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Genetic Characteristic of Indonesian Local Ducks Based on Single Nucleotide Polymorphism (SNP) Analysis in D-loop Region Mitochondria DNA

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Abstract. The aim of the study was to know the genetic characteristic and polymorphysm of Indonesian local ducks including Magelang, Tegal, Mojosari, Bali and Alabio duck based on Single Nucleotide Polymorphism (SNP) analysis in D-loop region mtDNA. The long term aim was to set the spesific genetic marker based on SNP D-loop region mtDNA which could differentiate local ducks in Indonesia. In the future, it could be used as selection tool for local duck conservation, and refinement strategy as well as the improvement of genetic quality by utilizing the available native duck germplasm. There were 20 ducks for each duck population and were taken 3 ml of its blood as sample. DNA Isolation Kit high pure PCR template preparation (Geneaid) was uded for Genome DNA isolation. Amplification with PCR technique used primer *DL-AnasPF* (L56) as forward and *DL-AnasPR* (H773) as reverse. Next, PCR product or amplicon were sequenced. Sequence result were analyzed with SNP technique and observed the similarity and difference of its nucleotide sequence between individual and population. The result of the study showed that genome DNA from local duck in Indonesia was successfully isolated. DNA fragment of 718 bp was amplified with primer pair of *DL-AnasPF* and *DL-AnasPR.* Nucleotide sequence was 469 nt and analyzed with SNP technique. It was compared with standard nucleotide sequence of *Anas* platyrhynchos (HM010684.1) in *Gen Bank*. The result of nucleotide sequence similarity percentage was 99.68±0.56%. Single Nucleotide Polymorphism D-loop region mtDNA Indonesian local duck was 0.32±0.56%. Some SNP was found in Magelang duck C (*Klawu blorok)*, F (*Cemani* black), G (*Gambiran*), H (*Jarakan kalung*), I (*Jowo* plain) and K (Plain white) also Tegal duck 8, 1, 2, 5, 2, 8 and 2 SNP respectively. It could be concluded that polymorphic genetic characteristic similarity were existed in Indonesia local duck populations which was shown by its big standard deviation SNP in D-loop region mtDNA. Magelang duck with different feather color relatively more polymorphic to another local duck in Indonesia. Single Nucleotide Polymorphism which was achieved could be used as genetic marker that differentiate genetic characteristic of Indonesian local ducks.

Key words: genetic characteristic, local duck, Single Nucleotide Polymorphism (SNP), *D-loop* mtDNA

Abstrak. Penelitian ini bertujuan untuk mengetahui karakteristik genetik dan polimorfisme itik lokal Indonesia yaitu itik Magelang, Tegal, Mojosari, Bali dan Alabio berdasarkan analisis *Single Nucleotide Polymorphism* (SNP) daerah *D-loop* mtDNA. Tujuan jangka panjangnya adalah menetapkan marker atau penanda genetik berdasarkan SNP daerah *D-loop* mtDNA spesifik yang dapat membedakan itik-itik lokal yang ada di Indonesia. Selanjutnya digunakan sebagai alat bantu seleksi untuk konservasi, pembibitan dan pengembangbiakan itik lokal. Populasi masing-masing jenis itik lokal yang digunakan sebanyak 20 ekor untuk diambil 3 ml sampel darahnya. Isolasi DNA genom menggunakan DNA *Isolation Kithigh pure PCR template preparation* (*Geneaid*). Amplifikasi dengan teknik PCR menggunakan pasangan primer *DL-AnasPF* (L56) sebagai *forward* dan *DL-AnasPR* (H773) sebagai *reverse.* Produk PCR atau amplikon yang diperoleh disekuensing. Hasil sekuensing dianalisis dengan teknik SNP dan diamati kesamaan dan perbedaan urutan nukleotida antar individu itik dan antar populasi. Hasil penelitian menunjukkan bahwa DNA genom dari itik lokal di Indonesia berhasil diisolasi. Amplifikasi dengan teknik PCR berhasil memperoleh fragmen berukuran 718 bp. Urutan nukleotida hasil sekuensing sebesar 469 nt dianalisis dengan teknik SNP dan dibandingkan dengan urutan nukleotida standar dari itik *Anas platyrhynchos (*HM010684.1) yang ada di *Gen Bank*, diperoleh persentase kesamaan urutan nukleotid sebesar 99,68±0,56%. *Single Nucleotide Polymorphism* daerah *D-loop* mtDNA pada itik lokal di Indonesia sebesar 0,32±0,56%. Sejumlah SNP ditemukan pada itik Magelang C (*Klawu blorok)*, F (Hitam *cemani*), G (*Gambiran*), H (*Jarakan kalung*), I (*Jowo polos*) dan K (Putih polos) serta itik Tegal masing-masing 8, 1, 2, 5, 2, 8 serta 2 SNP. Kesimpulan dari penelitian ini adalah terdapat karakteristik genetik yang polimorfik pada populasi itik lokal di Indonesia, ditunjukkan dengan adanya

