



Genetic Characterization of the Vang Cattle Population using mtDNA Chromosomal, Y Chromosomal Haplotypes and Polymorphisms of Genes Associated with Economic Traits

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Abstract | The local Vang cattle currently live primarily in the An Giang Seven Mountains, Vietnam. In some of the villages of the ethnic minority, cattle have phenotypes of Vietnam native Vang cattle, with very poor milk yield, carcass weight, and limited information about fertility. Consequently, cattle are at risk of becoming extinct, and the genetic diversity and polymorphism of genes linked to economically important traits have not yet been examined. This study centered on examining the genetic traits of Vang cattle by utilizing molecular markers. We investigated the nucleotide sequences of mitochondrial DNA and the *SRY* gene on the Y chromosome, as well as performed genotyping of the *DGAT1*, *NCAPG*, and *RNF212* genes related to economically important traits such as milk yield, carcass weight, and fertility within the Vang cattle population. The analysis of the mitochondrial DNA sequences indicated that the Vang cattle carry the *Bos indicus* type I1 haplotype, which implies a significant level of genetic diversity within their maternal lineage. According to the *SRY* gene sequence study, all of the males have a zebu-type haplotype, which could have resulted from a hybrid between *Bos javanicus* and zebu. Based on the genotyping analysis of the functional genes, the *DGAT1* gene exhibits polymorphism, whereas the *NCAPG* and *RNF212* genes display monomorphism. This result may have important implications for improving milk yield of the Vang breed based on molecular markers of the *DGAT1* gene. Therefore, the present study may provide useful information for future breeding and conservation efforts of this Vang breed.

Keywords | Vang cattle, Genetic diversity, mtDNA, Polymorphisms, SNPs, Y chromosome

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INTRODUCTION

There are more than six unique indigenous cattle breeds and more than fourteen imported cattle breeds in Vietnam (Pham *et al.*, 2013; Hoang Kim Giao, 2009). Of

which, the Vietnamese Vang (Yellow) cattle is a native breed, and there is no purebred breed. These animals are referred to by various names depending on the region and ethnic group. They are adaptable to harsh environments, resistant to diseases, low feed requirements, and have a beau-

tiful coat color with a bright yellow color. Therefore, this was the main breed of cattle, raised for a long time in Vietnam (Huyen Le Thi and Karen, 2020). The specific type of cattle focused on in this research is known as the Vang cattle among the ethnic minorities residing in the Seven Mountains area of An Giang Province. Accordingly, the milk production and carcass weight of the cattle are notably low, and there is also a scarcity of data regarding their fertility. As a result, the Vang cattle are at risk of extinction. Therefore, the decline in genetic diversity is happening rapidly, while information on population genetic diversity and the phylogenetic relationship of the An Giang Vang cattle has not been clearly published.

Since livestock's economically important characteristics, like milk yield, carcass weight, and fertility, are impacted by a combination of genes, the genetic profiles of these genes can be utilized to assess the livestock's genetic potential. For instance, studies by Ripol *et al.* (2006); Hoashi *et al.* (2007); Kong *et al.* (2008), Yamada *et al.* (2008), Lindholm-Perry *et al.* (2013); Cardoso *et al.* (2014) have demonstrated the impact of genetic variations on beef cattle productivity. Additionally, research by Nishimaki *et al.* (2016); Okuda *et al.* (2017); Le *et al.* (2018) explored genotype distribution and frequency in cattle breeds to assess their genetic capabilities. Since genetic polymorphisms can be applied to marker-assisted selection for economically valuable traits, investigating genetic polymorphisms in Vang cattle could aid in both genetic characterization and conservation efforts of this traditional breed, as well as enhancing their genetic potential. In this study, we analyzed the polymorphisms of three key genes in the Vang cattle. These included the diacylglycerol O-acyltransferase 1 (*DGAT1*) gene, which is linked to milk production and fat content (Grisart *et al.*, 2002), the non-SMC condensin I complex subunit G (*NCAPG*), associated with carcass weight (Eberlein *et al.*, 2009), and the ring finger protein 212 (*RNF212*) gene, which plays a significant role in controlling crossover events during meiosis and is related to high fertility (Sandor *et al.*, 2012). These were genotyped using PCR-RFLP methods.

