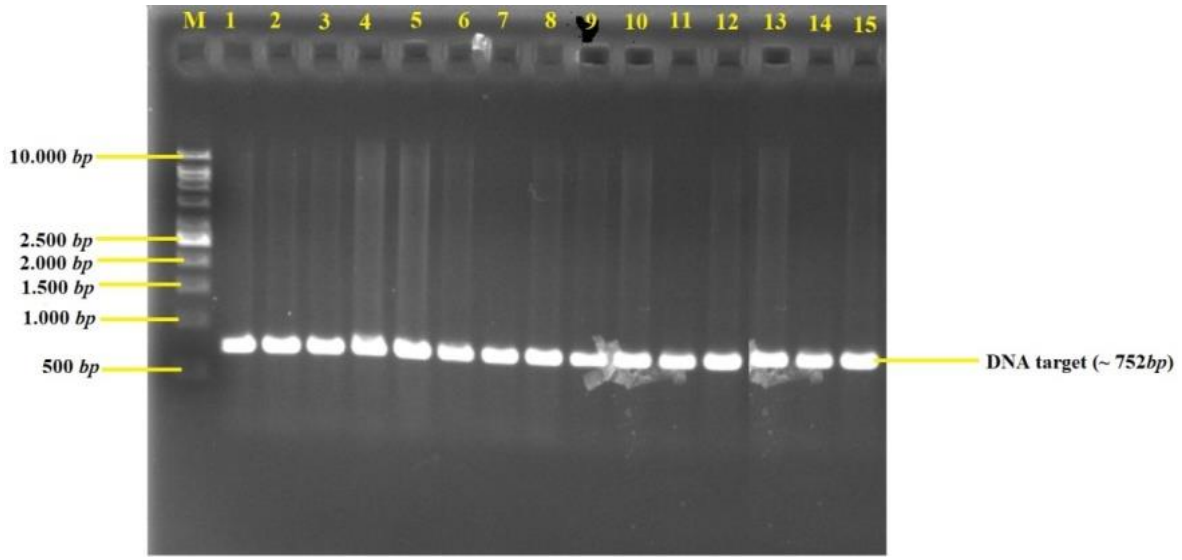

Figure 1. Mitochondrial DNA COI gene band of Bengkulu's Burgo chicken. Notes: M = ladder DNA, 1-15 (Burgo chicken individual) with BRCOI code

