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Polymorphism of D-Loop Mitochondrial DNA Region and Phylogenetic in Five Indonesian Native Duck Population

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Abstract: This research aimed to determine mitochondrial DNA (mtDNA) D-loop region polymorphism and phylogenetic in five Indonesian native ducks population namely Magelang duck, Tegal duck, Mojosari duck, Bali duck and Alabio duck. The significance of this research was applicable to conservation and refinement strategy as well as the improvement of genetic quality by utilizing the available native duck plasma nutfah. It further concerned with determining the genealogy of family, channel, breed or maternal inheritance that bred individuals in native duck population in Indonesia and discovering phylogenetic relations with *Mallards* duck (*Anas platyrhynchos*) and the other *Anas* ducks. Fragment of 718-bp was amplified by PCR and determined the nucleotide sequence of 701-bp. The sequence was then analyzed using Single Nucleotide Polymorphism (SNP) and compared to the standard nucleotide sequence from *Anas Platyrhynchos* complete genome (HM010684.1) in GenBank Accession. It obtained nucleotide percentage equation of 93.59±8.23%. Phylogenetic investigation used sequencing products and was analyzed using MEGA5 software. Indonesian native ducks have a relatively close genetic relationship with *Anas Platyrhynchos* and *Anas zonorhyncha* shown by the genetic distance varying from 0.000-0.786 compared to the other *Anas* ducks in the world (0.073-1.037) or to *Cairina moschata* (2.972-5.776). Highly distant genetic variation was found in Magelang duck compared to the other native ducks ranged from 0.000-0.950 to 0.000-0.312. The research concluded that polymorphism of mtDNA D-loop region was found in Indonesian native ducks and had relatively similar maternal inheritance with *Anas platyrhyncos* dan *Anas zonorhyncha*.

Key words: Polymorphism, D-loop DNA mitochondria, phylogenetic, native ducks in Indonesia

INTRODUCTION

Ducks as known today are the result of wild duck (*Anas Boscha* or *Wild Mallard*). Domesticating process has taken place for centuries in which South East Asia as one of the centers. This type of ducks is extensively used as layer and meat producer (Wu *et al*., 2011). Duck egg production in Indonesia comprises 20% of domestic products and is the second biggest production after layer chicken (65%) (Yudohusodo, 2003). The ducks however do not belong to pure breed and still share a highly common genetic uniformity due partly to nomadic farming or shepherd system, leading to a likely random crossbreeding which may bring unfavorable effect on genetic structure on the duck type. It is observed from the high diversity in morphology and productivity rate (Purwantini *et al*., 2005). Various native ducks in Indonesia are named according to the location with particular morphological traits. Native ducks in Java Island, for instance are known as Tegal duck and Magelang duck in Central Java, Mojosari duck in East Java, Cihateup duck in West Java and Turi duck in Yogyakarta. Native ducks developed as genetic resource in Sumatra Island particularly in West Sumatra province are Pitalah duck, Kamang duck and Bayang duck (Purwanto, 2012). They are also called Bali duck in Bali and Alabio duck in Kalimantan especially in South Kalimantan province. This type of duck is the crossbreed of native and import ducks, resulting in various duck names (Hetzel, 1985 and Wilson *et al*., 1997 in Yuwanta *et al*., 2001). Ducks in the east end of Java Island, Bali and Lombok are the crossbreed between Indian Runner from East Hindia and wild domestic ducks through a long period of evolution or phylogenesis (Rudolph, 2002).

Information on molecular genetic identification using mt DNA D-loop region to reveal phylogenetic of native ducks in several part of Indonesia is still limited. Wu *et al*. (2011) stated that mtDNA D-loop region map has been used as the most popular genetic marker to support species conservation that has close genetic relationship with wild *Mallard* duck and domesticated duck (*Muscovy*) and to understand the inheritance, domestication process, genetic diversity and domestic duck difference. Polymorphism of mtDNA D-loop region with SNP technique is subject to alternative method development in analyzing genetic characteristics and individual genetic diversity within population, estimating genetic distance and reconstructing phylogenetic characteristics

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between individual in duck population in China (Nenzhu *et al*., 2009; Wang *et al*., 2011; Wang *et al*., 2012). It is used as well to evaluate phylogenesis of seven types of birds and as genetic marker in *Gallus Anser* (Feng *et al*., 2009), in dove (*Columba livia*) (Tsai *et al*., 2009) and in *bubbler* from China and Taiwan (Li *et al*., 2010). The formation of pylogenetic tree in mtDNA D-loop region in ducks in China has been published (Donne-Gousse *et al*., 2002; Peters *et al*., 2005). The sequence of mtDNA D-loop region is used to define the root and diversity of native chicken in Nigeria as well, identifying 36 polymorphic sites producing 35 haplotype (Adebambo *et al*., 2010).

This research aimed to figure polymorphism of mtDNA D-loop region and phylogenetic of five Indonesian native ducks namely Magelang duck, Tegal duck, Mojosari duck, Bali duck and Alabio duck. The significance of this research was applicable to conservation and refinement strategy as well as the improvement of genetic quality by utilizing the available native duck plasma nutfah. It further concerned with determining the genealogy of family, channel, breed or maternal inheritance that bred individuals in native duck population in Indonesia and discover phylogenetic relations with *Mallards* duck (*Anas platyrhynchos*) and the other *Anas* ducks.

