



Genetic Divergence of Two Vietnamese Swamp Buffalo Populations using Microsatellite Markers

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Abstract | This study evaluated the genetic diversity of two Vietnamese swamp buffalo populations, LangBiang (LB) in Lam Dong and Thanh Chuong (TC) in Nghe An Provinces of Vietnam. Ear tissue samples were collected from 96 buffaloes including 64 LB (32 LB-LD and 32 LB-LC) and 32 TC and a set of 18 microsatellite markers was used to analyze their genetic diversity. Results showed that all the microsatellite markers were polymorphic in the two local swamp buffalo populations. The average number of alleles of each microsatellite marker in the two native buffalo populations varied from 6.11 (in LB-LD and LB-LC sub-populations) to 7.67 (in the TC population), with mean values of expected heterozygosity (H_e), observed heterozygosity (H_o), and polymorphic informative content (PIC) 0.743, 0.271, and 0.738, respectively. The two local swamp buffalo populations were separated into two main branches. One branch came from the TC local buffalo population while the other from the LB buffalo population subsequently separated into two sub-clades (LB-LD and LB-LC). Most of the polymorphic markers used in this study were applicable for investigating the genetic relationships among Vietnamese local swamp buffalo populations. Inbreeding among the two Vietnamese native buffalo populations was indicated, the separation of PB into the two LB sub-populations and bull exchange policy among populations requires further study.

Keywords | *Bubalus bubalis*, Genetic divergence, Native buffalo, Microsatellite marker, Polymorphism, Swamp buffalo

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INTRODUCTION

Local swamp buffaloes are domesticated ruminant animals and become a traditional symbol of Vietnamese

culture and poetry (Nguyen *et al.*, 2022), they have been raising throughout the highlands and lowlands and are used for meat and agriculture-forestry. Buffalo-derived by-products such as skin and horns are used as handicrafts

while buffalo manure is useful organic soil fertilizer. Buffaloes play a significant role in the livelihood of farmers and are considered as safe finance when needs arise. Local buffalo breeds are well-adapted to cost-effective production systems within a broad range of environments. Vietnam has six eco-economic regions with rich biodiversity and about 2.25 million buffaloes (Department of Livestock Production, 2022), classified into large frame in the south and medium frame in the north of Vietnam (Berthouly *et al.*, 2009; Nguyen *et al.*, 2022). Analysis of the genetic structure of provincial buffalo populations is a prerequisite to any conservation or improvement project. Authors have also suggested that buffaloes from the southern districts could be utilized to improve the frame size of buffaloes from the northern districts of Vietnam (Berthouly *et al.*, 2009). In Indonesia, Prihandin *et al.* (2023) suggested mating Aceh buffalo bulls with southeast Sulawesi female buffaloes to produce Indonesian swamp buffaloes with a larger frame size. Evaluating the genetic divergence and structure of buffalo populations at the provincial level is essential to any conservation or improvement projects (Berthouly *et al.*, 2009; Prihandin *et al.*, 2023). Thanh Chuong (TC) and Lang Biang (LB) are indigenous Vietnamese buffaloes, which are representative buffaloes for the mountain areas of Vietnam, that can adapt to extreme climate and ecological conditions central coastal and highland regions. The most valuable feature of TC and LB buffaloes is their outstanding ability to confront diseases. However, due to the recent industrialization in agriculture, the need for draught power has reduced and the number of buffalo has decreased in several Southeast Asian countries. Breed selection is also less focused, resulting in common inbreeding. Thus, to support conservation and develop the genetic resources of TC and LB buffaloes effectively, a genetic diversity analysis was conducted for TC and LB buffalo populations. Microsatellite markers were used because they offer co-dominant inheritance, high diversification, wide genome coverage and good reproducibility (Xu *et al.*, 2020). Microsatellite techniques have been widely applied to evaluate the degree of inbreeding, genetic characteristics, genetic distance, and genetic structure of different livestock in Vietnam (Pham *et al.*, 2013; Ba *et al.*, 2020; Pham *et al.*, 2022). Several studies have investigated the genetic diversity of native buffalo populations in Vietnam based on microsatellites (Berthouly *et al.*, 2009) to analyse the genetic diversity between buffalo population in low land area of southern Vietnam (Tay Ninh province) and mountain area of northern Vietnam (Thanh Chuong and Ha Giang provinces) or mtDNA D-loop sequencing (Nguyen *et al.*, 2022). This study evaluated the genetic diversity of TC and LB buffalo populations, which are representative for local buffaloes in the mountain areas of south and north of Vietnam based on microsatellite markers to provide basic data to develop a future breeding strategy.

SAMPLE COLLECTION

Samples were collected according to the standard of animal care in Vietnam, following guidelines based on EU directive 2010/63 for the best practice of using animals in research. Ninety-six buffalo ear tissue samples were collected from households in Thanh Chuong District, Nghe An Province, which is representative for medium frame of buffalo (TC= 32 Thanh Chuong buffalo samples) and in Lac Duong District, Lam Dong Province, which is representative for large frame of buffalo for southern Vietnam in highland area (LB-LD: 32 Lang Biang buffalo samples in Lac Duong Town and LB-LC: 32 Lang Biang buffalo samples in Lat Commune), and both of them are raised in the mountain areas of Vietnam (Figure 1). The samples were stored in tubes containing 1.5 ml of absolute alcohol (99%), transported to the laboratory and stored at -20°C until genomic DNA extraction was performed.

