



Genomic Characterization of Gaddi breed of Dog to Envisage Molecular Signatures

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Dog has been one of the loyal and preferred tamed animals since prehistoric times. They have been associated with hunting and herding activities, as guard dogs for homes, protectors of the family, and affectionate pet. Although more than 350 distinct breeds of dogs have been identified worldwide, *the species have been left neglected in developing countries*. Our history indicates that Indian dogs have been of great importance to Indian society, Indian kings, and British rulers. The forgotten glory of the indigenous canines has been emphasized to be revived in the recent talk aired by the honorable Prime Minister on 30th August 2020, where he urged on “*Bringing home the indigenous dog breeds*”. Four Indian dog breeds (Rampur Hound, Himalayan Sheep Dog/Gaddi, Mudhol Hound, and Rajapalayam) were featured on a set of postage stamps to celebrate India’s canine heritage in 2005. Genetic characterization of these breeds needs to be prioritized to conserve and safeguard the indigenous germplasms and make use of them for the betterment of humanity. The focus on indigenous canines has been quite rare in India. The National Bureau of Animal Genetic Resources (NBAGR), of ICAR, Government of India, has registered three indigenous breeds, namely, Rajapalayam, Chippiparai, and Mudhol Hound. Howbeit, the Dog Registry of America, Inc. (DRA) has recognized five native Indian dog breeds, namely, Rajapalayam, Gaddi dog, Combai, Rampur Hound, and Indian Spitz. Indian dogs have been locally identified as distinct genetic groups/breeds due to their diverse looks, behavioral features, and special traits due to their distinctive geographical conditions. In this section, we will unveil unique canine germplasm which is truly a treasure due to its certain features, like, herding ability, faithfulness, agility, boldness, and reliability.

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The indigenous Gaddi breed of dog, also called Himalayan Mastiff or Himalayan Sheepdog, or Himalayan Hound, originated from the Himalayan region. A local tribe, known as “Gaddi”, mostly relies on their livestock for their livelihood. Gaddi dogs (named after this tribe) play a major role in herding and protecting their stocks. However, these dogs have been ignored due to mere negligence. There is a long Indian border in the Himalayan region, so these dogs can survive well in those harsh conditions. The trained Gaddi dogs can be deployed in the armed forces in the Himalayan regions for patrolling and detection of arms and ammunitions beneath the ice. Since, Gaddi dog is a robust and healthy breed, native to harsh Himalayan terrains. The identification of various molecular markers for better adaptability to hypoxic conditions, disease resistance, sniffing attributes, and other characteristics suitable for enhanced utility of canines needs to be done with prime emphasis. The other stakeholders like dog breeders and farmers will also be benefited from the breed characterization and behavioral studies. Thorough research work on behavioral genetics, physiological, morphological through molecular perspectives are required to be focused to underscore the importance of the Gaddi dog breed for herding, protecting farms, and livestock as well as household protection.

Research on canine germplasm has now been emphasized by Government funding agencies. The Department of Biotechnology, Government of India has funded a mega-research project on canines that also includes germplasm characterization of Gaddi dogs. Our Animal Genomics Lab at the Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana, has been working on whole genome

sequencing and annotation of Gaddi with an aim to deep sequence (30X) and assemble the nuclear genome of indigenous dogs and bio-computational characterization of coding and non-coding sequences through *in silico* analysis of whole-genome sequence (WGS) data. The whole genome sequencing has been done using the NovaSeq 6000 system of Illumina (150 paired-end sequencings). On average 98 GB of raw data per sample was analyzed, with a total of 493 GB. The error rate of 0.03% was observed in the raw data of all five samples. Gene identification resulted in 41K to 49.5K gene transcripts in the Gaddi dog genome. Further analysis of the Gaddi dog genome depicted various gene sequences of known origin like canine MYH4- a myosin 4 protein, LOXH1 (sensory perception of sound), EFNMT (methyltransferase), and ARNT (Aryl hydrocarbon receptor nuclear translocator, a transcriptional regulator of adaptive response to hypoxia), etc. The gene sequences similar to human, mouse, dog, bovine, horse, and many more proteins have been found in the Gaddi dog genome. Putative microRNAs, ranging from 3 to 6 in number in those 5 whole genome sequences, a total of 22 putative miRNA sequences have been identified through the homology and structure-based biocomputational analysis of the indigenous *Gaddi* dog genome. Further, 7 unique and novel miRNA sequences have been predicted from the Gaddi dog genome. Thirty thousand long non-coding RNA (lncRNA) transcripts have been identified in the Gaddi dog genome. Genome-wide microsatellite sequences identification evaluated the mono, di, tri, tetra, penta, and hexameric repeats as well as the total SSR count and compound SSRs (3358579, 3359895, 3369192, 3313512, and 3299350 in number in five Gaddi dog genome samples) in the Gaddi dog genome coverage of 0.32%. A comparative study between the indigenous Gaddi dog breed and exotic dog breeds has been taken into consideration, to explore the differential repertoire of protein-coding and non-coding RNA specific genes.