Research Article

Mitochondrial D-loop Diversity and Morphometric Characterization of the Indigenous Van Linh Chicken, Vietnam

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Abstract | The Van Linh chicken, an indigenous breed from Lang Son Province, Vietnam, exhibits distinct sexual dimorphism in phenotypic characteristics. Hens (average body weight: 1,213.0 g at 18 weeks of age) are characterized by small combs and cream, pale-yellow, or pale-brown plumage with black markings on the wings and tail. In contrast, cocks (average body weight: 1,590.5 g) possess large combs and predominantly red-orange or orange-brown plumage, with dark-blue feathers on the wing tips, hackles, and tail. Despite these distinct phenotypic characteristics, the genetic diversity and evolutionary history of this breed remain poorly understood. To address this knowledge gap, a 657-bp fragment of the mitochondrial DNA (mtDNA) D-loop region was sequenced from 38 individuals. Sequence analysis identified 11 haplotypes defined by 22 polymorphic sites, revealing high genetic diversity (Hd = 0.817 ± 0.042; π = 0.00920 ± 0.00058). Phylogenetic analysis revealed a complex evolutionary pattern and a polyphyletic origin, with the haplotypes grouped into four major clades, predominantly B and E. These lineages clustered closely with Southeast Asian red junglefowl subspecies (*Gallus gallus spadiceus* and *Gallus gallus gallus*) and native chicken populations from India and Laos. Neutrality tests (Tajima's D = 0.51295; Fu and Li's D* and F* > 0; *P* > 0.10) suggested a stable demographic history under mutation–drift equilibrium, with no evidence of recent population expansion or positive selection. These findings demonstrate that Van Linh chicken represents a valuable genetic resource that should be prioritized for conservation and sustainable breeding programs.

Keywords | mtDNA, Control region, Genetic variation, Phenotypic traits, Vietnam native poultry

Received | June 22, 2026; **Accepted** | July 20, 2026; **Published** | August 21, 2026
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Citation | Thao DTP, Tuyen TA, Cuong DN, Hanh NV, Nam TT, Giang NTC, Luc DD, Anh NT, Pha PH, Thinh NH (2026). Mitochondrial D-loop diversity and morphometric characterization of the indigenous Van Linh chicken, Vietnam. Adv. Anim. Vet. Sci., 14(9):1972-1981.
DOI | https://dx.doi.org/10.17582/journal.aavs/2026/14.9.1972.1981
ISSN (Online) | 2307-8316

INTRODUCTION

Sequencing the mitochondrial DNA (mtDNA) control region (D-loop) is a widely used and cost-effective approach. Owing to its strict maternal inheritance, absence of recombination, and relatively high mutation rate compared with nuclear DNA (5-10× that of nuclear DNA), the D-loop locus enables high-resolution haplotype discrimination and robust haplogroup assignment across indigenous populations. First established by Fumihito *et al.* (1994) for phylogenetic inference and maternal lineage tracing between domestic and wild *Gallus* taxa,

this approach has been extensively validated in subsequent studies across diverse avian species (Haig *et al*., 2011). The D-loop's proven utility in resolving population structure, demographic history, and evolutionary relationships has established it as a standard marker in avian conservation genetics and is therefore employed in the present study. Elucidating the evolutionary origins and population genetic architecture of the domestic chicken (*Gallus gallus domesticus*) is essential for developing effective breeding programs, conserving genetic resources, and supporting the sustainable management of poultry germplasm. The domestic chicken exhibits complex ancestry shaped by adaptive introgression with sympatric junglefowl and localized adaptation, generating region-specific genetic signatures (Miao *et al*., 2013; Wang *et al*., 2020; Lawal and Hanotte, 2021). Extensive phylogeographic analyses across Southeast Asia reveal highly structured maternal lineages reflecting diverse evolutionary trajectories. Indigenous populations in Indonesia, Laos, Thailand, and the Philippines harbor substantial maternal diversity, characterized by multiple haplogroups (predominantly clades A and B) alongside distinct regional lineages (Sulandari *et al*., 2008; Kawabe *et al*., 2014; Teinlek *et al*., 2018; Godinez *et al*., 2021, 2022). Collectively, these findings indicate that chicken dispersal in Southeast Asia was driven by anthropogenic migration and extensive cross-regional introgression rather than a single localized domestication event.

Vietnam, recognized as a primary center of chicken domestication sharing a border the ancestral gene pools of southern China, serves as a critical reservoir of avian genetic diversity. Previous assessments of Vietnamese indigenous breeds have consistently demonstrated elevated haplotype richness and pronounced population structure. Studies across various native breeds have identified numerous haplotypes distributed across multiple clades (A through I, and V), with clades A and B remaining predominant, while specific breeds exhibit restricted, unique lineage combinations (Cuc *et al*., 2011; Nguyen *et al*., 2022; Giang *et al*., 2023). Notably, the recent discovery of clade V in northern Vietnamese breeds highlights the region's role in harboring undocumented maternal lineages linked to the lower Mekong River basin (Giang *et al*., 2023).

