

Molecular Characterization for Identification of Endangered Sri Lankan Leopard: A Forensic Application

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Sri Lankan Leopard (*Panthera pardus kotiya*) is an endemic subspecies and listed under endangered species by the International Union for the Conservation of Nature (IUCN). Even though killing of leopard is illegal, few incidences have been reported recently in Sri Lanka. To implement protection law effectively, precise forensic declaration of suspected body parts is necessary. Current investigation was conducted to identify the species from meat and fur samples suspected to be of a Sri Lankan Leopard using molecular sequence data. DNA from meat (n=2) and fur (n=2) were extracted using a commercial genomic DNA extraction kit (Qiagen, Germany). PCR was performed using previously published mitochondrial 16S rRNA primers and amplicons were sequenced bi-directionally by Sanger-sequencing method. Consensus sequences were generated in BioEdit 7 and MEGA 10, where identical sequences resulted from all samples. A phylogenetic tree was constructed in MEGA 10 under maximum parsimony criterion at 500 bootstrap replicates. National Center for Biotechnology Information (NCBI) BLAST search for consensus sequence showed 99.58% identity and 100% query cover with *Panthera pardus* mitochondrion complete genome with an E-value of 0.0 showing near complete match. Obtained phylogeny based on mitochondrial 16S rRNA sequence further suggested that *P. p. kotiya* is closely related to leopard subspecies from Russia (*P. p. orientalis*) and China (*P. p. japonensis*). However, mitochondrial 16S rRNA sequence of Indian subspecies *P. p. fusca* was not available in GenBank for comparison. This is the first reported genotypic identification of Sri Lankan subspecies *kotiya* by 16S rRNA primers and sequence alignment. Sequences generate in this study for *P. p. kotiya* were submitted to GenBank under the accession number MW703705. Present study provided new data on 16S rRNA sequence of subspecies *P. p. kotiya* in Sri Lanka.

Keywords: *Panthera pardus kotiya*, 16S rRNA, PCR, Sri Lanka