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Phylogenetic Studies on Red Junglefowl (*Gallus gallus*) and Native Chicken (*Gallus gallus domesticus*) in Samar Island, Philippines using the Mitochondrial DNA D-Loop Region

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A study was conducted to provide genetic information on the matrilineal phylogeny and genetic diversity of Red junglefowl (RJF) and native chickens in Samar Island, Philippines and to identify the genetic distance between Philippine junglefowls and other RJF species in Southeast Asia using complete mitochondrial DNA D-loop sequences. A total of 5 RJFs and 43 native chickens from Samar Island were included in this study. The results showed that Samar RJFs had a nucleotide diversity of 0.0050±0.0016, which was lower than those of three subspecies of *Gallus gallus*: *G. g. gallus*, *G. g. spadiceus*, and *G. g. jabouillei*. Meanwhile, Samar native chickens showed lower nucleotide diversity (0.0056±0.0004) than domestic fowls in some neighboring Southeast Asian countries, but higher than those in African and European countries. Phylogenetic analysis showed that 3 haplotypes of Samar RJFs clustered to haplogroup D1, and that 2 haplotypes clustered to haplogroup D2. Chickens native to Samar Island showed 100% resemblance to those in the haplogroup shared by domestic chickens and RJFs. Haplogroups A and B and sub-haplogroups D1 and E1 were the more widely distributed matrilineal lineages in Samar Island. Phylogenetic analysis of Samar RJFs showed that they were closely related to Myanmar RJFs (99.6%), Indonesia RJFs (99.5%), and Thailand RJFs (99.1%). This study is an initial investigation estimating the matrilineal phylogeny and genetic diversity of chicken populations in Samar Island, Philippines for developing strategies aimed at the future conservation and improvement of valuable genetic resources.

Key words: mtDNA, native chicken, phylogenetic, Red junglefowl, Samar Island

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Introduction

The domestication of various wild animals, particularly chickens, contributes largely to the sustenance, cultural development, and heritage of mankind. Although, humans gain much benefit from domestic chickens (*Gallus gallus domesticus*), its history of domestication remains controversial. Different contending views about the origin of the domestic fowl intrigued the interest of several researchers exploring the long history of the junglefowl species.

Indeed, archaeological discoveries of Red junglefowls (RJFs) spotted in the Indus Valley (Harappan culture) about 2,500 years BC (Zeuner, 1963) and in the Hebei Province, China 5,400 years BC (West and Zhou, 1988) gave rise to the monophyletic theory (Fumihito *et al*., 1994) that hypothesizes that RJFs are the sole progenitor of the domesticated chicken. However, different findings by Nishibori *et al*. (2005) gave molecular evidence that other junglefowl species in the genus *Gallus* could also have been progenitors of domestic chickens, making this issue more complicated.

The use of maternally inherited mitochondrial DNA (mtDNA), especially its complete displacement-loop (D-loop) region, has increased over the past decade. The nucleotide sequence of the mitochondrial D-loop region is one of the most important and powerful molecular tools used to track genetic information of chicken ancestral breeds, showing the phylogenetic relationship, genetic distance, and variability within and between populations (Nishibori *et al*., 2004). MtDNA is maternally inherited and there is no direct evidence that it can recombine with other mtDNA molecules (Clayton, 1992; Nishibori *et al*., 2005). This means that

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vertebrate mtDNA is passed on through female lineages in a clonal fashion with no horizontal mixing and this makes it more straightforward to reconstruct an evolutionary history of this molecule than for the nuclear genome (Mindell, 1997). Currently, a substantial mtDNA analysis is urgent to generate the baseline genetic information on matrilineal phylogeny, genetic diversity and distance, and variability within and between populations of RJFs and native chickens in Samar Island, Philippines.

In this study, complete mtDNA D-loop sequences from RJFs and native chickens in Samar, Philippines were determined to assess the matrilineal phylogeny, genetic diversity, evolutionary relationship, and genetic distance from other established junglefowl species in Southeast Asia.

