別紙４

学位論文の要旨

論文題目　Morphological and Phylogenetic studies on the Genetic Diversity and Origin of Philippine Red Junglefowl

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Red junglefowl (RJF) is an important animal resource domesticated long ago for the use of man in different aspects of life. Current reports suggested that the *Gallus gallus spadiceus* and not the *Gallus gallus gallus* is the maternal origin of the domestic chickens based on the 863 genomes sampled worldwide. In the Philippines (PH), RJFs still exist in the forests, however, their subspecific classification is still in question, given that the published results are often inconclusive and at times contradicting. To address this concern, an in-depth molecular study and analysis encompassing the whole Philippine archipelago could help us understand it genetic identity, maternal origin, and diversity, including their ecology. Furthermore, due to identification limitations, the need to use unconventional methods and DNA sources was explored as well. Connecting taxonomic classification and phylogenomic result of this study could help us address the possible conservation of this animal in the Philippines. This will also help us address one of the Sustainable Development Goals of the United Nations.

Initial identification of RJFs could serve as a vantage point towards a deeper understanding of this animals. Currently, due to the ambiguous subspecific classification of PH RJFs, the establishment of baseline data based on the morphological characteristics of the

RJFs found in the different areas of the Philippines could provide us an initial perspective on the identity of these animals. Thus, evaluation of RJFs through genotyping of phenotypic morphological expressions and morphometric assessment was conducted. This study revealed that there are still RJFs in the Philippines that exhibited the wild-type morphology. Moreover, in comparing the wild-type RJFs from the hybrid RJF, shank color, and eclipse plumage expression in male RJFs appeared to be the most important identification index. In determining the subspecific classification of RJFs, earlobe color, hackle, and mantle in males and females appeared to be the most significant parameters to assess. To bridge the gap between morphology and genetics, the genetic analysis conducted in this study supported the morphological classification of Philippine RJFs under *G.g.gallus* and *G.g.bankiva*. Consequently, this research supported the idea that identifying a wild RJF from a hybrid RJF is possible through morphological examination. Furthermore, the observed hybrid RJF in this study is a threat to the genetic diversity and integrity of RJFs.

Moreover, taxonomic classification through genotyping and morphometric analysis is not enough to verify the subspecific classification and determine the evolutionary history of the PH RJFs. Thus, phylogenetic analysis utilizing the genomic and matrilineal tracing advantage of mtDNA in addressing the past classification ambiguity of the PH RJFs, as well as evaluate its present ecological diversity status, and its complex evolutionary relationship with the RJFs in Asia was conducted. To address these concerns, we analyzed the mtDNA of the PH RJFs that were collected from the different mountainous areas in the Philippines. This study revealed that the Philippine RJFs analyzed were classified under haplogroups D, Y, and E. The result revealed multiple maternal origin of the PH RJFs, possibly from China and Indonesia. The haplotype sharing of the investigated RJFs with the domestic chickens of Laos, China, and India also showed prevalence of domestic chicken gene introgressed RJFs in the Philippines. It was also observed that two *Gallus gallus* subspecies were present in the Philippines, the *G.g.gallus* and *G.g. bankiva*, thus confirming the morphological analysis result of this research. The genetic analysis also revealed high genetic and nucleotide diversity, and population expansion of the PH RJFs. Moreover, considering the close genetic relationship of RJFs from the Philippines and Indonesia as revealed in this study, the need to conduct phylogenetic analysis comprising the RJFs from these two countries could help us understand their complex evolutionary relationship.

Initial phylogenetic analysis suggested that the RJFs in the Philippines shared the same haplogroup and haplotype with a *G.g.bankiva* RJF from Indonesia. However, to further clarify the phylogenetic relationship of the RJFs from the Philippines and Indonesia, a phylogenetic and divergence time analysis was conducted. The results revealed that the Indonesian RJFs were solely classified under haplogroup D, in contrast to the haplogroups D, Y, and E classification of the PHRJFs. Moreover, Indonesian RJFs only shared the same haplotype with a *G.g.bankiva* from Indonesia, aside from the domestic chickens from Laos and China. Furthermore, both the Philippine and Indonesian RJFs shared the same haplotype, thus, supporting common maternal ancestry. The coalescent divergence time analysis of this study also supported the close genetic relationship of the RJFs from these two countries. The divergence analysis of this study also suggested that the RJFs from the Philippines diverge first from least most common RJF ancestor than the Indonesian RJFs.

Philippines is one of the most biodiverse countries globally, which forms a global hotspot of species diversity, including RJFs. The collective result of this study reported continued existence of RJFs in the wild. However, the haplotype sharing of the Philippine RJFs with domestic and commercial chickens observed in this study is of biodiversity concern. This could mean genetic admixture of the wild and domestic chicken population, thereby jeopardizing the genetic integrity of the RJFs. Translating the result of this study in addressing global concerns, this study suggested that conservation consideration based on the phylogenomics must be incorporated for a more structured conservation and endangerment classification to address the SDG 15: Life on land.

In conclusion, this study revealed that differentiation of wild RJFs from RJFs introgressed with domestic chicken gene is possible through qualitative and quantitative morphological assessment. However, though the diversity and ecological status of Philippine RJFs through morphological assessment could be used as an initial evaluation approach, the need to conduct DNA analysis could provide us a better understanding of its the taxonomic classification, biodiversity status, and its evolutionary history. This study also highlighted the genomic advantage of mtDNA in determining the matrilineal ancestry, phylogenetic diversity, and endemism of the Philippine RJFs. mtDNA also highlighted the areas that can be targeted for protection and be given priority in any planning process for improved biodiversity conservation, leading to the sustainability of life on land.