Additionally, we studied mitochondrial DNA (mtDNA) and Y-chromosome haplotypes in Vang cattle. Since mtDNA is maternally inherited and Y-chromosomal sequences follow paternal inheritance, these markers have been widely used to trace the maternal and paternal phylogenetic lineages of livestock. (Edwards *et al.*, 2007; Mohamad *et al.*, 2012; Syed *et al.*, 2013; Le *et al.*, 2018). Notably, bovine mtDNA and Y-chromosome haplotypes are divided into *Bos taurus* and *Bos indicus* (zebu) haplogroups (Achilli *et al.*, 2009; Verkaar *et al.*, 2003).

MATERIALS AND METHODS

SAMPLE COLLECTION

Due to budget limitations as well as the suitable sample sizes in the studies of Okuda *et al.* (2017); Le *et al.* (2018), a total of thirty blood samples were randomly collected from healthy Vang cattle aged between 2 and 5 years, comprising fifteen females and fifteen males, from a household in the villages of the ethnic minority in the Seven Mountains area of An Giang Province, Vietnam. In adherence to ethical animal practices, approximately 2 mL of blood was drawn from the jugular vein, and the blood samples were preserved at 4°C. Genomic DNA extraction involved standard procedures for isolating white blood cells through centrifugation, followed by protein digestion using Proteinase K, DNA extraction with a combination of phenol, chloroform, and isoamyl alcohol, and DNA precipitation using ethanol (Barker, 1998). The concentration of the total DNA yield was assessed with a Nanodrop 2000 spectrophotometer (ThermoFisher Scientific, USA).

EXAMINATION OF mtDNA AND SRY GENE HAPLOTYPE DIVERSITY

To assess the haplotype diversity of mitochondrial DNA (mtDNA) in Vang cattle, a 650-bp mtDNA fragment was amplified using specific primer pairs (Table 1), based on the forward primer described by Loftus *et al.* (1994) and the reverse primer outlined by Le *et al.* (2018). The resulting

Table 1: Sequences of primers, lengths, and the annealing temperatures for functional genes.

Gene	Primer sequences (5'- 3')	Lengths (bp)	Annealing temperatures (°C)	Resources
mtDNA	F: CTGCAGTCTCACCATCAACC	650	58	Loftus <i>et al.</i> (1994) Thu <i>et al.</i> (2018)
	R: CCTTTGACGGCCATAGCTGA			
SRY	F: CCGGGCTATAAATATCGACCT	1062	58	Nijman <i>et al.</i> (2008)
	R:GATGAAACCTTGGGTCTCACAG			
DGAT1	F: GCACCATCCTCTTCCTCAAG	411	66	Grisart <i>et al.</i> (2002)
	R: GGAAGCGCTTTCGGATG			
NCAPG	F: ATTTAGGAAACGACTACTGG	129	51	Eberlein <i>et al.</i> (2009)
	R: ATTTGTATTCTCTTATTATCATC			
RNF212	F: GGGTCACCACAGTCCAGAGT	567	58	Sandor <i>et al.</i> (2012)
	R: GCTGCCTGTAAGGAGGTTCT			

Table 2: Restriction enzymes and genotyping.

Gene	Cut positions	Restriction enzymes	Temperature (°C)/time	Genotype	Allele size (bp)
<i>DGAT1</i> (K232A)	5'...AA/GC...3'	<i>EaeI</i>	37/24 hours	KK	411
				KA	411; 203; 208
				AA	203; 208
NCAPG (A>G)	5'...AA/TT...3'	<i>Tsp509I</i>	65/45 minutes	GG	129
				GT	129; 66; 63
				TT	66; 63
RNF212 (C>T)	GGATC(N)4 CCATG(N)5	<i>AlwI</i>	37/24 hours	CC	386; 168; 13
				CT	386; 203; 168; 183; 13
				TT	203; 183; 168

amplified fragment was subsequently sequenced directly using these primers. The polymerase chain reaction (PCR) was conducted with a total volume of 10 µl DNA, containing 0.2 µM of each primer, 0.2 µmol/l dNTPs, 2×PCR buffer, and 1U of Kod FX Taq DNA polymerase (Toyobo, Osaka, Japan). The PCR protocol consisted of 35 cycles involving denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, and extension at 72°C for 90 seconds. Following amplification, the PCR products were run on an agarose gel in TAE buffer, stained with 6X GelRed® dye (Biotum, USA), and separated through electrophoresis on a 2% agarose gel using 0.5X TAE buffer at 100V for 25 minutes, with HaeIII serving as a standard molecular weight ladder. The results from electrophoresis were analyzed using the Gel DocTM XR+ system (Bio Rad, USA).

