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Full Paper

Diversity of northern Thai native pigs determined by microsatellite analysis

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Abstract: Thai native pigs are traditional local breeds in rural areas and highland areas of Thailand. They are better adapted to the climate and part of the cultural heritage of communities in these areas. However, they are at risk of loss of genetic diversity because of modern agriculture and globalisation. We evaluated genetic diversity within and between five native pig populations from four river basins (n = 49) based on 10 microsatellite markers including eight markers from the FAO/ISAG panel. All microsatellites exhibited high degrees of polymorphism and allelic diversity. The mean polymorphism information content and observed and expected heterozygosity values were 0.725, 0.624, and 0.759 respectively. Thai native pigs in Mae Hong Son province exhibited high diversity and phenotype variation. Based on Nei's genetic distances, the five populations were classified into two main groups, in which native pig population from Pang Dang Nai, Chiang Mai was separated from the other four populations. We further evaluated 15 microsatellites for detailed diversity and clustering analyses of individuals with different phenotypes from Mae Hong Son (n = 17) and Nan (n = 5). The unweighted pair group method with arithmetic mean dendrogram indicated three major clusters, in which pigs with the same coat colour pattern were grouped together. However, some complex phenotypes could not be resolved by this set of markers alone. These results improve our understanding of current phenotypic and genetic diversity in native pigs in northern Thailand.

Keywords: Thai native pigs, microsatellites, genetic diversity, phenotypic diversity, breed identification

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INTRODUCTION

Thai native pigs have been classified into four breeds, namely Raad, Puang, Kwai and Hailum, based on morphological traits and region [1]. The native pigs are important for small-scale rural farmers and hill tribes on account of their resistance to diseases such as foot and mouth disease and internal parasites, their adaptability especially to hot and humid climate [2], low management requirements, their consumption of low-quality feed with high fibre content [3], and their involvement in local customs and religion. They are a valuable genetic resource for breed improvement and for sustainable agriculture. However, commercial pigs have been used to improve the genetic background for lard-type pigs with slow growth rates and low reproductive rates and the Chinese Meishan pig has been introduced to improve maternal traits. A number of pig populations in different regions of Thailand have declined and it is difficult to identify breeds by phenotypic characteristics. The Thai native pigs account for 6.4% of all pigs in Thailand and are reared by communities in northern areas under the Fifth Regional Livestock. This region, consisting of eight provinces, harbours approximately 31% of all Thai native pigs [4]. Therefore, a lack of a conservation strategy will lead to loss of genetic diversity and breed identity.

Microsatellites are widely used because they are highly polymorphic, follow Mendelian inheritance, and can be used for comparative studies of genetic distances between breeds, populations or individuals [5]. The use of different sets of microsatellites limits comparisons among breeds. The Food and Agriculture Organization and the International Society for Animal Genetics (FAO-ISAG) recommended a microsatellite panel to measure genetic diversity in various animal taxa including pigs [6]. This set of microsatellite markers has been used to analyse genetic diversity and classify breeds. For example, it has been applied to Chinese indigenous pig populations [7, 8], pig breeds in Korea and China [9], native Indian pigs [10, 11, 12], southern African domestic pigs [13], Brazilian pig breeds [14], Vietnamese native breeds [15] and Philippine native pigs [16].

Genetic variation in northern (n=22) and north-eastern (n=27) Thai native pigs was first evaluated using 15 microsatellite markers by Chaiwatanasin et al. [17] and the genetic relationship of this native group with the other three commercial breeds was studied [18]. The study did not report the specific location of the native pigs sampled. In 2007 Yang [19] has characterised genetic properties of north-eastern and southern Thai native pigs in comparison with a wild boar and Chinese Qianbei black pig populations based on 12 microsatellites, as recommended by FAO/ISAG. Recently, 26 microsatellite markers covering all porcine chromosomes, including 22 markers recommended by FAO/ISAG [20], were used to compare the genetic backgrounds of indigenous Thai pigs in six locations in northern Thailand (Figure 1) together with exotic and/or commercial pigs [21]. All of these markers exhibit a high degree of polymorphism and allelic diversity. Thai native pigs and wild boars have higher degrees of diversity than those of other breeds [21]. However, the previous study was based on a limited number of Thai pig populations, did not cover all regions of northern Thailand and consisted of insufficient phenotypic observations. For a better understanding of the genetic properties of Thai native pigs and to supply the missing data of the previous study especially in remote locations, we characterised the genetic diversity and their relationships in Thai native pigs from four river basins in northern Thailand using a small set of microsatellite markers together with phenotypic information. We further evaluated the utility of these markers for predicting pig phenotypes.

