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GENETIC DIVERSITY OF VIETNAMESE NATIVE CHICKEN BREEDS BASED ON MITOCHONDRIAL DNA D-LOOP SEQUENCE

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ABSTRACT

Genetic diversity of four Vietnamese indigenous chicken breeds including Lien Minh (GLM), Dong Tao (GDT), Nhan (GNH) and Chin Cua (G9C) were evaluated using mitochondrial DNA (mtDNA) D-loop sequence polymorphism. 1,050 bp of D-loop region of 66 individuals was amplified, sequenced, and analyzed. Comparative alignment results showed that the four local chickens shared 96.6% - 99.9% nucleotide identity with each other. Results of D-loop sequencing analysis revealed 53 variable sites (gaps included) in native Vietnamese chickens as compared to reference sequence (AB268540). Among them, there were six nucleotide deletions, ten nucleotide insertions (positions) and 37 nucleotide substitutions (sites). The estimated haplotype diversity (Hd) within breed was relatively high, ranged from 0.824 (GNH) to 0.913 (GLM). Phylogenetic study based on hypervariable region of 455 bp D-loop showed that all Vietnamese native chicken breeds were distributed over 16 haplotypes belonged to five haplogroups A, B, C, D and E, of which clade B was dominant. Individual of GLM, GDT, and G9C breeds clustered mainly in clade B (34/48) and GNH chicken (18 individuals) belonged to clade C and D. This study shows the relatively high genetic diversity of native Vietnamese chicken breeds and contributes to the conservation and breeding strategy of indigenous chicken in Vietnam.

Key words: DNA D-loop sequence, genetic diversity, Vietnamese indigenous chickens.

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INTRODUCTION

Domestic chicken is the most widely distributed species of all livestock in the rural households in Southeast and East Asia. Diversity of the chicken genetic resource is important for the better conservation of indigenous chicken breeds. Mitochondrial DNA sequence is an useful tool to investigate the genetic background of both closely related species and individuals within species (Harpending *et al*. 1998). Previous studies used mtDNA showed the high genetic diversity of local chickens (Liu *et al*. 2006; Oka *et al*. 2007; Cuc *et al*. 2011) and multiple origins of domestic chicken in East Africa (Mwacharo *et al*. 2011), Bangladesh (Bhuiyan *et al*. 2013), Oman (Al-Qamashoui *et al*. 2014) and Sweden (Englund *et al*. 2014). In Asia, Liu *et al*. (2006) were the first to demonstrate that several sub-species of red jungle fowl from South and Southeast Asia and surrounding areas were involved in the genetic makeup of modern chickens. Asian native chicken representatives revealed into nine divergent clades from A to I (Liu *et al*. 2006). While Japanese native chickens and some varieties were from other parts of Southeast Asia identified into seven clades from A to G (Oka *et al*. 2007). The classification of Oka *et al*. (2007) showed that the four clades E, B, C, and A were associated with four clades B, A, D and E according to the study of Liu *et al*. (2006) and Cuc *et al*. (2011). Cuc *et al*. (2011) analyzed the 455 bp sequence of D-loop of nine indigenous Vietnamese chicken breeds (H'mong, Mia, Ri, Ho, Dong Tao, Te, Choi, Ac, Tau Vang) and two chickens of Chinese origin (Luong Phuong, Tam Hoang) showed that Vietnamese native chickens spanning in eight clades (A-I), in which, they were distributed in two main branches (A and B).

Native Vietnamese chicken possesses various good characteristics such as: adaptability to harsh conditions, high disease resistance, adaptation to poor nutrition, the quality of meat and eggs, and many rare genetic resources. However, due to traditional poultry farming with small-scale production and to the imported chicken breeds, may have led to introgression of exotic chicken breeds into Vietnamese gene pool, so rare genetic resources are at risk of being hybridized. The GLM is one of native breeds, with a number of good characteristics and contribution to the economic value of rural citizens of Lien Minh, Tran Chau, and Cat Hai districts (Cat Ba Island). G9C breed is a multi-toes chicken (5-9 toes) with decreasing quantity and have been conserved at the National Park of Xuan Son, Tan Son District, Phu Tho Province. The GNH chickens are aggressive and brave so people wish to have them in chicken fighting games. GNH's legs are green colors, whereas, GDT is a special chicken species with yellow big and rough legs (Su *et al*. 2004). In recent years,

Vietnam has a program to conserve indigenous genetic diversity, in which GLM, G9C, GNH and GDT chickens are on the preserved list. Therefore, our study on the genetic diversity of these chicken breeds has significant contribution to the national program of conservation, development, and trademark registration of local breed.

MATERIALS AND METHODS

Animals: Four Vietnamese indigenous chickens, including GLM, GDT, GNH and G9C were used for analysis in our study (Table 1). Sampling sites were primitive origin of the variety or at the conservation site of the variety. Samples were taken from different families to minimize related individuals. Number of samples and sampling locations are presented in Table 1.

Table 1. Sample information.

