

# Goose and Muscovy

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## Mitochondrial *D-loop* sequence of domesticated waterfowl in Central Java: goose and muscovy duck

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**Abstract.** This study aims to determine the genetic characterization of domesticated waterfowl (goose and Muscovy duck) in Central Java based on a *D-loop mtDNA* gene. The *D-loop* gene was amplified using PCR technique by specific primer and sequenced using dideoxy termination method. Multiple alignments of *D-loop* gene obtained were 710 nucleotides at position 74 to 783 at the 5' end (for goose) and 712 nucleotides at position 48 to 759 at the 5' end (for Muscovy duck). The results of the polymorphism analysis on *D-loop* sequences of muscovy duck produced 3 haplotypes. In the *D-loop* gene of goose does not show polymorphism, with substitution at G117A. Phylogenetic trees reconstructions of goose and Muscovy duck, which was collected during this research compared with another species from *Anser*, *Chairina* and *Anas* was generated 2 forms of clusters. The first group consists of all kind of Muscovy duck together with *Chairina moschata* and *Anas*, while the second group consists of all geese and *Anser cygnoides* the other. The determination of Muscovy duck and geese identity can be distinguished from the genetic marker information. Based on the phylogenetic analysis, it can be concluded that the Muscovy duck is closely related to *Chairina moschata*, while geese is closely related to *Anser cygnoides*.

### 1. Introduction

Biodiversity in agriculture consist of domesticated animals that can be used by human to produce foods, stuffs and other products. More than 40 species of animals contribute in the chain livestock that shaped by a history of human kind in domesticated animal. Selection pressure from environmental stress, reproduction control proses, and farm shaping for consuming has produced various kind of breeding technic that inheriting stable gene in animals off spring [1].

Domestication is a complex and sequentially processes that changes animal behavior and morphological characteristic, makes it different from the ancestor [2, 3]. The history of domestication is possibility related with human needs of hunting and taming wild animals [2]. It is only few species can be domesticated until now. From 10.000 species of fowl, only 10 species (chickens, domestic



ducks, Muscovy ducks, domestic geese, guinea fowl, ostriches, pigeons, quails, and turkeys) that has already domesticated. Current research has stated that, the diversity of poultry in the world today is declining rapidly influencing uncharacterized the uniqueness of the genetic resources. When a breed of poultry or its population becomes extinct, the contribution of uniqueness of its adaptation capability is lost. Several mutually interacting genes, as well as the results of complex interactions between genotype and environment usually control the uniqueness of such adaptations [1].

The geese and muscovy duck are two species of domestic waterfowl in Indonesia. The population of both species is less than ducks and chickens population. Smaller populations are more susceptible in decreased of poultry's genetic diversity due to inbreeding effects [4] and the fixation of certain alleles in population. This effect results in increasing monomorphic waterfowl and decreasing its ability to evolve or adapt to environments changing that will lead a break of gene flow and increase genetic drift [4]. One effort that can be applied to prevent species decline is by gene characterization. A gene characterization contributes to the success of population conservation. Therefore, gene characterization of geese and Muscovy duck is an important step that must be conducted to obtain an efficient management of genetic resources [5]. Genetic characterization is an important aspect to maintain breed quality and manage genetic resources [6].

A mitochondria DNA (mtDNA) is known for having high-acquired mutation rates, which are 10 times higher than nuclear genomic DNA. It is commonly believed that high mutation rates of mtDNA are caused by lack of protective histones protein, inefficient DNA repair systems and continuous exposure of oxygen radicals from oxidative phosphorylation [7]. As demonstrated in this study, recent developments in molecular techniques have resulted in an abundance of data on genetic characterization from DNA analysis.

The data will offer better understanding of genetic variation within and between domesticated waterfowl in the nature. For conservation purposes, study of the genetic characterization between domesticated waterfowl is very important. Adaptation ability to their different environment conditions may have resulted in a unique combination of specific alleles to certain breeds that difficult to recreate [8]. The breeds that are very different to others may need to be conserved, since the carried genes and its combinations may be useful to agriculture and livestock improvement in the future. The successfully identified mitochondria will be stored in The Cell Bank as a reference of genetic resources and may strongly support identification of animals in the future analysis of mtDNA regarding phylogenetic studies.

