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Mitochondrial D-loop sequence variation among Central Javanese Duck in Indonesia

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ABSTRACT. This study was realized to determine the genetic variation of Central Javanese duck based on the D-Loop mtDNA gene. D-loop gene was amplified using PCR technique by specific primer and sequenced using dideoxy termination method with ABI automatic sequencer. ClustalW from MEGA-6.06 software program was employed for multiple alignments of nucleotide sequences. Nucleotide sequences of D-loop gene of mtDMA from the Central Javanese duck were aligned together with other Anas isolates from Genbank using ClustalW of MEGA-6.06 program. The estimation of genetic distance and phylogenetic tree construction were analyzed by Neighbor-Joining method, whereas the calculation of distance matrix was performed using Kimura 2-parameter. Multiple alignments obtained were 720 nucleotides at position 56 to 779 at the 5 'end. The results of the polymorphism analysis on D-loop sequences produced 23 haplotypes. However, this haplotype information does not represent the relationship among the geographical origins of duck with the certain duck species name. Moreover, a total number of 32 variable sites were identified. Insertions were detected in four sequences (126, 155, 771 and 779 nucleotide number). In the phylogenetic analysis, it is safe to conclude that the Central Javanese duck is closely related to *Anas platyrhynchos* and *Anas zonorynchos*.

Keywords: mtDNA, D-loop, Central Javanese duck, genetic variation.

Variação de sequências de D-Loop Mitocondrial entre Pato Central Java na Indonésia

RESUMO. Este estudo foi realizado para determinar a variação genética do pato de Java Central baseado no *D-Loop mtDNA*. A D-*Loop* foi amplificada utilizando a técnica de PCR com *primer* específico e sequenciado usando o método de terminação dideoxi com sequenciador ITB automático. ClustalW do programa software de MEGA-6.06 foi utilizado para alinhamentos de algumas sequências de nucleótidos. Sequências de nucleótidos de *D-loop* gene da *mtDMA* do pato de Java Central foram alinhados em conjunto com alguns isolados de *Anas* que foram obtidos de *Genbank* utilizando o programa *ClustalW* do programa *MEGA*-6.06. A estimativa da distância genética e a estrutura da árvore filogenética foram analisadas com o método *Neighbor-Joining*, enquanto que o cálculo da matriz de distância foi utilizado o parâmetro-2 *Kimura*. Os alinhamentos múltiplos obtidos foram 720 nucleótideos na posição 56 a 779 na extremidade 5'. As análises do polimorfismo sequencial do *D-loop* resultaram em 23 haplótipos. No entanto, esta informação haplótipo não representa a relação entre a origem geográfica de pato com o nome de determinadas espécies do pato. Além disso, num total de 32 sites favoráveis foram identificados. As inserções foram detectadas em quatro sequências (126, 155, 771 e 779 números de nucleotídeos). Na análise filogenética é seguro concluir que o pato de Java Central está estreitamente relacionada com a *Anas platyrhynchos* e *Anas zonorynchos*.

Palavras-chave: mtDNA, D-loop, javanês duck, variação genética.

Introduction

The breed conservation needs a genetic characterization to obtain an efficient management of genetic resources (Bjørnstad & Røed, 2002). Managing genetic diversity is one of the primary goals in conservation programs (Toro, Barragan, & Ovillo, 2003). Molecular analysis provides a reliable tool which can be used together with the quantitative approach and traditional breeding strategies for na efficient design of preservation strategy (Dovc, Kavar, Solkner, & Achmann, 2006). Genetic distances can also be used to determine the population structure and genetic distinctiveness of a population or breed (MacHugh, Loftus, Cunningham, & Bradley, 1998).

Local ducks in Indonesia are distributed over a wide geographical range. They are reared by communal farmers across the country under extensive production systems. Within households,

different age groups are raised as one flock. The collective ownership of the scavenging feed resources results in mixing of flocks from different households within communities. Although on average every household owns a cock, mixing with duck results in sharing of cocks among neighboring flocks. As a result, contiguous villages within districts would more or less constitute one breeding flock. On the other hand, because of the large geographic area of Indonesia and the environmental differences, genetic variation is expected among domestic ducks from different agro-ecological zones. On the other hand, domestic waterfowl is the favorite protein source by Indonesian people. Duck meat and egg have the typical nutrient content and taste at affordable prices (Matitaputy, 2014). However, there is a depopulation policy by mass extermination for waterfowls and poultry declared by the Indonesian government when there is an evidence of avian influenza (AI) virus outbreak although the identification of AI virus outbreak has not been developed well in Indonesia (Rahardjo, 2014). In addition to economic loss, the policy is also biologically detrimental because of its potential in annihilating the breed germplasm, is the wealth of breeds. Based on those explanations, the aim of genetic determination plays a crucial role in the germplasm conservation.