Materials and Methods

Blood Sample Collection

A total of five blood samples were collected from the wing vein of RJFs: 1 from Calbiga, Western Samar; 2 from Lawaan, Eastern Samar; and 2 from Lavezares, Northern Samar. These RJFs were captured in the wild by hunters living near the identified sampling sites. For the native chicken, a total of forty-three blood samples were collected randomly, mostly in the upland areas without the same family selection: 9 from Calbiga, Western Samar; 18 from Basey, Western Samar; 13 from Lawaan, Eastern Samar; and 3 from Salcedo, Eastern Samar. All 48 blood samples were used as DNA sources in this study (Table 1).

DNA Extraction, mtDNA Amplification and Sequencing

Genomic DNA was extracted from the stored whole blood of Philippine RJFs and native chickens using the phenol-chloroform method following the recommended protocol demonstrated by Nishibori *et al*. (2003).

The 5.0 kilobase pairs (kbp) mtDNA control region fragment and the 1.3 kbp mtDNA D-loop region fragment were amplified using a long and accurate – PCR (LA-PCR) kit (Takara Shuzo, Otsu, Japan) using chicken DNA as a template and an established primer set: *Cytb*-Forward: 5′-TACACGAATCAGGCTCAAACAACCCCCTAGGCATC-3′, *16S*-Reverse: 5′-TGCACCATTAGGTTGTCCTGATC-CAACATCGAGGT-3′, recommended by Nishibori *et al*. (2003). The reaction began with a preliminary denaturation at 94℃ for 2 min, followed by 30 cycles of DNA denaturation at 98℃ for 10 s, annealing of primers at 57℃ for 30 s, and primer extension at 68℃ for 2 min and 30 s, and an 8 min final extension of primers at 15℃ using a GeneAmp PCR System 9700 (Applied Biosystems, Foster City, CA, USA). The PCR products were electrophoresed on a 1.0% agarose gel and visualized following staining with ethidium bromide via an 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) to degrade the residual PCR primers and dephosphorylate the remaining dNTPs, respectively. Subsequently, samples were sent to FASMAC Corporation, (Midorigaoka, Atsugi-shi, Kanagawa, Japan) for direct DNA sequencing and fragment analysis.

DNA Analysis

The complete mtDNA D-loop sequences obtained from the sequencing company were edited manually and analyzed using GENESTUDIO Professional (sequence analysis software). Multiple sequence comparison by log-expectation (MUSCLE) was used for multiple sequence alignment and aligned nucleotide sequences were viewed using the BioEdit Sequence Alignment Editor. The diversity measures such as the number of polymorphic (segregating) sites (S), haplotype diversity (Hd), and nucleotide diversity (π) were estimated by the DnaSP 5.10 software (Librado and Rozas, 2009).

Phylogeny reconstruction using the neighbor-joining method (Saitou and Nei, 1987) by molecular evolutionary genetics analysis (MEGA) version 6.0 (Tamura *et al*., 2013) were used to estimate the genealogy of RJFs and native chickens in Samar Island, Philippines together with the 61 reference sequences representing different junglefowl and domestic chicken clades in the East, South, and Southeast Asian regions. The nomenclatures of the 13 clades (clades A to I and clades W to Z) reported by Miao *et al*. (2013) were used as references for the clade notation. The list of haplotypes used and the corresponding GenBank accession numbers are provided in the supplementary data.

