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THE ANALYSIS OF MICROSATELLITE DNA POLYMORPHISMS AS A BASE OF CONCERVATION OF LOCAL SWAMP BUFFALO OF SOUTHEAST SULAWESI

MUH. RUSDIN

MINISTRY OF NATIONAL EDUCATION AND CULTURE
SECRETARIAT GENERAL
SEAMEO SEAMOLEC
SOUTHEAST ASIAN REGIONAL CENTRE FOR TROPICAL BIOLOGY
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APPROVAL SHEET

1. Research Title : The Analysis of Microsatellite DNA Polymorphisms as a Base of Concervation of Local Swamp Buffalo of Southeast Sulawesi
2. Researcher
   a. Name : Muh. Rusdin
   b. Sex : Male
   c. Occupation : Postgraduate Students of IPB
3. Institution
   a. Name of Institution : Department of Animal Production and Tecnology, Faculty of Animal Science, Bogor Agricultural University
   b. Address : Jalan Agatis. Kampus IPB, Darmaga, Bogor 16680 Indonesia
   c. Telephone/fax/HP : 0251-8626024/0251-8628379/082196039103
   d. Email Address : departemeniptp@gmail.com, mrusdin74@yahoo.com
4. Research Schedule : 9 (nine) months
5. Research Budget :

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Endorsed by,
Deputy Director for Programme
SEAMEO BIOTROP

Researcher,

Dr. Jesus C. Fernandez

Muh. Rusdin, S.Pt, M.Si
Student ID. D161140031

Approved by,
Director of SEAMEO BIOTROP

Dr. Irdika Mansur, M.For, Sc
NIP. 19660523 199002 1 001

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ABSTRAK

Populasi kerbau di Asia Tenggara mengalami penurunan drastis selama periode 1990-2013. Oleh karena itu, perlu upaya konservasi sumber daya genetik, perbaikan mutu genetik dan optimalisasi pemanfaatan potensi rumpun kerbau lokal. Tujuan penelitian ini adalah untuk mengidentifikasi polimorfisme DNA mikrosatelit, penanda genetik, dan jarak genetik populasi kerbau rawa lokal Sulawesi Tenggara dan perbandingannya dengan tiga populasi kerbau rawa lainnya berdasarkan DNA mikrosatelit. Sebanyak 40 sampel darah kerbau rawa lokal Sulawesi Tenggara dan 30 sampel darah yang berasal dari tiga populasi lain di Indonesia digunakan dalam penelitian ini. Sampel darah dikoleksi dari vena jugularis sebanyak 5 ml di dalam tabung vacutainer yang mengandung EDTA. Penanda DNA mirosatelit yang digunakan adalah lokus CSSM047, ILSTS011, dan BM1706. Ekstrasi DNA, amplifikasi DNA, dan elektroforesis dikerjakan di Laboratorium Geneika Molekuler Fakultas Peternakan, Institut Pertanian Bogor. Sedangkan analisis Fragment DNA menggunakan jasa dari *Malaysia Genome Institute* di Selangor Malaysia. Sampel DNA diamplifikasi dengan teknik *Polymerase Chain Reaction.* Produk PCR dilabel dengan primer FAM-labeled M13 (-21) universal. Hasil penelitian menunjukkan bahwa tiga lokus DNA mikrosatelit berhasil diamplifikasi. Ditemukan 10 alel pada lokus CSSM047 (A, B, C, D, E, F, G, H, I, dan J). Ukuran alel berkisar 132 – 168 bp, dan frekuensi alel berkisar 0,000 – 0,5000. Kerbau rawa lokal Sulawesi Tenggara memiliki 8 alel (A, B, C, D, E, F, G, dan J). Sedangkan genotipe yang ditemukan pada seluruh sampel sebanyak 15 genotipe (AJ, BB, BD, BF, CC, CD, CHIJ, CJ, DD, DF, DG, DI, DJ, EE, dan JJ). Frekuensi genotipe berkisar 0,000 – 0,400. Kerbau rawa Sulawesi Tenggara memiliki 11 genotipe (AJ, BB, BD, CC, CJ, DD, DF, DG, DJ, EE, dan JJ). Hasil penelitian disimpulkan bahwa kerbau rawa lokal Sulawesi Tenggara memiliki keragaman DNA mikrosatelit yang cukup tinggi, dimana frekuensi alel berkisar 0,000 – 0,500, dan frekuensi genotipe berkisar 0,000 – 0,400.

Kata Kunci : DNA mikrosatelit, keragaman genetik, kerbau rawa, Sulawesi Tenggara

1. INTRODUCTION

1.1. Background

Total of buffalo population in the world in 2015 is 199.7 million heads, and 97% of them are in Asia. The highest populations of Buffalo in the world is in India. That is 56.5% of total buffalo populations in the world, followed by China, Pakistan, Philippines, Vietnam, Bangladesh, Thailand and Indonesia. In Southeast Asia, Indonesia has the highest populations of buffalo (FAOSTAT, 2015).

The domesticated and wild buffalo can be found in all region countries of the Southeast Asia sub region, particularly the swamp buffalo type (FAO, 2007). For hundreds of years, the buffalo in Southeast Asia region has been a part of the human development. It commonly has functions as a draft power in opening new farmlands, tillage, transport, and food resources. The next developments of animal husbandry were resulted in the specialized use of Buffalo as important sources of milk and meat (Cruz, 2015). The Buffalo is one of the animal husbandry that is often used by the Indonesian people's. Utilization of Buffalo is not only as food, but also as a working animal especially in the agriculture area, savings, and a ritual of society culture (Sumantri *et al.* 2017). In addition, the Buffalo has significance important related to the culture or beliefs of the people in Indonesia, including Toraja-South Sulawesi, Sumba-East Nusa Tenggara, Sumbawa-West Nusa Tenggara, Minangkabau-West Sumatra, and Pampangan-South Sumatra. In addition, the Buffalo has developed into the international tourist attraction (Talib, 2014).

However, the buffalo population in Southeast Asia during the period 1990-2013 has decreased drastically, especially in Indonesia, Malaysia, and Thailand. The higher decrease of buffalo population in Southeast Asia occurred in Thailand is 76.07%, followed by Indonesia 55.50% and Malaysia 41.51%, while Buffalo Philippines population has slightly increased around 5.35% (FAOSTAT, 2015). In 2016, the local buffalo population in Indonesia is 1,386,280 heads, more than cattle population 16,092,561 heads. The potential of buffalo as national meat resources is only 1.17% in 2016, while beef cattle had reached 16.51% (Dirjen of PKH, 2016). The decrease of local Buffalo population in Indonesia occurs in the several provinces, including in Southeast Sulawesi. Total of local buffalo populations in this province is 7,614 heads in 2006, and it has decreased 2,713 heads in 2016 or it has decreased 64.37% during the period 1990-2013 (BPS of Southeast Sulawesi, 2011; Ditjen of PKH, 2016).

The decrease of population of local buffalo in Indonesia were caused by utilization as working animal has been subtututed by hand tranctor. The decrease of local buffalo populations

also cause by they price is chaper that cattle, and increasing of rate inbreeding (Tiesnamuri and Talib, 2011). In addition, the reduction agricultural land to an industry area has caused low feed quality of Buffalo. This trend of the Buffalo population is very worrying, and if this problem are allowed to continue, it can be threaten the sustainability of local Buffalo genetic resources in the short time. Therefore, it needs efforts to improve genetic resources conservation of local Buffalo, and also genetic quality and optimize of using the potential of local Buffalo breeds. The efforts of conserve and improvement of the genetic quality of swamp buffalo will impact on increasing of meat production domestic and supporting of national meat self-sufficiency programs.

Genetic diversity information is a major concern in a program of animal genetic resources conservation. According to the FAO (2000), conservation of farm animal genetic resources refers to all human activities including strategies, plans, policies, and actions undertaken to ensure that the diversity of farm animal genetic resources is maintained to contribute food and agricultural production and productivity, now and in the future.

