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Assessment of genetic diversity of Wua-lan in Thailand

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Abstract

Wua-lan (Khao-Lan) is one of the indigenous cattle (*Bos indicus*) in Thailand that needs immediately scientific data of animal breeding plans for this cattle management. Genetic characterization is the first step in the development of proper management strategies for preserving genetic diversity and preventing undesirable loss of alleles. Thus, in this study, we investigated genetic diversity and relationship of Wua-lan among different areas using 20 microsatellite markers. In this study, the analysis of autosomal DNA was performed on 76 Wua-lan which exhibited sufficient diversity across all the areas. The mean observed heterozygosity across all loci in this population was 0.484. Wua-lan had the allele size larger than both Thai native cattle (CSSM66 and TGLA153) and domestic cattle (BM6117 and CSSM66), but some allele size of domestic cattle (BM6445) was larger than Wua-lan. The genetic diversity was identified by expected heterozygosity *(He)*, observed heterozygosity *(Ho)* and inbreeding coefficient (f) that was identified by areas as follows: Phetchaburi (0.546, 0.484 and 0.126), Ratchaburi (0.532, 0.478 and 0.134) andPrachuap Khiri Khan (0.508, 0.488 and 0.067), respectively. The *Ho* and He did not show differences between each area ($\triangle$*He-Ho*), the Wua-lan in Phetchaburi was the most different value, and Wua-lan in Prachuap Khiri Khan was the lowest difference value. The total of the observed heterozygosity and inbreeding coefficient (f) of Wua-lan in 3 areas were equal to 0.484 and 0.129. A broad variation of inbreeding coefficient (f) was found among all loci, ranging from -0.875 (CSSM66) in Phetchaburi to 0.894 (BM848) in Ratchaburi Province. The genetic differentiation of Wua-lan ranged from 0.930 (Rat-Pet pair) to 0.965 (Pra-Pet pair) that was genetically differentiated by a similar magnitude. The shortest of Nei's genetic distances was observed between the Wua-lan from Ratchaburi (Rat) and Phetchaburi Province (Pet) (0.072) followed by Wua-lan from Prachuab Khiri Kahn (Pra) and Phetchaburi Province (Pet) (0.036). The Wua-lan from Prachuap Khiri Khan Province (Pra) was the most distant population, displaying the largest of Nei's genetic distances (0.119) when compared with the Wua-lan from Phetchaburi Province (Pet). The bootstrap values among this breed ranged from 2.99 to 4.78. Thus, the low values in this study were caused by the high genetic similarity between areas of animal husbandry. This result of the change in interred clusters ($\triangle$K) values peaked at K=4, which exhibited a reliable grouping pattern. These points suggest a distant relationship between the populations of a single geographic area, with minimal appearance of shared genetic materials between the three areas. The finding in the current study point origins of Wua-lan in Phetchaburi, Ratchaburi, and Prachuap Khiri Khan and their close relationship has been supported, in this study that showed relatively more admixture within the west region of Thailand.

Keywords: microsatellite, observed heterozygosity, inbreeding coefficient

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1. Introduction

Over 20 years ago, livestock production in Thailand was switched from backyard systems to industrialized husbandry by introduction of highly productive breeds [1] [2]. Thus, the population of indigenous animals has not been concerned and consequently their populations have been steadily declined. Demographic pressures have been not only accelerated to the loss of advantage traits or decrease in population of local breeds, but also, in relation to less biodiversity [3]. Naturally, indigenous livestock commonly is a reservoir of genes such as genes resistance of tropical diseases, external parasites, and utilization of the sustenance on low quality of feed [4]. The study of the benefit local livestock genes would be valuable for future researches [3] [5]. FAO [6] reported

