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THE MOLECULAR GENETIC CHARACTERISTICS OF 15 VIETNAMESE DOMESTIC PIG BREEDS

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INTRODUCTION

Livestock genetic resources are vital for global food security and livelihoods, especially for developing countries. The demand for animal products has increased significantly, leading to changes in livestock production systems and replacements or crossbreeding between local breeds with higher performed exotic breeds in many developing countries. As a result, many indigenous breeds have become extinct, leading to a significant biodiversity loss (Rege and Gibson 2003). Moreover, it is well documented that maintaining sufficient diversity of animal genetic resources is crucial to ensure adaptation for uncertain future such as climate change, disease. Using biotechnology to research and evaluate molecular genetic characteristics of livestock breeds for conservation purposes for supporting selections of high-yield and high-quality livestock breeds were conducted in many countries around the world, such as analysis of microsatellite markers, sequencing and analysis of the mitochondrial DNA, SNP ….

According to the FAO (http://dad.fao.org/, access on July 201 8), Vietnam has 16 indigenous breeds, which are important genetic resources for the local farmers. The local pig breeds are economically crucial for regions in Vietnam and have adapted to specific habitats. Although not sufficient genetic information hindered genetic conservation efforts, only a few studies have been conducted on Vietnamese indigenous breeds. The current study was performed for all existing indigenous breeds better to understand local breeds' diversity for conservation purposes. Therefore, we carried out the project "**The molecular genetic characteristics of 15 Vietnamese domestic pig breeds**" to evaluate genetic diversity, genetic structure, and relationships; genetics, phylogenetic origin and genotype frequency characteristics number of molecular markers to support conservation, breeding and exploitation of genetic domestic pig breeds resources effectively.

2. Objectives of the study

The objectives of this study are,

To study genetic diversity, genetic distance and genetic structure of 15 domestic pig breeds were based on 19 microsatellites.

To determine the phylogenetic relationships among 15 domestic pig breeds and some pig breeds worldwide using sequence polymorphism of *Cyt b* gene.

To figure out the genotype and allele frequency of the candidate gene (*MX1* and *MX2*) related to vesicular stomatitis virus and porcine reproductive and respiratory syndrome virus resistance in 15 domestic pig breeds.

3. Research content

1. Research on genetic diversity, genetic distance and genetic structure of 15 domestic pig breeds based on 19 microsatellites.

2. Research on genetic diversity of *Cytochrome b* genes in 15 domestic pig breeds and phylogenetic relationships with some pig breeds globally.

3. Research on genetic polymorphism of *MX1* and *MX2* genes in 15 domestic pig breeds.

4. Scientific and Practical values

This study was the first systematic scientific document that provided information on the diversity, distance, and genetic structure based on microsatellites and phylogenetic origin of 15 Vietnamese domestic pig breeds. Some pig breeds in the world are using *Cytochrome b* gene sequence.

This study was the first systematic work in Vietnam providing information on genotype frequency, allele frequency of the *MX1* and *MX2* genes involved in disease resistance of 15 domestic pig breeds.

Articles published in domestic and foreign journals were valuable references for research and teaching.

Research results have contributed to the database of domestic pig breeds' genetic resources in Vietnam, thereby enabling further study to selected pig breeds with immune capacity (disease-resistant).

The thesis provides valuable information for conservation, breeding, orientation of breeding, exploitation, and use of domestic pig breeds' genetic resources effectively.

5. New contributions of thesis

The first systematic report investigated 15 Vietnamese domestic pig breeds' molecular genetic characteristics using markers of both the nuclear genome (microsatellites, *MX1*, and *MX2* genes) and the mitochondrial DNA (*Cytochrome b* gene) in Vietnam.

The thesis evaluated the genetic diversity, phylogenetic relationship, and genetic structure of 15 domestic pig breeds based on 19 microsatellites.

The phylogenetic tree was constructed using *Cytochrome B* gene sequences obtained from 15 Vietnamese domestic pig breeds and some pig breeds worldwide.

Polymorphisms of *MX1* and *MX2* genes were identified - the candidate genes related to 15 domestic pig breeds' disease resistance.

Chapter 1. LITERATURE REVIEW

Vietnam's domestic pig breeds are very diverse and distributed throughout the country, each with its own breeds. According to Ta Thi Bich Duyen et al. (2013), Vietnam has about 26 domestic pig breeds, raised mainly in rural and highland areas. Economic conditions are poor, harsh climate, and transportation is a problematic towel. The number of indigenous pigs in the regions has decreased rapidly in recent years.

Litt and Luty introduced the term microsatellite in 1989 to describe short tandem repeats sequences (2-6 bp) with high polymorphism. These molecular markers are valuable tools for assessing the degree of genetic polymorphism and genetic variation within and between populations.

Mitochondria are cellular organelles within the cytoplasm of eukaryote cells and are inherited in the maternal line, independent of the nucleus's genes. Mitochondria generate most of the cell's supply of adenosine triphosphate (ATP), used as a chemical energy source. The approximately 1140 bp *Cytochrome b* gene encodes the Cytochrome B protein, which functions as an electron transporter (proton H +) in the redox reaction chain for ATP synthesis. Many studies on population genetics and taxonomy apply *Cytochrome B* gene sequencing information in studies of the origin and maternal kinship relationships between species and breeds of animals.