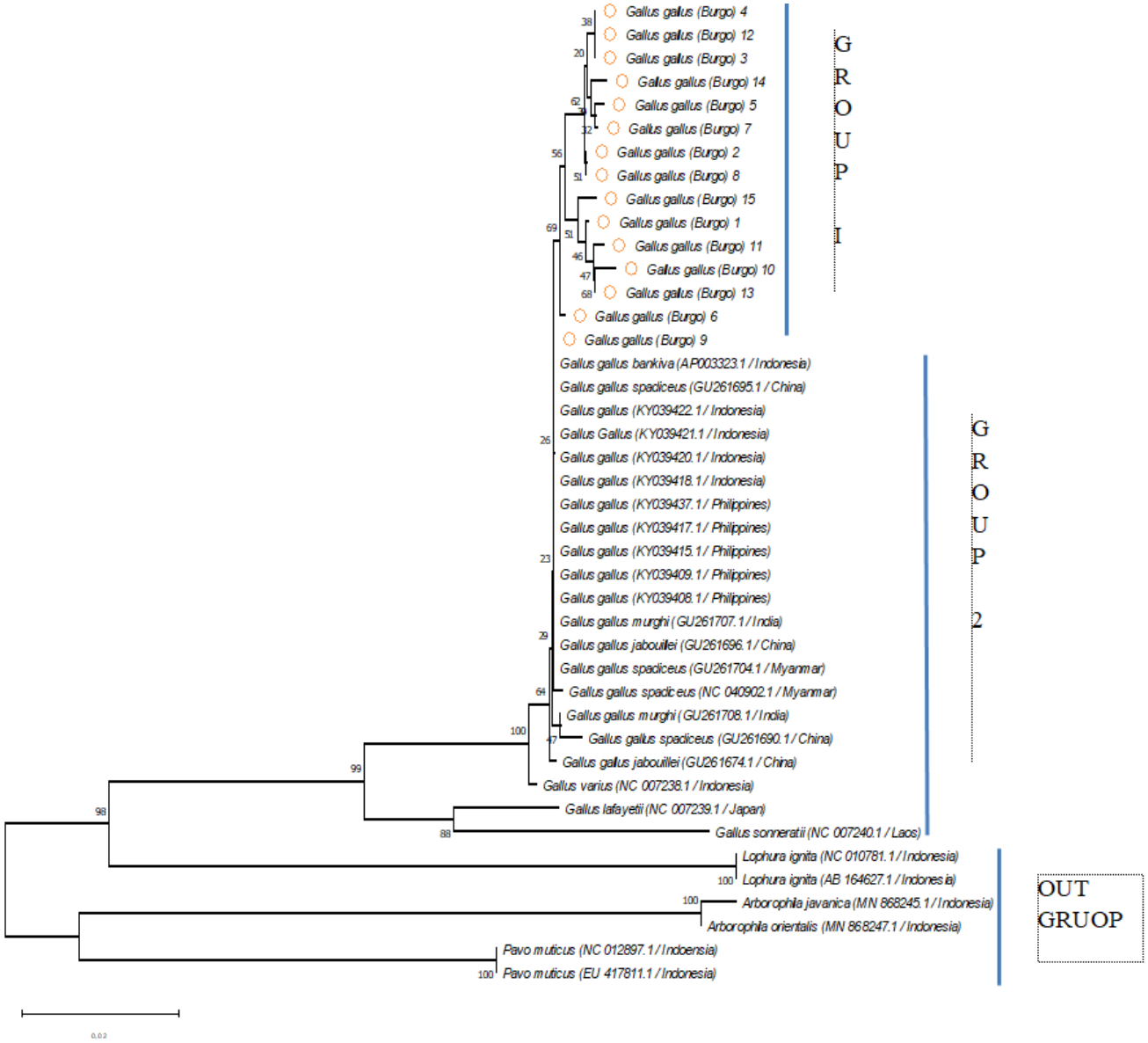

Figure 2. Phylogeny construction using Neighbor Joining (NJ) modeling of 15 individuals of Bengkulu's Burgo chicken using the K2P model and 1000 times bootstrap based on the COI gene (752 bp)

Discussion

The respective sequencing results obtained band lengths of 757 and 769 bp for forward and reverse 745 and 765 bp in the COI gene. The length of the nucleotide sequence obtained is in line with previous researchers (Jarulis et al. 2022; Päckert 2022; Yohanna et al. 2022). Table 2 shows the details of SNPs for each individual studied as follows: individual 1 has 3 specific sites of 27, 29 and 31. Individuals 2 and 8 have the same 3 specific sites, namely 26, 28 and 29. In individual 3, individual 4 and individual 12 both have 4 spe sites of 26, 28, 29 and 745. In individual 5, there are 5 specific sites of 26, 28, 29, 30 and 745. In individual 6, only 1 specific site was found, namely 745. In individual 7, there were 4 specific sites of 26, 29, 30 and 745. In individual 9, no specific site was found. In individual 10, there were 6 specific sites found 27, 29, 31, 32, 228 and 745. In individual 11, there were 5 specific sites of 27, 29, 31, 43 and 745. In individual 13, there were 4 specific sites at sites 27, 29, 43, and 745. This shows that individual Burgo chickens also have base nucleotide differences at several sites, indicating the degree of sequence diversity.

Single nucleotide polymorphism (SNP) sites are inseparable from the process of transition and transversion mutations, where transition mutations are mutations that occur due to substitutions between purine bases (adenine and guanine) or between pyrimidine bases (thymine and cytosine) and transversion mutations are substitutions, between purine and pyrimidine bases and vice versa. In Table 2, there are 6 transition mutations at sites 27, 28, 30, 31, 43 and 228 and 3 transversion mutations at sites 26, 32 and 745, while at site 29 there are 2 mutations, namely the transition from base A>G and transversion from base A>T.

