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Breed Determination for Indonesian Local Chickens Based on Matrilineal Evolution Analysis

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Abstract: Assessing the molecular characteristic of chickens is the powerful method to differentiate chicken breeds. This study analyses the matrilineal evolution of the Indonesian local chickens based on mitochondrial DNA D-loop region to reveal their genetic diversity and phylogenetic relationships. The estimated genetic diversity of Indonesian local chickens ranged from 0.00171 to 0.01800. Six (6) groups from 67 D-loop haplotypes of Indonesian chickens are observed in this study. The Indonesian local chickens can be differentiated from Saudi Arabian, ornamental and commercial line chickens. However, maintaining genetic variation of certain chicken population will be useful to conserve local genes for future utilization.

Key words: Breed determination, Indonesian chickens, mtDNA D-loop

INTRODUCTION

Local chickens in Indonesia are classified as non chicken breed (*bukan ras/buras*) to differentiate them from commercial modified genetic chickens such as Cobb, Lohmann, Ross, Hubbard etc (FAO, 2008). Determination of chicken breeds can be done by evaluating the phenotypic and molecular characteristics of chickens. This is very important for future utilization and conservation of biological material of chickens. Based on its phenotypic characteristics, some Indonesian local chickens such as kampung (KPg), kedu (KD), nunukan (NNK), sentul (STL) are officially established as different breed by Indonesian government (Ministry of Agriculture of Republic Indonesia, 2014). However, each chicken breed is very diverse in its phenotypic traits since the intensive breeding program for certain purposes (egg, meat, dual purposes as egg and meat or ornamental purposes) of Indonesian local chickens is generally not well developed yet.

Recently, some local breeders have been starting to apply breeding and selection program on local chickens to support the high consumer demand on local chicken products. The introgression of some commercial lines (Sulandari *et al*., 2008; Jakaria *et al*., 2012; Ulfah *et al*., 2012) and exotic chickens (Sulandari *et al*., 2008) might be found in Indonesian chicken gene pool since some commercial and exotic chicken breeds were intensively imported from other countries during recent years. The highest egg producer of Indonesian local chicken, arab chicken, might been introduced from western Asia (Riztyan *et al*., 2011a,b), mainly from Saudi Arabia. However, Sulandari *et al*. (2008) found that arab chicken is identic with other Indonesian local chickens. Little data is known to reveal the identical characteristics of arab chicken sampled in Indonesia to chickens from Saudi Arabia. There is also a possibility that arab chicken in Indonesia is genetically different from chickens in Saudi Arabia.

Using chicken samples from traditional farming which had been applying uncontrolled breeding programs might influence the different result of chicken breed determination. Rusdin *et al*. (2011) showed that a high diversity of phenotypic traits of local chickens can not be used to determine chicken breed. Based on blood group analysis, Yamamoto *et al*. (1996) had difficulty to differentiate some Indonesian local chickens. Based on molecular data, recent findings (Sartika *et al*., 2004; Sulandari *et al*., 2008; Riztyan *et al*., 2011) also found that the most Indonesian local chickens observed were very close each other and could not be determined as a certain distinctive breed. This study, therefore, assess the molecular characteristic of Indonesian local chickens sampled from local breeder which have been applying intensive breeding program for food and ornamental based on matrilineal analysis of mtDNA D-loop region. This study clarify some determination of local chicken breeds in Indonesia that could be useful for future utilization and conservation.

MATERIALS AND METHODS

Chicken population and ethic statement: This study used 8 types of Indonesian local chickens: arab golden line (ARGb and ARGt), arab silver line (ARS), cemani

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Table 1: Chicken samples used in this study