The electrophoresis outcomes following purification with Exo sap enzymes were subjected to Sanger sequencing. The resulting sequence data were aligned utilizing MEGA11 software (Tamura *et al.*, 2021), ultimately producing a 240-bp dataset spanning from position 16,023 to 16,262 of the mtDNA gene sequence. This was compared with sequences and accession codes in GenBank (NCBI), specifically V00654 for *Bos taurus* and L27733 for *Bos indicus* (Troy *et al.*, 2001; Chen *et al.*, 2010).

To examine the Y-chromosomal haplotypes of fifteen male Vang cattle, a 1,062 bp fragment of the *SRY* gene located in the male-specific region of the Y chromosome was amplified using a primer pair listed in Table 1 (Nijman *et al.*, 2008). These amplified fragments were sequenced simultaneously using the same primers. The PCR reactions were conducted in 10 µL mixtures, each containing 10 ng of genomic DNA, 0.2 µM primers, 0.25 µmol/L dNTPs, 2×PCR buffer, and 1 U of Kod FX Taq polymerase (Toyobo, Osaka, Japan). The amplification protocol consisted of 35 cycles, featuring a denaturation step at 94°C for 15 seconds, annealing at 58°C for 30 seconds, and an extension phase at 72°C for 45 seconds (Verkaar *et al.*, 2004). The resulting sequences were aligned using Clustal W, and a phylogenetic tree was constructed using the Neighbor-Joining method in MEGA11 software (Tamura *et al.*, 2021).

DETERMINATION OF GENETIC RELATIONSHIPS BETWEEN CATTLE POPULATIONS

An investigation into the genetic relationships among different cattle populations was conducted by assessing the sequence polymorphisms of the mitochondrial D-loop region. This analysis utilized the Tamura and Nei method (1993). A phylogenetic tree for the An Giang Vang cattle was created using 240 base pairs of mtDNA derived from 127 sequences, which comprised 30 sequences from An Giang Vang cattle and 97 sequences from native cattle found in South Asian nations, China, Southeast Asia, and India. The regions represented include Bhutan (Chen *et al.*, 2010; Lin *et al.*, 2007), Nepal (Takeda *et al.*, 2004; Fujise *et al.*, 2003), Bangladesh (Bhuiyan *et al.*, 2007), India (Chen *et al.*, 2010), Pakistan (Chen *et al.*, 2010), China (Lei *et al.*, 2006), Myanmar (Chen *et al.*, 2010), Cambodia (Chen *et al.*, 2010), Laos (Chen *et al.*, 2010), and Vietnam (Chen *et al.*, 2010). The construction of the tree was performed using the Neighbor-Joining (NJ) method via MEGA11 software (Tamura *et al.*, 2021).

Genetic relationships between populations were identified by evaluating the *SRY* gene sequence polymorphisms of the An Giang Vang cattle and exotic cattle populations by referencing sequences from Genbank DQ336526 (*Bos taurus*), DQ336527 (*Bos indicus*), and DQ336528 (*Bos javanicus*) (Namikawa *et al.*, 1983).

IDENTIFICATION OF FUNCTIONAL GENOTYPES

To genotype the functional genes *DGAT1*, *NCAPG*, and *RNF212*, amplification was performed using the primer pairs listed in Table 1. Genotyping was conducted through PCR-RFLP utilizing the restriction enzymes *EaeI*, *Tsp509I*, and *AlwI*, in accordance with the methodologies outlined by Grisart *et al.* (2002); Eberlein *et al.* (2009); Sandor *et al.* (2012), respectively (Table 2). The PCR reactions occurred in 10 µl mixtures consisting of 10 ng of genomic DNA, 0.2 µM primers, 0.25 µmol/L dNTPs, 2 × PCR buffer, and 1 U of Kod FX Taq DNA polymerase (Toyobo, Osaka, Japan). The cycling conditions included 35 to 40 cycles of denaturation at 94°C for 30 to 120 seconds, the appropriate annealing temperatures as indicated in Ta-

ble 1 for 30 to 60 seconds, followed by extension at 72°C for 30 to 120 seconds.