MATERIALS AND METHODS

Blood sample of 3 ml was taken from each 130 native ducks consisted of 50 Magelang ducks of 11 various feather colors, namely A. *Jarakan polos* (plain brown), B. *Bosokan* (dark brown), C. *Klawu blorok* (light brown and white), D. *Kalung ombo* (brown with wide white collar), E. *Kalung ciut* (brown with thin white collar), F. *Cemani* (plain black), G. *Gambiran* (dark brown and white), H. *Jarakan kalung* (brown with white collar), I. *Jowo polos* (brown with specific pattern), J. *Wiroko* (black and white), K. Plain white (yellow bill and feet) and other native ducks (Tegal, Mojosari, Bali and Alabio duck) comprising 20 heads each.

Laboratory apparatus used in this study were stationery, disposable syringe, vacutainer filled with EDTA, icebox, analytical scale, measure cup, micropipette, blue type, yellow type, white type, sterile tube (conical), eppendorf tube, GD column, centrifuge, DNA Isolation Kit (Geneaid), water bath/incubator, microwave, PCR machine, horizontal electrophoresis/Submarine Electrophoresis (Hoefer, USA) and digital camera.

Chemical reagents used to extract DNA were RBC (Red Blood Cell) Lysis Buffer, GB (Guanidin Buffer), W_1 Buffer, Wash Buffer and Elution Buffer or TE (Tris Edta), PBS (Phosphate Buffer Saline). Agarose gel was made of agarose powder, buffer 0.5x TBE and good view. PCR was done using KAPA (Kit PCR), primer Forward (DL-*Anas*PF), primer Reverse (DL-*Anas*PR), dH_2O free nuclease, loading day and DNA ladder. Experiment method was performed for (1) DNA extraction from blood sample (2) primer design and amplification in mtDNA D-loop region with PCR technique and (3) sequencing PCR product.

DNA extraction from blood sample: Blood sample was taken using disposable syringe from vena axillaries then put into vacuntainer filled with EDTA as anticoagulant. Total DNA genome was extracted using DNA Isolation Kit Geneaid according to protocol. DNA extraction was used as PCR template without purification process to obtain reproducible PCR product. PCR product in specific measurement appropriate with the primer, resulted from optimum PCR process.

Primer design and amplification in mtDNA D-loop region using PCR technique: Primer design of specific oligonucleotide in mtDNA D-loop region was administered based on GeneBank database (GenBank: HM010684.1, 2010) using Clustal X program. Primer pair was chosen on conserved region for observation. Oligonucleotide primer was then analyzed with Software Design Oligoprimer. The mtDNA D-loop region was amplified by PCR using primers DL-*Anas*PF (L56) 5'-GTTGCGGGGTTATTTGGTTA-3' and DL-*Anas*PR (H773) 5' CCATATACGCCAACCGTCTC-3'. PCR was used to amplify with GeneAmp^R PCR system thermocycler 2400 (Perkin Elmer). PCR reagent consisted of 12.5 µl KAPA (Kit PCR), 1 µl primer Forward (DL-AnasPF) 10 pmol, 1 µl primer Reverse (DL-AnasPR) 10 pmol, 9.5 µl dH_2O free nuclease and 1 µl DNA template. PCR cycle comprised pre-denaturation at 94°C for 5 minutes, denaturation at 94°C for 30 seconds, annealing at 55°C for 45 seconds, elongation (extension) at 72°C for 1 minute and post elongation at 72°C for 5 minutes. PCR was repeated 35 cycles to get optimum result. PCR product was separated by electrophoresis in low melting agarose gel 1.5% using buffer 0.5x TBE in Submarine Electrophoresis (Hoefer, USA).

PCR product sequencing: PCR products was done by PT Genetica Science Indonesia and Bio SM Indonesia. Sequencing product was analyzed by Sequence Scanner software v1.0 in form of electropherogram consisted of nucleotide sequence from mtDNA D-loop region in Magelang, Tegal, Mojosari, Bali and Alabio duck samples.

Data analysis: Polymorphism was analyzed with Single Nucleotide Polymorphism (SNP) technique by comparing sequenced products to standard nucleotide sequence from *Anas Platyrhynchos* complete genome (HM 010684.1) in GenBank Accession. Phylogenetic analysis was done by MEGA5 software program (Molecular Evolution Genetic Analysis 5) (Tamura *et al*., 2011) in which sequenced products were compared and aligned using ClustalW 183 program (Thompson *et al*.,

