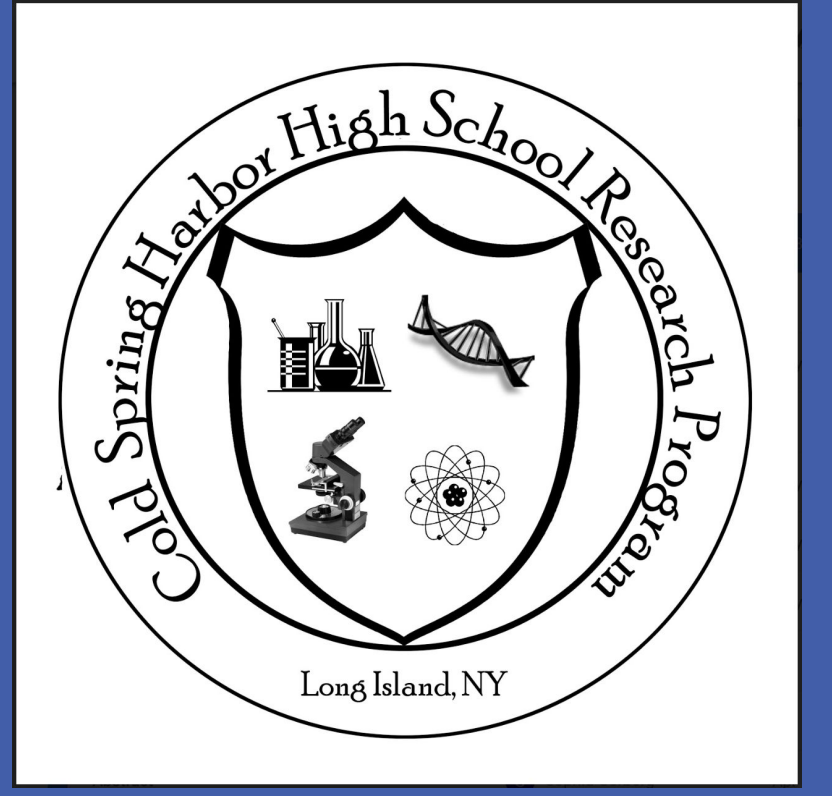




Biodiversity Among Aquatic Invertebrates

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Abstract

The purpose of this lab was to further our knowledge of aquatic invertebrates in Cold Spring Harbor and investigate the effects they have on humans. We first collected our specimens at the streams and ponds by the Cold Spring Harbor Laboratory. In order to do so we needed gloves, test tubes and filters. After collecting multiple specimens, we examined them by isolating their DNA in order to get their DNA sequences. From there we were able to determine what species each of the invertebrates were. Our results showed that we found Laccophilus sp., Ceratopogonidae sp., amphipods and Lumbriculus sp. None of these organisms contained parasites so we do not believe that these specific aquatic invertebrates have any negative effects on human lives. We, however, did find that the ceratopogonidae sp. bite livestock and can transmit a Blue Tongue virus which can make them very sick or die. This may have a major impact on the lives of those specimens, but they are of no harm to humans who simply want to explore the ponds that embody the Cold Spring Harbor Labs.

Introduction

The Cold Spring Harbor Laboratory has played a major role in the development of molecular genetics and biology. The great diversity of organisms occupying the ponds on the laboratory grounds allowed for the investigation of many different species. After obtaining all seven aquatic invertebrates and subsequently determining their DNA sequences, we were able to research each organism. None of the research yielded concern for any type of parasite that would be at all harmful to humans. We, however, did find that many freshwater flatworms are parasitic. These may have not been collected, but they are quite common aquatic invertebrates.

BACKGROUND INFORMATION

Many aquatic invertebrates are known to carry parasites which can affect other aquatic organisms and humans. According to the department of environmental conservation, some aquatic invertebrates found in NY are Flatworms (Platyhelminthes), Crayfish (Decapoda,) Mussel, Clams (Mollusca: Pelecypoda), Water Beetles (Coleoptera), Snails (Mollusca: Gastropoda), Worms (Oligochaeta) and Scuds (Amphipoda). Not all of these organisms carry parasites but Leung discussed how Mollusca carries diseases such as Mastigophora, Sarcodina, Haplosporidia, Labyrinthomorpha, Microsporidia and Microcytos. According to Leung, Oligochaeta carries diseases such as Microsporidia, Apicomplexa, Myxozoa, Digenea, Eucestoda, Rotifera, Nematoda, and Nemertea. Our samples did not seem to carry any parasites, but in New York there are two organisms Oligochaeta and Mollusca which are carriers of them.