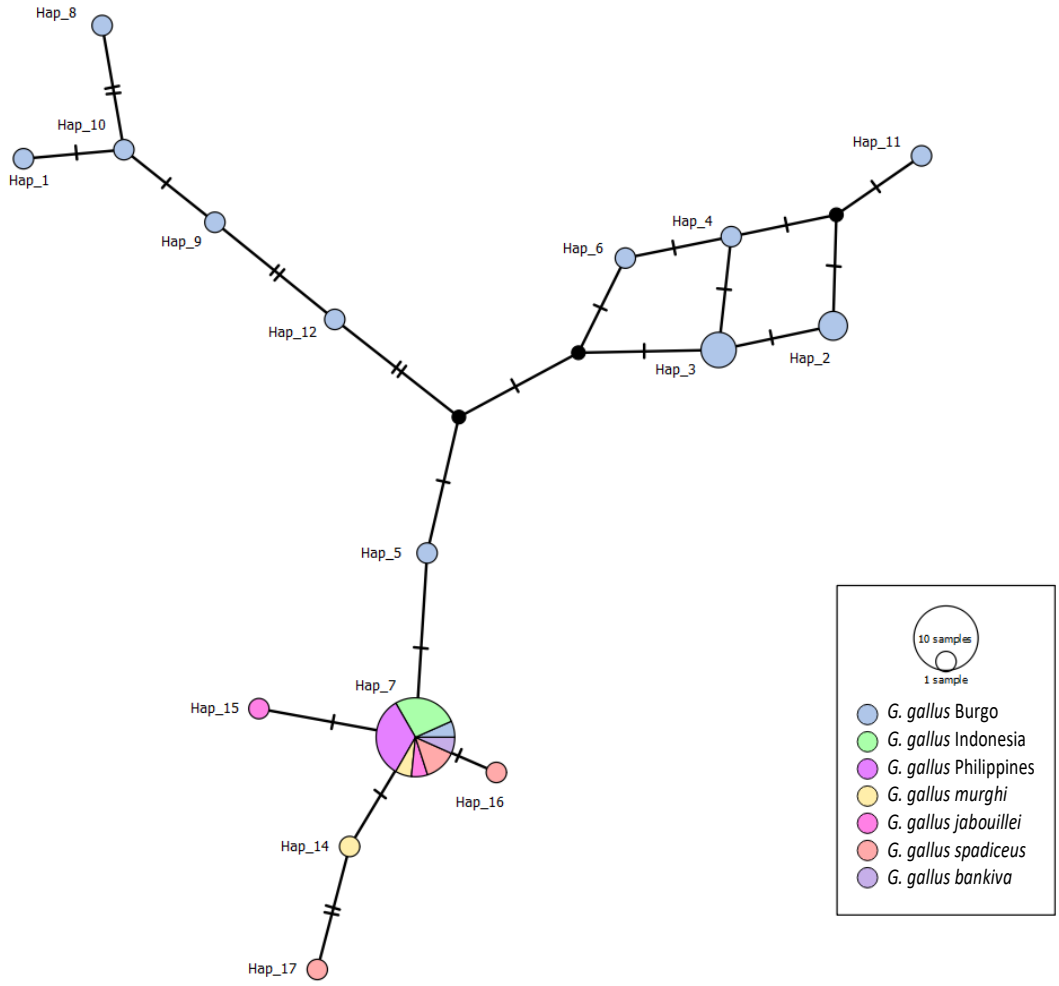

Figure 3. Population haplotype network of *Gallus gallus* spp. based on the COI gene of Mitochondrial DNA

Table 2. Genetic distance between species in Burgo chickens based on the COI gene (752 bp)

Genetic distance	Maximum	Minimum	Average
Intraspecies *Gallus gallus* (Burgo)	1.3%	0.0%	0.6%
Interspecies *G. gallus* (Burgo) with *Gallus* spp.	7.5%	0.0%	1.2%
Intergenus *Gallus* sp with outgroup	17.1%	12.7%	14.5%

According to Warmadewi et al. (2020), mutations can increase adaptability compared to the original traits so that they can eliminate the original traits and not new mutations where individuals or populations with these traits will experience decline and eventually extinction. In the COI gene sequence of shorebirds (Aves, Charadriiformes: *Charadrius*), there are 28 variable sites, 6 of which are highly informative sites (Päckert et al. 2022). Jarulis et al. (2022) reported that DNA barcoding research on *G. gallus* (Aves: Phasianidae) from 20 individuals identified 6 SNP sites (730 bp) and AT and GC compositions of 51.7% and 48.3%, respectively.

