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Genetic diversity and maternal origin of Vietnamese indigenous chicken breeds inferred from complete sequences of mitochondrial DNA D-loop region

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Abstract: Indigenous chickens play a significant role in food security, income growth and socio-cultural life of rural households in Vietnam. This study was conducted to assess the genetic diversity as well as the phylogenetic relationships of Vietnamese indigenous chickens (*Gallus gallus*) to support the conservation of their genetic resources for sustainable rural farming. In this study, the genetic diversity and the phylogenetic relationships of 10 Vietnamese indigenous chicken breeds were analyzed using complete sequences of mitochondrial DNA (mtDNA) displacement-loop (D-loop) region. The average nucleotide and haplotype diversities of Vietnamese chickens were 0.006 3 ± 0.000 22 and 0.918 ± 0.010, respectively. A total of 39 polymorphic sites and 29 haplotypes were identified. The maximum likelihood tree classified these haplotypes into seven haplogroups (A, B, C, D, E, G and V), with haplogroups A and B being the two predominant maternal lineages of Vietnamese indigenous chickens, while haplogroups C, D, E, G and V were found in the remaining chickens. Several haplotypes from different haplogroups were shared among some chicken breeds. These results suggested that Vietnamese indigenous chicken breeds have multiple maternal origins, mainly from Chinese, Southeast Asian and Indian chickens, and that these breeds share common maternal lineages. The high level of genetic diversity in Vietnamese chickens demonstrates significance of conservation for future use.

Keywords: haplogroups; mtDNA; non-coding region; phylogenetic relationship

In underdeveloped and developing countries mostly in Asia and Africa, indigenous chicken breeds are important resources for rural households' living conditions and culture, providing a high-quality source of animal protein, cash income, and playing a significant role in the socio-cultural life of the rural community (Padhi 2016). South and Southeast Asian countries are endowed with a diversity of native chicken breeds (Bett et al. 2014). Vietnam is a country located in Southeast Asia that also owns a diversity of indigenous chicken breeds despite the variability in the reported number of breeds. According to the International Livestock Research Institute, Vietnam owns more than 18 indigenous chicken breeds (Birhanu et al. 2021). A study conducted by Moula et al. (2011)

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shows that there are 14 indigenous chicken breeds in Vietnam while Phuong et al. (2015) concluded that there are about 30 breeds. In Vietnam, indigenous chickens are predominantly used in backyard non-intensive productions practiced by most rural households, which have significant contributions to the livelihood of smallholder producers. However, producers get foundation and replacement flocks from local markets or their own hatched flock (Hanh et al. 2007), which could lead to breed degeneration. In addition, during the past few decades, some high-producing chicken breeds were imported such as Ross, Cobb, Leghorn, Brown Nick, ISA, Tam Hoang, Luong Phuong and so on. Some hybrid chickens were established from crossing between indigenous cocks (mainly Ho, Choi, Dong Tao and Mia) and exotic hens (Luong Phuong, Tam Hoang, Isa Brown, JA57, Redbro, Sasso, Kabir and others) (Dien et al. 2023), which resulted in the introgression of exotic breeds into the Vietnamese gene pool, decreasing the genetic diversity of indigenous chickens.

The Food and Agriculture Organization (FAO) warned of the erosion of farm animal genetic resources and confirmed that conservation and sustainable use of these resources are required to change trends in genetic erosion (Scherf 2000; Rischkowsky and Pilling 2007). It is suggested that data from molecular studies of livestock domestication can be used to assist in the conservation of diversity of farm animal genetic resources (Bruford et al. 2003).

Mitochondrial DNA (mtDNA) is considered an ideal molecular marker to track the ancestry of breeds back hundreds of generations (Harpending et al. 1998). By using partial mtDNA displacement-loop (D-loop) sequences, the genetic diversity and maternal origin have already been studied for the chicken population in Ha Giang Province (Berthouly-Salazar et al. 2010), H'mong, Mia, Ri, Ho, Dong Tao, Te, Choi, Ac, Tau Vang (Cuc et al. 2011), To, Sau Ngon, Mong Tien Phong (Do et al. 2019), Lien Minh, Dong Tao, Nhan and Chin Cua (Nguyen et al. 2022).

