



DNA Barcode of Mekong Stingray (*Hemirtrygon laosensis*) For Species Identification



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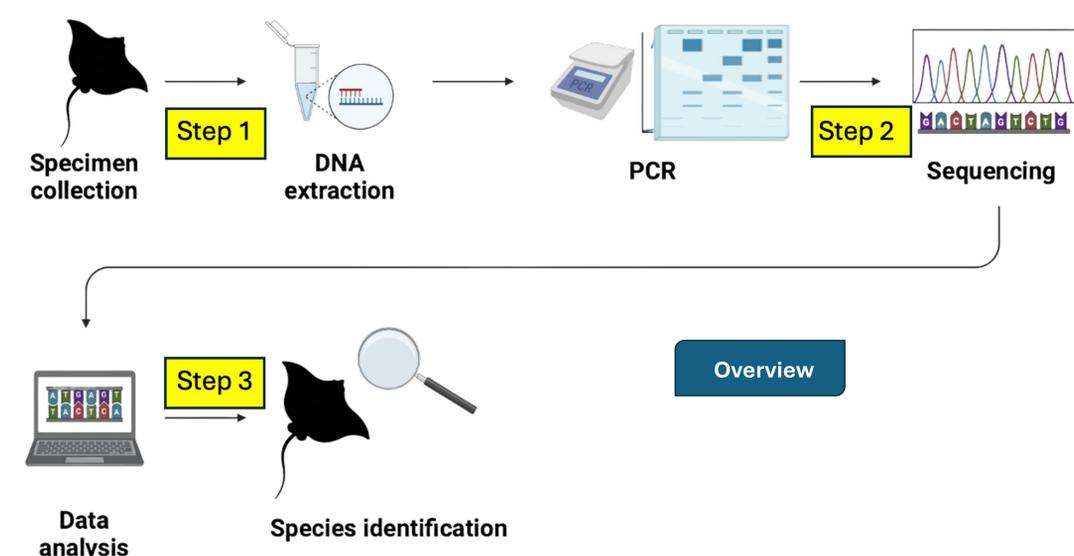
Abstract

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 *Hemirtrygon laosensis*, with 99.8% sequence identity to GenBank reference sequences. Spectrophotometric analysis revealed that mucus samples provided high purity ($A_{260}/A_{280} = 1.973 \pm 0.012$) and adequate concentration (314.31 ± 197.24 ng/ μ L), comparable to tissue samples ($A_{260}/A_{280} = 2.011 \pm 0.015$; 361.03 ± 80.47 ng/ μ L) though lower than fin samples ($A_{260}/A_{280} = 1.795 \pm 0.083$; 1851.24 ± 236.87 ng/ μ L). One-way ANOVA revealed significant differences in DNA concentration among sample types ($p < 0.001$) but not in DNA purity ($p < 0.01$), supporting mucus as a viable alternative.

Introduction

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.

Methods



Step 1. Specimen Collection & DNA Extraction

- 1.1) Samples Collection**
 - Collected **3 mucus** samples
 - Collected **3 fin** samples
 - Collected **3 tissue** samples
- 1.2) Extracts DNA by CTAB method**
- 1.3) Used Nanodrop to check**
 - i) **Concentration** by OD **A260** value
 - ii) **Purification** by OD **A260/A280** and OD **A260/230** ratios

Step 2. PCR & DNA Sequencing

- 2.1) DNA Amplification through PCR** Used **GLU1L (F) & THRIH (R)** primers - **CytB** region (Sezaki *et al.*, 1999)
- 2.1) Gel Electrophoresis**
- 2.3) Sequencing** - By Bio company

Step 3. Sequencing Trimming & BLAST

- 3.1) Sequencing Trimming** - By MEGA11
- 3.2) BLAST on NCBI (database)** - Species Identification