MX is a gene that stimulates the production of an interferon-a protein that plays an antiviral role. Unlike birds, mammals typically have two isoforms of *MX* genes, *MX1* and *MX2*. The *MX1* gene encodes the MX1 protein molecule, first known in mouse flu resistance studies, and then considered an important candidate gene for resistance to viral infection in animals.

Chapter 2. MATERIALS AND METHODS

2.1. Objectives of the study

Fifteen domestic pig breeds distributed nationwide: Mong Cai, Ha Lang, Huong, Tap Na, Hung, Lung Pu, Muong Khuong, Lung, Ban, Meo, Van Pa, Co, Chu Prong, Soc and Ba Xuyen. Besides, an exotic breed (Landrace) and Vietnamese and Thai wild boar samples were also used for the thesis's research contents.

2.2. Period and location of the study

Ear tissue samples of 15 domestic pig breeds were collected from 2012 to 2013. The polymorphic analysis experiments of markers were conducted at the Key Laboratory of Animal Cell Technology - National Institute of Animal Science for four years (from 2014 to 2018).

2.3. Methods

2.3.1. Methods of sample collection and preservation

Use a specialized sampling pincer to cut a piece of ear tissue about 0.5 g, then place in an Eppendorf tube containing 1.5 ml of alcohol (99%). Samples were transported to the laboratory and stored in a deep refrigerator (-20^{0}C) until DNA extraction.

2.3.2. Methods of extraction and determination of the concentration of DNA

DNA was extracted from ear tissue samples using the *AccuPrep*® Genomic DNA Extraction Kit (K3132-Korea), according to the manufacturer's instractions.

Determining the concentration and purity of extracted DNA: Using 1% agarose gel electrophoresis method to assess the qualitative of DNA extraction results. Next, each sample's DNA concentration was checked using NanoDrop 2000 UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., Waltham, MA, USA) to confirm the extracted DNA quality and quantity.

2.3.3. Research methods for genetic diversity, genetic distance and genetic structure by microsatellites

Samples used for analysis: 608 samples of 15 domestic pig breeds were analyzed genotyping, together with 15 samples of Landrace pigs and two wild boar breeds (Vietnam-6 samples and Thailand-9 samples) were used for comparison.

Microsatellite markers: All 19 pairs of microsatellite primers in swine recommended by FAO/ISIG in 2004 were carried out in 4 multiplex PCR.

Analysis of polymorphism of microsatellite indicators: The genotypes of pigs were separated by a capillary sequencer (Beckman Coulter CEQ8000).

Estimating the number of indicators related to genetic diversity: The number of alleles per locus, the average number of alleles per locus of breed, and the allele richness were estimated using FSTAT software version 2.9.3.2 (Goudet J. 2002). Expected (He) and observed heterozygosity (Ho), the F-coefficient (Fis, Fst) of locus and the mean of all microsatellites per breeds were estimated by Genetix software (version 4.0.5.2). The polymorphic information content value (PIC) of loci was calculated according to Botstein et al. (1980).

Testing Hardy-Weinberg equilibrium: The Genpop 1.2 software was used to test the exact Hardy-Weinberg equilibrium in breeds.

Determining the genetic distance between populations: Nei's standard genetic distance (Nei's, 1972) among breeds was calculated using Genetix software (version 4.0.5.2).

Determining the genetic structure: The genetic structure of 15 pig breeds was analyzed by a Discriminant Analysis of Principal Components (DAPC) using the "adegenet" package (Jombart et al. 2008) for R software " (Team, 2013).

2.3.4. Research method for Cytochrome B genetic diversity and phylogenetic relationships.

Samples used for analysis: This study was performed on 284 samples of 15 domestic pig breeds and a wild boar sample. The haplotype sequences have been published in the gene bank used for comparison: 2 haplotype Japanese pig; 3 Chinese pig haplotype; 1 haplotype of European pigs (Duroc); 2 haplotypes of Asian wild boar; 4 haplotypes European wild boar.

Polymerase chain reaction amplification: The *Cytochrome b* gene (1140 bp) was amplified by PCR reaction using H6 and L7 primers.

Purification of PCR products and sequencing: The PCR products were purified using the Invitrogen purification kit. The purified PCR products were then sequenced by direct sequencing on AB-3130 of Applied Biosystem.

Analysis of results: In this study, raw data of 285 cytochrome b gene sequences were processed using BioEdit 6.0 software. DnaSP 5.10 software was used to analyze the polymorphism of nucleotides and haplotypes. An unrooted Neighbor-Joining tree was built in MEGA 7.0 software to determine the phylogenetic relationships between breeds.

2.3.5. Methods to study the genetic diversity of gene locus MX1 and MX2

Samples used for analysis: 608 samples from 15 domestic pig breeds were used for genotyping *MX1* and *MX2*.

