# The Utilization of DNA Barcoding in order to Determine the Biodiversity of Submerged and Unsubmerged Aquatic Vegetation in The Moriches Bay

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## ABSTRACT:

Approximately 90% of aquatic vegetation, such as *Zostera marina* have decreased across Long Island. This vegetation increases habitat complexity, which influences predator prey relationships. Increased complexity, increases biodiversity, which decreases mortality rates of organisms such as juvenile shellfish. Biodiversity of submerged and unsubmerged aquatic vegetation was analyzed throughout Smith Point Beach and Moriches Bay (Carroll, J., Jackson, L., & Peterson, B., 2014). Approximately, 19 different species of vegetation were collected between the ocean and the bay. Twelve samples were unlabelled sequences under BOLD or NCBI databases.

## RESEARCH QUESTION:

Has speciation occurred between unsubmerged and submerged aquatic vegetation in both the ocean and bay side at Smith Point Beach?

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## REFERENCES:

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Peterson, B., Bricker, E., Brisbin, S., & Furman, B. (2013). Genetic diversity and gene flow in Zostera marina populations surrounding Long Island, New York, USA: No evidence of inbreeding, genetic degradation or population isolation. Aquatic Botany, 61-63. doi:10.1016/j.aquabot.2013.05.003

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## INTRODUCTION:

Biodiversity is the variety of species in a given ecosystem. As biodiversity increases the stability of an ecosystem increases. Another factor that increases ecosystem stability is habitat complexity. Pollution is one factor that can cause instability in an ecosystem, but with the help of Oysters added to the Moriches Bay it has reduced the amount of pollution from the bayside (Carroll, J., Jackson, L., & Peterson, B., 2014).

Submerged and unsubmerged aquatic vegetation increase habitat complexity. Habitat complexity plays a major role in marine ecosystems by creating third dimensional aspect for juvenile organisms to hide from predators (Carroll, J., Jackson, L., & Peterson, B., 2014). Habitat complexity affects how predators and prey interact with one another, and more complex webs create more stable ecosystems. It can also alter predator prey relationships and impact population dynamics. For example *Zostera marina* provides a nursery for juvenile shellfish which protects them from crabs. This allows Zostera's three dimensional canopy to make it easier for shellfish to hide from predators. This has caused prey survival to increase dramatically, leading to an increase in population for several different species. Submerged and unsubmerged aquatic vegetation is critical to habitat complexity because it's three dimensional canopy adds complexity and increases survival rates expanding the biodiversity of its ecosystem (Carroll, J., Jackson, L., & Peterson, B., 2014).

Unsubmerged aquatic vegetation such as *Spartina flora* and *Ammophila breviligulata*, also creates habitat complexity. Submerged aquatic vegetation like *Zostera marina* reproduces through the process of vegetative propagation and pollen dispersal. This demonstrates phenotypic plasticity which may lead to variation. This demonstrated phenotypic plasticity by having the ability to change its phenotype in response to environmental changes (Peterson, B., Bricker, E., Brisbin, S., & Furman, B., 2013).

Over the past few years the Moriches Bay has deteriorated due to red tides. Red tides are caused by large numbers of certain microscopic dinoflagellates. Water pollution has increased mortality rates in both fish and other shellfish (Watkins, S., Reich, A., Fleming, L., & Hammond, R., 2008). The pollution has had detrimental effects on life on the bay but with help of Submerged and Unsubmerged aquatic vegetation many fish have lived longer than expected (Watkins, S., Reich, A., Fleming, L., & Hammond, R., 2008). Aquatic vegetation such as *Zostera marina* plays a major role in protecting several marine species, and unfortunately it has declined 90% on Long Island. The Moriches Bay has several amazing components, containing a variety of species of shellfish and aquatic vegetation across the Bay (Peterson, B., Bricker, E., Brisbin, S., & Furman, B., 2013).

By using DNA barcoding, the biodiversity of submerged and unsubmerged aquatic vegetation can be analyzed. The specific sequence of DNA that will be analyzed for aquatic vegetation is rbcL. rbcL also refers to the primer that will be used during PCR amplification, ITS and tufA primers will also be used.

## MATERIALS AND METHODOLOGY:

The samples will be collected from different locations along Moriches Bay and Smith Point Beach. Data will be collected from each location with the necessary permits from Smith Point parks that will provide as our organisms that will be used for in genetic testing.

A DNA analysis will be performed by first extracting the DNA sample from a leafy part of the plant. This will be done by taking a small portion of the leaf or a small section of the root and then break through the cell membrane by grinding the samples using a pestle and lysis solution, then centrifuging to remove the proteins from the DNA. Then PCR will be used, which copies one segment of DNA, primers such as rbcl, ITS and tufA will be utilized. Then gel electrophoresis will be used in order to determine how strong the samples are. Darker bands will indicate a strong samples and once the darker bands are determined they will be sent to genewiz. Once genewiz uploads the nucleotide sequencing to DNA subway, the DNA subway will then be used to create several cladograms that will be used to analyze the biodiversity of the submerged and unsubmerged aquatic vegetation. Then using DNA subway, the DNA can be analyzed and compared through its sequencing according to the Cold Spring Harbor Protocol (CSHL). Once sequenced, we will use this data to help determine the biodiversity of Moriches Bay and Smith Point beach. We will evaluate the genetic biodiversity both at the locations as whole to evaluate the stability of the ecosystem (Lloyd, J.,2014).