Despite the extensive characterization of widely distributed major indigenous breeds, geographically restricted and endemic local populations remain largely genetically unexplored. The Van Linh chicken, an endemic breed native to the mountainous Lang Son Province, represents one such distinct genetic resource. Given its geographic isolation and unique phenotypic traits, it is hypothesized to harbor undocumented maternal genetic signatures. Therefore, this study aims to evaluate the maternal genetic diversity, phylogenetic relationships, and demographic history of the Van Linh chicken using mitochondrial DNA D-loop sequencing. By elucidating its genetic architecture, this research will fill a critical knowledge gap regarding Vietnam's endemic poultry resources and contribute to the comprehensive conservation of the regional poultry gene pool.

Van Linh chickens has been present and traditionally reared in Lang Son Province for centuries. This region is enclosed by extensive limestone mountain ranges, forming an ecologically isolated basin that restricts external gene flow, thereby limiting genetic introgression from surrounding poultry populations. Hu *et al*. (2025) demonstrated significant genetic differentiation between chickens reared in high- and low-altitude habitats, which form distinct genetic clusters. Specifically, the geographic and altitudinal disparities in the sampling regions (>3000 m vs. <600 m) act as ecological barriers, driving localized selective signatures and pronounced genetic divergence among chicken breeds. Van Linh chicken is distinguished by exceptional environmental adaptability and superior meat quality traits. However, recent investigations on Van Linh chicken have predominantly focused on phenotypic evaluation, growth performance, and reproductive parameters (Duong *et al*., 2023, 2024), leaving a substantial gap in molecular genetic characterization.

MATERIALS AND METHODS

Tissue samples from wing feathers of 38 chickens were obtained in Van Linh commune, Lang Son province, Vietnam (located at 21°39′10″N 106°28′17″E- Figure 1). All procedures were conducted in accordance with the ethical guidelines for animal care and use approved by the Faculty Council, Faculty of Animal Science, Vietnam National University of Agriculture under approval number FAS-VNUA-2024/01.

DNA extraction and Mitochondrial D-loop Amplification: The samples were collected and placed in sterile, tightly sealed 1.5-mL Eppendorf tubes and stored at 4°C until DNA extraction. DNA was extracted from tissue samples following the method described by Sambrook and Russell (2006). DNA integrity was assessed by 1% agarose gel electrophoresis with RunSafe nucleic acid stain. Concentration and purity were quantified using NanoDrop spectrophotometer (Thermo Scientific, USA). Samples with A260/A280 ratios between 1.8 and 2.0 were selected for further analysis. The mitochondrial D-loop control region was amplified via polymerase chain reaction (PCR) using species-specific primers: Forward primer (5′-AGGACTACGGCTTGAAAAGC-3′) and reverse primer (5′-CATCTTGGCATCTTCAGTGCC-3′) as described by Oka *et al*. (2007). Each 25 µL reaction contained: 1× PCR buffer, 2.5 mM $MgCl_2$, 200 µM of

each dNTP, 0.5 µM of each primer, 1.0 U Taq DNA polymerase (Thermo Scientific), and 50ng template DNA. Thermal cycling parameters comprised an initial denaturation at 95°C for 5 min, followed by 35 cycles of 95°C for 45s (denaturation), 60°C for 45s (annealing), and 72°C for 90s (extension), with a final extension at 72 °C for 7 min. Amplification products were checked on 1.0% agarose gels.

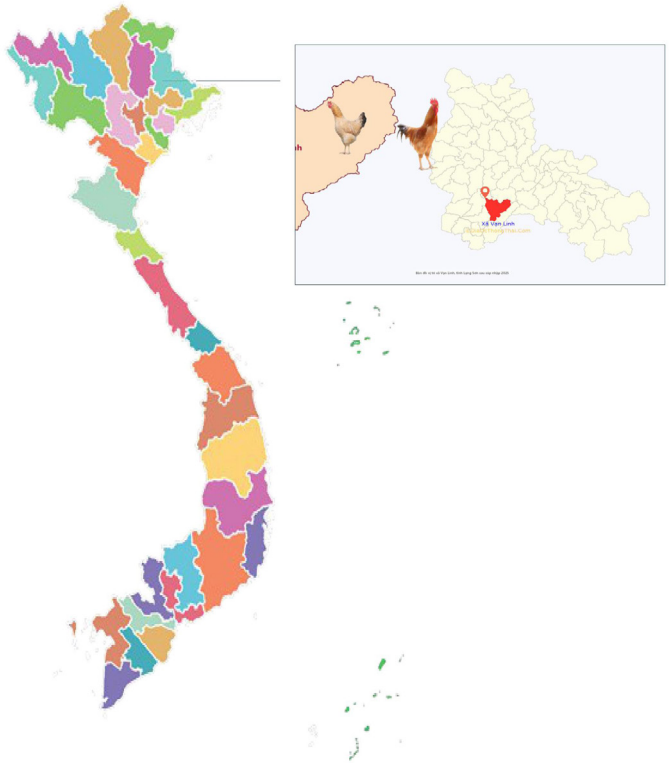

Figure 1: Map showing the DNA sampling location in Van Linh Commune, Lang Son Province, Notheasyern Vietnam (21°39′10″N 106°28′17″E).