simpang baku SNP pada daerah *D-loop* mtDNA yang relatif besar. Itik Magelang dengan warna bulu yang berbeda relatif lebih polimorfik dibandingkan dengan itik lokal lainnya di Indonesia. *Single Nucleotide Polymorphism* yang diperoleh dapat digunakan sebagai penanda genetik yang dapat membedakan karakteristik genetik yang dimiliki oleh itik lokal di Indonesia.

Kata kunci: karakteristik genetik, itik lokal, *Single NucleotidePolymorphism* (*SNP*), *D-loop* mtDNA

Introduction

Local ducks are part of biodiversity that calls for serious attention on their sustainability. Accordingly, efforts are essential to maintain and improve the population size through conservation, either in-situ or ex-situ. However, the main obstruction in local duck conservation is the restricted information on its genetic status. The previous researches only cover morphological aspect, and little is on molecular study.

Morphological aspects, body measurement and production performance among individual within population of local duck still vary extensively, assumed due to different genetic quality among individuals within and across duck population. It hinders the determination of pure specification, morphological characteristics and production performance of Magelang, Tegal, Mojosari, Bali and Alabio ducks for germ plasm conservation of local duck in various part of Indonesia. Equating the desired duck genetic quality is not easy, because each individual does not have definite genealogy record. The solution is through basic study to find specific marker for equating genetic quality of local ducks. Genetic analysis approach by observing morphology phenotype has inaccurate result due to difficulty to tell between homozygote and heterozygote individual. Morphological measurement is also difficult to identify genetic mutation because only 0.1% mutant allele is detectible (Urich, 1990). Single Nucleotide Polymorphism (SNP) in mtDNA D-loop region is one of molecular marker commonly used to identify genome in mitochondria across individuals to identify genetic relation proximity and tracing the oldest ancestral hen in various species and subspecies of local ducks in many countries. The polymorphic *Deoxyribonucleic acid* mitokondria is gene-compact DNA almost without intron in Mallard order or lineage (*Anas platyrhynchos)* measuring 16.604 pair base (pb), with one sealed circle whose nucleotide sequence was completely recognized and a non coding region called displacement loop (D-loop) measuring 1.049 pb (Tu et al., 2009). mtDNA can be utilized for genetic analysis in popolation because it does not undergo recombination in gamet forming. Genome in mitochondria is single DNA unit or haplotype hereditary from hen and is a single locus gene with many allele, therefore it is an easy subject to genetic variations (Sudoyo, 2004).

In fowl groups, mtDNA analysis has been conducted to commercial and non-commercial chicken, but restricted to figure out the origin of hen (Akishinonomiya et al*.,* 1994 ; Liu et al*.,* 2006 ; Niu et al*.,* 2002), and only few analysis on local ducks. Leekaew et al. (2008) has successfully applied mtDNA analysis technique in D-loop region to trace phylogenetic of local ducks in Thailand.