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that the cattle are the species with the highest number or reported extinct species (209 species). Wua-lan is one breed of native cattle in Thailand which are presently a small number of population and dwells in the west region-Thailand, that is very popular in Phetchaburi, Ratchaburi, Prachuap Khiri Khan and there are some in Kanchanaburi; Supanburi, Samut Sakhon, and Nakhon Pathom. The local breed characteristic is under changed over time, as a consequence of their adaptation to local environment. Thai cattle breed is classified to zebu cattle *(Bos indicus)* that has been evolved over centuries under low levels of selection following with traditional animal husbandry. In the past, Wua-lan was used as labor and during post-harvest rice. They were then tied to the poles arranged in a row in front ground, and encouraged to trample on the rice helped separate the grain from the ears. This activity of tying the Wua-lan to the poles developed the traditional culture of cow racing. Evidently, cow racing will take place after post-harvest rice every year. Regarding the estimation of Wua-lan population in Phetchaburi Province, 73% of beef cattle farming are Thai-native cattle which are approximately 93,962 cattles [7]. Due to a small number of Wua-lan population, inbreeding will cause decreased their numbers. Consequently, their genetic diversity has been disappearing. Moreover, the Who-Lan cattle population has been decreasing due to the impact of crossbreed with exotic cattle which have been imported to farm in the west of Thailand. Therefore, genetic resource is important for managing animal breeding program and prevention of inbreeding that is reduced the genetic variability, fertility, and survival rate, which lead to an increase in the extinction risk [8] [9]. Microsatellites markers have been widely used as a marker of molecular genetic for studying conservation genetics, investigating forensic identification, profiling DNA fingerprints [8] [10] [11][12]. Microsatellites have been effective in evaluating differences of highly discriminatory at the individual level to genetically similar groups (population, breed, or species level) and still constitute the classical markers for identification and pedigree testing. There are now more microsatellite-based studies to develop probabilistic approaches for breeding plans in livestock such as pigs, wild boar, cattle, and sheep. The approaches described to obtain breed or variety assignments are generally based on the fact that different breeds or varieties can have differences in the frequencies of fixed alleles[13]. The aim of this study was to investigate the genetic diversity and structure of Wua-lan, using microsatellite marker information, to gather baseline information essential to design an appropriate conservation strategy.

2. Materials and Methods

Animals and Sample collection:

Genomic DNA was extracted from blood samples of 76 Wua-Lan. They have been maintained from Central of Thailand: 16 from 5 farms in Ratchaburi Province, 40 from 8 farms in Phetchaburi Province, and 20 from 4 farms in Prachuap Khiri Khan. Blood samples were collected from jugular vein in 5 ml of EDTA blood sample and kept at -20oC until DNA extraction.

DNA extraction:

Genomic DNA was isolated from blood using Phenol-chloroform method as described by [14]. Concentration and purity of the extracted DNA was measured using a spectrophotometer (SmartSpecTMPlus Spectophotometer, BIORAD, USA).

DNA amplification:

Twenty microsatellite markers for domestic cattle were used for this study (Table 1) [15]. Research reported the efficacy of 20 microsatellite primers for Wua-lan that the polymorphism informative content values (*PIC*) of each locus were 0.135-0.813 and the average *PIC* was 0.504. Although, a *PIC* higher than 0.6 indicates high polymorphism and can be used to characterize individuals, a value of average *PIC* value between 0.30-0.59 is considered to be moderated informative [16]. Therefore, 20 microsatellite primer studies were sufficiently efficient for use in the study of the genomicity of Wua-lan. These microsatellite markers were spread all over the genome and with the ability to co-amplify in PCR reactions [17]. Twenty microliters of the PCR mixture (Invitrogen®, Brazil) was composed of 50 ng of DNA template, 2 μl of PCR buffer (10xbuffer), 1 μl of 50 mM MgCl2, 0.4 μl of 10 mM dNTPs, 0.1 μl of each forward and reverse primers (100 μM), 0.1 μl of Taq DNA polymerase and DNase-free water. The PCR conditions were modified from those described earlier. An initial denaturation at 94°C for 3 min; followed by 35 cycles of 94°C for 30s, 58°C for 30s, and 72°C for 45s and a final extension at 72°C for 5 min. The PCR products were electrophoresed using 1.5% agarose gel (ADVANCE, Japan) and visualized under ultraviolet illumination. DNA fragment analysis from the separation of amplified PCR product of twenty microsatellite DNA by capillary gel electrophoresis using QIAxcel device with BioCalculator Analysis Software (Qiagen Inc., Valencia, CA, USA).

Statistical analysis:

The size of alleles for cattle loci was analyzed using PowerMarker Version 3.25. Genetic diversity, often referred to as *He*, is defined as probability with randomly chosen alleles from the differenpopulation.

