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Phylogenetic relationships among domestic waterfowl using cytochrome C oxidase I Gene Variations in mtDNA

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Abstract. The current study aimed to analysis of genetic diversity and relationships of Indonesia domestic waterfowl based on cytochrome C oxidase I gene sequence. Nucleotide sequences of COI gene of domestic waterfowls in this research together with other waterfowls isolates from GenBank were aligned with ClustalW of MEGA 6.06 program. Estimation of genetic distance and phylogenetic tree construction were analyzed with Neighbor-Joining method and calculation of distance matrix with Kimura 2 -parameter. Result of phylogenetic analysis indicated that all waterfowls form three distinct sublineages. Two lineages is located in Indonesia cluster and one lineage in world cluster. Based on the neighbor-joining (NJ) method, waterfowl species used in this study can be well differentiated. Based on our results, waterfowl species can be discriminated with *COI* gene sequences, and this can be effectively used for an appropriate conservation program for the domestic waterfowl breeds in Indonesia.
Key words: *COI* gene, domestic waterfowl, mtDNA

Introduction

The variation in mtDNA has been investigated to determination of phylogenetic relationships among the domestic duck species at the molecular level. The mitochondrial genome is maternally inherited. The sequences of mtDNA have been extensively used in biodiversity studies of vertebrates (Baker & Marshall 1997; Mindell et al. 1997; Moore & Defilippis 1997; Wayne et al. 2002). Compared with nuclear genome, using mitochondrial genome have advantages which has faster nucleotide substitutions and does not allow recombination for phylogenetic analysis (Aquadro & Greenberg 1983; Lansman et al. 1983; Cann et al. 1984). Usually, coding genes of the mtDNA are used for phylogenetic studies to distinguish species (Moore & Defilippis 1997), whereas the COI (Cytochrome c oxidase subunit I) gene is considered more suitable for interspecific population studies (Hebert et al. 2003; Kevin et al. 2009).

Cytochrome c enzyme is a small, highly conserved, heme-containing bifunctional protein. It has been studied extensively, not only for its role in electron transport, but also for its role in apoptosis, it releases from the mitochondria in response to specific apoptotic stimuli (Wang 2001). Cytochrome c oxidase is an oligomeric enzymatic complex located in the inner membrane of mitochondria. Cytochrome c oxidase consists of three large primarily catalytic subunits encoded by the mtDNA and 10 smaller subunits encoded by nuclear genes. The nuclear encoded subunits are thought to modulate enzyme function. In vertebrates, cytochrome c oxidase is composed of 13-subunits, and a further 30 proteins are required for proper cytochrome c oxidase assembly (Ettickan et al. 2004). In eukaryotes, this enzyme complex is located in the inner membrane of mitochondri. It is considered to be a major site of mitochondrial oxidative phosphorylation regulation. This rate-limiting enzyme is also implicated in the production of reactive oxygen species (ROS) under oxidative stress conditions (Lee et al. 2001; Lee et al. 2003; Vijayasarthy et al. 2003). COI encodes an important enzyme involved in the oxidation phosphorylation pathway to energy production.

The duck industry in Indonesia has dramatically increased. The conservation of the genetic resources is very important not only for conservation perspectives but also the possible use of the wild breeds in the future. The waterfowl are considered to be a natural reservoir for AIV; they can carry various subtypes of AIV with little or no impact on their health (Webster et al. 1992). Moreover, the HPAI viruses have rarely been isolated from waterfowl even on farms experiencing HPAI outbreaks in poultry (Swayne & Suarez 2000). However, there were deaths of many waterfowl recorded in the outbreaks of H5N1 started in Hong Kong in 2002 (Sturm-Ramirez et al. 2004). In addition, Sakoda et al. (2010) successfully isolated the highly pathogenic avian influenza (HPAI) subtype H5N1 from dead wild waterfowl found in Mongolia. Therefore, it is very important to identify the origin and genetic relationships of domestic waterfowl in Indonesia. In this study, the *COI* gene is applied for designing breeding and conservation strategies for the Indonesia domestic waterfowl species.

Materials and Methods

Specimen Collection and DNA Extraction

DNA from a total of 6 domestic waterfowl (2 duck, 2 muscovy duck and 2 swan) were extracted from blood. Blood samples were collected from wing veins in an EDTA contained tube was used. DNA was extracted according to guidelines using the nucleospin Blood DNA extraction kit . Extracted DNA was stored at 4°C until use.