The information of genetic diversity of local Buffalo of Southeast Sulawesi has not been reporting. One of the molecular biology approach to identify animal genetic diversity is based on microsatellite DNA polymorphisms. The microsatellite markers are highly polymorphic distributed throughout the genome locus specific and codominant. They have an edge over other genetic markers for comparative studies of evolution, genetic variation, parentage assessment and gene flow. Microsatellite markers have been used for studying polymorphisms and genetic diversity in many livestock species. They are simple DNA sequences (e.g. AC), usually 2 or 6 bases long repeated variable numbers of times in tandem. They are easy to detect with PCR and a typical microsatellite marker that has more variants than other marker systems (Martin-Burriel at al., 1999; Sangwan, 2012).

The research of genetic diversity based on microsatellite (autosomal) markers including Y chromosome microsatellite has been developed rapidly in several of breed animal, such as in Vietnamese native cattle (Pham et al., 2013), Chinese native cattle (Zhang et al., 2007), breeds cattle in Spain (Cortes et al. 2011), and local Indonesian cattle (Handiwirawan et al 2003; Winaya et al. 2011; and Septian et al., 2015). It is similar to another, such as river buffalo in Cuba (Acosta et al 2014), Turkish local Buffalo (Ünal et al. 2014), Iraqi native Buffalo (Jaayid et al., 2013), Chinese buffalo breed and hybrids with Murrah and Nili-Ravi buffalo in China (Yang et al. 2011), Murrah buffalo in India (Bhuyan et al. 2010), Azakheli buffalo in Pakistan (Babar et al. 2009), Guilan buffalo in Iran (Aminafshar et al. 2008), swam buffalo and river buffalo in China (Zhang et al. 2008),

Anatolian buffalo in Turkey (Soysal et al. 2007), and swamp buffalo in Thailand (Triwitayakorn et al. 2006; Sraphet et al. 2008).

The results of the previous research were reported that microsatellite DNA polymorphisms of swamp buffalo in Central Lombok of West Nusa Tenggara Province were low relatively at loci of HEL09, INRA023, INRA032, and ILSTS005, which PIC value are 0.24; 0.35; 0.38; 0.31 respectively (Sukri et al. 2012). Microsatellite DNA polymorphisms of local swamp buffalo of Aceh, North Sumatra, Riau, Banten, Central Java, West Nusa Tenggara, and South Sulawesi were high relatively at loci of ILSTS61 and ILSTS17, which PIC (Polymorphism Information Content), values were 0.74 and 0.36 respectively and the CSMM66 locus have been low diversity with PIC value was 0.05 (Saputra, 2013). While the information of microsatellite DNA polymorphisms of other local Indonesian Buffalo such as red swamp buffalo in Madura, Kalang buffalo in South Kalimantan, Moa Buffalo in Maluku and local swamp buffalo in Southeast Sulawesi have not been reported.

1.2. Objectives

The objective this research are:

a. Indentify microsatellite DNA polymorphisms of local swamp buffalo of Southeast Sulawesi.
b. Identify genetic markers of local swamp buffalo of Southeast Sulawesi based on three locus of microsatellite DNA,
c. Estimate genetic distances among the local swamp buffalo subpopulation of Southeast Sulawesi and their comparison with three of other populations of local Indonesian swamp buffalo based on microsatellite DNA polymorphisms.

1.3. Expected Output

The specific output that was expected of this research is to find out information of genetic diversity, genetic markers, phylogeny relationships, and genetic distances among subpopulations of local swamp buffalo of Southeast Sulawesi, and their will be compared with three of other populations of local Indonesian swamp buffalo based on microsatellite DNA polymorphisms. In addition, the output of the research will be published in International Journal indexed by scopus, so that it can be a reference for other researchers. The specific outcomes that are expected of this research that are available sources of reference to be used as the basis in formulating conservation programs, improving the breeding management and support the establishment of the new breed of local swamp buffalo in Southeast Sulawesi. The

research is very important to do because in addition to supporting the completion process of the Doctoral of student proposer, is also very beneficial for the government of Southeast Sulawesi Province in coserving its unique buffalo.

2. BENEFIT AND IMPORTANCE OF RESEARCH

The results of this research specifically were expected can use a base in formulating conservation programs and improving breeding management, and also supporting the establishment of a new breed of local swamp buffalo of Southeast Sulawesi. Other than that the research is very important for it can support the completion of doctoral studies for proposer student, and it is also very useful for the government of Southeast Sulawesi Province in the conservating of its unique buffalo.

3. METHODOLOGY

3.1. Time and Location

This research will be conducted from March to December 2018. Blood samples of local swamp buffalo of Southeast Sulawesi were collected from four located (sub population), namely island and mainland regions of Bombana Regency, Konawe and Kolaka Regency Province of Southeast Sulawesi. Molecular genetics Analysis (DNA extraction to PCR) were conducted in the Molecular Genetic Laboratory di Faculty of Animal Sains, Bogor Agricultural University. While DNA fragment analysis by services of Malaysian Genome Institute in Selangor Malaysia.

3.2. Blood sample

A total of 40 blood samples of local swamp buffalo were collected from differents subpopulation in Southeast Sulawesi will be used in this research. The blood samples consists of 10 from Bombana Regency land area, 10 from Bombana Regency island area, 10 from Konawe Regency, and 10 from Kolaka Regency. A total of 30 blood samples from three other populations of Indonesia swamp buffalo were used in this research as a comparison from out group. A total of blood samples based on swamp buffalo populations will be used in this research can be see in Table 1.

Table 1. Total of blood samples based on buffalo populations

No.	Populations (Province)	Sample code	Total
1	Bombana land area (Southeast Sulawesi 1)	BD	10*
2	Bombana island area (Southeast Sulawesi 2)	BK	10*
3	Kolaka (Southeast Sulawesi 3)	KL	10*
4	Konawe (Southeast Sulawesi 4)	KN	10*
5	Toraja (South Sulawesi)	TR	10*
6	Sumbawa (Southeast West Nusa)	NTB	10**
7	Lebak (Banten)	BTN	10**
	Total		70

Note: *Blood samples were collected by proposer in 2016.
**Blood samples were collected by team of Kerjasama Kemitraan Penelitian Pertanian dengan Perguruan Tinggi (KKP3T) in 2007.

3.3. Research Procedure

a. Sample Collection

Blood samples of swamp buffalo wiil be used in this research are whole blood. They taken from the jugular vein as much 5 ml by using a vacutainer tube which containing anticoagulant (EDTA). An absolute ethanol is added into vacutainer tube with a ratio on 1:1 and they shak to form an eight figure. Blood samples are stored at room temperature before being analyzed further.

b. DNA Isolation

The procedure of DNA extration by using the standard phenol-chloroform extraction protocol, was modified from Sambrook and Russel (2001). A total of 100 µL of buffalo blood in the tube *eppendorf* was added to 1000 µL of 0.2% NaCl and then homogenized by vortex. The mixture were centrifuged at 8000 rpm (20 ^{o}C) for 5 min and the supernatant part removed. The precipitate was added to 1 x STE up to 350 µL, 40 µL of 10% SDS, and 10 µl proteinase-K 5 (5 mg/µL), and then incubated at 55 ^{o}C for 2 hours. Then, phenol solution (400 µL), CIAA (400 µL), and NaCl 5M (40 µL) were added to the mixture and shaken slowly at room temperature for 1 hour, end then mixture were centrifuged at 12000 rpm (20 ^{o}C) for 5 min. A total 200 µl of supernatant (DNA molecule) was moved in the tube of 1.5 ml, and was added to 800 µL EtOH absolute and 40 µL NaCl 5M, then frozen in the freezer at -20 ^{o}C for 12 hours. Sampel of DNA molecules was centrifuged at 12000 rpm (20 ^{o}C) for 5 min and supernatant part was removed. Then, DNA precipitate was added 800 µl of EtOH 70%, and were sentrifuged at 12000 rpm (20 ^{o}C) for 5 min, and supernatant was removed. Then, DNA

precipitate was air-dried in the open condition to lost of alchohol (DNA sampel was dry), and then added to 100 µL of 80% TE or Elution Buffer, and was stored in the freezer at -20°C.

c. Microsatellite Selection

This research use three microsatellite markers of autosome DNA (CSSM047, ILSTS011, and BM1706). Microsatelitte markers were selected from the panel of standardized markers recommended for buffaloes by the Molecular Genetic Characterization of Animal Resources (FAO, 2011), and based on the level of polymorphism that are adapted from various international journals. Information of the three microsatellite primer to use in the research were presented in Table 2.

