Using DNA barcodes to measure biodiversity of insects captured in NJ light traps

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Abstract

We used DNA barcoding to ID insects caught in NJ light traps. After DNA extraction and PCR, Sanger sequencing was employed. We achieved a high rate of amplification with good quality sequences. With our 20 samples, 19 of them had quality reads, with visible bands and Phred scores above 20. Moving forward, we are looking to perform a similar experiment to see how biodiversity of mammals impacts the amount of ticks that transmit Lyme Disease.

Introduction

Mosquitos are vectors of human diseases and monitoring the traps on Long Island, testing their feasibility. ranges of species present is important during outbreaks[1]. Identifying them by examining external structures or morphology is difficult and requires advanced training. DNA barcoding offers a quick and less ambiguous method of identification at a molecular level[2]. Coupled with currently deployed light traps it could help monitor species' ranges. Our goal was to use DNA barcoding to measure species diversity at several

Figure 1

Figure 2

Materials & Methods

The insects were collected by Suffolk Vector Control on 9/23/16 and 9/26/16. Identification at Connetquot High School was done using a PARCO TMX, a Celestron Digital Microscope Imager, and the Peterson Field Guide to Insects[3]. The DNA was extracted using the silica protocol, provided by Cold Spring Harbor Laboratory on 1/25/17, at CHS. The COI gene was amplified, by PCR using the LCO1490 primer and Taq beads, following CSHL protocol[2]. Gel Electrophoresis was performed on 2% agarose gels with 150V until the leading marker reached 10cm in order to confirm the presence of the PCR products. Samples were sent to Genewiz for sequencing. We used the blue line of DNA Subway to trim and align the sequences and then used BLAST to find species matches[4].

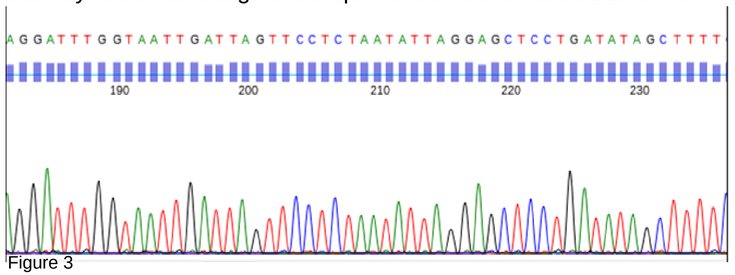


Figure 3

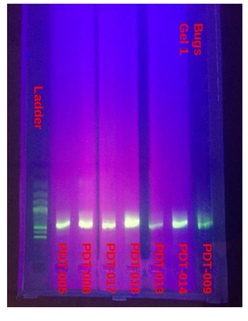


Figure 5

Results

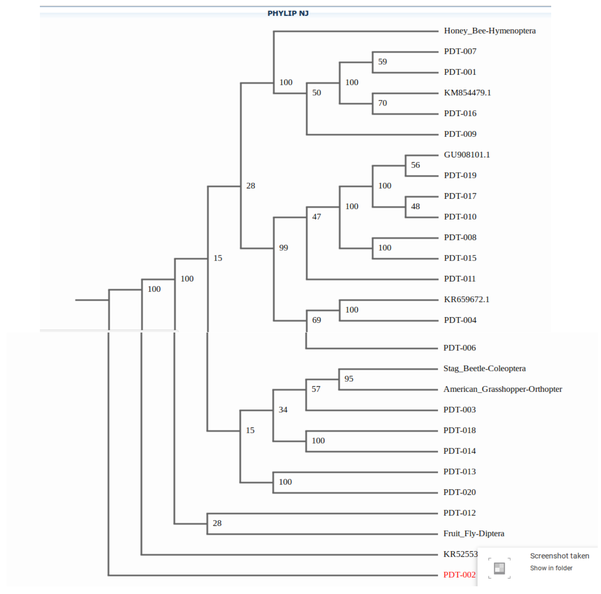
Visible bands appeared for all samples after gel electrophoresis indicating PCR amplification. 95% of our sequences had Phred scores above a 20 and a majority of them are about double, as can be seen in the Figure 3. Only sample PDT-005 was discarded because of the poor results on the reverse sequence. As you can see in Figure 4, we have seven different genuses: *Nephrotoma, Rhipidia, Dicranomyia, Goeldichironomus, Aedes, Limoniidae, Culex, and Cecidomyiidae*. The samples listed all had an E Score of 0, and a Bit Score of at least 800. Only the top match for each sample is listed in Figure 4.

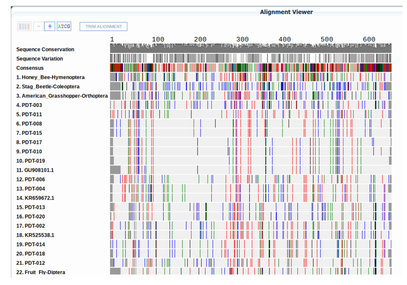
Sample	Best Match	Seq Length	Mismatches	E Score	Bit Score
PDT-001	Cecidomyiidae	585	3	0	1031
PDT-002	Nephrotoma ferruginea	651	18	0	1094
PDT-003	Goeldichironomus devineyae		9	0	1113
PDT-004	Aedes vexans	651	4	0	1148
PDT-005	Poor results				
PDT-006	Aedes sollicitans	629	1	0	1130
PDT-007	Cecidomyiidae	583	3	0	1038
PDT-008	Culex restuans	629	3	0	1121
PDT-009	Cecidomyiidae	633	0	0	1142
PDT-010	Culex salinarius	653	30	0	1059
PDT-011	Culex territans	640	3	0	1141
PDT-012	Dicranomyia	629	42	0	946
PDT-013	Dicranomyia	593	50	0	1047
PDT-014	Rhipidia	659	68	0	883
PDT-015	Culex restuans	629	1	0	1130
PDT-016	Cecidomyiidae	583	0	0	1052
PDT-017	Culex salinarius	655	30	0	1063
PDT-018	Rhipidia	649	66	0	874
PDT-019	Culex salinarius	649	28	0	1061
PDT-020	Dicranomyia	594	4	0	1054

Figure 4

Discussion

There were multiple different species barcoded in this project that bring about further questions. The samples with only a few mismatches may have been a result of a few of the bases not confidently barcoded. It would be interesting to continue research and lineage of samples that were nowhere near similar to any of the other species listed. Our success rate from sequencing was surprisingly high, with 19 out of 20 of our results being good. By simply incorporating more samples and having high school students work on this project this could be used to measure biodiversity across Long Island and monitor important vectors. We hope to move forward with this project and do a similar one where we use ticks to see how many of them carry Lyme Disease and how it correlates with what mammals they feed on.





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4. Fast Track to Gene Annotation and Genome Analysis - DNA Subway. Fast Track to Gene Annotation and Genome Analysis - DNA Subway. [accessed 2017 Mar 29].

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