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Genetic Variation of mtDNA Cytochrome Oxidase Subunit I (COI) in Local Swamp Buffaloes in Indonesia

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ABSTRACT

The objective of this research was to identify genetic variation of mitochondria DNA especially in cytochrome oxidase subunit I (COI) among population of Indonesian buffaloes. Samples of swamp buffaloes were collected from Aceh (n= 3), North Sumatra (n= 3), Riau (n= 3), Banten (n= 3), Central Java (n= 3), West Nusa Tenggara (n= 3) and South Sulawesi (n= 3), and riverine buffalo from North Sumatra (n= 1) out of group for comparison. Sequence of COI was analyzed using MEGA 5.10 software with neighbor-joining method kimura 2-parameter model to reconstruct phylogeny tree. The result showed that three haplotypes for swamp buffalo and one haplotype for riverine buffalo in Indonesia resulted from 41 polymorphic sites. This finding showed that the COI gene could be considered as a marker to distinguish among swamp buffaloes in Indonesia.

Key words: COI, filogeny, genetic variation, Indonesian buffaloes

ABSTRAK

Tujuan penelitian ini adalah untuk mengidentifikasi keragaman DNA mitokondria, khususnya pada daerah *cytochrome oxidase* subunit I (COI) pada delapan populasi kerbau lokal Indonesia. Sampel kerbau lumpur berasal dari Aceh (n= 3), Sumatra Utara (n= 3), Riau (n= 3), Banten (n= 3), Jawa Tengah (n= 3), Nusa Tenggara Barat (n= 3) dan Sulawesi Selatan (n= 3); dan sebagai pembanding digunakan kerbau sungai yang berasal dari Sumatra Utara (n= 1). Sekuen gen COI dianalisis menggunakan program MEGA 5.10 *software* dengan metoda *neighbor-joining* substitusi kimura 2 parameter untuk merekonstruksi pohon filogeni. Hasil penelitian menunjukkan adanya 41 situs polymorfime dan dapat dikelompokkan kedalam tiga haplotipe untuk kerbau lumpur dan satu haplotipe untuk kerbau sungai. Gen COI dapat dipakai sebagai penciri genetik untuk membedakan antar populasi kerbau di Indonesia.

Kata kunci: COI, filogeni, kerbau Indonesia, variasi genetik

INTRODUCTION

Indonesian animal genetic resources for buffaloes consist of swamp buffalo, riverine buffalo, spotted buffalo (toraja buffalo) and kalang buffalo (borneo buffalo) (Director General of Livestock Services, 2003). Riverine buffaloes were introduced into Indonesia from India. Spotted buffalo and kalang buffalo are Indonesian swamp buffalo breed. Indigenous buffalo in Indonesia is swamp buffalo. Characterization of buffalo breeds in Indonesia are usually just based on the phenotype information. Study of the genetic distance estimation of local swamp buffaloes through morphology analysis has been done (Anggraeni *et al*., 2011) and growth hormone genes variation (Sumantri *et al*., 2010). Genetic information is needed to assist in determining of buffalo breeds. Recent investigations have suggested mitochondrial DNA (mtDNA) as a tool for studying the taxonomy and evolution of animal populations. The cytochrome oxidase subunit I (COI) gene is a part of mtDNA. This gene was chosen because of its main role in metabolism and its presence is almost in all *eukaryotes*. Additionally, the size and structure of COI gene have been well conserved in the animal groups, a feature which makes it especially suitable for evolutionary studies (Lunt *et al*., 1996). COI is one of the most conserved mitochondrial protein-coding genes in animals (Mueller, 2006), and thus displays a better phylogeny signal (Wilson, 2010). COI has been known to be as DNA barcoding that been used very successfully in many animal. For examples, it has been differentiated species in birds (Herbert *et al*., 2004), chicken (Gao *et al*., 2011), cattle (Syed-Shabthar *et al*., 2013),

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Cricula trifenestrata (Suriana *et al*., 2012), lepidoptera (Wilson, 2010), beetles (Funk *et al*., 1995), some insect pests (Toda & Murai, 2006) *Mermerodes hamona* moth (Hulrc *et al*., 2007), mosquito (Cywinska *et al*., 2006), and *Thrips tabaci* (Karimi *et al*., 2010).