Heterozygosity is referred to as the *Ho*. The inbreeding coefficient (f) is relative measure, in that there will be a certain level of homozygosity within the population and it estimates the increase from that initial level as a result of recent inbreeding. Nei's unbiased measures of genetic identity and genetic distance of three populations of Wua-lan were estimated using POPGENE 32, following [18] [19] [20]. The most popular model-based clustering technique was STRUCTURE program [21] [22] [23]. The STRUCTURE analysis at the chromosomal level to investigate genome-wide distribution of the Wua-lan backgrounds among the population and assumes a model with *K* populations; *K* may be unknown. It is assumed that within populations loci are in linkage equilibrium and Hardy–Weinberg equilibrium; STRUCTURE program assigns individuals to populations to achieve this.

3. Results and Discussion

All 20 microsatellite markers for domestic cattle could be assessed for the genetic diversity of 76 Wua-lan. From previous study, Arlai et al., [14] found a total of 110 alleles of Wua-lan (Phetchaburi, Ratchaburi and Prachuap Khiri Khan) by analysis on the basis of the same microsatellite markers, with average allele equal to 5.5 alleles per position. The main allele frequencies (MAF) were ranging from 0.225 to 1.000 and one of this MAF, BM6445 has MAF equal to 1. Only 1 allele was found monomorphic in cattle from Ratchaburi. The total number of alleles found in each local area from all used to study ranged from 1-9. Mean alleles of all 20 loci in Wua-lan were found the lowest at 4.100 alleles in Ratchaburi and the highest at 4.950 alleles in Phetchaburi. The average *PIC* was 0.504. The genetic markers in this study showing *PIC* values higher than 0.5 are normally considered as informative in population genetic analyses (Botstein et al., 1980). Next, the present studies were found the size of these allele sizes for locus as shown in Table 1. In this study, Wua-lan had the allele size larger than both those of Thai native cattle such as Kho Lan (Wua-lan), Kho Khaolumpoon, Kho Isaan, and Kho Chon (CSSM66 and TGLA153) [24][25] and domestic cattle (BM6117 and CSSM66) [17][26] but some allele size of domestic cattle had larger than (BM6445) Wua-lan. These loci of Who-lan cattle showed different alleles with no overlapping size ranged for other Thai native cattle and domestic cattle. The previous report by Duangjinda and Phasuk [25] conducted the analysis on the basis of the same microsatellite markers (BM6117, ETH225, CSSM66 and TGLA53). The obtained results are different that could probably be explained by the higher number of samples and also wide-ranged sampling localities in the present study [27]. Part of other microsatellites remain in these studies were overlapping all in domestic cattle. So, these markers are highly conserved in Bovidae.

The genetic diversity was identified by *He*, *Ho* and f values. The averages of the Wua-lan genetic diversity (expected heterozygosity; *He*), heterozygosity (Observed heterozygosity: *Ho*) and inbreeding coefficient (f) that was justified by areas are as follows: Phetchaburi (0.546, 0.484 and 0.126), Ratchaburi (0.532, 0.478 and 0.134) and Prachuap Khiri Khan (0.508, 0.488 and 0.067), respectively. The *Ho* and *He* did not show differences between each local (△He-Ho, Total observed heterozygosity and inbreeding coefficient (f)) of Wua-lan in 3 areas, the values were equal to 0.484 and 0.129, respectively. The results in each locus are shown in Table 2 and figure 1. In general terms, all of the areas presented moderate genetic diversity. The observed heterozygosity was lower than the expected heterozygosity in every area: in Phetchaburi, the *Ho* value (0.484) ¡ *He* value (0.546); in Ratchaburi, the *Ho* value (0.478) ¡ *He* value (0.532) and in Prachuap Khiri Khan, the *Ho* value (0.488) ¡ *He* value (0.508). The *Ho* value lower than *He* value meant the strong condition of Hardy-Weinberg disequilibrium found for the majority of the loci analyzed, which may reflect the narrow genetic base of the current population of this breed [27][28]. According to Sharma et al., [3] it found the observed value (0.459) and expected heterozygosity (0.594) in Ongole cattle. While in the previous studies, the overall *He* and *Ho* of Thai native cattle were ranged from 0.79-0.85 and 0.17-0.31, respectively. *He* and *Ho* values of Wua-lan were equal to 0.8 and 0.17, respectively. But, for this study, the *He* of Wua-lan was lower and the *Ho* was higher when compared to the study of Duangjinda and Phasuk, [3]. Sharma et al., [3] found that Shahabadi cattle breed had the highest heterozygosity values of Indian cattle (0.735), which could be explained by the occurrence of low selection pressure due to the lack of breeding programs. Moreover, in the past, the gene flows between different populations and a large number of alleles were presented in all breeds [29]. While, happen in Tharparkar cattle in India (0.64) [30], Orissa cattle (0.62-0.66) in India [31], Creole cattle in Brazil (0.61) [32] and Chinese cattle (0.62) [33]. However, the differences in the estimates can also attribute to the different numbers of markers and/or individuals analyzed [34]. It is also relevant in a long-term perspective, as selection limits are determined by the initial allelic composition rather than by heterozygosity [35]. The inbreeding coefficients were derived from an additive relationship matrix for the whole population and within the above-mentioned genetic groups [36]. The total f value of Wua-lan, in this study was higher than zero (0.129). A broad variation of inbreeding coefficient (f) was found among all loci, ranging from -0.875 (CSSM66) in Phetchaburi to 0.894 (BM848) in Ratchaburi. Highly heterozygote deficit of Wua-lan of all of the 3 populations investigated were the highest in Ratchaburi (0.134), followed by Phetchaburi (0.126)

