DNA Barcoding of Marine Organisms from Brooklyn Bridge Park and a Local Seafood Market

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Abstract

Marine organisms were collected through seining in the East River off the shore of the Brooklyn Bridge Park. Atlantic silversides, mud snails, comb jellies or ctenophores, and the invasive Asian shore crab were collected. DNA was isolated several different ways from frozen mud snails (Chelex method, a Qiagen spin column method, and a silica bed method. Amplification of a cytochrome b mitochondrial DNA product was conducted using primers that were specific to invertebrates. However, no DNA was visualized for unknown reasons. In order to attempt to get positive results, we switched our tactic a bit and focuses on fresh seafood from a local market. We were able to obtain sequences , that, through a BLAST search identified with Atlantic salmon (Salmo salar) and Japanese bay scallops.

Atlantic salmon: *Salmo salar*

Asian shore crab *Hemigrapsus sanguineus*

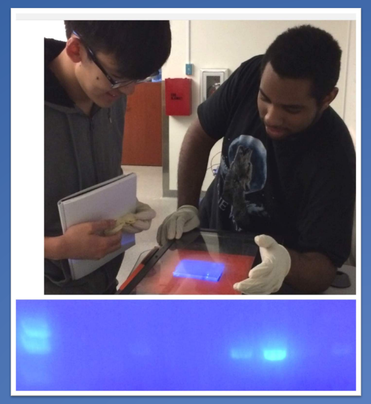
Eastern mud snail Tritia obsoleta

Introduction

New York City has a vast marine biodiversity in the estuarine ecosystem, mostly located in the fusion of the saline Atlantic Ocean waters and the fresh waters of the Hudson River. Of the marine organisms that live in the ecosystem, we will primarily study the silverside fish, Mud Snail, Asian Shore Crab, and Comb Jelly.

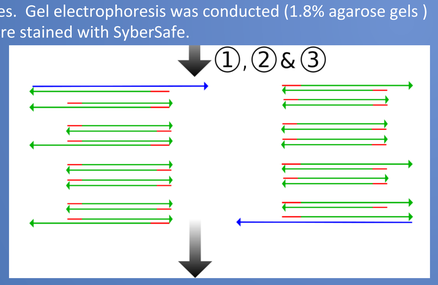
The organisms collected from Brooklyn Bridge Park shore will be a good representation of the species in the surrounding marine area. The first of these species is the silverside fish. They are common subjects for scientific research because they are sensitive to extreme environmental conditions such as low oxygen levels, drastic temperature changes, and contaminants in the water.
In addition to the above, we will look at fresh marine fish that can be bought at a fish store, such as scallops and salmon. Smoked seafood products will be explored as well.

Besides revealing biodiversity, DNA barcoding can reveal if fish has been mislabeled of not. Sometimes a cheaper cut of fish is "substituted" for a more expensive piece. DNA barcoding can also reveal if one is trying to sell something that is endangered or is on the CITES list. Unfortunately in some species, such as sturgeon an even more endangered fish is marketed that what it is "substituting" for.



Material and Methods

Marine organisms were collected by seining as illustrated above. They were frozen until further use. Three different methods (Chelex, Qiagen spin columns, and silica beads were used in an attempt to extract DNA from these organisms. We were not successful with the mud snails for unknown reasons. We then used the silica method with the mammal primers on the farmed and smoked salmon samples. Insect primers were used for the mollusks. (bay and sea scallops). The silica method began with lysis of cells, proteinase K digestion, further lysis, and then separation of DNA from other components of the cell through adherence to silica beads and alcohol washings. DNA was eventually eluted from the beads. A polymerase chain reaction (PCR) was set up to amplify cytochrome b mitochondrial DNA with the appropriate primers —mammalian for the fish vertebrates, and insect primers for the scallop invertebrates. Gel electrophoresis was conducted (1.8% agarose gels) and gels were stained with SyberSafe.



PCR and electrophoresis--NCSTS

C S H L D N A

Results

We did not obtain any results with the mud snails, even after three different types of extractions. So we tried a different tact. We wanted to get a positive result, so we turned to fresh fish and scallop tissue. DNA from 4 samples yielded positive results (Figure 1). After running a BLAST in NCBI, the salmon has over 600 Contiguous Read Length (CRL),. and 99% similarity with *Salmo salar* isolate (Atlantic salmon) cytochrome oxidase subunit I gene, mitochondrial (Figure 1a.). The scallop has 268 Contiguous Read Length, as indicated by the second figure. It has 97% similarity with *Mizuhopecten yessoensis*, which is a type of Japanese scallop (haplotype hap 12 cytochrome).

