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Qualitative traits and genetic characterization of native chicken (*Gallus gallus domesticus*) in selected areas of Eastern and Western Samar, Philippines

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ABSTRACT

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The Philippines has a number of chicken genetic groups, mostly of non-descript and indigenous type. In view of the need to expand the information on native chicken diversity, this study was conducted to identify distinct qualitative traits and estimate genetic diversity and relationship among native chicken populations in selected areas of Eastern and Western Samar, Philippines. A total of 100 native chickens were qualitatively analyzed using a non-parametric test, and 43 generated mtDNA sequences were used in the genetic analysis. Results revealed significantly different distributions of plumage color among male native chickens and shank color in female native chickens (p<0.05). The occurrence of plumage pattern, earlobe color and shank color for male native chickens and plumage color, plumage pattern, and earlobe color for female native chickens across Samar Island is not different (p>0.05). The genetic relationship showed 41.2% native chicken populations clustered to a group shared by Red junglefowl and native chicken, 29.4% clustered to a group closer to White Leghorn, and White Plymouth Rock chicken breeds, 17.6% clustered to a group shared by *G. g. spadiceus* and a commercial line, and 11.7% clustered to a group closer to Rhode Island Red and a commercial egg layer line. Samar native chickens had red (wild-type, e^+) laced (*lg*) and brown (e^b) pencilled (*Pg*) plumage in rooster and hen, respectively. The phenotypic and genetic information concluded that there is considerable diversity of native chickens in Samar, Philippines. There is a tremendous opportunity to work with larger sample size in the areas where a number of indigenous chickens have not yet been characterized.

Keywords: qualitative traits, genetic diversity, mtDNA, native chicken, Samar Philippines

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INTRODUCTION

Domestic chickens are widely farmed around the world, especially in Southeast Asia, as protein sources in the form of meat and eggs, providing food security for rural households (Shand 1997). Other important roles of chickens range from food to entertainment, ornamental purposes, and religious practices. The Philippines is considered as one of the biodiversity hotspots in the Indo-Australian Archipelago (Myers et al 2000). It was reported that 6 out of 243 recorded local poultry breeds in Asia could be found in the Philippines (DAD-IS 2011). The Philippines, like many countries in the world, has a number of chicken genetic groups, mostly non-descript, indigenous types, and commonly referred to as traditional chickens (FAO 2012).

In recent years, there has been a renewed interest in the identification, documentation, and utilization of Philippine native chickens. Information on the phenotypic and molecular genetic characteristics of these chicken populations is very important for strategic decision-making regarding conservation and/or improvement (Boettcher et al 2010). It is essential to design livestock conservation, development, and breeding programs for the management of animal genetic resources at the local, national, regional, and global levels (FAO 2012). The findings of Bejar et al (2012) and Picardal et al (2015) both intensively characterized the Samar native chickens phenotypically, which gave important information on its diversity. However, genetic characterization, especially identifying ancestral lineages of the native chicken populations in the area is limited.

The nucleotide sequence of the mitochondrial D-loop region is an important and powerful molecular tool used to track genetic information about the ancestral breeds of chicken; showing the phylogenetic relationship, genetic diversity, and differentiation within and between populations (Nishibori et al 2004, FAO 2011, Miao et al 2013). The use of maternally inherited mitochondrial DNA (mtDNA), especially its complete displacement-loop (D-loop) region, has been increasingly used over a decade. Hence, this study was conducted to identify distinct morphological traits and estimate the genetic relationship and diversity of native chickens raised locally in Samar, Philippines.

MATERIALS AND METHODS

Phenotypic Characterization

A total of 100 samples were collected in geographically selected upland areas of Calbiga, Western Samar (*n*=25), Basey, Western Samar (*n*=25), Lawaan, Eastern Samar (*n*=25), and Salcedo, Eastern Samar (*n*=25) (Table 1).

Discrete phenotypic characters were determined by an actual examination of every adult animals following identification indices set by Nishibori et al (2005) and FAO (2012). The data were analyzed for descriptive statistics using frequency procedures of Statistical Packages for Social Sciences (SPSS) version 22. Data on plumage color and pattern, earlobe color, and shank color were analyzed using the Kruskal-Wallis test to examine differences in the morphological characteristics across sampling areas. Significant differences in the frequency of occurrences among sampling areas were analyzed using the least significant differences (LSD) for ranks.

Table 1. List of Samar native chicken populations used in the study

Species	Phenotype sample	Blood/DNA sample	Source of sample*
Native chicken	25	9 (3♂, 6♀)	CWS
	25	18 (9♂, 9♀)	BWS
	25	13 (4♂, 9♀)	LES
	25	3 (♂)	SES

*CWS=Calbiga Western Samar, LNS=Lavezarez Northern Samar, LES=Lawaan Eastern Samar, BWS=Basey Western Samar, SES=Salcedo Eastern Samar

Genetic Characterization

DNA extraction, mtDNA amplification and sequencing

A total of 43 native chicken blood samples were collected randomly, mostly in the upland areas ensuring chickens were not selected from the same family: 9 from Calbiga, Western Samar, 18 from Basey, Western Samar, 13 from Lawaan, Eastern Samar and 3 from Salcedo, Eastern Samar. All blood samples were used as DNA materials in this study (Table 1).