Currently, technology in the field of molecular biology continues to experience new developments and breakthroughs in efforts to identify species using genetic markers. Taylor and Harrist (2012) stated that DNA Barcoding can be used for taxonomic identification, species determination and grouping. The sequence length obtained is still within the range of sequence lengths commonly used in DNA barcoding analysis (Gonçalves et al. 2015; Zein 2018). Based on this statement, it can be concluded that the use of DNA barcodes can indirectly facilitate the recognition of certain species because each species or individual has a unique sequence. Table 3 shows 22 sites showing population-specific barcodes of the *Gallus* spp. genus only found in Indonesia (green), which can be a distinguishing characteristic between Indonesian *Gallus* spp. and *Gallus* spp. in the world.

Table 3. DNA barcode of the species *G. gallus* (Burgo) and the genus *Gallus* spp. based on the mitochondrial DNA COI gene

Individual	Sequnce number																									
	3	**22**	**62**	**74**	**179**	**182**	**203**	**212**	**218**	**308**	**434**	**449**	**455**	**458**	**459**	**551**	**557**	**563**	**572**	**575**	**584**	**587**	**602**	**647**	**746**	**750**
G. sonnerati (NC007240)	**C**	**C**	**C**	**T**	**C**	**C**	**T**	**A**	**C**	**C**	**A**	**A**	**T**	**G**	**T**	**C**	**T**	**C**	**T**	**T**	**C**	**T**	**T**	**G**	**-**	**C**
G. lafayetii (NC007239)	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	**.**	-	**.**
G. varius (NC007238)	.	.	.	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	-	T
G. gallus (KY039421)	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	-	T
G. gallus (Burgo) 1	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 2	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 3	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 4	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 5	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 6	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 7	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 8	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 9	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 10	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 11	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 12	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 13	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 14	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T
G. gallus (Burgo) 15	T	T	T	C	A	T	C	G	T	T	C	G	C	A	C	T	C	T	C	C	T	C	C	A	A	T

Notes: Blue = Species barcode; Black = Genus barcode; Green = Genus barcode in *Gallus spp.* populations in Indonesia. Number 1 nucleotide sequence in Burgo chicken same with number 166 complete sequence of the COI gene *Gallus gallus* from Genbank (KY039421)

Genetic distance can describe the degree of similarity between individuals or populations of a species based on nucleotide differences (Abinawanto et al. 2022). However, in this study, the genetic distance results were divided into 13 groups, which were expected to obtain more specific results regarding the genetic distance of Burgo chickens with comparative genetic data. The 13 genetic distance groups are as follows, genetic distance between individuals (interspecies), genetic distance between Burgo chickens and Indonesian *G. gallus*, genetic distance between Burgo chickens and Philippines *G. gallus*, genetic distance between Burgo chickens and the subspecies *G. gallus murghi*, genetic distance between Burgo chickens and *Gallus gallus* subsp. *jabouillei* Delacour & Kinnear 1928, genetic distance between Burgo chickens and *Gallus gallus* subsp. *spadiceus* Bonnaterre 1792, genetic distance between Burgo chickens and *Gallus gallus* subsp. *bankiva* Temminck 1813, genetic distance between Burgo chickens and *G. varius*, genetic distance between Burgo chickens and *G. lafayetii*, genetic distance between Burgo chicken and *G. sonneratii*, genetic distance between Burgo chicken and *Pavo muticus* Linnaeus 1766 (outgroup).