COI variations between bird species averaged 7.93%, whereas variation within species averaged 0.43% (Herbert *et al*. 2004). Genetic diversity of COI gene in Chinese chicken breed found seven haplotypes (Gao *et al*., 2011). Genetic diversity of COI gene in Indonesian buffalo has not been explored yet. This study was therefore aimed to identify genetic variation of mitochondria DNA especially in cytochrome oxidase subunit I (COI) among population of local swamp buffaloes in Indonesia.

MATERIALS AND METHODS

DNA Materials

Samples of blood swamp buffaloes were collected from Aceh (n= 3), North Sumatra (n= 3), Riau (n= 3), Banten (n= 3), Central Java (n= 3), West Nusa Tenggara (n= 3) and South Sulawesi (n= 3); and riverine buffalo from North Sumatra (n= 1) out of group for comparison. All samples were stored in vacutainer tube with EDTA. DNA extraction was performed by using phenol chloroform method (Sambrook & Russel, 2001) and modified by Andreas *et al*. (2010), with the following procedure:

Sample preparation. The blood in the alcohol was as much as 200 µL. Sample was inserted to a 1.5 mL tube. Alcohol was eliminated from the sample by adding distilled water until 1000 µL, and left in room temperature for 20 min. Then it was precipitated by centrifugation at a speed of 8000 rpm for 5 min.

Protein degradation. The samples were cleared from alcohol and added by 200 µL 1x STE (sodium tris EDTA), 40 µL sodium dosesil sulfate 10%, and 20 µL proteinase K (5 mg/mL). The mixture ware incubated overnight at 55 °C temperature while shaken gently.

Organic material degradation. After incubated, samples were added by 400 µL phenol solution, 400 µL choloform: isoamyl alcohol (24:1), and 40 µL 5M NaCl. Then, the mixture was shaken at room temperature for one hour.

DNA precipitation. Samples were centrifuged at a speed of 5000 rpm for 10 min to separate the water phase with phenol phase. Water phase was transferred in a new tube with the volume measured. DNA molecules deposited by adding a 2x volume of alcohol absolute and 0.1 x volume of 5M NaCl. Then the mixture was incubated at a temperature of -20 °C over night. Subsequent DNA precipitation was by centrifugated at a speed of 12000 rpm for 10 min. Obtained DNA precipitate was washed by 70% alcohol, and then precipitated again. Precipitated DNA clean from alcohol restored by adding 100 µL TE (Tris-EDTA). DNA samples were stored at -20 °C and ready for use.

PCR Amplification and Sequencing

Two pair of primer was designed by using Primer-Blast (http://www.ncbi.nlm.nih.gov/tools/primer-blast/) with reference of genbank accession numbers of NC_020615, EF536351, AY488491, AF547270, NC_006295 and AY702618 (Table 1). PCR amplification was carried out with 35 µL PCR reaction containing of 50-100 ng sample DNA, 10 pmol Primer (Alpha DNA), 10 mM dNTPs (Fermentas), 25 mM $MgCl_2$ (Fermentas), 10xBuffer (Fermentas), and 2.5 U Taq Polymerase (Fermentas). The condition of thermal cycling consisted of pradenaturation at 95 °C for 5 min, followed by 35 cycles of denaturation 95 °C for 40 s, annealing 60 °C for 45 s, and extension 72 °C for 1 min. The final extension step was at 72 °C for 5 min. Amplification was carried out in a thermal cycler (*Geneamp* 9700, AB System). PCR amplicons were visualized on 1.5% agarose gels in 0.5 x TBE buffer containing 10% ethidium bromide at 100 volt for 45 min and visualized by UV transiluminator.

Sequencing analysis was carried out by sending PCR amplicon samples to a commercial service (1st BASE, Malaysia). Sequences were analyzed using MEGA 5.10 software (Tamura *et al*., 2011). Method of neighbor-joining with kimura 2-parameter model was applied for reconstructing phylogeny tree.