Genomic DNA was extracted from the stored whole blood of the native chickens using phenol-chloroform method.

The amplification for complete mtDNA control region sequence - fragment 5.0 kilobase pairs (kbp) and mtDNA D-loop region, 1.3kbp fragment was amplified using long and accurate – PCR (LA-PCR) kit (Takara Shuzo, Otsu, Japan) with chicken DNA as a template, following established primer set, *Cytb-Forward*: 5'-TACA CGAATCAGGCTCAAACAACCCCCTAGGCATC-3', 16S-Reverse: 5'-TGCACCATTAG GTTGTCCTGATCCAACATCGAGGT-3' recommended by Nishibori et al (2003). The reaction began with a preliminary denaturation at 94°C for 2min, followed by 30 cycles of DNA denaturation at 98°C for 10s, annealing of primers at 57°C for 30s, primer extension at 68°C for 2min and 30s and 8min final extension of primers at 68°C using a GeneAmp PCR System 9700 (Applied Biosystems, Foster, USA). The PCR products were electrophoresed on a 1.0% agarose gel, and visualized by staining with ethidium bromide via ultraviolet transilluminator (UVP Transilluminator – BioDoc-It Imaging System). The PCR products from the segmental amplification were cleaned and purified using Exonuclease 1 (Exo1) and Shrimp Alkaline Phosphatase (SAP) which degrades the residual PCR primers and dephosphorylates the remaining dNTPs, respectively. After purification, the samples were sent to FASMAC Corporation, (5-1-3 Midorigaoka, Atsugi-shi, Kanagawa, Japan) for direct DNA sequencing and fragment analysis.

Data Analysis

The complete mtDNA D-loop sequences obtained from sequencing companies were initially edited using GENESTUDIO Professional (sequence analysis software) and aligned using ClustalW (Thomson et al 1994). Aligned nucleotide sequences were edited and viewed using the BioEdit sequence alignment editor (Hall 1999).

Phylogeny reconstruction using Neighbor-Joining method (Saitou & Nei 1987) by Molecular Evolutionary Genetics Analysis (MEGA) version 6.0 (Tamura et al 2013) were used to estimate genetic relationships within and among native chickens on Samar Island, Philippines together with reference sequences representing different haplogroups. Nomenclatures of the 13 clades (clades A to I & clades W to Z) reported by Miao et al (2013) were used as reference for the clade notation. The list of haplotypes used and the corresponding GenBank accession numbers are provided in the supplementary data. Bootstrap values were estimated with 1,000 repetitions.

RESULTS AND DISCUSSION

Phenotypic Characteristics of Samar Native Chicken

Plumage color

In this study, 41 male and 59 female native chickens were characterized phenotypically (Table 2). The results showed that among male native chickens, six allelomorphic plumage colors were observed across sampling areas (Figure 1). The most observed plumage colors among male native chickens were red (wild-type, e^+) and silver sex-linked (S). Unique plumage color of domestic chickens also spotted in the island. The birchen (E^R) plumage color (4.9%) having dark wild-type with finely stippled wing bays and black sex-linked ($B/W/b^+/b^+$) plumage color (9.8%) which is believed to be due to the sex-linked crosses between Barred Plymouth Rock females and Rhode Island Red or New Hampshire males (Crawford 1990).

In hens, 9 different allelomorphic plumage colors across sampling areas were observed. Brown (e^b) plumage color was observed the highest (23.7%), followed by recessive wheaten (e^y) (16.9%). (Table 2; Figure 2). However, statistical analysis revealed that the distribution of each plumage color assigned with a relative gene constitution was only different among male native chickens ($p<0.05$) and not in female native chickens ($p>0.05$). Male native chickens from Calbiga, Western Samar, were observed to be different between sampling areas.

The higher occurrence of red plumage color (wild-type, e^+) in roosters and brown plumage color (e^b) in hens agreed with the findings of Cabarles et al (2012) in Western Visayas and Picardal et al (2015) in Eastern Samar that these chickens may be descended from their progenitor possibly through natural selection or to a lesser extent through artificial selection done by the local people.

According to Paxton (2009), the dominant red with combined gold and dark-maroon coloration in roosters and brown colorations in hens could be due to varying levels of two classes of melanin pigments, eumelanin and phaeomelanin. However, eumelanin gives rise to black and dark brown hues, and phaeomelanin produces a reddish-brown color (McGraw 2006). The wild-type (e^+) plumage color is reported to be completely dominant to brown and recessive wheaten. The degree of dominance appears to be influenced by modifying genes capable of enhancing and inhibiting the expression of eumelanin. The existence of two wheaten alleles with almost similar phenotypic effects, but marked differences in their dominance relationships, is unusual and interesting. However, consistent results revealed by Crawford (1990), indicated that either two separate alleles or a single wheaten gene are very closely linked to a modifier of eumelanic expression.

