ABSTRACT

MOLECULAR DIVERSITY OF INDIGENOUS POULTRY USING DNA BARCODING AND D-LOOP SEQUENCES IN THE STATE OF WEST BENGAL AND ASSAM

by

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Chicken and other poultry species being an integral part of human civilization immensely contribute to mankind providing animal protein, research model for biological research and many more. India which is the epicentre for the origin of this species possess 29 diverse varieties of indigenous poultry with some novel characters. Due to alarming decay of poultry genetic resources, biodiversity vis-à-vis its conservation is of utmost importance. In this outset the present study are undertaken with an objective to shed some light on physio-morphological characters and molecular diversity of the indigenous poultry from four distinct agro-climatic zones of West Bengal and three such zones of Assam. The physio-morphological studies revealed that both qualitative and quantitative morphological characters like plumage and skin color, body weight, egg production, broodiness etc did not vary significantly and all the birds possess novel characters of excellent scavenging characters with moderate to high disease and stress resistance. We also carried out physio-biochemical investigation of the birds in diverse agro-climatic conditions, but surprisingly it was found that haematological, hormonal, enzymatic, stress related enzymes and radicals along with serum micro and macro mineral status did not differ widely with minor significant changes and in congruence with the normal range of chickens, indicating their excellent adaptability to their natural habitat. To elucidate the molecular diversity, we approached two gold standard mitochondrial markers, DNA barcode (cytochrome oxidase I) for species level phylogenetic studies and D-loop control region by amplification with sequencing of cytochrome oxidase I (CoI) employing published primer for CoI gene and own designed primer for D-loop genes respectively. We successfully amplified and sequenced the above genes. The sequences are analyzed using relevant bioinformatics software and sequenced data submitted to global database with generation of seven CoI sequences reported for the first time in India (Accession No.- JQ812738, JQ812739, JQ812740, JQ812741, JQ812742, JQ812743 and JN793568) and 30 D-loop sequences (KF411004 to KF411033). The phylogenetic analysis of the indigenous Gallus species using own sequenced 6 indigenous poultry
with other 37 retrieved sequences of genus *Gallus* and 10 different genus of the same *Phasianinae* subfamily and closely related duck species from associated taxa, reveals genetic divergence increases with increased taxonomic level and all species possesses a distinctive set of CoI sequences showing low intra species divergences and most species separated into distinct clusters except *Gallus sonneratii* formed cluster with *Gallus gallus*. The tight clustering of conspecific mtDNA sequences not only bolsters the view that species are fundamental biological units, but also reveals that their identification is usually uncomplicated. In addition, the evolutionary analyses of our Red Jungle fowl sequence (JN793568) with other retrieved sequences suggest that the evolutionary clade of RJF clearly differentiated from other jungle fowls supporting that the DNA barcode can be effective to identify and differentiate red jungle fowl between and within clades. The diversity study of different population of poultry based on own D-loop sequences analysis in respect to the generated jungle fowl D-loop sequences (KF_411027) for population structure indicated phylogeographic isolation of the indigenous poultry in Assam and West Bengal resulting 19 distinct haplotypes clustered in two haplogroups viz. West Bengal cluster and Assam cluster with minor shift, reported for the first time from India. Molecular clustering of our D-loop sequences well distributed in Eastern and Northeastern India resulted three distinct haplogroups viz. B, D and E assigned distinctly for each haplogroups suggested by Liu *et. al* (2006) for global poultry haplogroups. The differentiation of each of the haplogroups predates the domestication pattern of chicken as a recent event. On the basis of this study conducted, it is anticipated that further growth in taxon and geographical coverage will not seriously alter the conclusions drawn from this study. We also provide evidence that there is little genetic exchange between *G. g. murghi* and *G. g. domesticus* from our study region. Our results expand the perspectives into the complex history of chicken domestication and dissemination. The result can be used to formulate strategies for future conservation of indigenous chickens from West Bengal and Assam, and from India as well through comprehensive genetic studies to unravel more details. Global analysis using combined D-Loop dataset resulted 69 distinct haplotypes and D-loop sequences from Eastern and Northeastern India molecularly clustered in previously described global haplogroups viz. B, D and E.

**KEYWORDS**: Poultry, physiobiochemical, species identification, DNA barcode, *cytochrome c oxidase subunit I*, CoI, D-loop, poultry diversity, phylogeny.