Genetic diversity assessment and phylogenetic tree construction: Purified PCR products were subjected to bidirectional Sanger sequencing using an ABI Prism 310 Genetic Analyzer (Applied Biosystems). The raw sequencing data obtained in this study were processed and aligned using ClustalW within the BioEdit Sequence Alignment Editor, after which DnaSP v6.12.03 was employed to evaluate mitochondrial DNA (mtDNA) D-loop diversity metrics, including number of polymorphic sites (S), number of haplotypes (h), haplotype diversity (Hd), nucleotide diversity (π), and total number of mutations alongside neutral evolution testing via Tajima's D. Phylogenetic analysis of 657 bp D-loop sequences from Van Linh chicken and GenBank references (Table 1) was performed using the neighbor-joining (NJ) method and the Kimura 2-parameter model to generate an unrooted tree. Phylogenetic relationships between the Van Linh chicken population and the reference breeds were reconstructed using MEGA v10.0, with topological reliability assessed through 1,000 bootstrap replicates.

Morphological and phenotypic evaluation: Morphological and productive characteristics of Van Linh chicken was evaluated at three developmental stages: day-old chicks, 8-week-old, and 18 weeks of age. Plumage characteristics were systematically recorded, while quantitative morphometric measurements including body length, chest circumference, wing length, and body weight were collected from 800 individuals (400 males and 400 females) at 8 and 18 weeks of age following standardized manual measurement protocols (TCVN 13474-1:2022; Livestock Department, Ministry of Agriculture and Rural Development of Vietnam, 2022).

Table 1: Haplotype designations and corresponding GenBank accession numbers for reference mitochondrial DNA (mtDNA) D-loop sequences of Asian chickens (Miao *et al*., 2013; Teinlek *et al*., 2017; Giang *et al*., 2023).

Serial	Haplotype code	GenBank record	Serial	Haplotype code	GenBank record
1	Vietnamese Ri NC	OR834460	14	RJFspa W	GU261706
2	RJFspa A	GU261695	15	RJFspa X	GU261692
3	RJFspa Bi	GU261704	16	RJFspa Y	GU261693
4	RJFspa Bii	NC_007235.1	17	RJFjab Z	GU261674
5	RJFgal C2	AB007725	18	ChiNC C1	GU261701
6	RJFspa C3	GU261716	19	ChiNC D2	GU261683
7	RJFmur C3	GU261707	20	IndNC D3	GU261697
8	RJFgal D1	NC_007236.1	21	LaoNC E1	AP003319
9	RJFban D1	NC_007237.1	22	IndNC E2	HQ857209
10	RJFmur E3	GU261708	23	ChiNC H	GU261715
11	RJFspa Fi	GU261702	24	JapNC	AB268543
12	RJFspa Fii	GU261703	25	IndNC I	GU261698
13	RJFspa G	GU261690	26	CJF	NC_007239

RESULTS

DNA purity and subsequent PCR success

Genomic DNA extracted from wing feathers of 38 chickens exhibited high molecular weight and integrity, with minimal degradation observed. Spectrophotometric analysis revealed OD260nm/280nm ratios ranging from 1.8 to 2.0, indicating high DNA purity suitable for downstream applications. DNA concentrations were adequate for PCR amplification. The mitochondrial D-loop region was successfully amplified from all samples. The gel electrophoresis image confirmed that the amplified PCR products had the expected fragment size, consistent with that reported by Oka *et al.* (2007). The amplicons appeared as sharp, high-intensity bands with no non-specific amplification or primer-dimers, indicating high specificity of the primers and optimal PCR cycling conditions. These results demonstrate that the DNA extraction from feather samples and the subsequent amplification protocols were highly effective, yielding high-quality templates for sequencing and genetic analysis (Figure 2).

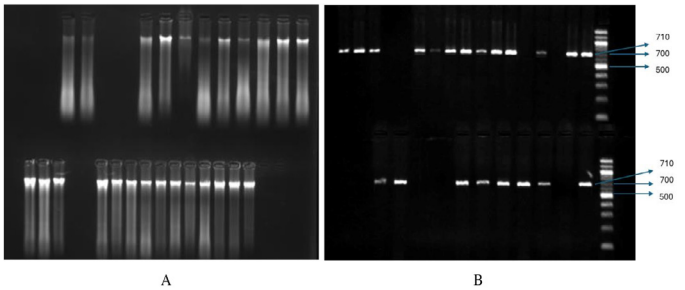

Figure 2: Representation of (A) chicken wing feather drived genomic DNA and (B) the resulting PCR amplicons of the mitochondril D-loop. Lane Marker denotes the GeneRuler 1kb DNA Ladder (Thermo Scientific).