Table 2. Number of individuals possessing allelomorphic characteristics in Samar native chickens

Sex	Trait	Locus	Genotype (Phenotype)	Sampling Sites								Total	
				Calbiga WS		Lawaan ES		Salcedo ES		Basey WS			
				freq	%	*freq*	%	*freq*	%	*freq*	%	*freq*	%
Male	Plumage color[a]	*E/e*$^{+}$	*E* (extended black)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
			E^{R} (birchen)[a]	0	0.0	0	0.0	2	4.9	0	0.0	2	4.9
			e^{Wh} (dominant wheaten)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
			e^{+} (wild type)	8	19.5	4	9.8	1	2.4	2	4.9	15	36.6
			e^{b} (brown)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
			e^{y} (recessive wheaten)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
		I/ii	*I* (white)	0	0.0	1	2.4	2	4.8	1	2.4	4	9.8
			ii (colored)	1	2.4	1	2.4	0	0.0	0	0.0	2	4.9
		S/s^{+} (sex-linked)	*S* (silver)	1	2.4	2	4.9	6	14.6	5	12.2	14	34.1
			s^{+}(gold)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
		B/W/b^{+}*b*$^{+}$*	*B/W/b*$^{+}$*b*$^{+}$ (black)	0	0.0	1	2.4	1	2.4	2	4.9	4	9.8
												41	**100**

[a]the occurrence of plumage color for male native chickens and shank color for female native chickens across selected areas in Samar Island, Philippines are different (p<0.05)
[b]the occurrence of plumage pattern, earlobe color and shank color for male native chickens and plumage color, plumage pattern and earlobe color for female native chickens across selected areas in Samar Island, Philippines are not different (p>0.05)

Table 2 continued

Sex	Trait	Locus	Genotype (Phenotype)	Sampling Sites								Total	
				Calbiga WS		Lawaan ES		Salcedo ES		Basey WS			
				freq	%	*freq*	%	*freq*	%	*freq*	%	*freq*	%
Male	Plumage pattern[b]		*Pg* (penciling)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
			Lg (lacing)	9	22.0	7	17.1	7	17.1	5	12.2	28	68.3
			Sp (spangling)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
			mo (mottling)	0	0.0	0	0.0	3	7.3	2	4.9	5	12.2
			Plain	1	2.4	2	4.9	2	4.9	3	7.3	8	19.5
												41	**100**
	Earlobe color[b]		(white)	0	0.0	3	7.3	3	7.3	3	7.3	9	21.9
			(red)	4	9.8	0	0.0	4	9.8	1	2.4	9	21.9
			(red with white)	6	14.6	6	14.6	5	12.2	6	14.6	23	56.1
												41	**100**
	Shank color[b]	*ID/id* (sex-linked)	*ID* (yellow/white)	7	17.1	6	14.6	5	12.2	5	12.2	23	56.1
			id (black/willow)	3	7.3	3	7.3	7	17.1	5	12.2	18	43.9
												41	**100**
Female	Plumage color[b]	E/e^{+}	*E* (extended black)	0	0.0	3	5.1	1	1.7	1	1.7	5	8.5
			E^{R} (birchen)[a]	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
			e^{Wh} (dominant wheaten)	3	5.1	1	1.7	2	3.4	2	3.4	8	13.6

[a]the occurrence of plumage color for male native chickens and shank color for female native chickens across selected areas in Samar Island, Philippines are different ($p<0.05$)
[b]the occurrence of plumage pattern, earlobe color and shank color for male native chickens and plumage color, plumage pattern and earlobe color for female native chickens across selected areas in Samar Island, Philippines are not different ($p>0.05$)

Table 2 continued

Sex	Trait	Locus	Genotype (Phenotype)	Sampling Sites								Total	
				Calbiga WS		Lawaan ES		Salcedo ES		Basey WS			
				freq	%	*freq*	%	*freq*	%	*freq*	%	*freq*	%
			e^+ (wild type)	0	0.0	1	1.7	0	0.0	1	1.7	2	3.4
			e^b (brown)	4	6.8	0	0.0	3	5.1	7	11.9	14	23.7
			e^y (recessive wheaten)	5	8.5	2	3.4	1	1.7	2	3.4	10	16.9
		I/ii	*I* (white)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
			ii (colored)	1	1.7	2	3.4	1	1.7	0	0.0	4	6.8
		S/s$^+$ (sex-linked)	*S* (silver)	0	0.0	1	1.7	4	6.8	0	0.0	5	8.5
			s^+(gold)	1	1.7	0	0.0	0	0.0	2	3.4	3	5.1
		B/W/b$^+$*b*$^+$*	*B/W/b*$^+$*b*$^+$ (black)	1	1.7	6	10.2	1	1.7	0	0.0	8	13.6
												59	**100**
	Plumage pattern[b]		*Pg* (penciling)	6	10.2	8	13.6	8	13.6	10	16.9	32	54.2
			Lg (lacing)	8	13.6	3	5.1	3	5.1	3	5.1	17	28.8

[a]the occurrence of plumage color for male native chickens and shank color for female native chickens across selected areas in Samar Island, Philippines are different ($p<0.05$)
[b]the occurrence of plumage pattern, earlobe color and shank color for male native chickens and plumage color, plumage pattern and earlobe color for female native chickens across selected areas in Samar Island, Philippines are not different ($p>0.05$)