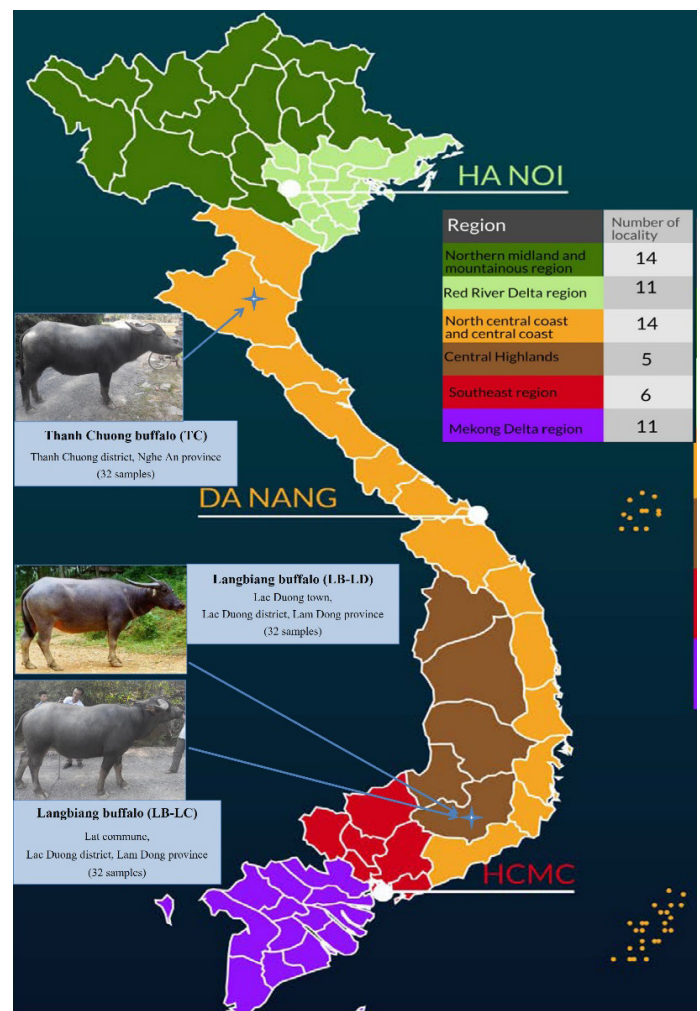


Figure 1: Representative images of buffaloes in the six eco-economic regions.

DNA EXTRACTION, PCR AMPLIFICATION AND MICROSATELLITE GENOTYPING

A DNeasy Blood and Tissue kit (Qiagen, Hilden, Germa-

ny) was applied to extract the ear tissue sample according to the manufacturer's instructions. The quality and quantity of genomic DNA were checked by electrophoresis (1% agarose gel), with determination of the OD value (NanoDrop 2000 Spectrophotometer-Thermo Fisher Scientific, Waltham, USA), respectively. Eighteen microsatellite markers were selected from list of 30 microsatellites that recommended by the International Society for Animal Genetics - Food and Agriculture Organization (FAO, 2009), applied and revealed polymorphic markers for evaluating of local buffalo at different areas of Vietnam by Berthouly *et al.* (2009) as indicated in Table 1. Optimization of PCR condition for each marker was tested by single PCR, then grouping for multiplex PCR and four sets of multiplex PCR was set up (Table 1). The combination of microsatellite markers was optimized according to the difference in three fluorescent dyes (D2, D3 and D4) and in the amplification of PCR primers (Table 1). The multiplex PCR was carried out in a 25 µl reaction mixture containing 1X PCR buffer, 200 µM dNTP mix, 1.5 units of Hot Start *Taq* DNA Polymerase, 10 pM each of forward or reverse primer and 25 ng to 50 ng template genomic DNA. The multiplex PCR was performed under the condition of initial denaturation at 95°C for 15 min, followed by 35 cycles of 95°C denaturation for 1 min, 55- 65°C annealing for 1 min, and at 68-72°C extension for 1 min and 20 s, and a final extension at 72°C for 10 min. The PCR products were labeled with fluorescent dyes and genotyped using a capillary sequencer (Beckman Coulter CEQ8000). The

alleles of each microsatellite marker were determined by an automated DNA fragment analyzer (Beckman Coulter CEQ8000).

STATISTICAL ANALYSIS

The values of number of alleles were estimated by FSTAT software version 2.9.3.2 (Goudet, 2002). The expected heterozygosity (He), the observed heterozygosity (Ho), and the F statistic (Fis, Fit and Fst), genetic distance and PCA (Principal Coordinate Analysis) were measured by GENETIX software version 4.05 (Belkhir *et al.*, 2002). The polymorphic information content (PIC) was obtained based on the formula of Bostein *et al* (1980). The dendrogram was generated using the neighbor-joining method with bootstrapping 1000 times by Population software version 1.2.28 (Langella, 2002) and TreeView software version 1.6.6 (Page, 1996).