Polymorphism analysis in mtDNA D-loop region with SNP technique is improvable into alternative method to analyze the characteristics and variation of individual genetic within population, to estimate genetic distance, and to reconstruct the shape of phylogenetic

characteristics among individuals in China duck population (Nenzhu et al., 2009; Wang et al., 2011; Wang et al., 2012), in pigeon (Tsai et al., 2009) and in bubbler in China dan Taiwan (Li et al., 2010). Analysis and comparison of mtDNA sequences in string format or list of mtDNA Single-Nucleotide Polymorphisms (mtSNPs) was designed with mtDNA profiler and Web application highly supporting the accuracy through automatic nomenclature and validity check (Yang et al., 2013)

The objective of this research was to (1) recognize the genetic characteristics of polymorphism of nucleotide sequence of mtDNA D-loop region in Indonesian local ducks (2) to recognize the polymorphic nucleotide sequence of mtDNA D-loop among individuals in Magelang, Tegal, Mojosari, Bali and Alabio ducks, and (3) to find the specific sequence of mtDNA D-loop as genetic marker for selection tools for duck conservation, breeding and multiplication of Indonesian local ducks. The relation of this research to dissertation accomplishment and the contribution in developing science and technology was: polymorphism analysis of nucleotide sequence in mtDNA D-loop region with SNP technique is improvable into alternative method to analyze genetic characteristics, to determine uniformity and diversity of individual genetics within local ducks population, to estimate genetic distance and to reconstruct the shape of phylogenetic characteristics across individuals in Magelang, Tegal, Mojosari, Bali and Alabio ducks population.

Materials and Method

Survey among farmers and laboratory observation were conducted in the folowing steps: blood sampling of farmers' local ducks, DNA genome isolation, DNA amplification with PCR, mtDNA sequencing, mtDNA analysis with SNP and nucleotide frequence, and evaluation to obtain the optimum genetic marker for local ducks diversity in Indonesia.

Blood sampling of farmers' local ducks. Survey was conducted to Magelang duck farmers in Keji village, Muntilan District, Magelang regency, Central Java Province; to Tegal duck farmers in Pakijangan village, Bulakamba district, Brebes regency, Central Java Province; to Mojosari farmers in Modopuro village, Mojosari district, East Java Province; to Bali duck farmers in Mengwi village, Denpasar district, Bali Province; and to Alabio duck farmers in alabio village, Amuntai district, Hulu Sungai Utara Regency, South Kalimantan Province. Three ml of blood was taken from 5 out of 20 ducks in each region, or 100 ducks in total.

Genomic DNA isolation. *Deoxyribonucleic acid* (DNA) genome from local ducks blood sample was isolated using DNA *Isolation Kit high pure PCR template preparation* (*Geneaid*). Quality of isolated DNA was observed using horizontal gel electrophoresis on 1% agarose gel in *Submarine Electrophoresis* (Hoefer, USA). Observation utilized UV radiation (ג = 300 nM) after gel was g*ood view*-colored.

DNA primer design and amplification using PCR. Primer design of specific *oligonucleotide* in mtDNA D-loop region was conducted based on *Gene Bank* database (HM010684.1). Base sequence of mtDNA D-loop region in Mallards (*Anas platyrhynchos*) was 1.049-bp. Primer pair was chosen on conserved or observed zone. Primer oligonucleotide was then analyzed using *Software Design Oligoprimer* with *Primer3 online*. It obtained primer pair of *DL-AnasPF* (L56) as *forward primer* with base sequence: 5' - GTTGCGGGGTTATTTGGTTA-3' and *DL-AnasPR* (H773) as *reverse primer*: 5' - CCATATACGCCAACCGTCTC-3'. DNA amplification

with PCR in this research used *GeneAmp*^{R}PCR *system thermocycler* 2400 (*Perkin Elmer*). Amplification of mtDNA D-loop region was conducted under the following condition: pre-denaturation at 94^0 C thermocycler for 7 minutes. After temperature reached 94^0 C stable temperature, PCR reaction process began. DNA template denaturation occured at 94^0 C for 30 seconds. Primer annealing on DNA template was at 56^0 for 45 seconds. Elongation or Extension occured when PCR reaction was about to stop, thermocycler was maintained at 72^0 C for 1 minute. Optimum result was obtained by conducting PCR reaction for 35 cycles. Final extension, to complete DNA elongation for the incomplete ones, was conducted at 72^0 C for 5 minutes.