Table 2. The three microsatellite primers that were used in the research

Locus	Chro-mosome position	Sequen primer (5'- 3')	Acces code *GenBank* /References	Size (bp)	Repetition motive
CSSM047	3q(8)	F: TCTCTGTCTCTATCACTATATGGC R: CTGGGCACCTGAAACTATCATCAT	U03821	127-162	(TG)12. TA.TG. TA(TG)4
ILSTS011	-	F: GCTTG CTACATGGAAAGTGC R: CTAAAATGCAGAGCCCTACC	Tantia *et al.* (2006)	264-272	-
BM1706	-	F: ACAGGACGGTTTCTCCTTATG R: CTTGCAGTTTCCCATACAAGG	Coulson *et.al.* (1998)	211-271	-

Note: F = Forward; R = Reverse

d. DNA Amplification and Electrophoresis

DNA samples were amplified by The Polymerase Chain Reaction Technique (PCR), in accord with Zachos et al. (2003). DNA amplification method accord with Schuelke (2000). The forward primer was merger among a sequence of forwarding primer in Table 2 with a sequence of universal M13 (-21) (TGTAAAACGACGGCCAGT). PCR products were labeled by a primer of FAM-labeled M13 (-21) universal (FAM-TGTAAAACGACGGCCAGT-3′). PCR reaction was conducted in a 25 µL reaction mixture, included 50 ng/µL of DNA template, 25 pmol of each primer (IDT, Singapore), 1 unit Go Taq® Green Master Mix (Promega, Madison, WI, USA), and water. The PCR qit from Qiagen product (Q5® Hot Start High-Fidelity Polymerase) was used for DNA sample containing heparin. PCR reaction was conducted in a 25 µL reaction mixture, included 50 ng/µL of DNA template, 25 pmol of each primer (IDT, Singapore), buffer, enhancer, dNTP, Taq polymerase and water which the PCR grade was 10 mM.

PCR reaction was conducted in the two-step. The first step for 30 cycles which PCR reaction conditions of predenaturation at 94 oC for 5 min, denaturation at 94 oC for 10 sec,

annealing at 52 to 55 oC for 20 sec, and extension at 72 oC for 30 sec. The second step for 8 cycles which PCR reaction conditions of denaturation at 94 oC for 10 sec, annealing at 53 oC for 20 sec, extension at 72 oC for 30 sec, and the final extension at 72 oC for 5 min. DNA amplification by used a thermocycler machine (GeneAmp® PCR System 9700, Applied Bio SystemTM, Foster City, CA, USA). The PCR product (amplicon) was verified by electrophoresis method (Sambrook & Russell 2001) in 1.5% agarose gel, included 0,45 g of agarose, 30 ml of 0,5 x TBE buffer, and 1,8 µL of fluoro safe. A total of 5 µl of the amplicon was electrophoresed at 100 V for 35-45 min. Then, band pattern of each PCR product was visualized by UV train simulator (Alpha Imager®EP).

e. Genotyping

Allele size of all samples were determined by the labelled fragment fluorescently method in Biosystem Genetic Analyzer. Microsatellite genotyping that was interpreted based on fragment analysis by software of GeneMapper version 4.0 base on services of Malaysia Genome Institute.

3.4. Data Analysis

a. Allele and Genotype Frequencies

The allele frequencies were analyzed based on number of gene in populations (Nei 1987; Nei and Kumar, 2000). Allele frequencies was calculated by formula:

$$\chi_i = \left(2n_{ii} + \sum_{j \neq i} n_{ij} \right) / (2n)$$

Genotype frequencies was calculated by formula:

$$\chi_{ii} = \frac{n_{ii}}{n}$$

Description:

X_i = ith allele frequencies

X_{ii} = iith genotype frequencies

n_{ii} = Number of sample of ii genotype (homozygous genotype)

n_{ij} = Number of sample of ij genotype (heterozygous genotype)

n = Total samples

b. Total of Allele Effective

The total of allele affective (Ne) was calculated in accord with Hedrick (2001) by formula:

$$N_e = \frac{1}{\sum_{i=1}^{n} P_i^2}$$

c. Hardy-Weinberg Equilibrium

Tes of Hardy-Weinberg Equilibrium (HWE) was conducted with chi-square test (Nei and Kumar, 2000) by the formula:

$$\chi^2 = \sum \frac{(O-E)^2}{E}$$

Description:

X^2 = Hardy-Weinberg Equilibrium test

O = Observed number of iith genotype

E = Expected number of iith genotype

d. Fixation Index

The fixation index was used to determine the structure of population breeding or selection method were associated with polymorphic alleles (Nei, 1987) by formula:

$$F_{is} = \frac{\chi_{ii} - \overline{\chi_i^2}}{\overline{\chi}_i - \overline{\chi_i^2}}$$

$$F_{it} = \frac{\chi_{ii} - \overline{\chi}_i^2}{\overline{\chi}_i - \overline{\chi}_i^2}$$

$$F_{st} = 1 - \frac{\overline{\chi}_i - \overline{\chi_i^2}}{\overline{\chi}_i - \overline{\chi}_i^2}$$

Description:

F_{is} = Inbreeding coefficient index

F_{it} = Corelation of two gamete on total of population

F_{st} = Genetic differentiation index

e. Observed and Expected Heterozygosity

Observed heterozygosity (Ho) was proportion of total heterozygous individuals, while expected heterozygosity (He) was obtained from allele frequencies (Hartl (1988). They were estimated by formula:

$$H_o = \sum_{i \neq j} \frac{N_{1ij}}{N}$$

Description:

N_{1ij} = Number of heterozygous individuals in locus 1th

N = Total sample

$$He = 1 - \sum_{i=1}^{n} p_i^2$$

$$I = 1 - \sum p_i InP_i$$

Description:

P_i = ith allele frequencies in locus 1th

n = Total allele

f. Polymorphism Information Content (PIC)

Polymorphism Information Content (PIC) was calculated in accord with Mateescu et al. (2005) by formula:

$$PIC = 1 - (\sum_{i=1}^{n} p_i^2) - \sum_{i=1}^{n-1} \sum_{j=i-1}^{n} 2 p_i^2 p_j^2$$

Description:

p_i = ith allele frequencies

p_j = ith ith allele frequencies

Data of allele frequencies, genotype frequencies, total of allele (Na), total of effective alleles (Ne), Hardy-Weinberg Equilibrium, observed heterozygosity (Ho), Expected Heterozygosity (He), fixation index (Fis, Fit, Fst), and Polymorphism Information Content (PIC) were analyzed by GENEPOP version 3.2 (Raymond and Rousset 2001).

g. Identical and Genetic Distances

Genetic identical (I) was a measurement of the genetic relationship between populations, and genetic distances (D) was a measurement of the genetic difference between populations. They were calculated based on the allele frequencies (Nei (1987) by formula:

$$I = \sum_{i=1}^{m} (Pix \times Piy) \div \left[\left(\sum_{i=1}^{m} Pix^2 \right) \left(\sum_{i=1}^{m} Piy^2 \right) \right]^{0,5}$$

$$D = -Ln\ I$$

Description:

Pix = ith allele frequencies from X population

Piy = ith allele frequencies from Y population

Identical and genetic distances were analyzed based on the Kimura 2-Parameter, while the reconstruction of phylogenetic trees was conducted by Neibour-joining method with bootstraps 1000 repetitions in software of MEGA version 6 (Tamura *et al*. 2013).

4. RESULTS AND DISCUSSION

4.1. Amplification of Microsatellite DNA

Three-locus of buffalo microsatellite DNA (CSSM047, ILSTS011, and BM1706) success was amplificated with Polymerase Chain Reaction Technique (PCR). Size of microsatellite DNA fragment was amplificated in the locus of CSSM047, ILSTS011, and BM1706 in the research ranges from 132-168 bp, 264-272 bp, and 211-271 bp respectively. Visualization of PCR product band pattern in 1.5% agarose gel electrophoresis of tree locus of buffalo DNA microsatellite in the study was presented in figure 1 to 3.

