DNA Barcoding *Oryzias latipes, Haliotis, C. elegans, Drosophila melanogaster, and Homo sapiens* to find species proximity with *Haliotis cracherodii* using the COX1 gene

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Summary

- *Haliotis cracherodii*, or Black Abalone, is an endangered species off of the Pacific Coastline.
- We extracted, PCR amplified, sequenced and analyzed the DNA of 5 species: *Oryzias latipes, Haliotis, C. elegans, Drosophila melanogaster*, and *Homo sapiens*.
  - We compared the COX1 gene in each of the species to analyze the evolutionary relatedness to Black Abalone.
  - Black Abalone is most genetically similar to *C. elegans*.

Abstract

The Black Abalone, or *Haliotis cracherodii*, is a species of sea snail found along the Pacific coastline. It is crucial for the regulation of kelp forests, which in turn keeps the food web stable. However, in recent years, the population of Black Abalone has lowered significantly due to environmental factors and diseases. One such disease is withering shell disease, causing shrinkage of the foot, which causes black abalone to be susceptible to infection and death under warmer water temperatures. Phylogenetic categorization of the species can aid with research and conservation efforts. Samples of Black Abalone in the lab were tested and it's DNA was sequenced and then compared to other species such as C. elegans, fruit flies, humans and medaka. We used the COX1 gene specifically to compare species, as it has been proven to be a good tool for barcoding due to it's high evolution rate. Through our analysis and comparison of sequences, we found that the Abalone were most similar genetically to C. elegans.

Introduction

Hypothesis

- Black Abalone DNA sequence is most genetically alike to the DNA sequence of *C. elegans*.
- Black Abalone DNA is least genetically similar to the DNA sequences of Humans and Japanese Ricefish.

Black Abalone (*Haliotis cracherodii*)

- Black Abalone is part of the haliotidae (abalone family) and Gastropoda class, where they are related to Haliotis walallensis and haliotis rufescens. [2]
- A seawater gastropod mollusk. [2]
- Taxonomy categorizes them in the family Haliotidae. [2]
- A critically endangered species due to overfishing acidification of the ocean. [2]
  - Was once the most abundant abalone on the Pacific coast.
  - Suffers from Withering Syndrome, caused by the intracellular bacterium candidatus Xenohaliotis californiensis, where the abalone's digestive enzymes consume its own body mass. [1]
- Mostly resides along the intertidal west coast of California. [2]
  - Inhabits rock crevices of rocky shores.
- Distinguished by the dark black outer shell. [2]

COX1 Gene (Cytochrome c oxidase subunit I)

- Chosen for its reliability as the most commonly used genetic marker. [5]
  - Isoform expressed in most tissues under various conditions. [6]
  - Expressed as a housekeeping gene. [6]
  - Great for barcoding as they evolve quickly compared to other genes. This allows for a more accurate representation for barcoding and relationships.[5]
  - Helps find recognizable mutation variation within the species.
  - Analyzing DNA mutations within the COX1 gene as the genetic marker phylogenetically categorizes the species. [4]

Significance

- Black abalone are being used as the model organism because they are endangered, so phylogenetically categorizing the species will aid conservation efforts. [3]
- Optimizing these methods will help to adequately protect the abalone in their natural habitats.

Methods

  - Analyzing mitochondrial DNA and assessing its sequence similarities.
  - To identify the species, DNA barcoding will be used on the COX-1 gene which is a mitochondrially encoded gene.

Materials and Methods

Gathering and Extraction of DNA Samples

- Samples were gathered from *Haliotis cracherodii, Oryzias latipes, Haliotis, C. elegans, Drosophila melanogaster, and Homo Sapiens* tissue in the lab.
- Lyse cells with heat and chaotropic agents.
- Separate DNA from components by binding them to the NucleoSpin Tissue Column that contains silica.
- Wash isolated DNA with reagents that do not destroy DNA
- Place the column into a microcentrifuge tube and incubate, then centrifuge.

PCR and Gel Electrophoresis

- Use the extracted DNA, a buffer, primers, and TAQ polymerase to amplify the region of interest in order to create copies of the gene of interest (COX1).
- Prepare agarose gel apparatus and create a charge using a power supply and TAE buffer.
- Pipette the amplified DNA sample into the wells in the agarose gel apparatus and allow time to determine if PCR was successful.

Sequencing

- Using the Sanger Sequencing method, conduct PCR with fluorescent, chain-terminating ddNTPs using the amplified DNA sample, a buffer, one primer, and the DNA Taq Polymerase.
- Separate the DNA fragments by size using capillary gel electrophoresis
- Excite the fluorescent tags by using a laser and generate a chromatogram figure using the emitted colors.
- Manually correct any errors or background noise in the data.

