Philippine Red Junglefowl: A separate *Gallus gallus* subspecies or not?

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1. INTRODUCTION

Red junglefowls (RJFs) are believed to be the sole ancestor of today's domestic chicken as proposed by Darwin (1868) and supported by the first molecular mitochondrial DNA (mtDNA) analysis in RJF (Fumihito *et al.* 1994; 1996). Meanwhile, other hypotheses claimed multiple lineages of chickens (Nishibori *et al.* 2005; Eriksson *et al.* 2008), suggesting that other *Gallus* species also contributed to the gene pool of domesticated chickens that we see today through hybridization. The recognized RJF subspecies based on their morphology and geographical distribution are: *G. g. gallus*, *G. g. spadiceus*, *G. g. jabouillei*, *G. g. bankiva*, and *G. g. murgii* (Johnsgard 1999).

RJFs are an important animal resource domesticated long ago for the use of man in different aspects of life. This domestication was established during the long and close interrelationship between the junglefowl and human beings, as humans started to provide the RJFs with ample supply of feeds under favorable conditions. RJFs could be easily identified through their morphology as they possessed certain phenotypic characteristics such as the boat-shape body appearance, white or red ear lobe, and slender greyish blue leg. On the other hand, sexual dimorphism between male and female RJF is easy to distinguish as male RJFs exhibit seasonal eclipse plumage that is absent in females. In addition, male RJFs have prominent bright red combs while the females have very small single serrated combs (Syahar *et al.* 2014).

In the Philippines, RJFs are scientifically referred to as *Gallus gallus* and are locally called as “labuyo” or “manok ihalas”. Its local name literally translates into “wandering around freely” as these RJFs were found to be roaming freely and scattered in the forests and are distributed all over the country. However, due to habitat loss

and human migration into the forests, RJF sightings are becoming rare (Masangkay *et al.* 2010). Though investigations have already been made in the Philippines, specifically in the islands of Luzon, Palawan, Mindoro and Negros in the years 1971 and 1975 (Nishida and Masangkay 1978), the genetic purity of the chicken could have changed from that period onwards.

Moreover, subspecies classification of the Philippine RJFs is still in question given that the published results are often inconclusive and at times contradicting. Also due to insufficient studies providing evidence for the true genealogy of the Philippine RJFs, their true subspecific classification, maternal origin and domestication remain unclear to this day. To address this problem, an in-depth molecular study and analysis encompassing the whole Philippine archipelago could help in pinpointing their geographic and genetic identity, maternal origin and diversity including their ecology.

The purpose of this review is to discuss and summarize different taxonomic classifications used in identifying the Philippine RJFs as well as their breeding potentials under the confinement system. These methods include morphological, breeding, and genetic studies.

2. Morphological and genetic identification and breeding studies of Philippine RJFs

Morphological identification and classification

Hachisuka (1939) first classified the Philippine RJFs under a separate *Gallus gallus* subspecies which he called *G.*

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g. philippensis, denoting that this RJF was an endemic species widespread in the Philippines. Under a separate subspecies, he described the Philippine RJF to have small lappets, and golden hackle end more intensely colored than in *G. g. bankiva* and in Micronesian RJF subspecies. Parkes (1962) scrutinized the work of Hachisuka (1939) stating that the Philippine RJF was considered to be the same with *G. g. gallus*, adding that Hachisuka (1939) was faced with various limitations in conducting his work. There were however some inconsistent differences, as some avian characteristics were best developed in populations from the southern parts of the Philippines such as in Mindanao and Basilan, and lesser so towards the northern part of the country. However, Parkes (1962) weighed on the evidences and concluded that the RJFs found in the Philippines were indigenous and were not introduced by man. In addition, Parkes (1962) conclusion was also based on an earlier study conducted by Rand and Rabor (1960) whose main work was on the general avifauna of the Philippine Islands.