A median-joining (MJ) network was constructed to infer the evolutionary relationships of Samar RJF and native chicken haplotypes using NETWORK 4.6 software (Bandelt

Table 1. **List of species and populations used in the study**

Species	Abbreviations	Blood/DNA sample	Source of sample*
Red junglefowl	SPW	1 (♂)	CWS
		2 (1♂, 1♀)	LES
		2 (♀)	LNS
Native chicken	SPN	9 (3♂, 6♀)	CWS
		18 (9♂, 9♀)	BWS
		13 (4♂, 9♀)	LES
		3 (♂)	SES

* SPW=Samar, Philippine Wildfowl, SPN=Samar, Philippine Native chicken, CWS=Calbiga Western Samar, LNS=Lavezarez Northern Samar, LES=Lawaan Eastern Samar, BWS=Basey Western Samar, SES=Salcedo Eastern Samar

et al., 1999). This method calculates the net divergence of each taxon from all other taxa as the sum of the individual distances form variance within and among groups of Samar, Philippine RJFs and native chickens. Bootstrap values were estimated with 1,000 repetitions.

Results and Discussion

MtDNA D-loop Sequence Variation of Samar RJFs and Native Chickens

Five mtDNA D-loop sequences from the RJF populations and 43 from native chickens were generated in this study. The distribution of nucleotide positions and sequence variations of each haplotype are presented in Table 2. All sequences were deposited in the GenBank database (accession no. MK085033-MK085052). All Samar RJF haplotypes were found to have 17 base transition substitution single nucleotide polymorphism (SNP) sites with a single nucleotide deletion at base 859, except for the SPW2 (MK085034) haplotype. On the other hand, all Samar native chicken haplotypes showed 24 base transition substitution SNP sites and 3 base transversion SNP sites with 1 single nucleotide deletion at base 852, except for 4 haplotypes: SPN4 (MK085041), SPN5 (MK085042), SPN12 (MK 085049), and SPN14 (MK085051). However, sequencing of Samar native chickens revealed a transversion substitution in the reverse sequence of base 948 (A/C), 1,174 (A/T), and 1,190 (T/G). This result was in agreement with the findings of Miao *et al*. (2013) where Philippine RJF (NC_007236) showed close similarity in SNP sites with the haplotypes of Samar RJFs except at the bases 199, 309, 417, and 293. Samar native chicken haplotypes were more genetically diverse, showing differences at 13 SNP sites.

The number of polymorphic (segregating) sites, number of haplotypes, haplotype diversity, and nucleotide diversity of Samar RJFs and native chickens are presented in Table 3. The overall haplotype diversity of 5 Samar RJFs was 1.00±0.20, with almost the same Hd value across sampling areas, whereas its overall nucleotide diversity was highest at 0.0050±0.0016, 0.0081±0.0041 and 0.0081±0.0028 in Western Samar and Northern Samar, respectively, and the lowest at 0.0075±0.0031 in Eastern Samar. This revealed a lower nucleotide diversity compared to that shared by *G. g. gallus, G. g. spadiceus,* and *G. g. jabouillei* subspecies of *Gallus gallus*, 0.01080±0.00059, in China, India, and Indonesia (Liu *et al*., 2006). Moreover, the Samar native chicken nucleotide diversity of 0.0056±0.0004 was also lower than that of some Asian fowls including the Thailand native chicken, (0.0156±0.0082) (Pramual *et al*., 2013); Bangla-

Table 2. **Sequence variation of 5 haplotypes of Samar RJFs and 17 haplotypes of Samar native chickens derived from 5 and 43 individuals, respectively observed in the mtDNA D-loop region**

	167	190	199	212	217	225	243	246	256	261	281	293	296	306	309	310	315	342	396	399	417	446	466	792	*852*[a]	*859*[b]	948	1174	1190	1214	1215	GenBank Accession Number
Ref. seq.	N	T	T	T	A	T	C	T	T	T	C	A	T	C	T	T	C	T	A	T	G	C	C	G	G	C	C	A	A	T	C	A	NC_007235
SPW1	1	.	.	.	G	.	.	C	C	C	T	G	.	.	C	.	T	C	.	.	.	.	.	.	.		-	.	.	.	.	G	MK085033
SPW2	1	.	.	C	G	.	.	C	C	C	T	G	.	T	C	.	T	C	.	.	.	.	.	A	.		C	.	.	.	.	G	MK085034
SPW3	1	.	.	.	G	.	.	C	C	C	T	G	.	T	C	.	T	C	.	.	.	T	.	A	.		-	.	.	.	.	G	MK085035
SPW4	1	.	.	.	G	.	.	C	C	C	T	G	C	T	C	.	T	C	G	.	.	.	.	A	.		-	.	.	.	.	G	MK085036
SPW5	1	.	.	.	G	.	.	C	C	C	T	G	.	.	C	C	T	C	.	.	.	.	.	.	.		-	.	.	.	.	G	MK085037
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