Chicken	Sample code	n	Sampling location	Farming system	Utilization	HD	ð
Indonesian chicken							
Arab (golden red line)	ARGb†	20	West Java	Intensive	Egg	0.84562	0.00412
	ARGt‡	17	East Java	Intensive	Egg	0.90441	0.00691
Arab (silver line)	ARS	20	West Java	Intensive	Egg	0.87710	0.00401
Cemani	CMN	12	Center Java	Intensive	Ornamental	0.30303	0.00171
Kampung	KPGt¶	10	East Java	Intensive	Meat	0.96429	0.00846
	KPGg	10	Center Java	Scavenging	Dual purposes	0.98485	0.00787
	KPGb¶	15	West Java	Intensive	Meat	0.92513	0.00787
	KPGbd	15	West Java	Scavenging	Dual purposes	0.96667	0.01800
	KPGs	10	Sumatera	Scavenging	Dual purposes	0.96154	0.00554
	KPGsl	10	Sulawesi	Scavenging	Dual purposes	0.77780	0.00388
Black kedu	KDh	20	Center Java	Intensive	Egg	0.83330	0.00439
White kedu	KDp	18	Center Java	Intensive	Egg	0.89286	0.00597
Nunukan	NNK	10	East Kalimantan	Intensive	Meat	0.64444	0.00305
Sentul	STL	10	West Java	Intensive	Meat	0.60000	0.00677
Exotic chicken							
Bangkok	BGK§	8	West Java	Intensive	Fighting	0.85714	0.01182
Kate	KT‖	5	West Java	Intensive	Ornamental	1.00000	0.01692
Serama	SRM#	5	West Java	Intensive	Ornamental	0.00000	0.00000
Commercial chicken							
CP707	BRO	10	West Java	Intensive	Meat	-	-
Lohman brown light	L	10	East Java	Intensive	Egg	-	-
Total		235					

†ARGb and ARS are created by Trias Farm, Bogor, West Java, which have been bred for more than 10 years under itensive breeding and selection program for egg production.

‡ARGt have been bred by local chicken in East Java for egg type chicken, however, high variation of qualitative and quantitative traits were observed. Historically it was also crossed with other Indonesian chickens (KPG and KDh) and ornamental chicken (KT).

¶KPGt and KPGb have been created for meat type chicken. Historically, they were also crossed with fighting chicken and other Indonesian chicken, therefore they are diverse in qualitative and quantitative traits.

§BGK is originally from Thailand.

‖KT is a dwarf chicken, originally from China.

#SRM is a dwarf chicken, originally from Malaysia.

n: number of sample used.

HD: haplotype diversity.

ð: Nucleotide diversity. Genetic differentiation estimation: Hudson 2000, Snn (nearest neighbour statistic): 0.40592 PM test; p-value of Snn: 0.0000*** (this indicates significant genetic differentiation). Gene Flow Estimates: Hudson, Slatkin and Maddison 1992. Fst: 0.59585 Nm: 0.30 (indicates that exchange between populations is high)

(CMN), kampung (KPGb, KPGbd, KPGt, KPGg, KPGs, KPGsl), black kedu (KDh), white kedu (KDp), nunukan (NNK) and sentul (STL) which were collected from local breeders in many regions of Indonesia (Table 1). For better undestanding the contribution of exotic and commercial chickens which most abundantly kept in Indonesia, we also used fighting chickens (bangkok BGK), ornamental chicken (kate KT and serama SRM), commercial broiler CP707 (BRO) and layer Lohman Brown Light (L) which were kept in Indonesia. The genomic DNA of chickens were extracted by using combination method of phenol-chloroform (Sambrook and Russel, 2000) and DNeasy Blood Kit (Qiagen, Valencia, CA). All regulations and experiments in this study were under approval of Animal Care and Use Committee (ACUC), Bogor Agricultural University (IPB), Bogor, Indonesia (ACUC No: 17-2014IPB).

mtDNA amplification and sequencing: A pair of primer 5'-GGTTAGACCCCAAGGACTAC-3' and 5'-ATGTGCCTGACCGAGGAACCAG-3' was used to amplify a fragment 614 bp mtDNA D-loop. The PCR was performed with an initial denaturation at 95°C for 3 min, followed by 30 cycles consisting of denaturation at 95°C for 30 sec, annealing at 62°C for 30 sec, extension at 72°C for 1 min and final extension at 72°C for 5 min. The BigDye Terminator Kit on an ABI 3730xl DNA analyzer (Applied Biosystems, Foster City, CA) was used to sequence the amplicon in both directions with by using the same primer above.