In Table 3, genetic distances using the 752 bp COI gene obtained intraspecies distance of *G. gallus* (Burgo chicken) ranges from 0.0-1.3%, with an average genetic distance of 0.5%. In the genetic distance between Burgo chickens and *G. gallus* from Indonesia and the Philippines and the subspecies *G. gallus bankiva*, genetic distance values were obtained at 0.0-0.8% and an average genetic distance of 0.5%. Meanwhile, the genetic distance between Burgo chickens and the subspecies *G. gallus murghi* and *G. gallus jabouillei* has a genetic distance range of 0.9% and an average genetic distance of 0.6%. The genetic distance between Burgo chickens and the subspecies *G. gallus spadiceus* ranges from 0.0-1.1%, with an average genetic distance of 0.6%. The genetic distance between Burgo chickens between the spec and species *G. varius*, *G. lafayetii* and *G. sonneratii* obtained quite high genetic distance of 0.4-7.6%. While the outgroup genetic distance between the outgroup (Phasianidae family) and Burgo chickens obtained a high genetic distance of 12.7-17%. Jarulis et al. (2022) reported that the genetic distance between the red junglefowl of Bengkulu and South Sumatra ranges from 0.005-0.014. In the Labuhan Batu Village, the chicken population ranged from 0.048 to 2.736 (Rangkuti et al. 2014).

In Figure 2, all samples, namely *G. gallus* (Burgo), *G. gallus* Indonesia, *G. gallus* Philippines, *G. gallus murghi*, *G. gallus jabouillei*, *G. gallus spadiceus* and *G. gallus bankiva*, are indicated by the number of colors found on the haplotype 7 circle. Haplotype 7 (COI gene) is the genetic source of broodstock that becomes the source of other species of chickens through a gene flow process, which then undergoes an adaptation process based on habitat, thereby changing the nucleotide structure and triggering phenotypic changes. The analysis results showed that the Burgo chicken group marked with a circle is separated from other *Gallus* spp. Group 1 consists of 15 individual samples of Burgo chicken from Bengkulu, group 2 consists of 4 genetic databases of *G. gallus* from Indonesia, 5 genetic databases of *G. gallus* from the Philippines, 2 genetic databases of *G. gallus*. *G. gallus murghi* from India, 4 genetic databases of the subspecies *G. gallus spadiceus* from China and Myanmar, 2 genetic databases of the subspecies *G. gallus jabouillei* from China, 1 genetic database of the subspecies *G. gallus bankiva* from Indonesia, 1 genetic database of *G. varius* from Indonesia, 1 genetic database of *G. lafayetii* from Japan and 1 genetic database of *G. sonneratii* from Laos, while genetic databases of *Lophura ignita* Shaw 1798, *Arborophila javanica* J.F.Gmelin 1789, *Arborophila orientalis* Horsfield 1821 and *P. muticus* are included in the outgroup. All genetic databases on comparison individuals for groups 2 and outgroup come from Genbank. The use of outgroup groups in the phylogenetic tree reconstruction process is necessary because it can determine character or trait polarization (Hidayat 2016).

In Figure 2, using the COI gene, the bootstrap value between Burgo chickens was 69%. The bootstrap value is <70%, so the chance of changes in group composition is high so that when analysis is carried out, the branches and trees formed can still change and vice versa (Wirdateti and Semiadi 2017). Although the bootstrap value results present a value below 70%, the Burgo chicken species is separate from the *Gallus* spp. species, where in the results of the phylogeny tree Burgo chickens are grouped in group 1, *Gallus* spp. in group 2 and species from the genus Phasianidae are in the outgroup. The formation of a phylogenetic tree cannot be separated from the genetic distance values, both intraspecies and interspecies, so that the genetic distance values can be arranged to form a phylogenetic tree. In summary, Burgo chickens are closely related to other red junglefowl from the genetic distance. It was found to be 1.2% and 1 site, 746 sites were found to be Burgo chicken with a certain characteristic that makes them distinguish other breeds of chicken.

ACKNOWLEDGEMENTS

Universitas Bengkulu, Indonesia for research funding assistance through the flagship research scheme in 2021 with contract number No.: 1773/UN30.15/PG/2021. Thanks are also addressed to the Bengkulu Burgo chicken community for easy sampling and all parties who have helped with the research.

REFERENCES

Abinawanto A, Zulistiana T, Lestari R., Dwiranti A, Bowolaksono A. 2022. The genetic diversity of ayam ketawa (*Gallus gallus domesticus*, Linneaus, 1758) in Bangkalan District, Madura Island, Indonesia. Biodiversitas 22 (6): 3145-3155. DOI: 10.13057/biodiv/d220617.

