

**MOLECULAR CHARACTERISATION OF CANINE GENETIC  
RESOURCES OF TAMIL NADU**

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**CERTIFICATE**

This is to certify that the thesis entitled "**MOLECULAR CHARACTERISATION OF CANINE GENETIC RESOURCES OF TAMIL NADU**" submitted in partial fulfilment of the requirements for the degree of **MASTER OF VETERINARY SCIENCE** in **ANIMAL GENETICS AND BREEDING** to the Tamil Nadu Veterinary and Animal Sciences University, Chennai - 600 051 is a record of *bonafide* research work carried out by **HISHAM, A.** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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**ABSTRACT**  
**MOLECULAR CHARACTERISATION OF CANINE GENETIC  
RESOURCES OF TAMIL NADU**

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The indigenous dog breeds of Tamil Nadu have a long historical association with wars, hunting and social bondage and effective guarding of farm animals. Despite their unique qualities, the number of dogs under each breed is decreasing alarmingly. Hence, a study was conducted to characterize the native dog breeds of Tamil Nadu using microsatellite markers and to estimate the genetic relationship among these breeds; which will ultimately help in the conservation of these treasured germplasm.

A total of 50 samples each from Rajapalayam, Chippiparai and Kanni and 25 samples from Kombai breeds were collected from their respective breeding tracts and DNA isolated. PCR was carried out to amplify the genomic DNA using 29 canine-specific microsatellite markers and their products were resolved through automated DNA sequencer. The genotypes were scored and the number, size and frequency of alleles were calculated. Other microsatellite variability measures, genetic distance, phylogeny and population structure were also determined using suitable molecular genetics software.

In this study, a total of 297 alleles were observed across 29 microsatellite loci with an overall mean number of alleles as  $10.24 \pm 0.61$  per locus. The number of alleles in the markers differed substantially (5 in AHTk211 to 19 in LEI2D2) indicating that these microsatellite loci showed high variability in the native dog breeds of Tamil Nadu. Microsatellite allelic richness varied from 7.97 in Kanni to 8.48 in Chippiparai dogs with an average number of alleles per locus per breed as

8.17. The overall mean effective number of alleles was found to be  $5.38 \pm 0.32$  which ranged from  $4.42 \pm 0.29$  in Chippiparai to  $5.05 \pm 0.27$  in Kombai dogs. These microsatellite alleles were of size ranging from 80 (AHT121) to 484bp (FH2148). A total of 36 private alleles were detected across 18 loci with more number of private alleles in Kombai (13) followed by Rajapalayam (10), Chippiparai (9) and Kanni (4) dogs.

The overall PIC value observed in the present study ranged from 0.6337 (INU030) to 0.8914 (LEI2D2) with a mean value of  $0.7704 \pm 0.01$ . The average observed and expected heterozygosities pooled over different markers in the four dog breeds were  $0.7334 \pm 0.02$  and  $0.7997 \pm 0.01$  respectively, which ranged from 0.5389 (REN105L03) to 0.8869 (AHT137) and 0.6904 (INU030) to 0.9023 (LEI2D2). High heterozygosity values indicated the presence of high amount of genetic variation in all the four dog populations.

The results of the  $\chi^2$  test for goodness of fit revealed that among the 29 microsatellite loci studied 13, 13, 9 and 5 were in Hardy-Weinberg disequilibria in Rajapalayam, Chippiparai, Kanni and Kombai dogs respectively. The mean  $F_{IS}$  values obtained were 0.0494, 0.0472, 0.0207 and 0.0208 for Rajapalayam, Chippiparai, Kanni and Kombai dogs respectively. The overall  $F_{IS}$ ,  $F_{IT}$  and  $F_{ST}$  values observed in this study were 0.0319, 0.0785 and 0.0481 respectively for all the four dog populations.

In the present study, no mode-shift was detected in the frequency distribution of alleles and normal L-shaped curves were observed in all the four native dog breeds of Tamil Nadu strongly indicating that the populations are non-bottlenecked and maintaining sufficient amount of genetic diversity. An analysis of Nei's genetic distance revealed that genetic distance between Chippiparai and Kanni dogs was the shortest (0.1109), followed by Kanni and Kombai (0.2335), Chippiparai and Kombai (0.2558), Rajapalayam and Kanni (0.2612) and Rajapalayam and Kombai (0.2839); whereas the genetic distance between Chippiparai and Rajapalayam was the longest (0.3311).

Phylogenetic tree, principal component analysis and multidimensional scaling pinpointed the uniqueness of Rajapalayam and Kombai dogs as well as the closeness of Chippiparai and Kanni dogs. Structure analysis revealed that Chippiparai and Kanni dogs had more admixtures between them; while Rajapalayam and Kombai were more distinct with lesser amount of breed admixture.