Table 1. Characteristics of allele sizes in domestic cattle and Thai native cattle.

Microsatellite loci	Allele size range (bp)		
	Domestic cattle[1]	Thai native cattle[2]	In this study
		Kho Lan (Wua-lan), Kho Khaolumpoon, Kho Isaan, and Kho Chon	(Wua-lan)
BM1824	176-197	-	180-204
BM2113	116-146	123-147	140-158
INRA023	195-225	-	202-230
BM1260	120-133	-	128-146
ILSTS006	277-309	-	274-308
BM6117	110-114	-	124-146
ETH225	131-159	140-160	153-187
BM1237	187-223	-	195-223
BM6445	159-175	-	143-153
BMC1222	272-302	-	276-324
CSSM66	171-129	154-174	192-232
BM848	215-227	-	219-245
TGLA53	143-191	119-153	158-184
TGLA126	115-131	-	117-151
TGLA122	137-173	-	140-176
BM1818	258-272	-	245-295
BM226	128-164	-	142-182
BMC8012	197-215	-	205-229
BM2515	132-148	-	144-158
BM6436	198-208	-	198-226

[1] From FAO, 2011; Bishop et al., 1994

[2] From Mekchay et al., 2007; Duangjinda and Phasuk, 2008

and Prachuap Khiri Khan (0.067) respectively. This is consistent with the study of Colombian creole cattle, which was higher than zero, indicating a general deficit of heterozygous individuals in all analyzed local areas of animal husbandry [34]. Possible heterozygous deficit factors may include inbreeding, selection of small number of populations in each generation, or mating among closely related animals [3] [27] [37]. From the interviews with farmers, the breeder of Thai native animals is the main factor with genetic variation effect in Thai native animals. One of them was Wua-lan that the mating choice decided by the breeders such as mating between close relatives with a few superior animals (father and mother). Over reproduction in Wua-lan populations without control is the main cause attributed to inbreeding. Due to the fact in most Thai native cattle farms, crossed breeding with no records or controls are frequently occurred in related animals. This style of breeding leads to a decrease of heterozygous individuals in the population. Therefore, a higher inbreeding rate has been reported for dairy cattle populations [38] and horse populations [39], leading to increased precision of inbreeding evaluation, although the inbreeding coefficients were relatively low [40]. The positive effects of inbreeding may have been due to the purging of deleterious alleles in previous generations [41]. Moreover, several farmers said that some part of Wua-lan herds in the country has usually dominated by one or two sires over the entire herd, the above actions may increase the proportion of homozygous alleles that decrease *Ho* and increased f value in the population with the consequent decrease in the genetic variability of herds [29]. Most of Thai native animals' gene pools were limited in a local area and most of them can be found inbreeding of Thai native animals. Therefore, the opportunity of the new gene transfer into a population is usually limited [42]. In addition, the racing males of Wua-lan are castrated around the age of one year leading to a decrease of their gene in the population. Thus, the paucity of pure bulls, as well as management, seems to be the main reasons for heterozygote deficiency in these cattle.

