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GENETIC DIVERSITY OF MITOCHONDRIAL DNA D-LOOP SEQUENCE IN BANG TROI CHICKEN BREED

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ABSTRACT

Bang Troi chicken (GBT) is a local chicken that is free-gardening raised in Quang Ninh province. A 1,268 bp of mitochondrial DNA (mtDNA) D-loop of 17 Bang Troi chicken individuals was sequenced and analyzed. Comparative multiple sequence alignment revealed 17 base substitutions within 17 Bang Troi chicken individuals. Bang Troi chicken had a relatively high level of genetic diversity, expressed in the number of 13 haplotypes, haplotype diversity of 0.949. The Tajima's test indicated that the analysed population in balancing selection with a D-value of 2.39. A phylogenetic tree based on 455 bp of hypervariable mtD-loop sequence classified 17 Bang Troi chicken individuals into three clades A, B, and E, which are common groups of Vietnamese and Asia indigenous chickens. This result provides the first genetic information and maternal lineages of Bang Troi chickens and it can support for registration of local chicken breeds.

Keywords: Bang Troi chicken breed, mtDNA D-loop sequence, genetic diversity, haplotype, phylogeny.

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INTRODUCTION

Vietnam is one of the agricultural countries with developed poultry farming, especially for the high number of indigenous chicken breeds. Indigenous chicken breeds are increasingly interested not only because of their good adaptability to weather and nutritional conditions, but also have good egg quality and delicious meat favour that are preferred by the local people. Furthermore, they are valuable genetic resources for their better conservation and for crossbreeding with high-yielding chickens to improve the performance of local breeds.

Analysis of genetic diversity using different types of molecular markers have been applied for the assessment of indigenous livestock genetic resources. Several studies have used mitochondrial sequence in analysing the evolutionary origin, genetic variation of chicken breeds. Liu et al. (2006) presented an overview of the genetic distribution of domestic chicken breeds in the world with nine branches (named A->I), in which, there are many Asian chicken breeds. The research results of Oka et al. (2007) also showed seven genetic clades of the Japanese chicken breed (A–G), of which four clades coincided with the study of Liu et al. (2006). Several Vietnamese indigenous chickens have been evaluated for genetic diversity using the mtDNA D-loop sequence (Cuc et al., 2011; Nguyen et al., 2022).

Bang Troi chicken is an indigenous backyard chicken breed raised by local people for a long time, originating in Bang village of Thong Nhat commune and Troi village of Le Loi commune, Hoanh Bo district, Quang Ninh province. Currently, Bang Troi chicken has been interbred with others and a high rate of inbreeding, it leads to breed degeneration and low productivity (Phan Thanh Lam et al., 2019). For that reason, Bang Troi chicken has been included for research and conservation of indigenous chickens by the National Livestock Genetic Conservation Program. This study provides information on the diversity of endemic genetic resources, supporting the task of diversity conservation and development of indigenous genetic resources.

MATERIALS AND METHODS

Materials

Bang Troi chickens were selected based on typical morphological characteristics of the breed from ten communes of Hoang Bo district, Quang Ninh province. A total of 17 Bang Troi chicken individuals were randomly sampled from different farms to avoid inbreeding. About 3 mL of blood from individuals were taken from the wing vein and collected in anti-coagulant tubes with EDTA and stored at 4 ^{o}C.

mtDNA D-loop sequences of Cay Cum chicken (GCC), Lien Minh chicken (GLM), Nhan chicken (GN), Chin Cua chicken (G9C) and representative samples of clades A, B, C, D, E, F, G, H and I were used for phylogenetic analysis.

Methods

Genomic DNA was extracted by a standard procedure using Proteinase K digestion followed by phenol-chloroform extraction and precipitation with ethanol (Ausubel et al., 1995). The quantity and quality of genomic DNA were checked with a UV spectrophotometer and agarose gel electrophoresis.

