Research Article

Genetic Divergence of Local Goats in Ninh Thuan Province Vietnam

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Abstract | The hypervariable region (D-loop) of mtDNA (mitochondrial DNA) of local goat breeds in Ninh Thuan Province, Vietnam was studied to provide useful information on their origin. Blood samples were taken from two local goat breeds in Ninh Thuan (De Co = CO and Bach Thao = BT, 10 samples each) and three other exotic goats including Saanen (SA; 10 samples), Red Boer (RB; 10 samples) and White Boer (WB; 9 samples) raised at An Phu Dairy Company in Cu Chi District, Ho Chi Minh City. Extracted mtDNA was used to amplify the fragment of 687 bp in the D-loop by polymerase chain reaction (PCR) followed by sequencing to analyze nucleotide polymorphism, haplotype diversity and genetic distance to construct a phylogram. Results showed that the fragment of 687 bp was successfully amplified. Examination of 49 sequence variants in 599 bp of the control region revealed the nucleotide composition was Adenine (A) = 32.55%, Thymine (T) = 28.33%, Guanine (G) = 14.67%, Cytosine (C) = 24.87% and G+C content was 39.12%. Out of a total of 50 nucleotide polymorphic sites, 48 were transition and 2 were transversion, with 21 haplotypes observed. Nucleotide and haplotype diversity indices (π and Hd) were 0.03005 and 0.889, respectively. The genetic diversity of Saanen goats was highest at (π) = 0.03031 and (Hd) = 0.987, while the lowest genetic diversity was found in Bach Thao goats (π = 0.00089 and Hd = 0.533). Genetic distance between WB and CO or BT was higher (0.058) than between RB and CO or BT (0.056 or 0.055). The lowest genetic distance was found between CO and BT goats (0.001). Most local goat breeds were grouped in one clade, with exotic goat breeds concentrated in other clade, indicating clear maternal divergence between local and exotic goat breeds. D-loop mtDNA diversity within the two local goat breeds in Ninh Thuan Province of Vietnam was low, indicating a close genetic relationship with Indochina goats (Laos), separated into a sub-lineage from maternal lineage B. Further insights regarding the genetic diversity of Vietnamese local goats require detailed in-depth studies.

Keywords | Local goat, D-loop, Mitochondrial DNA, Genetic divergence, Phylogeny

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INTRODUCTION

Ninh Thuan is one province that is located in south central coast of Vietnam with major parts of province's population are Cham and Raglai ethnic groups. The semi-arid conditions allow the coexistence of ruminant species such goats, sheep, cattle and buffaloes. The existence of small ruminants plays a pivotal role in daily local life. Goats are domestic animals that provide meat and milk. They can readily adapt to different climatic conditions such as arid or semi-arid, mountainous areas, tropical conditions and cold winters (Ganbold et al., 2020; Febriana et al., 2021). In Vietnam, most of the goats belong to two local breeds as De Co plus the local geographic name (Le et al., 2017) and Bach Thao that originated in Ninh Thuan Province and later spread to the whole country as dual purpose breeds (meat and milk). However, information on the genetic source of these goat breeds, especially Bach Thao remains unclear. A good understanding of the genetic sources of domestic animals is a basic requirement to evaluate breed relationships and optimize both conservation and development management (Barker et al., 1997; Martin-Burriel et al., 1999). mtDNA sequence variation is used to analyze the genetic divergence and promote the evolution of husbandry livestock populations (Ganbold et al., 2020). The divergence of mtDNA is vital in evaluating population structure and phylogeny, as well as the maternal origin of domestic livestock (Beja-Pereira et al., 2006; Rocha et al., 2011; Ly et al., 2015; Teinlek et al., 2018; Touma et al., 2019). Here, the genetic divergence and maternal genetic relations of local goat breeds in Ninh Thuan Province were evaluated to provide a principle database for a detailed conservation and development program strategy.