Genetic characterization of Van Linh chicken population

Following the sequencing of 38 PCR amplicons from Van Linh chicken population, a 657-bp consensus sequence of the mitochondrial D-loop region was established. The G+C content was 43.4%, consistent with typical mitochondrial DNA composition in avian species. Sequence analysis revealed 22 polymorphic sites, corresponding to 22 mutations, including 3 singleton variable sites (304, 391, 393) and 19 parsimony-informative sites. The population exhibited a high level of genetic variation, evidenced by a nucleotide diversity (π) of 0.00920±0.00058 and an average number of nucleotide differences (k) of 6.03272, indicating a relatively low degree of sequence divergence across the sampled individuals. Watterson's theta per site (θw) was estimated at 0.00798, which was slightly lower than the nucleotide diversity estimate, suggesting a relatively stable population demographic history. A total of 11 distinct haplotypes were identified among the 38 sequences, yielding a haplotype diversity (Hd) of 0.817±0.042. This relatively high haplotype diversity indicates substantial haplotype variation within the studied population, with high genetic structuring (Table 2).

Table 2: Genetic diversity parameters of the Van Linh chicken population based on 38 mitochondrial D-loop sequences.

Parameter	Value	Notes
Number of sequences analyzed	38	
Analyzed region length (bp)	657	
G+C content	0.434	
Number of variable sites (S)	22	Indicates polymorphic positions
Total number of mutations (Eta)	22	
Nucleotide diversity (π)	0.00920	High diversity
Standard deviation of π	0.00058	
Average number of nucleotide differences (k)	6.03272	
Theta-W per site (from S)	0.00798	Based on Watterson's estimator
Number of haplotypes (h)	11	
Haplotype diversity (Hd)	0.817	High haplotype variation
Standard deviation of Hd	0.042	
Tajima's D	0.51295	Not significant, P > 0.10
Fu and Li's D*	0.74591	Not significant, P > 0.10
Fu and Li's F*	0.73762	Not significant, P > 0.10

Neutrality tests were conducted to assess deviations from neutral evolution and demographic equilibrium. Tajima's D value was positive (0.51295) but not statistically significant (P > 0.10). Similarly, Fu and Li's D* (0.74591) and F* (0.73762) tests showed positive, non-significant values (P > 0.10). These results suggest that the mitochondrial D-loop region evolves neutrally in this population and provide no evidence for recent population expansion, bottleneck events, or natural selection acting on this genomic region. The positive but non-significant neutrality test values are consistent with a population at or near mutation drift equilibrium. In terms of haplotype structure, 11 distinct haplotypes (Hap 01-Hap 11) were identified among 38 sequences. The haplotype diversity (Hd) was estimated at 0.817, indicating a high level of haplotype variation within the studied population. Hap 01 was designated as the reference haplotype. Sequence alignment revealed variation at 22 polymorphic nucleotide sites distinguishing the haplotypes (Table 3). While most haplotypes (Hap 02, 03, 05, 06, 09, 10, and 11) shared common substitutions relative to the reference such as A at

Table 3: Sequence variation among mtDNA D-loop sequences of four varieties of Van Linh indigenous chicken.

Hap	Polymorphic nucleotide sites																						Sam
	167	199	212	217	219	225	242	243	246	256	261	281	306	308	310	315	342	361	363	367	417	446	
Hap 01	T	T	G	C	C	C	C	C	C	C	T	A	T	C	T	C	A	A	C	T	C	T	11S
Hap 02	*	*	A	T	*	*	G	T	T	T	C	*	*	T	C	T	*	*	*	*	*	C	G2
Hap 03	*	*	A	T	*	*	G	T	T	T	C	*	*	*	C	T	*	*	*	*	*	C	12S
Hap 04	*	C	*	*	*	*	G	*	*	*	*	*	*	*	*	*	*	*	*	*	*	*	3S(1)
Hap 05	C	*	*	T	*	T	G	T	*	T	C	*	*	*	C	*	*	T	*	*	*	C	2S(2)
Hap 06	*	*	A	T	*	T	G	T	T	T	C	*	*	*	C	T	*	*	*	*	*	C	G10
Hap 07	*	*	*	T	*	*	A	*	*	*	C	G	*	*	*	*	G	*	T	C	*	C	2S(3)
Hap 08	*	C	*	*	*	*	G	*	*	*	*	*	*	*	*	*	*	*	*	*	T	*	3S(4)
Hap 09	*	*	A	T	*	*	G	T	T	*	C	*	*	*	C	T	*	*	*	*	*	C	G22
Hap 10	*	*	A	T	T	*	G	T	T	T	C	*	*	*	C	T	*	*	*	*	*	C	G29
Hap 11	*	*	A	T	*	*	G	T	T	T	C	*	C	*	C	T	*	*	*	*	*	C	G30