1994). Genetic distance estimation was analyzed with pairs of taxa scope and the estimation of genetic variation was analyzed with Bootstrap method with 1000 replication and the substitution model was Maximum Composite Likelihood (Kimura, 1980; Tamura and Nei, 1993 and Tamura *et al*., 2011). Phylogeny reconstruction used All selected Taxa scope and Neighbour-joining statistics. Test of Phylogeny used Bootstrap method with 1000 replication and substitution method of Maximum Composite Likelihood. Phylogenetic tree was arranged based on sequencing result of 701-bp from mtDNA D-loop region control using 'Neighbor-joining analysis' in MEGA5 software (Kimura, 1980 and Tamura *et al*., 2011). Sequencing analysis of several Indonesian native duck and several sequence from GenBank (NCBI) was used to establish phylogenetic tree. Data GenBank (NCBI) with access codes of *Anas platyrhynchos* complete genome *(*HM010684.1) and *Anas platyrhynchos* haplotype 35 (JN811041.1) *Anas zonorhyncha (*GU246018.1), *Anas rubripes* (AF382426), *Anas strepera* (AY112944.1), *Anas acuta* (HM063478.1), *Anas bahamensis* (AY112940.1), *Anas clypeata* (HM063479.1), *Anas crecca* (AY112942.1), *Anas sibilatrix* (AY112943.), *Anas americana* (HM063480.1) and Muscovy duck (*Cairina moschata*) (GQ922096.1) were used as out group. Phylogenetic analysis served to indicate two different genetics in *Pilophorustypicus* in Japan. The groups were divided into some geographical distance (Ito *et al*., 2011). Data analysis result used 'neighbour-joining analysis' comprised in phylogenetic tree dendrogram.

RESULTS AND DISCUSSION

PCR amplification from DNA extraction: Total genome DNA from blood samples of Magelang, Tegal, Mojosari, Bali and Alabio duck were well extracted using Isolation Kit Geneaid, showing relatively thick and bright bands. The brightness and thickness of the resulted DNA band determined the quality of DNA. Leekaew *et al*. (2008) reported that the quality of DNA obtained using commercial kit was better than that using K/SDS proteinase and alkali method. Amplification of mtDNA D-loop region with primer DL-*Anas*PF (L56) and DL-*Anas*PR (H773) acquired a 718-bp bright band as shown in Fig. 1, proving the primers were specific and well amplified DNA fragment in mtDNA D-loop region in several Indonesian native duck in Indonesia. Leekaw *et al*. (2008) has successfully amplified 710-bp fragment from mtDNA D-loop region in Thailand native duck namely Nakorn-Pathom (NP) dan ParkNam-(PN).

Sequencing mtDNA D-loop region: 718-bp PCR product (Fig. 1) was sequenced using primer DL-*Anas*PF (L56) dan DL-*Anas*PR (H773). The mtDNA D-loop region nucleotide sequence of 701-bp was mapped in Alabio duck sample (Fig. 2), while the electropherogram of sequencing product after editing was shown in Fig. 3.

Polymorphism mtDNA D-loop region with SNP analysis: SNP of mtDNA D-loop region of Indonesian native duck in this research was 6.44±8.51% (Table 1). Standard deviation showed a relatively high polymorphism of Indonesian native ducks. Duck population with the

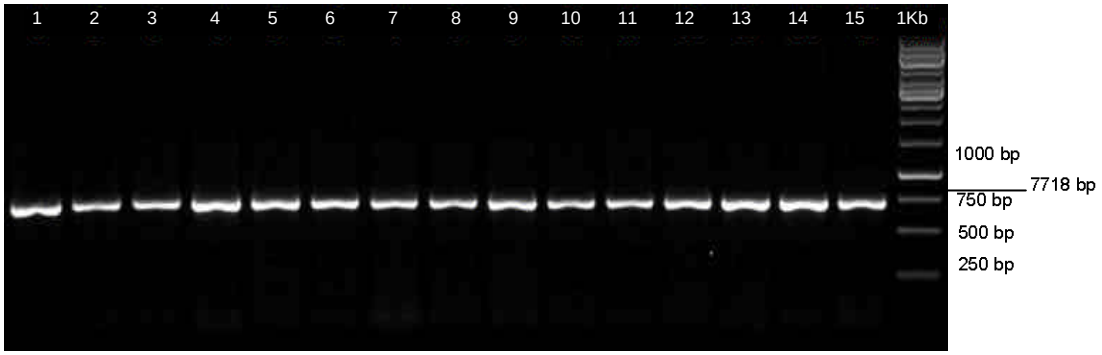

Fig. 1: Result of electrophoresis PCR product with primer pair of DL-*Anas*PF (L56) and DL *Anas*PR (H773) blood sample of native ducks in Indonesia used gel 1.5% Agarose

1	AAAACTTCCG	GGGGTCATAG	GCCAATATAT	TGGCCGATGT	GTCTCGTGTG	ATCGACACTG	CATAACCCAA	CATTCCCCCA	TGCACGGACT	90
91	AAACCCATCA	CATGTCAACG	GACATACCCT	ACCTATCGGA	CTACCCTCCC	AACGGACCCA	GAGTGAATGC	TCTAATACCC	AACACCTCAA	180
181	CACCACATAA	CATGCCCCCA	ACCAGAACAA	GGCCCCATAA	TGATGAATGC	TTGACAGACA	TACCCTACCA	ACACTCCAAA	TTCCTCTCCA	270
271	CCCACCCATT	ACTCATGAAG	CTGCGTACCA	GATGGATTTA	TTAATCGTAC	ACCTCACGTG	AAATCAGCAA	TCCTTGCACA	TAATGTCCGA	360
361	CGTGACTAGC	TTCAGGCCCA	TACGTTCCCC	CTAAACCCCT	CGCCCTCCTC	ACATTTTTGC	GCCTCTGGTT	CCTCGGTCAG	GGCCATCAAT	450
451	TGGGTTCACT	CACCTCTCCT	TGCCCTTCAA	AGTGGCATCT	GTGGAATACT	TCCACCATCT	CAATGCGTAA	TCGCGGCATC	TTCCAGCTTT	540
541	TTGGCGCCTC	TGGTTCCTTT	TATTTTTTCC	GGGGTTACCT	CACAGCTGGC	CCTTCCCAGT	GACTTCGGGG	GTCCCACAAT	CTAAGCCTGG	630
631	ACACACCTGC	GTTATCGCGC	TATCCTATAT	CTCAGGGATT	ACTCAATGAG	ACGGTTGGCG	TATATGGAAA	A		701

