Sequencing 12S mtDNA from Neotropical Knifefishes for Genetic Barcoding

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Introduction

- **Gymnotiformes** are an order of South American freshwater fishes known for their **electrosensory** systems[1]
- Over 244 species, mainly in the **Amazon Basin**, with species diversity enabled by electrocommunication[1]
- 5 families: Gymnotidae, Hypopomidae, Rhamphichthyidae, Apteronotidae and Sternopygidae
- Organisms shed **eDNA** that can be used to assess the impacts of climate change on species abundance[2]
- **12S rRNA gene** (~1 kb) provides excellent taxonomic resolution for species identification[3]
- **Objective:** Create a reference library of Gymnotiformes 12S sequences for use in genetic barcoding and eDNA studies

Figure 1. Electric eel (*Electrophorus electricus*). Image: Steven G. Johnson

Methods

PCRs were run to amplify the 12S gene from **150 species** using the following primers[4]:

Forward	5'-CAA AGG CTT GGT CCT GAC-3'
Reverse	5'-AGC ATT CCC TTG CGG TAC-3'

Table 1. Forward and reverse primers used for 12S PCR

Results were verified via gel electrophoresis for **30 minutes at 90V**, ensuring that a fragment of ~**1000 bp** had been amplified

If no band appeared for a particular sample, another sample from the same species was used to reattempt amplification

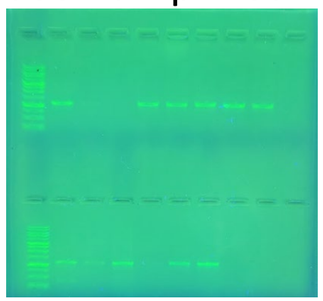

Figure 2. Illuminated gel showing 10 well-amplified 12S samples

Successfully amplified fragments were sent for sequencing at The Centre for Applied Genomics (TCAG)

Returned chromats were assembled and trimmed to form a **consensus sequence** for each species in Geneious Prime[5]

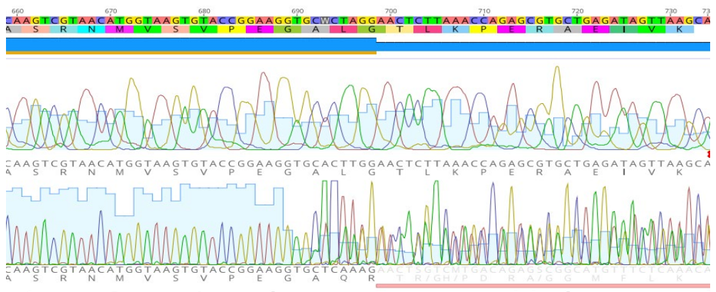

Figure 3. Portion of sequence assembly for *Gymnotus obscurus* (bp 650-735)

Results and Future Directions

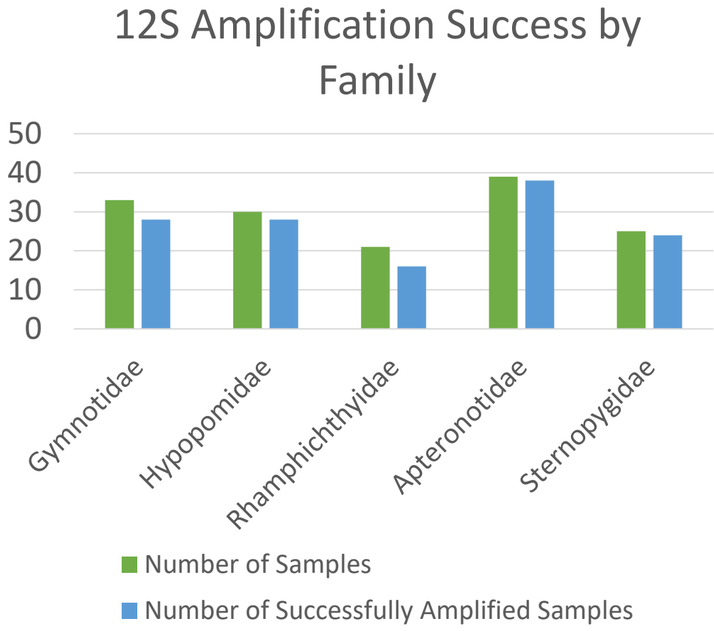

Figure 4. PCR success by family

- **103 sequences** obtained
- Average sequence length: **1001 bp**
- Family with lowest success rate (79.19%) was **Rhamphichthyidae**, which can be referenced to develop new primers that better target 12S in currently unamplified species

References and Acknowledgements

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4. Van Nynatten A, Gallage KS, Lujan NK, Mandrak NE, Lovejoy NR. 2023. Ichthyoplankton metabarcoding: An efficient tool for early detection of invasive species establishment. Mol Ecol Resour. 23(6): 1319-1333
5. Geneious Prime 2022.1.1 (https://geneious.com)

I would like to thank the Centre for Global Change Science for facilitating this research opportunity, in addition to the members of the Lovejoy Lab for their help and support. I would also like to thank Kaelyn Gomes for her assistance in running PCRs, and finally Dr. Nathan Lovejoy for providing me with the opportunity to participate in this project.