Note: In the alignment, dots indicate identity with the reference haplotype (Hap 01), while letters denote nucleotide substitutions at polymorphic sites. Hap= Haplotype; Sam = sample. 11S= G1, G3, G8, G13, G15, G24, G25, G26, G27, G33, G37; 12S = G4, G7, G9, G11, G16, G17, G19, G23, G31, G32, G34, G36; 3S(1) = G5, G20, G38;2S(2) = G6, G35; 2S(3) = G12, G14; 3S(4) = G18, G21, G28.

position 212 and T at position 217 others exhibited unique mutation patterns. For instance, Hap 07 displayed a highly distinct profile with multiple substitutions, including T at position 212, A at 243, C at 256, G at 281, G at 361, T at 367, and C at 417. Hap 04 and Hap 08 also presented specific variations, with Hap 04 showing a C at position 167 and Hap 08 showing a C at position 199. Among the 11 identified haplotypes, Haplotypes 3 and 1 were the most prevalent, comprising 12 (31.6%) and 11 (28.9%) individuals, respectively. The remaining nine haplotypes were observed at lower frequencies, with five of them identified as singletons occurring in only a single individual.

Phylogenetic relationships and maternal origins

Phylogenetic reconstruction based on mitochondrial D-loop sequences revealed that Van Linh chicken samples exhibit a polyphyletic topology, distributing across multiple well-differentiated clades interspersed with reference sequences from Red Jungle fowl (*Gallus gallus*) and regional domestic populations (Figure 3). The overall tree topology, supported by bootstrap values ranging from 40 to 98, indicates substantial maternal genetic heterogeneity within the Van Linh population. The predominant maternal lineage was recovered in Clade B (upper section), which harbored most of Van Linh samples 5416543/G17 (G17), G19, G16, G4, G9, G11, G7, G2, G1, G8, G10, G13, and others. This assemblage formed a cohesive clade (bootstrap = 54) with Southeast Asian Red Jungle fowl references, particularly *Gallus gallus spadiceus* (RJFspa Bii, RJFspa Bi, RJFspa A) and the Vietnamese Ri native chicken (Vietnamese Ri NC), corroborating a primary maternal ancestry derived from autochthonous jungle fowl subspecies consistent with the Southeast Asian domestication center. Some branches were supported by relatively low bootstrap values, indicating limited statistical support for the inferred phylogenetic relationships. This is not uncommon in studies of genetic diversity, particularly when closely related haplotypes or short mitochondrial DNA fragments are analyzed. Nevertheless, the sequenced D-loop region contained numerous polymorphic sites, revealing substantial genetic diversity and multiple maternal lineages within the Van Linh chicken population. Additional maternal lineages were resolved in distinct subclades. Specimens G6 and G1 clustered within Clade A alongside Ri Vang Rom and RJF spa A reference sequences, a grouping supported by a bootstrap value of 73 that signifies close genetic affinity. A separate subclade (Clade C; bootstrap = 76-81) united Van Linh genotypes with the Chinese-derived sample ChiNC C1, indicating discrete maternal introgression from East Asian gene pools.

A further distinct assemblage (Clade E) comprised Van Linh samples (G5, G20, G18, G21, G7, G1, G3, G4, G6, G12, G8, G13, G15) that grouped with Indian (IndNC E2, IndNC D3), Lao (LaoNC E1), and Chinese (ChiNC D2, ChiNC C1) reference lineages with high nodal support (bootstrap = 40-61). This phylogeographic pattern suggests historical gene flow mediated by anthropogenic dispersal, trade networks, or structured crossbreeding programs that introduced maternal haplotypes from South and East Asian populations. Notably, several Van Linh samples (G12, G14) formed a tightly supported cluster (bootstrap = 76-81) with Chinese native chicken (ChiNC C1, ChiNC D2), providing molecular evidence for specific maternal contributions from Chinese breeds, a finding congruent with documented historical livestock exchange between Vietnam and China.