The mtDNA analysis uses in phylogenetic and domestication studies and displays a high level of genetic variation [9-11]. The mitochondrial genome of animals is a better target for analysis than the nuclear genome because of its lack of introns, its limited exposure to recombination and its haploid mode of inheritance [12]. Past phylogenetic work has often focused on mitochondrial genes encoding ribosomal (12S, 16S) DNA, but their use in broad taxonomic analyses is constrained by the prevalence of insertions and deletions (indels) that greatly complicate sequence alignments [13]. Generally, mtDNA has become genetic markers for individuals identification and characterization. It is because of mtDNA structure is more specific per each individual than nuclear DNA.

In this study, mtDNA of the domesticated goose and muscovy duck breeds at Central Java were analyzed to provide a genetic based tool for effective conservation programs. The investigations was conducted using two mtDNA markers for robust and powerful genetic diversity for control region (*D-loop*) of mtDNA. It is because of its high mutation rate [14, 15], lack of recombination and maternal inheritance, a very useful marker system for population and evolutionary biology.

## 2. Methods

The waterfowl used in this study were local muscovy duck (*Chairina moschata*) and geese (*Anser cygnoides*) from Central Javanese poultry farms. The DNA was collected from wing's feather follicle of 5 muscovy duck and 5 geese. It is only calamus and the rachis about 4-5 cm which is filled with



marrow and tissue. The genomic DNA was extracted using gSYNC™ DNA Extraction Kit from (Geneaid) as instructed by the manufacturer.

The primer pair consists of forward primer DL-AnasPF (L56) 5'-GTTGCGGGGTATTGTTA-3' and reverse primer DL-AnasPR (H773) 5'-CCATATACGCCAACCGTCTC-3'. The *D-loop* gene amplification was performed using KAPA 2G HotStart Fidelity Mix Kit (Kapabiosystems). A final volume of 20 µl of DNA solution for PCR, consist of 12.5 µl of ready mix; 1.5 µl of forward primer; 1.5 µl of reverse primer, 7.5 µl of ddH<sub>2</sub>O, and 2.0 µl of DNA sample. There were three phases of PCR to amplify the DNA. They were denaturation step for 5 min 94°C, A total of 35 amplification cycles were adapted to 94°C/ 30 sec for denaturation, 56.1°C/ 45 sec for annealing, and 72°C/ 1 min for extension. After cycles had done, a final extension adapted at 72 °C for 5 minute. Optimal PCR conditions were adjusted with the gradient amplification process. 1.2% agarose gel visualized the expected 712 bp of PCR product. After it was collected enough concentration, the PCR product was sequenced using dideoxy termination method with ABI automatic sequencer (Applied Biosystems) using forward and reverse primers by 1<sup>st</sup> BASE Pte Ltd, Singapore via Genetika Science Indonesia Ltd.

The sequences were aligned by Clustal W of Molecular Evolutionary Genetics Analysis (MEGA) software version 6.06. A BLAST search in the NCBI database was used to determine their homology with the GenBank data. All nucleotide sequence were submitted to GenBank with an accession number. Identical sequences were considered as the same haplotype. Nucleotide sequences of *D-loop* gene of mtDNA from domesticated waterfowl at Central Java in this research together with other Chairina, Anser and Anas isolates from Genbank were aligned with ClustalW of MEGA-6.06 program [16]. Estimation of genetic distance and phylogenetic tree construction were analyzed with Neighbor-Joining method and calculation of distance matrix with Kimura 2-parameter model, and the bootstrap value was 1,000. At the same time, we selected the complete sequences of *Chairina moschata* and *Anser cygnoides* from GenBank as controls.

## 1 Results and discussion

Each PCR reaction showed clear images without extra contaminated DNA bands. This result indicated that all steps of the PCR process is going well. Optimization of annealing temperature in the PCR process was performed at a temperature of 56.1°C. By using of annealing temperature at 56.1°C, the PCR produced a clear band for target DNA fragment of mtDNA *D-loop*.