Polymerase chain reaction amplification: PCR was carried out with a total volume of 25 µl with primers specific to each gene according to previous publications (MX1 intron 6 - Li et al., 2007; MX1 promoter - Li et al. et al., 2015; MX2 - Sasaki et al., 2014).

RFLP and electrophoresis: The PCR product was cut with restriction enzymes according to the manufacturer's instructions. Gene polymorphism was examined via agarose gel electrophoresis (2.0%).

Chapter 3. RESULTS AND DISCUSSION

3.1. DNA extraction and concentration.

Six hundred and eight DNA samples were extracted from 608 ear tissue samples of 15 domestic pig breeds. Electrophoresis gel images showed that the DNA has uniform backgrounds, non smeared bands. After measuring on Nanodrop 2000, the DNA samples have a high purity with the rate A260 / 280 in the range of 1.79 - 2.03 and the total concentration of DNA range from 70-150µg / µl.

3.2 Genetic diversity, phylogenetic relationship, and genetic structure of 15 domestic pig breeds based on the 19 microsatellites

3.2.1. Results optimization *of 4 multiplex PCR*

Using Qiagen's PCR multiplex Kit to optimize multiplex PCR assemblies with the 19 fluorescently marked primer pairs in 4 multiplex PCR reactions. Summary of research results on Vietnamese pigs shows that 14/20 locus have different allele ranges from FAO / ISAG (2004) in world pigs: Sw122, Sw857, S0097, Sw72, S0026, S0155, Sw936, S0215, S0225, Sw2410, S0226, Sw2008, and Sw911.

3.2.2. Genetic polymorphism of the 19 microsatellites

A total of 280 alleles were detected in the 19 microsatellites of 638 pigs. The number of alleles ranged from 9 to 19, with a mean value of 14.7. The allelic richness ranged from 4.18 to 7.10. The Sw2410 also showed the highest importance for the allelic richness. The PIC value averaged 0.81 with a range of 0.68 to 0.90. The observed heterozygosity (Ho) ranged from 0.56 to 0.76, and a mean was 0.67. The expected heterozygosity (He) ranged from 0.61 to 0.81 and a mean of 0.73. The average inbreeding coefficient (Fis) of the 19 microsatellites was 0.13. The mean F-statistics (Fst =0.15) observed across loci indicating that approximately 15% of the total genetic variation corresponded to differences between breeds. In comparison, the remaining 85% were attributed to differences among individuals within a breed.

3.2.3. Genetic diversity of pig breeds

The average number of alleles in 15 domestic pig breeds ranged from 5.68 (Huong) to 10.68 (Tap Na), while in Landrace pigs, it was 4.84 and wild boar was 5.47-6.00. The allelic richness was the lowest in Co pigs (3.38) and highest in Tap Na pigs (5.09) while Landrace pigs were 3.59 and in wild boars was 4.55-5.64. The He ranges from 0.59 (Co) to 0.78 (Ha Lang), and Ho ranged from 0.58 to 0.78. Vietnamese wild boar has a higher He than the average domestic and Thai wild boar breeds. The values of the average number of alleles, the allelic richness, and the heterozygote frequency of Landrace pigs were lower than that of domestic and wild boars. The average inbreeding coefficient of 15 domestic pig breeds was 0.08 (ranging from -0.002 in Van Pa to 0.180 in Mong Cai). Very high Fis was found in Ha Lang, Hung. Mong Cai and Soc. The inbreeding coefficient in Vietnamese wild boar (0.142) is higher than in Thai wild boar (0.032). The Hardy-Weinberg equilibrium tests using Genepop software indicated the five breeds, namely: Huong, Lung Pu, Mong Cai, Co, Landrace and Ba Xuyen, were under heterozygote deficit ($p < 0.05$).

3.2.4. Genetic distances

Genetic distances between 15 domestic pig breeds, wild boar and Landrace pig were estimated according to Nei, 1972. The genetic distance is the lowest among the Ban-Meo (0.101) and the largest among the Huong-Thailands (1.498). Among 15 domestic pig breeds, Ba Xuyen pigs have a long genetic distance when compared to other domestic pig breeds (0.56-1.29). Pig breeds in the North, such as Mong Cai, Huong, Ha Lang, Lung Pu, Muong Khuong, Hung, Lung, Ban, and Tap Na, have close genetic distances (in the range of 0.2-0.7). According to Le Viet Ly et al. (1999), the Ba Xuyen pig was formed from crossbreeding between local pigs in the South Viet Nam and European pigs. This study also showed that Ba Xuyen pigs' genetic distance with Landrace pigs is the closest. Ishihara et al. (2018) said that most domestic pig breeds in Vietnam were distributed in groups similar to the geographical distances except for Ba Xuyen pigs. However, in this study, some breeds had genetic distances that do not comply with geographical distances. As the Meo pig in Nghe An, quite far from Son La, but the genetic distance is very close to the Ban pig; Ba Xuyen pig (the South) have the genetic distance relative to the Lung pigs and the Ha Lang pigs (the North) although they are far geographically distant. Phylogenetic trees built on genetic distances were shown in Figure 3.3.