PCR products or aplified/amplicon DNA fragments were separated by electrophoresis to observe the amplification success. Electrophoresis was conducted at 1.5% low melting agarose gel. Horizontal gel electrophoresis was conducted at 50volt for 45 minutes. Picture obtained were documented from *Electrophoresis Documentation and Analysis System* 290 (EDAS 290) with UV radiation aid (ג = 300 nm).

DNA sequencing. Amplified PCR products were sequenced with primer pair of *DL-AnasPF* and *DL-AnasPR.* Sequencing products were nucleotide seequences and electropherogram graphics with colorful peaks to differ the typical nitrogen bases (nucleotide). DNA sequencing process of Magelang duck and other local ducks were conducted by PT *Genetika* Science Indonesia and *Bio* SM Indonesia. Sequencing products were scanned using *software Sequence Scanner v1.0,* the result were scanned electropherogram consisting of nucleotide sequences. Each nucleotide had different colored peak, namely greem *Adenin* (A), purple *Guanin* (G), blue *Cytosin* (C) and red *Tymin* (T).

Data analysis with SNP and nucleotide frequency. Nucleotide sequences result of 469 nucleotide (nt) from Magelang, Tegal, Mojosari, Bali and Alabio ducks were administered for SNP analysis and nucleotide frequency. To determine polymorphism, the nucleotide sequences of local ducks in this research were compared with those of *Anas platyrhynchos* from (*Gen Bank* number HM 010684.1) as the standard (Tu et al., 2009). For instance, difference spotted in C16223T duck sample indicating C different nucleotide on nucleotide position 16223 by standard; in contrast, the sample indicated T. The result obtained could be used as genetic marker in Indonesian local ducks.

Nucleotide freuency was obtained from the proporsion or comparison of particular nucleotide amount in each sample. Nucleotide frequency analysis on mtDNA D-loop region used *software Maximum Likelihood Model Compute Nucleotide Composition* (Tamura and Nei, 1993) in MEGA5.1 *Beta*3 program (Tamura et al., 2011).

Result and Discussion

Blood sampling of Indonesian local ducks. Observation showed that feather color of Magelang ducks were relatively more varied than that of other local ducks. Purwantini et al. (2013) reported 11 feather color in Magelang duck population, namely A. *Jarakan polos* (solid brown with black spots), B. *Bosokan* (dark brown), C. *Klawu blorok* (grey mixed white), D. *Kalung ombo* (brown with wide white collar), E. *Kalung ciut* (brown with narrow white collar), F. *Cemani* (solid black), G. *Gambiran* (dark brown mixed white), H. *Jarakan kalung* (brown with white collar), I. *Jowo polos* (bright brown with dark brown spots), J. *Wiroko* (black mixed white) and

K. solid white (yellow bill and feet). Tegal ducks were relatively uniform in brown, Mojosari were white and brown, Bali were relatively similar to Mojosari with two feathers of brown and white also black, while Alabio were relatively uniform in white with black spots overall the body and white line around the eyes. Male Alabio had relatively darker feather, and shiny bluish green wing linings. Blood sampling on local ducks was conducted individually using *disposibel syrink* from wing vene (*vena axillaries*), as much as 3ml from each bird, then the blood sample was extracted through isolation to obtain DNA genome.

Result of DNA genome isolation. DNA isolation is basically the removal of DNA molecule from other component through lysis. This research had isolated DNA genome, indicated from DNA electrophoresis of blood sample of Indonesian local ducks as presented in Figure 1. The relatively bright and wide ribbons showed relatively high DNA concentration (Yuwono, 2006). DNA isolation result was DNA genome for DNA template in PCR amplification followed by sequencing.