Table 2 continued

Sex	Trait	Locus	Genotype (Phenotype)	Sampling Sites								Total	
				Calbiga WS		Lawaan ES		Salcedo ES		Basey WS			
				freq	%	*freq*	%	*freq*	%	*freq*	%	*freq*	%
			Sp (spangling)	0	0.0	0	0.0	0	0.0	0	0.0	0	0.0
			mo (mottling)	0	0.0	1	1.7	0	0.0	1	1.7	2	3.4
			Plain	1	1.7	4	6.8	2	3.4	1	1.7	8	13.6
												59	**100**
	Earlobe color[b]		(white)	2	3.4	1	1.7	7	11.9	4	6.8	14	23.7
			(red)	7	11.9	6	10.2	2	3.4	5	8.5	20	33.9
			(red with white)	6	10.2	9	15.2	4	6.8	6	10.2	25	42.4
												59	**100**
	Shank color[a]	*ID/id* (sex-linked)	*ID* (yellow/white)	10	16.9	2	3.4	3	5.1	8	13.6	23	39.0
			id (black/willow)	5	8.5	14	23.7	10	16.9	7	11.9	36	61.0
												59	**100**

[a]the occurrence of plumage color for male native chickens and shank color for female native chickens across selected areas in Samar Island, Philippines are different ($p<0.05$)
[b]the occurrence of plumage pattern, earlobe color and shank color for male native chickens and plumage color, plumage pattern and earlobe color for female native chickens across selected areas in Samar Island, Philippines are not different ($p>0.05$)

Figure 1. Six different allelomorphic plumage color in male native chickens in Samar Island, Philippines. (A) wild-type (e^{+}), (B) silver sex-linked (S), (C) plain-white (I), (D) black sex-linked ($B/W/b^{+}/b^{+}$), (E) birchen (E^{R}), and (F) colored (ii)

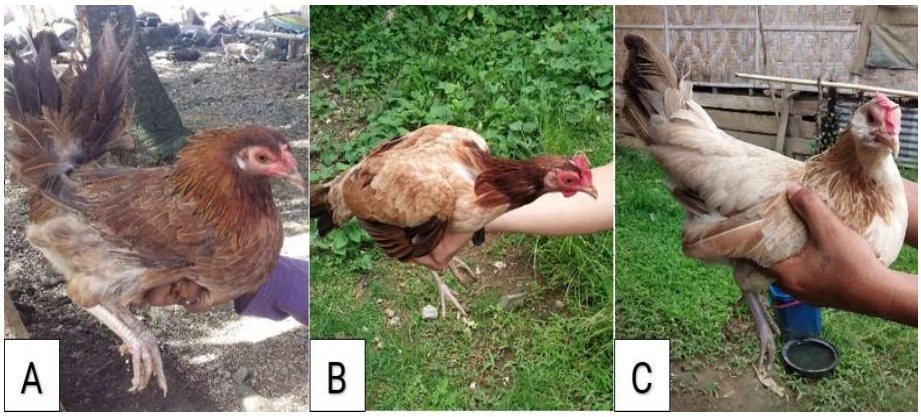

Figure 2. Nine different allelomorphic plumage color in female Samar native chickens. (A) brown (e^{b}), (B) recessive wheaten (e^{y}), (C) dominant wheaten (e^{Wh}), (D) black sex-linked ($B/W/b^{+}/b^{+}$), (E) extended black (E), (F) silver sex-linked (S), (G) colored (ii), (H) gold sex-linked (s^{+}), and (I) wild-type (e^{+})

Figure 2 continued

Plumage Pattern

Statistical analysis revealed that the distribution of each plumage pattern regardless of sex was not different (p>0.05) across sampling areas in Samar Island (Table 2). Result displayed that 68.3% of the 59 roosters had laced-plumage pattern and 19.5 percent plain-plumage pattern (Table 2; Figure 3). In hens, 54.2% had pencilled-plumage pattern, followed by laced-plumage pattern (28.8%), plain-plumage pattern (13.6%) and mottled-plumage pattern (3.4%) had the least. (Table 2; Figure 4).

Results coincided with the findings of Cabarles et al (2012) in Western Visayas native chickens where the laced-plumage pattern for roosters and pencilled-plumage pattern for hens were the most observed plumage patterning. Although this is opposed to the findings of Salces et al (2015) with predominant plain-plumage pattern in Boholano native chickens. The plumage of chickens is displayed by the arrangement of feathers with various pigmentation patterns. According to Smyth (1990), the color patterns are due to the distribution of eumelanin and the presence or absence of pheomelanin at the feather developmental stage. The position of feather on the body may also affect the expression of color pattern because of differences in the intensity of melanin pigmentation in the skin (Yu et al 2004).