Nevertheless, the data regarding mtDNA variation of Vietnamese indigenous chickens are still insufficient to accurately elucidate maternal origin because several Vietnamese chicken genetic resources have not been characterized yet and the analysis based on partial D-loop sequences is believed to represent less genetic information than the complete mtDNA D-loop sequence. The complete mtDNA D-loop sequence has been successfully used in phylogenetic relationship studies (Teinlek et al. 2018). The aims of this study, therefore, are to assess the genetic diversity and matrilineal evolution of 10 Vietnamese local chicken breeds based on the complete sequence of mtDNA D-loop region. In comparison with partial sequences, the complete mtDNA D-loop sequences provide more in-depth investigation on the genetic diversity and maternal lineage phylogeny of Vietnamese indigenous chickens.

MATERIAL AND METHODS

Ethical statement

Sample collection was carried out according to the standard animal care in Vietnam as per guidelines from the Vietnam National Institute of Animal Sciences (01/2012). Because there are no specific rules regarding animal welfare in Vietnam, we followed the rules in accordance with Vietnamese Law on Animal Health (2015, https://vanban.chinhphu.vn/default.aspx?pageid=27160&docid=180584) and Vietnamese Law on Animal Husbandry (2018, https://vanban.chinhphu.vn/?pageid=27160&docid=206100). However, these laws do not clearly explain how to use animals in research. Hence, sample collection in this study was conducted according to the guidelines for using animals in research based on EU Directive 2010/63.

Sample collection and genomic DNA extraction

A total of 150 samples representing 10 breeds of Vietnamese indigenous chickens were collected for this study (Table 1; Figure 1). Samples were taken randomly from different populations and based on the genealogical information from farmers' knowledge to minimize genetically related chickens. Approximately 1 ml of blood was collected from the wing vein of the chickens and stored in vials containing 0.5 M EDTA as anticoagulant at a temperature of 4 °C until use. Genomic DNA was extracted from the blood by using GeneJET Genomic DNA Purification Kit (Thermo Scientific™, Thermo Fisher Scientific, Waltham, MA, USA).

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Table 1. List and location of 10 Vietnamese indigenous chicken breeds

Chicken breeds	Location	*n*
Cay Cum	Cao Bang	15
Te	Lang Son	15
Lun Cao Son	Quang Ninh	15
To	Thai Binh	15
Kien	Binh Dinh	15
Lac Son	Quang Binh	15
Ma Da	Dong Nai	15
Long Xu	Gia Lai	15
H'mong	Ha Giang	15
Ri Vang Rom	NIAS	15
Total	–	150

n = number of samples; NIAS = National Institute of Animal Sciences, Hanoi, Vietnam

mtDNA D-loop region amplification and sequencing

The complete sequence of mtDNA D-loop region (1 231–1 232 bp) was specifically amplified by the polymerase chain reaction (PCR) using two pairs of primers designed by Bao et al. (2008): primer pair 1: forward: 5'-CTC GCC CTA CTT GCC TTC C-3'; reverse: 5'-TGC CTG ATA CCT GCT CCT TT-3'; and primer pair 23: forward: 5'-GAT TAG ACG CCA CAG CTA AA-3'; reverse: 5'-TTC GTG AAA AGT GAG AAA GTT C-3'. Primer pair 1 amplified about 925-bp fragment of mitogenome, including about 657 bp of D-loop region, and Primer pair 23 amplified about 721-bp fragment of mitogenome, including about 574 bp of D-loop. The PCR reaction was performed in a 25 µl reaction volume including 12.5 µl buffer DreamTaq PCR Master Mix 2X (Thermo Scientific™, Thermo Fisher Scientific, Waltham, MA, USA), 0.5 µl each primer (10 pM), 1 µl (25–100 ng) DNA template, and 10.5 µl nuclease-free water. The thermal cycling conditions began with initial denaturation at 94 °C for 3 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 60 °C and 63 °C for 30 s (depending on each primer pair), and extension at 72 °C for 1 min; then final extension at 72 °C for 7 minutes. The PCR products were mixed with 6X loading dye before electrophoresis using 1.5% agarose gel staining with RedSafe™ Nucleic Acid Staining Solution (iNtRON Biotechnology, Seongnam, Korea) for 35 min at 100 V and visualised under UV light. Subsequently, the amplicons were purified using PureLink™ PCR Purification Kit (Invitrogen™, Thermo Fisher Scientific, Waltham, MA, USA) and sequenced in both directions using ABI 3130 Genetic analyser (Applied Biosystems, Foster, CA, USA) using the same PCR primers.