PCR Amplification and DNA Sequencing

Extracted genomic DNA was used for the amplification of the *COI* gene in mtDNA. One forward primer (5'-TTCTCCAACCACAAAGACATTGGCAC-3') and one of two reverse primers (R1: 5'-ACGTGGGAGATAATTCCAAATCCTG-3', R2: 5'-ACTACATGT GAGATGATTCCGAATCCAG-3') were used to amplify the partial *COI* gene (Hebert *et al.*, 2004). PCR was performed in a thermal cycler with an initial denaturation step at 95°C for 5 min followed by 35 cycles of 30 sec at 95°C,30 sec at 60°C, 30 sec at 72°C and a final extension step at 72°C for 7 min. Sequencing of all PCR products was done by 1st BASE Singapore.

Data Analysis

Waterfowl *COI* sequence data were aligned using the ClustalW from MEGA 6.06 software program. Neighbor-Joining (NJ) phylogenetic tree was conducted by using MEGA software version 5 (Tamura et al. 2011). Also, nucleotide divergence, pair-wise distance using Kimura 2 parameter (K2P) model was calculated. For the analysis, published sequence of Anas, Cairina and Cygnus were downloaded from the Genbank (Table 1).

No	Spesies	No. Acces Genbank
1	*Anas acuta*	GU571714.1
2	*Anas americana*	DQ434269.1
3	*Anas bahamensis*	JQ174014.1
4	*Anas clypeata*	GU571716.1
5	*Anas crecca*	JN703196.1
6	*Anas sibilatrix*	FJ027108.1
7	*Anas strepera*	JN703210.1
8	*Cairina moschata*	JX160010.1
9	*Cygnus cygnus*	GU571854.1
10	*Cygnus columbianus*	GU571852.1

11	*Cygnus olor*	GU571856.1
12	*Cygnus buccinator*	AY666349.1
13	Cygnus melancoryphus	KM896299.1

Result and discusion

In this research, the DNA primers, birdF1 and birdR2, amplified a 150-bp region of the mitochondrial cytochrome oxidase subunit I gene from all domestic waterfowl (Fig 1). The results of this study differ from previous research. Research of Jin et al (2012) showed that amplification products of COI gene with the Bird-F1 and Bird-R2 primer pairs is 749bp. The difference results of amplification is probably due to the primer pairs is not compatible for waterfowl species in Indonesia. Need to be further explored compatible primer for domestic waterfowl in Indonesia.

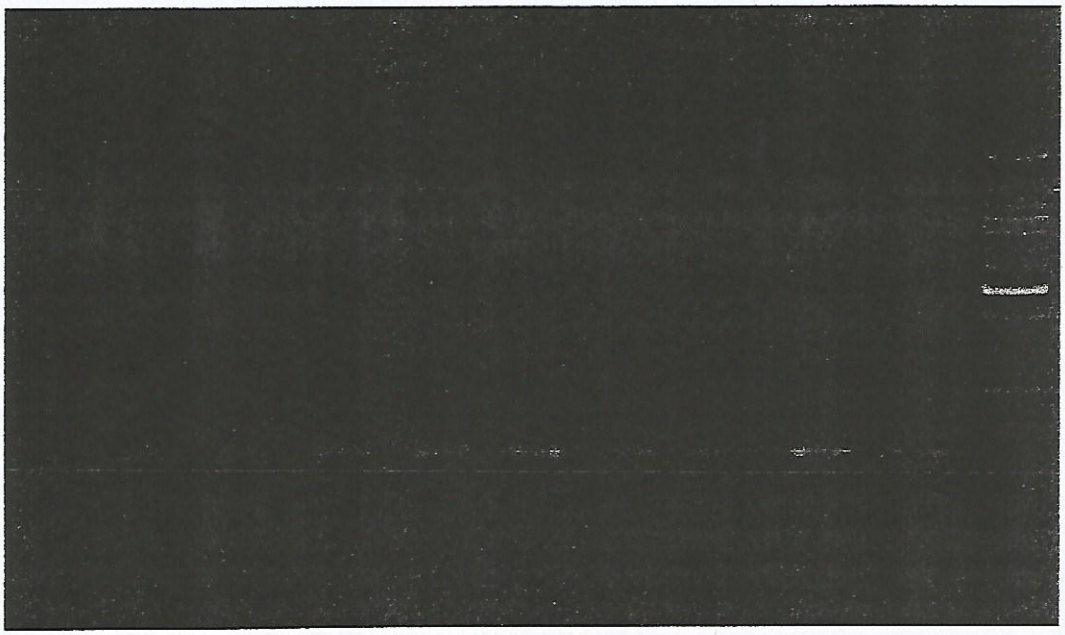

Figure 1. The visualization of the PCR amplification product in 0.8% agarose gel electrophoresis. Amplification using Bird-F1 and Bird-R2 primer pair, the positive-tested samples showed 150 bp PCR product. Lane 1,2,4,5,6 are duck, lane 3 and 10 are goose, lane 7-9 are muscovy duck. M= Marker of 100bp.