Based on the BLAST analysis, indicated that all samples of geese from Central Java farm had 99-100% homology with *Anser cygnoides* (GenBank Acc Number KU211647.1; KP943133.1; KP881611.1; KP 026178.1; KJ794189.1; KJ794188.1; KJ778677.1). Then the sequences generated from the amplified PCR products of Muscovy duck had 99-100% homology with the corresponding sequences of *Chairina moschata* (GenBank Acc Number EU431185.1; EU755254.1; AY112952.1; L16769.1) from Genbank data. All of the obtained sequences of domestic geese from Central Java were submitted to GenBank under accession number from KX826789 to KX826793 while sequences of domestic Muscovy duck were submitted under accession number KX826794 to KX826798.

The sequencing of mtDNA *D-loop* nucleotides from PCR product were performed with two primers. Multiple alignments of geese *D-loop* gene obtained was 710 nucleotides at position 74 to 783 at the 5' end. Compare with the reference sequence (GeneBank acc. number: KU211647.1), All samples of geese's *D-loop* gene showed that 1 base in the gene is haplotype with altogether 1 base substitution sites within 710 bp of sequence. Substitution were detected in G117A.

The absence of *D-loop* gene polymorphism, may not yet represent all domesticated geese in Central Java, due to limited sample size. The small amount of sample size this study, caused by low number of domestic geese population. Further research is needed to conduct using more samples, with more areas Conservation status of goose (*Anser cygnoides*) at the International Union of the Conservation of Nature (IUCN) red list of threatened species, are vulnerable. A vulnerable species, which is categorized by IUCN is likely become an endangered species, unless circumstances of its

survival and reproduction process can be improved. In these circumstances, the conservation initiatives of geese and Muscovy duck may only succeed where mutual relation of both animals and humans is considered [17, 18]. To reach successfully in conservation program, it is needed to ensure a beneficial value of poultry to local people connected to conservation initiatives [18-20]. Whilst the easiest indicator of it, is when goose population in the poultry farms increase from very small amount [18,21].

The domesticated geese are managed for over 6,000 years ago, making them the one of the first domesticated poultry. They are capable of rapid growth, disease resistance, and high liver lipid storage capacity, and can be easily fed with coarse fodder. After survive from the egg and juvenile stages, the adult geese can potentially life until 20 years, with an average lifespan is 9 years [22], this gives opportunities to a long reproductive period during the goose's life. Compared with other terrestrial poultry (for example, chicken and turkey), waterfowl, such as ducks and geese possess uniquely favorable economic traits and survival advantages. First, they prove a low susceptibility to certain avian viruses, presenting little or no symptoms while still acting as a virus carrier, making them a natural storehouse for certain avian viruses [22-24]. Second, compared to other birds, the goose liver has a high capacity for fat accumulation, although geese do not normally develop liver fibrosis or necrosis [25].

Multiple alignments of muscovy duck's *D-loop* gene was obtained using 712 nucleotides, from the first position is 48 to 759 base position at the 5' end. A total number of six variable sites is identified. Based on this polymorphic sites, all samples form 3 haplotypes was aligned (Table 1). The DNA base insertions are detected in two sequences (110 and 320 nucleotide number). This could be a reasonable explanation that a quite high sharing rate of haplotypes between breeds of Muscovy duck was determined, but it was too limited sample using in our study to be concluded as representative animals examined.