Genetic Differentiation and Genetic Distance

The genetic differentiation and Nei's genetic distance pairwise estimates between Wua-lan areas of animal husbandry (Phetchaburi, Ratchaburi, and Prachuap Khiri Khan Province) which the corresponding genetic tree was created using the neighbor-joining algorithm. The genetic differentiation between areas of animal husbandry is presented in Table 3. The genetic differentiation range of Wua-lan was from 0.930 (Rat-Pet pair) to 0.965 (Pra-Pet pair) by a similar magnitude. The shortest of Nei's genetic distances was observed between the Wua-lan from Ratchaburi (Rat) and Phetchaburi Province (Pet) (0.072), followed by Wua-lan from Prachuap Khiri Khan (Pra) and Phetchaburi Province (Pet) (0.036). The Wua-lan from Prachuap Khiri Khan Province (Pra) was the most distant population, displaying the largest of

Table 2. Observed heterozygosity, Expected heterozygosity and Inbreeding coefficient of 20 microsatellite markers in bovine from Phetchaburi (Pet), Ratchaburi (Rat) and Prachuap Khiri Khan Province (Pra).

Marker	**Observed heterozygosity (*Ho*)**			**Expected heterozygosity (*He*)**			**Inbreeding coefficient (f)**		
	Pet	**Rat**	**Pra**	**Pet**	**Rat**	**Pra**	**Pet**	**Rat**	**Pra**
BM1824	0.250	0.313	0.200	0.598	0.643	0.545	0.590	0.537	0.648
BM2113	0.500	0.625	0.450	0.569	0.607	0.441	0.134	0.003	0.006
INRA023	0.600	0.375	0.550	0.676	0.451	0.489	0.125	0.200	-0.100
BM1260	0.100	0.375	0.100	0.095	0.477	0.095	-0.040	0.244	-0.027
ILSTS006	0.550	0.750	0.600	0.685	0.732	0.736	0.209	0.008	0.210
BM6117	0.500	0.438	0.200	0.381	0.342	0.180	-0.301	-0.250	-0.086
ETH225	0.625	0.625	0.650	0.698	0.611	0.550	0.117	0.010	-0.157
BM1237	0.650	0.438	0.800	0.707	0.520	0.705	0.093	0.189	-0.109
BM6445	0.150	0.000	0.300	0.139	0.000	0.255	-0.068	NaN	-0.152
BMC1222	0.600	0.313	0.450	0.719	0.660	0.540	0.178	0.550	0.191
CSSM66	0.975	0.938	0.950	0.524	0.529	0.499	-0.857	-0.758	-0.900
BM848	0.175	0.063	0.150	0.490	0.557	0.229	0.650	0.894	0.367
TGLA53	0.425	0.375	0.450	0.456	0.322	0.520	0.080	-0.132	0.160
TGLA126	0.575	0.625	0.450	0.492	0.557	0.469	-0.156	-0.091	0.066
TGLA122	0.700	0.688	0.750	0.833	0.762	0.833	0.172	0.129	0.124
BM1818	0.550	0.375	0.500	0.584	0.486	0.644	0.070	0.259	0.248
BM226	0.775	0.875	0.700	0.758	0.770	0.641	-0.010	-0.105	-0.066
BMC8012	0.400	0.500	0.450	0.740	0.725	0.754	0.469	0.339	0.424
BM2515	0.250	0.438	0.550	0.219	0.342	0.399	-0.130	-0.250	-0.357
BM6436	0.333	0.429	0.500	0.560	0.541	0.644	0.415	0.243	0.248
Mean	**0.484**	**0.478**	**0.488**	**0.546**	**0.532**	**0.508**	**0.126**	**0.134**	**0.067**

NaN= Not a number

Nei's genetic distances (0.119) when compared with the Wua-lan from Phetchaburi Province (Pet).

