

# The COX1 gene sequence indicates *Oryzias latipes* is more closely related to the endangered black abalone, *Haliotis cracherodii*, than *Danio rerio*

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## Summary

- Extracted tissue from six different species and used the COX1 barcoding gene to look at the genetic differences among the species.
- Medaka shows a closer genetic relation to abalone than to humans or fruit flies.
- Zebrafish shows a closer genetic relation to humans, fruit flies, and roundworms than to medaka or abalone.

## Abstract

Black abalone are an endangered species. The conservation and rehabilitation of black abalone is important as they are a keystone species. In this study, we compared the relatedness of six different species, including the abalone, by analyzing their DNA sequences and creating a phylogenetic tree. We found that the abalones are closely related to humans and fruit flies based on the sequences from the NCBI database. From the in-lab samples that we collected, we found that zebrafish are more closely related to humans, fruit flies, and roundworms than to any abalone species.

## Introduction

### Hypothesis:

- Zebrafish and medaka are more closely related to abalones than fruit flies, roundworms, and humans genetically, because they are aquatic species and live in similar environments.



Red Abalone Shell

### Black Abalone

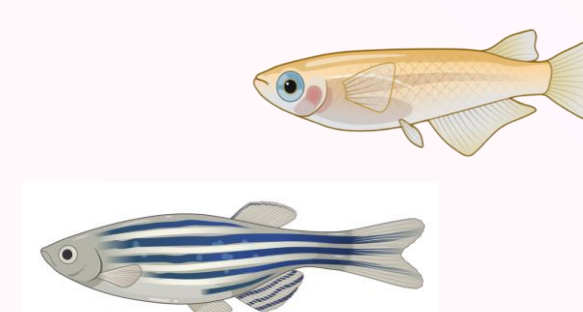
- Black abalone are an endangered species, with small populations left in the wild off the coast of California. (Gruenthal et al., 2008)
- Keystone species (NOAA Fisheries, 2021)



Black Abalone Shell

### Environmental Stresses

- Climate change exposes species to environmental stresses (Hader, Donat-P, 2019)
- Some species are unable to adapt and become endangered or extinct (Javeline et al., 2015)
- Studying and conserving black abalone is important due to their ecosystem contribution; studying related species is important because related species could have similar responses to changes in pH, temperature, salinity, pollution, stress, etc. (Raisuddin et al., 2007).



Medaka (top) and Zebrafish (bottom)

### COX1 Barcoding Gene

- COX1 gene is the Cytochrome c oxidase subunit I and is a part of complex IV of the electron transport chain
- Important for energy production within cell.
- COX1 has been used as a common barcoding gene to differentiate between species (Kher, Chandni, 2011), because it is conserved across species, while its sequence also varies

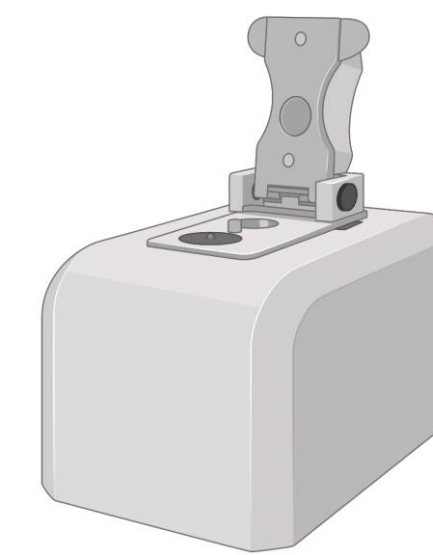
## Methodology

### DNA Extraction

- 25 mg of tissue for *Caenorhabditis elegans*, *Drosophila melanogaster*, *Danio rerio*, *Oryzias latipes*, *Haliotis rufescens*
  - except for *Homo sapiens*, where a cheek swab was used to collect cells
- QIAGEN DNeasy® Blood & Tissue Kit

### Nanodrop

- Used to measure the DNA yield
- Also for examining the purity of DNA (ratio of absorbance at A260 and A280)
- Carried out PCR with most viable DNA samples



### PCR

- GoTaq Green Master Mix
- Positive control: fruit fly and red abalone tissue DNA
- Negative control: replaced DNA volume with water