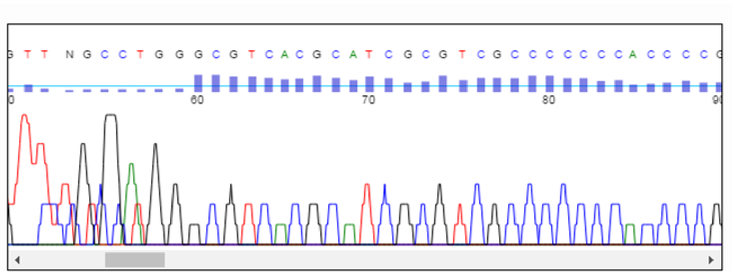



**Figure 1**:This is a close analysis of NQD-006s Phred scores. The blue line is indicated to be a marker that determines whether a nucleotide in the sample is accurate. If the bar falls above the Phred score line, it means that the nucleotide given is 100% accurate. The phred score quality will measure the quality and identification of all nucleotides in each sample which will be used to compare the impact red tides have on biodiversity and habitat complexity.This phred score demonstrates both high and low scores.

## RESULTS:

The following samples NQD 02, 04, 06, 07, 08, 09, 10, 11, 18 and 19 were not found in the BOLD and NCBI databases and were labeled as unidentified species. The specific genes, ITS and tufA for the samples were not found in either database which could lead to the indication that these genes for these specimen are not published.

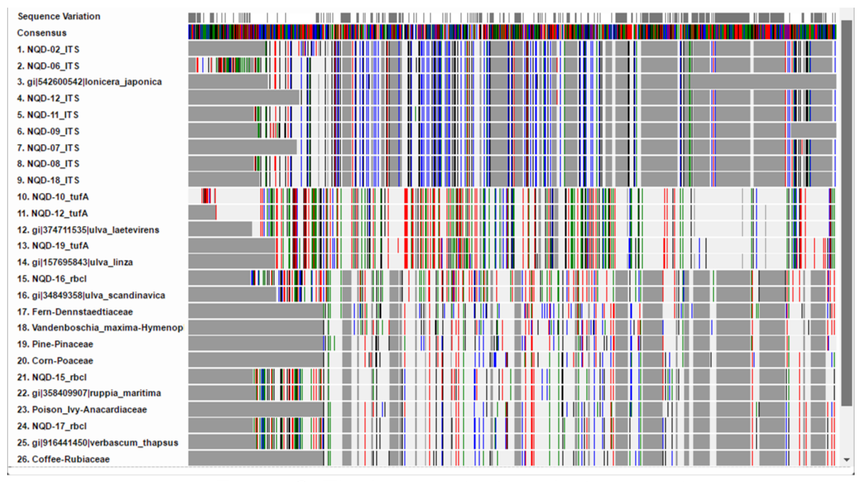
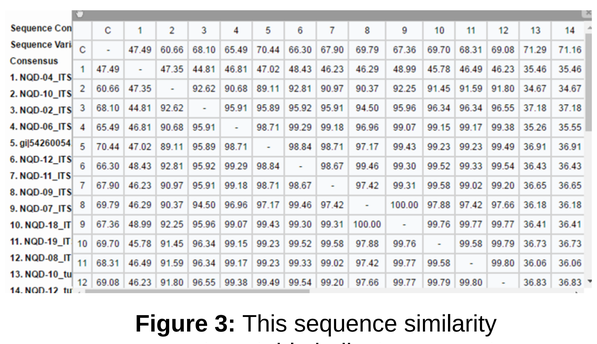



**Figure 2:** The barcode table indicates samples NQD 02, 04, 06, 07, 08, 09, 10, 11, 18 and 19 are unidentifiable on BOLD and NCBI. This table also shows the relativity between samples.