MATERIALS AND METHODS

Collection of whole blood samples and DNA extraction

The goats were handled by experienced workers and technicians to minimize the suffering of animals as the main priority. Blood samples were taken from the jugular vein of 49 individual goats comprising local goat breeds such as De Co (CO, n=10) and Bach Thao (BT, n=10) in Ninh Thuan Province. Three exotic goat breeds as Saanen (SA, n=10), Red Boer (RB, n=10) and White Boer (WB, n=9) raised at An Phu Dairy Cattle Company in Ho Chi Minh City were also sampled (Figure 1). DNA extraction was conducted using a TOPPURE® blood DNA extraction kit.

Primer design

A set of primers was designed using Primer3 software based on the retrieved sequence from GenBank with access number AB162216.1. A length of fragment with 687 bp in the hypervariable region was magnified by the primer set (5'-3') as forward (TCCCACTCCACAAGCCTAC) and reverse (TAGGTGAGATGGCCCTGAAG).

Figure 1: Representative images of goats. CO: Co goat; BT: Bach Thao goat; RB: Red Boer goat; WB: White Boer goat; SA: Saanen goat.

DNA amplification

The PCR technique was applied to amplify the fragment of 687 bp in the D-loop by a MasterCycler Pro S machine (Eppendorf, Germany). Reactions were conducted in a total of 25 µL that consisted of 50 ng/µL (2 µL) DNA templates, 0.8 µL primers (Phu Sa, Vietnam), 12.5 µL of Mytaq Mix 2X (Bioline) and 8.9 µL nuclease-free water. PCR was performed consisting of initial stage at 95°C for 4 min, then repeat as following steps for 35 cycles with 95°C-57°C-72°C for 30 seconds for each, and final step at 72°C for 5 min. Separation of the PCR products was applied by electrophoresis using agarose gel (1.5%, 30 min, 100V) with a 100 bp DNA ladder and observed under UV light.

Data analysis

The alignment of 49 sequenced samples with the mtDNA D-loop sequence selected in the GenBank database (AB162216.1) was applied using BioEdit (Version 7.2.5). Nucleotide haplotype diversity was calculated using DNA Sequence Polymorphism (Version 6.12.03 x64).

The genetic distance and phylogenetic tree using D-loop mtDNA sequences (576 bp) of 49 individual goats were applied using MEGAX software (Version 10.2.5). Eight sequences of D-loop mtDNA of *Capra hircus* retrieved from GenBank including KM279318.1 (India), AB162216.1 (Pakistan), LC484844.1 (Georgia), AB044299.1 (Laos), AB004081 (*Capra hircus*), AB110590.1 (*Capra aegagrus blythi*), AB004305.1 *(Capra falconeri*) and AJ317875.1 (*Capra caucasia*) were used as references for the ingroups, while *Bos taurus* (NC006853.1) was used as a reference for the outgroup to build the phylogenetic tree. Six retrieved sequences from GenBank representative for the maternal

Table 1: Nucleotide composition

Goat breed	N	Percentage (%)					
		A	T	G	C	A+T	G+C
CO	10	32.82	27.95	14.02	25.21	60.77	39.23
BT	10	32.82	27.95	14.02	25.21	60.77	39.23
RB	10	32.25	28.81	14.51	24.42	61.06	38.94
WB	9	32.33	28.58	14.41	24.67	60.91	39.09
SA	10	32.50	28.36	14.25	24.84	60.36	39.14
Average	31	32.55	28.33	14.67	24.87	60.88	39.12

CO: Co goat; BT: Bach Thao goat; RB: Red Boer goat; WB: White Boer goat; SA: Saanen goat

Table 2: Nucleotide substitution in 599 bp fragment length of the D-loop

Items		Number	Substitution	Variable sites
Two variants	Singleton	9	C/T	180
			T/C	186; 318; 491; 632
			A/G	598
			G/A	553; 651
			C/G	611
	Parsimony	41	C/T	493; 466; 544; 547; 570; 576; 615; 720
			T/C	207; 235; 377; 500; 517; 520; 535; 539; 542; 545; 546; 550; 551; 580; 581; 607; 610; 636; 653
			G/A	298; 413; 468; 483; 490; 543; 589; 597
			A/G	201; 206; 317; 552; 562
			T/A	737