Following PCR amplification and subsequent digestion with restriction enzymes, the PCR fragments were subjected to electrophoresis on an agarose gel using TAE buffer and then stained with Gelred. For size reference, HaeIII was employed, displaying bands measured at 2,000, 1,500, 1,000, 600, 500, 400, 300, 200, and 100 bp, with visualization achieved via a UV transilluminator. Allele and genotype frequencies were determined in accordance with the Hardy-Weinberg equilibrium (HWE) principle, calculating the variance between expected and observed values ($p = P + H/2$, $q = Q + H/2$), where p and q represent allele frequencies (Mader, 2001; Dino et al., 2018; Le et al., 2018).

The key research question of this study aims to investigate: What genetic diversity and phylogenetic relationships characterize the Vang cattle population in An Giang? Additionally, how do gene polymorphisms correlate with significant economic traits such as meat production, milk quality, and fertility?

RESULTS AND DISCUSSION

mtDNA HAPLOTYPE DIVERSITY IN AN GIANG VANG CATTLE

To evaluate the genetic diversity of mtDNA haplotypes, the Vang cattle samples were sequenced for the nucleotides of the D-loop region. Analysis of 240 bp mtDNA, from position 16023-16262. The results in Table 3 show that these cattle possess twelve nucleotide polymorphisms and seven haplotype groups (haplogroups) consisting of I1, I2, and five haplotypes belonging to I1. The nucleotide sequences of these five haplotypes have only one nucleotide substitution from haplotype I1, and therefore they are classified into group I1 (Figure 1) and are tentatively named: I1.V.NH1, I1.V.NH2, I1.V.NH3, I1.V.NH4, and I1.V.NH5. All these seven haplotypes were similar to the Bos indicus sequence, and were submitted to GenBank with accession numbers from OR449742 to OR449747 and OR449752. Nearly all mtDNA sequences from Bos taurus fall under the macro-haplogroup T, which comprises six sub-haplogroups: T (originating from the Near East), T1 (originating from Africa), T2 (also originating from the Near East), T3 (originating from both the Near East and Europe), T4 (originating from East Asia), and T5 (Troy et al., 2001; Mannen et al., 2004; Achilli et al., 2009). In contrast, all Bos indicus mtDNA sequences group together within macro-haplogroup I, which can be further categorized into two sub-haplogroups: I1 (based in the Indus Valley) and I2 (Chen et al., 2010). When compared with the standard reference sequence V00654 (Bos indicus), the D-loop mtDNA region in Vang cattle had twelve substitution polymorphisms ($G \rightarrow A$); and ($C \rightarrow T$); there

were seventeen Vang cattle individuals with mtDNA carrying the same genetic pattern as the reference sequence; 7 individuals (I1.V.NH1) had the same genetic pattern as the I1- 49; 2 individuals had the I1-2 haplotype; 1 cattle had mtDNA carrying the same genetic pattern as I2 (I2-28); and 3 individuals I1.V.ANH3, I1.V.ANH4, and I1.V.ANH5 carrying the haplotype belonging to I1, in which I1.V.ANH5 is a new haplotype and has not been observed in cattle breeds (accession numbers OR449752). This result suggests that An Giang Vang cattle belong to the Bos indicus I1 group.

Table 3: mtDNA haplotype diversity of Vang cattle.