SPW; SPN – refers to the abbreviations in Table 1

N, number of individuals 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; *859*[b] – Nucleotide base deletion specific for Samar RJFs

Table 3. **MtDNA diversity indices of Samar Red jungle fowl and native chicken populations and the number of haplotypes (SPW1-5 and SPN1-17)**

Population	Location	N	S	Ht	Hd	π	SPW1	SPW2	SPW3	SPW4	SPW5	SPN1	SPN2	SPN3	SPN4	SPN5	SPN6	SPN7	SPN8	SPN9	SPN10	SPN11	SPN12	SPN13	SPN14	SPN15	SPN16	SPN17
Red junglefowl	CWS	1	10	1	1.00±0.50	0.0081±0.0041	1																					
	LES	2	14	2	1.00±0.27	0.0075±0.0031		1	1																			
	LNS	2	15	2	1.00±0.27	0.0081±0.0028				1	1																	
Overall		**5**	**17**	**5**	**1.00±0.20**	**0.0050±0.0016**	**1**	**1**	**1**	**1**	**1**																	
Native chickens	CWS	9	20	6	0.91±0.08	0.0061±0.0011								1			3	1		1		1						2
	LES	13	20	8	0.91±0.06	0.0047±0.0007											2		1	2			1		1	4	1	1
	SES	3	13	3	1.00±0.18	0.0054±0.0024									1	1										1		
	BWS	18	21	8	0.86±0.06	0.0055±0.0005						3	1	1					1		1		6	1		4		
Overall		**43**	**27**	**17**	**0.92±0.02**	**0.0056±0.0004**						**3**	**1**	**2**	**1**	**1**	**5**	**1**	**2**	**3**	**1**	**1**	**7**	**1**	**1**	**9**	**1**	**3**

N – number of sequences; S – number of polymorphic (segregating) sites; Ht – number of haplotypes; Hd – haplotype diversity; π – nucleotide diversity; CWS, LES, LNS, SES and BWS – refers to the abbreviations in Table 1

deshi fowl (0.014±0.001) (Bhuiyan *et al*., 2013), and Laotian fowl (0.0102±0.0056) (Kawabe *et al*., 2014); however, it showed higher nucleotide diversity than in African and European chicken populations, namely Ethiopian (0.0032), Sudanese (0.0018), Ugandan (0.0009) (Mwacharo *et al*., 2011), Nigerian (0.0016) (Adebambo *et al*., 2010), and Hungarian (0.0049) (Revay *et al*., 2010), except Kenyan domestic fowls (0.0119) (Mwacharo *et al*., 2011). Thus, these results revealed that Samar RJFs and native chicken populations still showed higher genetic diversity than African and European domestic fowls and substantial diversity among Asian junglefowls and domestic chickens.

Phylogenetic Analysis of Samar RJFs and Native Chickens

The matrilineal phylogeny of Samar RJFs and native chickens were constructed together with 61 complete mtDNA D-loop sequences derived from GenBank using the neighbor-joining (NJ) method (Fig. 1). The results showed that 3 out of 5 identified Samar RJFs, SPW2 (MK085034), SPW3 (MK085035), and SPW4 (MK085036), confined at the sub-haplogroup D1, which is believed to be the identified sub-haplogroup classification of most junglefowls inhabited in Philippines and Indonesia. The results imply that these 3 Samar RJFs are not a unique local group, and thus may be derived from neighbors. These results agreed with Osman and Nishibori (2014) that Southeast Asian neighboring countries including Myanmar, Cambodia, Laos, Bangladesh, Indonesia, and Philippines had a close genetic relationship in terms of D-loop nucleotide positions. The updated perspective of chicken domestication had classified the wild fowls in the Philippines belonging to the D1 sub-haplogroup (Miao *et al*., 2013).