RESULTS AND DISCUSSION

DNA Amplification

COI gene of the riverine buffalo and swamp buffalo has 1545 bp in length (according genbank accession number AF547270 and NC_006295). Primer FS2 amplified at position 6364 in the Cytochrome Oxidase Subunit I (COI) to position 7411 in the $tRNA^{Ser}$ (according genbank accession number AF547270) with fragment prediction of 1047 bp in length (Figure 1). Primer FS3 amplified at position 5948 to position 6729 in the COI

Table 1. Overlapping primer for COI gene

Primer	Sequence (5′-3′)	Size	Position*	Name of gene
FS2	F: CAG CGG GGG GAG GAG ATC CTA TTC TAT ACC	1047 bp	6364-6393	COI
	R: GCC TAG TTG TAT AGG GTA TGC CAT ATG AGA		7383-7411	$tRNA^{Ser}$
FS3	F: TCC CTC TAA TAA TTG GCG CTC CCG	781 bp	5948-5971	COI
	R: GCC TAG GGC TCA CAT TAT AGC GGG		6706-6729	COI

Note: * according Genbank accession number AF547270

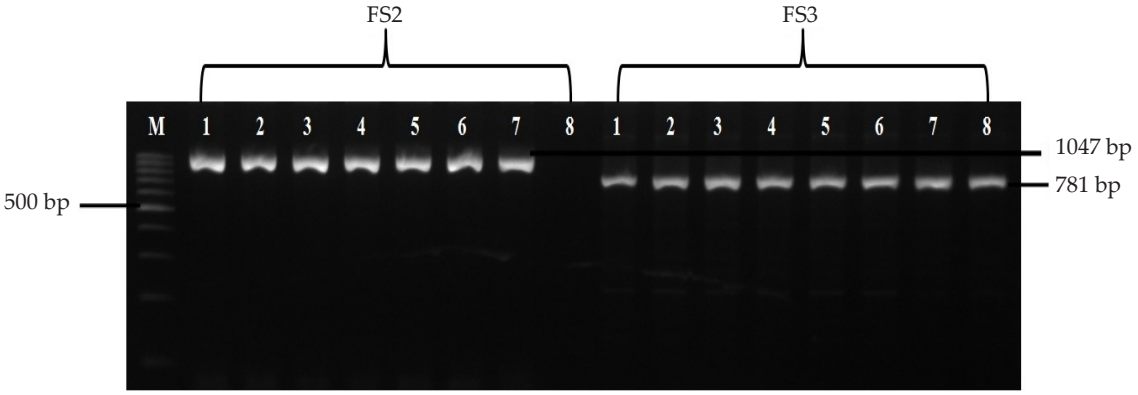

Figure 1. Cytochrome oxidase subunit I gene PCR product amplified from samples. M= 100 bp ladder size standard; 1= North Sumatra (river buffalo); 2= North Sumatra (swamp buffalo); 3= Riau (swamp buffalo); 4= Banten (swamp buffalo); 5= Central Java (swamp buffalo); 6= West Nusa Tenggara (swamp buffalo); 7= South Sulawesi (swamp buffalo); and 8= Aceh (swamp buffalo) (FS2 & FS3= name of primer).

Table 2. Nucleotide composition of sequences of seven population of Indonesian local buffaloes and Genbank accession number