The reports of Hachisuka (1939) and the generalization of Parkes (1962) based on collected evidence from the literature were all in disagreement with the findings of Nishida *et al.* (1985) and Nishida and Masangkay (1978). Through morphological identification of species, Nishida *et al.* (1985; 2000) reported that the RJF species found in the Philippines belong to the *G. g. gallus* subspecies of the *Gallus gallus* species. To support this claim, previous reports (Nishida *et al.* 1978; 1985; Nishida and Masangkay, 1978) documented nine Philippine RJF samples collected from different parts of Luzon, Mindoro, Negros, and Palawan during the first (1971) and second (1975) field investigations in the Philippines. These collected samples were all classified under the genus *Gallus* Linn. based on their white earlobes. With reference to the description suggested by Madoc (1956) and Wells (1999) on the RJFs in Malaysia as well as those described by Gilliard (1950) and Rand and Rabor (1958; 1960) on the morphological features of the Philippine RJFs, the nine collected samples were all categorized under the *G. g. gallus* subspecies. Nishida *et al.* (1985; 2000), on the other hand, did not mention about the existence of the *G. g. philippensis* subspecies, suggesting that this was really not considered as a subspecies of the *Gallus gallus* species even before. In addition, Johnsgard (1999) did not include *G. g. philippensis* as a recognized *Gallus gallus* subspecies. Figure 1 shows the distribution

Fig. 1. Map showing the distribution of the three subspecies of red junglefowl (*Gallus gallus*) and green junglefowl (*Gallus varius*) in Thailand, Indonesia, and the Philippines (Nishida *et al.* 1985).

map of the different *Gallus gallus* subspecies in Southeast Asia. It was also noted that in this map the RJF subspecies represented in the Philippines was the *G. g. gallus* subspecies (Nishida *et al.* 1985).

Genetic identification and studies of the Philippine RJF

To date, no defined molecular conclusion on the genetic identity of the Philippine RJF has been published yet. Though there were few literatures available, they however contradicted with each other. Some literature classified the Philippine RJF under *G. g. philippensis* (Bondoc 2013) while others recorded it under the *G. g. gallus* classification (Nishibori *et al.* 2005). In a comprehensive research of Bondoc (2013) subjecting 25 Philippine RJF samples coming from different mountainous areas in the Philippines for DNA barcoding technique, he suggested that these RJFs were classified under *G. g. philippensis*. In this research Bondoc (2013) used the *cytochrome c oxidase subunit I (COI)* of the mitochondrial genome for DNA barcoding to assess the diversity and genetic distances of RJFs obtained from the different mountains in the country and clustered these Philippine samples with the different RJF subspecies available in GenBank. His results had shown monophyletic branching of the Philippine RJF samples forming two separate clades different from the reference sequences. The monophyletic branching in his results suggested the classification of Philippine RJF to a different subspecies. However, upon checking the Barcode of Life Data System (BOLD) (<https://www.biosystem.org>), no DNA sequence data for the 25 RJFs

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analyzed by Bondoc (2013) were found. The availability of these sequences could somehow serve as basis for future studies and proper identification of wild caught and introgressed Philippine RJFs. The absence of these sequences made it difficult to justify the true existence of this subspecies. It was also noted that the subspecies *G. g. philippensis* was not included as a category of RJF classification in almost all genetic studies regarding RJFs (Nishibori *et al.* 2005; Miao *et al.* 2013; Godinez *et al.* 2019; Wang *et al.* 2020). In fact, the research of Bondoc (2013) was the only literature that genetically categorized the Philippine RJF under the *G. g. philippensis* subspecies. Moreover, due to the unavailability of the DNA barcode sequences of the RJFs studied by Bondoc (2013) in DNA data banks, further DNA barcoding is advised to be conducted to verify this claim. In addition, the DNA barcode sequenced must be made available to public for verification and as inference for future Philippine RJF studies.

On the other hand, the DNA barcoding technique used by Bondoc (2013) is useful in assigning unknown individuals to species and helps in the discovery of new species (Hebert *et al.* 2003; Stoeckle 2003). However, using DNA barcodes in identifying species have some drawbacks, and one of which is limited phylogenetic resolution which arises from confusion from the scope of inference (Craig and Cicero 2004). Also, the *COI* sequence is shorter as compared to the complete mtDNA sequence, making *COI* less informative as compared to the mtDNA D-loop. In addition, *COI* has the least variable mitochondrial gene, suggesting that it has slow evolutionary rate (Kerr 2011) and has lower non-synonymous substitution rate among the protein-coding mitochondrial loci (Eo and DeWoody 2010; Kerr 2011). In contrast, mtDNA is highly polymorphic with faster evolutionary rate with a higher nucleotide substitution rate (Brown *et al.* 1982).