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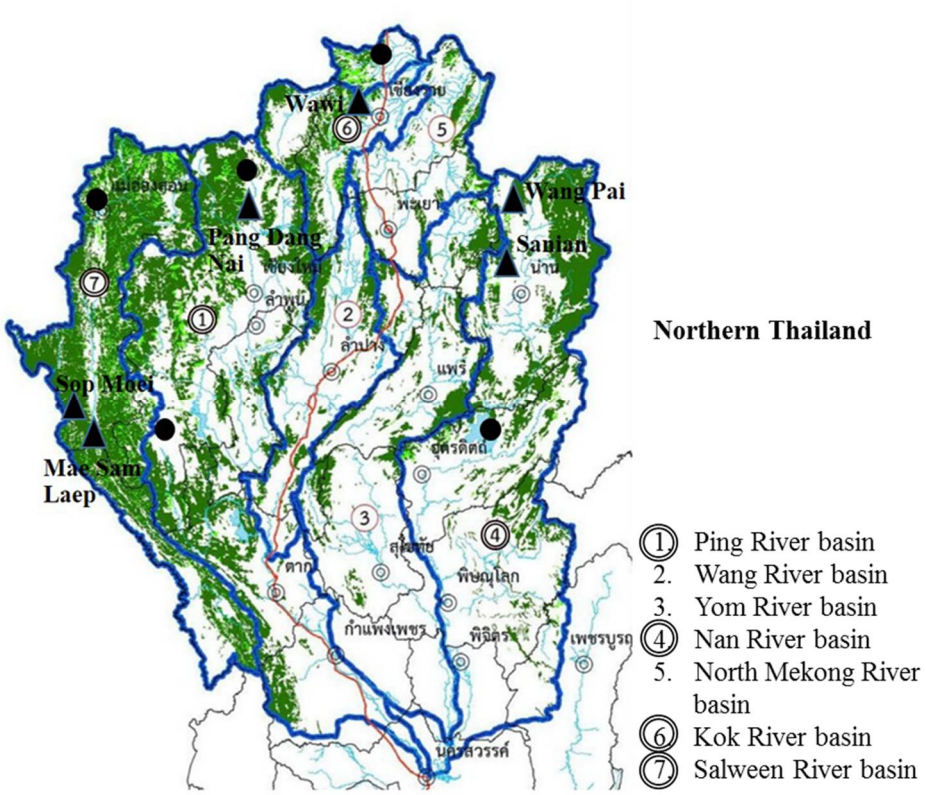

Figure 1. Sampling locations of Thai native pigs in this study (▲) and in Charoensook et al. [21] (●). Double rings indicate river basins where sample collection was done in this study.

MATERIALS AND METHODS

A total of 49 Thai native pigs were collected from four provinces in four river basins with distinct ethnic groups in northern Thailand (Table 1), covering a range of geographical regions and populations, ecological diversity, and pig phenotypes. Ear clip samples were collected by a veterinarian under Animal Restraint for Veterinary Professionals protocol [22]. Genomic DNA was extracted from ear clip tissues by the salting out method [23]. Morphological characteristics, viz. body size, face and ear shape, coat colour and teat number, were recorded. Body length was measured between the ears and the upper tail. Body height was measured from the fore leg hoof to the shoulder.

Microsatellite Analyses

Ten microsatellite markers including eight from the FAO/ISAG panel [20] were used to amplify DNA from 49 pig samples (Table 2) to clarify diversity within and between five populations. These markers have also been used by Yang [19] and Charoensook et al. [21]. Additionally, 15 microsatellite markers, 10 from previous studies and five additional ones, were used to identify variation in native pigs within the Mae Hong Son (SM) population and to differentiate among phenotypes of 17 individuals from SM and 5 pigs from Nan (SN1-5). Fluorescence-labelled primers (HEX and FAM) with overlapping fragment sizes were assigned to each multiplex polymerase chain reaction (PCR), with five markers per combination. PCR was performed in a 25-µL volume containing 1 µL (20 ng) of genomic DNA, 12.5 µL of Taq provided in the PCR Master Mix Kit (Qiagen, Germany), appropriate primer concentrations, and deionised water to make up the final volume. Multiplex PCR conditions were as follows: an initial denaturation at 95°C for 5 min., 35