OBJECTIVES/HYPOTHESIS

We predict that the species aquatic invertebrates collected are of no harm to human health. There has not been any concern in the ponds that embody the Cold Spring Harbor Laboratories.

MATERIALS/METHODS

We first purchased latex gloves, buckets, shovels and strainers in order to dig through the dirt and sand and retrieve the invertebrates. We then went to the Cold Spring Harbor laboratory in which there is a stream that goes into two ponds. We used a bucket to scoop pond water and strain it to see if there are any invertebrates. There were several test tubes in which we obtained eight different aquatic invertebrates. As each worm is collected, we labeled the test tube with a number 01-08 with the three letter code BBT before it and took pictures of all seven plates. We extracted the DNA and ran PCR to copy the CO1 region. Next, we ran a gel. For the samples that had a PCR product we sent them for sequencing. Finally, we used DNA to analyse the results. Refer to DNA Barcoding 101 protocol.

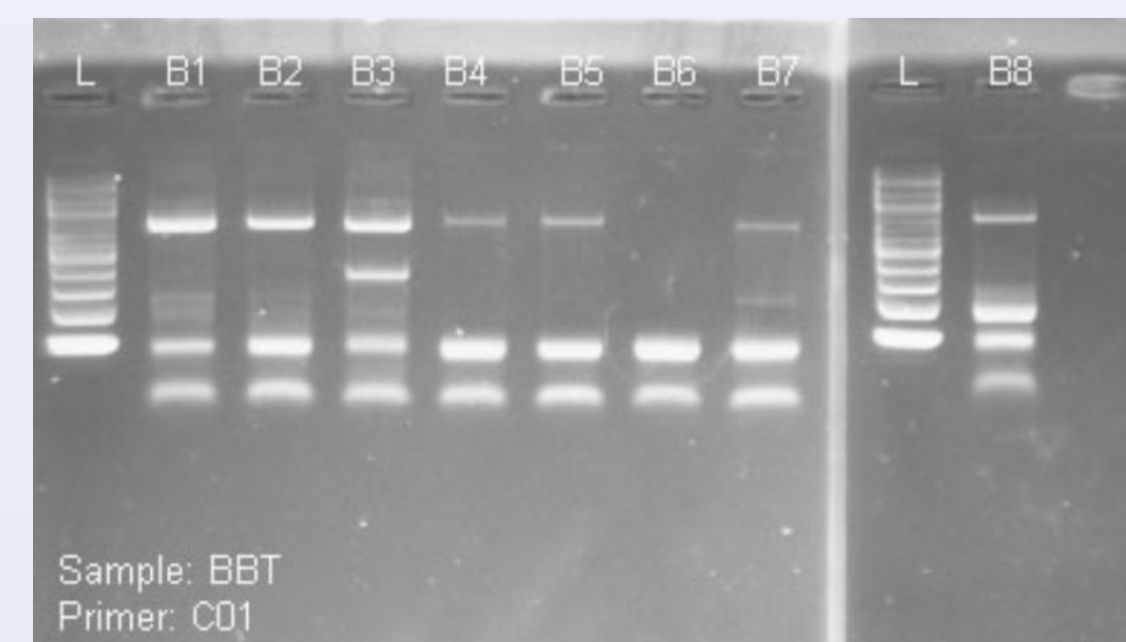


Figure 1: Gel Electrophoresis

PCR products for CO1 for samples B1-B8. We used gel electrophoresis on our eight samples to confirm that there was DNA of the predicted length. As shown in sample B6, one can infer that there was no amplification. This was also the case for both samples B7 and B8 as no sequence was available due to a low quality of the given DNA.