Table 3: Types of nucleotide substitution

Type of substitution	Number	Percentage (%)
Transitions		
C/T	32	64
G/A	16	32
Total	48	96
Transversions		
C/G	1	2
A/T	1	2
C/A		
T/G		
Total	2	4

Table 4: Haplotype and nucleotide diversity

Goat type	Number of samples	Number of haplotypes	Divergence index (mean ±SE)	
			Haplotype	Nucleotide
CO	10	3	0.644±0.0125	0.00156±0.0000003
BT	10	2	0.533±0.00896	0.00089±0.00
RB	10	6	0.778±0.01889	0.00723±0.0000035
WB	9	6	0.833±0.016	0.00686±0.0000034
SA	10	9	0.987±0.00292	0.03031±0.0000186

Total	31	21	0.889±0.00089	0.03005±0.00000008

CO: Co goat; BT: Bach Thao goat; RB: Red Boer goat; WB: White Boer goat; SA: Saanen goat

Table 5: Haplotype distribution

Haplotype (H)	Number	Individual goats	Haplotype index (±SE)
H-1	13	CO1, CO6, CO7, CO8, CO10, BT1, BT4, BT5, BT8, BT9, BT10, SA1, SA8	Hd= 0.889±0.00089
H-2	1	CO2	
H-3	9	CO3, CO4, CO5, CO9, BT2, BT3, BT6, BT7, SA9	
H-4	2	SA2, WB8	
H-5	1	SA3	
H-6	1	SA4	
H-7	1	SA5	
H-8	1	SA6	
H-9	1	SA7	
H-10	1	SA10	
H-11	5	RB1, RB2, RB8, RB9, RB10	
H-12	1	RB3	
H-13	1	RB4	
H-14	1	RB5	
H-15	1	RB6	
H-16	1	RB7	
H-17	1	WB1	
H-18	1	WB2	
H-19	4	WB3, WB4, WB7, WB9	
H-20	1	WB5	
H-21	1	WB6	

CO: Co goat; BT: Bach Thao goat; RB: Red Boer goat; WB: White Boer goat; SA: Saanen goat

Table 6: Genetic distance among goat breeds

	CO	BT	SA	RB	WB
CO		0.001	0.006	0.010	0.010
BT	0.001		0.006	0.010	0.010
SA	0.037	0.037		0.004	0.005
RB	0.056	0.055	0.026		0.003
WB	0.058	0.058	0.029	0.011	

Values above the diagonal show the margin of error of genetic distance, with genetic distance values below the diagonal. CO: Co goat; BT: Bach Thao goat; RB: Red Boer goat; WB: White Boer goat; SA: Saanen goat

lineage of domestic goats such as A (KR059184), B (KR059219), C (GU229280), D (KR059210), F (KR059213) and G (KR059226) were used to trace the maternal lineages of Ninh Thuan local goat breeds.

RESULTS AND DISCUSSION

Nucleotide and haplotype variation

Forty-nine sequences of D-loop fragment were analyzed, with average nucleotide composition for A, T, G and C 32.55, 28.33, 14.67 and 24.87% (Table 1). Base contents of C+G and A+T were 39.12 and 60.88%, respectively.

Ganbold et al. (2021) analyzed 174 sequences using a 452 bp fragment of hypervariable region 1 in mtDNA from 12 different Mongolian goat breeds. Results showed percentage of base content as A (31.0%), C (22.2%), G (16.5%) and T (30.3%), with the percentage of G+C (38.7%) lower than A+T (61.3%). The trend of low G+C content in this study was also observed in large ruminants such as buffaloes (Sari et al., 2021; Suhardi et al., 2021; Youssef et al., 2021).