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Table 1. Summary of sampling locations

River basin	Sampling location	Ethnic group	Spatial information	Symbol	No. of pig samples
Salween River basin	Sop Moei and Mae Sam Laep village, Sop Moei district, Mae Hong Son province	Karen-Pwo	17°57′43″N 97°56′0″E	SM	17
Nan River basin	Sanian village, Muang district, Nan province	Yao	18°47′37″N 100°43′46″E	SN	10
	Wang Pai village, Song Khwae district, Nan province	Yao	19°21′35″N 100°42′3″E	SQ	10
Ping River basin	Pang Dang Nai village, Chiang Dao district, Chiang Mai province	Palong	19°21′58″N 98°57′51″E	PD	10
Kok River basin	Wawi village, Mae Suai district, Chiang Rai province	Akha, Lisu	19°39′24″N 99°32′30″E	MN	2

cycles of 95°C for 90 sec., annealing at 57°C for 60 sec. for all markers, and extension for 30 sec. at 72°C, with a final extension at 72°C for 30 min. PCRs were performed using Biometra T-Gradient Thermocycler (Biometra, Germany). To check fragment integrity, PCR products were separated on 2% agarose gels stained with ethidium bromide. For genotyping of samples, the size separation and fragment analysis were performed using an ABI 3100® Capillary Analyzer (Applied Biosystems, Germany)

Statistical Analyses

Genetic diversity parameters, viz. the number of alleles (Na), effective number of alleles (Ne), observed and expected heterozygosity (Ho and He), total inbreeding coefficient (FIT), population differentiation (FST), within-population inbreeding coefficient (FIS), deviation from Hardy–Weinberg equilibrium (HWE) and Shannon's information index (I), were calculated using GenAlEx 6.5 [24]. Polymorphism information content (PIC) was calculated using Cervus 3.0 [25]. Genetic divergence between individuals and populations was inferred using the unweighted pair group method with arithmetic mean (UPGMA) method [26] based on Nei's genetic distances [27]. Evolutionary analyses were conducted using MEGA7 [28].

RESULTS AND DISCUSSION

Morphological Characteristics

Phenotypic variation was highest in the SM population. This population spanned two villages (separated by approximately 10 km) in the same district of Mae Hong Son province. Although 58.83% of pigs (10 of 17 samples) exhibited a black coat with white markings on the feet and other parts of the body such as belly, forehead and tail tip (Figure 2A), the other seven pigs showed different

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phenotypes such as white hair, large ears and curved wrinkled snouts. This population was classified as the small native pig type because the mature body length was 60 - 80 cm (76.47% of SM population) and the height was 30 - 40 cm (47.06% of SM population). The black coat with white markings on the feet and other body parts (Figure 2B) is also the main phenotype of native pigs in Nan province, Muang district (SN; 90% of pigs), and in Song Khwae district (SQ; 100% of pigs). Nevertheless, body sizes of pigs in SN and SQ were larger than those of native pigs in the SM population but smaller than those in Pang Dang Nai (PD) and Wawi village (MN). From our survey, all pigs from Pang Dang Nai village, Chiang Dao district had black skin and hair with large ears (Figure 2C) and body lengths of 101 - 120 cm. This can be explained by the cross-breeding of the Thai native with Meishan and Duroc breeds in this population. Only two Thai native pigs were sampled from Wawi village because most of these pigs were cross-bred. Owing to the physical difficulty of data collection, teat number was determined for 35 pigs; 30 pigs had five pairs and the others showed four (3 pigs) and six pairs (2 pigs).