The dendrogram of genetic distance matrix generated by UPGMA method[43] as well as bootstrapping proportions are shown in Figure 3. The Wua-lan is *Bos indicus* specie. In this study, Wua-lan are all within the same cluster. The genetic tree shows the different between Wua-lan from Ratchaburi, Phetchaburi, and Prachuap Khiri Khan. For Wua-lan breed of Prachuap Khiri Khan Province, the most distant breed could be observed when compared to Wua-lan breed of Ratchaburi Province. However, Wua-lan in Phetchaburi and Prachuap Khiri Khan are grouped under the same node, and this genetic closeness could be explained considering that both area exchange their genetic material. In the past, the breeder of Whua-Lan cattle in Phetchaburi and Prachuap Khiri Khan might share certain geographical areas of the Wua-lan territory, which might have favored genetic exchange between them. While breeders of Whua-Lan cattle in Ratchaburi might choose local breeders rather than from other areas. Wua-lan are spread in a large number of small herds in three different areas.The large within-herd mean relationship and low effective population size warn that inbreeding must be kept under control, by avoiding mating among close relatives and rotating animals among herds. The bootstrap values among this breed ranged from 2.99 to 4.78 (Figure 2). Thus, the low values in this study were caused by the high genetic similarity between areas of animal husbandry.

Population structure

This research used model-based clustering implemented in STRUCTURE to partition the genetic of each region of animal into a predefined number of clusters. As shown in Figure 3, for predefined K = 2, K = 3, and K = 4 populations of Wua-lan were clearly clustered into Phetchaburi, Ratchaburi, and Prachuap Khiri Khan provience, individual cattle were assigned to the given population, which was identified by color. Wua-lan is classified as a Zebu type. This result of change in intered clusters (△K) values peaked at K=4, indicating strong support for three populations. Each population area was independently assigned to its inferred cluster despite some evidence of admixture. These points suggest a distant relationship between the populations of a single geographic area, with minimal appearance of shared genetic materials between the three areas, hence indicating that Wua-lan from all populations have been very low levels of gene flow between the three areas. The finding in the current study point origins of Wua-lan in Phetchaburi, Ratchaburi, and Prachuap Khiri Khan. Their close relationship has been supported, in this study, that showed relatively more admixture within the central region of Thailand. This result may be due to the phylogenetic relationships between these breeds and/or migration of individuals between the different farms located in the distribution area of the different populations. Al-

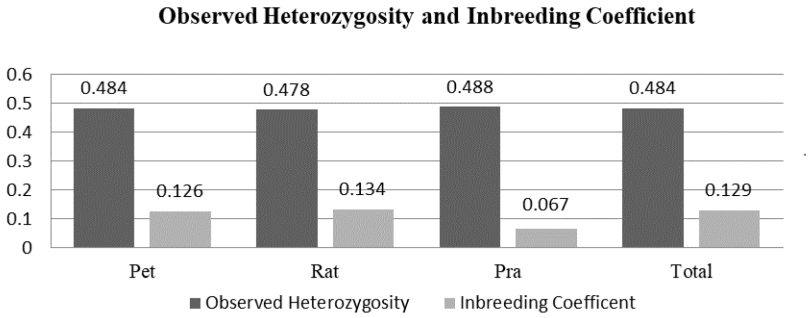

Figure 1: Observed heterozygosity and inbreeding coefficient of Wua-lan in Phetchaburi Province (Pet), Ratchaburi Province (Rat), Prachuap Khiri khan (Pra), and a total of three provinces.

Table 3. Nei's genetic differentiation (above diagonal) and genetic distance (below diagonal) of cattle from Phetchaburi (Pet), Ratchaburi (Rat) and Prachuap Khiri Khan Province (Pra). .

Pop ID	Pet	Rat	Pra
Pet	***	0.930	0.965
Rat	0.072	***	
Pra	0.036	0.119	***

though, the Thailand government created conservation plans for native cattle such as Wua-lan breeds though the Department of Livestock development. Nowaday, there is no conservation program in the country for Wua-lan and other native breeds. For this reason, the number of pure native breeds has been reduced over time. This situation could threaten the survival of the purebred and should be concerned.