Phylogenetic or population genetic studies have been focused on finding genetic variations in mitochondrial DNA (mtDNA) because mutations in this region contained five times higher than other genetic materials (Mannen et al., 2004; Pfeiffer, Voelkel, & Brenig, 2005). The triple stranded noncoding region of mtDNA with the high variation is called the displacement loop (D-loop) (Hill et al., 2007; Krause et al., 2010). Animal mtDNA is deemed to follow maternal inheritance strictly and is highly variable within a species. By this reason, mtDNA is an important material for phylogenetic inference and for analyzing genetic diversity (Wolf, Rentsch, & Hubner, 1999). Due to its strict maternal inheritance, individuals within a maternal family line should share the same mtDNA haplotypes, thereby allowing an evaluation of maternal line assignment accuracy (Wan, Wu, Fujihara, & Fang, 2004). Several investigations have shown that using two or more mtDNA markers might be more robust and powerful for genetic diversity analysis (Pedrosa et al., 2005). Within the framework of breed conservation, genetic characterization acts an important aspect of maintaining breed integrity and managing genetic resources (Glowatzki-Mullis et al., 2006). According to genetic structures and segregation patterns of mtDNA following material inheritance systems, the analysis of mtDNA can be used for tracing back the origins of breeds as well as identifying individual animals. Formation of mtDNA, which showed non-recombining patterns in nature of animals, revealed as a closed circular double helix DNA sizing approximately 16,500 bp encoding 13 hydrophobic polypeptides, 22 tRNAs, and 2 rRNAs (Brown, 1980; Anderson et al., 1981).

In general, searching genetic variants in mtDNA has been focused on the D-loop region that was mainly used to analyze genetic distances among breeds due to huge mutations (Christopher et al., 2001; Pfeiffer et al., 2005). Genes in mtDNA are also available to characterize breeds and individuals in phylogenetic studies (Hassanin & Douzery, 1999; Cai et al., 2007). However, only a few reports are available for SNPs from the coding regions because studies believed that SNPs in coding regions might not be available to explain genetic diversity with low frequencies of genetic variations. It is still, however, required that uses of the whole mtDNA sequences are needed to estimate the genetic relationships among breeds, to characterize the breed specificity, and to identify individuals.

Purity and uniqueness of each central Javanese duck are part of the germplasm. Conservation strategies difficult to determine because generally ducks reared for this comes from the seeds of unknown origins and do not have any genetic genealogy records (Purwantini, Yuwanta, Hartatik, & Ismoyowati, 2013). Identification of the molecular can be used as genetic markers to reveal the difference intraspecies, phylogeography and know the relationship between clumps that can be used to study the genetic diversity (Sulandari, Zein, Paryanti, & Sartika, 2007). This study aims to determine the genetic variation (haplotype, genetic distance, polymorphic sites) of Central Javanese duck based on the D-Loop mtDNA gene. In the framework of this study, mtDNA of the seven Central Javanese duck breeds was analyzed to provide a genetically based tool for effective conservation programs.

Material and methods

Samples in this research were 35 local ducks. Those local ducks consisted of seven types of javanese ducks in Central Java, namely Magelang duck, Peking duck, Pengging duck, Tegal Branjangan duck, Tegal Jarakan duck, Tegal Blorong duck, and Tegal Lemahan duck. Samples of duck feather with follicle was taken from the right and left wings. Feather samples used at the time of isolation only in the calamus and the rachis about 4-5 cm which is filled with marrow and had a lot of networks. DNA Isolation used gSYNC™ DNA Extraction Kit.