However, 2 other Samar RJFs, SPW1 (MK085033) and SPW5 (MK085037), revealed a unique genetic change in their D-loop sequences. The informative SNP sites at bases 296 and 686 are absent in both SPW1 and SPW5, which have been found to be common among the other 3 Samar RJFs. Instead, both SPW1 and SPW5 SNP sites appeared to have the same SNP sites in the D2 sub-haplogroup with accession No. GU261683 according to the findings of Miao *et al*. (2013). This D2 sub-haplogroup classification strongly agreed with the up-to-date haplogroup tree and standardized hierarchical haplogroup nomenclature system established by DomeTree (Peng *et al*., 2015).

On the other hand, the Samar native chicken mutational motif in the D-loop sequences showed haplogroups A and B, and sub-haplogroup D1 and E1 were widely distributed across areas of Samar, Philippines. These results were further supported by the findings of Thomson *et al*. (2014), where Philippine chicken populations confined at 4 distinct haplogroups A, B, D, and E with higher spread throughout haplogroup D with relatively high genetic diversity (Hd＝0.89).

Phylogeographic studies have identified that one mtDNA lineage (haplogroup D) was largely limited to the Asia-Pacific region and that haplogroups A, B, and E contain haplotypes from all over Eurasia (Liu *et al*., 2006). Haplogroup E was predominant among Indian, Middle Eastern, and European chickens with sub-haplogroup E1, which was the single most-common chicken haplotype found around the world (Gongora *et al*., 2008). It was postulated that there is a higher population of native chickens inhabiting in Samar, Philippines, which are descendants of RJFs with a considerable mixture of indigenous chickens and commercial breed lines. These results suggested that Samar native chickens still mingled with the RJF species within Samar Island, although others were already a product of crossbreeding of commercial breed lines or a combination of different breed lines. This lineage likely changes because of human dispersal and migration carrying their animals and most likely, because of natural and artificial hybridization of commercial hybrid lines done by the local people.

Network Analysis of Samar RJFs and Native Chickens

Median-joining network analysis (Bandelt *et al*., 1999) of Samar RJFs and native chickens clearly clustered into the 4

Fig. 1. **Phylogenetic tree of 109 complete mtDNA D-loop nucleotide sequences (61 derived from GenBank and 48 from this study) based on the neighbor joining method (Saitou and Nei, 1987).** The numeral at each branch indicates the bootstrap value of replications. Bootstrap values lower than 50% are not shown. The greg circle marker indicates Red junglefowl and the black triangle marker indicates native chicken samples. The scale bar (0.001) indicates the genetic distance (substitutions per site).