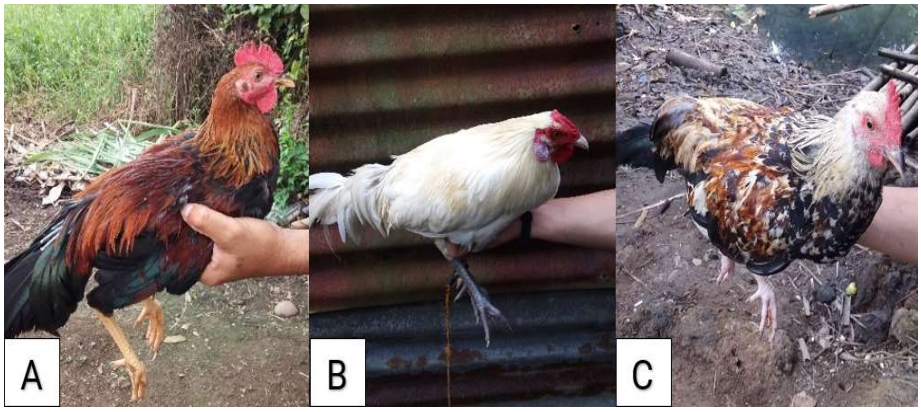

Figure 3. Three different allelomorphic plumage pattern in male Samar native chickens. (A) lacing (lg), (B) plain, and (C) mottling (mo)

Figure 4. Four different allelomorphic plumage pattern in female Samar native chickens; (A) pencilling (Pg), (B) lacing (lg), (C) plain, and mottling (mo)

Earlobe Color

It was observed that 56.1% of the 41 characterized male native chickens had red with white earlobes, followed by the same occurrences of the white earlobes and red earlobes at 21.9% each (Table 2; Figure 5). Among hens, 42.4% of the 59 characterized female native chickens had red with white earlobe color. This was followed by 33.9% red earlobe, while white earlobe occurred the least with 23.7% (Table 2; Figure 6). However, statistical analysis revealed that the distribution of each earlobe color regardless of sex was not different (p>0.05) across sampling areas in Samar Island.

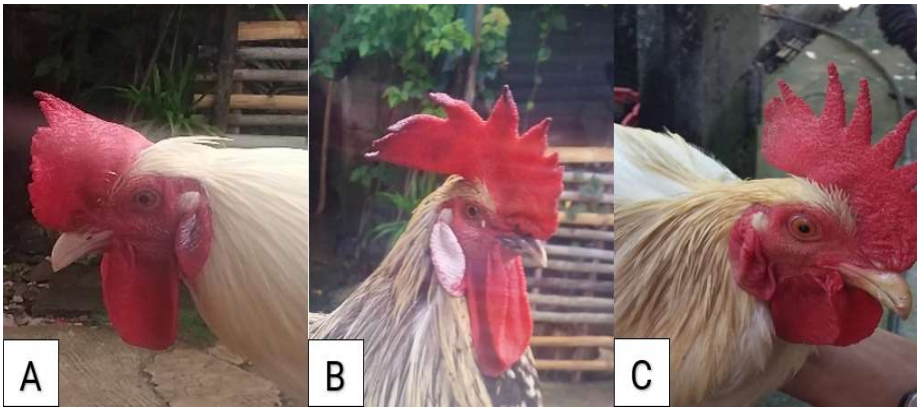

Figure 5. Earlobe color among male native chickens in Samar Island, Philippines: (A) red with white earlobe, (B) white earlobe and (C) red earlobe

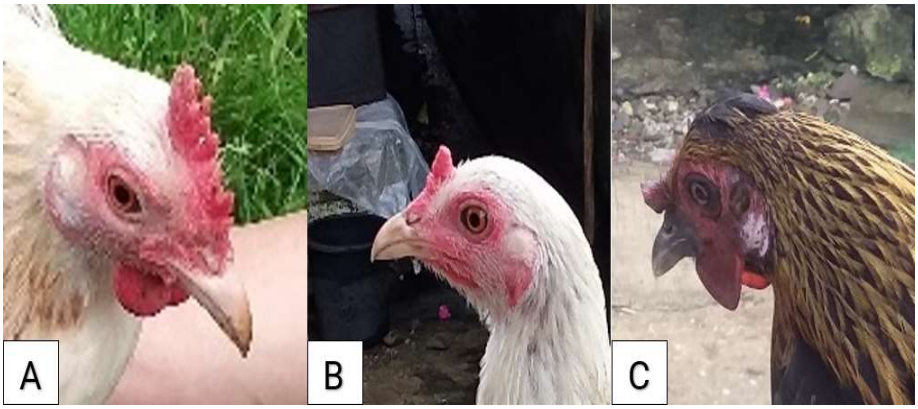

Figure 6. Earlobe color among female native chickens in Samar Island, Philippines: (A) red with white earlobe, (B) red earlobe and (C) white earlobe

The diversity of earlobe colors can be due to the variability of ancestral lineages and mutations which possibly occurred 1,000 years ago resulting from hybridization between subspecies of *Gallus gallus,* particularly *G. g. gallus* which carries white earlobes crossed with *G. g. spadiceus* and *G. g. jabouillei*, which possessed red color earlobes (Nishida et al 2000). The results of phylogenetic

analysis (Figure 8) revealed 17.6% of the characterized native chickens clustered closer to *G. g. spadiceus* species, which is extensively known to have red color earlobe, and 11.7% closer to a clade of Rhode Island Red and a commercial layer line. These findings demonstrated that the distinctly observed higher occurrences of red with white color earlobes among Samar native chickens were due to considerable crossbreeding interferences between indigenous native chickens and commercial breed lines.