Figure 2. Most common pig phenotype found in (A) Mae Hong Son province (SM), (B) Nan province (SN and SQ), and (C) Chiang Mai province (PD)

Microsatellite Polymorphism

All microsatellites had high degrees of polymorphism and allelic diversity (Table 2). The total number of alleles per locus (Na) varied from 5 (S0101) to 14 (S0005) and the total number of alleles across all populations was 97. The effective number of alleles per locus (Ne) ranged from 2.009 - 7.400. Observed heterozygosity (Ho) (mean 0.624) was lower than expected heterozygosity (He) (mean 0.759) except for the S0090 locus. Average heterozygosity in a population should be in the range of 0.3 - 0.8 to effectively measure genetic variation [29]. PIC is an indicator of genetic variation and is used to measure marker informativeness. It can vary from 0 to 1, where values close to 1 indicate a greater level of polymorphism [30] and values above 0.5 indicate high polymorphism levels. The mean PIC for 10 microsatellites in this study was 0.725, ranging from 0.458 (S0101) to 0.851 (SW240); this was lower than that for the pig population in Charoensook et al. [21] (0.789). The differences in genetic diversity can be attributed to the number and choice of markers and a small sample size. FIT, FST and FIS, used to evaluate inbreeding, were 0.138, 0.119 and 0.020 respectively when all loci were considered. FIS revealed a deficiency of heterozygosity at ten loci; we obtained negative values for six loci, indicating a lack of inbreeding, and positive values for four loci, suggesting inbreeding. S0386 exhibited the highest FIS value and was not consistent with the expectations of the HWE, revealing an excess of homozygous individuals, with a much lower Ho (0.200) than He (0.719).

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Table 2. Characterisation of ten microsatellite markers in five populations

PCR combi-nation	Locus	SSC	Allele size (bp)	N_a	N_e	Ho	H_e	PIC	FIT	FST	FIS	HWE
1	*SW240*	2	88–114	13	7.400	0.787	0.874	0.851	0.040	0.108	-0.076	ND
	SW951[a]	10	120–132	6	2.307	0.447	0.573	0.525	0.149	0.049	0.106	NS
	S0155	1	132–166	10	4.186	0.630	0.769	0.733	0.097	0.091	0.007	NS
	S0005	5	201–246	14	5.025	0.733	0.810	0.785	0.045	0.101	-0.062	NS
	S0090	12	226–249	11	5.068	0.826	0.812	0.782	-0.060	0.108	-0.189	NS
2	*SW24*	17	93–118	10	4.956	0.674	0.807	0.772	0.112	0.081	0.034	ND
	SW857	14	134–162	9	6.124	0.804	0.846	0.817	0.018	0.149	-0.153	ND
	S0386[b]	11	160–186	7	3.445	0.200	0.719	0.683	0.769	0.128	0.735	***
	S0101	7	203–213	5	2.009	0.432	0.508	0.458	0.076	0.199	-0.154	ND
	S0355	15	241–270	12	7.256	0.711	0.874	0.848	0.137	0.176	-0.047	ND
	Mean for all loci			**9.7**	**9.70**	**0.624**	**0.759**	**0.725**	**0.138**	**0.119**	**0.020**	

Note: *SSC* = *Sus scrofa* chromosome; N_a = total number of alleles per locus; N_e = effective number of alleles per locus; H_o and H_e = observed and unbiased expected heterozygosity; PIC = polymorphism information content; FIT = total inbreeding coefficient; FST = population differentiation; FIS = within-population inbreeding coefficient; HWE = deviation from Hardy–Weinberg equilibrium (***P < 0.001, NS = not significantly different, ND = not done). Locus superscripts indicate markers not in the FAO/ISAG panel: [a] from Oh et al. [16]; [b] from Wang et al. [31].

Genetic Population Structure

An analysis of five populations based on ten microsatellite markers is summarised in Table 3. The highest genetic diversity based on the effective number of alleles and number of alleles per locus was observed in the Sop Moei district (SM) population, consistent with the high phenotypic variation and the scale of the population, with two villages in the same district. The lowest diversity was found in Mae Suai district (MN) owing to the small sample size. The lowest Ho and He values were observed in the Song Khwae district (SQ) population, and Ho was much lower than He. These results indicate that a high level of inbreeding occurred in the SQ population. Shannon's information index, which describes variation within population [32], ranged from 1.061 (MN) to 1.582 (MN) with a mean value of 1.326.

In an analysis of population differentiation, we obtained negative FST values for the PD and MN populations; these values should be effectively interpreted as zero values, indicating complete sharing of genetic material. FST values for mammals generally range from 0 to 0.25 and are typically close to 0.1, as observed in the SM, SN and SQ populations. Values close to 0 indicate a high level of interbreeding. However, small sample sizes can lead to an overestimation of genetic differentiation [33].