Breed	Sample code	Special morphological characteristics	Study area	GenBank accession no.	Reference
Lien Minh (n= 24)	GLM 01 - 24	Feathers at the tip of the wing and tail are black	Lien Minh village, Cat Hai, Hai Phong City, Northern of Vietnam	KY172116 – KY172121; MH425591-MH425608	This study
Chin Cua (n= 6)	G9C 01 – 06	Many toes (5-9 fingers)	Phu Tho provincial, Northern of Vietnam	KR338974-KR338978	This study
Dong Tao (n= 18)	GDT 01 – 18	Yellow giant legs	Khoai Chau District, Hung Yen, Northern of Vietnam	MH447149-MH447166	This study
Ga Nhan (n= 18)	GNH 01 - 18	Green leg's color	Ca Mau provincial, Southern of Vietnam	MH425609-MH425626	This study
Reference samples					
Haplotype	**GenBank accession no**			**Reference**	
A1	AB114069			Liu *et al*., 2006	
B1	AB007744			Liu *et al*., 2006	
C1	AB114070			Liu *et al*., 2006	
D1	AY588636			Liu *et al*., 2006	
E1	AB114076			Liu *et al*., 2006	
F1	AF512285			Liu *et al*., 2006	
G1	AF512288			Liu *et al*., 2006	
H1	D82904			Liu *et al*., 2006	
I1	AB009434			Liu *et al*., 2006	
A1-A9	GU564361 - GU564369			Cuc *et al*., 2011	
B1-B8	GU564370 - GU564377			Cuc *et al*., 2011	
C	GU564378			Cuc *et al*., 2011	
D1-D5	GU564379- GU564383			Cuc *et al*., 2011	
E1-E9	GU564384- GU564392			Cuc *et al*., 2011	
F	GU564393			Cuc *et al*., 2011	
G1-G2	GU564394- GU564395			Cuc *et al*., 2011	
I1-I2	GU564396- GU564397			Cuc *et al*., 2011	

Sample collection: Blood samples were taken from the wing vein and collected in anti-coagulant tubes with EDTA (Macrogen) and stored at 4°C**.** 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 UV spectrophotometer and agarose gel electrophoresis. DNA samples were stored at -20°C until analysis.

PCR amplification and sequencing: Primer sequences used for amplification of the D-loop region are following: F: 5'-AGGACTACGGCTTGAAAAGC-3'; R:5'-CATCTTGGCATCTTCAGTGCC-3' (Eriksson *et al*., 2008). PCR was performed in a 25 µl reaction containing 1x PCR Buffer, 1.5 mM $MgCl_2$, 1.25 mM each dNTPs, 5 pM primers, 1U Taq-polymerase (Fermentas), and 100 ng genomic DNA. In PCR amplification, an initial denaturation at 94°C for three minutes followed by 35 cycles of denaturation at 94°C for 45 seconds, annealing at 56°C for 45 seconds and extension at 72°C for 90 seconds, and an additional extension of 72°C for seven minutes were applied. For DNA sequencing, PCR products were purified using a Agencourt AMpure XP Beads (Beckman Coulter). Purified PCR products were

used for sequencing on ABI Sequencer (3100 Genetic Analyzer, Applied Biosystems).

Analysis: Nucleotide sequences were identified with the Basic Local Alignment Search Tool (BLAST) (Altschul *et al*. 1990). Multiple nucleotide alignments were carried out with BioEdit (Hall, 1999). The diversity parameters, including the nucleotide diversity haplotypes, number of polymorphic sites, number of haplotypes, and haplotype diversity of all sequences were estimated using DnaSP v.5 software (Librado and Rozas, 2009). Neighbor Joining (NJ) phylogenetic tree was conducted using MEGA version 6.1 (Tamura *et al*. 2007). Phylogenetic tree analysis was constructed based on classification of Liu *et al*. (2006) with reference mtDNA sequences from the GenBank database as listed in Table 1.

RESULTS

1,050 bp mtDNAD-loop sequences from total 66 individuals of GLM, G9C, GNH, and GDT chickens were submitted in GenBank (accession numbers listed in Table 1). Pairwise comparison showed that the Vietnamese native chicken breeds shared high nucleotide identity with the domestic chicken, which deposited in the GenBank (Table 2). The four native breeds shared 96.6% - 99.9% nucleotide identity with each other. There were higher nucleotide identities (97.4% –99.1%) between GLM and GDT chickens, but only 96.6% – 97.3% identities between GNH and GDT chickens. In which, the GNH chicken breed shared the highest 99.8% - 100% nucleotide identity with each other, 99.6% - 100% for G9C chickens, 99.1% - 100% for GDT chicken, and the lowest for Lien Minh chickens 98.6% - 100%.

Nucleotide variations among mtDNA D-loop sequences of GLM, GDT, G9C, and GNH breeds, and representative sequence (AB268540) are shown in Figure 1. The results show that, the average percentage of polymorphic site was 5.1% (53/1,050). Out of 53 sequence variations, nucleotide deletions at positions (50-54 and 861), ten nucleotide insertion positions and 37 nucleotide substitution sites were observed. Among that, all GDT chickens shared nucleotide deletions at positions 50 – 54 as well as four nucleotide insertions at 838 (G), 859 (C), 866 (T) and 867 (T/A) sites, and nucleotide substitution at positions 56, 58, 62 (A↔G), 77 (T↔C) and 862 (A ↔ T). GNH characterized by nucleotide substitution at positions 1027, 1030 (A ↔ C), 1028 (C ↔ T), and 1029 (T ↔ A).

