

# Abalone COX-1 Gene Sequencing and Phylogenetic Analysis

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## Summary

- eDNA extracted from abalones tank water
- DNA collected from related species tissue
- Ammonia level measured
- Phylogenetic Trees constructed with eDNA revealed evolutionary relationship between alternative species

## Abstract

Animals infected with bacteria responsible for withering syndrome exhibit body shrinkage as the disease progresses due to a combination of reduced food intake and specific pathological changes in the structure of the digestive gland. These circumstances ultimately lead to death. One of the Physiological manifestations of withering syndrome is increased ammonia excretion. Traces of ammonia could allow detection of infections prior to onset of clinical signs in both black and red abalone. Phylogenetic analysis expressed evolutionary distance between related species.

## Introduction

### Hypotheses:

- 1.) "The genetic alignments of the medaka will be similar to that of the red abalone."
- 2.) "The ammonia levels for laboratory abalone will be the level expected of healthy abalone tank water."

### eDNA:

DNA extracted from tissues of abalones, amplified using primers in PCR

### Black Abalone (*Haliotis cracherodii*):

- Distributed along the Pacific Coast
- Critically Endangered.
- Vulnerable to bacterial attack: Withering Syndrome due to *Candidatus X. californiensis* [1]
- Natural habitat of abalone has ammonia (<0.1 mg L-1) [2]



*Haliotis cracherodii*

### Investigation Methods:

- Increased ammonia level in tank water
- eDNA amplification using PCR
- Ammonia level detection can possibly correlate to health of abalone[3]
- Construct a phylogenetic tree to find animals prone to a disease similar to WS

## Methodology

### Sample Collection:

- Water samples collected from tank (1L at a time from each tank, pooled to total 3L). Water contains tissues and feces of black abalones
- Filter disc processed through Mo Bio Power Water Kit
- Ammonia level quantified using an ammonia detection kit.



### DNA extraction:

- Target specific gene (*Cox1*) of *Haliotis cracherodii*
- Cell lysed with PW1 lysing agent (detergent)
- Filter Frozen at -80C for storage or processed through Mo Bio Power Water Kit for DNA Extraction
- Filtered through .45um MCE #HABG04700
- Extracted DNA : (100ul) Product

### Ammonia Test

- Replicates of three 1L bottles of abalone water
- 5ml water + 8 drops of Test Solution #1 & 2 added
- Compare color gradient



### Polymerase Chain Reaction:[4]

- Used materials: Buffer, DNA Polymerase, Taq Polymerase, 5ul of DNA Template, using Gotaq 2X protoc, Primer used for Universal COX1
- FWD: TGATCCGGCTTAGTCGGAAGCTGC**  
**RVS: GATGTGTTGAAATTACGGTTCGGT**
- Heated up to 95°C: Denaturation
  - Reduced to 55°C: Annealing
  - Nucleotides added at 72°C: DNA Elongation



### Gel electrophoresis:[5]

Extracted DNA (10ul of PCR rxn used + 2ul loading dye) added to the Agarose Gel wells → Electric current (90V for 55 Minutes) applied to the 1.5% Agarose Gel → DNA bands separated by size → DNA bands were stained → Visualized using SYBR Safe Stain and UV Box.



### DNA Sequencing and Analysis

- Nucleic acids sequenced using the Sanger Method
- Phylogenetic Tree construction and sequence alignment

## Results

### Phylogenetic Trees

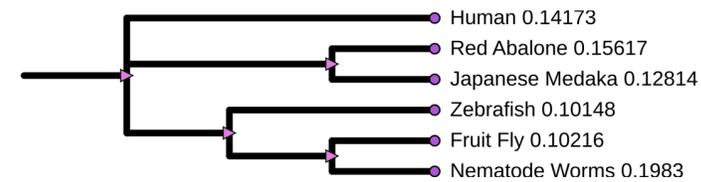


Figure-01: Tissue DNA: Human, Fruit Flies, Worms, Abalone, Medaka, Zebrafish COX1. Medaka and Abalone are have close evolutionary distance, whereas, Zebrafish are an outcast.\*Zebrafish COX-1 from NCBI

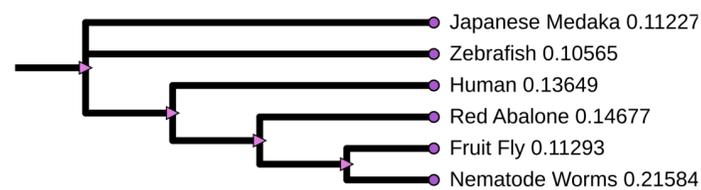


Figure-02: NCBI Ref Seq: Human, Fruit Flies, Worms, Abalone, Medaka, Zebrafish. Worms and Fruit flies have closer evolutionary distance and Medaka has a farther distance from Abalone.



Figure-03: eDNA Phylogenetic Tree: Medaka, Abalone, Zebrafish. Red Abalone is the most evolutionarily distant species



Figure-04: Phylogenetic Tree NCBI RefSeq: Abalone, Medaka, Zebrafish. Red Abalone is the most evolutionarily distant species

### Ammonia Results:

- Trial 1: 0.25 ppm
- Trial 2: 0.50 ppm
- Trial 3: 0.50 ppm



### Abalone and Medaka Sequence Alignment

#### Distribution of the top 1 Blast Hits on 1 subject sequences

Score	Expect	Identities	Gaps	Strand
75.2 bits(82)	9e-19	69/88(78%)	0/88(0%)	Plus/Plus
Query 17	GCCAGGGGCACTCTTGGGGGATGACCAACTCTATAACGTAATTGTAACAGCCACGCTTT	76		
Sbjct 69	GCCTGGGCTCTATTAGGTGACGACCAAACTATAATGTAATTGTAACCGCATGCCTT	128		
Query 77	CGTAATAANCTTCTTCTAGTATATCCCA	104		
Sbjct 129	CGTAATAATTTCTTTATAGTAATACCA	156		

Figure-05 (a,b): Abalone and Medaka Sequence COX1 Gene sequence alignment

## Discussion

- In comparison to all species sequenced, it appears that while all are similarly related, the species closest to the red abalone (and in turn black abalone) is the medaka. (fig.1)
- COX1 gene alignment of the medaka and the red abalone show substantial similarity between medaka and abalone genomics (fig.5); Percent Identity: 78.41%
- Ammonia levels supported the hypothesis that ammonia levels in infected tank water would be higher than the level found for healthy abalone i.e, 0.5 ppm > 0.1 ppm [2]

## Limitations

- Ammonia Test alone is not a reliable method to confirm Withering Syndrome in Abalone.
- The eDNA of Red Abalone was used instead of Black Abalone
- We did not extract ammonia from the feces, but from the water.

## Further Directions:

- Investigate whether Cecropins [6], a group of antimicrobial peptides with bactericidal activity against a broad spectrum of bacteria, (used to produce bacteria resistant transgenic medaka) could exhibit bactericidal activity towards *Candidatus Xenohaliotis californiensis*.
- Construct phylogenetic tree with more possibly related species.
- Perform Black abalone sequence alignment with Medaka
- DNA based detection of WS Pathogen

## References