Data Analysis

- A phylogenetic tree was generated using Clustal Omega based on the query DNA sequences extracted from the lab and were compared to subject sequences using the NCBI database.
- The proximity of *Haliotis cracherodii* to other species on the phylogenetic tree was studied.

Results

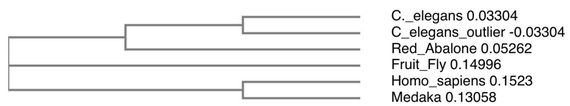

Figure 1. Clustal Omega Cladogram Phylogenetic Tree
Proximity on the tree represents relatedness. Nodes predict where the species evolved from a common ancestor. All sequences in this tree were gathered in the lab.

Figure 2. Clustal Omega Cladogram Phylogenetic Tree using NCBI Sequences
Proximity on the tree represents relatedness. Nodes predict where the species likely evolved from a common ancestor. All sequences in this tree were gathered in the NCBI Genome Database.

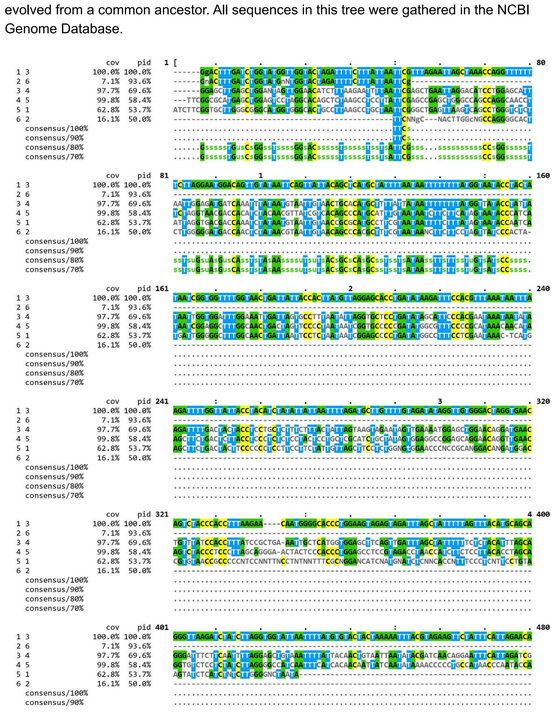

Figure 3. Clustal Omega Sequence Alignments
Matches are highlighted with either yellow, light blue or green. This figure was generated in MView, a section of Clustal Omega. Species 1 = Medaka, Species 2 = Abalone, Species 3 = C. elegans, Sequence 4 = Fruit fly, Sequence 5 = Homo sapiens, and Sequence 6 = C. elegans.

Discussion and Conclusion

- Results were consistent with hypothesis that black abalone were related to red abalone and the haliotides family [2]
- Since black abalone is not recorded in the NCBI database, red abalone is the closest match to it because they both belong in the Haliotis family.
  - According to NCBI BLAST, sequence 2 from the lab has a 100% percent identity and 100% query cover with *Haliotis rufescens* (red abalone).
- Figures 1, 2 and 3 used clustal omega to generate alignments for 5 to 6 sequences.
- As shown by Figure 1, 6 DNA query sequences gathered from the lab is on a phylogenetics tree. The phylogenetics tree indicates that *C. elegans* and *C. elegans* with E. coli clumps likely has the most similar DNA sequence to black abalone.
- DNA sequences gathered from the NCBI database in Figure 2 has drastically different results compared to figure 1.
  - The phylogenetics tree shows that abalone is most genetically similar to humans and Japanese Ricefish.
  - Black abalone are most genetically similar to *C. elegans*, both of which are invertebrates.
- Understanding species relatedness of Black Abalone compared with the DNA Sequences of Japanese Ricefish, red abalone, *C. elegans*, fruit fly, and humans is important as a reference when utilizing black abalone eDNA in the future to aid conservation efforts.
  - Because black abalone are an endangered species, it is essential to examine less of its tissue samples directly and analyze the eDNA produced.
- Recognizing black abalone's close species proximity to C. elegans on the phylogenetics tree will help us understand how certain mutations and diseases affect DNA and cause death.
  - Ex. If C. elegans was exposed to the same disease as black abalone, the DNA of the two species can be examined and analyze the genetic reaction and prevent the likely cause of death.

Study Limitations and Future Directions

- **Limitations:**
  - Could not sequence a variety of black abalone samples
  - Limited samples for other species
  - Sample sequences varied from other sequences in databases
  - Some inconsistencies between NCBI database sequences and gathered sequences were presented during analysis of the generated phylogenetic trees.
  - C. elegans DNA with E. coli clumps had an incomplete sequence.

- **Future directions:**
  - Gather data from other species of abalone
  - Gather more species to sequence and extract DNA from
  - Have a higher sample size for each species
  - Have more potentially related species versus species such as Homo sapiens, which do not share the same morphology, habitat or common ancestor

References and Acknowledgements

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