Moreover, molecular genetics literature categorizing Philippine RJF under *G. g. gallus* is very limited. One research using this subspecies classification was by Nishibori *et al.* (2005), wherein they used a male RJF from Manila, Philippines collected in 1998. For accessibility and future reference, the sequence of this sample was made public in DDBJ/EMBL/GenBank (Accession numbers: AP003322/ NC_007236). Their results presented phylogenetic trees based on amino acid sequence and based on the sequences of the first and second nucleotide/codon of

mtDNA and these results have shown the genetic relatedness of the Philippine RJF with *G. g. bankiva*. Using BLAST homology search this same Philippine RJF sequence was inferred with selected *Gallus gallus* subspecies reference sequences (Table 1) together with Philippine RJF sequences published in GenBank. The results have shown that it shares 99.84% identity with the *G. g. bankiva* (AP003323) which has an accession length of 1,232 bp. It also shared 99.59%-99.84% identity with the other RJFs collected by Godinez *et al.* (2019) with an accession length of 1,231bp, lower than *G. g. bankiva* (AP003323). Given the limited sample number, the result on the test of homology only showed close genetic relatedness and did not denote final classification of the sample of Nishibori *et al.* (2005) to *G. g. bankiva*. In addition, that sample was collected only from one area in the Philippines and therefore did not represent the general classification of all the RJFs found in the Philippines.

Recently, Godinez *et al.* (2019) collected RJF from Samar, Philippines that was classified under the species *Gallus gallus* and which was further categorized under *G. g. gallus* in GenBank. On the other hand, their sample was limited to only one island and did not significantly represent the rest of the Philippines. Moreover, their result was in agreement with the findings of Miao *et al.* (2013) wherein, the Philippine RJF (AP003322/ NC_007236) showed close similarity with the SNP sites with the haplotypes of Samar RJFs except at bases 199, 293, 309, and 417. In addition, the Philippine RJF sample of Miao *et al.* (2013) was classified under the *G. g. gallus* subspecies. Furthermore, their results also implied that the Samar RJFs were not a unique local group and might have been derived from neighboring countries. Also, their results agreed with Osman and Nishibori (2014) affirming close genetic relationship in terms of D-loop haplogroup classification among Southeast Asian neighboring countries including the Philippines. This result further added to the evidence that the Philippine RJF did not belong to a separate and unique taxon, further suggesting that it does not belong to a separate subspecies.

Furthermore, Thomson *et al.* (2014) using ancient DNA to study the dispersal of ancestral Polynesian chickens across the Pacific found modern specimens from the Philippines carrying similar haplotypes with the ancient Pacific samples providing clues about a potential homeland of the Polynesian chicken. In addition, the DNA sequence which provided clue for the relationship of the Polynesian

Table 1 List of reference sequence used for homology testing.

Accession number	Subspecies/breed	References
MK085033	<i>Gallus gallus gallus</i>	Godinez <i>et al.</i> 2019
MK085037	<i>Gallus gallus gallus</i>	Godinez <i>et al.</i> 2019
MK085036	<i>Gallus gallus gallus</i>	Godinez <i>et al.</i> 2019
MK085035	<i>Gallus gallus gallus</i>	Godinez <i>et al.</i> 2019
AB007725	<i>Gallus gallus gallus</i>	Miyake 1997
AP003323	<i>Gallus gallus bankiva</i>	Nishibori <i>et al.</i> 2005
GU261690	<i>Gallus gallus spadiceus</i>	Miao <i>et al.</i> 2013
GU261709	<i>Gallus gallus murghi</i>	Miao <i>et al.</i> 2013
GU261696	<i>Gallus gallus jabouillei</i>	Miao <i>et al.</i> 2013
NC_007239	<i>Gallus lafayetii</i>	Nishibori <i>et al.</i> 2005
AP003324	<i>Gallus varius</i>	Nishibori <i>et al.</i> 2005
NC_007240	<i>Gallus sonneratii</i>	Nishibori <i>et al.</i> 2005
AB007723	<i>Gallus gallus domesticus</i> /White Leghorn	Miyake 1997
AP003318	<i>Gallus gallus domesticus</i> /White Plymouth Rock	Nishibori <i>et al.</i> 2005

chickens and the chicken from the Philippines was also the sequence sample (AP003322) published by Nishibori *et al.* (2005). On the other hand, Peterson and Brisbin, Jr. (1998) suggested that Philippines together with other countries might have accepted introduced junglefowl brought by human settlements from different countries, and hence held junglefowl populations that were genetically mixed from the outset, thus contradicting the conclusion of Parkes (1962).

