Matanzas Biodiversity Initiative (MatBio)

Documenting our region's biological diversity using DNA barcoding

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Abstract/Introduction

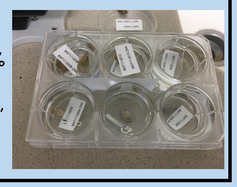
The Whitney Laboratory is located in the biologically diverse Matanzas River Basin. We are surveying the biodiversity of marine invertebrates in the area. The larval stages of organisms in the plankton look very different from the adult stages, making their identification difficult. The goal of this study was to match some of the larvae with their adult forms. The DNA sequences from the collected adult specimens were compared to sequences from planktonic larvae collected in the area in 2017 and 2018. In 2017, there were 74 species based on genetic distances, of those, 17 planktonic specimens were matched to their respective adults. The 2018 collections are currently being sequenced and should provide additional matches, with a focus on the phyla with the most missing matches.

Sampling

Samples of adult organisms were collected in the Matanzas River estuary near the Whitney lab by hand, with nets, cores and dredges. Collected material sifted through 2000 micron and 500 micron sieves. The specimens were sorted and labeled with a unique identifier that captured information on location and method and date of collection. Over 220 adult specimens have been collected so far.

Processing

The labeled specimens were relaxed and photographed. Sub-samples were obtained for DNA and preserved separately in 95% ethanol. The rest of the specimen was preserved in 75% ethanol and sent to the Florida Museum of Natural History in Gainesville, Florida, to be further curated and added to their invertebrate collections and database.

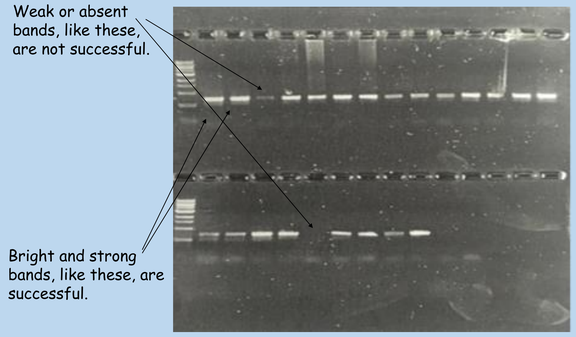


Extractions

DNA samples were extracted and incubated, overnight, in a 7.5% Chelex solution to prepare the specimen for PCR (Polymerase Chain Reaction).

PCR and Gel Electrophoresis

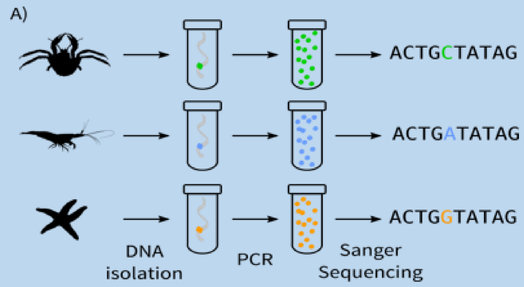
The Chelex extraction is then spun down and the supernatant is taken and added to a tube containing GoTaq Master Mix, H2O and primers. The tubes are then heated and cooled in a series of cycles in order to amplify the CO1 gene. The PCR product is run through a gel to check that the PCR was successful. Successful bands are strong, and bright because gel acts as a sieve, collecting the DNA fragments, which are stained providing a visual. If not successful, the process was repeated. The extractions are re-incubated and put through a new round of PCR. If still not successful the specimen was reextracted and put through the process again.



Gel with a 87.5% success rate.

Sequencing

The successful PCR products were prepared then sent away for sequencing.

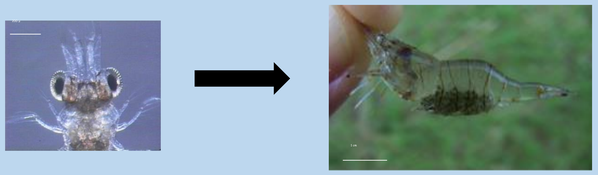


Editing

Once the sequences are received the process of editing and cleaning the sequences begins. The forward and backward runs of the sequences are lined up using a program called Geneious which helps to check for discrepancies. The program highlights all potential issues and then the user goes through to look at each case. If there is a discrepancy and one of the reads is very strong, then the discrepancy is amended to the stronger read. However if a section has many discrepancies, then the sequence is either discarded or cut down to the part with solid evidence. This provides clean and accurate sequences.