DATA

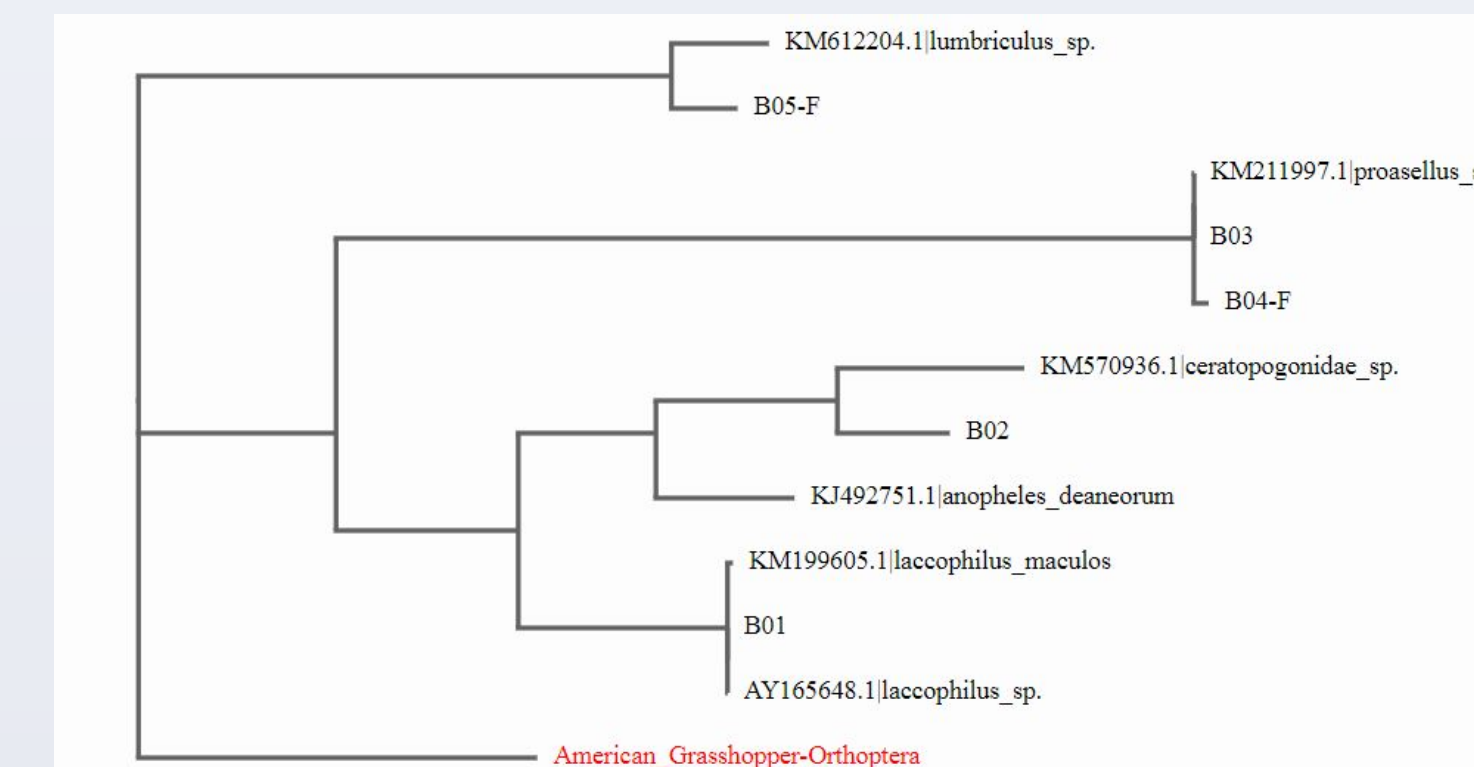


Figure 2: Phylogenetic Tree

ML Tree from DNA Subway showing relatedness of our samples with their closest matches.

| Sample ID | DNA Subway BLAST | Common Name | # Mismatches | % Similarity | BOLD Systems | % Similarity |
|-----------|-----------------------|-------------|--------------|--------------|-----------------------|--------------|
| BBT 001 | Laccophilus maculosus | Beetle | 1 | 99.82 | Laccophilus maculosus | 99.84 |
| BBT 002 | Ceratopogonidae sp. | Worm | 51 | 90.91 | Ceratopogonidae | 97.07 |
| BBT 003 | Proasellus sp. | Amphipod | 0 | 100 | Proasellus sp. | 100 |
| BBT 004 F | Proasellus sp. | Amphipod | 1 | 99.78 | Proasellus sp. | 100 |
| BBT 005 F | Lumbriculus sp. | Worm | 32 | 94.56 | Lumbriculus sp. | 99.28 |
| BBT 006 | No DNA | | | | | |
| BBT 007 | Poor Quality | | | | | |
| BBT 008 | Poor Quality | | | | | |