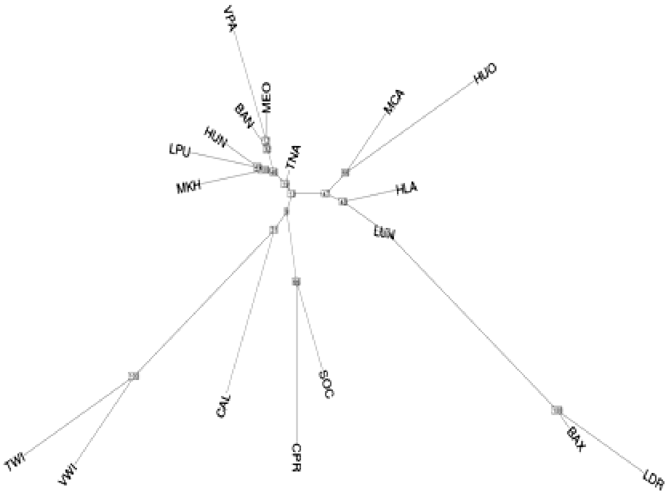

Fig 3.3. Genetic relationship among the eighteen populations analyzed in this study. Neighbor-joining (NJ) dendrogram based on Nei's 1978 genetic distances estimated with 19 microsatellites

The constructed phylogenic trees showed three separate branches: - The first branch included BAX, LDR, LUN, MCA, LPU and HUO breeds; The second branch consisted of VWI, TWI, CAL, SOC, and CPR and the remaining one included other local breeds. Moreover, the LPU, HUN, and MKH tended to group in a new subgroup, while VPA, MEO and BAN also tended to cluster in the same subgroup. Moreover, it is also worth mentioning that the lowest genetic distance among the local breed was found between the BAN and MEO breeds. It could be explained by the migration of the minority group of Thai or Hmong people from Son la province (original province for BAN breed) to the Nghean province (original province for MEO breed). The branches of the tree do not reflect the geographic distance of pigs completely. The high bootstrap value obtained indicated that the tree was robust.

3.2.5. Genetic structure

DAPC analysis results show that the number of groups identified corresponding to the minimum BIC value is K = 12. Details of the genetic structure of 18 breeds of pigs were shown in Figure 3.5.

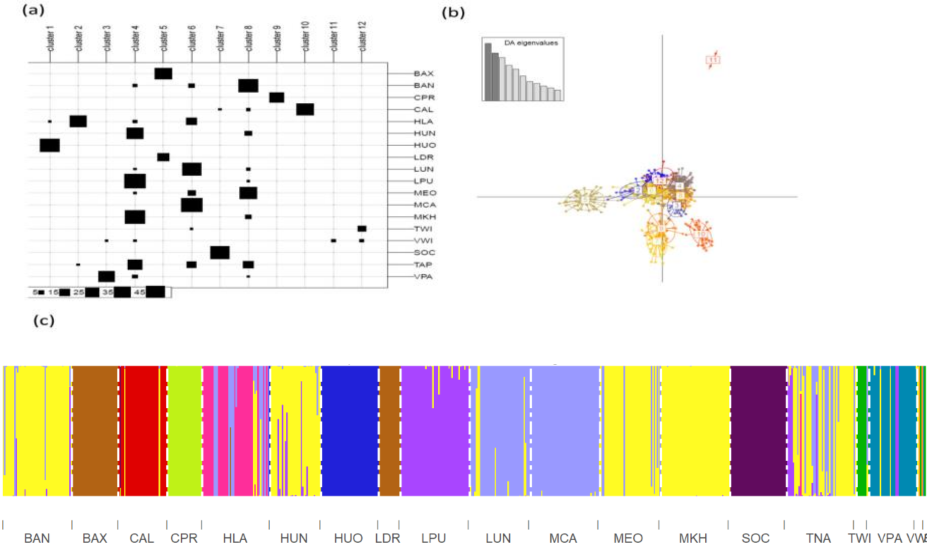

Fig. 3 Principal component analyses and clustering assignment of the studied populations obtained using the DAPC analysis

The genetic structure of 18 pig breeds distributed in 12 genetic clusters in Figure 3.5, clusters 4, 6 and 8 are pretty complex. Via the detailed image of 12 genetic clusters showed that some pig breeds have pure structure, some are relatively pure and complex. Ba Xuyen, Landrace, Huong, Mong Cai, Chu Prong and Soc are relatively pure, while Ha Lang, Tap Na and Vietnamese pigs were mixed.