D-loop gene amplification was performed using KAPA 2G HotStart Ready Mix Kit with the

composition of the 12.5ul ready mix; 1.5 ul of forward primer; 1.5 ul of reverse primer, 7.5 ul of ddH2O, and 2.0 ul of DNA samples. Primers used refers to Purwantini et al. (2013) using forward primer DL-AnasPF (L56) 5'-GTTGCGGGGTT ATTTGGTTA-3 'and reverse primer DL-AnasPR (H773) 5'-CCATATACGCCAACCGTCTC-3'. The PCR program was pre-denaturation 94°C for 5 minutes, denaturation 94°C for 30 seconds, annealing 56.1°C for 45 seconds, extension 72°C for 1 min., and a final extension 72°C for 5 minutes. The cycle is repeated 35 times. 1.2% agarose gel visualized the expected 718bp PCR product. And then, PCR product sequenced using dideoxy termination method with ABI automatic sequencer (Applied Biosystems) by 1st BASE Pte Ltd, Singapore via PT. Genetika Science Indonesia.

Multiple alignments of nucleotide sequences were analyzed using ClustalW of Molecular Evolutionary Genetics Analysis (MEGA) software version 6.06. The nucleotide sequence of D-loop gene was analyzed using Basic Local Alignment Search Tool (BLAST) NCBI to determine their homology with the GenBank data. All nucleotide sequence of D-loop gene of Central Javanese duck included in Genbank data. Identical sequences were considered as the same haplotype. Nucleotide sequences of D-loop gene of mtDNA from Central Javanese duck in this research together with other Anas isolates from Genbank were aligned with ClustalW of MEGA-6.06 program (Kumar, Tamura, Jakobsen, & Nei, 2001).

Estimation of genetic distance and phylogenetic tree construction were analyzed with Neighbor-Joining method and calculation of distance matrix with Kimura 2-parameter model, and the bootstrap value was 1,000. At the same time, we selected the complete sequences of *Anas platyrhynchos* and *Anas zonorhyncha* from GenBank as controls.

Results and discussion

Genomic DNA was obtained from follicle samples using gSYNC ™ DNA Extraction Kit. The good result of DNA extraction in this study is in line with Leekaew, Songserm, Choothesa and Boonyaprakob (2008) who reported that the quality of the DNA obtained using a commercial kit is better than using proteinase-K/SDS and alkali method. PCR product visualization shows the uniform band 718bp for all the samples (Figure 1). This product shows that the D-loop gene of Central Javanese duck can be amplified by primer DL-AnasPF (L56) and DL-AnasPR (H773), at nucleotide numbers 56-773. Clear and bold of the band indicates the optimal PCR conditions were achieved; therefore the PCR process can take place properly.

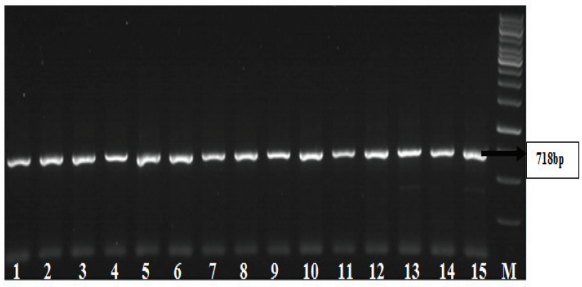

Figure 1. PCR amplicon of D-loop gene on central javanese duck DNA samples shows 718 bp in length product in 1.2% agarose gel.

The 718-bp fragment of the mtDNA D-loop region was sequenced. All nucleotide sequence of D-loop gene of Central Javanese duck included in Genbank data, with accession number in Table 1.

Table 1. Nucleotide sequence of D-loop gene of central Javanese duck.