Data analysis: The MUSCLE program (Edgar, 2004) in Molecular Evolutionary Genetic Analysis (MEGA) version 6 (Tamura *et al*., 2013) was initially used to align the 213 D-loop sequences of chickens sampled in Indonesia (included of sampled of BRO and L), with 37 published sequences consisted of white leghorn NC_001323 (Desjardins and Morais, 1990), AP003317 (Nishibori *et al*., 2003), barred plymouth rock AB007719 (Miyake, 1997), white plymouth rock AP003318 (Nishibori *et al*., 2003), rhode island red HQ836363 (Cho *et al*., 2010) and chicken from Saudi Arabia KC436009-KC436040 (Yacoub and Fathi, 2013). The insertion and deletion of nucleotides were discarded for further analysis. The haplotype diversity and genetic differentiation of chickens were estimated by using DnaSP software version 4.10.9

(Librado and Rozas, 2009). Phylogenetic tree was then constructed using Mr. Bayes version 3.0. 0 (Ronquist and Huelsenbeck, 2003). A general time reversible model (rates = invgamma, nst = 6) was performed in this analysis. Markov Chain Monte Carlo (MCMC) was run for one million generations each. Trees were sampled every 100 cycles from chain and visualized using FigTree version 1.4.0. (http://tree.bio.ed.ac.uk/software/figtree/). The D-loop sequence of white leghorn NC_001323 (Desjardins and Morais, 1990) was used as out group.

RESULTS

Genetic diversity:

Genetic Diversity and Haplotype Classification: A total of thirty seven variable sites were identified in this study (Table 2). The haplotype diversity and nucleotide diversity (genetic diversity) of chicken population are shown in Table 1. Scavenging chickens (KPGbd) with little or no selection program had the highest genetic diversity (0.01800) and followed by populations selected for quantitative traits of KPGt, KPGb, ARGt, STL, KDp, KDh, ARGb, ARS and NNK (0.00846, 0.00787, 0.00691, 0.00677, 0.00597, 0.00597, 0.00412, 0.00401 and 0.00305, respectively). The chicken conservation flocks of CMN had the lowest genetic diversity (0.00171).

Haplotype distribution and phylogenetic tree: Six groups from 67 haplotypes were observed in this study (Fig. 1). The representative of haplotypes of Indonesian local chickens were submitted to GenBank (accession No. KT853000-KT853016) (Approved by NCBI). Nodes with posterior probability values >50% clarify that chicken can be determined as different breed (Fig. 1). The Saudi Arabian chickens build the same group with commercial layer chicken (L) and ornamental chicken (KT). The monophyletic relatedness of barred plymouth rock (AB007719), white leghorn (AP003317) and white plymouth rock (AP003318) with ARS and NNK in group II was also observed in this study. Group III consisted of slow growing feather chicken (ARS33) and meat type chicken (BRO). Most Indonesian chickens and ornamental chickens in group VI became a sister group to white leghorn NC_001323 and rhode island red HQ836363 which formed the same group with ARGb (West Java population) and BGK in group V. Most of Indonesian local chickens in group VI, however, can't be determined as distinctive breed since they have very low posterior probabilities (<50%).

DISCUSSION

High level of genetic diversity (nucleotide diversity of 0.01800) were found in Indonesian chicken populations based 614 bp mtDNA D-loopsequences. Scavenging chickens for meat and egg production (dual purposes) posses high genetic diversity followed by intensively selected chickens for egg or meat production and conservation flocks for ornamental purpose as also mentioned by Granevitze *et al.* (2007). High level of chicken genetic diversity were also broadly reported from India (0.01800, Kanginakudru *et al.*, 2008), Vietnamese chickens (0.013, Berthouly-Salazar *et al.*, 2010), Thailand (0.0226, Pramual *et al.*, 2013) and Laotian chickens (0.014519, Kawabe *et al.*, 2014). However, low genetic diversity of Dutch (0.0063, Dana *et al.*, 2010) and African chicken (0.00745, Mwacharo *et al.*, 2011) were also reported. Since the genetic variation of local chicken in certain region might be important in considering the origin of chicken domestication, this study support the hypothesis that Indonesia was one of potential locations where multiple chicken domestication occurred together with India and other South East Asian countries (Liu *et al.*, 2006; Gongora *et al.*, 2008; Cuc *et al.*, 2011; Storey *et al.*, 2012; Miao *et al.*, 2013; Pramual *et al.*, 2013; Kawabe *et al.*, 2014).