To get the complete sequences of mtDNA D-loop (1 231–1 232 bp), sequences of two fragments amplified with two pairs of primers were assembled using Unipro UGENE software v40.1 (Okonechnikov et al. 2012). The 150 assembled sequences were edited manually to obtain 150 complete mtDNA D-loop sequences in FASTA file format.

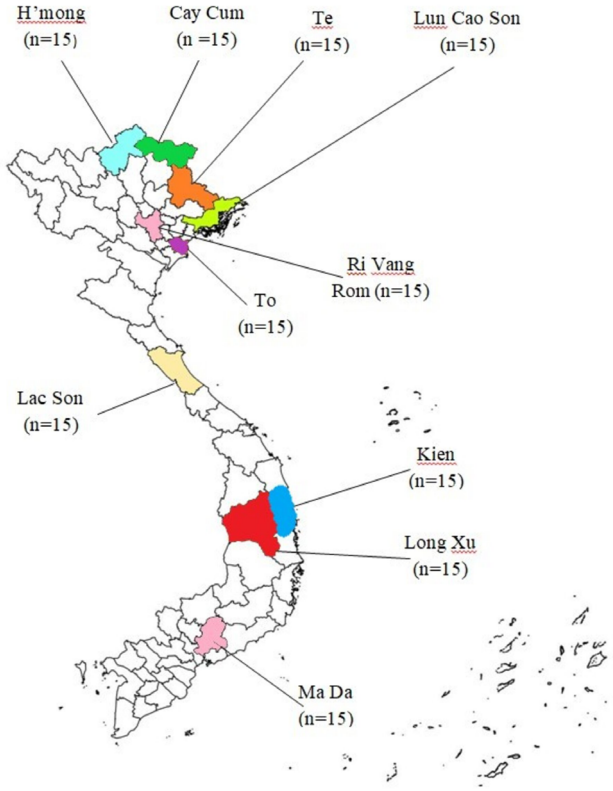

Figure 1. Sampling locations of 10 Vietnamese indigenous chicken breeds in this study

DNA sequence analysis

To investigate the genetic diversity of Vietnamese indigenous chickens, complete mtDNA D-loop sequences were aligned using MUSCLE program (Edgar 2004) in Molecular Evolutionary Genetic Analysis (MEGA) software vX (Kumar et al. 2018) and corresponding to a mtDNA reference sequence from the GenBank accession number NC_007235

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(Nishibori et al. 2005). MtDNA D-loop diversity indices: number of polymorphic sites (P), number of haplotypes (H), haplotype diversity (Hd), and nucleotide diversity (π) were estimated using DnaSP software v6.12.03 (Rozas et al. 2017).

A total of 65 mtDNA sequences representing 13 haplogroups, A to I and W to Z (Miao et al. 2013) and haplogroup V (Godinez et al. 2022), were used as references for the haplotype classification. An unrooted maximum likelihood tree was constructed based on complete mtDNA D-loop sequences of Vietnamese indigenous chickens and 65 reference mtDNA sequences by MEGA software vX (Kumar et al. 2018). The best-fit substitution model, HKY + G + I, was also determined by MEGA software vX (Kumar et al. 2018). Bootstrap values of the phylogenetic tree were estimated with 1 000 replicates.

