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Molecular genetic assessment of formalin-fixed ethanol-preserved King Cobra: A comparative study on pretreatment protocols and DNA extraction methods

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ABSTRACT

Four different pretreatment protocols for formalin-fixed ethanol-preserved (FFEP) King Cobra (*Ophiophagus hannah*) specimens were tested to remove excess formalin before DNA extraction. Either TE buffer or TE buffer combined with absolute ethanol seemed to be the most efficient protocol to pretreat FFEP specimens. Moreover, commercial DNA extraction kit appeared to be the most successful method for DNA extraction from FFEP King Cobra compared with modified phenol-chloroform isoamyl alcohol and Chelex DNA extraction methods, respectively. Due to the degraded nature of DNA found in FFEP tissues, amplification by overlapping segments, between 150 and 250 bp, within 737 bp of NADH dehydrogenase subunit2 gene was recommended to increase a success rate of amplification. DNA sequence of the FFEP King Cobra, from Phetchabun province, was closely related to the sequences of King Cobra shed skins from northern Thailand, Myanmar, and southern China. Meanwhile, DNA sequences of the other FFEP samples, with no locality information, were identified as King Cobra from southern Thailand. Thus, archival FFEP specimens could be a useful source for studying molecular genetics, biodiversity and conservation of the most dangerous snake, King Cobra, when properly prepared fresh shed skins were not available.

Key words: archival FFEP King Cobra, pretreatment protocols, DNA extraction methods, DNA sequencing