Shank Color

It was found that 54% of the 100 characterized native chickens had black or willow shank color (*id* gene constitution), while 46% had white or yellow shank (*ID* gene constitution) (Table 2; Figure 7). Among roosters, 43.9% possessed black or willow shank, while 56.1% had white or yellow. In hens, 61% had black color shank (*id*), while 39% had white or yellow shank (*ID*). However, statistical analysis revealed that the distribution of each shank color with a relative gene constitution was only different among female native chickens ($p<0.05$) and not in male native chickens ($p>0.05$). Female native chickens from Lawaan, Eastern Samar, were observed to be different between sampling areas.

The diversity in shank color can be due to interactions of major and modifier genes as pointed out by Smyth (1990). This is controlled by dermal melanin (*id+*), inhibition of dermal melanin (*Id*), black extension factor (*E*) and autosomal white (*W+*) genes located in the Z chromosome. *Id* and *id+* expression are confined in the dermis, whereas *E* and *W+* are in the epidermis. Homozygosity to *E* in chickens will express black shank. The interactions of *id+* and *E* with dominant white *(I)* chickens will express slate or willow shank. The presence of sex-linked barring, mottling and wheaten genes will inhibit the expression of *id+*. Barring makes the pigmentation of the shank lighter in roosters than in hens, whereas mottling expressed small black spots in the white shank. The presence of *W+* interacting with melanin will appear as blue or slate shank and the *w* for green or willow. Yellow shank is due to the interaction of homozygous recessive for *w* and *e+* with homozygous *Id*, whereas white shank is brought about by the accumulated effects of *W+/W+ Id/ Id e+/e+*.

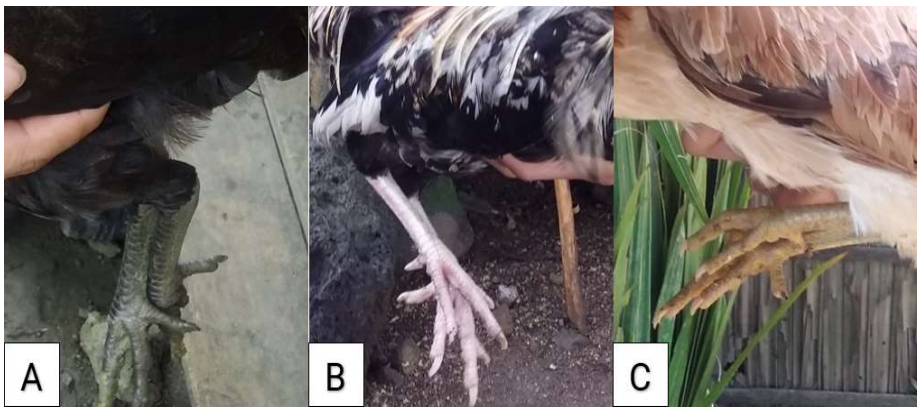

Figure 7. Shank color among native chickens in Samar Island, Philippines; (A) black/willow shank color, (B) white shank color and (C) yellow shank color