RESULTS

MtDNA D-loop sequence variability and genetic diversity

The complete mtDNA D-loop sequences obtained from 150 samples of Vietnamese indigenous chickens were deposited at the GenBank with accession numbers OP846528-OP846559 and OP882147-OP882264. The 1 232 bp haplotype had 81 sequences. The 1 231 bp haplotype was detected in 69 sequences due to nucleotide C deletion at position 859 bp. This insertion/deletion polymorphism was excluded from the analysis. A total of 39 polymorphic nucleotide sites were identified including 14 singleton polymorphic sites and 25 parsimony-informative polymorphic sites as shown in Table 2. The majority of variable sites were transitions, while positions 31 and 361 were transversions. The polymorphic sites at nt positions 256 and 296 were also determined to be transversions. However, they were rare transversion mutations, so this result should be presented with caution and might require further validation. From site 1 to 166 bp, one mutation was determined at site 31 bp, while the 167 to 446 bp region was considered a hypervariable region with 33 mutations. The rest of the mtDNA D-loop sequence, 447 to 1 232 bp region, was relatively conserved with five variable sites. These 39 polymorphic sites generated 29 haplotypes from 150 samples. Haplotype information is presented in Table S1 in electronic supplementary material (ESM; for ESM see the electronic version).

The calculated genetic diversity indices of Vietnamese native chickens are presented in Table 3. The highest number of haplotypes was found in Ri Vang Rom (seven haplotypes) while the lowest number was observed in Ma Da (one haplotype). The haplotype diversity had a wide range from 0 ± 0.000 for Ma Da to 0.905 ± 0.041 for Ri Vang Rom. The Hd of Lun Cao Son and H'mong breeds was lower than 0.3 (0.257 ± 0.142 and 0.248 ± 0.131, respectively). The overall Hd for 10 breeds was high with 0.918 ± 0.010. The nucleotide diversity in 10 studied breeds ranged from 0 ± 0.000 00 (Ma Da) to 0.006 44 ± 0.000 68 (Ri Vang Rom). The average nucleotide diversity within 150 mtDNA D-loop sequences was 0.006 3 ± 0.000 22. These results suggest that Vietnamese native chickens had relatively high genetic diversity.

Phylogenetic analysis and haplotype distribution

The maximum likelihood (ML) tree (Figure 2) revealed that 29 haplotypes of Vietnamese native chickens were clustered into seven haplogroups (A, B, C, D, E, G and V) out of 14 haplogroups of the nomenclature (A to I and V to Z) based on the complete mitochondrial genome and complete D-loop region (Miao et al. 2013; Godinez et al. 2022). Five haplogroups, A, B, C, D, and E, were observed to have multiple haplotypes, while haplogroups G and V contained only one haplotype. The distribution of 29 haplotypes in Vietnamese native chicken populations is presented in Table 4. Haplotypes belonging to haplogroups A, B, C, D and E were distributed in various indigenous chicken populations. Meanwhile, haplotype VNG01 of haplogroup G and haplotype VNV01 of haplogroup V were detected in one breed (Te and Long Xu, respectively). Haplogroups A and B were the two predominant haplogroups which accounted for nine and ten haplotypes, respectively. Nine haplotypes of haplogroup A were identified in five breeds (Cay Cum, Te, Lac Son, Long Xu and Ri Vang Rom), and ten haplotypes of haplogroup B belonged to nine chicken breeds (Cay Cum, Te, Lun Cao Son, To, Kien, Lac Son, Ma Da, Long Xu, and Ri Vang Rom). Both haplogroups C and D contained two haplotypes found in this study. Haplotypes of haplogroup C were found in three

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Table 2. Polymorphic nucleotide sites in complete mtDNA D-loop sequences of 29 haplotypes of Vietnamese native chickens