The phylogenetic analyses based on Bayesian analysis revealed that the mtDNA D-loop haplotypes of Indonesian chickens were dispersed in 6 groups (Fig. 1). Each chicken population posses specific mtDNA haplotype. This result was also consistent with high levels of observed mtDNA D-loop variation (Table 1). In group I, KT as ornamental chicken, which might be originated from China, was grouped with commercial layer line L25 and local chicken from Saudi Arabia. This may indicate a possible exchange of genetic material between Chinese chickens, commercial lines and Arabian chickens. Historical data mentioned that China as one of chicken domestication centre in the world (West and Zhou, 1988), therefore the Chinese chickens might spread worldwide. However, further study should be done to quantify the relationship of Chinese chickens with Indonesian chickens.

Group II consisted of NNK, ARS, barred plymouth rock (AB007719), white leghorn (AP003317) and white plymouth rock (AP003318). Sartika and Iskandar (2007) suggested that NNK was created in East Kalimantan during colonial era in 18th century which might be resulted from crossing between Indonesian local chicken and Chinese chicken. Based on the phylogenetic tree (Fig. 1), this study suggested that commercial chickens might also contributed to NNK creation. This study also suggested that the ARS was established as egg producer after the creation of NNK chickens. However, we lack data about its establishment history. In this study, the ARS chickens were provided by Trias Farm, Bogor, West Java, Indonesia. The first progenitor of ARS came from arab chicken population from local breeders in East, Center and West Java in 20th century. The intensively selection for more than 10 years by Trias Farm resulted a distinctive breed of ARS as slow growing feather (ARS33, group II) and fast growing feather (ARS35, group III) with posterior

Table 2: Identified haplotype in Indonesian local chicken population based on polymorphism of mtDNA D-loop sequence

		Nucleotide position																																		
Chicken breed	Group	11	15	48	49	50	167	197	199	210	212	217	221	222	225	243	246	256	261	281	296	306	309	310	315	330	342	391	396	446	463	464	483	484	485	514	518
AB007719	II	A	T	A	C	C	C	T	T	T	C	G	C	C	A	C	C	C	C	T	A	C	T	T	T	C	C	A	C	T	T	A	C	T	T	C	-
KC436010	I	.	A	-	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	-
KC436018	I	C	.	-	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	-
KC436020	I	C	.	-	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	-
KC436030	I	C	.	-	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	-
ARS33	II	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
ARS35	III	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	-
ARGb13	V	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	T	.	T	C	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	-
NC001323	V	.	.	.	-	-	-	C	.	.	T	.	T	.	.	T	T	.	T	C	.	.	.	.	C	.	.	.	.	C	.	.	.	.	.	.	-
HQ836363	V	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	T	T	T	C	.	.	.	.	C	T	.	.	.	C	.	.	.	.	.	.	-
ARGb14	IV	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	C	.	.	.	.	.	.	-
ARGb29	VI	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	C	C	.	.	.	.	.	C	.	.	.	.	.	.	-
ARGt1	VI	-	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	C	G	.	C	C	.	.	.	.	.	C	.	.	.	.	.	.	-
ARGt18	VI	C	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	C	C	.	.	.	.	.	C	.	.	.	.	.	.	-
ARGt5	VI	C	-	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	C	.	.	.	.	.	.	C	.	.	.	.	.	.	-
ARS3	VI	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	C	C	.	.	.	.	.	C	G	A	.	.	.	.	-
Kdp907	VI	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	C	.	.	.	.	.	.	G
KPGjb20	VI	.	.	.	.	.	.	.	.	.	.	.	T	T	.	.	.	.	.	.	G	.	C	C	.	.	.	.	.	C	.	.	.	.	.	.	-
KPGb653	VI	.	.	.	.	.	.	.	.	.	.	A	T	.	.	.	T	T	T	C	.	T	.	.	C	T	.	.	.	C	C	.	.	.	.	.	-
KPGb696	VI	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	C	C	.	.	.	.	.	.	C	.	.	G	G	T	-
KPGg808	VI	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	.	C	.	.	.	.	.	T	.	C	.	.	.	.	.	-
KPGt455	VI	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	G	T	C	.	.	.	.	.	T	.	C	.	.	.	.	.	-
KPGs160	VI	.	.	.	.	.	.	.	.	.	.	.	T	.	.	.	.	.	.	.	.	.	C	C	.	.	.	.	.	.	C	.	.	.	.	.	