The DAPC analysis results also showed that Ba Xuyen pigs belonged to the same group as Landrace pigs. The black and white pig breeds near the northern border (Ha Lang, Huong, and Mong Cai) were in 3 different clusters. Huong and Mong Cai pigs belong to two separate groups, while Ha Lang pigs have a mixture of genetic genes with four distinct clusters. In addition to the specific gene of cluster 2, Ha Lang pigs have the most mixing with Mong Cai pigs, followed by black pigs in Cao Bang, Ha Giang and Lao Cai. Of the 15 domestic pig breeds, apart from the Ba Xuyen pigs and three kinds of black and white Lang pigs, there are nine black pig breeds; the remaining is black and white. Northern black pig breeds were from two typical clusters (number 4 and number 8). Cluster 4 was a black pig in the North (Cao Bang, Ha Giang, Lao Cai), Cluster 8 was a black pig in the Northwest (Son La). The analysis of the genetic structure of cluster 6 showed the distribution of Mong Cai pig genetic resources mixed in Ban, Meo, Ha Lang, Tap Na, and Lung pigs. Because the Mong Cai breed has superior characteristics, the Mong Cai pig gene source's widespread influence on many other domestic pig breeds resulted from the productivity enhancement process in the 1980s. Although Co and Van Pa pigs were still partially mixed, Van Pa, Co, Soc and Chu Prong distributed four separate genetic clusters forming endemic breeds of the Central and Central Highlands.

3.3. The diversity of Cytochrome gene in 15 domestic breeds

3.3.1. PCR reaction results

Cytochrome b gene was successfully amplified by PCR reaction with H6 and L7 with 1140 bp fragment as expected.

3.3.2. The results of *cytochrome b* gene sequencing

The results of *cytochrome b* gene sequencing in each direction are exact up to 700 nucleotides. After removed the beginning and the end, consensus sequences approximately 975 bp long were retrieved for each individual by assembling sequences from both strands from 285 pig samples (284 domestic pigs and a wild boar)

3.3.3. The diversity of nucleotide and the polymorphism of cytochrome B gene sequence in each domestic pig breed in Vietnam.

In 15 domestic pig breeds in Vietnam, Ba Xuyen pig breed (Ke Sach- Soc Trang) and Meo pig breed (Ky Son- Nge An) had high haplotype diversity, seven different haplotypes were discovered with the haplotype (Hd) diversity (0,634), nucleotide (Pi) (0,00124) in Ba Xuyen and (0,792) (Hd), (0,00183) (Pi) respectively in Meo. In contrast, only a haplotype was found in Huong (Hoa An- Cao Bang).

With 28 detected haplotypes, haplotype 1 and haplotype 9 were the two most popular haplotypes found in 16 pig breeds with the rate of 166/285 and 60/285 respectively (Table 3.8), mainly collected wild boar samples which coincide in sequence with haplotype 1- popularly in 15 domestic pig breeds. These results were not high; on 16 analyzed breeds, there were only 28 different haplotypes identified. There is a significant difference when compared genetic differences between the 28 haplotypes. Meanwhile, there was only a sample of wild boar in the popular haplotype. Therefore, when building phylogenetic trees among breeds, sequence data of wild boar samples are removed.

The phylogenetic tree was divided into three main groups as follow:

- Group 1: Mong Cai and Ha Lang

- Group 2: Tap Na, Muong Khuong, Hung, Lung Pu, Co, Mezzan, Van Pa, Soc, Meo, Ban, Chu Prong and Ba Xuyen

- Group 3: Huong

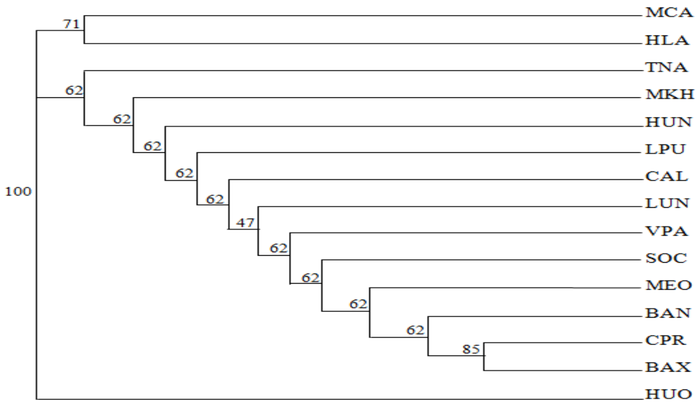

Figure 3.8. Phylogenetic tree of relationship among 15 Vietnamese domestic pig breeds based on Cytochrome B gene sequences (Neighbor-joining)

Figure 3.8 showed that Mong Cai pigs and Ha Lang pigs have a close genetic relationship. It is consistent with the previous results of genetic structure analysis based on 19 microsatellite markers. In this analysis, the Huong pig had a long genetic distance and was separated by a branch from the other breeds. Genetic structure analysis was based on 19 microsatellite markers in Huong having pure and separate genetic structure and the diversity at a low level. Four pig breeds in the Northern border mountainous areas in group 2 (Tap Na, Muong Khuong, Hung, Lung Pu) had close relationships with each other (figure 3.8), also included the black pig breeds in Lung, Van Pa, Co and Soc. They are black and high lean percentage (Ta Thi Bich Duyen et al., 2013). Ban pigs and Meo pigs are also close together. It is the same as Chu Prong and Ba Xuyen in group 2. It was molecular evidence confirming Ba Xuyen pig's origin that was a cross between European boar and southern native female pig.