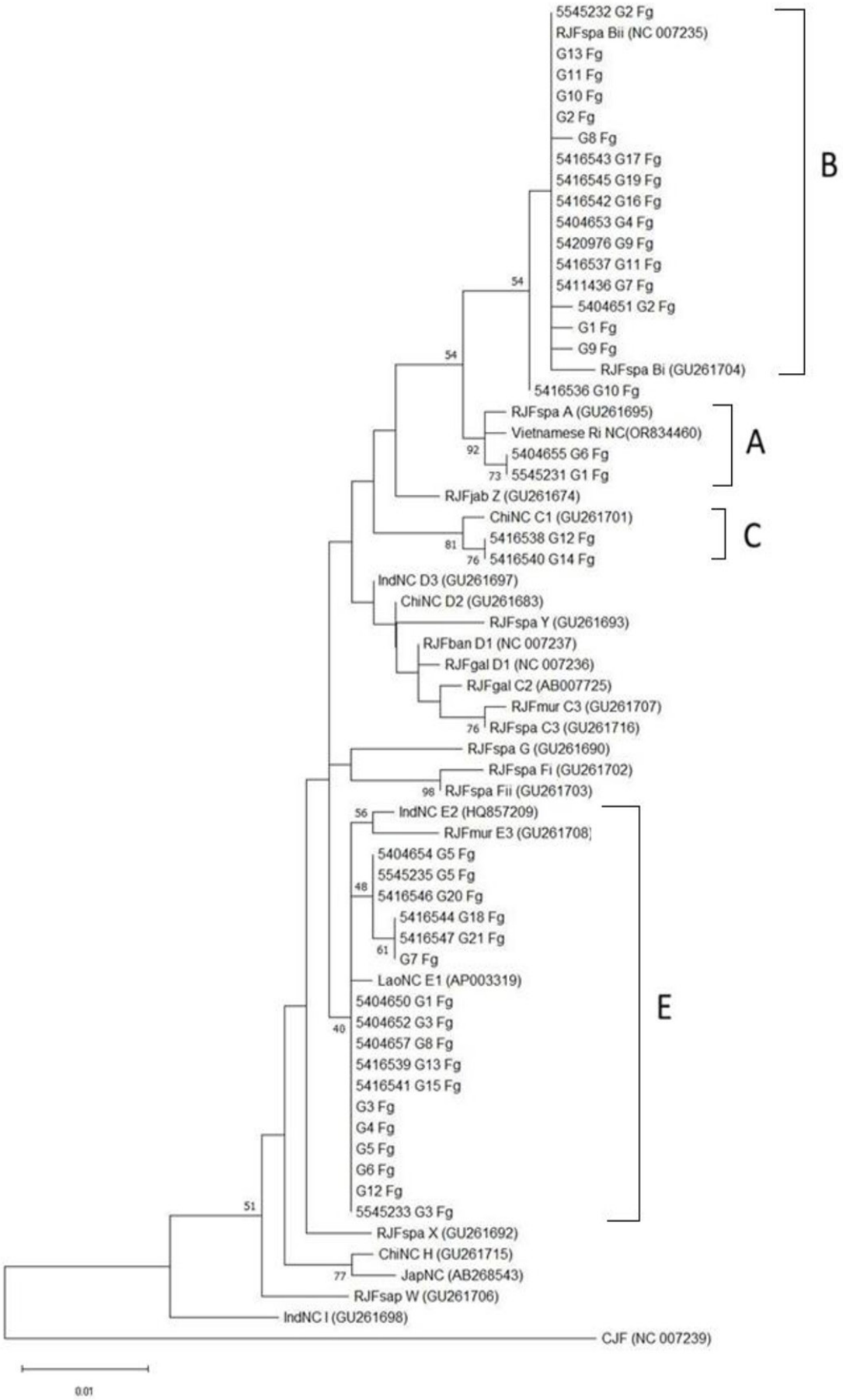

Figure 3: Neighbor-joining phylogenetic tree of Van Linh chicken based on mitochondril D-loop sequences. Samples labeled from G1 to G38 correspond to the Van Linh population. References sequences from various Gallus species were retrieved from GenBank for comparative analysis.

Table 4: Body weight, size of Van Linh chicken according to age (g, cm).

Trait	Week 8 (Mean ± SE)		Week 18 (Mean ± SE)	
	Male	Female	Male	Female
Body weight (g)	785.52^{a} ± 5.83	651.73^{ab} ± 4.45	1590.5^{a} ± 10.10	1213.0^{b} ± 17.00
Body length (cm)	16.50^{a} ± 0.57	14.80^{ab} ± 0.42	20.70^{a} ± 0.38	18.80^{b} ± 0.27
Chest circumference (cm)	23.58^{a} ± 0.12	21.00^{ab} ± 0.16	31.97^{a} ± 0.30	28.00^{b} ± 0.22
Wing feather length (cm)	41.04^{a} ± 0.75	37.77^{b} ± 1.18	52.21^{a} ± 0.84	43.79^{b} ± 0.36

Note: Within the same week of age, means in the same row with different superscript letters are significantly different between males and females ($P < 0.05$).

Outgroup taxa including *Gallus sonneratii* (RJFsap W), Japanese native chicken (JapNC), and other regional variants were phylogenetically segregated from the main Van Linh clades, thereby validating the taxonomic assignment of the study population as *Gallus gallus domesticus*. The polyphyletic distribution of Van Linh maternal lineages across the inferred phylogeny reflects three non-mutually exclusive evolutionary processes: (i) a complex domestication history in Southeast Asia characterized by multiple, potentially independent, domestication events; (ii) extensive historical gene flow facilitated by human-mediated migration and regional trade; and (iii) traditional smallholder production systems that promoted the admixture of genetically heterogeneous stocks. This inferred demographic complexity is quantitatively consistent with the elevated haplotype diversity (Hd= 0.817) and high nucleotide diversity (π= 0.00920) observed within the Van Linh population, collectively underscoring its value as a reservoir of indigenous avian genetic variation.