		Nucleotide position											
		1	1	1	1	1	1	1	1	1	1	1	1
		6	6	6	6	6	6	6	6	6	6	6	6
Cattle Haplotype		0	0	0	0	0	0	0	1	1	2	2	
		4	5	5	5	5	7	8	8	1	4	3	3
		9	0	1	6	7	4	4	5	0	1	0	3
L27733 ^a	I1	T	C	T	A	A	C	T	T	C	T	G	C
V00654 ^b	T3	C	C	T	A	G	T	C	T	C	T	A	C
Vang cattle(n=17) I1		.	.	.	.	.	.	.	.	.	.	.	.
Vang cattle (n=7) I1.V.NH1 ^c		.	.	.	.	.	.	.	.	.	.	.	T
Vang cattle (n=1) I1.V.NH2 ^c		.	.	.	.	.	.	.	.	.	.	.	C
Vang cattle (n=2) I1.V.NH3 ^c		.	.	.	.	.	.	.	.	.	.	T	.
Vang cattle (n=1) I1.V.NH4 ^c		.	.	.	.	G	.	.	.	.	.	.	.
Vang cattle (n=1) I1.V.NH5 ^c		.	.	.	G	.	.	.	.	.	.	.	.
Vang cattle (n=1) I2		.	.	.	.	.	.	C	C	.	C	.	T

^aBos taurus; ^b Bos indicus; ^cVang haplotype.

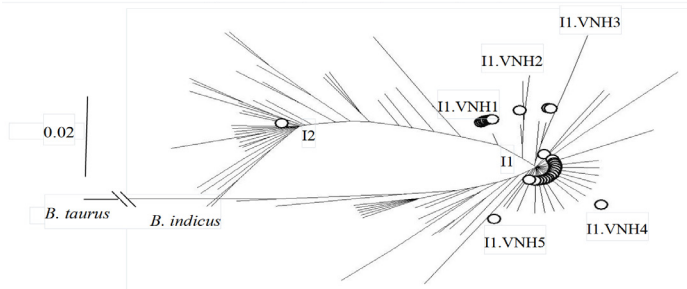


Figure 1: Phylogenetic tree of Vang cattle*; (*Constructed using the Neighbor-Joining (NJ) method by MEGA11 software, based on 240 bp mtDNA of 127 sequences, including 30 An Giang Vang cattle and 97 sequences of indigenous cattle in South Asia, China, Southeast Asia, and Europe. Circles indicate the seven haplotypes found in An Giang Vang cattle).

Okuda et al. (2017) analyzed 27 mtDNA samples from Laotian native cattle, and the results also found only 3 haplogroups belonging to I1, no new haplotypes, and no haplotypes belonging to the Bos taurus group. Moreover, analyzing 28 mtDNA samples from Pesisir native cattle in Indonesia, Dino et al. (2018) presented that there was 1

haplotype I2, and 1 out of 5 haplotypes were new, all belonging to group I1 of *Bos indicus*. The results of the study of Yellow cattle in Vietnam (Le et al., 2018) suggested that this Yellow cattle population belongs to the *Bos Indicus* I1 group. In Ha Giang Province of Vietnam, Berthouly et al. (2010) also reported that the Ha Giang indigenous cattle population (H'mong cattle) belongs to the *taurine-zebu* group. Most of the I2 haplotype occurred in Southeast Asia with very low rates, as shown not only in the above studies but also in studies by Dino et al. (2018); Le et al. (2018); Xia et al. (2019). Therefore, Chen et al. (2010) suggested that the near absence of the I2 group in Southeast Asia may be due to the later introduction of this group into the domestic cattle group in the country and possibly because the culture at that time prevented the spread of the I2 group. The later scattered appearance of I2 in the region may be explained by trade exchange.

As a result, the analysis of the An Giang Vang cattle in this study identified seven haplogroups, classified into groups I1, I2, and a novel haplotype. Their relative frequencies exceed those documented in previous research performed in Laos, Japan, Indonesia, and Vietnam (Mannen et al., 2000; Mannen et al., 2017; Dino et al., 2018; Le et al., 2018, respectively). This finding suggests that the An Giang Vang cattle population exhibits a significant level of genetic diversity within its maternal lineage.