Fig. 2: Result of mtDNA D-loop region sequence from Alabio duck sample

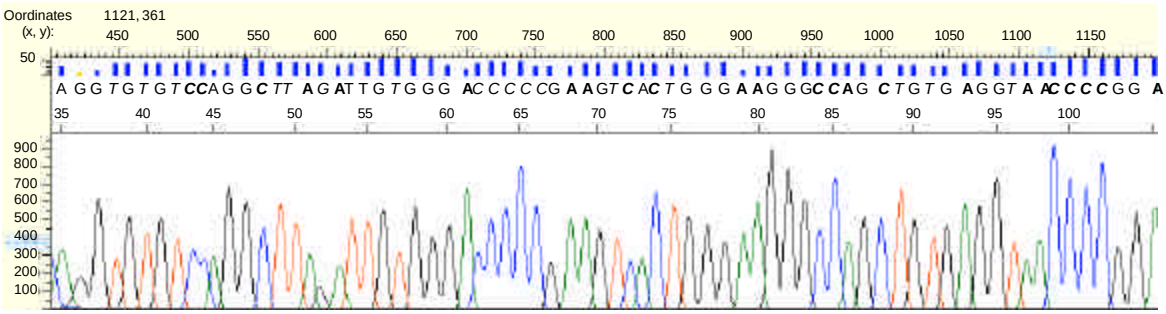

Fig. 3: Result of electropherogram mtDNA sequence in Alabio sample after editing

Table 1: Uniformity and diversity percentage of nucleotide (SNP) mtDNA D-loop region in native ducks in Indonesia with *Anas Platyrhynchos*

Native ducks	Amount of sequence/ nucleotide	Amount of nucleotide uniformity	Percentage of nucleotide uniformity (%)	Amount of nucleotide diversity (SNP)	Percentage of nucleotide diversity (%)
Magelang duck A	701	689	98.29	12	1.71
Magelang duck B	701	591	84.31	110	15.69
Magelang duck C	701	592	84.45	109	15.55
Magelang duck D	509	345	67.78	164	32.22
Magelang duck E	673	647	96.14	26	3.86
Magelang duck F	672	636	94.64	36	5.36
Magelang duck G	700	680	97.14	20	2.86
Magelang duck H	701	677	96.58	24	3.42
Magelang duck I	638	592	92.79	46	7.21
Magelang duck J	701	690	98.43	11	1.57
Magelang duck K	701	581	82.88	120	17.72
Tegal duck 1	701	680	97	21	2.99
Mojosari duck 1	701	696	99.29	5	0.71
Mojosari duck 2	701	696	99.29	5	0.71
Bali duck 1	701	691	98.57	10	1.42
Bali duck 2	701	697	99.43	4	0.57
Alabio duck 1	701	688	98.15	13	1.85
Alabio duck 2	701	698	99.57	3	0.43
Average			93.59		6.44
Standard deviation			8.23		8.51

highest nucleotide diversity was found in *kalung ombo* Magelang duck (D) namely 32.22% and respectively in plain white feathers (K) of 17.72%, *bosokan* (B) of 15.69%, *klawu blorok* (C) of 15.55%, *jowo polos* (I) of 7.21% and *cemani* (F) of 5.36%. Accordingly, Magelang duck with different feather colors showed relatively higher polymorphism than the other native ducks in Indonesia. Magelang ducks showed qualitative trait of diverse color (Ismoyowati and Purwantini, 2010) and quantitative trait of bigger body and higher egg production compared to the other native ducks (Purwantini *et al*., 2005). High polymorphism in Magelang duck population was assumed to derive from native duck crossbreeding in Indonesia, resulting in excellent genetic characteristics and morphology (Purwantini *et al*., 2005). Feather coloring component is melanin (Mundy, 2005) and the color formation of the feather, eyes and skin was affected by melanin pigment and the synthesis was catalyzed by tyrosinase enzyme (Liang *et al*., 2010). Single locus of *melanocortin-1 reseptor* (MC1R), is responsible to melanic polymorphism in at least three species, Bananaquit, Snow Goose and Skua Arctic. The role of MC1R in feather color pattern was different among species (Mundy, 2005). There was no significant correlation between MC1R gen polymorphism and feather color of native Chinese duck owing to the absence of amino acid change in SNP (Nenzhu *et al*., 2009). Duck's feather pattern color was defined by some factors concerning different seasonal phenomena and reproduction. Feather color was not a significant factor in defining color pattern uniformity (Pyle, 2005). The amount and the way feather color genetics interact are still unidentified (Stevens, 1991). Distinguished feather color pattern in bird species is still beyond explanation. Why closely related species show a highly distinctive feather pattern? On the contrary, why a very similar feather color pattern undergoes a frequent evolution as found in a relatively distant genetic relationship. (Price and Bontrager, 2001).

