The Use of DNA Barcodes in the Evolutionary Analysis of Domestic Breeds and Strains of Chicken (*Gallus gallus domesticus*) in the Philippines

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DNA barcodes (cytochrome c oxidase subunit I or COI in the mitochondrial genome) were used to differentiate 31 domestic chicken breeds and strains (*Gallus gallus domesticus*) and 25 red jungle fowls (*Gallus gallus philipensis* Hatchisuka) in the Philippines. Evolutionary analyses using Kimura 2-parameter model in MEGA5 and the Neighbour-Joining method that created a bootstrap consensus tree inferred from 1000 replicates indicated the existence of two evolutionary clades.

Based on 610 COI sequences, overall genetic diversity among domestic chickens was 50.6%. Average evolutionary divergence over sequence pairs within chicken groups was highest among 11 standard breeds (71.4%), followed by 7 native chickens (51.0%), 10 fighting cocks (38.3%), and lowest among 3 commercial hybrid chickens (2.5%). Our analyses indicated that DNA barcodes can effectively identify and differentiate chicken breeds and strains, but not distinguish between commercial hybrid chickens.

DNA barcoding is recommended to complement recently-developed molecular tools capable of calculating genetic distances between important chicken breeding populations and to determine those needing more detailed phylogenetic analysis. DNA barcoding, together with phenotypic performance data and history of populations, is recommended to describe the existing resources and should provide reliable guidelines for conservation decisions and for the design of local breeding programs for native chickens.

**Key Words:** DNA barcodes, domestic chicken breeds and strains, evolutionary analysis, genetic diversity

**Abbreviations:** COI – cytochrome c oxidase subunit I, K2P model – Kimura 2-parameter model, NJ tree – Neighbour Joining tree, MEGA5 – Molecular Evolutionary Genetics Analysis 5 software program

**INTRODUCTION**

In the Philippines, chickens (*Gallus gallus domesticus*) are the main source of poultry meat and eggs and are also popularly bred for cockfighting. The total chicken inventory in 2011 was 164.9 million head, of which 45.9% accounted for native or village chickens raised in backyard farms, 35.3% broilers and 18.8% layers. A total of 1,353.1 metric tons of chicken meat and 387.3 metric tons of eggs were produced in 2010 (Bureau of Agricultural Statistics 2012).

Native chickens are commonly raised under the free-range system or in semi-confinement for their meat and eggs and to provide extra income for many smallholder farmers. Most populations of native chickens have been subjected to little or no deliberate selection for higher productivity (Bondoc 1998). As in many developing countries (Tadelle et al. 2000; Safalaoh 2001; Besbes et al. 2007), locally adapted populations are often maintained in small populations and the loss of these genetic resources is still happening by replacement with modern industrial stocks. Most of these indigenous breeds are not well characterized, and monitoring them as poultry genetic resources will be difficult.

In contrast, commercial broiler and layer production is entirely dependent on imported grand parental stocks in the form of day-old chicks or hatching eggs from the United States, Canada, Netherlands, Israel, Thailand, Japan and Australia (Bondoc 2008). Commercial broilers as in many parts of the world are four-way crosses with paternal grandparents used as synthetic lines derived in part from White Cornish. The maternal grandparents are
also synthetic lines based heavily on White Plymouth Rock. Various strains have also been selected for rapid growth, early feathering, and good feed conversion. On the other hand, white egg layers virtually are White Leghorns comprising three-way or four-way crosses of selected grandparent lines. The brown egg layers are also bred in the same way as the white egg layers with a broader genetic base utilizing the Rhode Island Red, Barred Plymouth Rock, Australorp, and New Hampshire (Crawford 1990).

Fighting cocks are bred especially for sport purposes and consist of strains whose names originated from people that performed well in the cockpits, with the birds they made themselves through selective breeding. Strains are broken down further by other breeders who did well with a particular strain, which in turn, had a version of that strain named after them.