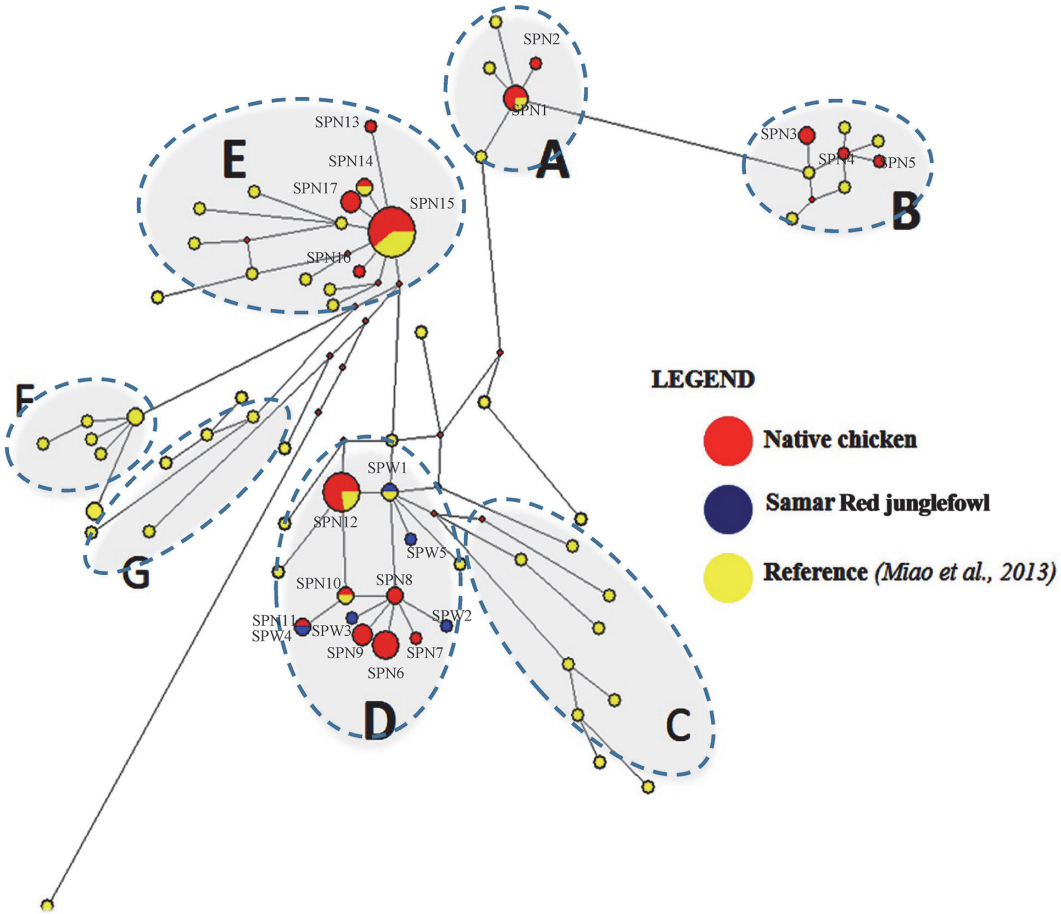

Fig. 2. **Median-joining network for 5 and 17 haplotypes of Samar, Philippines Red junglefowl and native chicken, respectively, based on the polymorphic site of the complete mitochondrial D-loop region (1,232 bp).** The area of each circle is proportional to the frequency of the corresponding haplotype. Different classes of haplotypes are distinguished by use of color codes.

main clades (A, B, D, and E) presented in Fig. 2. The results showed that all Samar RJF haplotypes are clustered into clade D, while Samar native chicken haplotypes are clustered 41.2% in clade D, 29.4% in clade E, 17.6% in clade B, and 11.8% in clade A. The results concur with the global mtDNA maternal lineage profile where haplogroup D occurred in Southeast Asia, South Asia, East Asia. and Africa with a high frequency of occurrence in the Pacific Islands (~77%). Southeast Asia likely served as the homeland of most domesticates spreading to the Pacific (Miao *et al*., 2013). Moreover, haplogroup E was distributed with high frequency in domestic chickens around South Asia, West Asia, Europe, South America, Africa, and among commercial breeds (Mwacharo *et al*., 2011, Miao *et al*., 2013, Osman *et al*., 2016), while the maternal lineages of commercial lines consisted of the three most common haplogroups A, B, and E1 (Miao *et al*., 2013).