No	Central Javanese duck (code)	Amount of nucleotide	GenBank accession no.
1	Tegal Blorong duck -1 (SSU-TB1)	720	KX756158
2	Tegal Blorong duck -2 (SSU-TB2)	719	KX756159
3	Tegal Blorong duck -3 (SSU-TB3)	675	KX756160
4	Tegal Blorong duck -4 (SSU-TB4)	721	KX756161
5	Tegal Blorong duck-5 (SSU-TB5)	721	KX756162
6	Tegal Branjangan duck-1 (SSU-Tbr1)	720	KX756163
7	Tegal Branjangan duck-2 (SSU-Tbr2)	718	KX756164
8	Tegal Branjangan duck-3 (SSU-Tbr3)	720	KX756165
9	Tegal Branjangan duck-4 (SSU-Tbr4)	721	KX756166
10	Tegal Branjangan duck-5 (SSU-Tbr5)	720	KX756167
11	Tegal Jarakan duck-1 (SSU-TJ1)	720	KX756168
12	Tegal Jarakan duck-2 (SSU-TJ1)	720	KX756169
13	Tegal Jarakan duck-3 (SSU-TJ1)	720	KX756170
14	Tegal Jarakan duck-4 (SSU-TJ1)	720	KX756171
15	Tegal Jarakan duck-5 (SSU-TJ1)	720	KX756172
16	Tegal lemahan duck-1 (SSU-TL1)	677	KX756173
17	Tegal lemahan duck-2 (SSU-TL2)	720	KX756174
18	Tegal lemahan duck-3 (SSU-TL3)	721	KX756175
19	Tegal lemahan duck-4 (SSU-TL4)	721	KX756176
20	Tegal lemahan duck-5 (SSU-TL5)	721	KX756177
21	Magelang duck-1 (SSU-M1)	721	KX712252
22	Magelang duck-2 (SSU-M2)	720	KX712253
23	Magelang duck-3 (SSU-M3)	720	KX712254
24	Magelang duck-4 (SSU-M4)	720	KX712255
25	Magelang duck-5 (SSU-M5)	721	KX712256
26	Peking duck-1 (SSU-PK1)	720	KX712257
27	Peking duck-2 (SSU-PK2)	721	KX712258
28	Peking duck-3 (SSU-PK3)	720	KX712259
29	Peking duck-4 (SSU-PK4)	721	KX712260
30	Peking duck-5 (SSU-PK5)	721	KX712261
31	Pengging duck-1 (SSU-PG1)	678	KX712262
32	Pengging duck-2 (SSU-PG2)	719	KX712263
33	Pengging duck-3 (SSU-PG3)	720	KX756155
34	Pengging duck-4 (SSU-PG4)	720	KX756156
35	Pengging duck-5 (SSU-PG5)	720	KX756157

Out of the 32 polymorphic sites, 28 were caused by transitions or transversions, and four were a consequence of an insertion. Based on these

polymorphic sites, all samples form 23 haplotypes (Table 2).

BLAST analysis results indicate that all samples had a 99-100% homology with *Anas platyrhynchos* Rongshui (GenBank Acc Number KJ833587.1) and 99% homology with *Anas zonorhyncha* isolate ZH-18 haplotype A-56 (GenBank Acc Number AY506969.1). The high percentage homology of all central Javanese duck with *Anas platyrhynchos*, indicating that the central Javanese duck in Indonesia in this study originated from duck *Anas platyrhynchos*. This result is consistent with the results of Purwantini et al. (2013) research that most of the local ducks in Indonesia come from *Anas platyrhynchos* duck.

Multiple alignments obtained were 720 nucleotides at position 56 to 779 at the 5 'end. The results of the analysis of polymorphism D-loop sequences produced 23 haplotypes (Table 2). This result indicates that a different hair color of central Javanese duck in Indonesia relatively polymorphic that can be used as a genetic marker that can distinguish central Javanese ducks with other local ducks. A total number of 32 variable sites were identified (Table 3).

Table 2. Haplotype names of central Javanese duck in this study.

No.	Haplotype	Ducks Samples
1.	A	Pengging 1 (SSU-PG1)
2.	B	Pengging 2 (SSU-PG2), Pengging 3 (SSU-PG3), Peking 1 (SSU-PK1), Peking 3 (SSU-PK3), Magelang 2 (SSU-M2), Magelang 3 (SSU-M3), Tegal Branjangan 5 (SSU-Tbr5), Tegal Jarakan 3 (SSU-TJ3), Tegal Jarakan 4 (SSU-TJ4), Tegal Jarakan 5 (SSU-TJ5), and Tegal Blorong 1 (SSU-TB1)
3.	C	Pengging 4 (SSU-PG4)
4.	D	Pengging 5 (SSU-PG5)
5.	E	Peking 2 (SSU-PK2)
6.	F	Peking 4 (SSU-PK4)
7.	G	Peking 5 (SSU-PK5)
8.	H	Magelang 1 (SSU-M1), Magelang 5 (SSU-M5), dan Tegal Lemahan 5 1 (SSU-TL5)
9.	I	Magelang 4 (SSU-M4)
10.	J	Tegal Branjangan 1 (SSU-Tbr1)
11.	K	Tegal Branjangan 2 (SSU-Tbr2)
12.	L	Tegal Branjangan 3 (SSU-Tbr3)
13.	M	Tegal Branjangan 4 (SSU-Tbr4)
14.	N	Tegal Jarakan 1 (SSU-TJ1)
15.	O	Tegal Jarakan 2 (SSU-TJ2)
16.	P	Tegal Blorong 2 (SSU-TB2)
17.	Q	Tegal Blorong 3 (SSU-TB3)
18.	R	Tegal Blorong 4 (SSU-TB4)
19.	S	Tegal Blorong 5 (SSU-TB5)
20.	T	Tegal Lemahan 1 (SSU-TL1)
21.	U	Tegal Lemahan 2 (SSU-TL2)
22.	V	Tegal Lemahan 3 (SSU-TL3)
23.	W	Tegal Lemahan 4 (SSU-TL4)

Table 3. The identified haplotype with the mitochondrial D-loop sequence polymorphism in central Javanese duck.