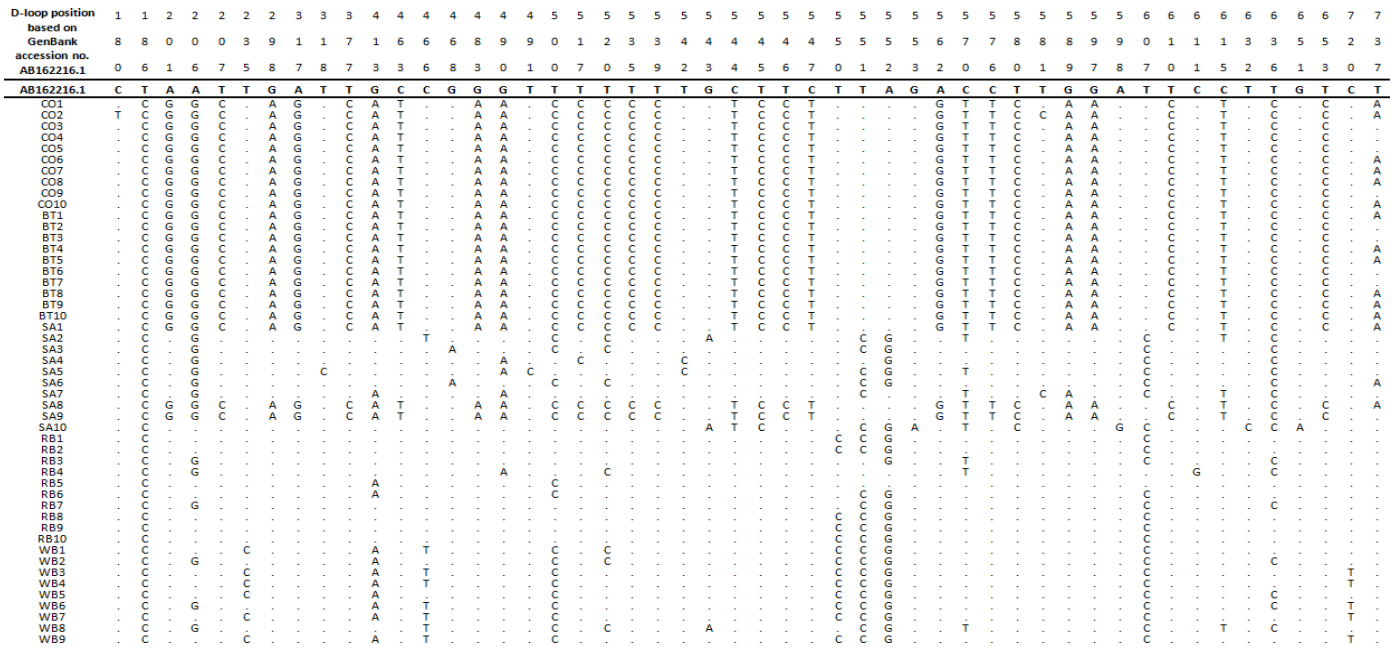

Figure 2: Sequence variation and polymorphic sites observed on the mitochondrial D-loop sequence. *AY488491.1: Ref. from GenBank;* CO: Co goat; BT: Bach Thao goat; RB: Red Boer goat; WB: White Boer goat; SA: Saanen goat. *A: Adenine; T: Thymine; G: Guanine; C: Cytosine; (.):*

Nucleotide polymorphism was analyzed from 49 sample sequences, with one WB sample discarded due to low sequencing quality. Results are presented in Figure 2 and Table 2. Fifty polymorphic sites were observed as 48 transitions and 2 transversions at 96 and 4%, respectively with no insertions or deletions (Table 3). The high level of mtDNA diversity of imported exotic goats in this study was due to the maternal effects of the forebears (Mannen et al., 2001). The proportion of transition/transversion was 25:1, indicating a strong bias of transition, higher than the 17:1 and 16.7:1 ratios documented in husbandry goat breeds (Luikart et al., 2001; Chen et al., 2005) or Indian local goats (Joshi et al., 2004) but lower than in Tanzanian indigenous goats (28.7:1) reported by Nguluma et al. (2021).

Many studies have documented that the trend of strong bias toward transitions is an obvious indicator for distinguishing mtDNA evolution, as observed in ruminant species such as buffaloes (Lau et al., 1998; Kierstein et al., 2004; Lei et al., 2007; Babar et al., 2011; Shaari et al., 2019; Raungprim et al., 2021), sheep (Guo et al., 2005; Ly et al., 2015), cattle (Lai et al., 2006) or chicken (Liu et al., 2004).