RESULTS AND DISCUSSION

MICROSATELLITE POLYMORPHISM

Microsatellite markers are a reliable technique for studying genetic diversity, lineage analysis, and characterization of animal breeds. Using multiplex PCR, a set of 18 microsatellite markers was utilized to amplify the target sequence, the optimized result for single PCR before generating of multiplex PCR did not show in this article. All the microsatellites exhibited polymorphic, non-ambiguous peaks for

Table 1: The group markers used for multiplex PCR.

Multiplex PCR	Locus	Chromosome location	Dye label	Allele size range (bp)	Annealing temperature (°C)	Reference
I	ETH003	3	D2	94-106	65	Berthouly <i>et al.</i> , 2009
	ILSTS068	20	D2	150-168	55	
	CSSM033	17	D3	154-174	65	
	ILSTS052	21	D4	153-167	55	
II	CSSM041	21	D2	129-141	55	
	CSSM022	4	D2	206-210	55	
	CSSM047	3	D3	125-148	55	
	CSSM032	1	D3	208-224	55	
	CSSM029	9	D4	176-194	55	
III	ILSTS061	15	D2	131-157	55	
	CSSM008	Unknown	D2	178-194	55	
	CSSM019	1	D3	129-160	55	
	CSSM046	11	D4	150-158	55	
	BMC1013	3	D4	234-250	55	
IV	ILSTS056	12	D2	154-162	55	
	ILSTS005	11	D3	178-186	55	
	CSSM043	1	D3	222-258	55	
	ILSTS028	11	D4	158-166	55	

Table 2: Mean number of alleles (Na), expected (He), observed heterozygosity (Ho) and polymorphic information content (PIC) estimates within buffalo populations.

Locus	Number of alleles			Observed heterozygosity (Ho)			Expected heterozygosity (He)			Polymorphic information content (PIC)		
	TC	LB-LD	LB-LC	TC	LB-LD	LB-LC	TC	LB-LD	LB-LC	TC	LB-LD	LB-LC
ETH003	6	7	7	0.719	0.750	0.781	0.766	0.806	0.826	0.713	0.765	0.787
ILSTS068	16	12	8	0.719	0.844	0.625	0.865	0.872	0.834	0.838	0.843	0.800
CSSM033	8	7	6	0.875	0.750	0.656	0.834	0.773	0.762	0.798	0.725	0.711
ILSTS052	9	8	9	0.719	0.688	0.906	0.801	0.847	0.859	0.764	0.813	0.827
CSSM041	5	3	3	0.719	0.719	0.719	0.616	0.585	0.657	0.554	0.504	0.571
CSSM022	3	3	3	0.406	0.344	0.188	0.484	0.489	0.432	0.427	0.392	0.386
CSSM047	8	5	5	0.656	0.563	0.563	0.775	0.696	0.702	0.730	0.632	0.636
CSSM032	7	6	7	0.406	0.656	0.719	0.588	0.764	0.777	0.542	0.709	0.729
CSSM029	6	5	5	0.406	0.688	0.500	0.519	0.633	0.559	0.433	0.583	0.516
ILSTS061	11	7	7	0.844	0.875	0.781	0.867	0.841	0.826	0.838	0.806	0.789
CSSM008	7	6	6	0.750	0.875	0.688	0.770	0.780	0.801	0.720	0.730	0.755
CSSM019	13	9	10	0.813	0.875	0.656	0.831	0.843	0.836	0.805	0.809	0.804
CSSM046	5	4	4	0.719	0.531	0.406	0.738	0.671	0.627	0.683	0.611	0.565
BMC1013	7	4	6	0.656	0.594	0.500	0.681	0.666	0.697	0.642	0.599	0.640
ILSTS056	7	6	7	0.875	0.719	0.781	0.845	0.772	0.770	0.810	0.719	0.722
ILSTS005	4	4	4	0.375	0.656	0.656	0.446	0.663	0.641	0.393	0.585	0.565
CSSM043	9	7	7	0.813	0.656	0.781	0.798	0.767	0.747	0.754	0.717	0.691
ILSTS028	7	7	6	0.781	0.563	0.656	0.727	0.750	0.760	0.684	0.700	0.707
Average	7.67	6.11	6.11	0.681	0.686	0.642	0.720	0.734	0.729	0.674	0.680	0.678
SE	0.75	0.53	0.45	0.040	0.033	0.039	0.032	0.024	0.026	0.034	0.028	0.028

TC: Thanh Chuong buffalo population; **LB-LD:** LangBiang buffalo in Lac Duong Town sub-population; **LB-LC:** LangBiang buffalo in Lat Commune sub-population.

allele assignation, and reproducibility, as shown in Figure 2. Loci polymorphism and the mean number of alleles of each buffalo population are listed in Table 2.