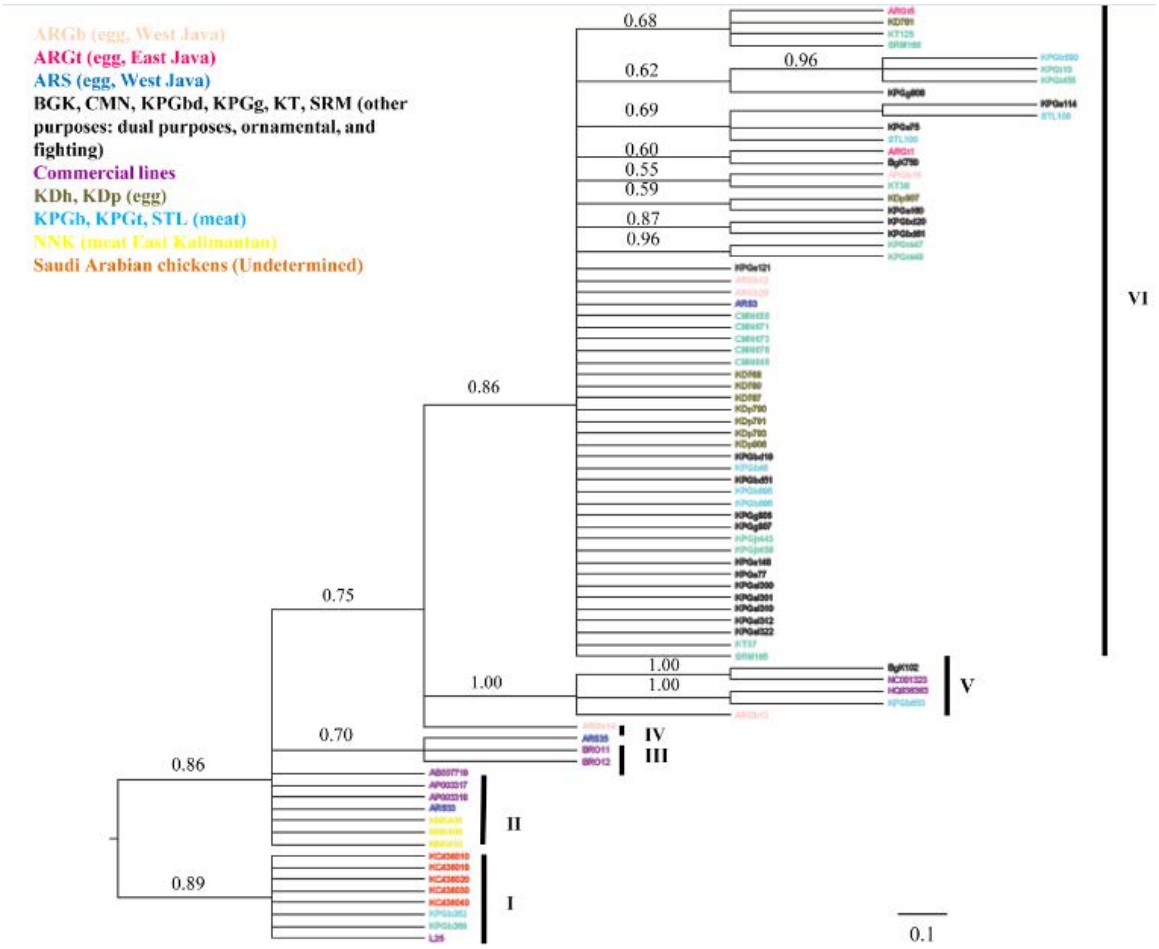

Fig. 1: A strict consensus tree of the mtDNA D-loop sequence region resulted from Bayesian analysis. Posterior probabilities are shown above the branch. The chickens were distributed to 6 groups: group I (Saudi Arabian chicken), II (NNK, ARS, commercial lines), III (ARS), IV (ARG), V (ARG and commercial lines), VI (other Indonesian local chickens and ornamental chickens)

probability of 70%. The NNK were also recognized to have slow feather gene (K gene) as described by Sartika and Iskandar (2007). Therefore, this is the first finding that explained clearly the relationship of ARS slow growing feather (ARS33) with NNK (group II). However, they are differ on egg production (250 eggs/hen/year for ARS and 40-60 eggs/hen/year for NNK). The further studies, therefore, need to be done to quantify their relationship.

Interestingly, the Bayesian analysis (Fig. 1) also revealed a distinctive group of ARS (group II, III) and ARG (group IV and V). A closer relationship of ARG to commercial line of rhode island red HQ836363 in group IV could be also interpreted by their similarity in Columbian (golden red) feather. However they were differed in shank and egg shell color. The rhode island red had white or yellow shank and brown eggshell, while ARS had black or/and green shank and white eggshell color.

The selection of chicken sex maturity also established 3 distinctive breeds of ARG14 (group IV), ARG13 (group V) and other ARG in group VI with posterior probability 100% and 86%, respectively. Historically, local breeder of arab chicken in East Java had been crossed arab chicken with exotic chicken breeds for certain traits, e.g., KT for reducing feed consumption ratio and BGK for higher body weight. Attempts also had been done by local breeders to cross arab chicken with local chickens, mainly KDh. These resulted arab chicken distributed in group VI together with other Indonesian local chickens and exotic chickens. Based on Fig. 1, we didn't find any introgression of Saudi Arabian chickens (group I) to arab chickens from Indonesia populations. The arab chicken, therefore, must be considered to have a different name since they do not have any relationship with Saudi Arabian chickens.