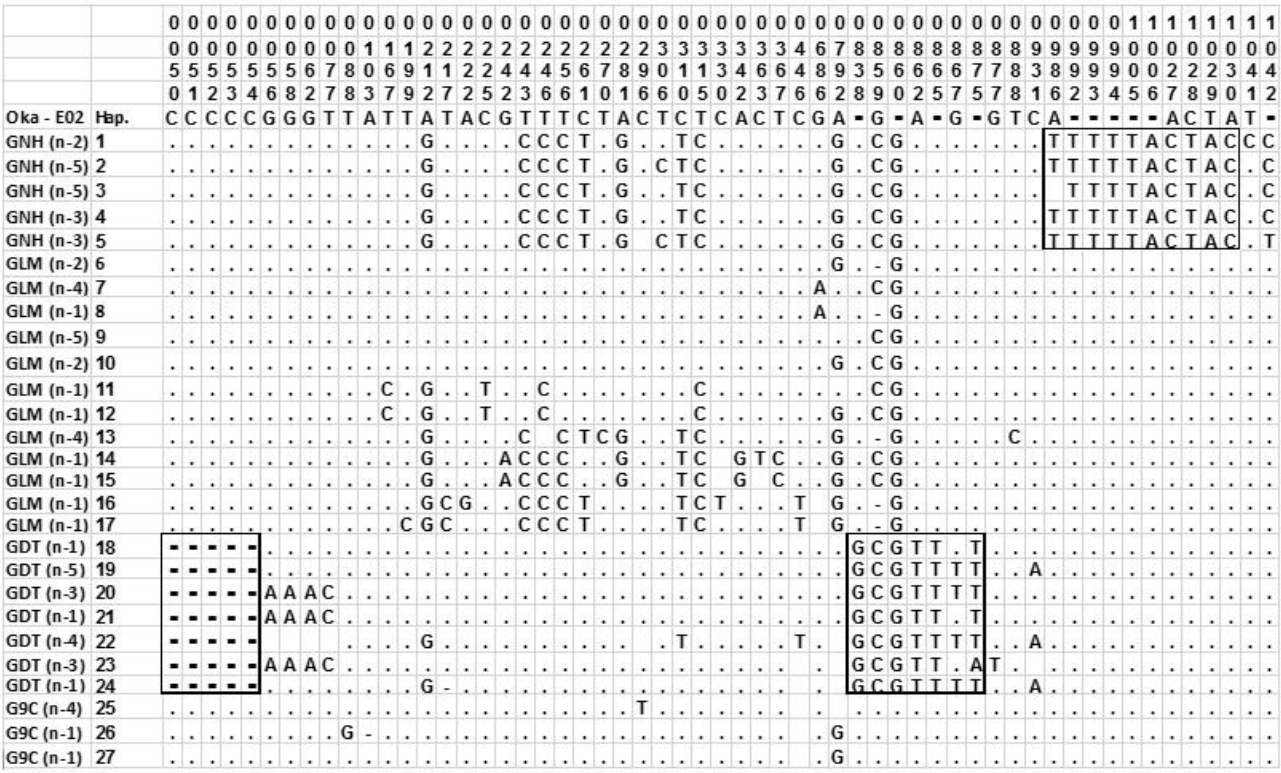

Fig. 1. Nucleotide polymorphisms observed in the D-loop of the Vietnamese chicken sequences (1,050 bp). The first column signifies identification number of samples and references chickens. Vertically oriented numbers indicate the variable sites position. Dots (.) indicate identity with the reference sequence (GenBank accession number AB268540) while different base letters denote substitutions. Black line – nucleotide deletion, continue black line ____ no information on nucleotide sequence.

Table 2. Nucleotide identity of D-loop sequence among samples.