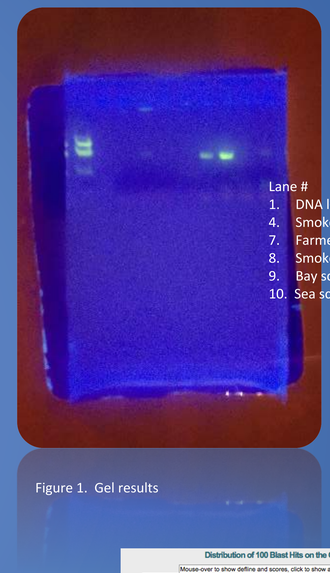


Figure 1. Gel results

Salmo salar (Atlantic salmon)
Sequence File : 2-M13F(-21).seq
>2-M13F(-21)_B11.ab1
NNNNNNNNNNGGCNNNGNAGTGTCATANCTGTTTTCTGNTTC
TTCGGTCCCCTGAGGGGTCATANNTGTTNCNGGNNNNN
ANNCAGCCAGCCTGGCGCCCTTCTGGGAGATGACCAAATTTAT
AACGTAATTGTTACAGCCCATGCCTTCGTCATAATTT
TCTTTATAGTCATACCGATTATGATCGGCGGCTTTGGAAACTGA
TTAATTCCTCTTATAATCGGGGCCCCCGACATAGCA
TTCCCCCGAATGAATAACATAAGTTTTTGACTTCTCCCTCCCTCC
TTTCTTCTCCTCCTGGCCTCATCTGGAGTTGAAGC
CGGCGCTGGCACCGGATGAACAGTCTACCCCCCCTCTAGCAGGT
AATCTTGCCCACGCAGGAGCTTCCGTTGACTTAACTA
TTTTTTCCCTCCATTTGGCTGGTATTTCTTCAATTCTTGGGGCCAT
TAATTTTATTACAACCATTATTAATATAAAACCC
CCAGCTATCTCTCAGTATCAAACCCCACTTTTTGTTTGAGCTGTA
TTAGTCACTGCCGTCCTTTTGTTACTCTCCCTCCC
TGTTCTAGCAGCAGGCATTACCATACTACTTACAGACCGAAATC
TAAATACCACTTTCTTTGACCCGGCANGCGGAGGAG
ANCCAATCTTGTACCAACATCTCTTCTGATTCTTCGGNCACCCTA

AGTGTCATANNNGTTTCCTNNA

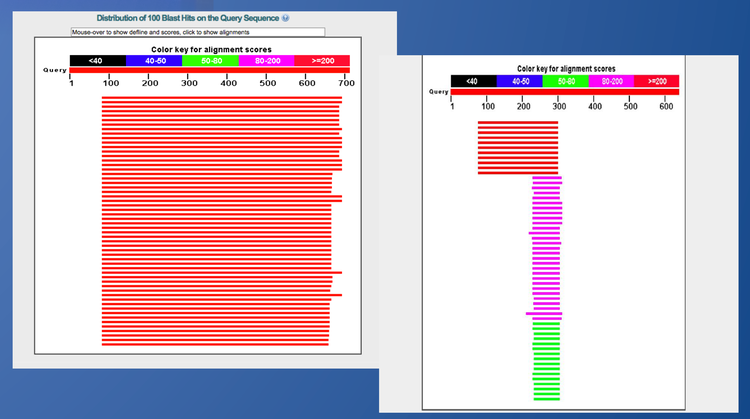


Figure 1a. BLAST results of fresh and smoked salmon (both were similar) and Figure 1b. BLAST results for sea scallop sample.

Seining in Brooklyn Bridge Park

scallop

Bay and sea scallops; fresh farmed and smoked salmon

Discussion

We do not know why we were not able to discern mud snail DNA. We would like to experiment with this and perhaps change the primers or the PCR amplification conditions. It was interesting to us that the smoked salmon revealed as much if not more DNA than the fresh salmon. We were pleased to see that smoked salmon DNA amplified, and see a line of future study of other smoked products. Also, the sea scallops were an aquaculture product; we were under the impression that they were produced locally (Gillespie et al., 2012). Since over fifty percent of our fish is now farmed, it perhaps should have not been a surprise (Nolan, 2011). Using DNA barcoding techniques in the future should ensure that the food we are eating is properly labeled with the correct species.

Selected References

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