DIVERSITY OF SRY HAPLOTYPE IN VANG CATTLE

We also identified the Y chromosomal haplotype of the An Giang Vang cattle by sequencing a 1,062 bp fragment of the *SRY* gene found on the Y chromosome. In their study on the Y-chromosomal haplotypes among cattle, Nijman et al. (2008) categorized these haplotypes into three groups: *Bos taurus*, *Bos indicus*, and Southeast Asian Banteng-*Bos javanicus*, based on five SNPs within this region. As outlined in Table 4, all fifteen male cattle displayed a unique haplotype defined by 10 polymorphic positions. Of these, seven nucleotide positions matched the haplotype nucleotide sequences in *Bos indicus*, while the other seven were aligned with the haplotype nucleotide sequences of *Bos javanicus*, with no representation of the *Bos taurus* haplotype. This Vang haplotype has been provisionally designated as VAGY. Genetic studies on local breeds of Indonesian native cattle have revealed that there is gene flow from Banteng into several of these breeds (Namikawa et al., 1983; Nijman et al., 2003; Tanaka et al., 2011; Mohamad et al., 2012). Conversely, other research indicates that male cattle in Indonesia and Vietnam display zebu traits (Dino et al., 2018; Le et al., 2018), with a limited occurrence of *Bos taurus* type also noted in Vietnam (Le et al., 2018). These findings suggest a possible paternal gene flow from Banteng into Vang cattle. Consequently, the examination of mtDNA gene nucleotypes and polymorphisms of the Y chromosome *SRY* gene revealed that An Giang Vang cattle are

derived from zebu cattle on both the maternal and paternal sides. Notably, the paternal lineage may represent a hybrid of Southeast Asian wild cattle and zebu cattle, offering valuable insights into the origin and historical development of An Giang Vang cattle in Vietnam.

Table 4: Haplotype diversity of the *SRY* gene in Vang cattle.

Haplotype	Nucleotide position									
	1	1	2	2	2	2	2	2	2	2
	7	7	0	0	1	1	2	2	3	3
	0	9	1	5	0	1	2	3	0	1
	7	0	8	9	3	6	9	8	7	4
AB039748 ¹	G	T	A	C	T	A	A	C	C	C
AY079145 ²	T	C	A	T	T	G	G	T	T	T
AY079146 ³	T	T	G	C	C	A	A	T	T	T
(VAGY ⁴ , (n=15)	T	T	A	T	T	A	A	T	T	T

¹*Bos taurus*; ²*Bos indicus*; ³*Bos javanicus*; ⁴An Giang Vang cattle.

Table 5: Allele frequencies and genotypes associated with economic traits.

Gene	n	Genotype			Allele frequency		Chi-square values
DGAT1	30	K/K	K/A	A/A	K	A	
	27	3	0		0.95	0.05	0.77
NCAPG	30	T/T	G/T	G/G	T	G	
	30	0	0		1	0	NA
RNF212	30	CC	CT	TT	C	T	
	30	0	0		1	0	NA

NA: Not applicable; does not follow Hardy-Weinberg equilibrium.

POLYMORPHISM OF GENES ASSOCIATED WITH ECONOMIC TRAITS

We then investigated the genetic polymorphisms linked to specific economic traits in cattle, focusing on the *DGAT1* gene, which influences milk fatty acid composition. The *K* allele of this gene is associated with increased fat content (Grisart et al., 2002). We also examined the *NCAPG* gene, known for its connection to carcass weight, where the *G* allele enhances carcass weight (Eberlein et al., 2009). Additionally, we looked at the *RNF212* gene, crucial for regulating crossover during meiotic recombination; the *T* allele of *RNF212* is recognized for boosting the meiotic recombination rate in cattle (Reynolds et al., 2013; Qiao et al., 2014). According to the data presented in Table 5, the distribution of the genotypes *KK* and *KA* within the *DGAT1* gene shows 27 and 3 individuals, respectively, highlighting the presence of both *K* and *A* alleles in the An Giang Vang

cattle population. In a study examining 27 local cattle samples from Laos and 30 samples of Central Vietnam Yellow cattle, it was found that all cattle in these two regions displayed a single genotype, *KK* (Okuda *et al.*, 2017; Le *et al.*, 2018). As a result, allele A of the *DGAT1* gene was identified in the An Giang Vang cattle population, indicating a low-frequency polymorphism for this gene ($A = 0.05$).

The *NCAPG* I442M is a mutation that replaces T with G at position g.38245413 (exon 9) and changes the amino acid isoleucine to methionine at position 442 of the *NCAPG* protein (Setoguchi *et al.*, 2011). In this study, the analysis of *NCAPG* gene polymorphism by the PCR-RFLP method showed that there was only one genotype, *TT*, proving that the *NCAPG* I442M gene does not show polymorphism in this cattle population. It means that this population has not detected the $T > G$ substitution mutation related to carcass weight. This result is similar to the result of Okuda *et al.* (2017) where 100% of the research samples had the *TT* genotype. Thus, there is no presence of the G allele of the *NCAPG* gene in the An Giang Vang cattle population, meaning there is no polymorphism in this gene.

