Brackish Barcoding to Determine Biodiversity of Marine Invertebrates

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Abstract

Barcoding of marine invertebrates is necessary to determine the diversity of species inhabiting ecosystems. Our aim 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 in Gardiner Park, NY. 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, then 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. The 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 types of species (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).

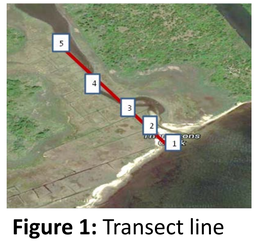

Figure 1: Transect line and quadrats are shown where organisms were collected.

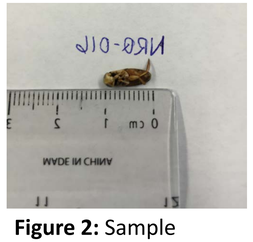

Figure 2: Sample NRQ-016 is being investigated as a novel barcode.

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.

Sample ID	Collector Name	Water Salinity (ppt)	Habitat Description	Common Name	Blast result from Gen-Bank				Results from BOLD	
					E value	Bit Score	Mis-match	Genus and Species	% Similarity	Genus and Species
NRQ-001	Shannon	26	Edge of Bay	Shrimp	0	443	28	Palaemontes vulgaris	93.76	Palaemontes vulgaris
NRQ-003	Jamie	26	Edge of Bay	Snail	0	1151	3	Ilyanassa obsoleta	100	Ilyanassa obsoleta
NRQ-004	Nicolette	26	Edge of Bay	Snail	0	1155	4	Ilyanassa obsoleta	99.8	Ilyanassa obsoleta
NRQ-007	Jamie	24	Mud flat	Snail	0	1056	1	Ilyanassa obsoleta	99.83	Ilyanassa obsoleta
NRQ-008	Shannon	24	Mud	Crab	0	1052	2	Dyspanopeus sayi	99.84	Dyspanopeus sayi
NRQ-014	Nicolette	24	Mud flat	Snail	0	1150	3	Ilyanassa obsoleta	99.63	Ilyanassa obsoleta
NRQ-016	Nicolette	0	Pond	Bug	0	836	58	Hesperocorixa interrupta	90.62	Hesperocorixa interrupta
									95.98	Hesperocorixa lucida

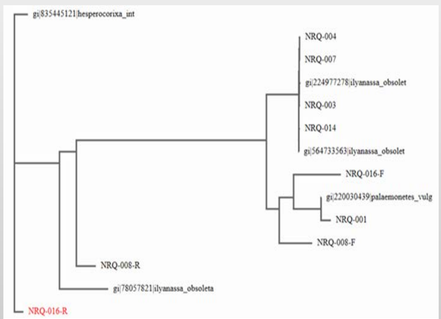

Figure 3: The closer together the samples are, the more closely related they are. NRQ-016 was chosen as an out-group because it was least similar to the other organisms collected.

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.

References

Hebert, P. D., Cywinska, A., & Ball, S. L. (2003). Biological identifications through DNA barcodes. Retrieved June 2, 2016, from http://www.urbanbarcodeproject.org/images/pdf/Biological-identifications-through-DNA-barcodes.pdf

Snelgrove, P. (1997). The importance of marine sediment biodiversity in ecosystem processes. Retrieved June 02, 2016, from http://www.academia.edu

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