The primer pair F: 5'-AGGACTACGGCTTGAAAAGC-3' and R: 5'- CATCTTGGCATCTTCAGTGCC-3' (Eriksson et al., 2008) was used for specific amplification of the mitochondrial D-loop region. PCR was performed using 2x DreamTaq master mix with 10 nM of each primer and 100 ng genomic DNA. A thermal cycle was set as follows: initial denaturation at 95 ^{o}C for three minutes followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 60 ^{o}C for 30 seconds, extension at 72 ^{o}C for 30 seconds, and an additional extension of 72 ^{o}C for 10 minutes. A PCR reaction was carried out on the Veriti™ 96-Well Thermal Cycler (Applied Biosystems). The expected length of the PCR product was about 1,300 bp.

The PCR product was two-way directly sequenced using the Sanger method by ABI-3100 Avant Genetic Analyzer (Macrogen, Korea).

Obtained nucleotide sequences were identified with the BLAST Tool on NCBI (Alschul et al., 1990). Multiple nucleotide alignments were carried out with BioEdit (Hall, 1999).

The diversity parameters, including the nucleotide and haplotype diversity were estimated using DnaSP v.6 software (Rozas et al., 2017). Neighbor Joining (NJ) phylogenetic tree was conducted using MEGA version 7.1 (Tamura et al., 2007). Phylogenetic tree analysis was constructed based on the classification of Liu et al., (2006) with representative reference mtDNA D-loop sequences from the GenBank.

RESULTS AND DISCUSSION

Analysis of mtDNA D-loop sequence

About 1.3 kb mtDNA D-loop sequence was successfully amplified by PCR using specific primer pairs from 17 Bang Troi chicken individuals. Figure 1 shows the PCR product as a clear band with the estimated molecular size of 1.3 kb corresponding to theoretical calculation.

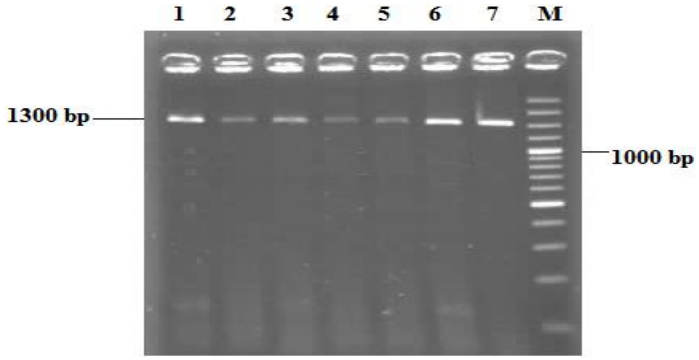

Figure 1. PCR product of mtDNA D-loop region of Bang Troi chicken. M: 1 kb DNA ladder (Thermo); 1–7: PCR product of mtD-loop sequence of Bang Troi chickens (≈1.3 kb)

Direct sequencing results of the entire mtD-loop region of 17 Bang Troi chicken individuals showed a sequence of 1,268 bp. These DNA sequences were compared with each other and with other domestic chicken mtDNA D-loop sequences available on GenBank. Comparative sequence alignment results revealed 17 base substitutions within individuals. Using the BLAST tool, all sequences shared high similarity (moore than 99%) with the mtDNA D-loop reference sequences of domestic chicken *Galus galus* belonging to branches A, B and E (according to the classification of Liu et al., 2006).

Haplotype analysis of Bang Troi chickens

DnaSP software was used to analyze the polymorphisms in the mtDNA D-loop region of the Bang Troi chicken breed. The following parameters were included: number of polymorphic sites (S); the number of haplotypes (number of haplotype - h); haplotype diversity (haplotype diversity - Hd); nucleotide diversity (π), average number of nucleotide differences (k); Tajima's test - D). The results were presented in Table 1.

17 Bang Troi chicken individuals used in this study were assigned to the 13 haplotypes and their distribution was shown in Table 2.