Table 1: Sample Details

Table showing BLAST hits and percent similarities

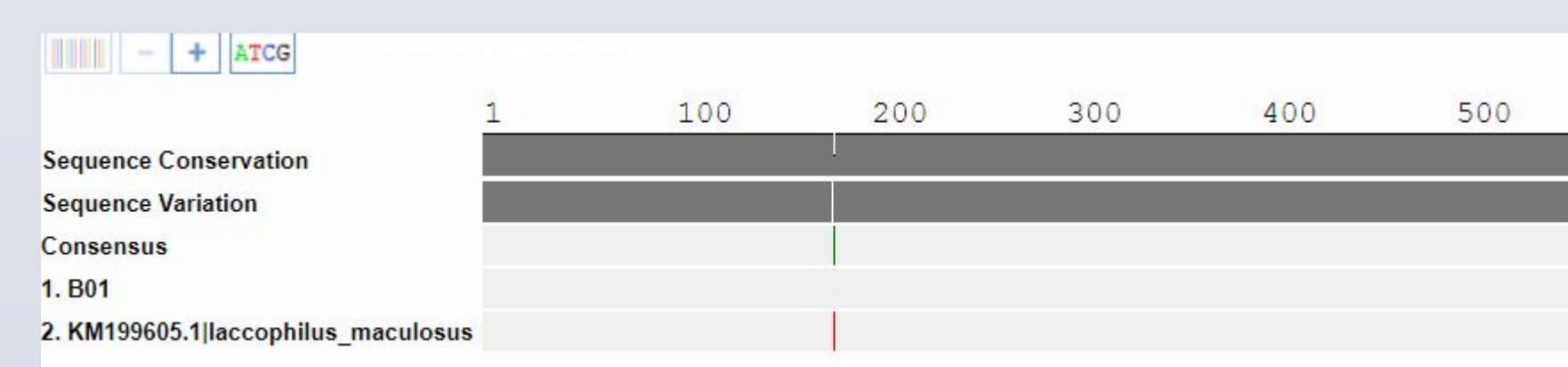


Figure 3: Sequence Alignment for B01

Sample B01 aligned with its closest match from DNA Subway.

| Phylum | Class | Order | Family | Genus | Species | Subspecies | Similarity (%) | Status |
|------------|---------|---------|-----------------|-------------|---------|------------|----------------|-----------|
| Arthropoda | Insecta | Diptera | Ceratopogonidae | | | | 97.62 | Published |
| Arthropoda | Insecta | Diptera | Ceratopogonidae | | | | 97.07 | Published |
| Arthropoda | Insecta | Diptera | Ceratopogonidae | Sphaeromyia | | | 90.84 | Published |