Bajpai N, Tewari RR. 2010. Mitochondrial DNA sequence-based phylogenetic relationship among flesh flies of the genus *Sarcophaga* (Sarcophagidae: *Diptera*). J Genet 8 9 (1): 51-54. DOI: 10.1007/S12041-010-0010-5.

Barbato M, Hailer F, Orozco-terWengel P, Kijas J, Mereu P, Cabras P, Mazza R, Pirastru M, Bruford MW. 2017. Genomic signatures of adaptive intogression from european mouflon into domestic sheep. Sci Rep 7 (1): 1-3. DOI: 10.1038/s41598-017-07382-7.

Cock MJW, Beseh PK, Buddie AG, Cafa G, Crozier J. 2017. Molecular Methods to Detect *Spodoptera frugiperda* in Ghana, and implications for monitoring the spread of invasive species in developing countries. Sci Rep 7: 4103. DOI: 10.1038/s41598-017-04238-y.

Gering E, Johnsson M, Willis P, Getty T, Wright D. 2015. Mixed ancestry and admixture in kauai's feral chickens: Invasion of domestic genes into ancient red juglefowl reservoirs. Mol Ecol 24 (9): 2112-2124. DOI: 10.1111/mec.13096.

Gonçalves PFM, Marques ARO, Matsumoto TE, Miyaki CY. 2015. DNA barcoding identifies illegal parrot trade. J Hered 106: 560-564. DOI: 10.1093/jhered/esv035.

Hall T. 2011. BioEdit: An important software for molecular biology. GERF Bull Biosci 2 (1): 60-61.

Hidayat, Saleh E, Aulawi DANT. 2016. Identifikasi keragaman gen bmpr-1b (Bone Morphogenetic Protein Receptor Ib) pada ayam arab, ayam kampung dan ayam ras petelur menggunakan PCR-RFLP. Jurnal Peternakan 13 (1): 1-11. DOI: 10.24024/jupet.v12i1.2383. [Indonesian]

Jarulis J, Nurmeiliasari N, Haryanto H, Vilanda I. 2022. DNA barcode of red junglefowl *Gallus gallus* L, 1958 (Aves: Phasianidae) of sumatra based on Mitochondrial COI DNA Gene. Biosaintifika: J Biol Biol Edu 14 (2): 200-210. DOI: 10.15294/biosaintifika.V14i2.36530.

Kononov A, Ustyantsev K, Wang B, Mastro VC, Fet V, Blinov A, Baranchikov Y. 2016. Genetic diversity among einght *Dendrolimus* species in Eurasia (Lepidoptera: Lasiocampidae) inferred from Mitochondrial COI and COII, and nuclear ITS2 markers. BMC Genet 17: 157. DOI: 10.1186/s12863-016-0463-5.

Kumar S, Stecher G, Li M, Knyaz C, Tamura K. 2018. MEGA X: Molecular Evolutionary Genetic Analysis across computing platforms. Mol Biol Evol 35: 1547-1549. DOI: 10.1093/molbev/msy096.

Madduppa H, Taurusman AA, Subhan B, Anggraini NP, Fadillah R, Tarman K. 2017. Short Communication: DNA barcoding reveals vulnerable and not evaluated species of sea cucumbers (Holothuroidea And Stichopodidae) from Kepulauan Seribu Reefs, Indonesia. Biodiversitas 18 (3): 893-898. DOI: 10.13057/biodiv/d180305.

Ni'mah A, Kartikasari Y, Pratama AD, Kartikasari LR, Hertanto BS, Cahyadi M. 2016. Detection of pork contaminantion in fresh and cooked beef using geetic marker Mitochondrial-DNA Cytochrome b by duplex-PCR. J Indon Trop Anim Agric 4 (1): 88-100. DOI: 10.14710/jitaa.41.1.7-12.

Otim MH, Tay WT, Kanyesigye D, Adumo S, Abongosi J, Ochen S, Sseumaga J, Alibu S, Abalo G, Agona A. 2018. Detection of sister-species in invasive populations of the fall armyworm *Spodoptera frugiperda* (Lepidoptera: Noctuidae) from Uganda. PLOS ONE 13 (4): e0194571. DOI: 10.1371/journal.pone.0194571.