All diversity parameters listed in Table 1 revealed the richness in the genetic diversity of Bang Troi chickens. As shown in Table 1, the Bang Troi chickens (n = 17) have a higher level of haplotype diversity (Hd = 0.949) than all three Vietnamese indigenous chicken breeds used in the study of Nguyen et al. (2022). The study of Cuc et al. (2011) used a

455 bp sequence of the mtD-loop region of Vietnamese indigenous chicken breeds showed that the number of haplotype (h) observed ranged from 8 to 24, haplotype diversity (Hd) was in the range of 0.615–0.942 with the lowest Hd was the Ho chicken breed and the highest value was the Tau Vang chicken breed.

Table 1. Haplotype diversity of Bang Troi chicken and other reference breeds

Breed	N	S	h	Hd	π	k	D	Reference
Liên Minh	24	23	12	0.913	0.0062	6.47	0.187	Nguyen et al., 2022
Dong Tao	19	11	7	0.854	0.0038	4.67	1.736	
Nhan	18	4	5	0.824	0.0011	1.22	0.153	
Bang Troi	17	17	13	0.949	0.0064	8.12	2.39	This study

Note: N: Number of sample.

Table 2. Haplotype distribution of Bang Troi chickens

No.	Haplotype	Number of individual	Sample	No.	Haplotype	Number of individual	Sample
1	Hap_1	1	[GBT1]	8	Hap_8	1	[GBT15]
2	Hap_2	1	[GBT2]	9	Hap_9	1	[GBT16]
3	Hap_3	1	[GBT3]	10	Hap_10	2	[GBT18 GBT19]
4	Hap_4	4	[GBT7 GBT10 GBT21 GBT22]	11	Hap_11	1	[GBT23]
5	Hap_5	1	[GBT8]	12	Hap_12	1	[GBT4a]
6	Hap_6	1	[GBT9]	13	Hap_13	1	[GBT6a]
7	Hap_7	1	[GBT12]				

The Tajima test is used to evaluate the state of the population (Tajima, 1989). There are three cases: i) D-value can be 0, which means that the observed polymorphism is similar to the expected polymorphism, without the influence of selection; ii) D-value < 0: rare alleles appear with high frequency, which means the influence of selection is in the direction of breed segregation; iii) D-value > 0: rare alleles appear at low frequency - the effect of selection remains balanced. In this study, Tajimma's test showed that the D-value was 2.39, which means rare alleles appear at low frequency and the analysed population is in balancing selection.

Phylogenetic tree of Bang Troi chickens

455 bp of the hypervariable region of mtDNA D-loop sequence of Bang Troi chickens, other Vietnamese native chickens, and *Galus galus* domestic chickens has been used to construct the phylogenetic tree using MEGA 6.0 software. Liu et al., (2006)'s classification was applied. The results were presented in Figure 2.

The phylogentic tree showed the very clear result that 17 Bang Troi chicken individuals were classified into three clades A, B and E. The Vietnamese indigenous chicken breeds were distributed in five clades A, B, C, D and E (Cuc et al., 2011; Nguyen et al., 2022). In addition, a small number of Vietnamese native chickens were also classified into less common groups such as F, G and I (Cuc et al., 2011). The above three branches (A, B and E) are also the most common branches of indigenous chicken breeds in Asian countries such as China, Laos, Thailand, Tibet, and Indonesia (Liu et al., 2006; Oka et al., 2007; Wattanachant et al., 2004). Similar to most other Vietnamese indigenous chicken

breeds, the Bang Troi chickens used in this study had multiple maternal origins (branches A, B and E). This is the first genetic information and maternal lineages of Bang Troi chicken and it can support for registration of local chicken breeds.