Matching

Once the sequences are cleaned up, they are run against the data from the plankton tows and against data in GenBank, an annotated collection of all publically available DNA sequences. The matches found between plankton tows and these sequences help link larvae to adult specimens, making it easier to identify species.



Larval and adult stages of *Palaemonetes pugio*

Example Sequence

TACACTATACTTCATTTTCGGAGCGTGAGCAGGAATAGTAGGCACTTCTCTTAGGTTGTTAATTCGAGCA
GAGTTAGGGCAGCCAGGAACTCTTATTGGTAATGACCAAATCTATAATGTGATCGTTACAGCCCACGCCT
TTGTTATAATCTTCTTCATGGTAATACCAATTATGATTGGAGGATTTGGAAACTGGCTAGTCCCACTAAT
ACTAGGAGCCCTGATATAGCTTTTCCTCGTATAAATAATATAAGATTCTGACTTCTTCCTCCAGCTCTT
ACACTTCTACTCTCCAGAGGTATAGTAGAAAGTGGTGTAGGCACAGGGTGAACCGTTTATCCTCCACTGG
CTAGTGGGCTAGGACATGCTGGAGCCTCAGTAGATTTAGGCATTTTCTCACTGCACCTAGCAGGAGTATC
TTCGATTTTAGGGGCCGTAAATTTTATTACTACAGTAATCAATATACGAGCACCAGGCATAACTATAGAC
CGAACTCCCCTATTTGTTTGAGCAGTATTCTTAACTGCTATTTTACTTCTCCTTTCTCTACCCGTCCTAG
CAGGGGCAATCACTATACTTCTTACAGACCGTAATCTAAATACTTCATTCTTTGACCCTGCCGGAGGAGG
TGATCCCATTCTTTATCAACATTTATTC

Plankton Results

Taken from plankton tows in the Matanzas River estuary.

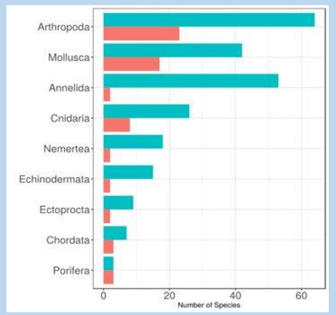


Figure 1. Number of species from plankton tows by phyla (shown in teal) vs. number of matches found on GenBank (shown in red).

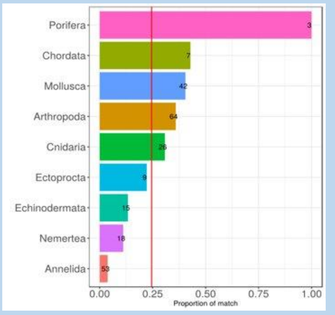


Figure 2. Number of species in decreasing phyla order. The numbers at the end of the bars show the total number of species found in plankton tows. The red line represents the mean proportion of matches across all phyla.

Results/Conclusion

In 2017, there were 74 adult, sequenced species (based on genetic distances) which yielded 17 matches with the plankton sequences. As a result of that, collections in the summer of 2018 were focused around phyla that were missing many matches, such as Annelida. These samples will be sent away for sequencing this spring (2019). Once received, the results will show if focusing efforts effectively increased the matching success. If not, methods of collection should be revisited, and more current plankton collections could be taken to compare. There is always the possibility that some planktonic larvae don't settle in the areas of adult collection, or that they have limited recruitment success in the areas surveyed.

This project has served to expand invertebrate collections at the Florida Natural History Museum (accessible online), has increased understanding of the invertebrate biodiversity of the Matanzas River estuary, and has contributed new DNA sequences to GenBank.

The Carl and Marcella Matthaei Ecological Scholarship

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