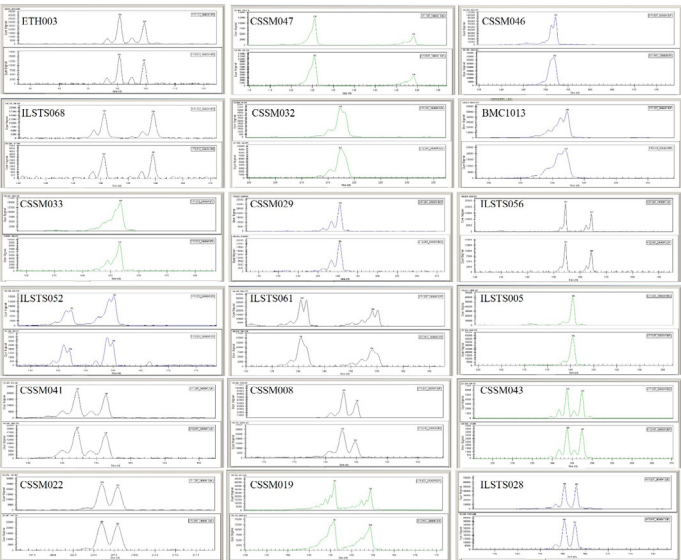


Figure 2: Representative images of the polymorphic microsatellite sites used in this study.

A total of 138 alleles were detected in the TC buffalo population, higher than the number of alleles in each LB buffalo sub-population (110 alleles for sub-populations LB-LD and LB-LC).

The allele number in each locus varied from 3 for CSSM022 or CSSM041 to 10 for CSSM019 in the LB-LC sub-population, from 3 for CSSM041/CSSM022 to 12 for ILSTS068 in the LB-LD sub-population, and from 3 for CSSM022 to 16 for ILSTS068 for TC population. For all data, the number of alleles ranged from 3 to 16. The lowest value was found in CSSM022/CSSM041 and the highest in ILSTS068. Results suggested that the microsatellite markers used were polymorphic and also allele-rich. The average number of alleles was 6.1 in the LB-LD and LB-LC sub-populations and 7.67 in the TC population. The same MS markers were used to analyze the Ha Giang buffalo population located in Northern Vietnam, with the average number of alleles reported as 6.24 (Berthouly *et al.*, 2009). Another study in LangBiang buffalo by Nguyen *et al.* (2018) reported that two loci (ETH003 and CSSM032) were determined as non-polymorphic while the number of alleles in three loci (BMC1013, CSSM033 and CSSM043)

was lower than in the current study. Similarly, other research on Thailand native buffalo breeds also reported that average allele numbers were low when genotyping by polyacrylamide electrophoresis (Triwitayakorn *et al.*, 2006). These differences may be due to different genotyping methods such as agarose-based and/or poly-acrylamide electrophoresis vs. capillary sequences applied to detect alleles with size variation below 10 bp or populations/breeds of animals.

Studies on the genetic diversity of Egyptian buffalo populations (Merdan *et al.* 2020) or local buffaloes in Jammu and Kashmir (Singh *et al.*, 2017) used 12 or 15 microsatellite markers, respectively and reported that the average number of alleles was 5.33 in five Egyptian buffalo breeds and 6.86 in Jammu and Kashmir local buffaloes. Recently, the genetic divergence of 17 provincial populations of river buffalo in Turkey was analyzed using 20 microsatellite markers. Results showed high polymorphism with the mean number of alleles 7.28 (ranging from 6 in ILSTS005 to 17 in ETH003) (Unal *et al.*, 2021). Similarly, 25 microsatellite loci were used to genotype three buffalo populations (Murrah, Nili-Ravi, and Gojri) in India. Vohra *et al.* (2021) mentioned that 22 out of 25 loci were polymorphic for all three populations, and 22 polymorphic loci were used for further downstream analysis with 145, 138, and 173 alleles recorded across 22 loci in 128 individuals sampled from the Murrah, Nili-Ravi, and Gojri buffaloes, respectively. These findings had minor dissimilarities compared to the current study due to the different number of markers as well as the different buffalo breeds examined. Vohra *et al.* (2021) also mentioned that the type of breed, utilization of different microsatellite markers, methods of genotyping, and genetic polymorphism within the breed itself influenced the number of alleles. In short, regarding the morphological characteristic, the native buffaloes in Vietnam are classified in to three types based on the frame side of buffaloes in different ecological areas. the different allele numbers or mean of allele between LB and TC buffalo populations may be due to the different of breed line in different areas, matting system applied, the new males introduced in each region. This could be considered for further strategy of development.