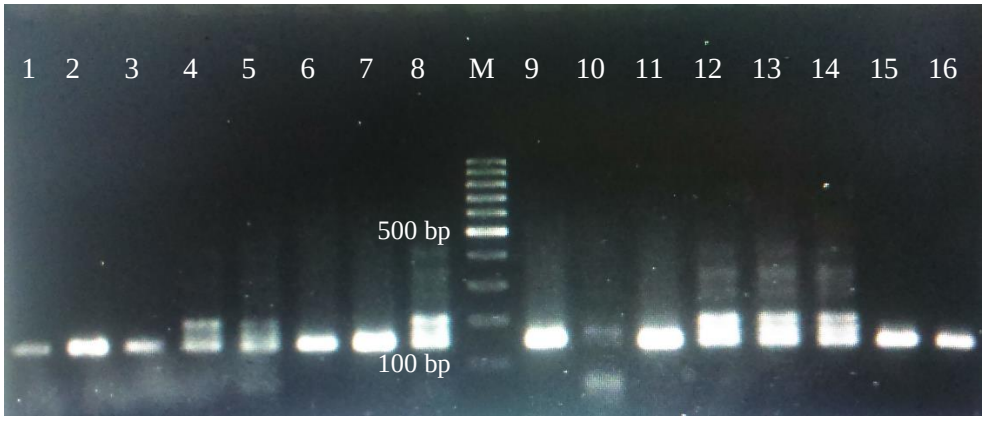

Figure 1. Visualization of PCR product band pattern in 1.5% agaros gel electrophoresis of CSSM047 locus. M: Marker; 1-16: samples.

Figure 1 to 3 showed that PCR product samples for sample 1 to 16 success were amplified. All samples of PCR product has single and double band pattern. While the example of DNA microsatellite genotyping of CSSM047 locus has shown in figure 4.

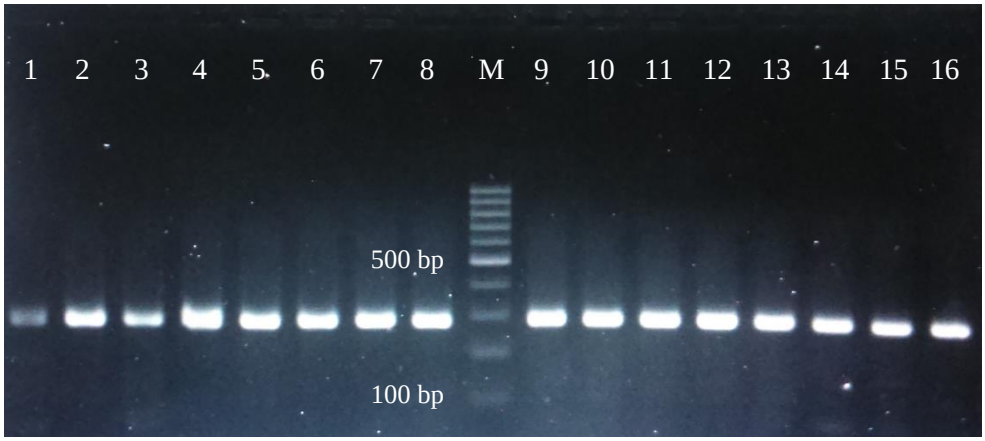

Figure 2. Visualization of PCR product band pattern in 1.5% agaros gel electrophoresis of ILSTS011 locus. M: Marker; 1-16: samples.

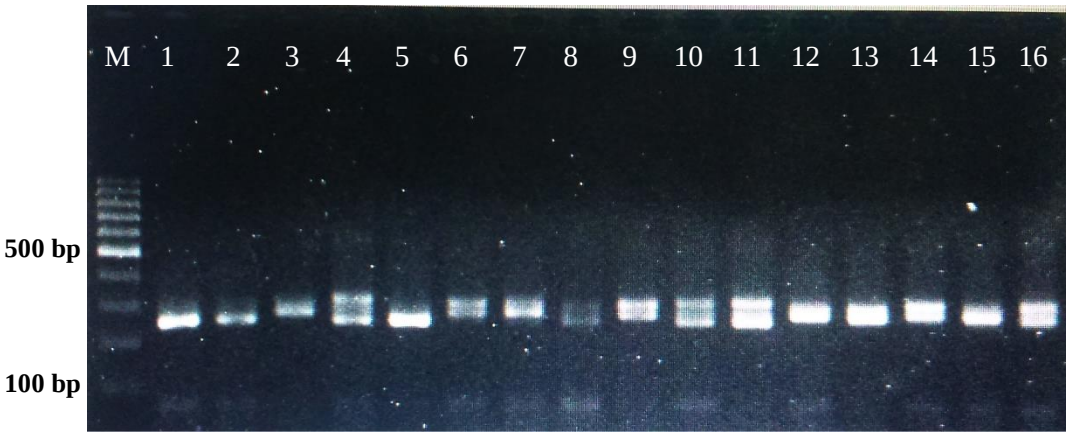

Figure 3. Visualization of PCR product band pattern in 1.5% agaros gel electrophoresis of BM1706 locus. M: Marker; 1-16: samples.

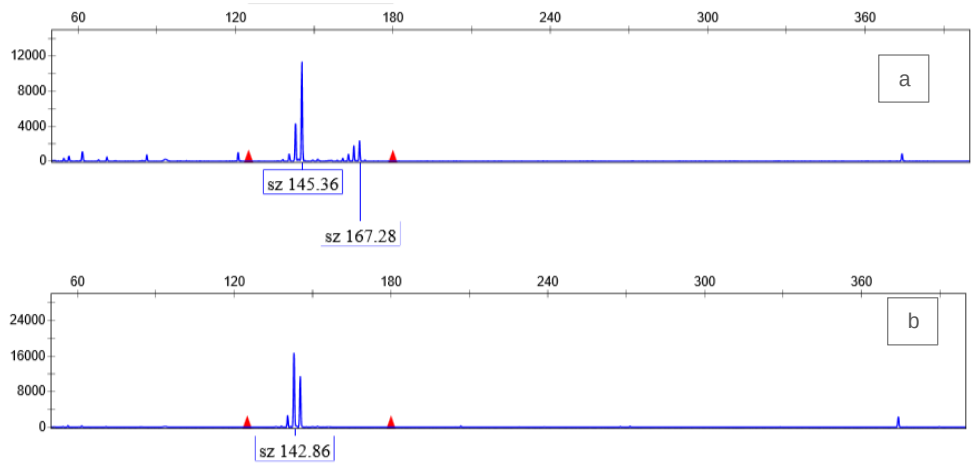

Figure 4. The example of DNA microsatellite genotyping of CSSM047 locus. (a): 146/168 heterozygote genotype; (b); 142/142 homozygote genotype.

4.2. Allele and Genotype Frequencies

Allele frequencies are one of the genetic parameters that are can descriptions of the genetic status of the animal population. Genetic diversity information is major attention in the conservation program of animal genetic resource. Allele frequencies of CSSM047 locus base swamp buffalo population were shown in Table 3.

Table 3. Allele frequencies of CSSM047 locus base on swamp buffalo population

Allele symbol	Populatios						
	Bombana island area (n=10)	Bombana land area (n=10)	Kolaka (n=10)	Konawe (n=10)	Toraja (n=10)	NTB (n=10)	Banten (n=10)
132 (A)	0.000	0.000	0.000	0.100	0.000	0.000	0.000
142 (B)	0.250	0.000	0.000	0.000	0.000	0.100	0.000
144 (C)	0.150	0.100	0.200	0.200	0.300	0.150	0.300
146 (D)	0.300	0.400	0.500	0.400	0.400	0.400	0.500
148 (E)	0.100	0.000	0.000	0.000	0.000	0.000	0.000
150 (F)	0.000	0.000	0.000	0.100	0.000	0.150	0.000
152 (G)	0.000	0.100	0.000	0.000	0.000	0.000	0.000
164 (H)	0.000	0.000	0.000	0.000	0.100	0.000	0.000
166 (I)	0.000	0.000	0.000	0.000	0.000	0.100	0.000
168 (J)	0.300	0.450	0.250	0.250	0.150	0.350	0.100

The result of the study in the Table 3 showed there were 10 alleles in CSSM047 locus that were alleles A, B, C, D, E, F, G, H, I, and J allele. AlleleS size range were 132 to 168 bp, which alleles frequencies range were 0,000 to 0,5000. From of 10 alleles that found there were 8 alleles CSSM047 locus of South East Sulawesi swamp buffalo, that were alleles of A, B, C, D, E, F, G, and J. While genotypes frequencies were shown in Table 4. There were 15 genotypes were found in CSSM047 locus, that were genotypes AJ, BB, BD, BF, CC, CD, CHIJ, CJ, DD, DF, DG, DI, DJ, EE, and JJ.