Seq->	GNH1(n=2)	GN H2(n=5	GNH 3(n=5	GNH 4(n=3	GNH5(n=3)	GLM1(n=5)	GLM2(n=2)	GLM3(n=4)	GLM4(n=1)	GLM5(n=2)	GLM6(n=1)	GLM7(n=1)	GLM8(n=4)	GLM9(n=1)	GLM10(n=1	GLM11(n=1	GLM12(n=1	GDT1(n=1)	GDT2(n=5)	GDT3(n=3)	GDT4(n=1)	GDT5(n=4)	GDT6(n=3)	GDT7(n=1)	G9C1(n=4)	G9C2(n=1)	G9C3(n=1)
GNH1(n=2)	1	0.998	**0.999**	0.998	0.998	0.98	0.98	0.979	0.978	0.981	0.981	0.981	0.984	0.983	0.984	0.982	0.983	0.971	0.969	**0.966**	0.967	0.97	**0.966**	0.97	0.977	0.979	0.977
GNH2(n=5)		1	0.999	0.998	0.998	0.98	0.98	0.979	0.978	0.981	0.981	0.981	0.984	0.983	0.984	0.982	0.983	0.971	0.969	**0.966**	0.967	0.97	**0.966**	0.97	0.977	0.979	0.977
GNH 3(n=5)			1	0.999	0.999	0.981	0.981	0.98	0.979	0.981	0.981	0.982	0.985	0.984	0.985	0.983	0.984	0.972	0.97	0.967	0.968	0.971	0.967	0.971	0.978	0.98	0.978
GNH 4(n=3)				1	0.998	0.981	0.981	0.981	0.98	0.982	0.982	0.983	0.986	0.985	0.986	0.984	0.985	**0.973**	0.971	0.968	0.969	0.972	0.968	0.972	0.979	0.981	0.979
GNH5(n=3)					1	0.981	0.981	0.98	0.979	0.981	0.981	0.982	0.985	0.984	0.985	0.983	0.984	0.972	0.97	0.967	0.968	0.971	0.967	0.971	0.978	0.98	0.978
GLM1(n=5)						1	0.998	0.999	0.998	0.999	0.995	0.994	0.989	0.988	0.989	0.987	0.988	0.991	0.989	0.986	0.987	0.986	0.986	0.988	0.997	0.997	0.995
GLM2(n=2)							1	0.997	0.998	0.999	0.993	0.994	0.991	0.988	0.989	0.989	0.99	0.989	0.987	0.984	0.985	0.984	0.984	0.986	0.996	0.998	0.996
GLM3(n=4)								1	0.999	0.998	0.994	0.993	0.988	0.987	0.988	0.986	0.987	0.99	0.988	0.985	0.986	0.985	0.985	0.987	0.996	0.996	0.994
GLM4(n=1)									1	0.997	0.993	0.992	0.989	**0.986**	0.987	0.987	0.988	0.989	0.987	0.984	0.985	0.984	0.984	0.986	0.996	0.996	0.994
GLM5(n=2)										1	0.994	0.995	0.99	0.989	0.99	0.988	0.989	**0.99**	0.988	0.985	0.986	0.985	0.985	0.987	0.996	0.998	0.996
GLM6(n=1)											1	0.999	0.988	0.989	0.99	0.988	0.989	0.986	0.984	0.981	0.982	0.983	0.981	0.985	0.992	0.992	0.99
GLM7(n=1)												1	0.989	0.99	0.991	0.989	0.99	0.985	0.983	0.98	0.981	0.982	0.98	0.984	0.991	0.993	0.991
GLM8(n=4)													1	0.991	0.992	0.992	0.993	0.98	0.979	0.976	0.977	0.98	0.976	0.98	0.987	0.989	0.987
GLM9(n=1)														1	0.999	0.989	0.99	0.98	0.978	0.975	0.976	0.979	0.975	0.979	0.985	0.987	0.985
GLM10(n=1)															1	0.99	0.991	0.98	0.979	0.976	0.977	0.98	0.976	0.98	0.986	0.988	0.986
GLM11(n=1)																1	0.997	0.979	0.977	**0.974**	0.975	0.98	0.974	0.978	0.985	0.987	0.985
GLM12(n=1)																	1	0.98	0.978	0.975	0.976	0.98	0.975	0.979	0.986	0.988	0.986
GDT1(n=1)																		1	0.998	0.995	0.996	0.995	0.994	0.997	**0.988**	0.988	0.986
GDT2(n=5)																			1	0.995	0.994	0.997	0.992	0.999	0.986	0.986	0.984
GDT3(n=3)																				1	0.999	0.992	0.997	0.994	0.983	0.983	0.981
GDT4(n=1)																					1	0.991	0.998	0.993	0.984	0.984	0.982
GDT5(n=4)																						1	0.989	0.998	0.983	0.983	0.981
GDT6(n=3)																							1	0.991	0.983	0.983	**0.981**
GDT7(n=1)																								1	0.985	0.985	0.983
G9C1(n=4)																									1	0.998	0.996
G9C2(n=1)																										1	0.998
G9C3(n=1)																											1

GNH: Nhan chicken; GLM: Lien Minh chicken; GDT: Dong Tao chicken; G9C: Chin Cua chicken.
(n =): The number of individuals, which share 100% nucleotide identities

A total of 27 haplotypes in 66 individuals from four Vietnam native chickens based on 1,050 bp of mtDNA D-loop sequence, and all these haplotypes belong to five clades. In which, GLM chicken had 12 haplotypes, GDT chicken had seven haplotypes, GNH chicken had five haplotypes, and G9C chicken belonged to three haplotypes. Non-significant Tajima's D were observed in all breed chickens of this studied (Table 3), indicating that neither balancing selection nor purifying selection occurred in all chicken populations. There by suggesting neutral selection is involved. The native Vietnam chickens have been free-range, in which random mating is permitted without farmer's interest in programmed breeding.

Table 3. Polymorphic sites, haplotype and nucleotide diversity of Vietnamese and Asian chicken breeds-based D-loop sequences.