| | C | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| C | - | 47.49 | 60.66 | 68.10 | 65.49 | 70.44 | 66.30 | 67.90 | 69.79 | 67.36 | 69.70 | 68.31 | 69.08 | 71.29 | 71.16 |
| 1 | 47.49 | - | 47.35 | 44.81 | 46.81 | 47.02 | 48.43 | 46.23 | 46.29 | 48.99 | 45.78 | 46.49 | 46.23 | 35.46 | 35.46 |
| 2 | 60.66 | 47.35 | - | 92.62 | 90.68 | 89.11 | 92.81 | 90.97 | 90.37 | 92.25 | 91.45 | 91.59 | 91.80 | 34.67 | 34.67 |
| 3 | 68.10 | 44.81 | 92.62 | - | 95.91 | 95.89 | 95.92 | 95.91 | 94.50 | 95.96 | 96.34 | 96.34 | 96.55 | 37.18 | 37.18 |
| 4 | 65.49 | 46.81 | 90.68 | 95.91 | - | 98.71 | 99.29 | 99.18 | 96.96 | 99.07 | 99.15 | 99.17 | 99.38 | 35.26 | 35.55 |
| 5 | 70.44 | 47.02 | 89.11 | 95.89 | 98.71 | - | 98.84 | 98.71 | 97.17 | 99.43 | 99.23 | 99.23 | 99.49 | 36.91 | 36.91 |
| 6 | 66.30 | 48.43 | 92.81 | 95.92 | 99.29 | 98.84 | - | 98.67 | 99.46 | 99.30 | 99.52 | 99.33 | 99.54 | 36.43 | 36.43 |
| 7 | 67.90 | 46.23 | 90.97 | 95.91 | 99.18 | 98.71 | 98.67 | - | 97.42 | 99.31 | 99.58 | 99.02 | 99.20 | 36.65 | 36.65 |
| 8 | 69.79 | 46.29 | 90.37 | 94.50 | 96.96 | 97.17 | 99.46 | 97.42 | - | 100.00 | 97.88 | 97.42 | 97.66 | 36.18 | 36.18 |
| 9 | 67.36 | 48.99 | 92.25 | 95.96 | 99.07 | 99.43 | 99.30 | 99.31 | 100.00 | - | 99.76 | 99.77 | 99.77 | 36.41 | 36.41 |
| 10 | 69.70 | 45.78 | 91.45 | 96.34 | 99.15 | 99.23 | 99.52 | 99.58 | 97.88 | 99.76 | - | 99.58 | 99.79 | 36.73 | 36.73 |
| 11 | 68.31 | 46.49 | 91.59 | 96.34 | 99.17 | 99.23 | 99.33 | 99.02 | 97.42 | 99.77 | 99.58 | - | 99.80 | 36.06 | 36.06 |
| 12 | 69.08 | 46.23 | 91.80 | 96.55 | 99.38 | 99.49 | 99.54 | 99.20 | 97.66 | 99.77 | 99.79 | 99.80 | - | 36.83 | 36.83 |

**Figure 3:** This sequence similarity percentage table indicates percent similarity between samples collected.

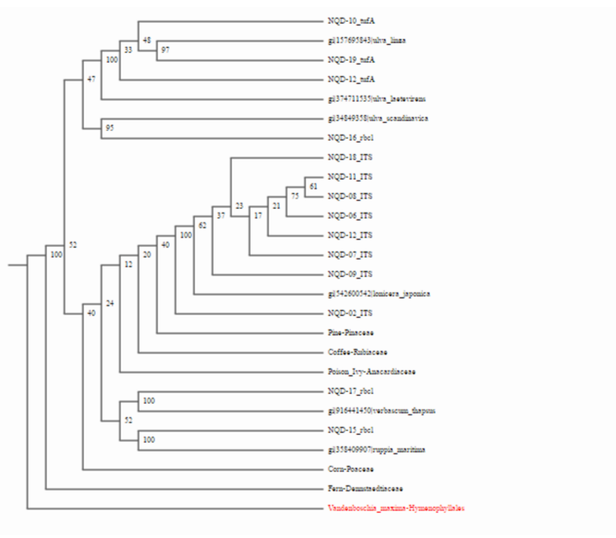



**Figure 4:** The above image shows the relationships/relativity between each of the unidentified species in both the BOLD and NCBI databases. The unidentified samples are compared to common plants that could have a close association to each other.

## DISCUSSION:

The results indicate that speciation may have occurred because the ITS and tufA genes were unlabelled in both the BOLD and NCBI genbank data bases for twelve out of the twenty species. Figure 4 demonstrates the relationships between each of the samples, for example samples NQD 011-ITS and NQD 8-ITS are placed a 61 bracket because their DNA sequencing were attached 61 times out of 100 and thus are very similar. Figure 4 demonstrates differences and similarities in the barcode of the organisms studied. For example, NQD 11 and 8 have similar barcode with distinct differences, indicating that they have similar genetic sequences. The results of this project indicate that the two genes: ITS and tufA, in 12 species, were found unlabeled in both genetics databases. Figure 2, figure 3, and figure 4 demonstrate a noticeably similar barcodes with some significant differences. For example, all 12 samples analyzed had ITS and tufA genes similar to *Lonicera japonica.* The highest mismatch was approximately 10, which indicates the samples come from a common ancestor.

## FUTURE IMPLICATIONS:

In order to improve with this scientific research, the sample size from the Moriches Bay and Smith Point Beach should be increased and continuation of identifying the level of habitat complexity throughout the Smith Point Beach area.