Amplification on mtDNA D-loop region of Indonesian local ducks with PCR. Primer pair of DL-*AnasPF* (L56) as *forward primer* and *DL-AnasPR* (H773) as *reverse primer* with duck DNA genome as DNA *template* were used in PCR process. Amplification success was shown from PCR products or amplicon in form of DNA fragment separated by electrophoresis in 1% agarose gel. PCR product of mtDNA D-loop region of Indonesian local ducks as much as 718 bp presented in Figure 2 showed that all components in PCR reaction were in optimum condition and served well.

SNP Analysis on mtDNA D-loop region of Indonesia local ducks. SNP analysis was conducted after all sequenced nucleotide and *Anas platyrhynchos* taken from *Gen Bank* number HM 010684.1 were subject to multiple alignment (*multiple alignment*) using *Clustal W* 1,83 program (Thompson et al*.,* 1994). Result of multiple alignment was nucleotide sequences of mtDNA D-loop region in Indonesian local ducks, scoring 469 nucleotide (nt) at position 160 through 629 from 5' end. Nucleotide sequence scheme of mtDNA D-loop region resulted from multiple alignment is presented in Figure 3.

Kimura (1980) and Tamura and Nei (1993) reported that all nucleotide sequence positions with gap and missing data were discarded. Mirza and Kurniasih (2002) stated that nucleotide sequence from one species was not different, but different species had different nucleotide sequence. The amount of SNP based on nucleotide difference matrix of Indonesian local ducks compared to *Anas plathyrhynchos* from *GenBank* number HM 010684.1 as presented in Table 1.

Tabel 1. Shows that mean and standard deviation of SNP mtDNA D-loop region of Indonesian local ducks was 0.32±0.56%. Standard deviation showed that SNP in Indonesian local ducks was relatively polymorphic. Some SNP found in Magelang duck C (*Klawu blorok)*, F (Hitam *cemani*), G (*Gambiran*), H (*Jarakan kalung*), I (*Jowo polos*) and K (solid white) and Tegal duck was 8, 1, 2, 5, 2, 8 and 2 SNP, respectively. Duck population with the highest SNP was Magelang C (*Klawu blorok)* and K (solid white) ducks, namely 1.71%.

SNP location in Indonesian local duck population indicated that Magelang C (*Klawu blorok*) had 8 SNP on A-347G, C-367T, G-384C, C-390T, A-430G, G-433T, C-447G and G-463C; Magelang F (Hitam *cemani*) had 1 SNP on A-299C; Magelang G (*Gambiran*) had 2 SNP on A-347G and A-430G; Magelang H (*Jarakan kalung*) had 5

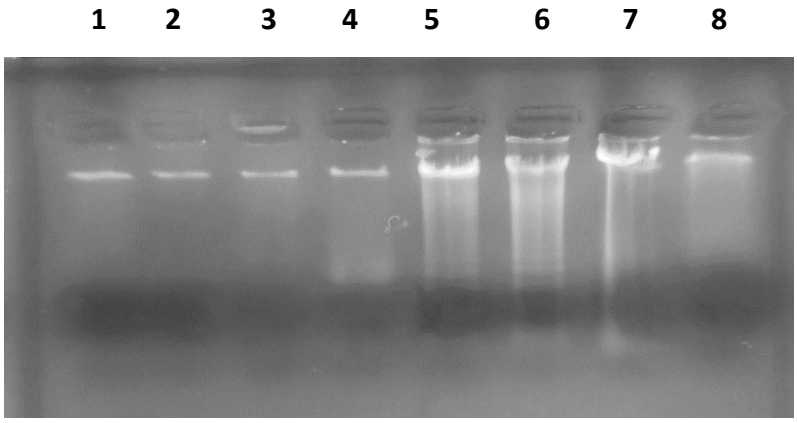

Figure 1. Result of DNA genome electrophoresis of blood sample of Indonesian local ducks using 1% agarose gel
Note: In sequence (1) Magelang A (*Jarakan polos*), (2) B (*Bosokan*), (3) C (*Klawu blorok*), (4) D (*Kalung ombo*), (5) Tegal, (6) Mojosari, (7) Bali, (8) Alabio ducks