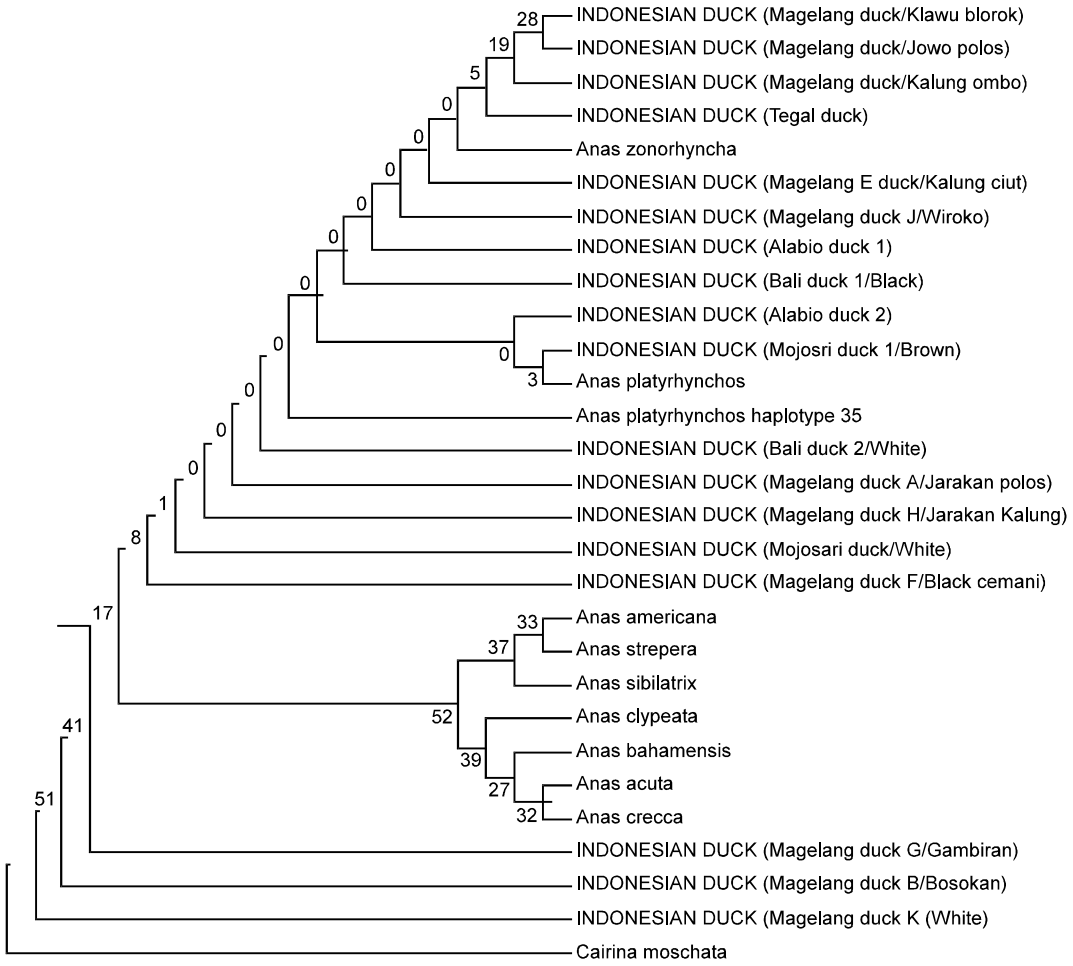

Fig. 4: Phylogenetic tree of mtDNA D-loop region in native ducks population in Indonesia and *Mallard* duck (*Anas platyrhyncos*) and other *Anas* duck in the world

SNP analysis mtDNA D-loop region on of native Chinese duck prolactin polymorphism (*Shanma*, *Shaoxing*, *Youma*, *Jinyun*, *Jingjiang* and crossbred population (F2) of *Liancheng* white duck and white *Kaiya*) was successfully identified (Wang *et al*., 2011), connected with the characteristics of Beijing duck carcass (Zhang *et al*., 2010). Three SNP from growth genetics hormones were found in several Chinese native ducks, among which were Beijing duck, *Xihu Mallards*, *Cherry Valley duck*, *Jinding duck*, *Shan Partridge*, *Jingjiang duck*, *Shaoxing duck* and *Partridge Jinyun* related to duck's productive trait (Hai *et al*., 2007). Five single polymorphism (SNP) was significantly related with body weight on the first laying, egg production in 360 days and 420 days (Wang *et al*., 2012) and can be potential genetic marker to improve some reproductive traits in ducks (Nenzhu *et al*., 2009).