### Gel Electrophoresis

- 1.5% gel (1.5 g agarose in 100 mL 1X TAE Buffer)
- 110 V for 1 hour

### Sequence Alignment

- NCBI Blast Database
- Compared sample sequences to database sequences to check query coverage and percentage identity

### Phylogenetic Tree Creation

- Clustal Omega Multiple Sequence Alignment tool
  - Used DNA sequences obtained from samples
  - Used DNA sequences from NCBI database

### Tree Comparison

- Comparison of common ancestry and branch arrangement in phylogenetic tree with respect to *Haliotis rufescens*

## Results

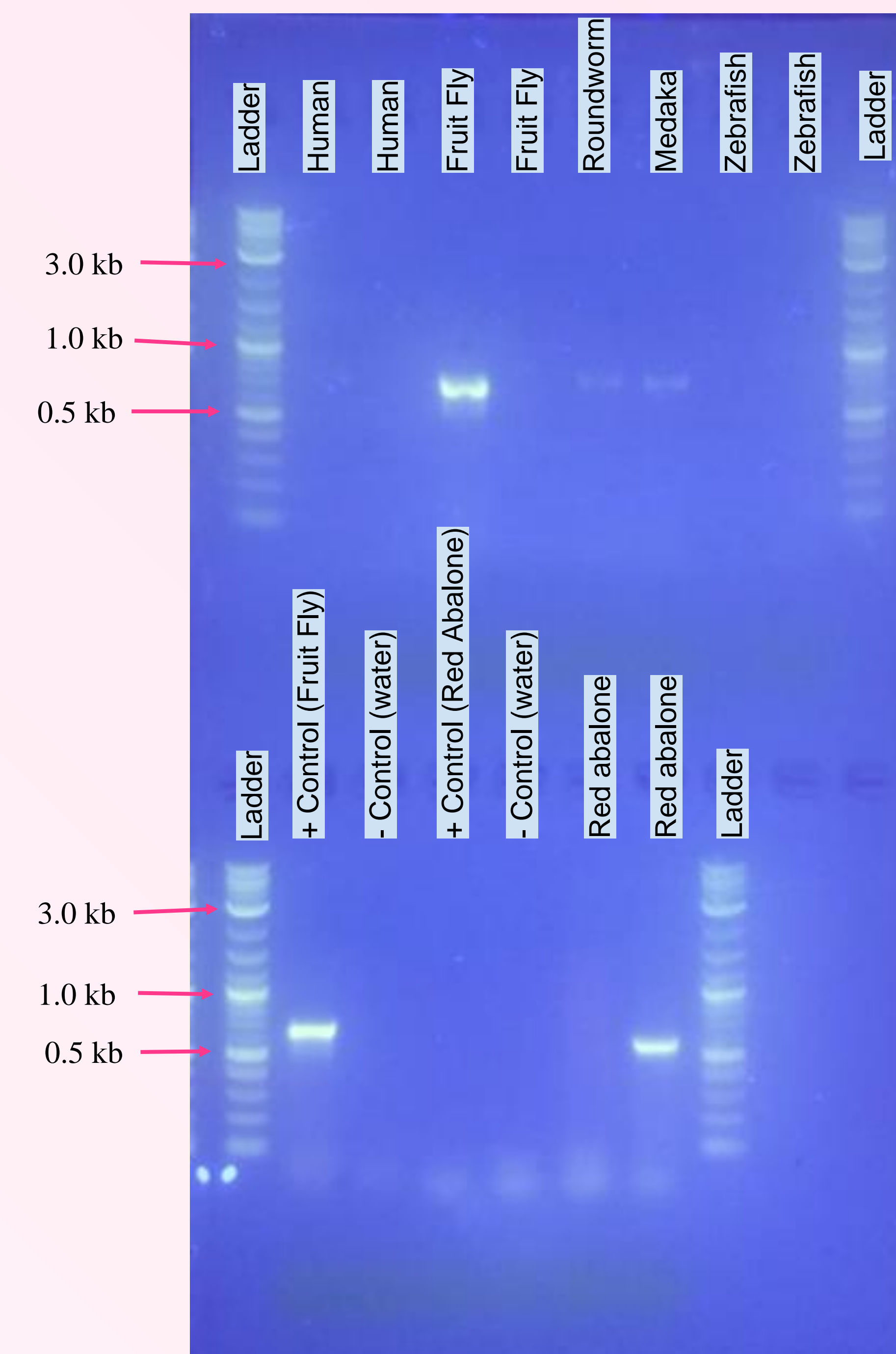


Fig 1. Agarose gel of in-lab samples. 1.5% agarose gel was used. Ladder sizes are indicated on the left. Type of sample loaded into the well was indicated above each lane.

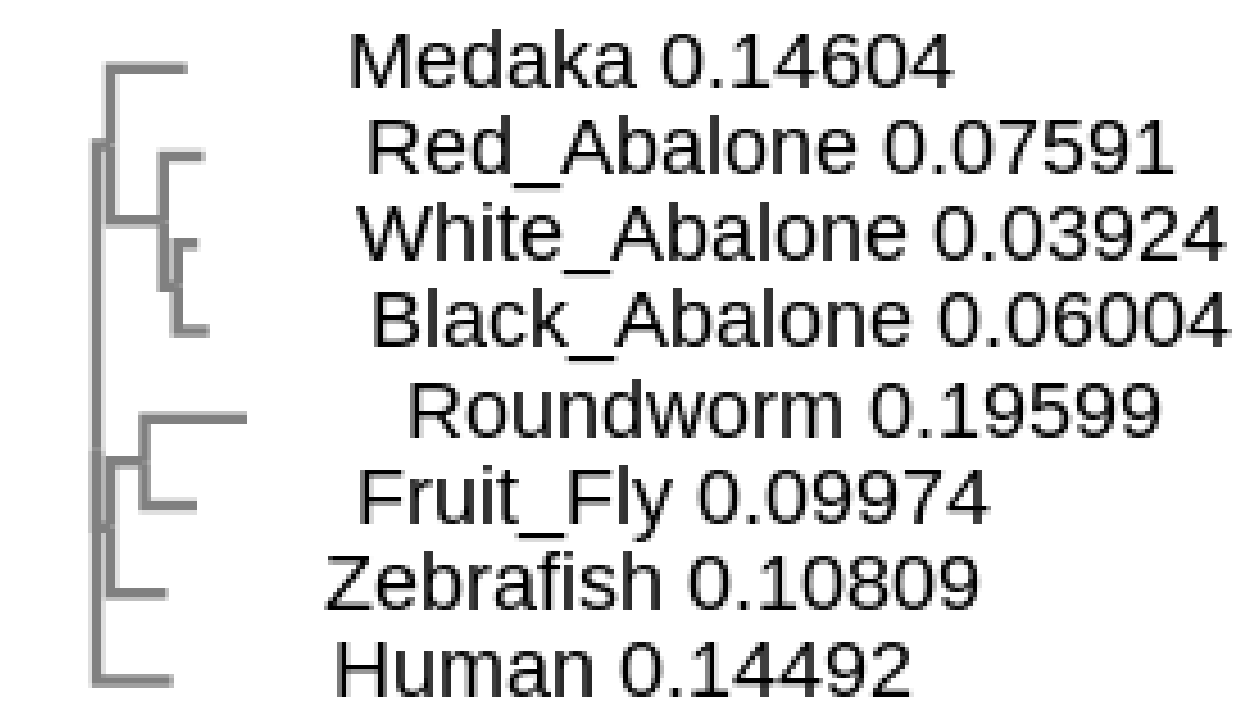


Fig 2. Phylogenetic tree using in-lab samples. Sanger sequencing results from in-lab samples were compared in Clustal Omega, and a neighbor-joining phylogenetic tree was made. Numbers next to the species indicate the distance from the most common ancestor.