Evaluation of genetic diversity and distance

The diversity of nucleotide (π), haplotype (Hd) of two local goat breeds and three exotic goat breeds were evaluated. Highest value of nucleotide diversification (π) was recorded in SA goats (π = 0.03031±0.0000186), with moderate values in RB and WB (0.00723 and 0.00686, respectively), a lower value in CO goats (0.001561) and the lowest value in BT goats (0.00089). Similarly, haplotype divergence (Hd) ranged from 0.987 in SA to 0.533 in BT (Table 4). Average diversity values of Hd and π were 0.889±0.000899 and 0.03005±0.00000008, respectively. These results were similar to haplotype and nucleotide diversity of Mongolian or Tanzanian indigenous goats (Ganbold et al., 2021; Nguluma et al., 2021) but haplotype and nucleotide divergence within the local goat population in our study was lower.

The DNA sequences from 49 individual goats revealed 21 different haplotypes (Table 5). Most CO and BT were placed in haplotypes 1 (H-1) and 3 (H-3). Five Red Boer goats were placed in H-11, while four White Boer goats appeared in H-19. Saanen goats in this study showed higher diversity than CO and BT goats. By contrast, local goat breeds such as CO and BT showed reduced diversity compared to exotic goat breeds, possibly because exotic goats originated from diverse maternal sources. Average values of haplotype diversity indicated lower genetic divergence in the two local goat breeds. This was attributed to low mutation rates in the D-loop region as a result of reduced maternal gene sources from different geographical regions; however, further detailed studies are required to improve the current hypotheses.

As shown in Table 6, genetic distances between local goat breeds were smaller (0.001) when compared with imported exotic goat breeds (ranging 0.037-0.058). Genetic distances among local goat breeds were similar (0.001). By coincidence, genetic distances between SA and CO or BT were the same (0.037), between WB and CO or BT 0.058, and between RB and CO or BT 0.056 or 0.055, respective-

ly. Sari et al. (2021) stated that a genetic distance value of less than 0.5 indicated closely related genetic livestock. The genetic relationship among local goats was similar to WB or RB goats. Febriana et al. (2021) examined the control region sequences of Indonesian local goats (Kacang, Kejobong and Senduro) and found that the genetic distance among these local goats was also low (0.0047-0.0061).

Analysis of phylogeny

The dendogram in Figure 3 indicated that most of the local goat breeds were grouped in one clade, while the exotic goat breeds were concentrated in other clades, indicating clear maternal divergence between local and exotic goat breeds. The phylogenetic tree also revealed that local goat breeds were closer to Laos goat breeds originating from the Indochina region. By contrast, most of the exotic goat breeds, except SA, developed into one clade and then separated into many small clusters that were closely related to *Capra hircus* maternal origin from India, Pakistan and Georgia. Evidence from the phylogenetic tree also revealed that nearly 50% of SA individual goats were placed in local goat breeds.

Identification of maternal lineages has been documented in many reports (Sultana et al., 2003; Sardina et al., 2006; Kamalakkannan et al., 2018). In our study, most of the Ninh Thuan local goat breeds in sub-lineage B developed from maternal lineage B, while all imported exotic goat breeds displayed multiple maternal lineages as A, D and F (Figure 3).

Naderi et al. (2007) analyzed mtDNA on a large scale and identified six haplogroups of domestic goats, while Ganbold et al. (2020) reported that sub-lineage B was also found in Chinese and Mongolian goats, similar to our results.

Many studies reported lineage A as an old lineage, widely distributed in Asian, European and African goat breeds (Pereira et al., 2005; Royo et al., 2009; Vacca et al., 2010). Recently, investigations of domestic goats in Brazil (Silva et al., 2019) and Arabia (Al-Araimi et al., 2017) reported that most belonged to maternal lineage A. More than 90% of the world's goats were identified as belonging to lineage A (Wang et al., 2015; Deng et al., 2018; Ganbold et al., 2020; Nguluma et al., 2021). Maternal lineage B showed limited distribution and was only found on the Asian continent (southern and eastern areas), and in South Africa, while other lineages (C, G, F and D) were recorded in Southern Europe, Southwest Asia, Northern Africa and Italy, respectively (Pakpahan et al., 2016; Nguluma et al., 2021).