Sample	%						Total
	T(U)	C	A	G	T+A	C+G	
NC 020615.1|*Bubalus depressicornis*	29.4	26.0	27.6	17.0	57.0	44.6	1243
EF536351.1|*Bubalus depressicornis*	29.4	26.0	27.6	17.0	57.0	44.6	1243
AY488491.1|Riverine Buffalo	29.6	25.7	28.0	16.7	57.6	42.4	1243
AF547270.1|Riverine Buffalo	30.1	25.3	27.9	16.7	58.0	42.0	1243
Riverine Buffalo North Sumatra 8	29.6	25.7	28.0	16.7	57.6	42.4	1243
NC 006295.1|Swamp Buffalo	29.2	26.1	28.2	16.5	57.4	42.6	1243
AY702618.1|Swamp Buffalo breed Haikou	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo Aceh 7	29.1	26.4	27.6	16.9	56.7	43.3	711
Swamp Buffalo Aceh 8	29.1	26.4	27.6	16.9	56.7	43.3	711
Swamp Buffalo Aceh 73	29.1	26.4	27.6	16.9	56.7	43.3	711
Swamp Buffalo North Sumatra 16	29.0	26.4	28.2	16.4	57.2	42.8	1243
Swamp Buffalo North Sumatra 21	29.0	26.4	28.0	16.7	57.0	43.0	1243
Swamp Buffalo North Sumatra 26	29.2	26.1	28.2	16.5	57.2	42.8	1243
Swamp Buffalo Riau 14	29.0	26.4	28.2	16.4	57.0	43.0	1243
Swamp Buffalo Riau 26	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo Riau 30	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo Banten 7	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo Banten 41	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo Banten 50	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo Central Java 4	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo Central Java 20	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo Central Java 16	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo West Nusa Tenggara 7	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo West Nusa Tenggara 13	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo West Nusa Tenggara 23	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo South Sulawesi 102	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo South Sulawesi 103	29.2	26.1	28.2	16.5	57.4	42.6	1243
Swamp Buffalo South Sulawesi 117	29.2	26.1	28.2	16.5	57.4	42.6	1243

Table 3. Haplotype and polymorphic site

H	Nucleotide position* (bp)																																							
	336	348	354	373	375	378	382	384	398	402	410	415	421	423	456	458	475	510	546	555	603	618	663	717	738	750	800	804	847	876	930	1032	1038	1083	1131	1197	1401	1413	1422	1452	1515
H1	C	C	T	G	T	G	G	A	C	T	C	G	G	T	G	C	T	C	C	A	A	C	T	G	T	A	T	A	C	C	C	T	C	C	C	G	A	C	A	C	T
H2	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	-	-	-	-	-	-	-	-	-	-
H3	.	.	G	C	G	.	C	T	G	C	G	C	C	.	.	G	.	.	.	.	.	T	C	.	.	.	.	G	T	.	.	C	.	.	.	.	.	.	.	.	.
H4	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	C	.	.	.	.	C
H5	T	T	.	.	.	A	.	.	.	.	.	.	.	C	A	.	C	T	T	G	G	T	.	A	C	G	C	.	T	T	T	C	T	T	T	.	G	T	G	.	C

Note: *according buffalo COI gene (H is haplotype)

(according genbank accession number AF547270) with fragment prediction of 781 bp in length (Figure 1). Aceh samples not amplified using primer FS2 but amplified using primer FS3 (Figure 1).

Sequence Variation and Haplotype Sharing

Nucleotide composition of sequences (Table 2) showed a slight difference in the nucleotide composition of the sample. It is especially in Swamp Buffalo North Sumatra 16, Swamp Buffalo North Sumatra 21 and Swamp Buffalo Riau 14. Genetic variation at mitochondrial D-loop region in Egyptian river buffalo showed 28 different haplotypes resulting from 77 polymorphic sites (Hassan *et al*., 2009). In this research at mitochondrial Cytochrome Oxidase subunit I (COI) showed four haplotypes for local swamp buffaloes in Indonesia resulted from 41 polymorphic sites (Table 3).

The first haplotype consisted of Banten Samples, South Sulawesi Samples, West Nusa Tenggara Samples, Central Java Samples, Riau 30, Riau 26, North Sumatra 26, NC_006295.1 and AY702618.1. The second haplotype consisted of Aceh samples. The third haplotype was North Sumatra 21. The fourth haplotype consisted of North Sumatra 16 and Riau 14. The fifth haplotype consisted of North Sumatra 8 (River Buffalo) and AF547270 (River Buffalo). A unique sequence of North Sumatra 21 (Swamp buffalo) has many nucleotide differences (Table 3). As a result, the sample is separated from the swamp buffalo group (Figure 2).

