



Forensic Identification of ornate flying snake (*Chrysopelea ornata*) through COX2

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Abstract: Wildlife forensics deals with the identification of the animals and animal products that are confiscated during smuggling, trading, and illegal possession of the animals. Though identification of species can be done physically, it is not possible at all times. However, identification at molecular level is one of the fail proof method to make sure of the animal in possession. *Chrysopelea ornata* also known as gliding snake, golden tree snake and ornate flying snake is unique in reptiles, as it is the only snake species from Colubridae family that can modify its body to glide from one tree to another. This makes the snake one of the exotic pet in trade. Over the years, researchers have standardised the identification of any animal species and its by-products with the help of DNA barcoding. Animal by products that are caught during smuggling or possession of those by products that are illegal to possess with oneself are at times hard to identify from the fake ones. Among the genes that are used to identify the animal are COX1 or COI, CYT-B, 12s and 16s. Along with the above-mentioned genes, it has proven that COX2 gene can also be used to identify the species. This research for the first time has isolated COX2 region of *Chrysopelea ornata* and has submitted it to NCBI. Which will be helpful in identification of *Chrysopelea ornata*.

Keywords : COX2 , COII , DNA barcoding , wildlife forensics, *Chrysopelea ornata*

I. INTRODUCTION

Chrysopelea ornata is also famous as Indian flying snake, Golden tree snake and gliding snake is a near threatened species in family of Colubridae [1]. It has gathered its attention for its gliding asset [2]. *Chrysopelea ornata* is a snake that oblige surplus wide-ranging investigation, because present information shows that exploitation for trade presently can be a hazard. Species that are believed to currently have no forensic significance may later have a forensic significance when consequently their harvesting ecosystem is better-understood [3]. Mitochondrial region encoding the COII or COX2 gene is useful in evolution studies, population genetics due to its high degree of variation in the region [4]. Mitochondrial genes are used for identification of organisms due to the conserved nature and universal existence. The COI–COII genes are composed of interspersed, highly conserved membrane bound regions and variable extra membrane loops [5][6] Sequences of the mitochondrial genes that are obtained by standardized protocols, which are helpful in identification of an animal in trade, are universally known as (FINS) ‘Forensically Informative Nucleotide Sequencing’ [7] [8] [9].

Materials and Methodology:

Snake shed skin was collected from the Zoological garden, Alipore, Kolkata with the sanction of Office of the Principal Chief Conservator of Forests, Wildlife & Chief Wildlife Warden, West Bengal. DNA has been extracted using [10]. Extracted DNA was transferred to Sandoor Laboratories, Hyderabad. With the help of *QIAseq FX DNA Library kit* libraries were prepared and arranged using 100ng DNA. Fragmentation of DNA into smaller parts was done with the help of enzymes by a end-repair process, where an ‘A’ is added to the 3’ end, which made the DNA fragments prepared for adapter ligation. At this point, platform-specific adapters ligated both ends of DNA fragments. These adapters comprise sequence, for binding dual-barcoded libraries to a flow cell, which helps in the process of sequencing, allowing PCR amplification of adapter-ligated fragments, and binding standard Illumina sequencing primers. To get results from the sample; a high-fidelity amplification step was accomplished using HiFi PCR Master Mix. The mitochondrial genome were assembled using NOVOPlasty version 3.5 [11] and further annotated using MITOS Web Server version 1 [12].

Results:

ATGCCACATGCAGCTCAAATATCACTACAAGAAGCAACAGGGCCCACTATAGAGGAGGTA
GTATTCCTACATGACCACGTCCTACTACTAACCTGNCTAATATCAATGGTAATTATAATA
TTTGCACTAACAGCAACAACAACAGCTNTAACTCACAATGACCCAGCAGAAGAAGTGGAA
CAACTGGAAGCAGCCTGAACAGCAGCCCAATTATAATCTTAANTCTTACAGCCCTCCCA
TCAGTACGATCTTTATACCTCATAGAAGAAGTATTCAACCCATATCTAACTATTAAGCA
ACTGGACATCAATGATACTGAAACTACGAATATTCAGATGAAACTCAAATCTCATTTCGAT
TCTTACATAGTACAAACAAGGACCTACAAAGTGGATCACCACGGTTATTAGAAGTAGAT
AACCGCATAGTAATACCAGCANAACACTACAAACCCGGATTGTAGTGACTGCAGAAGATGTA
CTACACTCATGAACAATCCCATCACTAGGTGTAAAAGTAGACGCAGTGCCTGGACGACTA
AACCAACTACCATTAGCCACATCACGAGTTGGAGTGTCTTCGGCCAATGCTCAGAAATC
TGCGGAGCTAACACAGCTTTATACCAATCGCAATAGAAGCAACACCACTACACCACTTT
GAACAATGA

A total length of 669 base pair sequence of COX2 has been generated using the following process.

Conclusion:

The following sequence has been uploaded into the NCBI with an Genbank number : ON854910. This sequence is the first COX2 sequence that has ever been generated of the *Chrysopelea ornata* snake and this is useful in identifying the Snake

Conflict of Interest:

The authors state that there is no conflict of interest.

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