Haplogroup	Haplotype	Polymorphic nucleotide sites																																					
		31	167	199	207	212	217	221	223	225	228	237	239	242	243	246	256	261	281	296	302	306	310	315	320	322	330	341	342	355	361	363	367	391	446	686	792	1 075	1 214	1 215
	NC-007235[1]	A	T	T	A	A	T	C	T	C	C	A	A	G	T	T	T	C	A	C	C	T	C	T	A	T	C	T	A	T	A	C	T	C	C	G	G	A	C	A
A	VNA01	.	C	.	.	G	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
A	VNA02	.	C	.	.	G	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.	G
A	VNA03	.	C	.	.	.	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
A	VNA04	.	C	C	.	G	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
A	VNA05	.	C	.	.	G	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
A	VNA06	.	C	.	.	G	.	.	.	T	.	.	G	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
A	VNA07	.	C	.	.	G	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	G
A	VNA08	.	C	.	.	G	.	.	C	T	.	.	.	.	.	C	.	.	.	.	.	.	.	C	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
A	VNA09	T	C	.	.	G	.	.	.	T	.	.	.	.	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
B	VNB01	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
B	VNB02	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
B	VNB03	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	.
B	VNB04	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	.	.	.
B	VNB05	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	A	G	.	.
B	VNB06	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
B	VNB07	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.
B	VNB08	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
B	VNB09	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
B	VNB10	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
C	VNC01	.	.	.	.	G	.	.	.	.	.	.	.	A	C	C	C	.	G	.	.	.	T	C	.	.	.	.	G	.	.	T	C	.	.	.	.	.	.	G
C	VNC02	.	.	.	.	G	.	.	.	.	.	.	.	A	C	C	C	.	G	.	.	.	T	C	.	.	.	C	G	.	.	T	C	.	.	.	.	.	.	G
D	VND01	.	.	.	.	G	.	.	.	.	.	.	.	.	C	C	C	T	G	.	.	C	T	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	G
D	VND02	.	.	.	.	G	.	.	.	.	.	.	.	.	C	C	C	T	G	.	.	C	T	C	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	G
E	VNE01	.	.	.	.	G	C	.	.	.	.	.	.	.	C	C	C	.	.	.	.	.	T	C	.	.	T	.	.	.	.	.	.	.	T	.	.	.	T	G
E	VNE02	.	.	C	.	G	C	.	.	.	.	.	.	.	C	C	C	T	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	G
E	VNE03	.	.	.	.	G	C	.	.	.	.	.	.	.	C	C	A	T	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	G
E	VNE04	.	.	.	.	G	C	.	.	.	.	.	.	.	C	C	C	T	.	.	.	.	T	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	G
G	VNG01	.	.	.	.	.	.	.	.	.	.	.	.	.	C	C	C	T	.	A	T	.	T	.	.	C	.	.	.	.	.	.	.	.	T	.	.	.	T	G
V	VNV01	.	.	.	.	.	.	.	.	.	T	G	.	.	C	.	C	.	G	.	.	.	T	C	.	.	.	.	.	C	.	T	.	T	.	.	.	.	.	G

[1]The complete mitochondrial genome sequence with GenBank accession number NC_007235 was used as the reference sequence for determination of nucleotide position

Dots (.) indicate identity with the reference sequence and different base letters denote substitutions

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Table 3. Diversity indices in 10 breeds of Vietnamese native chickens based on complete mtDNA D-loop sequences

Chicken breeds	*n*	P	H	Hd ± SD	π ± SD
Cay Cum	15	12	6	0.743 ± 0.094	0.003 20 ± 0.000 54
Te	15	19	7	0.800 ± 0.083	0.003 88 ± 0.001 00
Lun Cao Son	15	2	3	0.257 ± 0.142	0.000 22 ± 0.000 12
To	15	13	4	0.619 ± 0.120	0.004 87 ± 0.000 93
Kien	15	18	4	0.600 ± 0.113	0.005 73 ± 0.000 92
Lac Son	15	19	5	0.752 ± 0.092	0.006 03 ± 0.000 69
Ma Da	15	0	1	0 ± 0.000	0 ± 0.000 00
Long Xu	15	18	6	0.790 ± 0.079	0.005 74 ± 0.000 65
H'mong	15	1	2	0.248 ± 0.131	0.000 20 ± 0.000 11
Ri Vang Rom	15	23	7	0.905 ± 0.041	0.006 44 ± 0.000 68
Total	150	39	29	0.918 ± 0.010	0.006 3 ± 0.000 22

π = nucleotide diversity; H = number of haplotypes; Hd = haplotype diversity; *n* = number of sequences; P = number of polymorphic sites

indigenous chicken breeds (Kien, Lac Son and Ri Vang Rom) and those of haplogroup D also occurred in three indigenous chickens (Kien, Lac Son and Long Xu). Haplogroup E also contained four haplotypes which were identified in other four indigenous chickens (To, Kien, H'mong and Ri Vang Rom).