Genetic variation was found in swamp Buffalo population in Aceh (with blue highlight color) at position 555 bp in cytochrome oxidase subunit I (Figure 2). Nucleotide at positition 555 bp encoded with thymine (T) called haplotype 2. This haplotype only found in Swamp Buffalo population in Aceh (Figure 2 and Table 3). Swamp Buffalo in Aceh is possibility of indigenous buffalo in Indonesia although only one nucleotide difference. This requires further research to prove this hypothesis. Phylogeny tree (Figure 3) showed no genetic

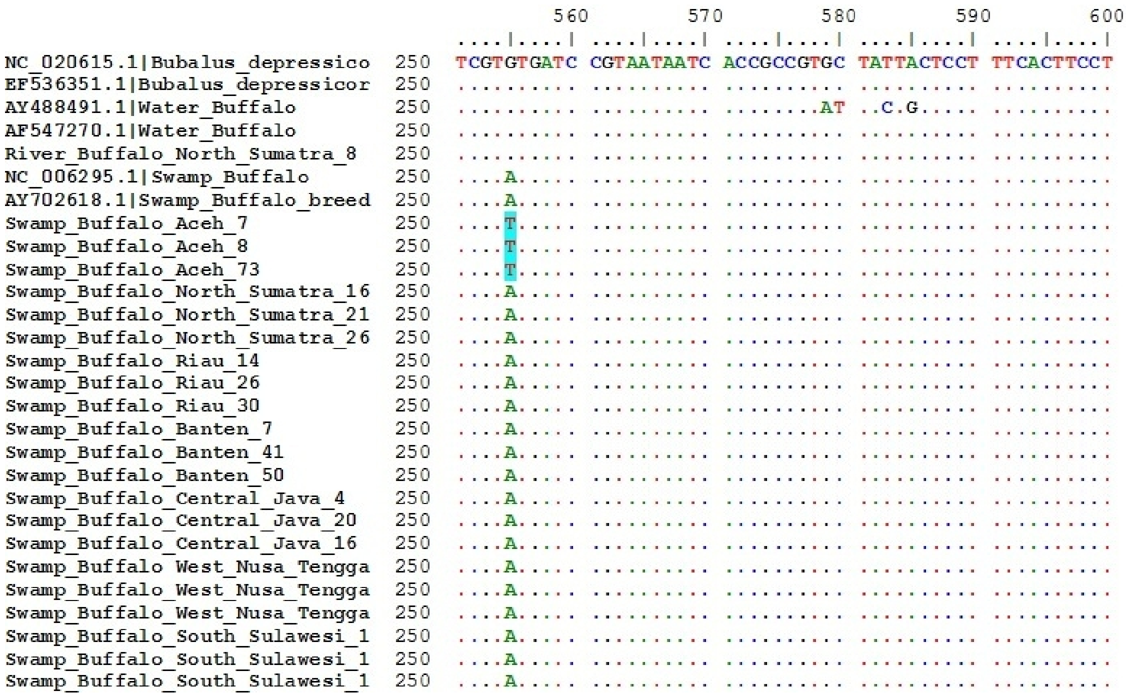

Figure 2. Sequence of cytochrome oxidase subunit I gene

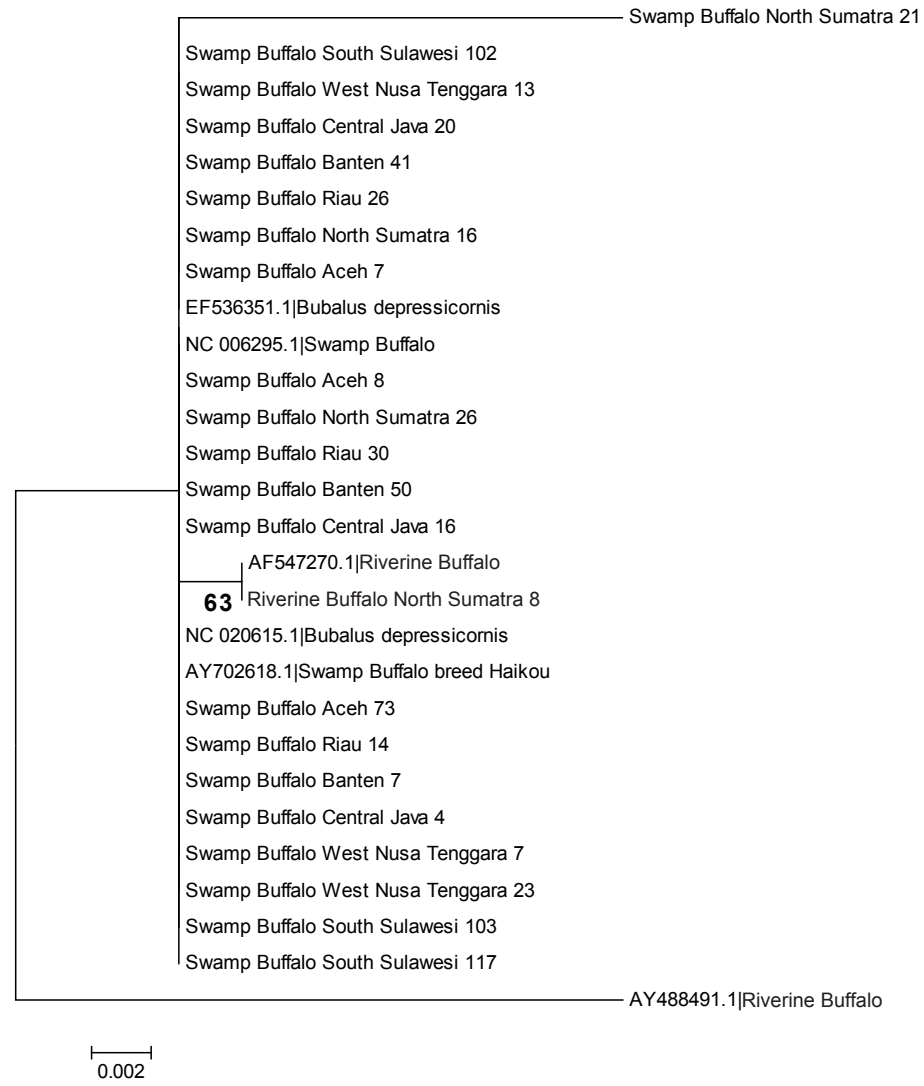

Figure 3. The phenogram tree according to cytochrome oxidase subunit I of eight population of Indonesian local buffaloes

differences between Indonesian swamp buffaloes with Chinese swamp buffaloes. This finding suggests those Indonesian swamp buffaloes are very closely related to swamp buffaloes in China. This present result supports that swamp buffaloes are the result of buffalo domestication in China (Kumar *et al*., 2007). Historical data of local swamp buffaloes in Indonesia are not available, so that it cannot explain the genetic relationship between the buffaloes in Aceh, Banten, Central Java, West Nusa Tenggara and South Sulawesi with swamp buffalo in China. Genetic variation at mitochondrial DNA D-loop and cytohrome b sequence showed eight haplotypes for D-loop mitochondria in Bogor and South Sulawesi and one haplotype for cytochome b in Bogor and South Sulawesi (Lau *et al*., 1998). This results support the following