GENETIC DIVERSITY

The genetic diversity within the studied populations was indicated by the average number of alleles (Na) per locus, expected heterozygosity (He), observed heterozygosity (Ho), and the inbreeding coefficient. In each population and across loci, the allele numbers, Ho, He, and the PIC were obtained and are presented in Table 2. The values of Ho varied from 0.375 (ILSTS005) to 0.875 (CSSM033; ILSTS056) with average mean (0.681) in the TC population, from 0.344 (CSSM022) to 0.875 (ILSTS061, CSSM008) with average mean (0.685) in the LB-LD sub-population, and from 0.188 (CSSM022) to 0.906 (ILSTS052) and average mean (0.642) in the LB-LC sub-population. The He value

varied from 0.446 (ILSTS005) to 0.867 (ILSTS061) and average mean (0.720) in the TC population, from 0.498 (CSSM022) to 0.872 (ILSTS068) and average mean (0.734) in the LB-LD sub-population, and from 0.432 (CSSM022) to 0.859 (ILSTS052) with average mean value (0.729) in the LB-LC buffalo sub-population.

The PIC value, varied from 0.393 (ILSTS005) to 0.838 (ILSTS068, ILSTS061) with average mean (0.674) in the TC buffalo population, from 0.392 (CSSM022) to 0.843 (ILSTS068) with average mean (0.680) in the LB-LD buffalo sub-population, and from 0.386 (CSSM022) to 0.827 (ILSTS052) with average mean (0.678) in the LB-LC buffalo sub-population.

Table 3: Genetic diversity parameters for the three buffalo populations.

Buffalo population	Na	He	Ho	F _{is}	PIC
LB - Lac Duong Town sub-population	6.11	0.72	0.64	0.12	0.68
LB - Lat Commune sub-population	6.11	0.72	0.68	0.07	0.68
Thanh Chuong	7.67	0.72	0.68	0.05	0.67
Average	6.63	0.72	0.67	0.08	0.68

LB: LangBiang buffalo; Na: average number of alleles; He: expected heterozygosity; Ho: observed heterozygosity; FIS: inbreeding coefficient within a population.

For the three buffalo populations the mean value of allele number, He, Ho, and PIC are presented in Table 3. The mean value of allele number was 6.38 (ranging from 6.11 in the LB-LD and LB-LC sub-population to 7.67 in the TC population), the mean He was 0.72 in each population/ sub-populations, the mean Ho was 0.67 (ranging from 0.64 in LB-LD to 0.68 in LB-LC or TC population), and the mean Fis value was 0.08 (ranging from 0.05 in TC to 0.12 in the LB-LD population). The allele numbers, Ho, He, F-statistics and PIC were obtained across the loci in all populations, as presented in Table 4.

The average allele number was 8.4, ranging from 4 in ILSTS005 to 18 in the ILSTS068 locus. The Fis had a positive value in all loci with average value 0.0081 and ranging from 0.0071 (CSSM022) to 0.0093 (CSSM041). The mean values of Fit and Fst were 0.014 (0.106 in CSSM022 to 0.127 in CSSM041) and 0.037 (0.032 in CSSM032 to 0.039 in ETH003/ ILSTS068), respectively. The mean values of Ho and He were 0.668 (0.302 in CSSM022 to 0.854 in ILSTS061) and 0.744 (0.451 in CSSM022 to 0.876 in CSSM019), respectively. The mean value of PIC was 0.706 (0.404 in CSSM022 to 0.859 in CSSM019).

In this study, the estimated values of He were higher than Ho for each population or sub-populations as well as for

all populations. The values of H_e and H_o obtained in this study were higher than reported by [Berthouly et al. \(2009\)](#) for Vietnamese local buffalo populations in Ha Giang, Nghe An, and Tay Ninh Provinces (H_e : 0.637-0.677; H_o : 0.531-0.608) and by [Merdan et al. \(2020\)](#) in Egyptian river buffalo populations (H_e : 0.59-0.67; H_o : 0.19-0.26). Similarly, an investigation of the genetic diversity among 17 water buffalo populations in 17 provinces of Turkey using 20 microsatellite markers, [Unal et al. \(2021\)](#) also found that the mean values of H_o and H_e across all polymorphic loci in all studied buffalo populations were 0.61 and 0.70, respectively. The value of H_o varied from 0.55 in the Bursa buffalo population (Marmara region) to 0.70 in the Mus buffalo (Eastern Anatolia Region), and the H_o value was lower than H_e in most of the populations, with a deviation from HWE (Hardy-Weinberg Equilibrium). The different of H_e value among populations might be due to different conditions of production systems, lack of male buffalo (or a good young male has sold out of herd as early stage of age due to high price for meat purpose), random mating after several generations without selection resulted in increasing of H_e as well as inbreeding in the population.

The mean value of the F_{is} (inbreeding coefficient) was 0.081, ranging from 0.05 in TC buffalo to 0.12 in LB-LD (data not shown). A positive F_{is} value indicates inbreeding within a population. [Berthouly et al. \(2009\)](#) reported that the F_{is} values among Ha Giang buffalo populations varied from 0.10 to 0.175. The F_{is} value of buffalo in Nghe An Province was 0.104 and buffalo in Tay Ninh Province had the highest value (0.19). For seven populations of Vietnamese cows, the average F_{is} was 0.07 based on 27 microsatellites ([Pham et al., 2013](#)) while for 27 Vietnamese indigenous pig breeds based on 20 microsatellites, the average F_{is} was 0.09 ([Ba et al., 2020](#)).