Breed	N	S	h	Hd	Pi	NC	D	References
Vietnamese chickens								
GLM	24	23	12	0.913± *0.034*	0.007±*0.00082*	4	0.187	This study*
GDT	18	11	7	0.856±*0.047*	0.004±*0.00041*	1	1.721	
GNH	18	4	5	0.824±*0.045*	0.001±*0.00013*	1	0.153	
G9C	6	4	3	0.867± 0.129	0.002±0.00040	1	0.562	
Total	***66***	***37***	***27***	***0.963±0.009***	***0.009 ±0.00041***	***5***	***0.513***	
Other Vietnamese chickens								
H'Mong	20	23	6	0.778		5		Cuc *et al*., 2011
Mia	20	10	7	0.737		2		
Ri	20	22	12	0.911		5		
Ho	20	8	4	0.615		2		
Dong Tao	20	20	7	0.768		3		
Te	20	14	5	0.716		2		
Choi	19	15	4	0.754		4		
Ac	21	13	5	0.767		3		
Tau Vang	20	24	13	0.942		6		
Total	***222***	***43***	***37***	***0.849***		***8***		
HG population	106	-	25	0.860	0.013	6	-	Bethouly *et al*., 2009
Chinese chickens								
Beijing Youkei	4		3	0.830	0.006	2		
Luke	9		4	0.810	0.013	3		
Dwarf Wugu	10		5	0.860	0.012	3		
Taihe Silky	14		3	0.650	0.010	3		
Jiangbian	28		11	0.850	0.013	4		
Xuchuan	50		10	0.869	0.009	4		Guo *et al*., 2017
Yunyang	24		9	0.812	0.011	4		
Lushi	28		17	0.942	0.013	4		
Silkia	30		12	0.894	0.012	4		
Black- bone	132	-	28	0.916	0.012	4	-	
Laotian chickens								
Vientiane	54	23	20	0.880	0.009	3		Kawabe *et al*., 2014
Luang Prabang	42	10	7	0.815	0.008	2		
Pakse	33	26	10	0.847	0.015	5		
Total	***129***	***37***	***29***	***0854***	***0.010***	***5***		
Tibetan chickens								
Aba	36	21	11	0.860	0.011		0.050	Zhang *et al*., 2017
Diqing	25	21	9	0.760	0.011		-0.403	
Lasa	49	33	15	0.904	0.014		-0.348	
Shannan	33	21	8	0.642	0.008		-0.871	
Total	***276***	***42***	***42***	***0,925***	***0.016***	***7***	***-0.138***	
Thai chickens								
Pra-dhu-hang-dam	80		11	0.832	0.005	4	1.319	Piyanat *et al*., 2018
Leun-hang-khao	76		13	0.818	0.006	5	0.762	
Chee	34		4	0.640	0.005	3	1.370	

Dang	30		8	0.782	0.005	5	-0.031	
Total	***220***	***-***	***23***	***0.861***	***0.006***	***6***	***0.403***	
Indonesia chickens								
Bangkok	7	53	6	0.952	0.039			Ulfah *et al*., 2017
Bekisar	13	44	9	0.936	0.029			
Burgo	14	6	6	0.791	0.004			
Cemani	12	3	2	0.303	0.002			
Nunukan	10	5	4	0.733	0.004			
Total	***204***	***76***	***93***	***0.840***	***0.017***			
Iranian chickens								
Iranian	168	22	17	0.00-0.601	0.002			Meydan *et al*., 2016

N: number of samples, S: number of polymorphic sites (excluding sites with gaps), h: Number of haplotypes, Hd: Haplotype diversity, Pi: nucleotide diversity, NC: number of clades, D: Tajima's test, *)1,050 bp of D-loop sequence had been used for analysis of diversity parameters.

To use Vietnamese chicken sequences as references, Cuc *et al*. (2011) used 31 D-loop sequences (455 bp) of native Vietnamese chicken, therefore, only 455 bp belonged to hypervariable of D-loop sequence had been used for phylogenetic analysis in our study. Phylogenetic tree was constructed using 66 D-loop sequences along with 41 reference sequences that corresponded to the nine clades defined by Liu *et al*. (2006) (Fig. 2). The four native breeds of Vietnam used in this study were assigned to the 16 haplotypes of five clades A, B, C, D, and E. Most of GNH chickens were distributed in clades C, which was observed in the majority of fighting and bantam chickens sampled in Indonesia (Oka *et al*. 2007). However, most of samples (70.8%) of three remaining breeds clustered in clade B. All individuals of G9C chickens were observed in clade E (6/6 samples). However, 24 GLM chickens were distributed in five clades A, B, C, D, and E, but mainly in clade B (58.3%). In particular, no Vietnamese chicken in this study was not found in clades F, G, H, and I.

