Brackish Barcoding to Determine Biodiversity of Marine Invertebrates

Jamie Hufford¹, Shannon Lafferty¹, Nicolette Nigro¹, Mary Kroll¹, Cristina Fernandez-Marco, Ph.D.², Sharon Pepenella, Ph.D.²

¹West Islip High School; ²Cold Spring Harbor Laboratory’s DNA Learning Center

Abstract

Barcoding of marine invertebrates is necessary to determine the diversity of species inhabiting ecosystems. Our purpose was to identify marine invertebrates adapted for fresh versus marine ecosystems, including those that survive in the brackish water where a stream meets the bay. DNA was extracted from each organism and gel electrophoresis was used to confirm the presence of an amplified CO1 gene after PCR. Results were sequenced, processed in DNA Subway, and compared to GenBank and BOLD systems. Organisms identified in bay water (salinity 24-26ppt) included marsh shrimp, mud snails, and mud crabs. Organisms collected in brackish water (salinity 18ppt) were not able to be sequenced. In freshwater (salinity 0ppt), a water bug was collected. This water bug will be further investigated as a possible novel sequence because its analysis resulted in 58 mismatches in GenBank and only a 90.62% similarity to sequences in BOLD systems.

Introduction

• DNA Barcoding is used to identify different species of (Hebert, Cywinska and Ball, 2003). This can be used to show why organisms live where they do and potentially, how they affect their environment.
• By barcoding marine organisms at Gardiner Park, different species can be found and tested for taxonomic identification.
• The ability to study marine biodiversity is limited because we do not have much knowledge of the many organisms that we know of and those that have not been discovered yet (Snelgrove, 1997). This project attempted to determine which species of marine invertebrates are adapted for and living in each water ecosystem (fresh stream) vs. marine (Great South Bay) and which species of marine invertebrates can also survive in the brackish water where the stream meets the bay.

• Our purpose was to identify different marine invertebrate species that live in Gardiner Park which contains water that flows from a fresh water stream into the salty Great South Bay.
• The hypothesis of this experiment was that some fresh water invertebrates and marine invertebrates will also inhabit the brackish water. We can identify this with DNA barcoding.

Materials & Methods

• Sample Collection: Transect line and quadrats were set up at Gardiner Park, NY.
• Sample Documentation: Habitat description, latitude, longitude, elevation, salinity and pictures were recorded at each quadrat. Sample pictures including the sample number were taken.
• DNA Collection: A small piece of the organism was removed.
• DNA Barcoding: DNA extraction ➔ PCR ➔ Electrophoresis ➔ Visualize gels on a UV Trans-illuminator ➔ Send results for sequencing ➔ DNA Subway ➔ GenBank ➔ BOLD

Results

• Eight samples were able to be sequenced and identified out of the twenty samples collected. One of these samples (NRQ-006 suspected to be ilyanassa obsoleta) did not have quality DNA and was therefore not analyzed.
• The organisms identified in this experiment: NRQ-001- Palaemonetes vulgaris; NRQ-003 - ilyanassa obsoleta; NRQ-004 - ilyanassa obsoleta; NRQ-007 - ilyanassa obsoleta; NRQ-008 - Dyspanopeus sayi; NRQ-014 - ilyanassa obsoleta; NRQ-016 - Hesperocorixa interrupta (Table).

Discussion

• Four samples were identified as ilyanassa obsoleta, which are commonly known as mud snails. Figure 3 shows the lack of diversity in the CO1 gene among the mud snails we collected.
• We hypothesized that some fresh water invertebrates and seawater invertebrates would inhabit the brackish water. The hypothesis could not be tested because while we collected invertebrates in the brackish habitat, none of their DNA showed up in the gel electrophoresis. The salinities of the organisms in the bay water (26 ppt) and the mud flat (24 ppt) were too similar to make a conclusion about the hypothesis or ultimately answer our research question.
• NRQ-016 has a possibility of being a novel barcode but research is still being conducted to support this.
• In this experiment, there were uncontrolled factors and errors including: the amount of marine invertebrates present at each quadrat could not be controlled, the gene amplifications from the brackish water were not visible after PCR. This may have been due to changing the loading dye from SYBR-Green to SYBR-Safe. The original loading dye SYBR-Green worked correctly but there was not enough to use for all of our samples.
• Another place of error could have been during the actual barcoding process. When extracting the DNA from an organism, you need to use a small piece of tissue but if the tissue is too large or too small, the organism may not be able to be identified. Also, if the tissue is not mashed up enough the DNA will not break loose and the chosen gene will not be able to be amplified.
• In repeating the experiment, we would collect more samples and retest the samples that were unable to be identified.

Table: Samples that were sequenced and the environments for each organism collected are described. The E value, Bit Score, number of Mismatches in GenBank and % similarity in BOLD are shown as well.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>Collective Name</th>
<th>Water habitat (ppt)</th>
<th>Habitat Description</th>
<th>Common Name</th>
<th>Bit score</th>
<th>E value</th>
<th>GenBank</th>
<th>BOLD</th>
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<tr>
<td>NRQ-001</td>
<td>Eristalis aquatic</td>
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<td>Stream</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>NRQ-002</td>
<td>CX-2</td>
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<td>Stream</td>
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<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>NRQ-003</td>
<td>Ilyanassa obsoleta</td>
<td>0</td>
<td>Stream</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>NRQ-004</td>
<td>Ilyanassa obsoleta</td>
<td>24</td>
<td>Bay</td>
<td></td>
<td></td>
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<td></td>
<td>90.62%</td>
</tr>
<tr>
<td>NRQ-005</td>
<td>Ilyanassa obsoleta</td>
<td>26</td>
<td>Bay</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>90.62%</td>
</tr>
</tbody>
</table>

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References


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