In all the populations, the average F_{is} value was 0.081 (ranging from 0.071 in CSSM022 to 0.093 in CSSM041). The average F_{it} value was 0.114 (ranging from 0.106 in CSSM022 and CSSM032 to 0.127 in CSSM041). The mean F_{st} value was 0.037 (ranging from 0.032 in CSSM032 to 0.039 in ETH003 and ILSTS068).

The F_{is} value is a good statistical method to assess alternative forms within a population that suggests depletion in heterozygosity through nonrandom mating ([Uffo et al., 2017](#)). Higher levels of inbreeding in an animal population result in a positive F_{is} statistic while outbreeding leads to a negative F_{is} statistic. A F_{is} value of zero designates inbreeding in line with the prognosticated amount based on allele frequencies in the population ([Wright, 1965](#)). Applying microsatellite techniques to evaluate the genetic diversity in cattle (HF crossbred and Sahiwal) and buffalo (Murrah and Nili Ravi), [Karthickeyan et al. \(2009\)](#) indicated that mean F_{is} values for the cattle breeds were -0.058

and -0.057 in HF Crossbred and Sahiwal, respectively. For buffalo breeds, the F_{is} values were -0.156 and 0.065 in the Murrah and Nili Ravi buffalo populations, indicating inbreeding at a low to moderate level. The Nili Ravi breed exhibited a positive F_{is} value indicating inbreeding within the population. The inbreeding value from this study also reflected the situation of increased of H_e compared to H_o in all populations, the excess of homozygosity in sub- or populations, with reference to molecular markers informs the pattern of reduction in diversity formed on several factors that existed in the populations, the positive value of F_{is} value obtained from this study showed deficiency of heterozygotes within populations, might be because of inbreeding and deviation of Hardy-Weinberg equilibrium.

The coefficient of genetic differentiation of a population is analyzed by estimation of the F_{st} value. In this study, the distribution of F_{st} was regarded as low genetic diversity ($0.000 < F_{st} < 0.037$) among all the populations, with the average proportion of genetic differentiation 3.2% among breeds. The F_{st} value in this study was lower than found in a genetic evaluation of Asian buffalo (16.8%) by [Barker et al. \(1997\)](#), Indian buffalo (3.4%) by [Kumar et al. \(2006\)](#), Turkish water buffalo (6.2%) by [Gargani et al. \(2010\)](#), Cuban and Brazilian buffaloes (7.5%) by [Marrero et al. \(2015\)](#), and Pakistan buffalo (7%) by [Hussain et al. \(2017\)](#) but higher than in Chinese buffalo (2.8%) reported by [Zhang et al. \(2008\)](#), Iranian buffalo (1%) by [Darestani et al. \(2019\)](#), and [Unal et al. \(2021\)](#) in Turkish buffalo (1%). Differences in F_{st} values between the buffalo populations were due to different breeds, the geographical distance between breeds, and/or bull exchanges between regions. According to the proposition for interpretation by [Wright \(1965\)](#) the range of F_{st} value can be classified such as: $0 \leq F_{st} < 0.05$ means small differentiation; $0.05 < F_{st} \leq 0.15$ means moderate differentiation; $0.15 < F_{st} \leq 0.25$ means important differentiation and $F_{st} > 0.25$ means very important differentiation. Regarding our result it indicate the genetic differentiation among buffalo population examined is moderate level.

The analysis of genetic diversity in Kangayam cattle (*Bos indicus* of Tamil Nadu) [Karthickeyan et al. \(2009\)](#) gave a F_{st} value of zero for all loci across all breeds, indicating the absence of notable genetic subdivisions between these cattle populations, with negligible gene flow across the breeds.

The F_{it} value can be used to estimate the heterozygosity loss of individuals in the overall population. In this study, the average F_{it} value was 0.114, speculating a lack of heterozygous individuals in the Vietnamese native swamp buffalo populations examined about 12%. This result showed that the F_{it} value was much lower than for buffalo populations in Pakistan ([Hussain et al., 2017](#)), Cuban water buffalo breeds ([Uffo et al., 2017](#)) or Turkish water buffalo populations ([Soysal et al., 2007](#); [Gargani et al., 2010](#)) but

higher than the F_{it} values reported in the Cuban and Brazilian buffaloes (Acosta *et al.*, 2014; Marrero *et al.*, 2015), Iranian buffalo (Darestani *et al.*, 2019), and Rumanian buffalo (Popa *et al.*, 2020). According to the explanation by Wright (1965), the value of F_{it} ranges from 0 (no isolation) to 1 (total isolation), however, Kanaka *et al.* (2023) also interpreted those lower values (for example 0.5) may signify complete isolation across populations when there is a tendency toward polymorphic loci.

Table 4: Genetic diversity parameters across loci in all populations.

