

Discrimination of Three Species of Hump-Nosed Pit Vipers (Genus: *Hypnale*) using Genetic Markers

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Hump-nosed pit vipers of the genus *Hypnale* are the most common cause of venomous snakebite in Sri Lanka. There are 3 species of the genus *Hypnale* named *H.hypnale*, *H.zara* and *H.nepa*. While the latter two are endemic the former one is found both in Sri Lanka and the Western Ghats of India. These species are looked alike to the naked eye. The objective of this study was to discriminate the 3 species using mitochondrial genes: cytochrome b (Cyt b) and NADH dehydrogenase subunit 4 (ND4) gene and adjacent tRNA. Fourteen tissue samples (tail tips and liver tissues) were collected from 3 climatic zones (wet, dry and intermediate) in Sri Lanka. Their DNA was extracted using a commercial kit. PCR amplification was carried out using forward and reverse primers of Cyt b and ND4 gene and adjacent tRNA with a commercial PCR kit. PCR products were purified and sequencing of DNA was done for 8 samples (*H.hypnale*-4, *H.zara*-2, *H.nepa*-2). Sequence similarity search was performed using Nucleotide BLAST program in the National Center for Biotechnology Information utilizing the reference GenBank sequences as a basis. Multiple sequence alignment was done using ClustalW v 2.0 and Multiple Sequence Alignment Editor with default parameters. Sequence alignment scores were calculated. From the generated sequence alignments, the evolutionary relationships were inferred using the Molecular Evolutionary Genetics Analysis (MEGA) v 7.0.20 by employing the maximum-likelihood method. *High nucleotide identities were observed among the three species, ranging from 86% to 88% (H.hypnale and H. zara 86%, H.hypnale and H.nepa 87% and H.zara and H.nepa 88%) in the Cyt b gene. In ND4 and adjacent tRNA region, the highest nucleotide identity (88%) was observed between H.zara and H.nepa and nucleotide identities of 87% and 86% were observed between H.hypnale and H.zara and H.hypnale and H.nepa respectively. In the phylogenetic tree obtained for Cyt b and ND4 sequence alignments, the 3 species showed distinct lineages indicating molecular differences at the species level. Thus, analysis of mitochondrial gene sequences indicates that 3 species of the genus Hypnale are distinct from each other.*

Keywords: *Cyt B, Hypnale, Mitochondrial genes, ND4 gene and adjacent Trna*