3.3.4. The additional relationship between Vietnamese pigs and domestic pigs, Asian and European wild boars

The relationship appeared between 28 haplotypes of 16 Vietnamese pig breeds and *Cytochrome b* gene haplotype samples from GenBank. The total number of 40 haplotypes *cytochrome b* gene was analyzed and compared sequences. The results showed that a Japanese domestic haplotype (AB015078) coincided with haplotype number 1 in 28 haplotypes in 16 pig breeds in Vietnam. Therefore, there are only 39 haplotypes analyzed. Basing on 39 different haplotype sequences, the genetic diversity assessment results identified 51 polymorphism points and nucleotide sequence diversity (Pi) among haplotypes 0.00157.

The phylogenetic tree of 39 haplotypes based on the neighbour-joining was showed in Figure 3.9.

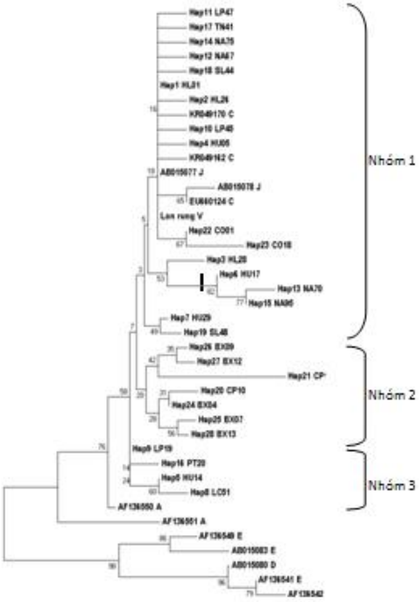

Figure 3.9. Phylogenetic tree of relationship among Vietnamese domestic pig and other pig breeds in the world (Neighbor-joining).

It shows that the Vietnamese pig's haplotype samples have a close genetic relationship with Asian wild boars, utterly separate from domestic pigs and European wild boars. Significantly, the pig samples raised in Vietnam were divided into three main branches. One branch was close to the Chinese and Japanese swine patterns. One intermediate and the other was close to the Asian wild boar samples.

The haplotype identified in domestic pig breeds, including those at Gia Lai and Dak Lak (Chu Prong pigs, Soc pigs), were genetically close to Asian wild boars. This result was consistent with the study of Le Thanh Long et al. (2014). The study results did not find the European haplotype in Vietnamese native pig samples. Meanwhile, Clop et al. (2004) found both Asian and European haplotypes when analyzed in European breeds (Canarian black, German Pietrain, Belgian Pietrain and Landrace pigs). According to the content section 3.1, Ba Xuyen pig had a close relationship with Landrace pigs. However, the genetic relationship analysis based on the *Cytochrome b* gene sequence showed no haplotype of Vietnamese pigs located in the European pig clade, including Ba Xuyen pig. However, the genetic relationship analysis based on the *Cytochrome b* gene sequence showed no haplotype of Vietnamese pigs located in the European pig clade, including Ba Xuyen pig. It can be assumed that Ba Xuyen pigs were formed during the crossbreeding process between native Vietnamese pigs and European pigs, so the genetic part (mitochondrial DNA) of Vietnamese pigs was still preserved in this pig breed. The results confirmed that 15 domestic pig breeds do not have the same maternal lineage as European pigs.

3.4. The genetic polymorphisms *MX1* and *MX2* genes in 15 domestic pig breeds

3.4.1. The results PCR

<u>3.4.1.1. Mx1 intron 6</u>

According to Li et al. (2007), the PCR product of the *Mx1* gene had about 1133 bp, consistent with the size published in the gene bank (AH015318.2). The results PCR of Mx1 gene on domestic pig breeds in Vietnam have a larger size than the results of Li et al. (2007); Feng et al. (2012) in Chinese indigenous pigs (911 bp).

3.4.1.2. Mx1 promoter

Mx1 promoter was successfully amplified and identified three genotypes polymorphism with specific primers corresponding to 2 alleles: A (461 bp) and B (736 bp). The genotype AA had size 461 bp, AB – 736 bp and 461 bp, BB - 736 bp.

3.4.1.3. Mx2

According to Sasaki et al. (2014) primer, Mx2 was successfully amplified with a fragment of about 211 bp.

3.4.2. Genetic polymorphism MX1 and MX2 genes

3.4.2.1. Mx1 - intron 6

After Mx1-intron 6 (1133bp) was amplified to determine genotype polymorphism, PCR reaction product Mx1 - intron 6 was cut with restriction enzyme *SnaB*I. The results identified three genotypes: AA, AB and BB with two alleles, A (1133 bp) and B (764 and 369 bp). The results of the analysis of the Mx1- intron 6 genes of 15 domestic pig breeds were shown in Table 3.9.