Results & discussion

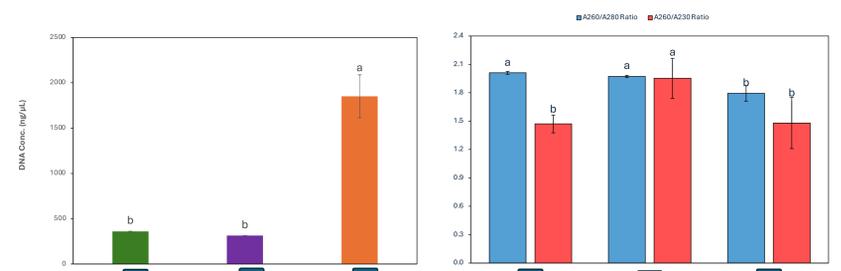


Table 1 mean of DNA concentration from different sample types indicated that all sample types are suitable for the PCR process. The fin sample showed the highest DNA yield, consistent with the findings of Eschbach (2012), which support this result.

Table 2 Showed mean of OD A260/A280 (blue graft) and OD A260/A230 (red graft) of each sample. In blue graft all of samples were good purification from protein and RNA contraminate. In red graft the mucus sample was most good purification from organic compounds. The mucus sample most purify than fin and tissue samples

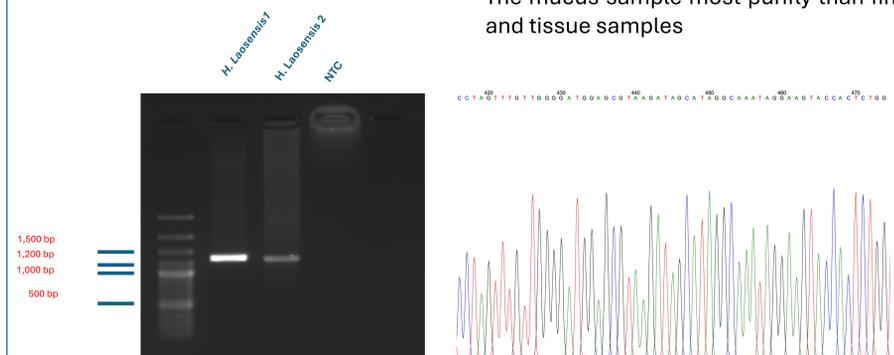


Figure 1 shows an estimated amplicon size of 1,200 bp, which corresponds to the cyt region reported in the research by Sezaki *et al.* (1999), where the amplicon size was 1,223 bp.

Figure 2 presents a chromatogram of nucleotides with good quality sequencing data, showing no N bases or peak overlaps.

Table 3 result of similarity of nucleotide that can identified successfully identified the specimen as *Hemirtrygon laosensis*, with 99.91% sequence identity to GenBank reference sequences.

Species	Accession	Query cover	E-value	Per Ident
<i>Hemirtrygon laosensis</i>	AB021504	100 %	0	99.91%
<i>Dasatis sp.</i>	AB021496	100 %	0	97.55%
<i>Dasatis nevarae</i>	EU870494	100 %	0	97.55%
<i>Dasatis bennetti</i>	KC833222	100 %	0	97.46%
<i>Dasatis bennetti</i>	NC_021132	100 %	0	97.46%
<i>Hemirtrygon akajei</i>	AB021501	100 %	0	96.68%
<i>Dasatis akajei</i>	NC_021132	100 %	0	96.59%
<i>Batrycoxia centroura</i>	NC_059937	100 %	0	91.51%
<i>Dasatis pastinaca</i>	NC_057976	100 %	0	90.81%
<i>Pteroplatytrygon violacea</i>	OP057115	100 %	0	90.81%

Acknowledgements

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References

- Sezaki, K., Begum, R., Wongrat, P., Srivastava, M. P., Srikantha, S., Kikuchi, K., Ishihara, H., Tanaka, S., Taniuchi, T. and Watabe, S. 1999. Molecular Phylogeny of Asian Freshwater and Marine Stingrays Based on the DNA Nucleotide and Deduced Amino Acid Sequences of the Cytochrome b Gene. *Fisheries Science*, 65(4), 563-570. doi:10.2331/fishsci.65.563
- Eschbach, E. 2012. Ascertaining optimal protocols for DNA extraction of different qualities of pike (*Esox lucius*) tissue samples – a comparison of commonly used solid phase extraction methods. *Environmental Biotechnology*, 8(1), 7-14.

Conclusion

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.