Figure 5: Sample B05 and BOLD matches

Sample 2 has a 97.63% match to the genus Ceratopogonidae in BOLD

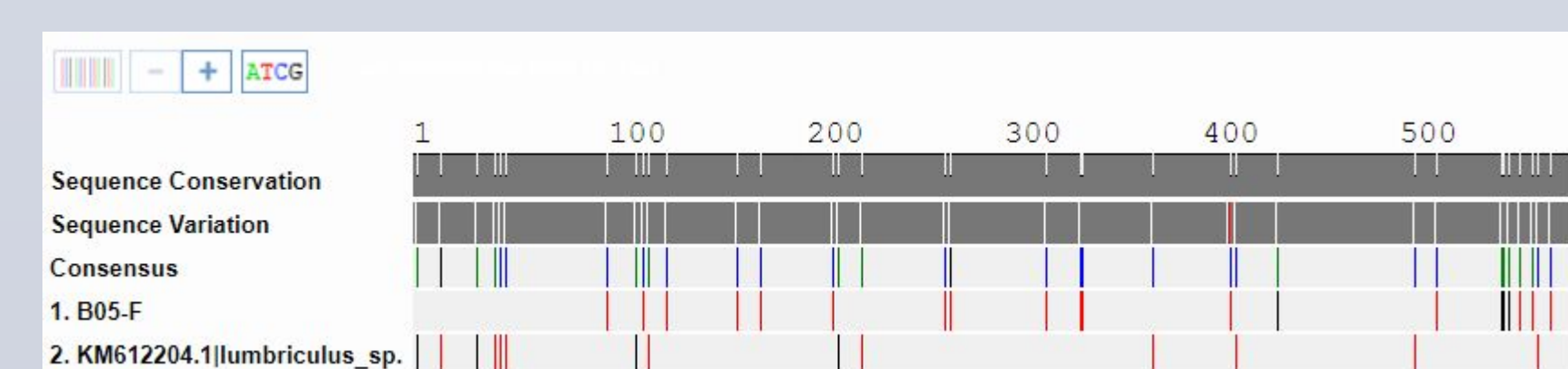


Figure 7: Sequence Alignment of B05-F from DNA Subway

The closest match to Sample 5 was the genus Lumbriculus

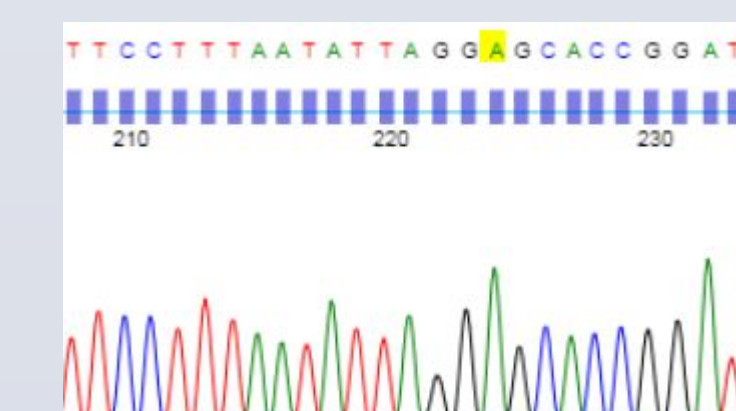


Figure 4: Electropherogram for Sample B01

Sample 1 has a polymorphism at position 227

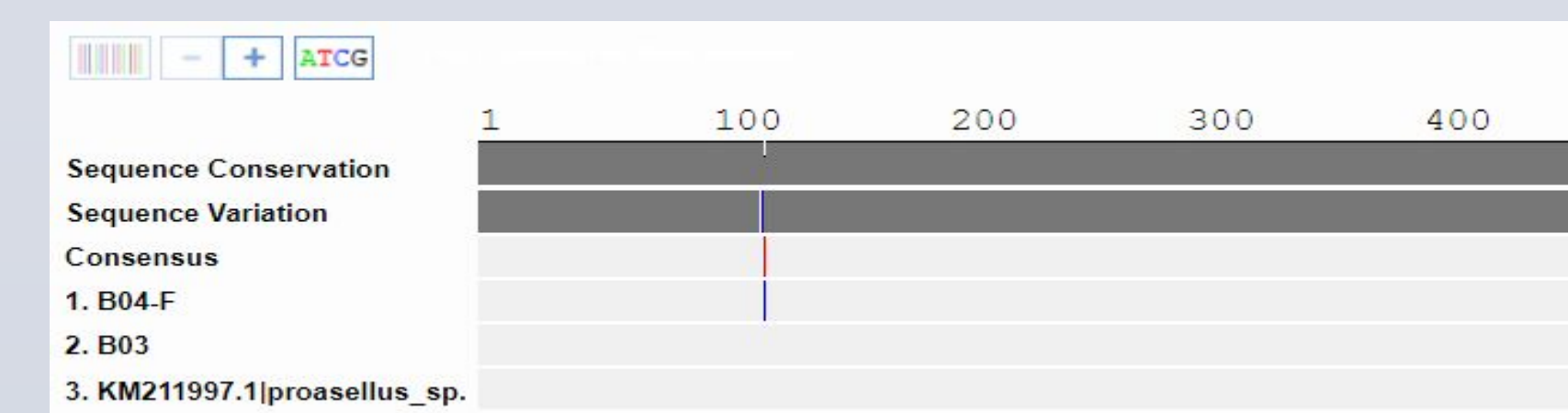


Figure 6: Sequence Alignment for B04-F and B03

Sample 3 and 4 have the same DNA Sequence. The closest match was only down to the genus Proasellus.

CONCLUSIONS

Samples 1,2, 3, 4, 5, 7 and 8 all had good amplification of the CO1 region. Our samples 1,3,4 and 5 obtained good sequence results. We found common aquatic invertebrates for these samples, but for Samples 2-5 we only found the genus and not the species from the DNA sequences. In order to verify the species we have to use morphophonology from the pictures we took. For sample 2 it looks like a Ceratopogonidae larvae. Samples 3 and 4 are in different stages of life. For samples 2-5 we can not tell the species from the pictures because all of the species are very similar looking. Since we didn't identify the species for these organisms from CO1 and morphology we would have to look at a different region of the DNA. For sample 6 there was nothing shown on the gel, therefore, we did not send it to be sequenced. Samples 7 and 8 we obtained results on the gel but the sequences were low quality. The samples identified were Laccophilus sp., Ceratopogonidae sp., Proasellus sp. and Lumbriculus sp. Two of our samples, 3 and 4, both turned out to be Proasellus sp. Sample number 3 look liked a younger and undeveloped specimen. Sample number 4 appeared to be an adult of the species.

We were searching for parasites that the aquatic invertebrates carry, but our results shows that the specimens we collected do not carry any parasites. The results support our hypothesis that the aquatic invertebrates in this area are not harmful to human health. There are aquatic invertebrates found in New York that carry parasites that could affect our health such as Oligochaeta or worms and Mollusca or mussels. We did not find any of these in our results which shows that the ponds near the Cold Spring Harbor Laboratory are safe and the aquatic invertebrates found there do not harm humans.

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