Haplotype	Nucleotide polymorphism																																
	56	58	59	61	65	67	68	69	71	76	77	82	84	86	90	97	98	100	102	103	126	155	161	162	206	222	275	325	610	771	778	779
KJ833587.1	G	T	T	C	G	T	A	T	T	A	T	A	C	T	T	A	T	T	C	C	-	-	G	C	C	C	C	T	T	-	G	
Haplotype A	.	.	.	-	-											T	A	A	G	G											A	
Haplotype B	T	-	-	-	-																										A	
Haplotype C	T	G	-	A	-			A																							A	
Haplotype D	T	-	-	G	-		T	A		T	A			G	A																A	
Haplotype E	T	-	-	G	-		T	A																						T	A	
Haplotype F	T	-	-	-	-			A																						T	A	
Haplotype G	T	-	-	-	-			A																							-	A
Haplotype H	T	-	-	-	-																									T	A	
Haplotype I	T	-	-	-	-																								C		A	
Haplotype J	T	-	-	-	-																				A						A	
Haplotype K	T	-	-	-	-																										-	-
Haplotype L	T	-	-	G	-		T	-	-			T	T										-	-		-	-	C			-	A
Haplotype M	T	-	-	-	-		-	-	-			-	-										-	-		T	T	-			A	
Haplotype N	T	-	-	G	-		-	A	G			T	-										-	-		-	-	-			A	
Haplotype O	T	-	-	T	-		-	-	-			-	-										-	-		-	-	-			A	
Haplotype P	-	-	A	G	T	A	T						A	G							C	C								A	A	
Haplotype Q	-	-	-	-	-														T												A	
Haplotype R	T	-	-	T	-		-	-	-			-	-										-	-		-	-	-		T	A	
Haplotype S	T	-	-	-	-		-	-	-			-	-										C	A		-	-	-			-	A
Haplotype T	-	-	-	-	-														T											A	A	
Haplotype U	T	-	-	-	-																		C	A		T					A	
Haplotype V	T	-	-	-	-		-	-	-			-	-										C	A		-	-	-		T	A	
Haplotype W	T	-	-	-	-		-	A	-			-	-										-	-		-	-	-		T	A	

Insertions were detected in four sequences (126, 155, 771 and 779 nucleotide number). Distribution of D-loop mitochondrial DNA sequences polymorphic sites in this study are shown in Figure 2. D-loop has two regions with a high rate of polymorphism; therefore the sequence is highly variable between individuals, the hypervariable I (HVSI) and hypervariable II (HVSII). The noncoding region also contains a control region because it has the origin of replication for the H-strand (OH) and promoter transcription to H and L strands (PL and PH) (Anderson et al., 1981; Gill et al., 1994). Also, the noncoding region also contains three areas of sustainable called conserved sequence block (CSB) I, II, III. The sustainable region is thought to have a major role in mtDNA replication (Anderson et al., 1981; Gill et al., 1994; Bellwood, Tevenson, Dizon, & Anderson, 2003).

At the base sequence between 56-100 contained 18 polymorphic sites (56.2%), 101-200 there are 6 polymorphic sites (18.7%), 201-300 there are 3 polymorphic sites (9.3%), 301-400 there is one polymorphic site (3.1%), 401-500 there are polymorphic sites (0%), 501-600 there are polymorphic sites (0%), 601-700 there is one polymorphic sites (3.1%), and 701-774 are 3 polymorphic sites (9.3%). The percentage of the polymorphic site is calculated from the total polymorphic sites (32) in this study.