Within haplotypes, VNA05, VNB01, VNB10, VNC01, VND01, VNE02 and VNE04 were shared among different chicken populations, in which VNA05, VNB01 and VNE04 were the major haplotypes, representing frequencies of 10%, 18.7%, and 12.7%, respectively.

Lun Cao Son and Ma Da populations were clustered in haplogroup B, in which 86.67% of Lun Cao Son population belong to haplotype VNB01 and 100% of Ma Da population belong to haplotype VNB07. Meanwhile, all individuals of the H'mong breed were in haplogroup E, with 86.67% in haplotype VNE04. The majority of Cay Cum and Ri Vang Rom populations were clustered in haplogroup A and those of Te, To, and Kien breeds were in haplogroup B with frequencies of 66.67%, 40%, 73.33%, 66.67%, and 60% respectively. Most Lac Son individuals were in haplogroup C (46.67%) and those of Long Xu in haplogroup D (40%) and haplogroup V (40%).

DISCUSSION

In this study, 150 complete mtDNA D-loop sequences from 10 Vietnamese native chicken breeds were identified.

A total of 39 polymorphic sites were detected. The overall haplotype and nucleotide diversity of Vietnamese indigenous chickens were estimated to be 0.918 ± 0.010 and 0.006 3 ± 0.000 22, respectively. These values showed higher genetic diversity than in Thai indigenous chicken populations (0.860 7 and 0.005 79) (Teinlek et al. 2018), Philippine native chickens (0.904 ± 0.012 and 0.004 47 ± 0.000 27) (Godinez et al. 2021), Cambodian chickens (0.889 ± 0.019 and 0.005 85 ± 0.000 30) (Godinez et al. 2022) and Egyptian native chickens (0.81 ± 0.03 and 0.004 5 ± 0.001 3) (Osman et al. 2016) which were also calculated based on the complete mtDNA D-loop sequences. These indices were also higher than the Hd of Vietnamese native chickens calculated based on a 455-bp fragment of mtDNA D-loop region (0.849 ± 0.184) (Cuc et al. 2011). These results indicated that Vietnamese native chickens had high genetic diversity.

In terms of phylogenetic analysis, as revealed by the ML tree reconstructed with 65 haplotypes representing 14 maternal haplogroups of domestic and wild chickens in Chinese and other Eurasian regions, 10 Vietnamese native chicken breeds were distributed into seven maternal haplogroups (A, B, C, D, E, G, and V). This result confirmed previous genetic evidence that Vietnamese native chicken breeds have originated from multiple maternal lineages from several regions of China and surrounding regions (i.e. Burma, Thailand, and India) (Cuc et al. 2011; Do et al. 2019). In this study, two haplogroups A and B were identified as two

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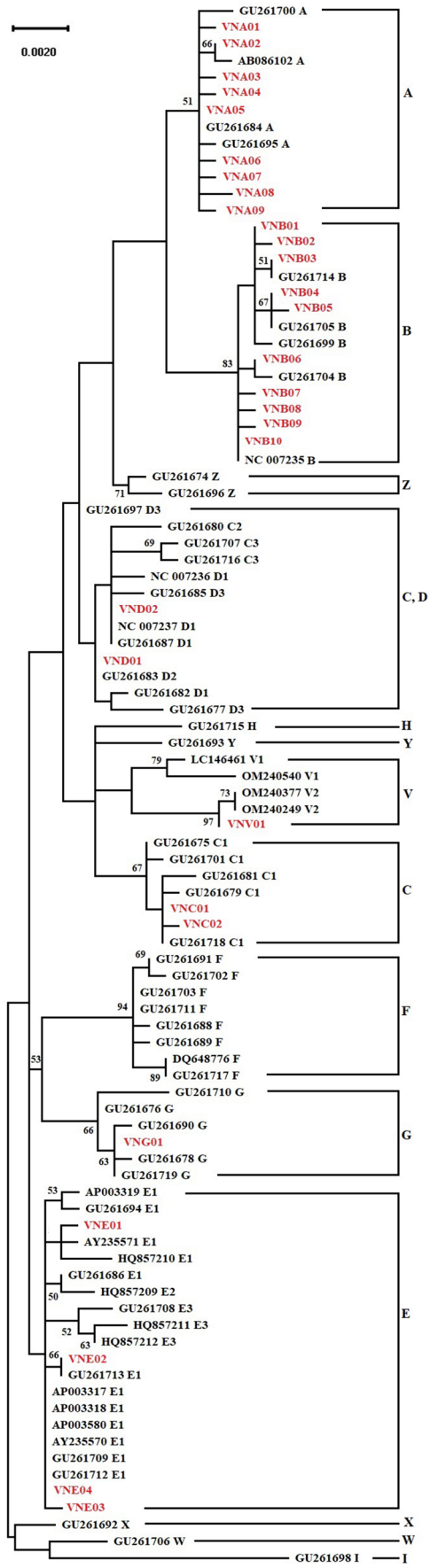