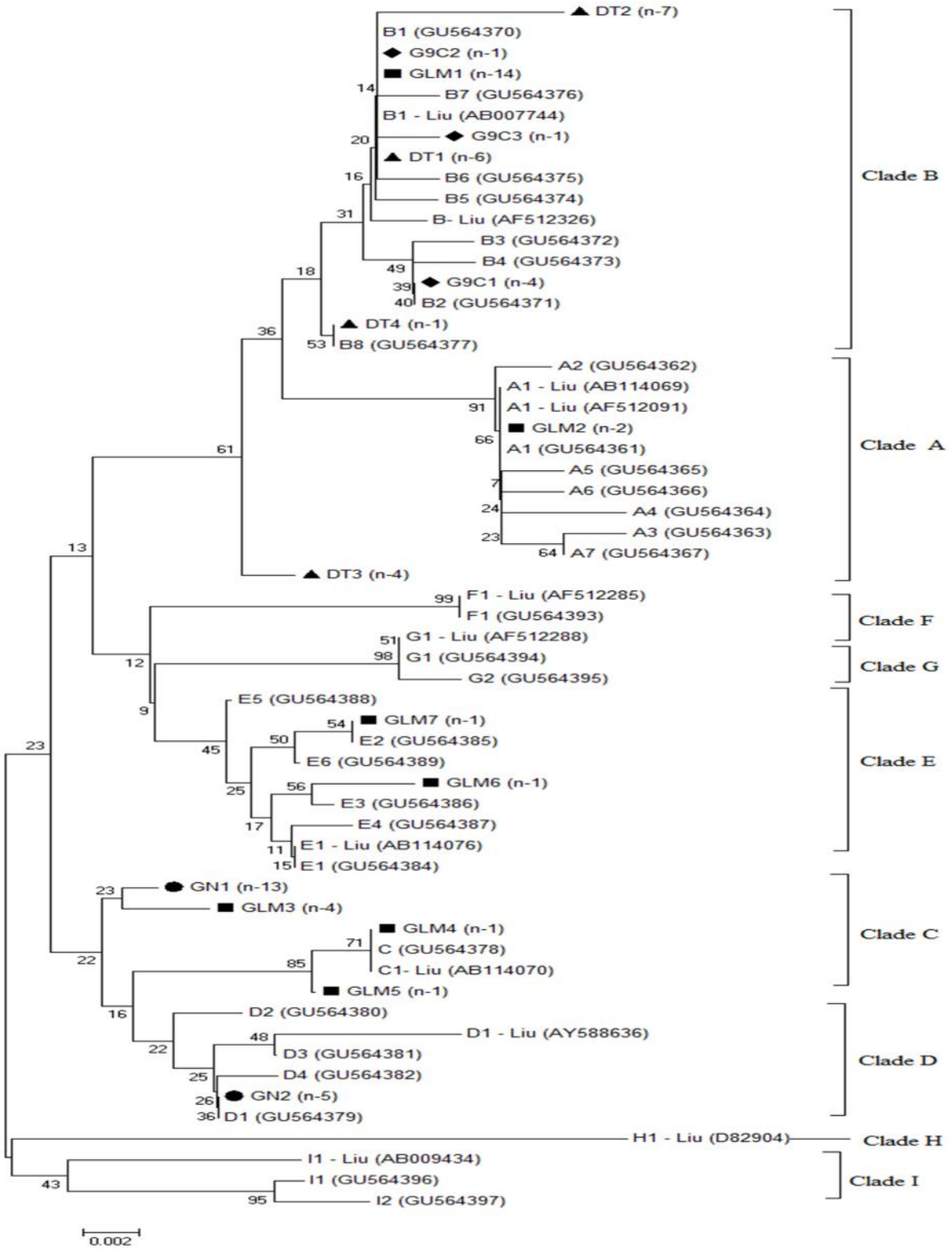

Fig. 2. Phylogenetic tree of Vietnamese native chicken breeds based on 455 bp of D-loop sequence. It was generated by the neighbor-joining method using MEGA6.1 with bootstrap values for interior clades after 1,000 replications. Different clade and haplotype are indicated. GLM, G9C, GDT and GNH breeds are marked (circle, tetragular, triangular, and square, respectively). Numbers in bracket are number of samples appeared at identical haplotype.

DISCUSSION

In this study, 1,050 bp mtD-loop of Vietnamese chickens had been sequenced and used for analysis of genetic diversity, whereas, phylogenetic tree had been constructed based on 455 bp belonged to hypervariable region.

Comparative alignment results showed high nucleotide identity within four local chickens and other Vietnamese chicken breeds (Cuc *et al*. 2011). There was also a high nucleotide similarity of D-loop sequences among Vietnamese native chickens and other the local chickens originated from Asian countries (Liu *et al*. 2006; Oka *et al*. 2007). Supporting for that, the analysis result also showed higher level of diversity of GLM, GDT, GNH and G9C chickens, which expressed by analyzing parameters S, h, Hd, and Pi (Table 3). The estimated haplotype diversity values (Hd) within breeds ranged from 0.824 (GNH) to 0.913 (GLM) and were similar to those of Asia native chickens. GDT's Hd index from 455 bp is 0.725 ± 0.055, the results of this analysis are quite similar to the results of Cuc *et al*. (2011) (Dong Tao 0.768). Considering to the number of polymorphic sites, number of haplotypes, haplotype diversity and number of clades, Vietnamese native chickens in this study had relatively high genetic diversity in comparison to other Vietnamese and Asian chicken breeds as shown in Table 3. The genetic information of this study may serve as molecular information for the characterization, conservation and improvement of valuable genetic resource of Vietnamese chickens.