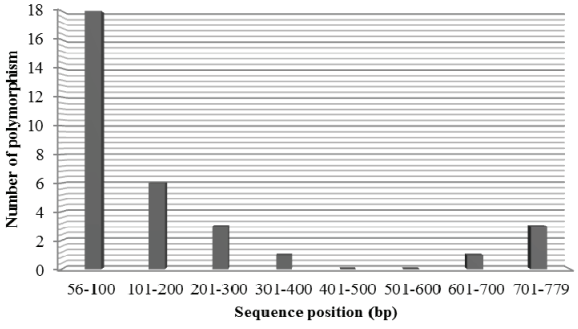

Figure 2. Distribution of polymorphic sites of mtDNA D-loop in central Javanese duck in this study.

The highest proportion of polymorphic nucleotide sequences found in 56-100 which 18 polymorphic sites (56.2%). This result indicates that D-loop sequences have high genetic variation in the HV segment-1 (hypervariable-1) at 397 bases in the first part of the D-loop segment. This result is consistent with the research of Zein and Sulandari (2009) that the HV-1 is an area that is more polymorphic than other regions of the D-loop gene. Variation in the D-loop fragment of mitochondrial DNA may affect the other site of mitochondrial DNA fragment. D-loop fragment is a non-coding region that does not encode proteins. However, according to Lindberg (1989), D-loop fragment is the initiator the process of transcription and replication. Variations of D-loop fragment will be followed by a variation on the other mitochondrial fragments.

Relations genetic variation of mitochondrial DNA D-loop with phenotype was also observed in other animals. Schutz, Freeman, Lindberg, Koehler and Nbitz (1994) reported that substitutions of nucleotide D-loop genes in the number of 169, associated with the production of milk and fat percentage of dairy cattle. Variations in D-loop also affect the reproduction of Hereford cattle and Composite (Sutarno, Cummins, Greef, & Lymbery, 2002). To study the influence of mitochondrial DNA to a particular phenotype required data records of several generations.

Variations on D-loop fragments of mitochondrial DNA can arise by mutation, natural selection, are also marriage. The amount of genetic variation in populations of ducks in Indonesia could be caused due to the level of random mating, migration, and selection in the population. The high amount of inbreeding in the population, there will be restrictions on the exchange of genes from species. In a population with much outbreeding, the level of genetic diversity will be high. Knowledge of the genetic variation can be used for wildlife conservation. Adaptation to the environment can produce unique and specific combinations of allele's genes and combinations of genes likely to be very helpful in the future (Christianti, Sutarno, & Etikawati, 2003).

Genetic distance in this study was analyzed using the Pairwise Distance Calculation (Tamura, Stecher, Peterson, Filipski, & Kumar, 2013). Nucleotides of central Javanese duck D-loop mtDNA aligned with the another Anas in the world that is *Anas zonorhyncha* (AY506969.1), *Anas acuta* (HM063478.1), *Anas platyrhynchos* (KJ833587.1), and *Cairina moschata* (GQ922096.1) as out of the group. Genetic distances ranging from 0:00 to 0:11 (Table 4). The smallest genetic distance is by *Anas platyrhynchos* and *Anas zonorhyncha*, namely 0.00, whereas with Anas acuta of 0.07-0.08. The highest genetic distance was in *Cairina moschata* as out of group 0.10-0.11 due to *Cairina moschata* is joined as *Muscovy duck*.

Leekaew et al. (2008) explained that there are similarities ancestors (91%) between the first duck

Thailand (Narkom-Pathom and the Park-Nam) with Mallard ducks (*Anas platyrhynchos*). Li et al. (2010) reported that there are similarities ancestors (81%) among the eight local ducks breed of Chinese with Mallard ducks (*Anas platyrhynchos*). Purwantini et al. (2013) reported that there are polymorphisms of D-loop mtDNA in five duck populations in Indonesia (Magelang, Tegal, Mojosari, Bali, and Alabio duck), with the closest genetic distance with *Anas platyrhynchos* i.e., 0000-0677.

Reconstruction of phylogenetic trees of central Javanese duck with another Anas forms two clusters (Figure 3). The first cluster consists of all kinds of central Javanese duck in Indonesia, *Anas platyrhynchos*, and *Anas zonorynchos*. In the first group, all Central Javanese duck form 4 distinct sublineages. The proximity pattern is characterized by high boostrap value of 100%. That is, all kinds of central Javanese duck grouped by *Anas platyrhynchos* and *Anas zonorynchos* in trials with 1000 bootstrap replications will show the results (positions) are the same. This result indicates that the central Javanese duck is closely related to *Anas platyrhynchos* and *Anas zonorynchos*. The second cluster consists of *Anas creca, Anas americana, strepara Anas, Anas bahamensis, Anas sibimatrix, and Anas acuta* with the bootstrap value of 43%.