Locus	N	Na	F_{is}	F_{it}	F_{st}	H_{Obs}	H_{Exp}	PIC
ETH003	96	8	0.082	0.117	0.039	0.750	0.805	0.772
ILSTS068	96	18	0.076	0.112	0.039	0.698	0.862	0.842
CSSM033	96	9	0.083	0.115	0.034	0.760	0.834	0.807
ILSTS052	96	11	0.081	0.116	0.038	0.792	0.837	0.815
CSSM041	96	5	0.093	0.127	0.037	0.740	0.624	0.552
CSSM022	96	3	0.071	0.106	0.037	0.302	0.451	0.404
CSSM047	96	8	0.075	0.107	0.035	0.604	0.757	0.714
CSSM032	96	7	0.076	0.106	0.032	0.594	0.770	0.732
CSSM029	96	6	0.081	0.115	0.037	0.500	0.567	0.524
ILSTS061	96	11	0.085	0.120	0.037	0.854	0.860	0.839
CSSM008	96	8	0.085	0.120	0.038	0.771	0.790	0.754
CSSM019	96	14	0.082	0.114	0.035	0.771	0.876	0.859
CSSM046	96	5	0.075	0.110	0.038	0.552	0.676	0.628
BMC1013	96	7	0.077	0.112	0.037	0.573	0.687	0.648
ILSTS056	96	8	0.086	0.119	0.037	0.792	0.814	0.783
ILSTS005	96	4	0.083	0.116	0.036	0.552	0.602	0.538
CSSM043	96	10	0.084	0.117	0.036	0.760	0.790	0.754
ILSTS028	96	9	0.079	0.110	0.034	0.667	0.789	0.751
Average		8.4	0.081	0.114	0.037	0.668	0.744	0.706
SEM		0.85	0.001	0.001	0.001	0.033	0.028	0.031

In this study, most of the 18 microsatellite loci had PIC values greater than 0.5, except for CSSM022 (Table 4), making them an advantageous tool in the field of study on genetic diversity. The PIC value is a commonly used parameter to indicate the level of informativeness of a microsatellite. The PIC values range from 0 to 1, the marker with a PIC value less than 0.25 is considered less informative while markers with PIC value greater than 0.50 are considered highly informative for the study of genetic variation (Botstein *et al.*, 1980). The mean PIC value obtained in this study was 0.706 (0.404–0.859) and comparable with values reported in the Indonesian swamp buffalo (Suputra *et al.*, 2020) or other water buffalo from Turkey, Cuba, Columbia, Iran, Egypt or Romani or Iraqi buffaloes (Unal *et al.*, 2014; Marrero *et al.*, 2015; Darestani *et al.*, 2019; Merdan *et al.*, 2020; Popa *et al.*, 2020). Several researchers also

mentioned varied PIC values for several markers, ranging between moderate (> 0.25 to < 0.5) and high (> 0.5). The variation in PIC values observed in the literature might due to different microsatellite markers used in the investigated populations (Angel-Marín *et al.*, 2010; Kathiravan *et al.*, 2012; Sing *et al.*, 2017; Unal *et al.*, 2021). Chesnokov and Artemyeva (2015), also mentioned that heterozygosity and polymorphic information content (PIC) are commonly used to evaluate the quality and informativeness of a polymorphism as a genetic marker. The high values of PIC and heterozygosity indicate a greater level of genetic variation and the informativeness of the marker in distinguishing between different genotypes or alleles within the population. The variation of the PIC values reported in the published papers may be due to different microsatellite markers and/or buffalo breeds used in the studied populations. In this study, our results showed allele-rich in each MS and high PIC values, which implied that the microsatellite markers used were highly polymorphic and useful for analyzing the genetic diversity of native buffalo in next step for other region of Vietnam.

GENETIC RELATIONSHIP

The result of the genetic distance analysis (Table 5) showed that the genetic differentiation (F_{st}) of TC and LB buffalo populations varied from 0.056 to 0.057, with pairwise Nei's genetic distance (D_s) from 0.21 to 0.22. The genetic distances between the two LB buffalo sub-populations were 0.006 (F_{st}) and 0.029 (D_s).

Table 5: Genetic differentiation (F_{ST}) and genetic distance (D_s) estimates among the three buffalo populations.

Buffalo population	LB-LD	LB-LC	TC
LB-LD		0.006	0.056
LB-LC	0.029		0.057
TC	0.21	0.22	

LB-LD: LangBiang buffalo in Lac Duong Town sub-population; **LB-LC:** LangBiang buffalo in Lat Commune sub-population; **TC:** Thanh Chuong buffalo population. Above the diagonal: genetic differentiation (F_{st}) data and below the diagonal: Nei's pairwise genetic distance (D_s).

The estimated result of the genetic distances among the three buffalo populations (Thanh Chuong: TC, Bao Yen: BY and Lang Biang: LB) by Nguyen *et al.* (2018) showed that the genetic distance between TC and BY populations was the lowest (0.12), followed by TC and LB (0.169) and the highest (0.177) was found between TC and BY. Vijn *et al.* (2008) used 22 microsatellites to determine the genetic distances between Indian buffaloes and the results revealed that the genetic distance among breeds varied from 0.044 to 0.598. The genetic distance between the two LB populations was small (F_{st} 0.006) compared with previous studies while the TC population showed a large genetic distance

from the LB population. Although the genetic distance between TC and LB was higher as compared to LB-LD or LB-LC but the genetic differentiation between two populations is still moderate to low level based on interpreted by Wright (1965) and the effect of geographical factor may be involved in genetic differentiation between TC and LB buffalo populations.