Further research is required to determine the relationship and position of Ninh Thuan local goats against other Vietnamese goat breeds and goats of Asian origin.

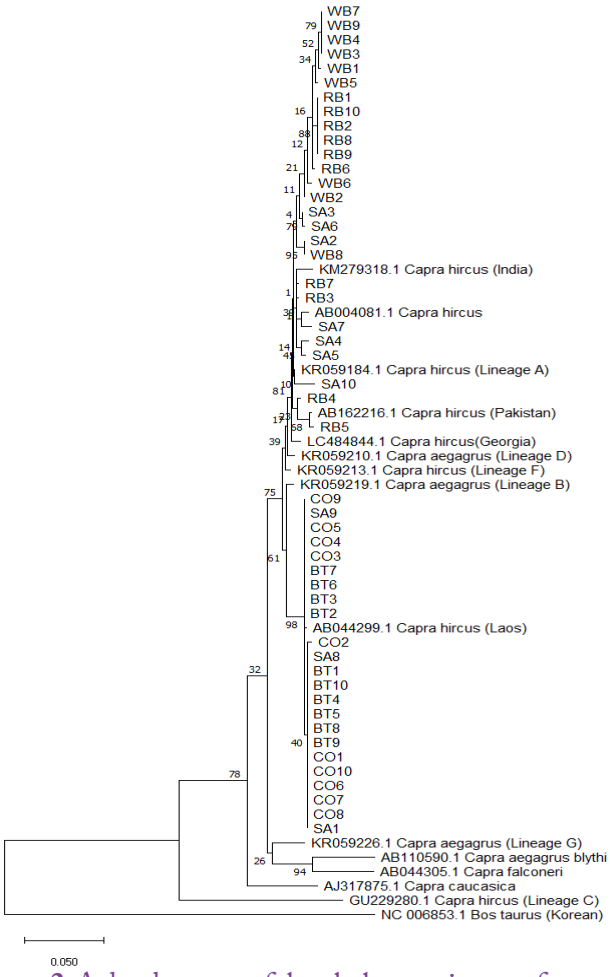

Figure 3: A dendrogram of the phylogenetic tree of sampled goats. CO: Co goat; BT: Bach Thao goat; RB: Red Boer goat; WB: White Boer goat; SA: Saanen goat *(References from GenBank: KM279381.1; AB162216.1; LC484844.1; AB044299.1; AB004081; AB110590.1; AB004305.1 and AJ317875.1); Bos taurus (GenBank: NC006853.1). Maternal lineage of domestic goats: A (KR059184), B (KR059219), C (GU229280), D (KR059210), F (KR059213) and G (KR059226)*

CONCLUSIONS AND RECOMENDATIONS

We, for the first time, report on mtDNA genetic variation of local goats in Ninh Thuan Province, Vietnam. The D-loop mtDNA diversity within the two local goat breeds was low, indicating a close genetic relationship with Indochina goats (Laos), separated into a sub-lineage from maternal lineage B. Further insights regarding the genetic diversity of Vietnamese local goats require further detailed in-depth studies.

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CONFLICT OF INTERESTS

No conflicting interests were declared by the authors regarding any financial sources or materials discussed in this manuscript.

NOVELTY STATEMENT

To our knowledge, this is the first study in Vietnam to report the genetic diversity of native goats in Ninh Thuan province based on mtDNA D-loop, and we strongly believe that our study will contribute to the scientific literatures on molecular genetic of Vietnamese native goat genetic resources and to provide as a useful database to develop the native goats conservation and development strategy.

AUTHORS CONTRIBUTION

All authors generally contributed to design the experimental works, read and approved the manuscript in each step. NGUYEN, N.T: covered all the research, writing the manuscript. TRAM, M.T. and PHAM, T.T. contributed the work equally on DNA extraction, design primer and amplification of target gene. LE, T.L. and NGUYEN, T.K.L. contributed the work equally on sequence analysis. HOANG, T.T. and PHAM, C.T. shared the work equally for sample collection. DUONG, N.T. contributed to evaluate the manuscript and check the plagiarism as well as revised the manuscript.

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