Table 3.9. Genotypes and alleles frequency of the gene Mx1-intron 6

Breeds	Genotypes and alleles frequency (%)					χ^2
	AA	AB	BB	Allele A	Allele B	
Ban	41.7	37.5	20.8	60.4	39.6	*2.24*
Ba Xuyen	96.8	3.2	0.0	98.4	1.6	*0.01*
Co	53.6	21.4	25.0	64.3	35.7	7.96
Chu Prong	52.6	31.6	15.8	68.4	31.6	*2.75*
Hung	37.1	20.0	42.9	47.1	52.9	12.54
Ha Lang	65.2	28.3	6.5	79.4	20.7	*0.87*
Huong	28.2	43.6	28.2	50.0	50.0	*0.64*
Lung Pu	19.2	27.7	53.2	33.0	67.0	6.59
Lung	63.6	27.3	9.1	77.3	22.7	*1.65*
Mong Cai	52.1	33.3	14.6	68.8	31.3	*2.41*
Meo	53.9	28.2	18.0	68.0	32.1	4.85
Muong Khuong	29.8	21.3	48.9	40.4	59.6	14.65
Soc	84.6	10.3	5.1	89.7	10.3	7.65
Tap Na	34.1	36.4	29.6	52.3	47.7	*3.24*
Van Pa	84.9	9.1	6.1	89.4	10.6	8.94
Average	53.2	25.3	21.6	65.8	34.2	5.00

According to the results of the analysis, there was a difference among 15 domestic pig breeds. Ba Xuyen, Soc, and Van Pa had a high AA genotype rate and low rate of BB (high frequency of allele A, low allele B). An allele frequency (98.4%) and 96.8% of samples have AA homozygous genotype appeared in Ba Xuyen. The A allele frequency was as high as Landrace (Li và cs., 2007; Ze và cs., 2012). Also, a domestic Vietnamese breed originated from European pigs. In contrast, Lung Pu and Muong Khuong had low allele A and high allele B frequency. Mong Cai, Ban, Meo, Tap Na have an average frequency of alleles A and B. The total result of AA is 53.2%, AB is 25.3%, BB is 21.5%. The average frequency of allele A and B are 65.8% and 34.2%, respectively. According to Hardy-Weinberg's law about the frequency of Mx1 - intron 6, Ban, Ba Xuyen, Chu Prong, Ha Lang, Huong, Lung, Mong Cai and Tap Na are the same ($\chi 2 < \chi 2$ (1; 0.05) = 3.81) except Co, Hung, Lung Pu, Meo, Muong Khuong, Soc and Van Pa. The reason might be that in these varieties under pressure selectively according to specific tendencies or inbreeding.

3.4.2.2. Gen Mx1- promoter

According to Li et al. (2015), by PCR method with specific primer pair, Mx1- promoter was successfully amplified and identified three genotypes polymorphism corresponding to 2 alleles A (461bp) and B (736bp) with the sizes as follow: AA- 461bp, AB- 736 bp and 461 bp, BB- 736 bp. There was a difference between allele A and allele B because at the position - 547 of the *Mx1* gene, a short repeating sequence polymorphism was inserted into the promoter region of the *Mx1* gene (SINE sequence) of size 275 bp. The results of Mx1 - promoter among Vietnamese domestic pig breeds were shown in Table 3.11. The results showed a big difference between allele A and B's frequency among 15 domestic pig breeds in Vietnam. The average rate of allele B is very high (87.8%), while that of allele A is low (12.2%). However, Ba Xuyen (crossbreed of European pigs with domestic pigs) have the highest allele A frequency (88.1%), with the majority of individuals carrying genotype AA (81.0%), AB and BB (14.2% and 4.8%).

Table 3.11. Genotype rate and allele frequency of Mx1-promoter of 15 domestic pig breeds

Breeds	Genotypes and alleles frequency (%)					χ^2
	AA	AB	BB	Allele A	Allele B	
Ban	0.0	4.7	95.3	2.3	97.7	0.024
Ba Xuyen	81.0	14.2	4.8	88.1	11.9	4.272
Co	0.0	0.0	100.0	0.0	100.0	N
Chu Prong	0.0	11.1	88.9	5.6	94.4	0.125
Hung	0.0	0.0	100.0	0.0	100.0	N
Ha Lang	8.9	17.8	73.3	17.8	82.2	6.911
Huong	0.0	0.0	100.0	0.0	100.0	N
Lung Pu	0.0	0.0	100.0	0.0	100.0	N
Lung	4.8	52.3	42.9	31.0	69.0	2.135
Mong Cai	0.0	5.3	94.7	2.6	97.4	0.028
Meo	0.0	16.7	83.3	8.3	91.7	0.397
Moung Khuong	0.0	0.0	100.0	0.0	100.0	N
Soc	0.0	13.0	87.0	6.5	93.5	0.224
Tap Na	2.5	10.0	87.5	7.5	92.5	3.12
Van Pa	0.0	27.3	72.7	13.6	86.4	1.097
Average	6.5	11.5	82.0	12.2	87.8	

N: unidentified

Except for Pigs, Van Pa and Ha Lang), the domestic pig breeds have a low allele A, very high allele B (over 90%). BB's rate was relatively high, and if excluding Ba Xuyen breed, BB is the genotype for domestic pig in Vietnam. According to Hardy-Weinberg's Law of allele frequency of Mx1-promoter, Ban, Chu Prong, Lung, Mong Cai, Meo, Soc, Tap Na, and Van Pa are the same ($\chi2 < \chi2$ (1; 0.05) = 3.81), while Ba Xuyen and Ha Lang are different. According to Li et al. (2015), allele B is considered to be typical of the Chinese native pig breed and has a very high frequency (0.88 - 0.92), whereas A is characteristic for European pigs with high frequency (over 0.90). The study results show that allele B (containing the SINE)- is associated with resistance to the PRRS virus. This gene segment was nominated as a crucial potential marker related to the resistance to the PRRS virus.