In conclusion, using neutral microsatellites markers showed a level of genetic diversity in Wua-lan population despite its had selective breeding by the breeder. Previous studies on the genetic diversity of Wua-lan have been limited to only one population [25]. However, no study has been done at the genetic level; hence necessitated the need to assess the genetic diversity of all existing Wua-lan. Wua-lan are indigenous cattle that are adapted to the tropical environmental condition, utilized by poor quality grazing, parasite infestation, and challenges of tick-borne diseases. However, Wua-lan is clustering solutions of sharing of diversity and present of genetic structure between the populations of a single geographic area. Moreover, inbreeding was detected in some provinces, suggesting the need for appropriate measures to avoid the negative effects. The results presented here can acknowledge all stakeholders. A wide range of genetic diversity is required in the future for generating transgressive variation for providing a valuable source of genetic material that may be used for meeting the demands of Wua-lan breeding programs. Thai native cattle breeders in Thailand did not have a scientific data system for mating and breeding selection. However, it raises the awareness to conserve the pure Wua-lan breed. The information on the inbreeding situation should be provided to the Thai native cattle breeders in order to collaborate together to prevent inbreeding depression in the future. Moreover, crossbreeding with free-ranging domestic cattle or Thai is usually an accidental finding especially in the urban area. If the owner ignore it, it was one of the main threats to their purebred status. Breeding management and pedigree recording should be considered to prevent the incorporation of native cattle genes into the Thai purebred cattle gene pool, avoid future inbreeding, and maintain their genetic integrity [44]. It is anticipated that such knowledge will not only help to develop the value of Wua-lan populations as an indigenous animal genetic resource, but will also contribute towards informed livestock production improvement strategies, to ensure national food security and enhance economic growth.

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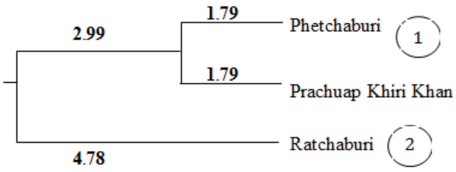

Figure 2: Dendrogram of genetic distance between Whua-Lan cattle from (Phetchaburi) (Pet), Ratchaburi (Rat) and Prachuap Khiri Khan (Pra).

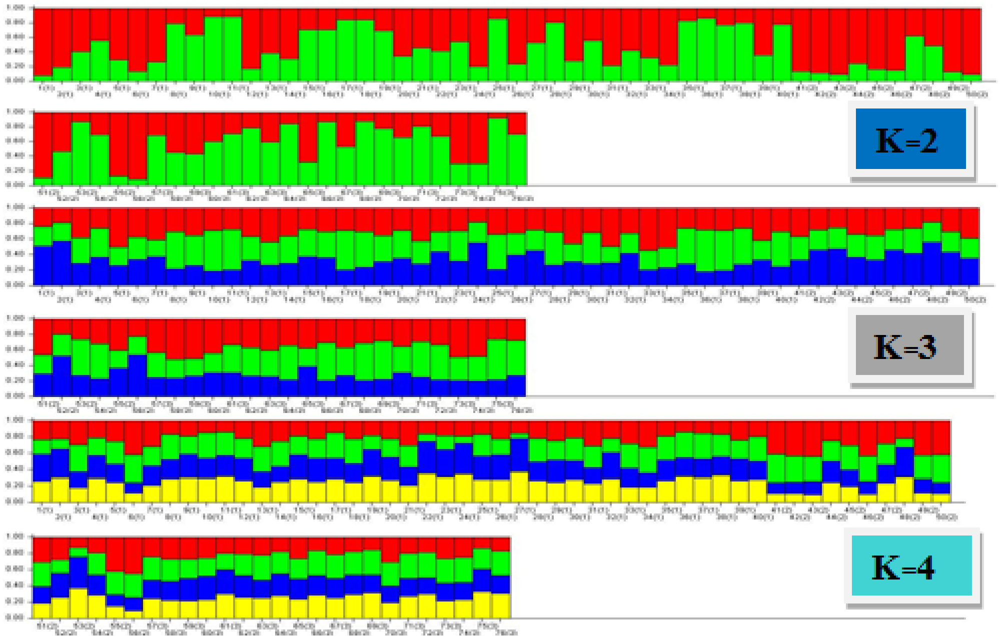

Figure 3: Population structure and membership fraction at K = 4, K = 3 and K = 2 for 76 accessions of Wua-lan base on microsatellite markers analysis (Phetchaburi Province: 1(1) - 40(1), Ratchaburi Province: 41(2) – 56(2) and Prachuap Khiri Khan Province: 57(3) – 76(3)). Each column accession generated by cutting the Ward dendrogram into a specified number of clusters/groups; the numbers of clusters from top to bottom were 2, 3, and 4. The clusters are represented by different colors.

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