

SERB Grant No. SB/SO/AS/077/2013

**GENETIC ASSESSMENT OF SAMBAR (*Rusa unicolor*) POPULATIONS
IN NORTH-EAST INDIA**

Project Compilation Report

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SCIENCE AND ENGINEERING RESEARCH BOARD

Project Team

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**भारतीय वन्यजीव संस्थान
Wildlife Institute of India**

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Executive Summary:

Deer family Cervidae, known as true deer, is characterized by the antlers (secondary sexual characteristics) present in males; however, in *Hydropotes* antlers are absent. Cervidae is the second most diverse group among terrestrial artiodactyls with 55 extant species. The available systematics of Cervidae is primarily based on morphological characters, in which antlers are considered the major characteristic feature. Sambar (*Rusa unicolor*, Kerr-1792) is the largest deer species in Asia and most widely distributed among the nine cervid species in India. However, due to several sub-species of this species, it is necessary to explore the different lineages of sambar from its distribution range in India.

Molecular phylogeny is an unambiguous approach for evaluating the phylogenetic structure of closely related species. The taxonomic frailty, which persists in closely related taxa, can be potentially resolved using mitochondrial and nuclear markers. It was evident from the study that multiple mitochondrial lineages of Sambar are present in the Indian sub-continent. The Northeastern population emerged as a distinct lineage with more affinity towards the Southeast Asian subspecies of *Rusa unicolor*. Western Ghats population was also identified as a distinct cluster based on high levels of genetic differentiation from the other populations of Sambar. Phylogenetic reconstruction based on mitochondrial cytochrome b gene revealed interesting clustering pattern of the Northeast Indian and other residual Indian sambar populations. The northern, central and southern populations were clustered together in a single clade. However, an interesting finding in the molecular phylogeny of Indian Sambar was the distinct clustering pattern of Northeast Sambar populations with the Southeast Asian populations of *Rusa*. Also, a distinct evolutionary lineage was observed in the Southern population of sambar in India, which is differentiated from the Indian Sambar clusters by *Rusa timorensis* clade. This unique clustering indicates towards the genetic relatedness of Northeast Sambar with Southeast Asian Sambar. The comparative estimates of evolutionary divergence among Sambar populations in India indicated towards the phylogenetic relatedness of Northeast Indian Sambar with Southeast Asian Sambar. The evolutionary divergence estimates suggested that the northeast sambar population is genetically more closely related with north and central Indian population as compared to the southern population of Indian Sambar.

Variation of repeat number was observed among populations of Sambar with most of the populations exhibiting five copies of the repeat. In the populations of Meghalaya, Manipur, Mizoram, and Nagaland, the 40 bp repeat motif was duplicated for five times resulting in an insertion of 40 bp in the mtDNA control region of Sambar. The mtDNA CR region with 40 bp insertion has already been reported from the Western Ghats in India. Moreover, the sequence analysis using GenBank sequences of *R. u. swinhoei* from Southeast Asia revealed the presence of 40 bp insertion in SE Asia. In addition, a localized insertion of 80 bp in the individuals of

Kaziranga Np and Itanagar Zoo was observed. The insertion resulted as a duplication of the identified 40 bp repeat tandem repeat in these populations. However, the 80 bp insertion was restricted to the Kaziranga TR and nowhere else observed in wild in the study area. The captive population of Itanagar zoo was the only population to exhibit the 80 bp insertion other than Kaziranga TR.

Interestingly, sequence alignment of the 80 bp insertion positive individuals with the mtDNA CR region sequences of *R. u. swinhoei* revealed the presence of 80 bp longer sequences in this subspecies of Sambar. However, the 80 bp insertion was observed only in two sequences and remaining sequences used in this study using the NCBI database exhibited different copy number of the 40 bp tandem repeat. The presence of 80 bp long sequences in *R. u. swinhoei* extended the distribution of 80 bp insertion in the mtDNA CR region to Southeast Asia along with Kaziranga TR in Northeast India. Detection of insertion of 40 and 80 bp in the sambar populations in this study is an interesting finding enlightening the high levels of genetic variability in Sambar deer. However, we have studied the populations of Sambar from Northeast India and utilized the information using previously published sequences of the mtDNA control region from India and Southeast Asia.

The presence of 40 bp insertion in Northeast India, Western Ghats along with the SE Asia is an interesting finding of this study potentially indicating towards the historical connectivity of these landscapes. The preliminary results of this study unwrap a scope for future studies focusing on the variation patterns of the tandem repeats in Sambar deer across its distribution range. Microsatellite markers developed on a variety of cervid and bovid species were successfully genotyped for the sambar populations of India. The selected markers revealed high mean number of alleles per locus (9.71) and were found appropriate for the population genetic study of this cervid. The study revealed occurrence of high levels of genetic diversity in the Sambar populations with three major genetic clusters viz. North-eastern cluster, Western Ghats cluster and the major Indian cluster including populations from North, Central and southern part of India. It is suggested to manage these clusters using the genetic differentiation information generated in this study.