Figure 2. Maximum likelihood phylogenetic tree for complete mtDNA D-loop sequences of 29 haplotypes of Vietnamese indigenous chickens (in red) and representative sequences of 14 haplogroups (in black; Miao et al. 2013; Godiner et al. 2022)
Node labels correspond to bootstrap values estimated with 1 000 replicates. The scale bar (0.002) indicates the genetic distance

predominant maternal lineages in Vietnamese indigenous chickens. These haplogroups are also mainly distributed in South and East China, Japan and Southeast Asia (Liu et al. 2006; Oka et al. 2007; Miao et al. 2013). Most Vietnamese indigenous chicken sequences and haplotypes assigned to haplogroups A and B suggested that the present Vietnamese indigenous chicken breeds shared common maternal ancestors with chickens from China and Southeast Asian countries. This sharing of maternal lineages could be related to the geographic location of Vietnam. Vietnam is located in Southeast Asia and has a long border line with China, which advantages the introduction of chickens between Vietnam and these countries through trading and human migration. There were migration waves of Southern Chinese people into Vietnam in the 3rd century B.C. and from the 17th to the 19th century A.D. along with their animals (Cuc et al. 2011). The four haplotypes accounting for 17.3% of the studied chickens were assigned to haplogroup E. This haplogroup was supposed to have originated from the Indian subcontinent and was widespread in commercial lines, European, Middle Eastern and Indian chickens (Liu et al. 2006; Miao et al. 2013). Moreover, it was also found in Philippine, Thai, Australian chickens and Norfolk Island ferals (Langford et al. 2013; Godinez et al. 2021; Hata et al. 2021). The occurrence of haplogroup E in Philippine chickens is believed to be the result of hybridization between current chickens and commercial breeds, while in Norfolk Island it is the result of British colonization (Langford et al. 2013; Godinez et al. 2021). The way haplogroup E contributes to the Vietnamese chicken gene pool is not clear, but some haplotypes of Vietnamese chickens assigned to haplogroup E indicated the genetic relationship between this maternal lineage and Vietnamese chickens. This study also found two haplotypes in subhaplogroup C1, one haplotype in subhaplogroups D1 and D2. Subhaplogroup C1 was observed in chickens from Southeast China: Henan, Hunan and Yuanan, while subhaplogroup D1 and D2 occurred in chickens from Southeast Asian countries and China, respectively (Miao et al. 2013). Haplogroups G and V were each represented by only one haplotype of Vietnamese native chickens in this study. Haplotype VNG01 (in Te chicken) was found in Lang Son province close to China, while haplotype VNV01 (in Long

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Table 4. Distribution of mtDNA D-loop haplotypes in 10 Vietnamese indigenous chicken breeds

