DNA Barcoding; An In Depth Study

By: Madisen Rosso, Veronica Scherer

Mentor: August Eberling

Abstract

The stability of the environment has been a major concern for many ecologists. A change or addition of species in an ecosystem can alter the health of the surrounding vegetation and organisms. By keeping a record of the history of an environment, ecologists can determine how stable the environment may be. In this experiment, we visited the Massapequa Preserve and collected 10 different samples of macroinvertebrates. By barcoding the DNA of the samples collected, we will be able to determine the species of the macroinvertebrates present. This will allow us to create an aquatic macroinvertebrate base line of the Massapequa Preserve that can be monitored in future years as well. Comparing this information to previous year's results we can investigate any change in the biological diversity of the area.

Introduction

Studying the biodiversity of an environment gives an overview of the species living in the area, certain aspects of the environment, and the overall stability of the ecosystem. By being able to observe the biodiversity of an area, scientists can gain a deeper understanding of what factors can affect the equilibrium of the environment.

An essential organism to the biosphere of the earth are aquatic macroinvertebrates. Spending the majority, if not all of their lives in the water, these small insects serve as an indicator for the quality of the body of water. Macroinvertebrates recycle nutrients by scavenging dead insects or decaying bacteria and reprocess them back into the environment.

One method of determining the species of the invertebrates present in the samples taken is by using DNA barcoding. By performing PCR and running the samples through a gel using electrophoresis, then BLASTN them using DNA Subway. This allowed for viewing of sections of DNA sequences' and for the comparison of nucleotides between the different samples.

Method

1.0- Go to Massapequa Preserve to obtain specimen.

2.0- Use various equipment (such as high wader boots) to obtain aquatic macroinvertebrates.

3.0- Once specimen are located, place in ethyl alcohol and store in a refrigerador at -20° celsius.

4.0-After specimen are taken out of the refrigerador, take photos of the specimen, observe, and note any features found on the specimen.

5.0- Identify any macroinvertebrates (using taxonomy) using various sources. Such as the internet, textbooks, online searches, etc.

6.0- Take specimen to Cold Spring Harbor Laboratory to barcode the specimen and perform PCR.

7.0- Specimen DNA was extracted using silica-gel beads.

7.1- PCR was preformed to amplify CO1 region.

7.2- Sample was sequenced.

8.0- Once the specimen have been barcoded, review the similarities and differences with the species using DNA Subway. This provides a small sample of the biodiversity within the river.

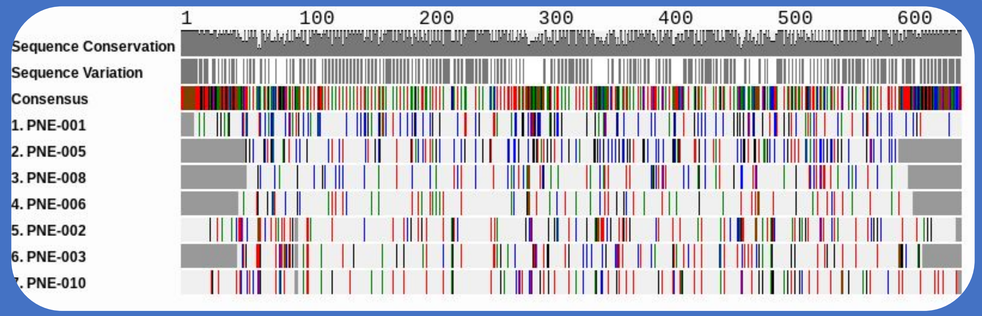

Figure #1: A MUSCLE Alignment comparing each sample of DNA taken from the Massapequa Preserve.

PIN #	Computed Result	# of Mismatches
PNE-001	Eiseniella tetraedra	1
PNE-002	Succineidae sp.	11
PNE-003	Physella acuta	1
PNE-004	X	X
PNE-005	Proasellus sp.	0
PNE-006	Chalcosyrphus nemorum	0
PNE-007	X	X
PNE-008	Ancyronyx procerus	56
PNE-009	X	X
PNE-010	Lymnaea humilis	1

Table #1: Collected sample DNA computed through DNA Subway compared to existing entries in the database.

Results

The samples taken above were computed through the database using DNA Subway and compared to existing DNA compositions. The results were shown in Table #1…

- PNE-006 was identified to be Chalcosyrphus nemorum, a common species of syrphid fly.
- PNE-001 was also recognized to be Eiseniella tetraedra, or the square-tail worm.
- PNE-010 was identified as Lymnaea humilis, a common gastropod.
- Samples PNE-002 and PNE-005 were only able to be identified by their suspected genus, that being Succineidae and Proasellus respectively.
- Sample PNE-008 is an outlier in terms of accuracy of comparison from existing results. DNA Subway's database identifies it as Ancyronyx procerus, the spider water beetle. Even so, this is done with 56 mismatches in the DNA sample taken compared to existing information taken from similar organisms.

Discussion

One result that seemed odd was the outcome of sample PNE-008. The sample did match with the species Ancyronyx procerus on DNA Subway, however, there were 56 mismatches to this data. Searches where then conducted on traits of the cricket to identify the species. Other methods of modern taxonomy were also used. By looking up the species name of Google, there were many differences between the two species. After contacting a taxonomist, traits of the specimen were compared to beetles within the genus. The specimen was then identified to be Ancyronyx variegatus. However, the markings on the beetle seemed to be different. This brings up the possibility that Ancyronyx variegatus could be a dimorphic species.

While there were multiple species collected among the samples shown, these results can't be considered a full overview of the biodiversity of the preserve. There are hundreds of thousands of species in a freshwater ecosystem. Collecting a small portion of the environment leaves many other species unidentified. In future years, collection at different spots across the river at different locations should occur (ie. the edges at the beginning of the river versus the middle and the end of the river). Sample sizes should also be collected from other areas of the preserve, not just aquatic. Terrestrial areas would be able to provide an insight to the quality of soil, nutrients found in the ground, etc. Gathering samples from different parts of the preserve provides a general overview of the state of the environment as well as the potential presence of pollution.

In modern day, damage towards the environment can be proven to be deadly towards the organisms inhabiting it and the neighboring populations. Being able to observe the impact of human activity on an environment using the macroinvertebrates in the area shows a clear overview of the state of the natural area. Based on the results, the river in the Massapequa Preserve seems to be in a relatively healthy condition.

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