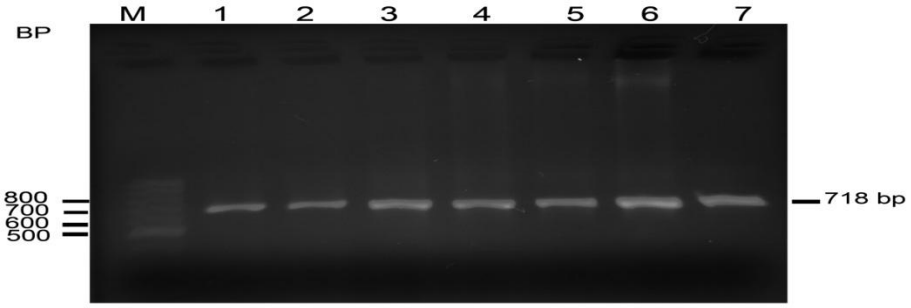

Figure 2. Electrophoresis result was 718bp PCR products with primer pair *DL-AnasPF* (L56) and *DL-AnasPR* (H773) from blood sample of Indonesian local ducks using 1% agarose gel. Note: in sequence (M) DNA marker, (1) Magelang, (2) Tegal, (3) white Mojosari, (4) brown Mojosari, (5) Bali putih, (6) black Bali and (7) Alabio ducks

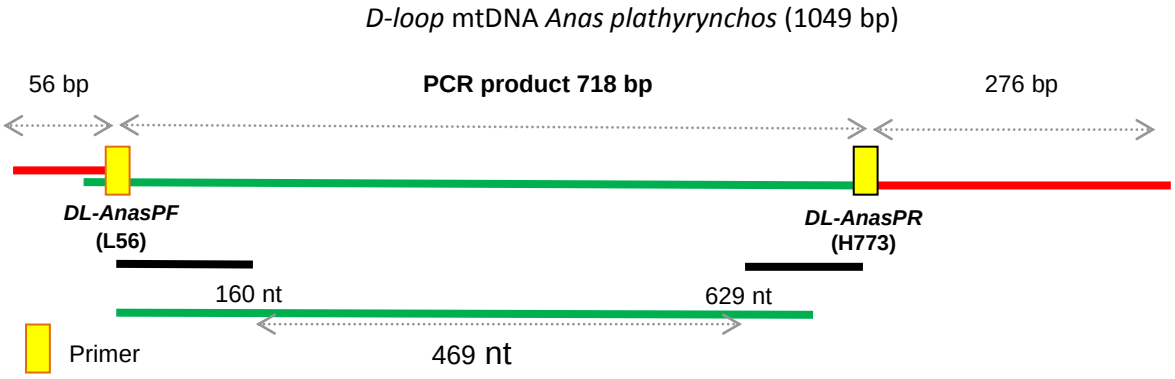

Figure 3. Multiple alignment scheme (469 nt) for SNP analysis in Indonesian local ducks

Table 1. Percentage of nucleotide (SNP) similarity and difference of mtDNA D-loop region of Indonesian local ducks compared to *Anas platyrhynchos* from *GenBank* number HM 010684.1)

Local ducks	Nucleotide amount (nt)	Similar nucleotide	Percentage of similar nucleotide (%)	Different nucleotide (SNP)	Percentage of SNP (%)
Magelang A	469	469	100.00	0	0.00
Magelang B	469	469	100.00	0	0.00
Magelang C	469	461	98.29	8	1.71
Magelang D	469	469	100.00	0	0.00
Magelang E	469	469	100.00	0	0.00
Magelang F	469	468	99.77	1	0.21
Magelang G	469	467	99.57	2	0.42
Magelang H	469	464	98.93	5	1.07
Magelang I	469	467	99.57	2	0.42
Magelang J	469	469	100.00	0	0.00
Magelang K	469	461	98.29	8	1.71
Tegal 1	469	469	100.00	0	0.00
Tegal 2	469	467	99.57	2	0.42
Mojosari 1	469	469	100.00	0	0.00
Mojosari 2	469	469	100.00	0	0.00
Bali 1	469	469	100.00	0	0.00
Bali 2	469	469	100.00	0	0.00
Alabio 1	469	469	100.00	0	0.00
Alabio 2	469	469	100.00	0	0.00
Mean population			99.68		0.32
Standard deviation			0.56		0.56