Päckert M. 2022. Free Access of Published DNA sequences facilitates regular control of (Meta-) data quality - an example from shorebird mitogenomes (Aves, Charadriiformes: Charadrius). Ibis: Intl J Avian Sci 164 (1): 336-342. DOI: 10.1111/ibi.13005.

Putranto HD, Hasibuan GP, Yumiati Y, Setianto J, Brata B, Kurniati N, Hakiki FF. 2017. The estimation of dynamical distribution of domesticated Burgo chicken population in Bengkulu Coastal Area, Indonesia. Biodiversitas 18 (2): 458-464. DOI: 10.13057/biodiv/d180203.

Putranto HD, Setianto J, Santoso U, Warnoto W, Nurmeliasari N, Zueni A. 2012. Estradiol-17î^2 Hormone concentration and follicles number in exotic Burgo chicken supplemented by *Sauropus androgynus* leaves extract. Biodiversitas 13 (1): 1-6. DOI: 10.13057/biodiv/d130101.

Rangkuti NA, Hamdan, Daulay AH. 2014. Identification of morphometrics and genetic distance of Kampung chickens in South Labuhanbatu. J Integrated Anim Husb 3 (1): 96-119. DOI: 10.32734/jpi.v3i1.2748.

Setianto J, Prakoso H, Sutrioyono S. 2013. Domestication of red jungle fowl: A case study of red jugle fowl poaching by communities in North Bengkulu. Pros Sem Nas Masy Biodiv Indon. 1 (2): 207-212. DOI: 10.13057/psnmbi/m010207.

Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. Mega6: Molecular Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol 30 (12): 2725-2729. DOI: 10.1093/molbev/mst197.

Taylor HR, Harris WE. 2012. An emergent science on the brink of irrelevance: A review of the past 8 years of DNA barcoding. Mol Ecol Resour 12 (3): 377-388. DOI: 10.1111/j.1755-0998.2012.03119.x.

Wang MS, Thakur M, Peng MS, Jiang Y, Frantz LA, Li M, et al. 2020. 863 Genomes reveal the origin and domestication of chicken. Cell Res 30 (8): 693-701. DOI: 10.1038/s41422-020-0349-y.

Warmadewi DA, Ardika I, Putra IA, Bidura IGN. 2020. The variation of phenotypics Bali cattle in Bali Province, Indonesia. Intl J Fauna Biol Stud 7 (4): 44-47. DOI: 10.22271/23940522.

Wirdateti, Semiadi G. 2017. Variasi genetik trenggiling sitaan di Sumatra, Jawa, dan Kalimantan berdasarkan control region DNA mitokondria. Jurnal Veteriner 18 (36): 181-191. DOI: 10.19087/Jveteriner.2017.18.2.181. [Indonesian]

Wu MY, Low GW, Forcina G, Van Grouw H, Lee BP, Oh RR, Rheindt FE. 2020. Historic and modern genomes unveil a domestic introgression gradient in a wild red junglefowl population. Evol Appl 13 (9): 2300-2315. DOI: 10.1111/eva.13023.

Yohanna, Irham M, Gunawan I, Saryono A, Tawakal Y, Prawiradilaga DM, Dharmayanthi AB. 2022. DNA Barcoding reveals the Identity of Bird remains from the Bird Strike Incident in Indonesia. Zoo Indonesia 31 (2): 92-100. DOI: 10.52508/zi.v31i2.4471.

Zahradden D, Butswar ISR, Kalla DJU, Sir SM, Bukar MT. 2005. Effect of frequency of ejaculation on semen characteristics in two breeds of turkeys (*Meleagris gallopavo*) raised in a tropical environment. Intl J Poul Sci 4: 217-221. DOI: 10.3923/ijps.2005.217.221.

Zein MSA. 2018. Barkoding DNA burung elang (Famili Accipitridae) di Indonesia. Jurnal Ilmu-Ilmu Hayati 17 (2): 165-173. DOI: 10.14203/beritabiologi.v17i2.3108. [Indonesian]