Identifying Endangered *H. crocherodii,* the Black Abalone, through Environmental DNA Isolation and COX1 DNA Barcoding

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Summary

- We investigated if DNA barcoding the COX1 gene can differentiate *Haliotis cracherodii, Haliotis rufescens,* and *Haliotis sorenseni.*
- We performed PCR amplification on the tissue DNA and eDNA collected and examined the products through AGE.
- Sanger sequence results were used to generate the phylogenetic tree that shows that COX1 of *H. cracherodii* was differentiated from *H. rufescens* and *H. sorenseni.*

Abstract

Decades of overfishing and terminal withering disease have caused the *Haliotis cracherodii* (black abalone) population of the California shores to become endangered. Traditional methods used to track invertebrates like the abalone are considered costly and damaging. To help detect the dwindling population, we developed and validated the method of COX1 barcoding on black, white, and red abalone tissue DNA and tank water eDNA samples. Through Sanger sequencing of the COX1 PCR products, we found that COX1 barcoding was able to effectively distinguish black abalone DNA sequences from the others. Our next step is to apply this method to marine eDNA samples and mature this affordable and non-invasive method for tracking endangered invertebrates.

Introduction

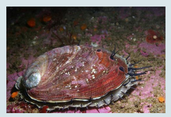
Hypothesis: *H. cracherodii* COX1 sequence can be differentiated from that of *H. rufescens* and *H. sorenseni* through DNA Barcoding.

The Abalones

Haliotis cracherodii, commonly known as **black abalone**
- inhabits the intertidal zone[4]
- population has drastically declined over the past 40 years[1]
- considered an endangered species[1]

Black Abalone[1]

Haliotis rufescens, commonly known as **red abalone**
- inhabits the low intertidal to subtidal zone[4]
- population currently unthreatened[2]



Red Abalone[2]

Haliotis sorenseni, commonly known as **white abalone**
- inhabits the subtidal zone[4]
- population currently endangered[3]

White Abalone[3]

DNA Barcoding
- Method of fast and accurate species identification by targeting and comparing a specific part of the genome[5]

Cytochrome c oxidase subunit 1 (COX1)
- Critical component of the Electron Transport Chain in the mitochondria[6]
- Provides discriminating power for differentiating closely related organisms via DNA barcoding[6]

Materials and Methods

Tissue Collection
- <25 mg tissue samples
  - *Caenorhabditis elegans*
  - *Oryzias latipes*
  - *Drosophila melanogaster*
  - *Homo sapiens*
  - *Haliotis rufescens*
  - *Haliotis cracherodii*
  - *Danio rerio*

Water Collection
- Three replicates of 3L samples collected from both *H. cracherodii* & *H. sorenseni* tank
- One 3L sample collected from the *Danio rerio* tank

3L water sample collection jug

Tissue DNA Isolation
- DNeasy Blood and Tissue Kit

eDNA Isolation
- MoBIO Laboratories Powerwater DNA Isolation Kit

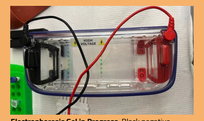
Millipore filter membrane (left) Filtration system (right)

Polymerase Chain Reaction
- GoTaq Master Mix
- Primers used:

Universal COX1 primers:
Forward: 5'-GGTCAACAAATCATAAAGATATTGG-3'
Reverse: 5'-TAAACTTCAGGGTGACCAAAAAATCA-3'

Abalone-specific COX1 primers:
Forward: 5'-TGATCCGGCTTAGTCGGAACTGC-3'
Reverse: 5'-GATGTGTTGAAATTACGGTCGGT-3'

Gotaq Master Mix buffer



Electrophoresis Gel in Progress. Black negative electrode, red positive electrode.

Agarose Gel Electrophoresis
- 1.5% gel
- 100 volts
- Quick-load Purple 1 kb Plus DNA ladder

DNA Sequencing & Sequence Analysis
- Sanger sequencing
- Phylogenetic tree built using Clustal Omega DNA sequence alignment tool

DNA sequencing result of *H. rufescens*. 200 bases of sequencing results shown by SnapGene Viewer.