SNP on A-347G, C-367T, A-383C, A-387C and A-430G; Magelang I (*Jowo polos*) had 2 SNP on A-299C and G-463A; and Magelang K (solid white) had 8 SNP on A-47C, A-48C, C-49A, T-401C, C-406T, C-417G, A-418C and T-424G. In other local ducks namely Tegal ducks, there were 2 SNP on G-234C and G-385T. Mojosari, Bali and Alabio ducks showed similar nucleotide sequence with that of *Anas plathyrhynchos.*

The occurrence of SNP was assumed due to unequal mutation in different nucleotide in each individual. Many SNP found in Magelang duck showed that population of Magelang duck with different feather colors was relatively more polymorphic than other Indonesian local ducks. The obtained *Single Nucleotide Polymorphism* in mtDNA *D-loop* region could be used as genetic marker to differ between Magelang and other local ducks.

SNP analysis on prolactin gene polymorphism of China native duck (*Shanma, Shaoxing, Youma, Jinyun, Jingjiang* and the crossbred population (F2) white *Liancheng* X white *Kaiya*) successfully detected 12 SNP (Wang et al., 2011). Four SNP detected in ATP6 gene with frequency of 0.12, 0.23 and 0.65, respectively were found in quality of Beijing duck carcass ($P<0.05$) (Zhang et al*.,* 2010). Three SNP were found in exon 4 of growth hormone gene in various China local duck that were related to some duck production characters (Hai et al., 2007). Five SNP in *complete coding region* (CDS) from *dopamine D1 receptor* ducks (DRD1) were identified. This result showed that DRD1 gene was a potential genetic marker to

improve some reproduction characters in ducks (Wang et al., 2012 and Nenzhu et al., 2009).

Mean and standard deviation of nucleotide similarity percentage between Indonesian local ducks and *Anas platyrhynchos* was 99.68±0.56%. Indonesian local ducks have similar characteristics with *Anas platyrhynchos*, or have 0% SNP percentage in population of Magelang A *(Jarakan polos*), B (*Bosokan*), E (*Kalung ciut*) and J (*Wiroko*), Tegal 1, Mojosari, Bali dan Alabio ducks.

High percentage of nucleotide uniformity between Indonesian local ducks and *Anas platyrhynchos* indicated that most Indonesian local ducks were derived or originated from *Anas platyrhynchos.* Purwantini et al. (2013) reported sequencing result namely 701 nt obtaining SNP mtDNA *D-loop* region in five Indonesian local ducks as much as 6.437±8.510% and the nucleotide uniformity percentage with A*nas platyrhynchos* was 93.594±8.225%. The different result was assumed due to different magnitude of nucleotide sequence used.

Nucleotide frequency of mtDNA D-loop region in indonesian local ducks. Result of sequencing PCR products namely 469 nt was also used to observe the Nucleotide frequency of mtDNA *D-loop* region of Indonesian local ducks using *software Maximum Likelihood Model Compute Nucleotide Composition* (Tamura and Nei, 1993) in MEGA5.1 *Beta*3 program (Tamura et al., 2011), as presented in Table 2.

Nucleotide frequency proportion in Indonesian local ducks were relatively similar to that of Anas in the world and *Cairina moschata* taken from *GenBank*. Nucleotide frequency in Anas in the world is presented in Table 3.