Haplogroup	Haplotype	Cay Cum	Te	Lun Cao Son	To	Kien	Lac Son	Ma Đa	Long Xu	H'mong	Ri Vang Rom	Total	%
A	VNA01	1	–	–	–	–	–	–	–	–	–	1	0.7
	VNA02	–	1	–	–	–	–	–	–	–	–	1	0.7
	VNA03	–	–	–	–	–	–	–	1	–	–	1	0.7
	VNA04	1	–	–	–	–	–	–	–	–	–	1	0.7
	VNA05	7	1	–	–	–	3	–	1	–	3	15	10.0
	VNA06	–	–	–	–	–	1	–	–	–	–	1	0.7
	VNA07	–	1	–	–	–	–	–	–	–	–	1	0.7
	VNA08	1	–	–	–	–	–	–	–	–	–	1	0.7
	VNA09	–	–	–	–	–	–	–	–	–	3	3	2.0
B	VNB01	4	–	13	9	–	2	–	–	–	–	28	18.7
	VNB02	–	–	1	–	–	–	–	–	–	–	1	0.7
	VNB03	–	4	–	–	–	–	–	–	–	–	4	2.7
	VNB04	–	–	–	–	–	–	–	–	–	2	2	1.3
	VNB05	–	1	–	–	–	–	–	–	–	–	1	0.7
	VNB06	1	–	–	–	–	–	–	–	–	–	1	0.7
	VNB07	–	–	–	–	–	–	15	–	–	–	15	10.0
	VNB08	–	–	–	–	9	–	–	–	–	–	9	6.0
	VNB09	–	–	–	–	–	–	–	1	–	–	1	0.7
	VNB10	–	6	1	1	–	–	–	–	–	–	8	5.3
C	VNC01	–	–	–	–	4	–	–	–	–	2	6	4.0
	VNC02	–	–	–	–	–	7	–	–	–	–	7	4.7
D	VND01	–	–	–	–	1	2	–	4	–	–	7	4.7
	VND02	–	–	–	–	–	–	–	2	–	–	2	1.3
E	VNE01	–	–	–	–	–	–	–	–	–	1	1	0.7
	VNE02	–	–	–	2	–	–	–	–	–	2	4	2.7
	VNE03	–	–	–	–	–	–	–	–	2	–	2	1.3
	VNE04	–	–	–	3	1	–	–	–	13	2	19	12.7
G	VNG01	–	1	–	–	–	–	–	–	–	–	1	0.7
V	VNV01	–	–	–	–	–	–	–	6	–	–	6	4.0
Total	–	15	15	15	15	15	15	15	15	15	15	150	100

Xu chicken) was distributed in Gia Lai province close to Laos and Cambodia. These haplogroups have been found in other regions of Asia: haplogroup G was mainly concentrated in Southwest China: Henan and Yunnan, while haplogroup V was found in Thailand, Laos and Cambodia (Miao et al. 2013; Godinez et al. 2022). These results indicated that the dispersal of these maternal lineages to Vietnam is consistent with the geographical relationship of these populations.

As shown in Table 4, Vietnamese native chicken breeds had more than one haplotype from different haplogroups exclusively for H'mong and Ma Da. For instance, Ri Vang Rom chickens had seven haplotypes belonging to haplogroups A, B, C and E. Seven haplotypes of Te chickens belonged to haplogroups A, B, and G. Some haplotypes of Lac Son chickens belonged to haplogroups A, B, C and D. Haplotypes of Long Xu chickens belonged to haplogroups A, B, D and V. This result suggested that these breeds had complex genetic backgrounds and that the evolution of maternal lineages was not independent. In addition, the sharing of the same haplotypes among some chicken breeds indicated that these breeds

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could share common maternal ancestors. Mating between lineages could occur as Vietnamese indigenous chickens are mainly reared in backyard farms where various breeds can be raised as one flock and the breeding management is poor.

CONCLUSION

This study provides understanding of genetic diversity and phylogenetic relationships of 10 Vietnamese indigenous chicken breeds. The results revealed Vietnamese indigenous chickens originated from different regions, mainly from China, Southeast Asia and the Indian subcontinent. These breeds have complex genetic backgrounds and evolution of maternal lineages due to sharing common maternal ancestors. Vietnamese indigenous chickens have high genetic diversity. Given the economic and cultural values of these breeds, their conservation will be necessary for future use.

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Conflict of interest

The authors declare no conflict interest.

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