Title : DNA Barcode of Mekong Stingray (*Hemitrygon laosensis*) For Species Identification

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ABSTRACT

Morphological identification of stingrays presents significant challenges due to ontogenetic variations, cryptic species, and phenotypic plasticity. Although DNA barcoding has emerged as a powerful tool for species identification, limited reference data in existing databases poses ongoing challenges for stingray taxonomy. This study aimed to (1) identify the stingray species using cytochrome b gene sequence analysis and (2) evaluate the efficacy of non-invasive sampling methods through comparative analysis of DNA quality parameters. Genomic DNA was extracted from mucus, tissue, and fin samples (n=3 each) using the CTAB method, followed by PCR amplification and nucleotide sequence analysis. Molecular analysis successfully identified the specimen as *Hemitrygon laosensis,* with 99.8% sequence identity to GenBank reference sequences. Spectrophotometric analysis revealed the DNA concentration and quality extracted from mucus as 314.31 ± 197.24 ng/μL, A260/A280 = 1.973 ± 0.012, and A260/A230 = 1.953 ± 0.211; tissue as 361.03 ± 80.47 ng/μL, A260/A280 = 2.011 ± 0.015, and A260/A230 = 1.471 ± 0.094; and fin as 1851.24 ± 236.87 ng/μL, A260/A280 = 1.795 ± 0.083, and A260/A230 = 1.480

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± 0.270, respectively. One-way ANOVA revealed significant differences in DNA concentration and quality among sample types. The DNA sample extracted from mucus yielded a DNA concentration lower than that extracted from fin ($p < 0.001$), but the amount of DNA in the mucus sample was adequate to do the analysis process. Moreover, DNA quality (A260/A230) in the mucus sample was shown to be the best between the three types of samples ($p < 0.05$), supporting mucus as a viable alternative. These findings achieved both research objectives by confirming species identity and validating mucus as a reliable non-invasive sampling method for DNA analysis, establishing an ecologically sustainable approach for freshwater stingray identification. This protocol provides a foundation for future taxonomic studies while minimising the impact on studied specimens.

Title name guide.

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