Phylogenetic investigation of Indonesian native duck based on mtDNA D-loop region sequencing: Species kinship or interspecies relationship is recognizable through distance estimation and poultry origin is definable through phylogenetic tree of several species or groups based on polymorphism characteristics. Distance estimation result in Indonesian native ducks and their relation with *Anas* ducks in the world is presented in Table 2. Indonesian native ducks have a relatively close genetic relationship with *Anas platyrhynchos* and *Anas zonorhyncha* observed from the genetic distance ranging from 0.000-0.786 compared to other *Anas* ducks in the world (0.073-1.037) or with *Cairina moschata* (2.972-5.776). A considerable genetic distance was found in Magelang duck than the other native duck, ranging from 0.000-0.950 and 0.000-0.132. Magelang duck with feather of *jarakan polos* (A), *kalung ciut* (E) and *jarakan kalung* (H) had genetic distance 0.000 with other native ducks (Bali and Alabio ducks) and *Anas* (*Anas platyrhyncos* and *Anas zonorhyncha)*, while *wiroko* (J) showed 0.000 genetic distance with other native ducks (Bali and Alabio ducks) and *Anas*

Table 2: Genetic distance between various kinds of native ducks in Indonesia and the relations with Anas duck in the world

	Indonesian native ducks and Anas duck								
Native ducks	Tegal duck	Mojosari duck	Bali duck	Alabio duck	*Anas. Platyrhynchos*	*Anas. Zonor-hyncha*	*Anas. Ameri-cana*	*Anas. Clype-ata*	*Cairina moschata*
Magelang duck A	0.009	0.003	0.000	0.000	0.000	0.000	0.073	0.109	4.151
Magelang duck B	0.053	0.052	0.051	0.051	0.051	0.201	0.273	0.304	3.832
Magelang duck C	0.103	0.103	0.101	0.101	0.101	0.305	0.352	0.418	2.972
Magelang duck D	0.691	0.678	0.677	0.677	0.677	0.786	0.913	0.950	5.776
Magelang duck E	0.009	0.003	0.000	0.000	0.000	0.000	0.073	0.109	4.151
Magelang duck F	0.009	0.004	0.003	0.003	0.003	0.003	0.076	0.112	4.080
Magelang duck G	0.016	0.013	0.012	0.012	0.012	0.043	0.115	0.147	3.741
Magelang duck H	0.009	0.003	0.000	0.000	0.000	0.000	0.073	0.109	4.151
Magelang duck I	0.017	0.013	0.013	0.013	0.013	0.046	0.125	0.165	4.229
Magelang duck J	0.009	0.003	0.000	0.000	0.000	0.086	0.073	0.089	4.151
Magelang duck K	0.058	0.058	0.059	0.058	0.058	0.230	0.290	0.347	3.416
Tegal duck	0.000	0.028	0.025	0.025	0.025	0.025	0.090	0.132	4.277
Mojosari duck 1	0.010	0.000	0.003	0.003	0.003	0.003	0.076	0, 112	4.170
Mojosari duck 2	0.031	0.000	0.006	0.006	0.006	0.006	0.079	0.116	4.151
Bali duck 1	0.025	0.003	0.000	0.000	0.000	0.000	0.073	0, 109	4.151
Bali duck 2	0.025	0.003	0.000	0.000	0.000	0.000	0.073	0.109	4.151
Alabio duck 1	0.025	0.003	0.000	0.000	0.000	0.000	0.073	0, 109	4.151
Alabio duck 2	0.025	0.003	0.000	0.000	0.000	0.000	0, 073	0.109	4.151

(*Anas platyrhyncos)*. Morphologically, Magelang duck with *jarakan polos* (A) feathers was very similar with Alabio duck while the white collar as in *kalung ciut* (E) and *jarakan kalung* (H) was assumed to derive from the white feather Bali duck and Mojosari duck. Black and white colors in Magelang duck in *wiroko* (J), was assumed to derive from Bali duck and Mojosari duck with black and white feathers.

Result of genetic distance analysis showed that close genetic distance between Magelang duck featuring *bosokan* (B), *klawu blorok* (C), *kalung ombo* (D), *cemani* (F), *gambiran* (G), *jowo polos* (I) and plain white (K) feathers ranged from 0.003-0.691 and with *Anas* ducks ranged from 0.003-0.950, while with C*airina moschata* ranged from 2.972-5.776. It was emphasized with phylogenetic investigation of Indonesian native ducks using phylogenetic tree (Fig. 4).

Indonesian native ducks population (Magelang, Tegal, Mojosari, Bali and Alabio duck) was closely related to *Anas platyrhyncos* dan *Anas zonorhyncha*. It was proven by the entrance of *Anas platyrhynchos*, *Anas platyrhynchos* haplotype 35 and *Anas zonorhyncha* to Indonesian native ducks group. There was ancestral similarity of 93.59±8.23% among Indonesian native ducks with Mallard duck (*Anas platyrhynchos*). Native ducks with the highest percentage of nucleotide similarity with *Anas Platyrhynchos* was in Alabio population as much as 99.57% and respectively in Bali, Mojosari, Tegal and Magelang duck featuring *jarakan polos* (A), *kalung ciut* (E), *gambiran* (G), *jarakan kalung* (H) and *wiroko* (J) feathers (Table 1). Ancestral similarity (91%) was found in native Thailand duck, *Nakorn-Pathom* (NP) and *Park-Nam* (PN) with *Mallard* (*Anas platyrhynchos*), derived from haplotype Mallard (Leekaew *et al*., 2008). There was ancestral similarity (81%) between eight native Chinese ducks with *Mallard* (*Anas platyrhynchos*). *Youxian Sheldrake* had the closest similarity with *Anas platyrhynchos* showing genetic distance of 0, 00056-0, 00414 (Li *et al*., 2010).