Furthermore, the *RNF212* P259S mutation involves a C to T substitution at genetic position g.118327636 on Chromosome 6 (NC_032655.1), leading to a change from proline to serine at position 259 within the *RNF212* protein. This specific mutation results in an elevation of the meiotic recombination rate in cattle. Given that meiotic recombination rate directly influences reproductive efficiency, this mutation could serve as a significant marker associated with enhanced fertility in cattle, as highlighted by studies (Sandor *et al.*, 2012; Kadri *et al.*, 2016). To identify the presence of the $C > T$ mutation at position g.118327636 (located in exon 12) within the analyzed samples, we opted for the use of the restriction enzyme *AlwI*. This selection was based on the screening outcomes from the NEBcutter V2.0 tool (New England Biolabs; <http://nc2.neb.com/NEBcutter2/>), which identified *AlwI* as a suitable enzyme capable of recognizing and cleaving at the specific mutation site. The PCR-RFLP analysis results of the *RNF212* genotype indicated the presence of solely one *CC* genotype, indicating the absence of polymorphism in the cattle population for the *RNF212* gene. Consequently, the specific $C > T$ substitution mutation linked to enhanced fertility in An Giang Vang cattle was not identified.

The genotype analysis results of Vang cattle revealed the presence of the milk fat ratio gene polymorphism, specifically linked to *DGAT1*. These gene variations are associated with distinct characteristics in beef and dairy cattle of the *Bos taurus* species. However, information on the distribution of these genetic variations within the Zebu group remains limited. Previous studies have highlighted notable variations in allele frequencies of genes between *Bos tau-*

rus and Zebu cattle populations, as documented in studies by Kaneda *et al.* (2011); Yonesaka *et al.* (2016); Okuda *et al.* (2017). Our research indicates the presence of *DGAT1* gene polymorphisms in the An Giang Vang cattle population, mirroring findings from studies on *taurine* cattle.

So far, studies have indicated minimal variation in the *NCAPG* gene among Zebu cattle. Interestingly, the G allele of this gene was found to be absent in the An Giang Vang cattle population in this research. Despite displaying genetic diversity in genes linked to milk fat quality characteristics, the An Giang cattle do not exhibit diversity in genes associated with meiotic recombination crossover regulation or enhancing carcass weight. Consequently, An Giang Vang cattle in this study showed polymorphisms in genes related to milk fat quality. They did not show polymorphisms in genes related to high fertility and increased carcass weight. Therefore, it is possible that their small size and low carcass rate are due to the fact that they do not carry the G allele in the population. Therefore, the results of this study can be used as a reference in improving the Vang cattle breed in the direction of improving milk quality, which will be more feasible. However, the number of observed samples in this study is quite modest, which may have certain limitations.

CONCLUSIONS AND RECOMMENDATIONS

An investigation into mitochondrial DNA, SRY haplotype diversity, and genotypes of functional genes revealed that An Giang Vang cattle possess considerable genetic diversity inherited from *Bos indicus* cattle through both maternal and paternal lineages. The paternal lineage may be a hybrid derived from *Bos javanicus* and zebu cattle, along with the presence of beneficial alleles for economically significant traits. This study represents the first detailed genetic characterization of An Giang Vang cattle utilizing molecular markers, which may provide valuable insights for the future conservation and breeding of this breed. Nonetheless, it is important to note that the sample size for the Vang cattle population in this research was relatively small, which may impose certain limitations.

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NOVELTY STATEMENT

It is possible to evaluate population genetic diversity and

select milk trait in the Vang cattle herd based on these molecular markers.

AUTHOR'S CONTRIBUTIONS

Nguyen Ba Trung: Conceived, designed, performed the experiments, and analyzed the data.

Nguyen Ba Trung and Pham Thi Kim Phuong: Wrote the paper, all authors collected the samples, reviewed, and approved the final manuscript.

CONFLICT OF INTEREST

We certify that there is no conflict of interest.

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