Genotype frequencies in swamp buffalo in CSSM047 locus in this reserach range were 0,000 to 0,400. Southeast Sulawesi local swamp buffalo have 11 genotypes that were AJ genotype (Konawe = 0.100), BB (Bombana island area = 0.200), BD (Bombana island area = 0.100), CC for population of Bombana island area, Bombana land area, Kolaka and Konawe, wich genotype frequencies was 0.100 for all population. Then CJ genotype for Konawe population (genotype frequencies was 0.100), and DD genotype for the population of Bombana island area, Bombana land area, Kolaka, and Konawe. Their genotypes frequencies were 0.100; 0.200; 0.400 and 0.300, respectively. Then DF genotype for Konawe population (genotype frequencies was 0.100), DG genotype for Bombana land area population (genotype frequencies

was 0.100), DJ genotype for population of Bombana island area, Bombana land area, Kolaka and Konawe, wich genotypes frequencies were 0.300 for all populations, and then EE genotype for Bombana island area population (genotype frequencies was 0.100), and JJ genotype for Bombana land area and Kolaka population. Their genotypes frequencies were 0.300 and 0.100, respectively.

Table 4. Genotype frequencies of CSSM047 lokus base on swamp buffalo population

Genotype symbol	Populations						
	Bombana islan area (n=10)	Bombana land area (n=10)	Kolaka (n=10)	Konawe (n=10)	Toraja (n=10)	NTB (n=10)	Banten (n=10)
AJ	0.000	0.000	0.000	0.100	0.000	0.000	0.000
BB	0.200	0.000	0.000	0.000	0.000	0.000	0.000
BD	0.100	0.000	0.000	0.000	0.000	0.000	0.000
BF	0.000	0.000	0.000	0.000	0.000	0.100	0.000
CC	0.100	0.100	0.100	0.100	0.200	0.100	0.200
CD	0.100	0.000	0.200	0.100	0.100	0.100	0.200
CHIJ	0.000	0.000	0.000	0.000	0.100	0.000	0.000
CJ	0.000	0.000	0.000	0.100	0.000	0.000	0.000
DD	0.100	0.200	0.400	0.300	0.500	0.100	0.500
DF	0.000	0.000	0.000	0.100	0.000	0.100	0.000
DG	0.000	0.100	0.000	0.000	0.000	0.000	0.000
DI	0.000	0.000	0.000	0.000	0.000	0.100	0.000
DJ	0.300	0.300	0.200	0.200	0.100	0.300	0.100
EE	0.100	0.000	0.000	0.000	0.000	0.000	0.000
JJ	0.000	0.300	0.100	0.000	0.000	0.100	0.000

5. CONCLUSION

The result of this research was conclusion there were 10 alleles and 15 genotypes of CSSM047 locus in Indonesian local swamp buffaloes. Southeast Sulawesi local swamp buffalo has genetic diversity was hinger. They have 8 alleles from 10 alleles were found in the research include alleles A, B, C, D, E, F, G, and J, wich their alelles frequencies range were 0.000 – 0.500 , and they have 11 genotypes from 15 genotypes was found, include genotypes AJ, BB, BD, CC, CJ, DD, DF, DG, adn DJ, wich their alelles frequencies range were 0.000 – 0.500, and genotypes frequencies range were 0.000 – 0.400.

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7. APPENDICES

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MICROSATELLITE DNA POLYMORPHISMS ANALYSIS OF SOUTHEAST SULAWESI LOCAL SWAMP BUFFALO

Muh. Rusdin[1,2], Cece Sumantri[3*], Dedi Duryadi Solihin[4], Asep Gunawan[3], Chalid Talib[5]

[1]Student of IPB Graduated School, Bogor University Agricultural, Kampus IPB Darmaga Bogor 16680
[2]Southeast Asian Regional Centre for Tropical Biology. Jl. Raya Tajur Km. 6 Bogor 1613, Indonesia
[3]Department of Animal Production and Tecnology, Faculty of Animal Science, Bogor Agricultural University, Kampus IPB Darmaga Bogor 16680
[4]Departement of Biologi, Matematic and Natural Science Faculty, Bogor Agricultural University, Kampus IPB Darmaga Bogor 16680
[5]Reseacrh and Development of Livestock Centre, Jl. Raya Pajajaran Kav. E-59 Bogor 16151
[*]Corresponding author, e-mail: csumantri12@gmail.com

ABSTRAK

Populasi kerbau di Asia Tenggara mengalami penurunan drastis selama periode 1990-2013. Oleh karena itu, perlu upaya konservasi sumber daya genetik kerbau lokal. Tujuan penelitian ini adalah untuk mengidentifikasi polimorfisme DNA mikrosatelit, penanda genetik, dan jarak genetik populasi kerbau rawa lokal Sulawesi Tenggara dan perbandingannya dengan tiga populasi kerbau rawa lainnya berdasarkan DNA mikrosatelit. Sebanyak 70 sampel darah kerbau rawa lokal Indonesia yang berasal dari populasi yang berbeda digunakan dalam penelitian ini. Sampel darah dikoleksi dari vena jugularis sebanyak 5 ml di dalam tabung vacutainer yang mengandung EDTA. Penanda DNA mirosatelit yang digunakan adalah lokus CSSM047, ILSTS011, dan BM1706. Ekstrasi DNA, amplifikasi DNA, dan elektroforesis dikerjakan di Laboratorium Geneika Molekuler Fakultas Peternakan, Institut Pertanian Bogor. Sedangkan analisis Fragment DNA menggunakan jasa dari *Malaysia Genome Institute* di Selangor Malaysia. Sampel DNA diamplifikasi dengan teknik *Polymerase Chain Reaction.* Produk PCR dilabel dengan primer FAM-labeled M13 (-21) universal. Hasil penelitian menunjukkan bahwa tiga lokus DNA mikrosatelit berhasil diamplifikasi. Ditemukan 10 alel pada lokus CSSM047. Ukuran alel berkisar 132 – 168 bp, dan frekuensi alel berkisar 0,000 – 0,5000. Kerbau rawa lokal Sulawesi Tenggara memiliki 8 alel (A, B, C, D, E, F, G, dan J). Sedangkan genotipe yang ditemukan pada seluruh sampel sebanyak 15 genotipe. Frekuensi genotipe berkisar 0,000 – 0,400. Kerbau rawa Sulawesi Tenggara memiliki 11 genotipe (AJ, BB, BD, CC, CJ, DD, DF, DG, DJ, EE, dan JJ). Hasil penelitian disimpulkan bahwa kerbau rawa lokal Sulawesi Tenggara memiliki keragaman DNA mikrosatelit yang cukup tinggi, dimana frekuensi alel berkisar 0,000 – 0,500, dan frekuensi genotipe berkisar 0,000 – 0,400.