Structural characteristics and productivity metrics

Phenotypic observations indicate that hatched chicks are predominantly covered in pale yellow natal down. A subset of individuals exhibits darker yellow markings or brown barring on the head, back, and wing regions (Figure 4a, b, c, d). Sexual dimorphism in comb development and plumage pigmentation becomes apparent from 8 weeks of age. At 18 weeks, hens possess relatively small combs and display pale buff to cream, pale-yellow, or pale-brown plumage, featuring distinct black and brown spotting on the wing and tail feathers. In contrast, cocks develop prominent combs and exhibit body plumage ranging from deep crimson to predominantly red-orange or orange-brown plumage. Their wing and tail feathers feature iridescent blue-black or brown markings, with the wing plumage appearing distinctly darker than the body. Additionally, males display characteristic hackle (neck) feathers in shades of brown to reddish-brown. Compared to other Vietnamese native chicken breeds such as the Ri or Mia chickens, which typically exhibit uniform brown or sesame plumage with less pronounced sexual dimorphism, Van Linh cocks display a much more striking crimson-to-orange coloration (Le *et al*., 2024; Pham *et al*., 2023). In contrast to native Vietnamese duck breeds exhibit distinct waterfowl phenotypic traits, characterized by different coloration patterns, such as the iridescent green head of male Vit Co or the uniform dark-brown coat of Vit Bau (Nguyen *et al*., 2024; Tran *et al*., 2023).

Figure 4: Phenotypic characteristics of Van Linh chicken at day old chicks (a); week old chickens (b); male at 18 weeks old chickens (c); female at 18 weeks old chickens (d).

Significant age-related differences in body dimensions were observed in Van Linh chickens (Table 4). At 8 weeks, male/female body length, weight, chest circumference, and wing feather length were 16.5 and 14.80 cm, 785.52 g and 651.73 g, 23.58 and 21.00 cm, and 41.04 and 37.77 cm, respectively. By 18 weeks, these values reached 20.7 and 18.8 cm, 1590.5 g and 1213.0 g, 31.97 and 28.00 cm, and 52.21 and 43.79 cm. Under the study conditions, the chickens exhibited relatively uniform morphometric characteristics, as evidenced by the consistently small standard errors (SE). Sexual dimorphism was observed for most morphometric traits at 18 weeks of age ($P < 0.05$). However, such differences were generally not apparent at 8 weeks of age, with the exception of wing length.

DISCUSSION

Sequence of the mitochondrial D-loop region of Van Linh chicken exhibited high haplotype diversity and nucleotide diversity. When contextualized within regional datasets, the Hd of Van Linh chickens is considerably lower than the pooled average reported for ten Vietnamese indigenous breeds (Hd= 0.918; Giang *et al*., 2023) and falls below values documented for specific breeds such as Lien Minh (Hd = 0.913) and Chin Cua (Hd = 0.867; Nguyen *et al*., 2022). Conversely, the nucleotide diversity π = 0.00920 markedly exceeds the national average for Vietnamese native chicken π = 0.0063 reported by Giang *et al*. (2023) as well as estimates for Thai π = 0.00579 indigenous chickens by Teinlek *et al*. (2018) and Philippine π= 0.00434 populations by Godinez *et al*. (2021). This discordance between haplotype richness and nucleotide divergence indicates that while the absolute number of maternal lineages is comparatively limited, the extant lineages exhibit substantial sequence divergence, reflecting deeply rooted and phylogenetically distinct maternal origins. The highly skewed distribution of haplotypes, dominated by Hap 1 and Hap 3 (which together account for more than 60% of the sampled individuals), suggests a possible founder effect or historical breeding practices within local farming communities that may have contributed to shaping the current genetic structure of the Van Linh chicken population.

Do *et al*. (2019) characterized the maternal genetic diversity of three Vietnamese indigenous breeds (Mong, To, and Sau Ngon). Notably, the Mong and Sau Ngon populations exhibit a phylogenetic clustering pattern and haplotype composition (12 of 16 haplotypes concentrated in clades B and E) that closely parallels the maternal genetic structure of the Van Linh chicken observed in the present study. In contrast, the To breed was predominantly associated with clade E (90.48% of clade E), while clade A occurred at low frequency, represented by only a single haplotype in the Mong breed. This distribution, characterized by a few high-frequency maternal lineages alongside numerous low-frequency haplotypes (singletons), mirrors patterns frequently observed across Southeast Asian indigenous poultry, including populations in Laos and the Philippines. The retention and sharing of core haplotypes within the Van Linh population likely stem from traditional smallholder production systems, wherein maternal lineages are maintained locally across generations with minimal external introgression, thereby preserving ancestral genomic signatures. Tests of selective neutrality yielded non-significant results for both Tajima's D (0.51295) and Fu's Fs (1.208) statistics ($P > 0.10$). This outcome aligns with previous findings in four Vietnamese indigenous breeds (Nguyen *et al*., 2022) and Thai native chicken (Teinlek *et al*., 2018), collectively indicating that the Van Linh population resides in a mutation–drift equilibrium. The non-significant neutrality indices further suggest the absence of recent demographic expansions or severe genetic bottlenecks. Rather, the population appears to have evolved primarily under neutral evolutionary processes, consistent with conventional free-range management practices that promote random mating and minimize the intense artificial selection pressures characteristic of commercial poultry lines.