In this study, ducks (Anseriformes) might determine the role of waterfowl as a natural reservoir for avian influenza (AI) virus. Susanti, Soejoedono, Mahardika and Wibawan (2008a) state that waterfowl is allegedly act as a natural reservoir for avian influenza (AI) virus subtype H5N1, as evidenced by the high prevalence of H5N1 infection in the unvaccinated healthy waterfowl.

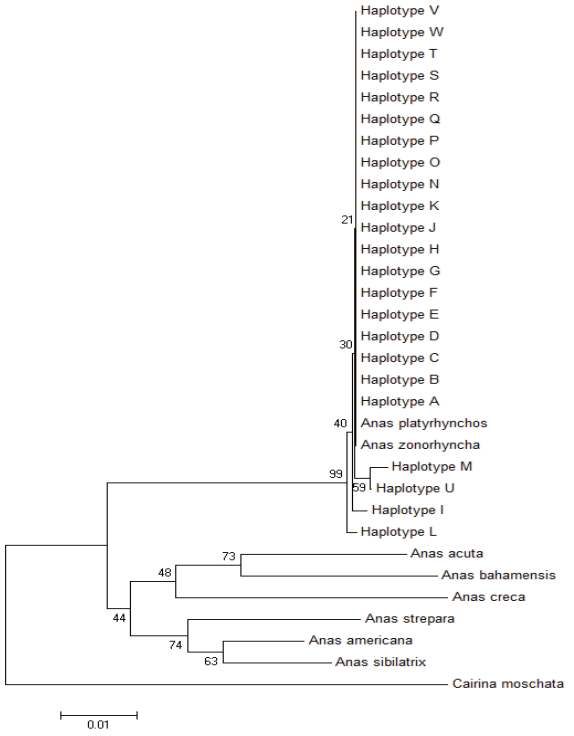

Figure 3. Neighbour-joining tree with 1000 bootstrap replication for central javanese duck and other Anas breeds from GenBank based on D-loop gene.

Table 4. Result of pairwise distance calculation.

Anas acuta																									
A. platyrhynchos	0.07																								
A. zonorhyncha	0.07	0.00																							
C. moschata	0.11	0.10	0.10																						
Haplotype A	0.07	0.00	0.00	0.10																					
Haplotype B	0.07	0.00	0.00	0.10	0.00																				
Haplotype C	0.07	0.00	0.00	0.10	0.00	0.00																			
Haplotype D	0.07	0.00	0.00	0.10	0.00	0.00	0.00																		
Haplotype E	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00																	
Haplotype F	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00																
Haplotype G	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00															
Haplotype H	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00														
Haplotype I	0.07	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00													
Haplotype J	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00												
Haplotype K	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00											
Haplotype L	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00										
Haplotype M	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01	0.00	0.00	0.01									
Haplotype N	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00								
Haplotype O	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00							
Haplotype P	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00						
Haplotype Q	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00					
Haplotype R	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00				
Haplotype S	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00			
Haplotype T	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00		
Haplotype U	0.08	0.00	0.00	0.11	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	
Haplotype V	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Haplotype W	0.07	0.00	0.00	0.10	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Waterfowl has reported to have no experience in pain, paralysis and death even though they are infected with AI virus subtype H5N1 which is shown to be molecularly and biologically pathogenic (Susanti, Soejoedono, Mahardika, & Wibawan, 2008b). However, the local duck is still necessary to be maintained to conserve its germplasm. By using the entire sequence information of the mtDNA D-loop, the genetic diversity of 7 breeds of Central Javanese duck was analyzed and then was inferred on their phylogenetic status. Therefore, the results are useful for the conservation and utilization of Javanese ducks genetic resources. The genetic information based on mtDNA typing has a great importance for the future breed conservation strategy, especially for the Central Javanese duck.

Conclusion

Based on the genetic variation analysis using D-loop mtDNA, a total number of 32 variable sites were identified. Insertions were detected in four sequences (126, 155, 771 and 779 nucleotide number). The results of the polymorphism analysis on D-loop sequences produced 23 haplotypes. However, this haplotype information does not represent the relationship among the geographical origins of duck with the certain name of duck species. This study remarks that all ducks in this study are identic with *Anas platyrhynchos* and *Anas zonorynchos*.

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