Nei's genetic distances were calculated between pairs of populations (Table 4). We obtained the highest genetic distance between SQ and MN populations (0.762) and the lowest distance between SQ and SN (0.196), the latter pair being located in Nan province, separated by approximately 110 km

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Table 3. Population genetic diversity[1] of Thai native pigs

Population	*N*	N_a	N_e	*I*	H_o	*He*	*F*
SM	17	7.20±0.72	4.17±0.51	1.58±0.12	0.61±0.06	0.75±0.04	0.17±0.06
SN	10	5.10±0.41	3.44±0.36	1.33±0.12	0.60±0.11	0.70±0.06	0.11±0.12
SQ	8	4.50±0.50	2.92±0.30	1.19±0.13	0.48±0.08	0.65±0.06	0.18±0.11
PD	10	5.60±0.31	3.91±0.40	1.47±0.08	0.75±0.04	0.76±0.03	-0.04±0.05
MN	2	3.10±0.23	2.89±0.27	1.06±0.09	0.85±0.11	0.83±0.05	-0.33±0.15
Mean	**47**	**5.10±0.28**	**3.47±0.18**	**1.33±0.06**	**0.66±0.04**	**0.74±0.02**	**0.02±.0.05**

[1] Diversity parameters were obtained for all populations. Note: *N* = no. of animals; *N*a = no. of alleles per locus; *N*e = effective no. of alleles per locus; *I* = Shannon's information index; H_o and H_e = observed and expected heterozygosity; *F* = fixation index

in the Nan River basin. In a UPGMA cluster analysis based on these genetic distances (Figure 3), the five populations were classified into two main groups. First, the PD population was separated from other groups; pigs in this group showed a cross-breed phenotype consistent with information from the head of the village. The second major group exhibited the native pig phenotype and was divided into two subgroups, one consisting of the SM and SN populations and the other the SN and SQ. In the previous study [21], six native pigs in Mae Hong Son province (Nam Piang Din village or long-neck Karen village, Muang district) were grouped with Jiangquhai Chinese pig, supporting a relationship between Thai and Chinese pigs.

Table 4. Pairwise population matrix of Nei's genetic distances

Population	SM	SN	SQ	PD	MN
SM	-				
SN	0.242	-			
SQ	0.263	0.196	-		
PD	0.337	0.414	0.317	-	
MN	0.326	0.662	0.762	0.654	-

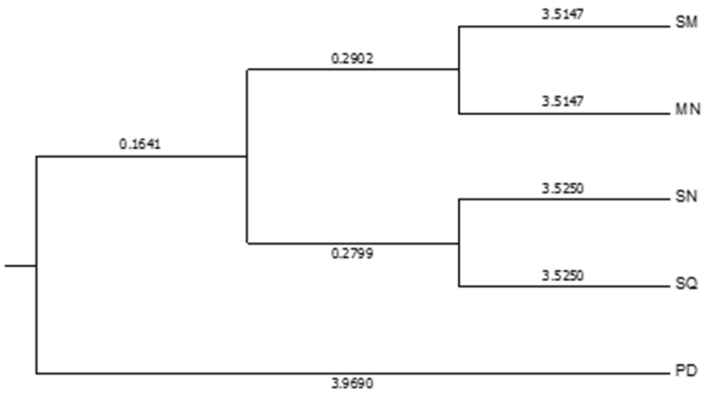

Figure 3. UPGMA tree based on Nei's genetic distances. Numbers indicate genetic distances

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Phenotypic Analysis

Based on the high genetic and phenotypic variation in the SM population and the lack of microsatellite information for native pigs in this area, we evaluated 17 individuals in this population in more detail using 15 microsatellite markers, with additional 5 markers in a third PCR combination (Table 5). Five phenotypes of pigs in Nan province (SN) were evaluated by a clustering analysis. The number of alleles per locus ranged from 4 (S0010) to 10 (S0005, S0090 and S0355) and the total number of alleles in the population was 117. The effective number of alleles per locus was lower than the expected number, except for SW857 and SO227. Heterozygosity values ranged from 0.3 to 0.8, indicating that the set of markers was useful for evaluating genetic variation. PIC ranged from 0.451 (SW951) to 0.830 (SW240). The mean fixation index within the population (0.088) was higher than the mean of total inbreeding estimate for five populations (FIT = 0.020), and the fixation index (F) value for S0386 was very high because this locus was not in HWE ($P < 0.05$). Calculating F statistics separately for each population, the positive value for SM (0.14) indicated inbreeding. These results can be explained by an environmental barrier within the sampling area, conserved ethnic groups, and/or small and closed populations.