This is the first report on analysis of haplotype diversity and phylogenetic of native chickens GLM, GNH, G9C at the mtDNA level. Liu *et al*. (2006) showed that, the Asian native chickens were grouped into nine divergent clades from A to I. The Asian chicken breeds also were classified into seven clades from A to G based on full D-loop sequence, (Oka *et al*. 2007). Vietnamese chickens were identified into eight haplogroups (A to G and I) (Cuc *et al*. 2011), and Lao chickens were spanned in five clades (A, B, D, F, and I) (Kawabe *et al*. 2014). In this study, phylogenetic tree analysis was constructed based on classification of Liu *et al*. (2006) using 455 bp D-loop sequence. A total 27 haplotypes observed in four Vietnam native chickens were grouped in five clades, which were defined by mutational motifs shared by the descendants (Fig. 2).

There were higher nucleotide identities (97.4% - 99.1%) between GDT and GLM, so most of samples of GDT and GLM grouped in the clade B, indicating close genetic closely related these breeds and individuals within breed. These sequence types resembled those observed in Shamo chicken (Oka *et al*. 2007) and in several Chinese native chicken populations, as reported by Niu *et al*. (2002). The differences between GLM, and GDT were small, indicating that the genetic diversity was very close in those breeds, the results of their close geographic distance (about 150 km), and because of economical-oriented selection pressure, genetic diversities of GLM and GDT chickens were low. Various studies showed that, clade B were observed in native chickens of South Asia, Chinese, Laotian, Japanese and Indian chickens (Liu *et al*. 2006; Oka *et al*., 2007; Cuc *et al*. 2011; Kawabe *et al*. 2014), and was not detected in Africa (Miao *et al*. 2013). The results showed that, G9C chickens like other Chinese breeds Tibetan, Henan cockfighting, Langshan chickens had a single maternal genetic background.

All samples of GNH collected in Ca Mau province, southern part of Vietnam was grouped in clade C and D, which were found in gamecocks from China, Japan, Madagascar and Vietnam. 50% the Choi chickens-game birds were observed in clade D (Cuc *et al*. 2011). Similar to that, this clade mainly consisted of game birds and 45% of Indian domestic chicken (Liu *et al*. 2006). With the same green color of shank, the Green-legged Partridgelike (GP) fowl was grouped in two clades, which associated with clade B and E according to classification of Liu (Siwek *et al*. 2013). On the other hand, the seven haplotypes of GLM in this study were spanned to five clades (A, B, C, D, E). However, most of them clustered in clade B, indicating clade B is predominant. As aforementioned, number of clades found in the Lien Minh chicken similar with other Vietnam breeds such as Choi (four clades), H'Mong (five clades), Ri (five clades) and HG (six clades) (Cuc *et al*. 2011; Berthouly *et al*. 2009). Due to traditional poultry farming with small-scale production, and farmers may not be aware of preserving precious genetic resources of indigenous chickens, so they can bring other chicken breeds to raise together. The above can lead to mixed maternal origins occurred in GLM chickens.

Only 3% Vietnamese chickens (GLM) in this study and 2.7% chickens of Cuc' study were assigned to the clade E. In contrast, the majority of Indian, Near Eastern and European breeds were found in this clade (Liu *et al*. 2006). Diversity of maternal lineages also was found in Africa, South Asia, Southeast Asia and East Asia (Miao *et al*. 2013). In addition, no number of Vietnamese chickens distributed in clades F, G, H and I.

Vietnamese native chickens had relatively high genetic diversity, in which GLM, GNH, GDT showed multiple maternal lineages, whereas G9C grouped in dominant clade B and had a single maternal origin (clade B). The results observed in our study are the genetic information supporting the registration of local breeds and also are valuable understanding of maternal lineages of population/breed. It can help for the improvement of production performance of local chickens by crossbreeding strategies within local or with exotic breeds.

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REFERENCES

Al-Qamashoui B., H. Simianer, I. Kadim and S. Weigend (2014). Assessment of genetic diversity and conservation priority of Omani local chickens using microsatellite markers. Trop. Anim. Health Prod. 10: 444-447.

Altschul S.F., W. Gish, W. Miller, E.W. Myers and D.J. Lipman (1990). Basic Local Alignment Search Tool. J. Mol. Bio. 215: 403-410.

Ausubel F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J. A. Smith and K. Struhl (1995). Short protocols in molecular biology, 3rd Ed. John Wiley & Sons, Inc.

Berthouly C., G. Leroy, T.N. Van, H.H. Thanh, B. Bed'Hom, B.T. Nguyen, C.C. Vu, F. Monicat, M. Tixier-Boichard, E. Verrier, J. Maillard and X. Rognon (2009). Genetic analysis of local Vietnamese chickens provides evidence of gene flow from wild to domestic populations. BMC Genetics, 10: 1.

Bhuiyan M.S., S. Chen, S. Faruque, A.K. Bhuiyan and A. Beja-Pereira (2013). Genetic diversity and maternal origin of Bangladeshi chicken. Mol. Bio. Reports. 40(6): 4123-4128.

Cuc N.T.K., H. Simianer, L.F. Groeneveld and S. Weigend (2011). Multiple maternal lineages of Vietnamese local chickens inferred by mitochondrial D-loop sequences. Asian Australasian J. Anim. Sci. 24: 155–161.