Nucleotide sequences of COI gene of domestic waterfowls in this research together with other waterfowls isolates from GenBank were aligned with ClustalW of MEGA 6.06 program. Estimation of genetic distance and phylogenetic tree construction were analyzed with Neighbor-Joining method and calculation of distance matrix with Kimura 2 -parameter. Result of phylogenetic analysis indicated that all waterfowls form three distinct sublineages. Two lineages is located in Indonesia cluster and one lineage in world cluster. Based on the neighbor-joining (NJ) method, waterfowl species used in this study can be well differentiated from another species in the world. The results of phylogenetic analysis shows that the ducks (BSB and BMB) not clustered with other Anas (species *A. americana, A. sibilatrix, A. strepera, A. acuta, A. bahamensis, A.crecca, A. clypeata*). Anas species from the GenBank data is not clustered in one group too. Muscovy duck (in the java language: entok) in this study (ESJ and EMB) are not clustered in one group with *Cairina moschata* (data from GenBank). Goose in this study (AMB) are not clustered in one group with *Cairina moschata*

(data from GenBank). Goose in this study (AMB) are not cluster with other Cygnus (*C. Olor, C. Columbianus, C. Buccinator, C. Cugnus, C. Melancoryphus*). Cycnus species from the GenBank data is not clustered in one group (Fig 2).

Based on our results, waterfowl species used in this study can be well differentiated with *COI* gene sequences, and this can be effectively used for an appropriate conservation program for the domestic waterfowl breeds in Indonesia. The results of Bondoc (2013) research indicated that cytochrome C oxidase I (COI) gene sequence can be effective to identify and differentiate between poultry families and between breeds and strains in most poultry species especially chickens, quails, turkey, ducks and pigeons. More COI sequences however should be determined from different poultry specimens to improve the reliability of using DNA barcodes to confirm the breed origin of an animal (Bondoc 2013).

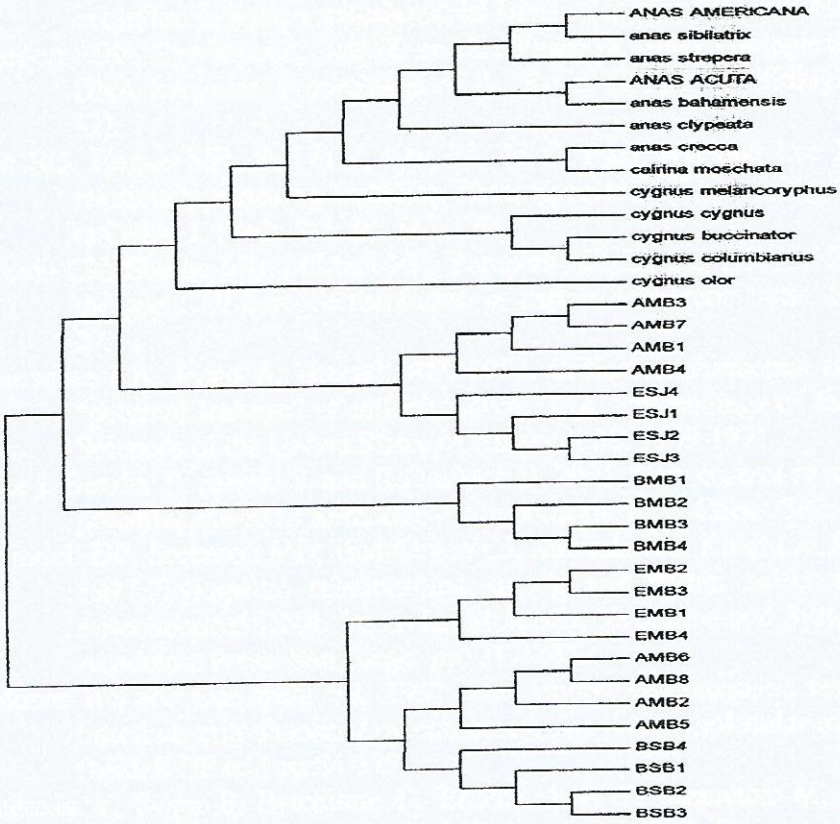

Fig 2. Phylogenetic tree construction of Indonesian domestic waterfowls in this research together with other waterfowls isolates from GenBank. All waterfowls form three distinct sublineages. Two lineages is located in Indonesia cluster and one lineage in world cluster

Conclusion

The partial mitochondrial DNA (mtDNA) *COI* gene sequences of waterfowl species used in this study can not be well differentiated. Domestic waterfowl species in this research can not be discriminated with Bird-F1 and Bird-R2 primer pair for *COI* sequences. Furthermore, should be exploration of primer pair for COI gene of domestic waterfowl in Indonesia.