Kata kunci : DNA mikrosatelit, keragaman genetik, kerbau rawa, Sulawesi Tenggara

ABSTRACT

The buffalo population in Southeast Asia has decreased drastically during the period 1990-2013. Therefore it needs conservation of genetic resources local Buffalo. The objective this research were indentify microsatellite DNA polymorphisms, genetic markers, and estimate genetic distances among the local swamp buffalo subpopulation of Southeast Sulawesi and their comparison with three of other populations based on microsatellite DNA polymorphisms. A total of 70 blood samples of Indonesian local swamp buffalo from difference population were used in the research. Blood samples were colected from the jugular vein as much 5 ml in a vacutainer tube which containing EDTA. Microsallite marker were used include CSSM047, ILSTS011, and BM1706 locus. DNA extraction, amplification and electrophoresis were conducted in the Molecular Genetic Laboratory in Faculty of Animal Science, Bogor Agricultural University. While DNA fragment analysis by services of Malaysian Genome Institute. DNA samples were amplified by The Polymerase Chain Reaction Technique (PCR). PCR products were labeled by universel primer of FAM-M13. Result of the research showed that three-locus of buffalo microsatellite DNA (CSSM047, ILSTS011, and BM1706) success was amplificated. There were 10 alelles were found at CSSM047 locus. Size of elleles were 132 to 168 bp, and alleles frequencies range were 0,000 – 0,500. Local swam buffalo of Southeast Sulawesi has 8 alleles (A, B, C, D, E, F, G, and J). While genotypes that were found in all samples were 15 genotypes. Genotypes frequencies range were 0.000 – 0.400. Local swamp buffalo of Southeast Sulawesi has 11 genotypes (AJ, BB, BD, CC, CJ, DD, DF, DG, DJ, EE, and JJ). The result of this research was conclusion that local swam buffalo of Southeast Sulawesi has genetic diversity was hinger, wich their alelles frequencies range were 0.000 – 0.500, and genotypes frequencies range were 0.000 – 0.400.

Keywords : microsatellite DNA, genetic diversity, swamp buffalo, Southeast Sulawesi

INTRODUCTION

Total of buffalo population in the world in 2015 is 199.7 million heads, and 97% of them are in Asia. The highest populations of Buffalo in the world is in India. That is 56.5% of total buffalo populations in the world, followed by China, Pakistan, Philippines, Vietnam, Bangladesh, Thailand and Indonesia. In Southeast Asia, Indonesia has the highest populations of buffalo (FAOSTAT 2015).

The Buffalo is one of the animal husbandry that is often used by the Indonesian people's are as food, a working animal especially in the agriculture area, savings, and a ritual of society culture. The Buffalo has significance important related to the culture or beliefs of the people in Indonesia, including Toraja-South Sulawesi, Sumba-East Nusa Tenggara, Sumbawa-West Nusa Tenggara, Minangkabau-West Sumatra, and Pampangan-South Sumatra. In addition, the Buffalo has developed into the international tourist attraction (Sumantri *et al.* 2017; Talib 2014). However, the buffalo population in Southeast Asia during the period 1990-2013 has decreased drastically, especially in Indonesia, Malaysia, and Thailand. The higher decrease of buffalo population in Southeast Asia occurred in Thailand is 76.07%, followed by Indonesia

55.50% and Malaysia 41.51%, while Buffalo Philippines population has slightly increased around 5.35% (FAOSTAT 2015).

In 2016, the local buffalo population in Indonesia is 1,386,280 heads, more than cattle population 16,092,561 heads. The potential of buffalo as national meat resources is only 1.17% in 2016, while beef cattle had reached 16.51% (Dirjen of PKH 2016). The decrease of local Buffalo population in Indonesia occurs in the several provinces, including in Southeast Sulawesi. Total of local buffalo populations in this province is 7,614 heads in 2006, and it has decreased 2,713 heads in 2016 or it has decreased 64.37% during the period 1990-2013 (BPS of Southeast Sulawesi 2011; Ditjen of PKH, 2016). This trend of the Buffalo population is very worrying, because it can be threaten the sustainability of local Buffalo genetic resources in the short time. Therefore, it needs efforts conservation of genetic resources of local Buffalo, improving genetic quality and optimize of using the potential of local Buffalo breeds.

Genetic diversity information is a major concern in a program of animal genetic resources conservation. However, the information of genetic diversity of local Buffalo of Southeast Sulawesi has not been reporting. One of the molecular biology approach to identify animal genetic diversity is based on microsatellite DNA polymorphisms. The microsatellite markers are highly polymorphic distributed throughout the genome locus specific and codominant. They have an edge over other genetic markers for comparative studies of evolution, genetic variation, parentage assessment and gene flow. Microsatellite markers have been used for studying polymorphisms and genetic diversity in many livestock species. They are simple DNA sequences (e.g. AC), usually 2 or 6 bases long repeated variable numbers of times in tandem. They are easy to detect with PCR and a typical microsatellite marker that has more variants than other marker systems (Martin-Burriel *at al.* 1999; Sangwan 2012).

The research of genetic diversity based on microsatellite (autosomal) markers including Y chromosome microsatellite has been developed rapidly in several of breed animal, such as in Vietnamese native cattle (Pham *et al.* 2013), Chinese native cattle (Zhang *et al.* 2007), breeds cattle in Spain (Cortes *et al.* 2011), and local Indonesian cattle (Handiwirawan *et al* 2003; Winaya *et al.* 2011; and Septian *et al.* 2015). Its similar to another, such as river buffalo in Cuba (Acosta *et al.* 2014), Turkish local Buffalo (Ünal *et al.* 2014), Iraqi native Buffalo (Jaayid *et al.* 2013), Chinese buffalo breed and hybrids with Murrah and Nili-Ravi buffalo in China (Yang *et al.* 2011), Murrah buffalo in India (Bhuyan *et al.* 2010), Azakheli buffalo in Pakistan (Babar *et al.* 2009), Guilan buffalo in Iran (Aminafshar *et al.* 2008), swam buffalo and river buffalo in China (Zhang *et al.* 2008), Anatolian buffalo in

Turkey (Soysal *et al.* 2007), and swamp buffalo in Thailand (Triwitayakorn *et al.* 2006; Sraphet *et al.* 2008).

The results of the previous research were reported that microsatellite DNA polymorphisms of swamp buffalo in Central Lombok of West Nusa Tenggara Province were low relatively at loci of HEL09, INRA023, INRA032, and ILSTS005, which PIC value are 0.24; 0.35; 0.38; 0.31 respectively (Sukri *et al.* 2012). Microsatellite DNA polymorphisms of local swamp buffalo of Aceh, North Sumatra, Riau, Banten, Central Java, West Nusa Tenggara, and South Sulawesi were high relatively at loci of ILSTS61 and ILSTS17, which PIC (Polymorphism Information Content), values were 0.74 and 0.36 respectively and the CSMM66 locus have been low diversity with PIC value was 0.05 (Saputra 2013). While the information of microsatellite DNA polymorphisms of other local Indonesian Buffalo such as red swamp buffalo in Madura, Kalang buffalo in South Kalimantan, Moa Buffalo in Maluku and local swamp buffalo in Southeast Sulawesi have not been reported.

The objective this research were indentify microsatellite DNA polymorphisms, genetic markers, and estimate genetic distances among the local swamp buffalo subpopulation of Southeast Sulawesi and their comparison with three of other populations based on microsatellite DNA polymorphisms.

MATERIALS AND METHODS

A total of 70 blood smples of local swamp buffalo from differents subpopulation in Indonesia. A total of blood samples based on swamp buffalo populations were used in this research can be see in Table 1.

Table 1. Total of blood samples based on buffalo populations

No.	Populations (Province)	Sample code	Total
1	Bombana land area (Southeast Sulawesi 1)	BD	10*
2	Bombana island area (Southeast Sulawesi 2)	BK	10*
3	Kolaka (Southeast Sulawesi 3)	KL	10*
4	Konawe (Southeast Sulawesi 4)	KN	10*
5	Toraja (South Sulawesi)	TR	10*
6	Sumbawa (Southeast West Nusa)	NTB	10**
7	Lebak (Banten)	BTN	10**
	Total		70

Note: *Blood samples were collected by proposer in 2016.
**Blood samples were collected by team of Kerjasama Kemitraan Penelitian Pertanian dengan Perguruan Tinggi (KKP3T) in 2007.

Blood samples were colected from the jugular vein as much 5 ml in a vacutainer tube which containing EDTA. Microsallite marker were used include CSSM047, ILSTS011, and BM1706 locus. Information of the three microsatellite primer to use in the research were presented in Table 2.