Results

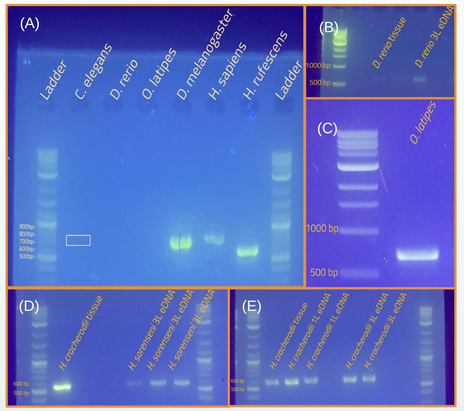


Figure 1: Agarose Gel Electrophoresis exhibiting PCR results. (A) One faint band returned for *C. elegans* sample. Bright bands observed for *D. melanogaster, H. Sapiens,* and *H. rufescens* samples. No bands appeared for *D. rerio and O. latipes.* **(B)** One faint band returned for *D. rerio* tissue sample, one bright band returned for *D. rerio* eDNA sample. **(C)** One bright band for *O. latipes* tissue sample. **(D)** Bright bands returned for *H. cracherodii* tissue sample and *H. sorenseni* eDNA samples. **(E)** Bright bands returned for *H. cracherodii* tissues and eDNA samples.

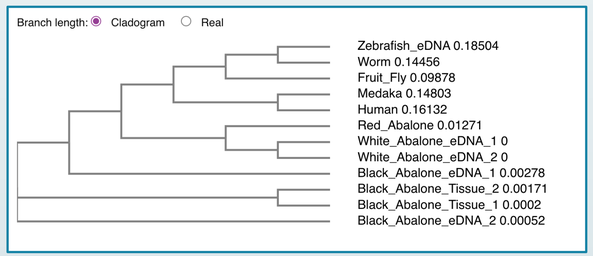


Figure 2: Phylogenetic Tree generated by Clustal Omega from all species' cleaned COX1 DNA sequences. All samples labeled by common name.

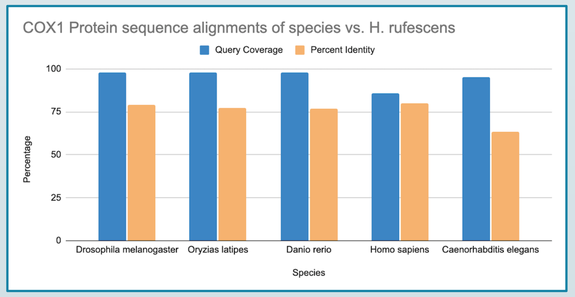


Figure 3: Query coverage and percentage identity of COX1 protein/amino acid sequences between *H. rufescens* and other species. Protein sequences of *C. elegans, O. latipes, D. melanogaster, H. sapiens,* and *D. rerio* were compared against *H. rufescens* through Basic Local Alignment Search Tools.

Discussion

- **Through COX1 DNA barcoding, *H. cracherodii* was differentiated from *H. rufescens* and *H. sorenseni.***
- No bands were observed from initial PCR results from *D. rerio*, and *O. latipes* tissue samples (Fig. 1 a).
- Only PCR products with clear bands observed were sequenced (Fig. 1).
- The abalone samples were all grouped together on the phylogenetic tree while each abalone species being clustered into smaller clades (Fig. 2).
  - The abalone COX1 DNA sequence were expected to be similar.
- COX1 DNA barcoding (Fig. 2) exhibit different species relatedness than COX1 protein/amino acid sequence alignment (Fig. 3).
  - DNA barcoding Vs. protein/amino acid sequence
  - Alternative splicing
- Successfully validated our method for detecting endangered black abalone in tank water samples.

Study Limitations and Future Directions

Limitations
- *O. latipes* tissue samples and *H. cracherodii* samples from the San Miguel Island failed to return results on PCR and gel electrophoresis, likely due to the abundance of inhibitors in the samples.
- The universal COX1 primer was not effective for *D. rerio* COX1 gene during PCR.
  - DNA sequencing results from *D. rerio* eDNA aligned 98% with *Pleuretra lineata*, a rotifer, indicating that the universal COX1 primer used is unfit for the *D. rerio* COX1 gene.

Future directions
- Alterations of dilution ratio (ex. 1:2, 1:5) for ocean environmental samples may potentially reduce inhibitor concentration in DNA samples, thus potentially yielding better PCR results.
- Condensing eDNA samples (ex. 10:1) after eDNA isolation may facilitate/improve primer binding during PCR.
- New primers need to be designed for zebrafish COX1 barcoding.

Sources