Table 5. Characterisation of 15 microsatellite markers in Mae Hong Son (SM) and Nan (SN) native pigs

PCR combination	Locus	SSC	Allele size (bp)	N_a	H_o	H_e	PIC	*F*
1	*SW240*	2	88–114	9	0.727	0.868	0.830	0.079
	SW951[a]	10	120–132	4	0.318	0.503	0.451	0.217
	S0155	1	132–166	8	0.591	0.688	0.641	0.071
	S0005	5	201–246	10	0.682	0.789	0.753	0.049
	S0090	12	226–249	10	0.773	0.826	0.785	0.031
2	*SW24*	17	93–118	8	0.714	0.812	0.763	0.048
	SW857	14	134–162	8	0.864	0.827	0.782	-0.041
	S0386[b]	11	160–186	7	0.200	0.722	0.679	0.562
	S0101	7	203–213	4	0.450	0.568	0.490	0.131
	S0355	15	241–270	10	0.750	0.873	0.835	0.065
3	*SW936*	15	88–122	8	0.773	0.847	0.807	0.036
	SO215[c]	13	132–184	9	0.682	0.809	0.769	0.094
	SW632	7	155–171	7	0.682	0.710	0.649	-0.002
	SO226	2	180–202	9	0.545	0.560	0.532	0.019
	SO227[d]	4	225–251	6	0.773	0.738	0.681	-0.032
	Overall mean			**7.8**	**0.635**	**0.743**	**0.696**	**0.088**

Note: SSC = *Sus scrofa* chromosome, N_a = no. of alleles per locus; N_e = effective no. of alleles per locus; H_o = observed heterozygosity; H_e = expected heterozygosity; PIC = polymorphism information content; *F* = fixation index. Locus superscripts indicate markers not in FAO/ISAG panel: [a] from Oh et al. [16]; [b] from Wang et al. [31]; [c] from Charoensook et al. [21]; [d] from Chaiwatanasin et al. [17] and Wang et al. [31].

A UPGMA dendrogram resolved 22 individuals into three major clusters (Figure 4). The first cluster consisted of pigs with phenotypic differences (SM11 and SN4). SM11 showed a similar body shape to that of Duroc cross-bred pigs. SN4 had native pig characteristics with long hair but a body

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size of 50 cm in height and 80 cm in length at age 5 years. The second cluster contained five pigs from the SM population with black skin and white markings on the legs. The third cluster was divided into two subclusters. One subcluster included only SM10, with a back coat, large ears, body length of 80 cm and four pairs of teats. The second subcluster was the biggest group, comprising 10 SM and 4 SN pigs. Although this group had many features of the native pig morphology, pigs with the same coat colour were grouped together, such as those with black coat, large ears but small body size (SM4 and SM12), white hair (SM1 and SM9), and white belly (SN1 and SN2). However, some differences in pig characteristics were not clearly separated by the markers, such as SM2 and SN5. Therefore, additional DNA markers, including mitochondria DNA analyses, could clarify the complex traits