Table 2. The three microsatellite primers that were used in the research

Locus	Chro-mosome position	Sequen primer (5'- 3')	Acces code *GenBank* /References	Size (bp)	Repetition motive
CSSM047	3q(8)	F: TCTCTGTCTCTATCACTATATGGC R: CTGGGCACCTGAAACTATCATCAT	U03821	127-162	(TG)12. TA.TG. TA(TG)4
ILSTS011	-	F: GCTTG CTACATGGAAAGTGC R: CTAAAATGCAGAGCCCTACC	Tantia *et al.* (2006)	264-272	-
BM1706	-	F: ACAGGACGGTTTCTCCTTATG R: CTTGCAGTTTCCCATACAAGG	Coulson *et.al.* (1998)	211-271	-

Note: F : Forward; R : Reverse

Microsatelitte markers were selected from the panel of standardized markers recommended for buffaloes by the Molecular Genetic Characterization of Animal Resources (FAO 2011), and based on the level of polymorphism that are adapted from various international journals. DNA extraction, amplification and electrophoresis were conducted in the Molecular Genetic Laboratory in Faculty of Animal Science, Bogor Agricultural University. While DNA fragment analysis by services of Malaysian Genome Institute. The procedure of DNA extration by using the standard phenol-chloroform extraction protocol, was modified from Sambrook & Russel (2001). DNA samples were amplified by The Polymerase Chain Reaction Technique (PCR), in accord with Zachos *et al.* (2003). DNA amplification method accord with Schuelke (2000). The forward primer was merger among a sequence of forwarding primer in Table 2 with a sequence of universal M13 (-21) (TGTAAAACGACGGCCAGT). PCR products were labeled by a primer of FAM-M13 (-21) universal (FAM-TGTAAAACGACGGCCAGT-3′). PCR reaction was conducted in a 25 µL reaction mixture, included 50 ng/µL of DNA template, 25 pmol of each primer (IDT, Singapore), 1 unit Go Taq® Green Master Mix (Promega, Madison, WI, USA), and water. The PCR qit from Qiagen product (Q5® Hot Start High-Fidelity Polymerase) was used for DNA sample containing heparin. PCR reaction was conducted in a 25 µL reaction mixture, included 50 ng/µL of DNA template, 25 pmol of each primer (IDT, Singapore), buffer, enhancer, dNTP, Taq polymerase and water which the PCR grade was 10 mM.

PCR reaction was conducted in the two-step. The first step for 30 cycles which PCR reaction conditions of predenaturation at 94 ^{o}C for 5 min, denaturation at 94 ^{o}C for 10 sec, annealing at 52 to 55 ^{o}C for 20 sec, and extension at 72 ^{o}C for 30 sec. The second step for 8 cycles which PCR reaction conditions of denaturation at 94 ^{o}C for 10 sec, annealing at 53 ^{o}C for 20 sec, extension at 72 ^{o}C for 30 sec, and the final extension at 72 ^{o}C for 5 min. DNA amplification by used a thermocycler machine (GeneAmp® PCR System 9700, Applied Bio SystemTM, Foster City, CA, USA). The PCR product (amplicon) was verified by electrophoresis method in 1.5% agarose gel (Sambrook & Russell 2001). Band pattern of each PCR product was visualized by UV train simulator (Alpha Imager®EP).

Data analysis include of allele and genotype frequencies (Nei, 1987; Nei & Kumar 2000), total of allele effective (Hedrick, 2001), Hardy-Weinberg Equilibrium (Nei & Kumar, 2000), fixation Index (Nei, 1987), observed and expected heterozygosity (Hartl 1988), Polymorphism Information Content (Mateescu *et al.* 2005), and identical and genetic distances (Nei, 1987). Data of allele frequencies, genotype frequencies, total of allele (Na), total of effective alleles (Ne), Hardy-Weinberg Equilibrium, observed heterozygosity (Ho), Expected Heterozygosity (He), fixation index (Fis, Fit, Fst), and Polymorphism Information Content (PIC) were analyzed by GENEPOP version 3.2 (Raymond & Rousset 2001). While identical and genetic distances were analyzed based on the Kimura 2-Parameter, and the reconstruction of phylogenetic trees was conducted by Neibour-joining method with bootstraps 1000 repetitions in software of MEGA version 6 (Tamura *et al*. 2013).

RESULTS AND DISCUSSION

Amplification of Microsatellite DNA

Three-locus of buffalo microsatellite DNA (CSSM047, ILSTS011, and BM1706) success was amplificated with Polymerase Chain Reaction Technique (PCR). Size of microsatellite DNA fragment was amplificated in the locus of CSSM047, ILSTS011, and BM1706 in the research ranges from 132-168 bp, 264-272 bp, and 211-271 bp respectively. Visualization of PCR product band pattern in 1.5% agarose gel electrophoresis of tree locus of buffalo DNA microsatellite in the study was presented in figure 1 to 3.

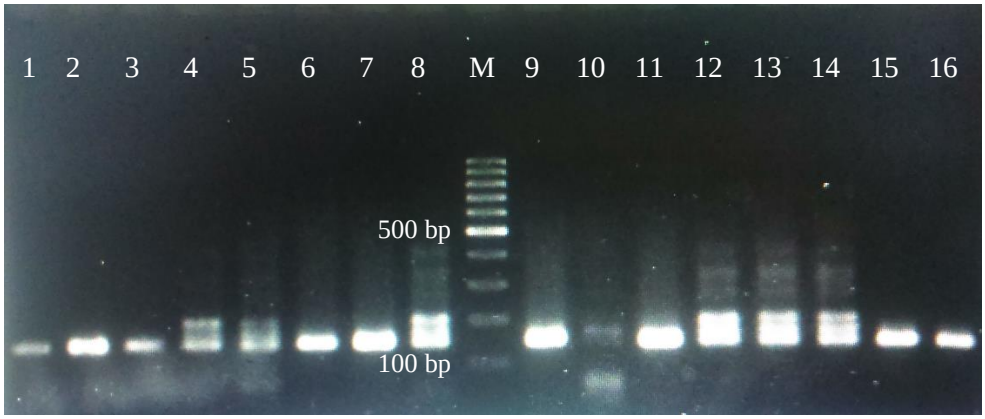

Figure 1. Visualization of PCR product band pattern in 1.5% agaros gel electrophoresis of CSSM047 locus. M: Marker; 1-16: samples.

Figure 1 to 3 showed that PCR product samples for sample 1 to 16 success were amplified. All samples of PCR product has single and double band pattern. While the example of DNA microsatellite genotyping of CSSM047 locus has shown in figure 4.

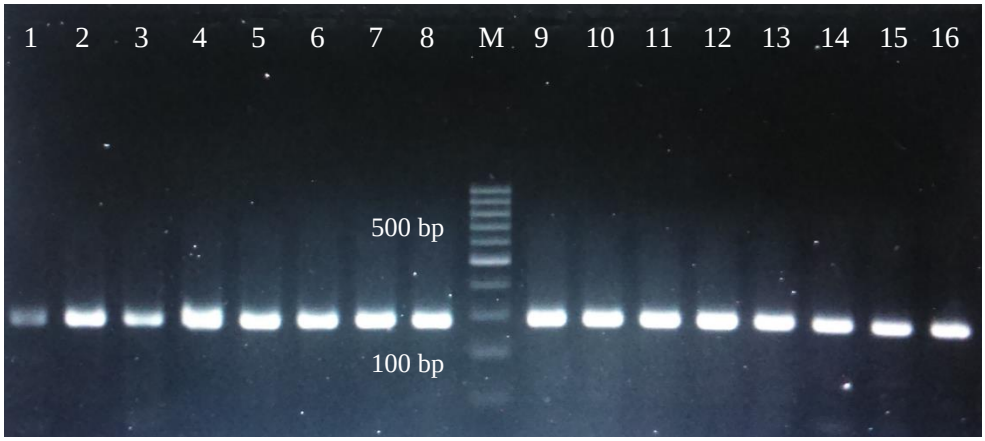

Figure 2. Visualization of PCR product band pattern in 1.5% agaros gel electrophoresis of ILSTS011 locus. M: Marker; 1-16: samples.

Allele and Genotype Frequencies

Allele frequencies are one of the genetic parameters that are can descriptions of the genetic status of the animal population. Genetic diversity information is major attention in the conservation program of animal genetic resource. Allele frequencies of CSSM047 locus base swamp buffalo population were shown in Table 3. The result of the study in the Table 3 showed there were 10 alleles in CSSM047 locus that were alleles A, B, C, D, E, F, G, H, I, and J allele. AlleleS size range were 132 to 168 bp, which alleles frequencies range were 0,000 to 0,5000. From of 10 alleles that found there were 8 alleles CSSM047 locus of South East Sulawesi swamp buffalo, that were alleles of A, B, C, D, E, F, G, and J. While genotypes frequencies

were shown in Table 4. There were 15 genotypes were found in CSSM047 locus, that were genotypes AJ, BB, BD, BF, CC, CD, CHIJ, CJ, DD, DF, DG, DI, DJ, EE, and JJ.