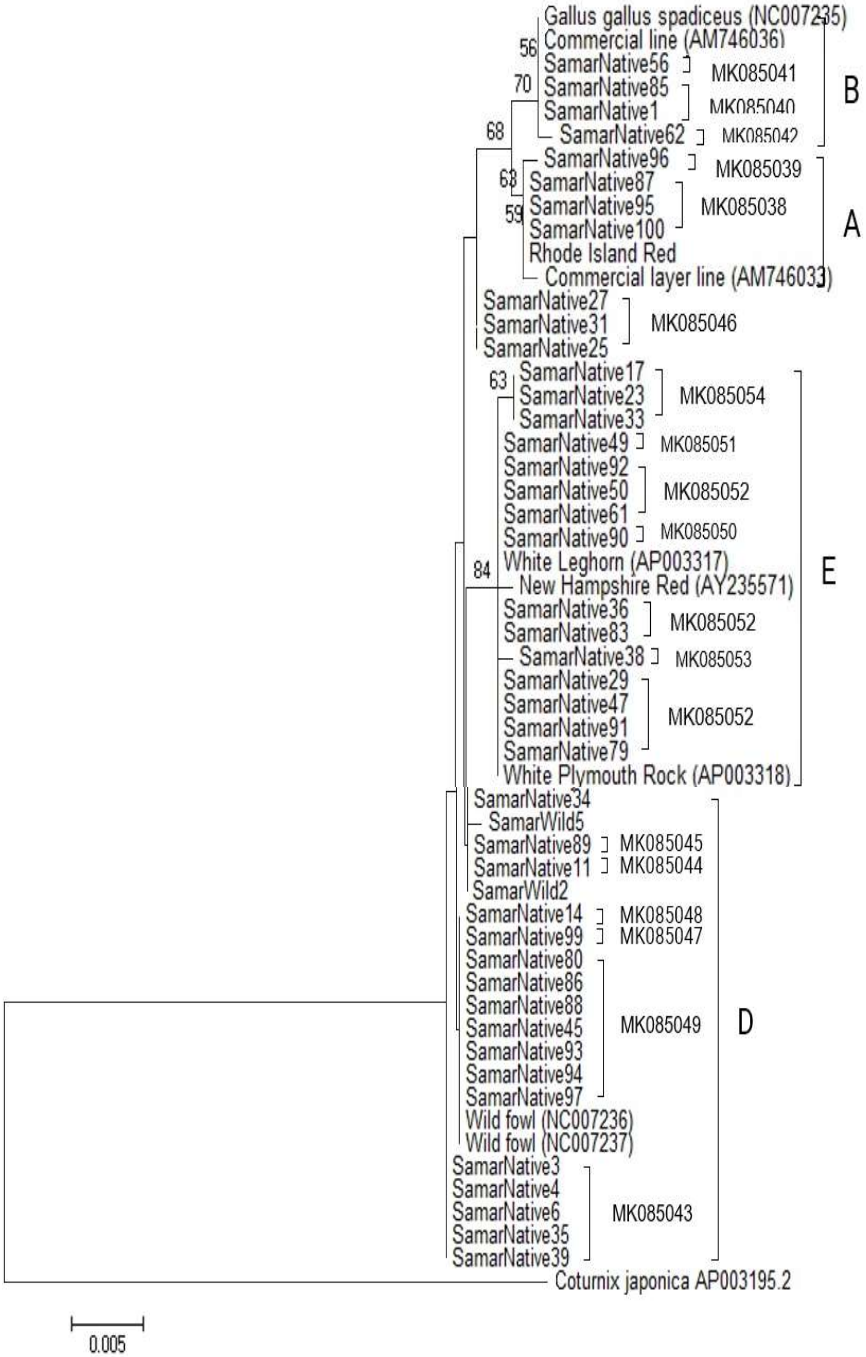

Figure 8. Phylogenetic tree of 55 complete mtDNA D-loop nucleotide sequences (45 from this study and 10 derived from Genbank) based on the neighbor joining method (Saitou & Nei 1987). The numeral at each branch indicates the bootstrap value of replications. Bootstrap values lower than 50% are not shown. The scale bar represents substitution rate per site. Coturnix japonica represent as outgroup. Letter notation A, B, D, and E represents haplogroups

Genetic Characteristics of Samar Native Chicken

Phylogenetic structure and sequence variation of Samar native chickens

Phylogenetic analysis of Samar native chickens was constructed along with the reference sequences derived from Genbank (Figure 8). The mutational motif in the D-loop sequences of Samar native chickens showed 41.2% clustered to a haplogroup shared by red junglefowl and native chicken, 29.4% clustered to a haplogroup closer to White Leghorn and White Plymouth Rock breeds, 17.6% clustered to a haplogroup shared by *G. g. spadiceus* and a commercial line, and 11.7% clustered to a haplogroup closer to Rhode Island Red and a commercial egg layer line.

Phylogeographic studies have identified that one mtDNA lineage (haplogroup D) was largely limited to the Asia-Pacific region and that haplogroup A, B, and E contain mtDNA control region haplotypes from all over Eurasia (Liu et al 2006). Haplogroup E is predominant among Indian, Middle Eastern, and European chickens, with the primary subhaplogroup *E1* which is the single most-common chicken haplotype found around the world, while haplogroup A and B predominate among South and Eastern Chinese and Japanese chickens (Gongora et al 2008). The updated perspective of chicken domestication had classified the wild fowls in the Philippines as belonging to *DI* subhaplogroup (Miao et al 2013). Haplogroups A, B, D, and E provide insights into the mtDNA signatures of ancient Asia-Pacific chickens and showed agreement with several genealogical findings done across Asia-Pacific region.

The result of this study strongly agreed with the haplogroup classification examined by Miao et al (2013) since Samar native chicken haplotypes revealed higher percentage clustered to subhaplogroup *D1*, where haplotypes observed to have 5 unique polymorphic sites at the nucleotide base 281, 296, 306, 342 and 686 (Table 3). The D-loop sequence information of 46.5% (20/43) of the Samar native chickens revealed subhaplogroup *D1* as the most widely distributed matrilineal lineage, which is believed to be the haplogroup signature of shared red junglefowls and domestic chickens. Moreover, 34.9% (15/43) of Samar native chickens classified into subhaplogroup *E1* showed 3 unique polymorphic sites at the nucleotide base 217, 446 and 1,214 (Table 3). This was believed to be the haplogroup signature of shared domestic chickens and commercial breed lines. An additional 9.3% (4/43) of Samar native chickens classified as haplogroup A which showed two unique polymorphic sites at the nucleotide base 167 and 225, and lastly, another 9.3% (4/43) classified as haplogroup B which showed one unique polymorphic site at the nucleotide base 792. This is believed to be the haplogroup signature of shared domestic chickens and commercial breed lines (Figure 8). These results were further supported by the findings of Thomson et al (2014), where Philippine chicken populations confined at 4 distinct haplogroup A, B, D, and E with higher spread throughout the haplogroup *D*.