The majority of Indonesian local chickens carried mtDNA haplotypes in group VI (Fig. 1) together with exotic

breeds. Even though most Indonesian local chickens had low value of posterior probabilities (below 50%), every type of chicken built their own sub groups. The Indonesian chickens resulted from intensively breeding program as meat producer (KPGb, KPGt and STL) and egg producer (ARGt, KDh and KDp) separated each other with posterior probability >50%. In this study, STL were have a closer relationship with KPG than other chickens as described also by Sartika *et al.* (2004). It is also reasonable if the Indonesian black chicken (CMN) has a close relationship with KDh as the KDh is the founder population of CMN (Sartika and Iskandar 2007). Moreover, breeding history to create certain chicken lines by crossing Indonesian local chicken with exotic chickens resulted harbour mtDNA haplotypes of exotic chickens in group VI. Further intensively breeding program for pure line establishment of Indonesian local chicken might be helpful for further breed determination of those chickens (Sartika, 2012). Genetic variation is important for chicken adaptation, selection and breeding program for certain trait improvement. As maintaining genetic variation of chicken population can counter the effect of genetic drift (Berthouly-Salazar *et al*., 2010), therefore conservation of genetic pool of Indonesian local chickens will be also very important to retain local genes for future utilization.

Conclusion: Matrilineal evolution analysis using mtDNA D-loop region indicated a clear genetic differentiation between NNK, ARS, ARG, KPG and other Indonesian local chickens, commercial line chickens and Saudi Arabian chickens. The history of chicken breeding and selection system applied by farmer must be further considered to determine chicken breeds. Further efforts to conserve genetic diversity of Indonesian local chickens must be focus on local populations which are distinctive from exotic and commercial breeds.

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