3.4.2.3. Gen Mx2

According to Sasaki et al. (2014), The Mx2 gene segment was amplified by PCR reaction with primers has a size of 214 bp. There are three genotypes obtained: AA (214 bp), AB (214, 191 and 23 bp) and BB (191 and 23 bp) after cutting with the *Xho*I restriction enzyme. However, the results of PCR-RFLP only obtained 100% AA genotype in all 15 domestic pig breeds. According to Sasaki et al., 2014, the A allele was related to the VSV virus's resistance. The role of the amino acid polymorphic allele 514 in the *Mx2* to the resistance virus is similar to the amino acid polymorphism of 631 in the *Mx* gene of chickens.

CONCLUSION AND RECOMMENDATION

CONCLUSION

1. The genetic diversity and structure of 15 pig breeds based on microsatellite markers

Fifteen Vietnamese domestic pig breeds studied all indicate high genetic diversity. Ban, Meo, and Tac Na pigs have high genetic diversity, followed by Ba Xuyen, Hung, Ha Lang, Muong Khuong, Mong Cai, Lung, Lung Pu, Soc and Van Pa pigs; Huong, Co and Chu Prong have lower genetic diversity.

According to the geographical distribution, between 15 domestic pig breeds, wild boars and exotic pigs, the genetic distances are similar. Eighteen breeds of pig were distributed in 3 big branches based on the genetic relationship between 15 domestic pig breeds with two wild boar and Landrace on the phylogenetic tree.

The genetic structure of 18 breeds of pigs was divided into 12 clusters. Ba Xuyen, Landdrace, Huong, Mong Cai, Chu Prong and Soc have pure structure. Some pig breeds have a relatively pure structure, such as Ban, Co, Hung, Lung, Lung Pu, Meo, Muong Khuong, Van Pa and Thai wild. Ha Lang, Tap Na and Vietnamese wild have a mixed structure.

2. Genetic diversity of mitochondrial Cytochrome b

Out of a total of 285 pig samples from 15 domestic pig breeds and 1 sample of Vietnamese wild boar, 28 haplotypes of *Cytochrome b* genes had been identified. Although the diversity of cytochrome b haplotypes in 15 domestic pig breeds is low, the genetic variation between haplotypes is relatively high and classified into three groups. The research results are molecular evidence of the phylogenetic relationship between domestic pigs in Vietnam and domestic pigs and Asian and European wild boars. It proved that Vietnamese domestic pigs had the same origin from Asian wild boars.

3. *MX1* and *MX2* gene

Gen Mx1 - intron 6 had a high polymorphism in 15 domestic pig breeds, in which the frequency of allele A (65.8%) was dominant compared to allele B (34.2%). Ba Xuyen, Soc, Van Pa have B allele low. It was in contrast to Lung Pu, Muong Khuong, Hung, Huong, Tat Na and Ban.

There was a difference in the frequency of allele Mx1 –promoter gene of 15 domestic pig breeds. The average frequency of an allele was low (12.2%), and allele B was very high (87.8%) appeared in Ban, Co, Chu Prong, Hung, Huong, Lung Pu, Mong Cai, Meo, Muong Khuong, Soc, Tap Na. Ba Xuyen has the lowest B allele frequency.

There is no polymorphism in the *Mx2* gene in 15 domestic pig breeds in Vietnam; all studied breeds only appeared AA genotype with the A allele frequency of 100%.

RECOMMENDATION

From the above research results, we give the following suggestions:

1. Hung, Ha Lang, Mong Cai and Soc with high inbreeding coefficients need a strict breed management policy to avoid the harmful effects of inbreeding.

2. Some pig breeds such as Hung, Ha Lang, and Tap Na with mixed genetic structures need to be selected and purebred.

3. To exploit the genetic characteristics of Vietnamese domestic pig breeds, it is necessary to have research to solve and analyze the entire genome sequence.

4. To find, evaluate, and breed domestic pigs resistant to diseases based on molecular markers, there should be a more in-depth research program.

PUBLISHED WORKS

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2. Nguyen Van Ba, Tran Thi Thu Thuy, Le Quang Nam, Nguyen Van Hau và Pham Doan Lan. Genetic polymorphisms of MX1 and MX2 genes in 15 Vietnamese native pig breeds. Journal of Animal Science and Technology No. 90, August 2018 p: 59 - 66.

3. Nguyen Van Ba, Le Quang Nam, Do Ngoc Duy, Nguyen Van Hau and Pham Doan Lan. An assessment of genetic diversity and population structures of fifteen Vietnamese indigenous pig breeds for supporting the decision making on conservation strategies. Tropical Animal Health and Production (2020) 52 p: 1033 - 1041.