Table 3. Sequence variation of 17 haplotypes derived from 43 individuals observed in the mtDNA D-loop region

Ref. seq.		167	190	199	212	217	225	243	246	256	261	281	293	296	306	310	315	342	396	399	446	486	492	852[a]	948	1174	1190	1214	1215	Genbank Accession Number
	N	T	T	T	A	T	C	T	T	T	C	A	T	C	T	C	T	A	T	G	C	G	G	C	A	A	T	C	A	NC_007235
SPN1	3	C	.	.	G	.	T	.	C	.	.	.	.	.	.	.	C	.	.	.	.	.	.	-	.	.	.	.	G	MK085038
SPN2	1	C	.	.	G	.	T	.	C	.	.	.	.	.	.	.	C	.	.	A	.	.	.	-	.	.	.	.	G	MK085039
SPN3	2	.	.	.	.	.	.	.	.	.	.	G	.	.	.	.	.	.	.	.	.	.	.	-	.	.	.	.	.	MK085040
SPN4	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	C	.	.	.	.	.	MK085041
SPN5	1	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	A	.	.	A	C	.	.	.	.	.	MK085042
SPN6	5	.	.	.	G	.	.	C	C	C	T	G	.	T	C	T	C	.	A	.	.	A	.	-	.	.	.	.	G	MK085043
SPN7	1	.	.	.	G	.	.	C	C	C	T	G	.	T	C	T	C	.	.	.	.	A	.	***-***	***C***	.	.	.	G	MK085044
SPN8	2	.	.	.	G	.	.	C	C	C	T	G	.	T	C	T	C	.	.	.	.	A	.	-	.	.	.	.	G	MK085045
SPN9	3	.	.	.	G	.	.	C	C	C	.	G	.	T	C	T	C	.	.	.	.	A	.	-	.	.	.	.	G	MK085046
SPN10	1	.	.	.	G	.	.	C	C	C	T	G	.	.	C	T	C	G	.	.	.	A	.	-	.	.	.	.	G	MK085047
SPN11	1	.	.	.	G	.	.	C	C	C	T	G	C	T	C	T	C	G	.	.	.	A	.	-	.	.	.	.	G	MK085048
SPN12	7	.	.	.	G	.	.	C	C	C	T	G	.	.	C	T	C	G	.	.	.	.	.	C	.	.	.	.	G	MK085049
SPN13	1	.	.	.	G	C	.	C	C	C	T	.	.	.	.	T	C	.	.	.	T	.	.	-	.	***T***	***G***	T	G	MK085050
SPN14	1	.	.	C	G	C	.	C	C	C	T	.	.	.	.	T	C	.	.	.	T	.	.	C	.	.	.	T	G	MK085051
SPN15	9	.	.	.	G	C	.	C	C	C	T	.	.	.	.	T	C	.	.	.	T	.	.	-	.	.	.	T	G	MK085052
SPN16	1	.	.	.	G	C	.	C	C	C	T	.	.	.	.	T	C	.	.	A	T	.	.	-	.	.	.	T	G	MK085053
SPN17	3	.	C	.	.	C	.	C	C	C	T	.	.	.	.	T	C	.	.	.	T	.	.	-	.	.	.	T	G	MK085054

SPN – Samar, Philippines native chicken, N - number of individuals is sharing the same haplotype. Vertically oriented numbers indicate the nucleotide position. Transversions are indicated by italic bold letters. Dots (.) indicate identity with the reference sequence. Dashes (-) indicate nucleotide deletions.
852[a] – Nucleotide base deletion specific for Samar native chickens

Results postulated that there are higher populations of native chickens inhabiting Samar, Philippines, which are descendants of shared Samar wild junglefowl and indigenous chickens with a considerable mixture of commercial breed lines. Further, results suggested that Samar native chickens still mingled with red junglefowl species, although others were already a product of crossbreeding of commercial breed lines or a combination of different breeds. This lineage likely changes because human dispersal and migration includes domestic animals and most likely, because of hybridization with commercial hybrid lines both natural and artificial, which is performed by the local people.

For future work, it is suggested to increase the sample size and modify the sampling design such that more areas and upstream villages can be included. Also, considering that Philippines is an archipelago comprising about 7,641 island and a number of islets, there is tremendous opportunity to work in areas where a number of different traditional chickens have not yet been characterized.

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SUPPLEMENTARY DATA

List of sequences obtained from Genbank used in the phylogenetic analysis of native chickens in Samar Island, Philippines

Source	Accession No.	Haplo-group	Origin	Reference
Commercial layer	AM746033	A	-	Muchadeyi et al 2008
Rhode Island red	AB268517	A	-	Oka et al 2007
Commercial line	AM746035	B	Japan	Muchadeyi et al 2008
Junglefowl	NC_007235	B	Laos	Nishibori et al 2005
Junglefowl	NC_007236	D1	Philippine	Nishibori et al 2005
Junglefowl	NC_007237	D1	Indonesia	Nishibori et al 2005
White leghorn	AP003317	E1	-	Nishibori et al 2003
White Plymouth rock	AP003318	E1	-	Nishibori et al 2003
New Hampshire red	AY235571	E1	-	Froman and Kirby 2005
Coturnix japonica	AP003195.2	outgroup	-	Nishibori et al 2001