Reference

Aquadro CF and Greenberg BD. 1983. Human mitochondrial DNA variation and evolution: Analysis of nucleotide sequences from seven individuals. Genetics. 103: 287-312.

Baker AJ and Marshall HD. 1997. Mitochondrial control region sequences as tools for understanding evolution. In: Avian MolecularEvolution and Systematics (Midell DP ed.) Academic Press. San Diego. pp. 51-82..

Bondoc OL. 2013. Genetic diversity and relationship of common poultry breeds and strains (Class Aves) in the Philippines based on cytochrome C oxidase I gene sequence. Asia life sciences *22*(1): 21-39

Ettickan B, Lenka N, Prabu SK, Fang JK, Wilkinson F, Atchison M, Giallongo A, and Avadhani NG. 2004. Regulation of Murine Cytochrome c Oxidase Vb Gene Expression during Myogenesis. J. Biol. Chem.279(34):35242-35254

Cann RL, Brown WM and Wilson AC. 1984. Polymorphic sites and the mechanism of evolution in human mitochondrial DNA. Genetics 106: 479-499.

Hebert PDN, Cywinska A, Ball SL and DeWaard JR. 2003. Biological identifications through DNA barcodes. Proceedings of the Royal Society B: Biological Sciences 270: 313-321

Jin SD, Hoque MR, Seo DW, Kim IK, Jo C, Paek WK and Lee JH. 2012. Phylogenetic relationships among Dabbling Duck Species in Korea using *COI* Gene Variations in mtDNA. J. Poult. Sci. 49: 163-170

Kevin CK, Sharon MB, Mikhail VK, Yaroslav AR, Eugeny AK and Hebert PD. 2009. Filling the gap-*COI* barcode resolution in eastern Palearctic birds. Frontiers in Zoology. 6: 29.

Lansman RA, Jcand A, Huettel MD. 1983. Critical experimental test of the possibility of "paternal leakage" of mitochondrial DNA. Proceedings of the National Academy of Sciences of the United States of America, 80: 1969-1971

Lee I, Bender E, Arnold S, and Kadenbach B. 2001. New control of mitochondrial membrane potential and ROS formation – a hypothesis. Biol. Chem. 382: 1629–1636.

Mindell DP, Sorenson MD, Huddleston CJ, Miranda HC, Knight A, Sawchuk SJ and Yuri T. 1997.Phylogentic relationships among and within select avian orders based on mitochondrial DNA. In: Avian Molecular Evolution and Systematics (Midell DP ed.) Academic Press. San Diego. pp. 211-247..

Moore WS and Defilippis VR. 1997. The window of taxonomic resolution for phylogenies based on mitochondrial cytochrome b. In: Avian molecular evolution and systematics (Midell DP ed.) pp.84-119.

Sakoda Y, Sugar S, Batchluun D, Erdene-Ochir T, Okamatsu M, Isoda N, Takakuwa H, Tsuda Y, Yamamoto N, Kishida N, *et al.* 2010. Characterization of H5N1 highly pathogenic avian influenza virus strains isolated from migratory waterfowl in Mongolia on the way back from the southern Asia to their northern territory. Virology. 406: 88-94

Swayne DE and Suarez DL. 2000. Highly pathogenic avian influenza. Revue Scientifique et Technique. 19: 463-482

Sturm-Ramirez KM, Ellis T, Bousfield B, Bissett L, Dyrting K, Rehg JE, Poon L, Guan Y, Peiris M. and Webster RG. 2004. Reemerging H5N1 influenza viruses in Hong Kong in 2002 are highly pathogenic to duck. Journal of Virology. 78: 4892-4901.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M and Kumar S. 2011. MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. Molecular Biology and Evolution 28: 2731-2739

Vijayasarthy C, Damle S, Prabu SK, Otto CM, and Avadhani NG. 2003. Adaptive changes in the expression of nuclear and mitochondrial encoded subunits of cytochrome c oxidase and the catalytic activity during hypoxia. Eur. J. Biochem. 270 (5): 871–879

Wang X. 2001.The expanding role of mitochondria in apoptosis. *J. Genes Dev*. 15:29-22

Wayne D, Ferguson JWH and Paulette B. 2002. Characterization and Evolution of the Mitochondrial DNA Control Region in Hornbills (Bucerotiformes). Journal of Molecular Evolution. 54: 794-806.

Webster RG, Bean WJ, Gorman OT, Chambers TM. and Kawaoka Y. 1992. Evolution and ecology of influenza A viruses. Microbiological Reviews. 56: 152–179