The relatively high genetic diversity observed in Van Linh chickens, particularly the elevated nucleotide diversity, underscores their value as a critical repository of indigenous avian germplasm. In contrast to several local breeds reported to exhibit declining diversity due to inbreeding and population fragmentation, the Van Linh population has successfully maintained substantial maternal variant richness. These molecular findings provide a robust empirical foundation for pursuing geographical indication (GI) registration for this local breed and for designing targeted breeding and conservation strategies aimed at preserving and enhancing its unique genetic architecture in future generations.

Comparative analysis indicates that Van Linh chickens exhibit superior growth performance relative to conventional Vietnamese indigenous lines. Comparative analysis revealed that Van Linh chickens exhibit a growth rate comparable to that of other prominent indigenous Vietnamese chicken breeds. Specifically, their body weight at 18 weeks was comparable to that of Ri Vang Rom and selected Ri chickens at 19–20 weeks (Ngo and Nguyen, 2018). Van Linh females reached a body weight of 1,213.0 g at 18 weeks of age, representing approximately 73.5% of the onset-of-lay body weight of Bang Troi females (1,650 g at 21 weeks of age), suggesting relatively rapid growth and favorable potential for meat production (Nguyen *et al*., 2020). The superior body length and chest circumference observed in Van Linh chickens compared to the Bang Troi breed underscore their enhanced skeletal framework and pectoral muscle development. These morphometric advantages confirm the breed's high potential for meat-oriented production systems, while females record a chest circumference of 28.00 cm. These metrics suggest superior skeletal development and pectoral muscle accretion, traits of high relevance for meat-oriented breeding. Consistent with patterns observed in Mong, Te, and Tien Yen breeds, Van Linh chicken display pronounced sexual dimorphism, collectively classifying them as a medium-to-large indigenous breed with significant potential for dual-purpose or specialized meat production systems.

CONCLUSION

This study delineates the maternal genetic architecture of Van Linh chicken via mitochondrial D-loop sequencing of a 657 bp fragment, identifying 11 haplotypes with 22 polymorphic sites, high haplotype diversity (Hd = 0.817), and elevated nucleotide diversity (π = 0.00920) significantly exceeding the national average for Vietnamese indigenous breeds. Tests for mutation-drift equilibrium indicate the absence of recent demographic bottlenecks or expansions, reflecting the stabilizing influence of traditional free-range management and underscoring the breed's phylogeographic uniqueness and genetic resilience through the coexistence of predominant and rare maternal lineages. Collectively, these molecular findings establish a robust baseline for geographical indication registration, evidence-based conservation prioritization, and the design of sustainable breeding strategies to preserve this valuable indigenous poultry resource.

CONCLUSIONS AND RECOMMENDATIONS

In this study, phenotypic characterization and genetic diversity analyses were conducted independently rather than on the same individuals. Consequently, it was not possible to determine whether individuals sharing the same haplotype also exhibit similar or different phenotypic characteristics. Further studies integrating phenotypic and genetic data from the same individuals are required to elucidate this relationship and provide more comprehensive conclusions.

ACKNOWLEDGEMENTS

This research was supported by the Science and Technology Research Fund of Hung Vuong University, Phu Tho, Vietnam under grant number HV30.2024.

NOVELTY STATEMENT

The novelty of this study lies in being the first comperhensive investigation to evaluate the genetic diversity and reconstruct the phylogenetic relationship of Van Linh chicken, an indigenous Vietnamses chicken breed, through comparison with several chicken breeds from different regions Wolrdwide.

AUTHOR'S CONTRIBUTION

To the best of our knowledge, this study represents the first comprehensive report on the genetic diversity and phylogenetic characterization of Van Linh chicken, a native Vietnamese chicken breed reared in Lang Son Province, Vietnam. All authors made substantial contributions to this study, including experimental design, data collection, and statistical analysis. All authors were actively involved in drafting, critically revising, and approving the final version of the manuscript for submission.

GENERATIVE AI AND AI ASSISTED TECHNOLOGY STATEMENT

We certify that this work has no conflicts of interest and no generative AI and AI assisted technology was used in the creation of this manuscript

STATEMENT OF CONFLICT OF INTEREST

The authors have declared no conflict of interest.

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