Some Magelang duck featuring *klawu blorok* (C), *jowo polos* (I) and *kalung ombo* (D) feathers had genetic relationship with Tegal duck (Fig. 5). While Magelang duck with j*arakan polos* (A), *kalung ciut* (E), *jarakan kalung* (H) dan *wiroko* (J) feathers had a closer genetic relationship with Bali, Alabio and Mojosari duck shown in the genetic distance of 0.000-0.003 (Table 2). Some Magelang duck featuring *cemani* (F) *gambiran* (G), *bosokan* (B) and white feathers had genetic relationship with either *Anas platyrhyncos* and *Anas zonorhyncha* or the other *Mallard ducks* (*Anas americana*, *Anas strepera*, *Anas sibilatrix*, *Anas clypeata*, *Anas bahamensis, Anas acuta* and *Anas crecca)*. Accordingly, Bali, Alabio, Tegal, Mojosari and some Magelang duck shared maternal inheritance with *Anas platyrhyncos* and *Anas zonorhyncha*. While some Magelang duck had different maternal inheritance, showing that Magelang duck derived not only from *Anas platyrhyncos* and *Anas zonorhyncha* but also from the crossbred of other *Mallard* or *Anas* ducks. Different maternal inheritance in Magelang ducks led to specific morphological characteristics to distinguish other native ducks. Genetic distance between native ducks in Indonesia and *Cairina moschata* showed the absence of direct kinship.

Some conclusions were drawn from this study. Polymorphism of mtDNA D-loop region was found in five Indonesian native ducks and shared maternal inheritance with *Anas platyrhyncos* and *Anas* shown in relatively close genetic relationship (93.59±8.23%) compared to the other *Mallard* or *Anas* in the world.

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REFERENCES

Adebambo, A.O., V.A. Mobegi, J.M. Mwacharo, B.M. Oladejo, R.A. Adewale, L.O. Ilori, B.O. Makanjuola, O. Afolayan, G. Bjørnstad, H. Jianlin and O. Hanotte, 2010. Lack of Phylogeographic Structure in Nigerian Village ChickensRevealed by Mitochondrial DNA D-loop Sequence Analysis. Int. J. Poult. Sci., 9: 503-507.

Donne-Gousse, C., V. Laudet and C. Hanni, 2002. A molecular phylogeny of anserifor-mes based on mitochondrial DNA analysis. Mol. Phylogenet. Evol., 23: 339-356.

Feng, T.J., H.Y. Hua, L.S. Feng, L. Ning and S. Key, 2009. Complete Sequence Determination and Analysis of Beijing Duck Mitochondrial Genome. Chinese J. Zool., 2009-02.

GenBank: HM010684.1, 2010. Anas platryrhynchos breed Shaoxing mitochondrion, complete genom.

Hai, X.S., B.W. Bin, C.J. Hua, H. Jun, Z. Hongxial and C.G. Hong, 2007. Polymorphism Analysis on Coding and Regulation Regions of Growth Hormone Gene in Duck. Chinese J. Anim. Vet. Sci. 2007, V38: 907-912.

Hetzel, D.J.S., 1985. Duck Breeding Strategies the Indonesia Example. In: Duck Production Science and World Practice, Farrell, D.J. and P. Stapleton (Eds.). University of New England, Armidale NSW., pp: 204-233.

Ismoyowati and D. Purwantini, 2010. An estimation of genetic variation in Indonesian native duck. Asian J. Poult. Sci., 4: 198-204.

Ito, K., H. Nishikawa, T. Shimada, K. Ogawa, Y. Minamiya, M. Tomoda, K. Nakahira, R. Kodama, T. Fukuda and R. Arakawa, 2011. Analysis of genetic variation and phylogeny of the predatory bug, Pilophorus typicus, in Japan using mitochondrial gene sequences. Journal of Insect Science 11:18 available online: insectscience.org/11.18.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol., 16: 111-120.

Leekaew, P., T. Songserm, A. Choothesa and Boonyaprakob, 2008. A Simple Method to Extract Mitochondrial DNA in a Non-invasive Phylogenetic Study of Domestic Native Thai Ducks. Kasetsart J. Nat. Sci., 42 : 41-50.

Li, S.H., C.K.L. Yeung, L. Han, M.H. Le, C.X. Wang, P. Ding and C.T. Yao, 2010. Genetic introgression between an introduced babbler, the Chinese hwamei Leucodioptron c. canorum and the endemic Taiwan hwamei L. Taewanus: a multiple marker systems analysis. J. Avian Biol., 41: 64-73.

Li, H.F., W.Q. Zhu, W.T. Song, J.T. Shu, W. Han and K.W. Chen, 2010. Molecular genetic diversity and origin of Chinese domestic duck breeds. Archiv. Tierzucht., 53: 609-617, ISSN 0003-9438. Leibniz Institute for Farm Animal Biology, Dummerstorf, Germany.

Liang, Z., C. Wang, H. Yu, X. Peng, Y. Feng, Y. Gong and S. Li, 2010. Molecular Cloning, Characterization and Expression Analysis of Duck Tyrosinase-Related Protein-1. J. Anim. Vet. Adv., 9: 2102-2108.