Table 2. Nucleotide frequency of mtDNA *D-loop* region in Indonesian local ducks

Local ducks	Nucleotide frequency of mtDNA *D-loop* region (%)				
	T(U)(*Tymin/ Urasil*)	C (*Cytosin*)	A (*Adenin*)	G (*Guanin*)	Total
Magelang A	23.8	16.2	25.7	34.3	463.0
Magelang B	23.8	16.2	25.5	34.4	462.0
Magelang C	24.2	16.2	25.1	34.6	463.0
Magelang D	23.8	16.2	25.7	34.3	463.0
Magelang E	23.8	16.2	25.7	34.3	463.0
Magelang F	23.8	16.4	25.5	34.3	463.0
Magelang G	23.8	16.2	25.3	34.8	463.0
Magelang H	24.0	16.4	24.8	34.8	463.0
Magelang I	23.8	16.4	25.7	34.1	463.0
Magelang J	23.8	16.2	25.7	34.3	463.0
Magelang K	23.6	16.5	25.1	34.8	462.0
Tegal 1	23.8	16.2	25.7	34.3	463.0
Tegal 2	23.8	16.2	25.7	34.3	463.0
White Mojosari (1)	23.8	16.2	25.7	34.3	463.0
Brown Mojosari (2)	23.8	16.2	25.7	34.3	463.0
White Bali (1)	23.8	16.2	25.7	34.3	463.0
Black Bali(2)	23.8	16.2	25.7	34.3	463.0
Alabio 1	23.8	16.2	25.7	34.3	463.0
Alabio 2	23.8	16.2	25.7	34.3	463.0
Mean population	23.82	16.25	25.55	34.39	462.89
Standard deviation	0.11	0.10	0.27	0.20	0.32

Table 3. Nucleotide frequency of mtDNA *D-loop* region in *Anas* in the world and *Cairina moschata*

Anas ducks	Nucleotide frequency of mtDNA *D-loop* region (%)				
	T(U)(*Tymin*/ *Urasil*)	C (*Cytosin*)	A (*Adenin*)	G (*Guanin*)	Total
Anas platyrhyncos	23.8	16.2	25.7	34.3	463.0
Anas zonorhyncha	23.8	16.2	25.7	34.3	463.0
Anas americana	22.7	17.6	26.9	32.8	454.0
Anas bahamensis	22.5	17.6	26.7	33.3	454.0
Anas clypeata	23.6	17.2	26.4	32.8	454.0
Anas crecca	22.8	17.5	27.1	32.6	457.0
Anas sibilatrix	22.2	18.2	26.6	33.0	455.0
Anas strepera	21.6	18.1	28.0	32.4	454.0
Anas acuta	22.7	17.4	26.2	33.7	454.0
Cairina moschata	24.2	17.4	25.1	33.3	454.0
Mean population	22.99	17.34	26.44	33.25	456.2
Standard deviation	0.83	0.68	0.83	0.67	3.71

According to Kimura (1980) nucleotide frequency recorded was A = 25.00%, T/U = 25.00%, C = 25.00%, and G = 25.00%, with 0.95 336 position on final dataset. Evolution analysis was performed in MEGA5 [1]. Tamura and Nei (1993) determined nucleotide frequency on mtDNA D-loop region of human and chimpanzee, namely A = 28.74%, T/U = 22.35%, C = 34.20%, and G = 14.71%. This analysis involved 228 nucleotide sequences obtained in final dataset. Evolution analysis was performed in MEGA5 [2]. Tu et al. (2009) reported that nucleotide composition in Beijing duck (*Anas platyrhychos)* in complete mitochondria genome was A = 29.19%, T/U = 22.20%, C = 32.81% and G = 15.80%. Different nucleotide frequency obtained was assumed due to different length and position of nucleotide used in data analysis.

Conclusion

Polymorphic genetic was found in Indonesian local duck population, indicated from relatively large SNP deviation on mtDNA D-loop region. Magelang ducks with different feather colors were relatively more polymorphic than other Indonesian local ducks. The obtained Single nucleotide polymorphism was viable genetic transverseness. Codon was included in the first, second, third and non coding. Position with gap and missing data would be eliminated, leaving marker to differ genetic characteristics among Indonesian local ducks.

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