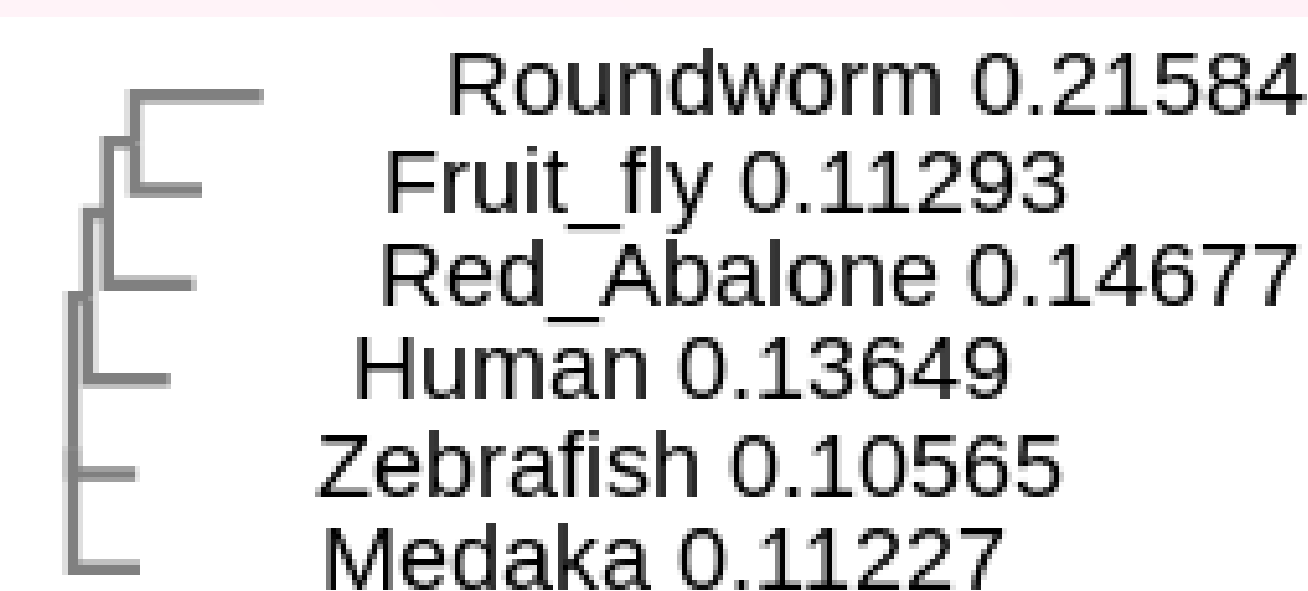


Fig 3. Phylogenetic Tree using database Sequences. DNA sequences were obtained from the NCBI database, and then compared in Clustal Omega to generate a phylogenetic tree. Numbers next to the species indicate the distance from the most common ancestor.

## Discussions & Conclusions

### Genetic Similarity

Our hypothesis was partially supported by our in-lab results.

- Medaka was more closely related to black, white, and red abalone than to humans, fruit flies, or roundworms (Fig 2).
- Zebrafish was unexpectedly more closely related to humans, fruit flies and roundworms than to medaka or any of the abalone species
- Database phylogenetic tree showed abalone more closely related to humans than to zebrafish or medaka (Fig 3).
- Different sequence lengths for database and lab samples could account for the differences in the phylogenetic trees (whole vs partial sequence) (Fig 2, 3)

Terrestrial and aquatic animals are phenotypically different due to the different environmental conditions they live in (Horne et al., 2015).

- Our results show that zebrafish are more genetically similar to humans, fruit flies, and roundworms compared to medaka or abalones, which was surprising considering they are aquatic organisms (Fig 2).

### Conclusions

- Being the most related to abalones, medaka would serve as good representative model to look at how abalone would response to different environmental changes or stressors.

## Study Limitations

- Only used one gene target to differentiate the species
- Limited sample size (10 individuals total): Looked at 1-2 individuals per species (fruit flies had multiple individuals) , which does not represent the variation and genetic diversity for an entire population
- Looking at lab populations (which have less genetic variation), rather than wild populations of species

## Future Directions

- Other species of abalone could be sequenced and compared (e.g. green abalone, pink abalone )
- Sequencing of non-abalone species and examining the most closely related species to black abalone to test whether their feedback to environmental factors (e.g. pollution) and diseases could indicate similar responses in abalone
- More marine species could be looked at to see the genetic similarities and how they differ

## References