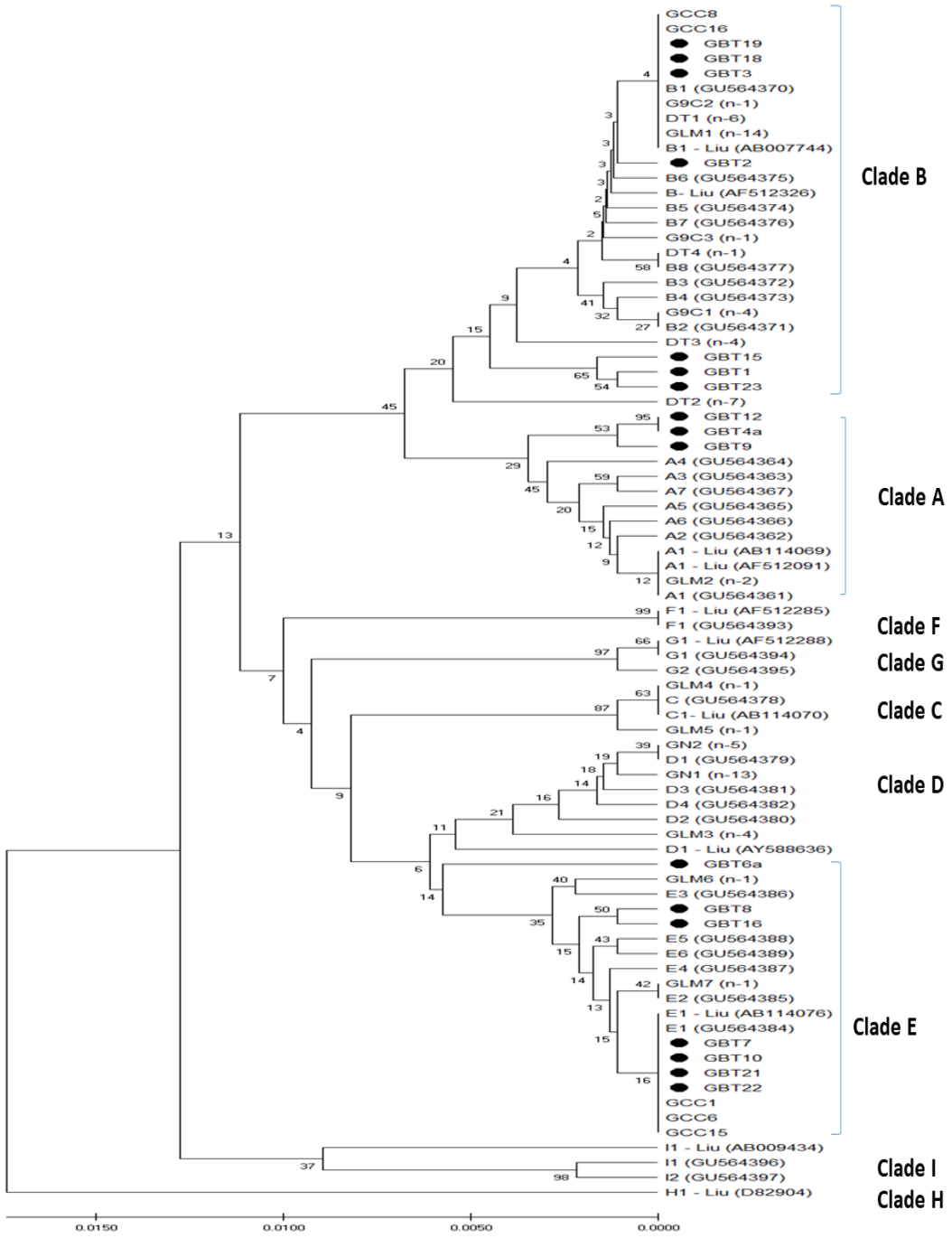

Figure 2. Phylogenetic tree constructed by MEGA X based on mtD-loop nucleotide sequence (455 bp) with bootstrap value of 1.000x. The black dots indicate the D-loop sequence of Bang Troi chicken individuals

CONCLUSION

The nucleotide sequence of the mtD-loop region of Bang Troi chicken with the molecular size of 1,268 bp has been analysed. A total of 13 haplotypes were observed in 17 Bang Troi chicken individuals and all these haplotypes belong to the three most common clades (A, B and E) of Vietnamese and Asian native chickens.

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