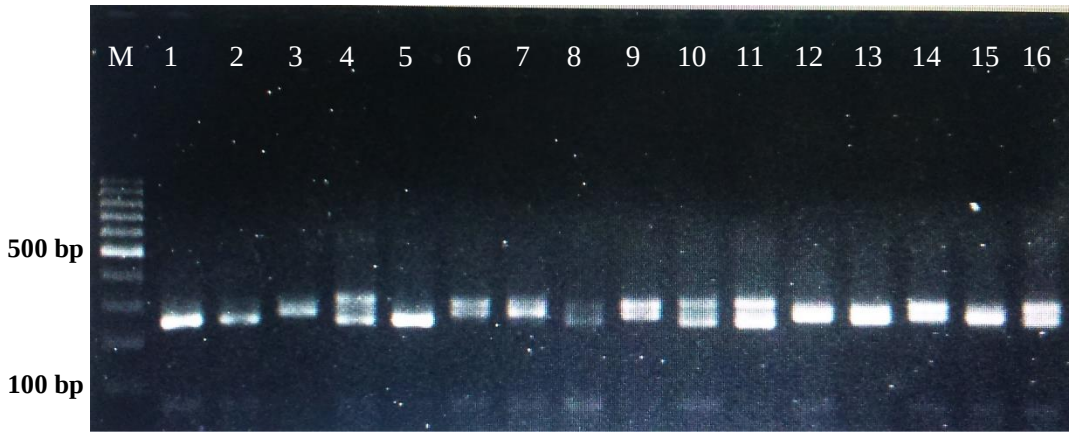

Figure 3. Visualization of PCR product band pattern in 1.5% agaros gel electrophoresis of BM1706 locus. M: Marker; 1-16: samples.

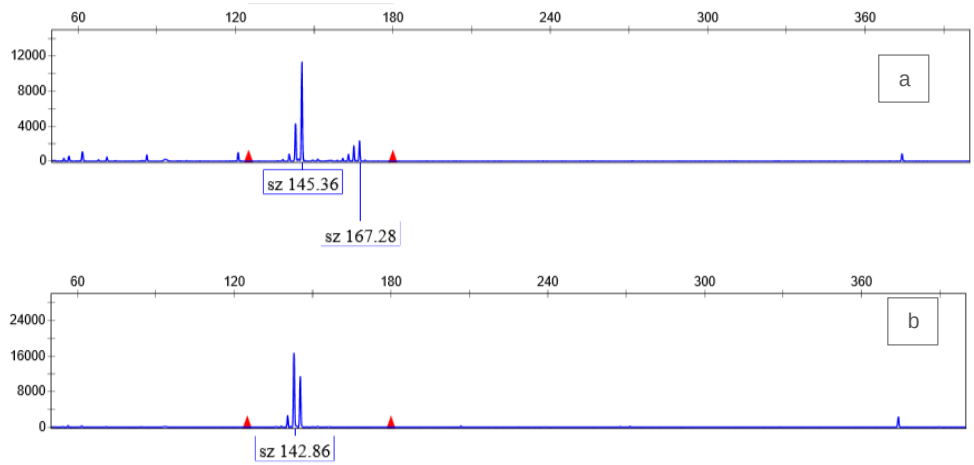

Figure 4. The example of DNA microsatellite genotyping of CSSM047 locus. (a): 146/168 heterozygote genotype; (b); 142/142 homozygote genotype.

Genotype frequencies in swamp buffalo in CSSM047 locus in this reserach range were 0,000 to 0,400. Southeast Sulawesi local swamp buffalo have 11 genotypes that were AJ genotype (Konawe = 0.100), BB (Bombana island area = 0.200), BD (Bombana island area = 0.100), CC for population of Bombana island area, Bombana land area, Kolaka and Konawe, wich genotype frequencies was 0.100 for all population. Then CJ genotype for Konawe population (genotype frequencies was 0.100), and DD genotype for the population of Bombana island area, Bombana land area, Kolaka, and Konawe. Their genotypes frequencies were 0.100; 0.200; 0.400 and 0.300, respectively. Then DF genotype for Konawe population (genotype

frequencies was 0.100), DG genotype for Bombana land area population (genotype frequencies was 0.100), DJ genotype for population of Bombana island area, Bombana land area, Kolaka and Konawe, wich genotypes frequencies were 0.300 for all populations, and then EE genotype for Bombana island area population (genotype frequencies was 0.100), and JJ genotype for Bombana land area and Kolaka population. Their genotypes frequencies were 0.300 and 0.100, respectively.

Table 3. Allele frequencies of CSSM047 locus base on swamp buffalo population

Allele symbol	Populatios						
	Bombana island area (n=10)	Bombana land area (n=10)	Kolaka (n=10)	Konawe (n=10)	Toraja (n=10)	NTB (n=10)	Banten (n=10)
132 (A)	0.000	0.000	0.000	0.100	0.000	0.000	0.000
142 (B)	0.250	0.000	0.000	0.000	0.000	0.100	0.000
144 (C)	0.150	0.100	0.200	0.200	0.300	0.150	0.300
146 (D)	0.300	0.400	0.500	0.400	0.400	0.400	0.500
148 (E)	0.100	0.000	0.000	0.000	0.000	0.000	0.000
150 (F)	0.000	0.000	0.000	0.100	0.000	0.150	0.000
152 (G)	0.000	0.100	0.000	0.000	0.000	0.000	0.000
164 (H)	0.000	0.000	0.000	0.000	0.100	0.000	0.000
166 (I)	0.000	0.000	0.000	0.000	0.000	0.100	0.000
168 (J)	0.300	0.450	0.250	0.250	0.150	0.350	0.100

Table 4. Genotype frequencies of CSSM047 lokus base on swamp buffalo population

Genotype symbol	Populations						
	Bombana islan area (n=10)	Bombana land area (n=10)	Kolaka (n=10)	Konawe (n=10)	Toraja (n=10)	NTB (n=10)	Banten (n=10)
AJ	0.000	0.000	0.000	0.100	0.000	0.000	0.000
BB	0.200	0.000	0.000	0.000	0.000	0.000	0.000
BD	0.100	0.000	0.000	0.000	0.000	0.000	0.000
BF	0.000	0.000	0.000	0.000	0.000	0.100	0.000
CC	0.100	0.100	0.100	0.100	0.200	0.100	0.200
CD	0.100	0.000	0.200	0.100	0.100	0.100	0.200
CHIJ	0.000	0.000	0.000	0.000	0.100	0.000	0.000
CJ	0.000	0.000	0.000	0.100	0.000	0.000	0.000
DD	0.100	0.200	0.400	0.300	0.500	0.100	0.500
DF	0.000	0.000	0.000	0.100	0.000	0.100	0.000
DG	0.000	0.100	0.000	0.000	0.000	0.000	0.000
DI	0.000	0.000	0.000	0.000	0.000	0.100	0.000
DJ	0.300	0.300	0.200	0.200	0.100	0.300	0.100
EE	0.100	0.000	0.000	0.000	0.000	0.000	0.000
JJ	0.000	0.300	0.100	0.000	0.000	0.100	0.000

CONCLUSION

The result of this research was conclusion there were 10 alleles and 15 genotypes of CSSM047 locus in Indonesian local swamp buffaloes. Southeast Sulawesi local swamp buffalo has genetic diversity was hinger. They have 8 alleles from 10 alleles were found in the research include alleles A, B, C, D, E, F, G, and J, wich their alelles frequencies range were 0.000 – 0.500 , and they have 11 genotypes from 15 genotypes was found, include genotypes AJ, BB, BD, CC, CJ, DD, DF, DG, adn DJ, wich their alelles frequencies range were 0.000 – 0.500, and genotypes frequencies range were 0.000 – 0.400.

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