Breeding studies on the Philippine RJF

In a study conducted by Buctot and Espina (2015) utilizing Philippine RJFs coming from selected areas in Leyte, they assessed the breeding performance of the RJF (♂) x native chicken (♀) and the quality of the egg produced under confinement system and under natural mating method. The result of their study had shown that the egg fertility and hatchability produced by the RJF (♂) x native chicken (♀) crossing is comparable with the native chicken (♂) x native chicken (♀) mating. In addition, though all of the RJFs used were collected from different areas in Leyte, Philippines, the results had shown no significant difference with each other with regards to breeding performance and egg production indices. On the other hand, Buctot (2016) also reported comparable production potential and egg quality traits between two RJFs sourced from the mountainous area in Southern Leyte, Philippines mated with the native chicken hens under confinement under natural mating method and compared it with the performance of native roosters mated with native chicken hens. These were assessed based on their breeding and production performance under confinement system. Their results have shown that the performance of RJFs were

comparable with the performance shown by the native chicken, though difference was observed only on the egg weight of due to genetic and non-genetic factors.

3. Supplementary information from different DNA data banks and ornithology websites

DNA data banks sources

With regards to the DNA sequence availability of *G. g. philippensis* there are no available sequences in GenBank. Another website which stores important DNA sequences is BOLD which was designed to support the generation and application of DNA barcode data of different species including those of chickens. However, in this website, no *COI* sequence or DNA barcode of *G. g. philippensis* or any Philippine RJF samples can be found. With the unavailability of the *G. g. philippensis* DNA sequence in any DNA public domain websites, we could not use this subspecies as basis for the molecular classification of the newly collected Philippine RJF samples, thus using this classification for future studies would be unlikely. On the other hand, Philippine RJFs classified under *G. g. gallus* (Nishibori *et al.* 2005; Godinez *et al.* 2019) are available online.

Other sources of information on the classification of the Philippine RJFs

There are several ornithology websites that did not catalogue *G. g. philippensis* as subspecies of *Gallus gallus*. These websites include “The Cornell Lab of Ornithology”, which contains comprehensive life histories of all bird families. Unfortunately, the subspecies *G. g. philippensis* is not among their list. Another reliable website is the Avibase

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which is an online database that organizes bird taxonomic and distribution data globally. Though *G. g. philippensis* is available in Avibase (<https://avibase.ca/CF6BDC57>), it was however, indicated with an invalid subspecies status. This could probably be attributed to the lack of morphology and molecular classification literatures that would support its existence.

On another account, one sample of Philippine RJF was catalogued in the Smithsonian Institution (SI) (https://www.si.edu/object/nmnhvz_4016513) which is a male RJF published as *G. g. philippensis* and recorded as nmnhvz_4016513. The specimen which was a whole skin preparation was collected in Palawan on July 19, 1888 by Dr. Platen. This record is perhaps the oldest known existing sample of this subspecies. However, despite its existence, no other description regarding its morphology and molecular identity were stated. Understandably only morphological information is available for this record.

4. CONCLUSION

To date, there are only very limited sources of morphological and molecular information, and no additional published studies that would advance our understanding of the identification of Philippine RJFs based on their morphology and genetics. Due to insufficient morphology and genetic studies supporting the existence of the *G. g. philippensis*, available data cannot be used for identification and classification of the Philippine RJFs. This subspecies will only become valid when DNA sequence can prove its existence. On the other hand, even if the Philippine RJF was classified under the *G. g. gallus* in some morphological, ecological and genetic studies we still cannot disclose that the Philippine RJF is classified under the said subspecies as it will be premature to do so.

To resolve the classification issue of the Philippine RJF, morphological identification must be supplemented with molecular analysis to bridge the gap between the morphological and molecular identification of this chicken. In addition, future studies must include large population number and expansive sampling sites that would represent the three major islands of the Philippines.

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