Biodiversity of Marine Worms

Ben Fagin and Elijah Gordon

Abstract

The town of Cold Spring Harbor, New York, contains a myriad of marine worms located along its coasts. The body of water behind Cold Spring Harbor Laboratory provides the opportunity to collect and examine a variety of species of marine worms. Our goal was to be able to use the data collected from DNA barcoding to observe marine worm biodiversity, and to determine if there are any invasive or foreign species in the Cold Spring Harbor waters. We did this by cross referencing our collected species with a database of known invasive species in North America.[2] Marine worms proved more difficult than expected to extract DNA from. We attempted to extract Extract the DNA using the DNA barcoding 101 protocol, but this method proved ineffective and only three out of our first fifteen samples had results. We then recollected around another 25 samples and used the Qiagen (DNeasy blood and tissue) kit. With this there were better quality sequences and many did not have any matches in existing databases. We believe we may have as many as 5 novel sequences. We were able to identify some worm species in our area including Leitoscoloplos fragilis, Buenoa macrotibialis, Lumbricus rubellus, Eukerria saltensis and Alitta virens.

Introduction

We aim to obtain 20-25 organisms from the marshlands near the CSH Labs, where the salt content varies. We will sift through samples of mud and water looking for marine worms with as many physical difference as possible. Then the samples will be frozen for until they are barcoded. Our goal is to identify the different types of species, in the mud and water, based on their DNA sequences. Barcoding these worms will reveal what species they are, and we will then be able to tell if their are any invasive or foreign species that could be harming the balance of the ecosystem. The potential damage to the ecosystems balance might then adversely affect the humans.

Objectives/Properties

The goal of our project was to collect as many worms as possible with varying phenotypes, and barcode the worms in order to determine their species. We will then compare the worms we find to known invasive species, and see if any could cause damage to the ecosystem. We predict that there will not be any species of worm that will cause any harm to the habitat or its inhabitants.

Materials/Methods

On October 12, 2017, we did our first collection on the shore of the Cold Spring Harbor Laboratory marshland. We used latex gloves, shovels, and plastic tubes to find and scavenge the marine worms in the mucky mess. After documenting and photographing the worms, we used the Barcoding 101 protocol to extract DNA, amplify the CO1 region using PCR, and analyze our results using gel electrophoresis. With only 4 out of the possible 15 samples collected, we deicded to do an additional collection and used the Qiagen DNeasy Blood and Tissue DNA extraction kit. On October 30, 2017, we collected an additional 23 samples, and 15 had a PCR product. We decided to do a third collection on April 30, 2018 using the Qiagen DNeasy Blood and Tissue DNA extraction kit again. Of the 11 additional samples, 4 had a PCR product. We sent a total of 24 samples out for sequencing to GeneWiz. We used DNASubway to analyze and compare our sequencing data.

Data

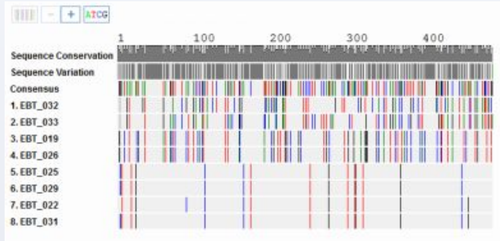
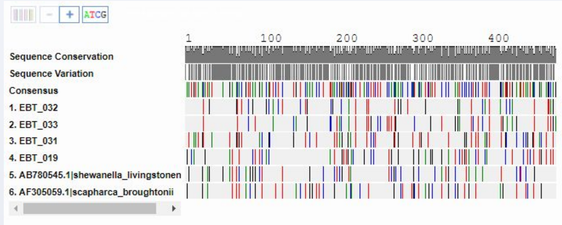


Figure 1: Sequence Alignment of Possible Novel Samples

The closest match for Samples 19 and 26 in the databases is only 83.10% The closest match for Samples 22,25,29 and 31 in the databases is only 81.06%. Interestingly, of these 4 samples, 50% show polymorphisms within the CO1 region. The closest match for Sample 32 in the databases is only 82.27%. The closest match for sample 33 in the databases is only 82.48%.



	C	1	2	3	4	5	6
C	-	89.76	89.86	86.99	88.19	89.41	87.93
1	89.76	-	89.03	81.35	80.86	82.28	82.27
2	89.86	89.03	-	81.72	81.67	82.48	80.99
3	86.99	81.35	81.72	-	82.69	81.06	79.71
4	88.19	80.86	81.67	82.69	-	83.10	82.46
5	89.41	82.28	82.48	81.06	83.10	-	87.56
6	87.93	82.27	80.99	79.71	82.46	87.56	-

Figure 2: Sequence Alignment and Similarities

Samples shown with their closet matches. Notice that all samples are well below 95% match leading us to believe these are novel.