hypothesis domestication of swamp buffalo in china spread with rice cultivation through two separate routes (a) through Taiwan, to the Philippines and to the eastern islands of Borneo and Sulawesi, (b) south through mainland south-east Asia (likely interbreeding with wild buffalo) to peninsular Malaysia and on to the western island of Sumatra and Java (Lau *et al*., 1998). Lei *et al*. (2007) found 12 haplotype of mtDNA D-loop Chinese swamp buffaloes and two maternal linages. Kierstein *et al*. (2004) found "unique group" based on mtDNA D-loop with assumpsion during migration to Indochina and South-East Asia occasional cross-breeding with wild buffaloes could have lead to those haplotypes now found in the "unique group", intermixing with the haplotypes of the founder population of domestic riverine buffaloes. Based on our results we hypothesize a "unique sequence" (Third haplotype) based on COI gene assuming the same happens with assumption of Kierstein *et al*. (2004). To clarify a "unique sequence", it is necessary to conduct more samples of local swamp buffaloes in Indonesia. Phylogeny tree shows the difference between river buffalo with swamp buffalo (Figure 3).

CONCLUSION

This research find three haplotypes in the cytochrome oxidase subunit I (COI) gene of local swamp buffaloes resulted from 41 polymorphic sites. The COI

gene could be considered as a marker to distinguish among swamp buffaloes in Indonesia. However, this study has to be validated in large buffaloes populations in order to evaluate its potential in selective breeding.

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