Mundy, N.I., 2005. A window on the genetics of evolution: MC1R and plumage colouration in birds. Proc. Biol Sci., 22: 1633-1640.

Nenzhu, Z., H. Honghong, C. Hui, Z. Zhiming, M. Zhongwei, L. Shenglin and D. Xiaoning, 2009. SNP Detection and PCR-RFLP Analysis of MC1R Gene in Mule Duck. J. China Poult., 2009-2018.

Peters, J.L., K.G. McCracken, Y.N. Zhuravlev, Y. Lu, R.E. Wilson, K.P. Johnson and K.E. Omland, 2005. Phylogenetics of wigeons and allies (Anatidae: Anas): The importance of sampling multiple loci and multiple individuals. Mol. Phylogenet. Evol., 35: 209-224.

Price, T. and A. Bontrager, 2001. Evolutionary genetics: The evolution of plumage patterns. Current Biology. Volume 11, Issue 10, 15 May 2001, Pages R405–R408. Department of Biology 0116, University of California at San Diego, La Jolla, California 92093, USA.

Purwantini, D., Ismoyowati, Prayitno and A.T.A. Sudewo, 2005. Menciptakan Bibit Unggul Itik Native Berproduksi Tinggi. Laporan Hibah Bersaing XII. Proyek Pengkajian dan Penelitian Ilmu Pengetahuan dan Teknologi, Direktorat Jenderal Pendidikan Tinggi Departemen Pendidikan Nasional.

Purwanto, H., 2012. Identifikasi DNA dan Gen Resisten Terhadap Virus AI (Avian Influenza) pada Itik Pitalah Sebagai Sumber Daya Genetik Sumatera Barat Dengan PCR (Polymerase Chain Reaction) Artikel. Program Pascasarjana Universitas Andalas Padang 2012 A.

Pyle, P., 2005. Molts and Plumages of Ducks (Anatinae). The Institute for Bird Populations, P.O. Box 1436, Point Reyes Station, CA 94956, USA Internet: ppyle@birdpop.org Waterbirds, 28: 208-219, 2005, Internet: ppyle@birdpop.org.

Rudolph, W., 2002.The Indian Runner Duck: A Historical Guide. Feathered World.Quoted in Ashton.

Stevens, L., 1991. Genetics and evolution of the domestic fowl. Departement of Biological and Molecular Sciences University of Stirling. Cambridge University Press. Cambridge. New York Port Chester Melbourne Sydney.

Tamura, K. and M. Nei, 1993.Estimation of the number of nucleotide substitution in the control region of mitochondrial DNA in humans and chimpanzees. Mol. Biol. Evol., 10: 512-526.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar, 2011. MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance and Maximum Parsimony Methods. Mol. Biol. Evol., 28: 2731-2739.

Thompson, J.D., D.G. Higgins and T.J. Gibson, 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignmentthrough sequence weighting, positionsspecificgap penalties and weight matrixchoice. Nucleic Acids Res., 22: 4673-4680.

Tsai, L.C., J.C.I. Lee, S.P. Liao, L.H. Wang, A. Linacre and H.M. Hsieh, 2009. Establishingthe mitochondrial DNA D-loop sructure of Columba livia. Electrophoresis: Emphasis on Proteins Proteomics, 30: 3058-3062.

Wang, C., Z. Liang, W. Yu, Y. Feng, X. Peng, Y.Gong and Shijun, 2011. Polymorphism of the prolactin gene and its association with egg production traits in native Chinese ducks. South African J. Anim. Sci., 2011, 41.

Wang, C., S. Li, C. Li, Y. Feng, X. Peng and Y. Gong, 2012. Molecular cloning, expression profile, polymorphism and the genetic effects of the dopamine D1 receptor gene on duck reproductive traits. Mol Biol Rep., 39: 9239-9246.

Wilson, B.J., D.M. Martin and H. Nott, 1997. Future genetic improvement in pekin type duck. Proceedings 11th European Symposium on Waterfowl, (ESW97), Nantes, Fraance, pp: 328-334.

Wu, Y.Z., S.C. Zeng., Y.Z. Luo and J.L. Han, 2011. A Mystery in Sequencing the Mitochondrial DNA D-loop from Blood Samples of Domestic Mallard Duck. J. Biol. Scie., 11: 181-188.

Yudohusodo, S., 2003. Agribisnis Berbasis Peternakan Menghadapi Era Perdagangan Bebas. Makalah yang Disampaikan pada Acara Peringatan Hari Ulang Tahun ke 37. Fak. Peternakan. UNSOED.

Yuwanta, T., Zuprizal, A. Musofie and N.K. Wardani, 2001. Produksi and Reproduksi Itik Bantul pada Sex Ratio, Lama Campur dan Sistem Pemeliharaan yang Berbeda. Pengembangan Peternakan Tropis. Universitas Diponegoro, Semarang, pp: 68-78.

Zhang, C.L., Y.H. Wang, M. Xie, M. Chen, X.H. Wang and S.S. Hou, 2010. Mitochondrial coding gene polymorphism associated with carcass traits in beijing duck. J. Anim. Vet. Adv., 9: 2522-2525.