Englund T., L. Stromstedt and A.M. Johansson (2014). Relatedness and diversity of nine Swedish local chicken breeds as indicated by the mtDNAD-loop. Hereditas, 151: 229–33.

Eriksson J., G. Larson, U. Gunnarsson, B. Bed'hom, M. Tixier-Boichard, L. Strömstedt, D. Wright, A. Jungerius, A. Vereijken, E. Randi, P. Jensen and L. Andersson (2008). Identification of the Yellow Skin Gene Reveals a Hybrid Origin of the Domestic Chicken. PLoS Genetics, 4: e1000010.

Guo H.W., C. Li, X.N. Wang, Z.J. Li, G.R. Sun, G.X. Li, X.J. Liu, X.T. Kang and R.T. Han (2017). Genetic diversity of mtDNA D-loop sequences in four native Chinese chicken breeds. Bri. Poul. Sci. 58(5): 490–497.

Hall T. (1999). BioEdit: a user-friendly biological sequences alignment editor and analysis program for Window 95/98/NT. Nucleic Acids Symposium Series, 41: 95-98.

Harpending H.C., M.A. Batzer, M. Gurven, L.B. Jorde, A.R. Rogers and S.T. Sherry (1998). Genetic traces of ancient demography. Proc. of the National Academy of Sciences of the United States of America, 95: 1961- 1967.

Kawabe K., R. Worawut, S. Taura, T. Shimogiri, T. Nishida, and S. Okamoto (2014). Genetic Diversity of mtDNA D-loop Polymorphisms in Laotian Native Fowl Populations. Asian-Australasian J. Anim. Sci. 27(1): 19-23.

Librado P. and J. Rozas (2009). DnaSP (version 5.0): A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25: 1451–1452.

Liu Y.P., G.S. Wu, Y.G. Yao, Y.W. Miao, G. Luikart, M. Baig, A. Beja-Pereira, Z.L. Ding, M.G. Palanichamy and Y.P. Zhang (2006). Multiple maternal origins of chickens: out of the Asian jungles. Mol. Phylogenet. Evol Mol. Phylo. and Evo. 38: 12–19.

Meydan H., C.P. Jang, M.A. Yildiz and S. Weigend (2016). Maternal Origin of Turkish and Iranian Native Chickens Inferred from Mitochondrial DNA D-loop Sequences. Asian Australasian J. Anim. Sci. 29(11): 1547–1554.

Miao Y., M. Peng, G. Wu, Y. Ouyang, Z. Yang, N. Yu, J. Liang, G. Pianchou, A. Beja-Pereira, B. Mitra, M. Palanichamy, M. Baig, T.K. Chaudhuri, Y. Shen, Q. Kong, R. Murphy, Y. Yao and Y. Zhang (2013). Chicken domestication: An updated perspective based on mitochondrial genomes. Heredity, 110: 277–82.

Mwacharo J.M., G. Biornstad, V. Mobegi, K. Nomura, H. Hanada, T. Amano, H. Jianli, O. Hanotte and K. Nomura (2011). Mitochondrial DNA reveals multiple introductions of domestic chicken in East Africa. Mol. Phylo. and Evo. 58(2): 374-382.

Niu D., Y. Fu, J. Luo, H. Ruan, X.P. Yu, G. Chen and Y.P. Zhang (2002). The origin and genetic diversity of Chinese native chicken breeds. Biochem. Genet. 40(5): 163-174.

Oka T., Y. Ino, K. Nomura, S. Kawashima, T. Kuwayama, H. Hanada, T. Amano, M. Takada, N. Takahata, Y. Hayashi and F. Akishinonomiya (2007). Analysis of mtDNA sequences shows Japanese native chickens have multiple origins. Anim. Genet. 38: 287–293.

Piyanat T., S. Kannika and T. Chanin (2018). Genetic diversity analysis of Thai indigenous chickens based on complete sequences of mitochondrial DNA D-loop region. Asian-Australasian J. Anim. Sci. 31(6): 804-811.

Siwek M., D. Wragg, A. Sławin´ska, M. Malek, O. Hanotte and J.M. Mwacharo (2013). Insights into the genetic history of Green-legged Partridgelike fowl: mtDNA and genome-wide SNP analysis. Anim. Genet. 44: 522–532.

Su V.V., N.V. Thien, D.T. Nhiem, L.V. Ly, N.V. Hai and H.V. Tieu (2004). Atlas of farm animal breeds in Vietnam. Agricultural Publishers, Hanoi, Vietnam.

Tamura K., J. Dudley, M. Nei, S. Kumar (2007). MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Bio. and Evo. 24: 1596-1599.

Ulfah M., D. Perwitasari, J. Jakaria, M. Mulado and A. Farajallah (2017). Multiple maternal origins of Indonesian crowing chickens revealed by mitochondrial DNA analysis. Mitochondrial DNA Part A, 28(2): 254-262.

Zhang L., P. Zhang, Q. Li, U. Gaur, Y. Liu, Q. Zhu, X. Zhao, Y. Wang, H. Yin, Y. Hu, A. Liu, D. Li (2017). Genetic evidence from mitochondrial DNA corroborates the origin of Tibetan chickens. PloS ONE, 1-11.