Genetic Distance Relationship among Samar RJFs and other SEA RJFs

A neighbor-joining tree was constructed using nucleotide sequences from 5 Samar RJFs and other SEA RJFs sequences including Myanmar RJF (LC146448), Thailand RJF (AB009432), Indonesia RJF (AB009438), Cambodia RJF (LC146456), Vietnam RJF (AB009434), and Philippine RJF (NC_007236) (Fig. 3). Genetic distance of Samar RJFs has shown that one haplotype SPW1 is closely related with the Cambodia RJF (LC146456), while four haplotypes (SPW2, SPW3, SPW4, and SPW5) were closely related to the Myanmar RJF, Thailand RJF, Indonesia RJF, and Philippine RJF. The pairwise similarity analysis revealed that RJF in Samar, Philippines were more closely related with the Myanmar RJF at 99.6% similarity, followed by 99.5%, 99.1%, 98.7%, and 98.5% similarity with Indonesia, Thailand, Vietnam, and Cambodia, respectively.

The present study revealed 3 haplotypes of Samar RJFs clustered to haplogroup D1 and 2 haplotypes to D2. Haplo-

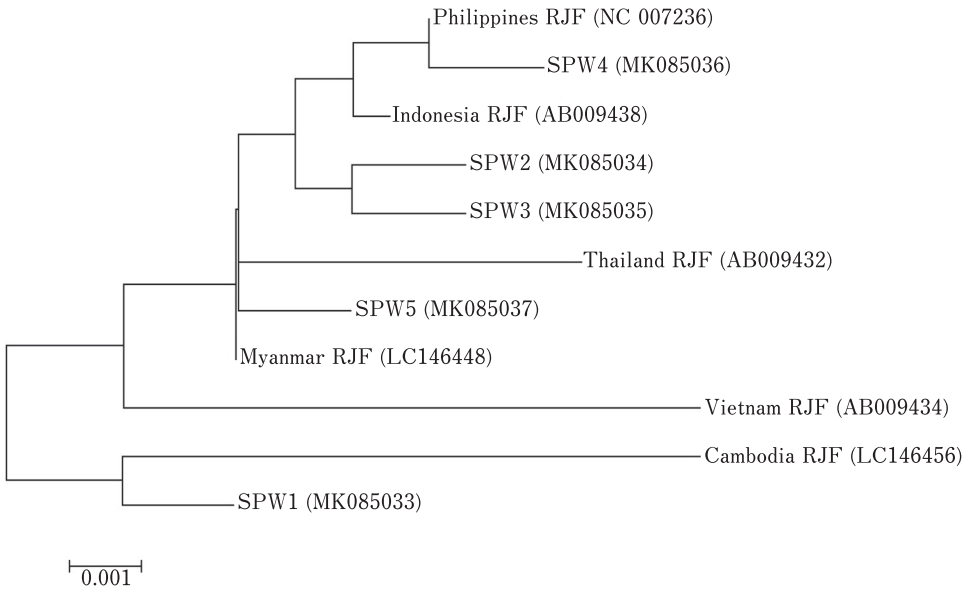

Fig. 3. **Neighbor-joining tree showing the close genetic distance of Samar Red junglefowl and other Red junglefowl subspecies across Southeast Asia region from complete mitochondrial DNA D-loop sequence.**

groups A and B, and sub-haplogroups D1 and E1 were the widely distributed matrilineal lineages of native chickens in Samar Island, Philippines. Samar native chickens revealed lower nucleotide diversity than other Asian fowls but higher than among African and European chicken populations. The genetic distance analysis of Samar RJFs showed close similarity to the Myanmar RJF, Indonesia RJF, and Thailand RJF. Thus, they were depicted to have a close genetic relationship among neighboring countries.

This is baseline genetic information to estimate evolutionary relationships, genetic diversity, and genetic distance of Samar RJFs among other RJF species inhabited in Southeast Asia, which is essential in developing future strategies for the characterization, conservation, and improvement of a valuable genetic resource in the country.

For future genetic studies, an in-depth molecular investigation is recommended that includes more representative sites and a larger number of samples. Modification of the sampling design to cover a larger area, focusing more on areas where the number of different domesticated chickens are not yet classified will facilitate a wider characterization of the domestic chicken breeds present in the different areas of the country.

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