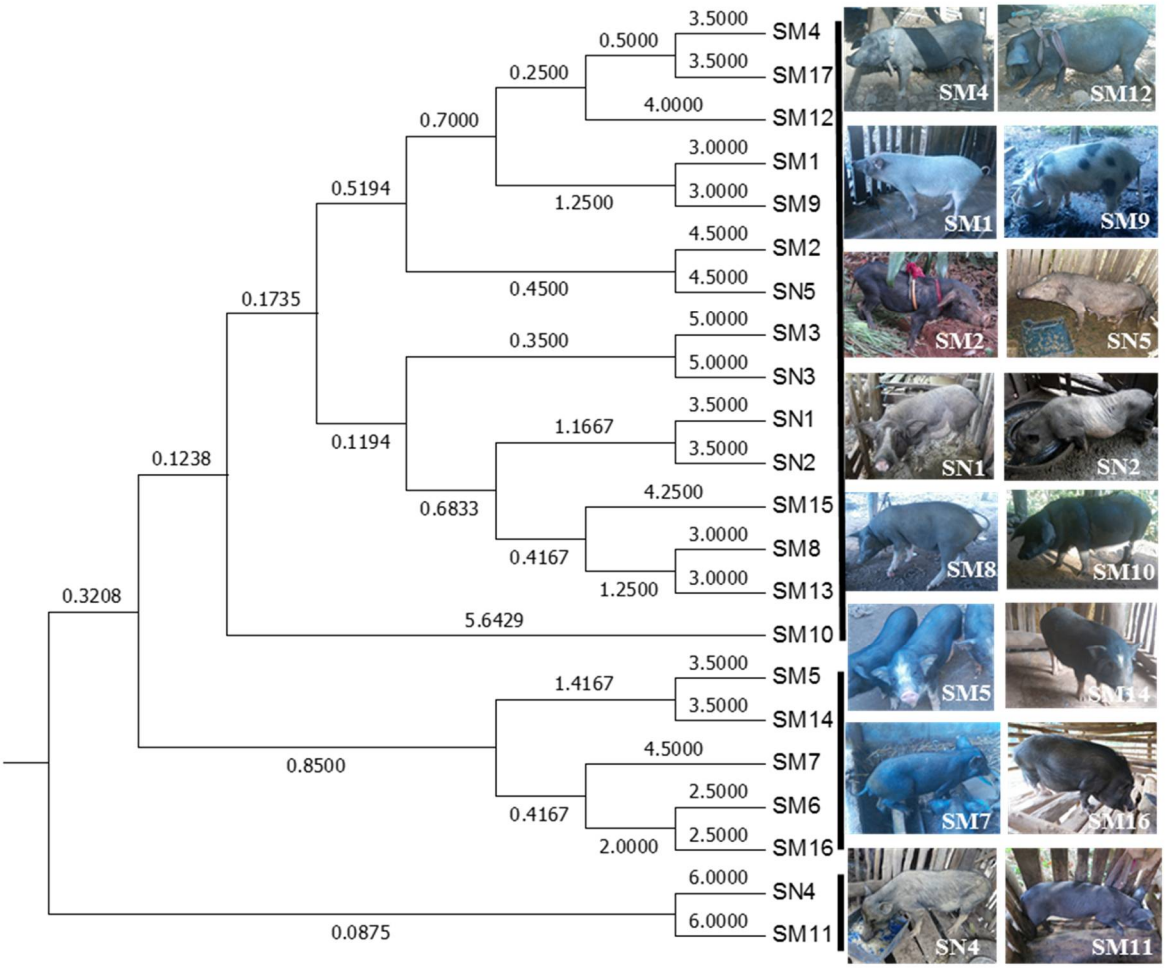

Figure 4. UPGMA dendrogram of 22 individuals, viz.17 from Mae Hong Son (SM) and 5 from Nan (SN) provinces based on 15 microsatellite markers. Sixteen pig phenotypes are shown. Black lines indicate the three main clusters.

These results demonstrate that pig phenotypes can be effectively distinguished by 15 microsatellite markers, consistent with previous findings showing that the efficiency of assignment to groups reaches 98% when 10 loci are used [34]. Small sets of microsatellites have been used to evaluate native Indian pigs (13 loci) [10] and Philippine native pigs [16]. This reduces the cost of materials in microsatellite analyses. However, markers in the FAO-ISAG set are strongly recommended to enable comparisons among studies.

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CONCLUSIONS

We have detected high genetic diversity and phenotypic variation in Thai native pigs, especially in the SM population, the most ethnically conserved and geographically distinct population. Our phenotypic analysis has indicated that the morphological features of native pigs in northern Thailand are highly similar to those of the Kwai and Hainan breeds [1,2] but the body size is smaller than previously reported. The genetic information inferred from this microsatellite analysis improves our understanding of the current status of native pigs in northern Thailand. Additional analysis such as that of mitochondrial DNA or coat colour genes might provide further insight into the population structure and genetic properties of Thai native pigs. Our results provide genetic information for the conservation of Thai native pigs, which could be used to optimise the characteristics of pigs for swine farming purposes.

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