Using DNA barcodes (i.e., cytochrome c oxidase subunit I or COI gene of the mitochondrial genome) initially proposed as a standard for species identification (Hebert et al. 2003), this study analyzed the evolutionary relationships, genetic diversity and distances among standard chicken breeds, commercial hybrids, native/indigenous strains, and fighting cocks commonly found in the Philippines. The practical applications of DNA barcoding in local programs for the genetic improvement and conservation of native chickens are also discussed.

MATERIALS AND METHODS

The taxonomic classification of domestic chickens is given as follows: Kingdom– Animalia, Phylum– Chordata, Class– Aves, Order– Galliformes, Family– Phasianidae, Genus– Gallus, Species/subspecies– G. gallus domesticus. Chickens contribute the greatest amount of genetic diversity of domesticated avian species, with over 400 identified genetic variations (Somes 1998). More than 100 chicken breeds and commercial varieties have been recognized depending on size, body type, production characteristics, posture, color, feather structure and location, comb shape, and behavior.

Field Sampling

Materials used for the present study came from 31 different chicken breeds and strains and are summarized in Table 1. At least one specimen representing a domestic chicken breed or strain was examined to ascertain COI sequence divergences between them. Closely related chickens of the same breed or strain are expected to have the same COI sequences. Demographic records (e.g., name of breed/strain, purpose or type, ID number, date of sampling) and morphological data (e.g., live weight, length of wing, neck, breast, shank, beak and egg weight), including digital images of pictures and video, were likewise taken for each animal specimen and contributed to the local DNA barcode library (Oliva and Rogado 2011).

Laboratory Analysis

Most analytic methods followed those described in the earlier study (Hebert et al. 2004). DNA sources for this study included blood samples extracted from male live specimens without harming them, using gauge 25 hypodermic needle on the wing vein. Fresh blood samples were placed in FTA cards and allowed to dry for 3 d under room temperature.

Laboratory protocols for DNA extraction, purification, elution, and amplification were developed for poultry (bird) specimens at the Animal Biotechnology Laboratory, Animal and Dairy Sciences Cluster, College of Agriculture, University of the Philippines Los Banos.

DNA extraction. A Harris 1.2 mm micropunch was used to collect at least 30 discs from each dried FTA card or sample; the disks were later collected and placed in labelled microcentrifuge tubes.

DNA purification. Sample discs were washed four to five times with 200 μL of FTA Purification Reagent and rinsed with 200 μL of sterile nanopure water. Sample discs were then dried in a laminar hood overnight.

DNA elution. Six dried sample discs were transferred in a sterile PCR tube and added with 55 μL of sterile nanopure water. DNA was eluted using Veriti 96 Well Thermal Cycler (Applied Biosystems) at 90 °C for 10 min. Eluted DNA was stored at -20 °C for further use.

DNA amplification. The COI gene was amplified using primers BirdF1 (5’ TTCTCCAACCACAAAGACATTGGCAC 3’) and BirdR1 (5’ ACGTGGGAGATAA TTCCAAATCCTG 3’). The 20 μL PCR reaction mix included 13.44 μL of sterile ultrapure water, 2.0 μL of 10X buffer, 1.0 μL of MgCl₂, 0.8 units of Taq polymerase, 0.4 μL (0.2 mM) of each forward and reverse primer and 2.0 μL of DNA template. The optimized PCR amplification program was composed of 3 min at 94 °C followed by five cycles of 40 s at 94 °C, 30 s at 56 °C and 45 s at 72 °C, followed by another 30 cycles of 40 s at 94 °C, 30 s at 58 °C, and 45 s at 72 °C, and finally 7 min at 72 °C.

PCR products were visualized in a 1.0% agarose gel with ethidium bromide. Post stained gels were viewed using BioRad GelDoc XR UV transilluminator. PCR products were purified using GF-1 PCR Clean Up Kit (Vivantis). In cases where multiple bands occurred (e.g., pseudogenes or short DNA sequences less than 200 bp), gels were excised and purified using GF-1 Gel DNA Recovery Kit (Vivantis). The DNA amplification regime was repeated four times for each sample specimen. The final PCR product for each sample specimen (about 30–50 μL final volume) was obtained from pooled amplicons of all four PCR reactions (replicates).

DNA sequencing. PCR products were sent to Macrogen Inc., Seoul, Korea for unidirectional

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