EBT- 19 EBT- 22 EBT- 25

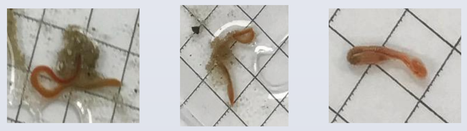


Figure 3: Photographs of of Samples EBT 019, 022, and 025

Samples 19, 22, and 25 are phenotypically similar, however Sample 19 differs genetically from Samples 22 and 25. This indicates the importance of DNA barcoding.

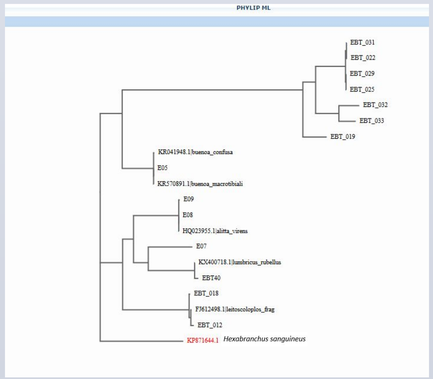


Figure 4: ML Phylogenetic Tree

Phylogenetic tree from DNA Subway showing the relationship between our samples and the closest matches. We used a flatworm (*Hexabranchus sanguineus*) as our outgroup.

Sample	Quality	Closest Species Match	% Identity
EBT-05	Good	Buenoa macrotibialis	100
EBT-07	Good	Eukerria saltensis (Early Release in BOLD)	100
EBT-08	Good	Alitta virens	100
EBT-09	Good	Alitta virens (One Polymorphism)	98.76
EBT-012	Good	Leitoscoloplos fragilis	98.91
EBT_018	Good	Leitoscoloplos fragilis	98.91
EBT_019	Good		83.1
EBT_022	Moderate		81.06
EBT_025	Moderate		81.06
EBT_029	Good		81.06
EBT_031	Good		81.06
EBT_032	Good		82.27
EBT_033	Good		82.48
EBT-040	Good	Lumbricus rubellus (Polymorphisms)	98.84

Table 1: Sample Details

Percent similarities to samples in Genbank and BOLD. Please note the 7 samples highlighted in yellow. These have no match in any databases.

Conclusions

The data suggests that Sample 5 is of the Buenoa macrotibialis species, Samples 8 and 9 are of the Alitta Virens species (8 is an exact match and 9 has one polymorphism), Samples 12 and 18 are of the Leitoscoloplos fragilis species, and Sample 40 is of the Lumbricus Rubellus species and has some polymorphisms.

Samples 19, 22, 25, 26, 29, 31, 32, and 33 had no matches in any of the databases and are possible novel sequences. We found that some of the samples had almost identical DNA sequences, which suggests that they are of the same species. These samples include 19 and 26, as well as 22, 25, 29, and 31. Sample 7 was an early release on BOLD, therefore we think we discovered a maximum of 5 potential novel species. From the identified species of worms we didn't find any known invasive or foreign species, but some of the worms had novel sequences. Therefore, one of the 5 novel species could be invasive, but this is unlikely.

We also found that marine worms are harder to barcode than some other species. We were only able to extract DNA (with good sequences) from 13 worms out of the 40 samples collected. Over the three collections we used both the DNA barcoding 101 protocol and the Qiagen (DNeasy blood and tissue) kit. The Qiagen kit worked the best, but we still didn't get as much product as hoped.

The results do not suggest that any of the worm samples cause any harm to the habitat or its inhabitants. There has been cases of marine worms having useful functions in medicines. So, a future direction could possibly consist of identifying these potentially useful medicines in these novel species of marine worms[1].

References

1. Berezow, A. (2017, May 9). Sea Snails and Marine Worms: New Antibiotics Found in Darndest Places. Retrieved May 24, 2018, from https://www.acsh.org/news/2017/05/09/sea-snails-and-marine-worms-new-antibiotics-found-darndest-places-11250

2. Irimia R, Gottschling M (2016) Taxonomic revision of Rochefortia Sw. (Ehretiaceae, Boraginales). Biodiversity Data Journal 4: E7720. https://doi.org/10.3897/BDJ.4.e7720. (n.d.). doi:10.3897/bdj.4.e7720.figure2f

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