The genetic distance between buffalo populations was analyzed using principal component analysis (PCA), simulated as a 3-dimensional space, as shown in Figure 3a. The three population/sub-populations of buffaloes were distributed into two main groups: Group 1 included the two sub-populations of Lang Biang buffalo which had a close distribution, and Group 2 contained only the Thanh Chuong buffalo population with an independent distribution from the two Lang Biang populations. As showed in Figure 3a, the first PC (Axe1) was responsible for 88.65% genetic variation, separated TC from examined population (group 2) and the second PC (Axe 2) represented 11.35% of genetic variation, separated LB-LC and LB-LD sub-population (group 1). Unal *et al.* (2021) analyzed the genetic divergence among and within 17 buffalo populations in Turkey using 20 microsatellite markers and the PCA analysis separated river buffaloes into two basic clusters involving different regional populations.

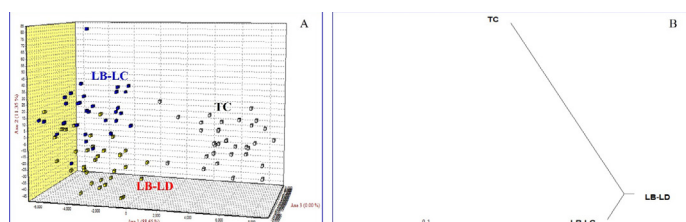


Figure 3: PCA result of TC and LB buffalo populations (A), and a neighbor-joining tree showing the genetic relationships between LB and TC buffalo populations (B) based on 18 microsatellite markers.

The genetic distance between buffalo populations was clearly illustrated by the phylogenetic tree (Figure 3b). Results showed that the three buffalo populations were distributed into two main clades. Clade 1 included the two LB buffalo sub-populations and Clade 2 consisted of the TC buffaloes. The TC buffalo population presented a valuable genetic distance compared to the LB buffalo populations and evolved in a separate direction. Based on the mtDNA analysis in D-loop, Nguyen *et al.*, (2022) also reported the separation of TC and LB buffalo populations. Combination the PCA and phylogenetic tree, it is revealed that PCA analysis can be considered as statistical tool to support the analyzing the genetic distance within and among populations.

Genetic diversity among and/or within populations is nec-

essary for well-organized, sustainable animal breeds and conservation planning. This study used 18 microsatellite markers and showed that local Vietnamese swamp buffalo populations exhibited noticeable genetic divergence from the low pressure of artificial selection and the possibility of non-random mating. Improvement of genetic quality can be carried out through reducing of inbreeding by providing good bulls at the regional area and rotating bulls among groups of female buffaloes. To develop the outbreeding programs, the genetic distance between buffalo sub- or populations within a district or province level can be determined through evaluating morphology and DNA markers (Berthouly *et al.*, 2009; Rusdin *et al.*, 2020; Saputra and Anggraeni, 2023). Therefore, a scientific solution to develop a practical production system is necessary to improve productivity without losing the significant genetic structure of these economically important animals in near future.

CONCLUSIONS AND RECOMMENDATIONS

Most of the microsatellite markers used in this study were highly polymorphic for detecting the genetic divergence of Vietnamese native buffalo. The allele-rich in the Thanh Chuong and Lang Biang buffalo breeds as well as inbreeding was found in all populations or sub-population of buffaloes. Two clusters of native buffaloes were recognized as the TC buffalo and the LB buffalo population which then separated into two sub-clades. These findings on the genetic diversity of Vietnamese local buffalo populations at LangBiang and Thanh Chuong contribute knowledge to preparing future strategies for conservation and breeding programs and the exchange of bulls, at least between two main populations can be considered.

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NOVELTY STATEMENT

Our results increase knowledge on the molecular genetic diversity and population structure of two Vietnamese local swamp buffalo populations. The findings have powerful implications in formulating future strategies for conservation and breeding programs to avoid inbreeding within and among buffalo populations, at least in part, through the exchange of buffalo bulls as suggested.

All the authors contributed to designing the experiments and reading as well as approving the manuscript at each step.

Van Dai Nguyen: Covered all the research, writing the research proposal.

Van Ba Nguyen: Covered all the data analysis, writing a draft manuscript.

Thi Thanh Nhan Giang, Cong Dinh Nguyen, Thi Lan Nguyen, Dinh Ngoan Vu, Duc Chuyen Nguyen, Van Can Ta, Khac Khanh Nguyen, and Thi Lien Cao: Shared the work equally for sample collection.

Doan Lan Pham and Khanh Van Nguyen: Contributed equally to the work on DNA extraction, primer testing, amplification of target genes, and sequence analysis.

Ngoc Tan Nguyen: As the corresponding author evaluated the manuscript, checked for plagiarism and revised manuscript.

CONFLICTS OF INTEREST

We certify that there are no conflicts of interest with any financial organization regarding the material discussed in this manuscript

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