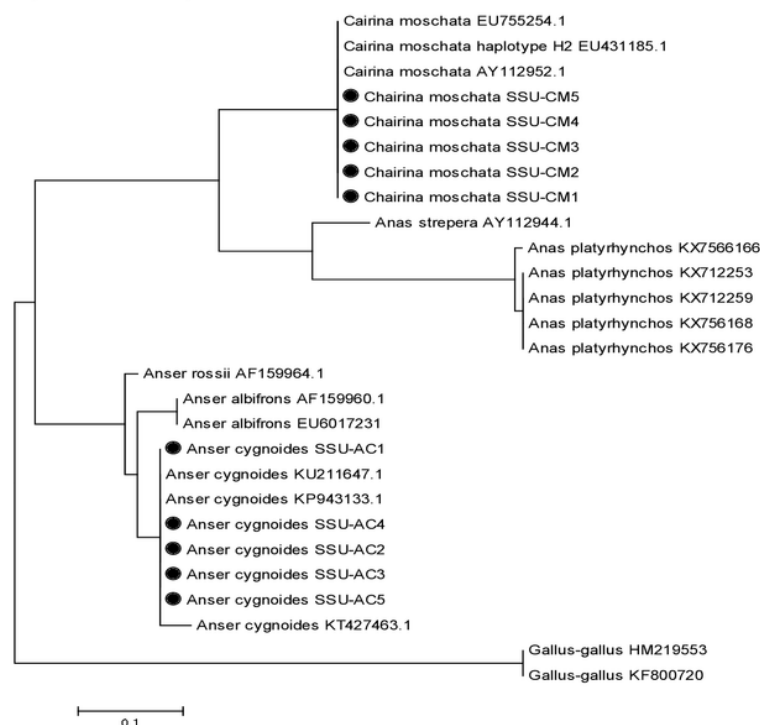
Conservation status of *Chairina moschata* at the IUCN red list of threatened species are LC (least concern). A least concern (LC) species is a species which has been categorized by the IUCN as evaluation in several period and population based calculation. Based on the IUCN calculation they not qualified to be categorized for any other status, such as they do not qualify to be placed in threatened, near threatened, or (before 2001) conservation dependent. Another characteristic of Muscovy duck is obviously expressed sexual dimorphism in the body weight. Yakubu *et al.* [26] reported that the canonical discriminant analysis on body weight and primary linear body measurements of African Muscovy ducks revealed that wing length was the most discriminating variable between the sexes, followed by body weight, neck circumference, total leg length, body length and foot length respectively [26]. The differences between sexes were significant between 7 and 12 weeks of age and at 14 weeks of age. The differences of Muscovy duck sexual dimorphism due to differences in circulating GH and IGF-I levels between sexes [27].

**Table 1.** Muscovy duck haplotype sequence of *mtDNA D-loop* variable region compared to the sequence GenBank EU755254.1

Haplotype	Position of nucleotide variable					
	66	76	110	260	295	320
GenBank EU755254.1 (48-759)	C	C	-	A	A	-
Haplotype A (SSU-CM1)	-	-	C	G	G	A
Haplotype B (SSU-CM2 and SSU-CM4)	C	C	C	G	G	A
Haplotype C (SSU-CM3 and SSU-CM5)	T	A	C	G	G	A

The reconstruction of phylogenetic trees of domesticated waterfowl with another species from genus *Anser*, *Chairina* and *Anas* was developed two clusters (Figure 1). The first cluster consists of all kinds of muscovy duck in this research with another *Chairina moschata* and *Anas*. The second cluster consists of all geese in this research with *Anser cygnoides* and another *Anser*. In the first group, all

waterfowl form 2 distinct sub lineages. The first sub cluster consists of all *Chairina*, and the second sub cluster consists of all accessed *Anas* from GenBank. The proximity pattern is characterized by high bootstrap value of 100%. With the result, all muscovy duck sample from central Java is grouped in *Chairina moschata* cluster in trials with 1000 bootstrap replications will show the results (positions) are the same. Then, all geese sample in this research is grouped in *Anser cygnoides* cluster. This result indicates that the muscovy duck in Central Java is closely related to *Chairina moschata*, while domesticated geese in Central Java is closely related to *Anser cygnoides*. Susanti *et al* [28] also determine the variation of Central Javanese duck using mitochondrial D-Loop genes. The whole mtDNA sequences are needed to estimate the genetic relationships among breeds, to characterize the breed specificity, and to identify individuals.



**Figure 1.** Neighbour-joining tree with 1000 bootstrap replication for domesticated waterfowl (●) and other *Chairina*, *Anser* and *Anas* breeds from GenBank based on *D-loop* gene

#### 4. Conclusion

The results of the polymorphism analysis on *D-loop* sequences of muscovy duck produced 3 haplotypes. In the *D-loop* gene of goose does not show polymorphism, with substitution at G117A. The determination of Muscovy duck and geese identity can be distinguished from the genetic marker information. Based on the phylogenetic analysis, it can be concluded that the Muscovy duck is closely related to *Chairina moschata*, while geese is closely related to *Anser cygnoides*.

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