Utilizing DNA Barcoding to Analyze Hymenoptera Biodiversity at Farmingdale State College

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ABSTRACT

Insect populations are currently decreasing globally and locally, threatening the sustainability of the ecosystem and the stability of food webs that are critical for human survival. The order of organisms, Hymenoptera, is composed of insects that play an important role in their ecological niche and contribute to agricultural growth. Biodiversity was measured by looking at the three types of biodiversity: genetic, species, and ecosystem biodiversity. Ecological biodiversity was studied through the organisms by analyzing the field vs forest traps. The specimens were collected in different locations and different habitats. The organisms were collected by Dr. Carly Tribull with malaise traps at the Farmingdale State College Teaching Garden. The specimens were then dissected and their heads and legs were preserved in ethanol until their DNA was amplified using the CHELIX isolation protocol. Gel electrophoresis then amplified the DNA, separating DNA by size, using positive and negative charges. The CO1 gene is one of the most popular markers used in barcoding. This experiment used the CO1 gene to barcode the collected species. Finally, using DNA Subway and the BOLD system, the DNA was trimmed, and compared with previously barcoded species to reveal the genetic makeup and identification of the insect. Further research identified novel species that have not yet been barcoded with the GenBank database. Samples CYC-011, CYC-016, and CYC-017 were identified as novel species as seen in figures 8,9, & 10 and CYC-018 was infected with a parasitic bacteria. A phylogenetic tree was then constructed to analyze the relationship between organisms.

RESEARCH QUESTION & HYPOTHESIS

RESEARCH QUESTION: Is biodiversity evident among the insect order Hymenoptera at Farmingdale State College?

HYPOTHESIS:

Ho: Biodiversity is not evident among the insect order Hymenoptera at Farmingdale State College.

Ha: Biodiversity is evident among the insect order Hymenoptera at Farmingdale State College.

INTRODUCTION

- Hymenopterans belong to the Phylum Arthropoda, Subphylum Labiata, Superclass Hexapoda, Class Insecta, and Order Hymenoptera. It is a large order of insects with over 150,000 described species. (Encyclopedia of Arkansas, 2022)
- This order of insects is considered to be the most beneficial to mankind of all the insects. (Smithsonian, 1999)
- The strongest benefit performed by most hymenoptera is the active pollination of plants, ensuring the proper development of many fruit and vegetable crops. Many kinds of Hymenoptera are also helpful in their actions of parasitism and predation on pest species of insects. (Smithsonian, 1999)
- Malaise traps are tent-like traps made of fine mesh material and used primarily for the collection of flies (Diptera) and wasps (Hymenoptera), although they also catch a great many other flying insects. (Mississippi State University, 2022)
- Emerging evidence suggests that insect populations may be declining at local and global scales, threatening the sustainability of the ecosystem services that insects provide. (Duffus, Echeverri et al., 2023)
- The CO1 gene is present in most eukaryotes. This gene is highly conserved across species where energy is generated from mitochondria. Because the gene is highly conserved, it can be copied from unknown organisms. (Science Learning Hub, 2009)
- The COI marker helped recognize misclassified species difficult to morphologically identify at the species level. (Gerard, Langerak et al., 2022)
- For DNA barcoding of animals, the CO1 gene can be used to identify individuals belonging to the same species, as well as to distinguish between individuals from different species. (Science Learning Hub, 2009)

Figure 1: Displays standard size dimensions for building a malaise trap.

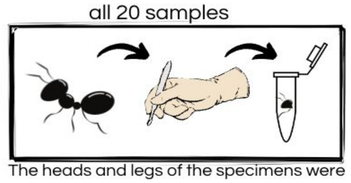
Figure 2: Displays various species of insects found in the order, Hymenoptera.

MATERIALS & METHODOLOGY

1. Collect, Document, and Identify Specimens

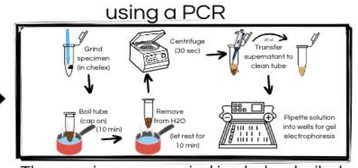
Specimens were collected with malaise traps at the Farmingdale State College Teaching Garden by Carly Tribull and stored in ethanol.

2. Isolate DNA from all 20 samples



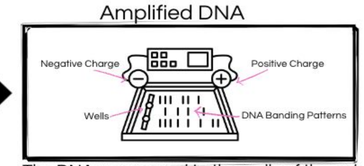
The heads and legs of the specimens were removed and stored in microcentrifuge tubes until their DNA was amplified.

3. Amplify DNA using a PCR



The specimen was grind in chelex, boiled, separated in a centrifuge, transferred into a clean tube using a micropipette, and moved to wells for gel electrophoresis.

4. Gel Electrophoresis of Amplified DNA



The DNA was moved to the wells of the gel and separated based on size by the positive and negative charges.

RESULTS

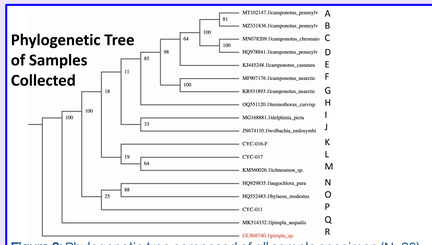


Figure 3: Phylogenetic tree composed of all sample specimen (N=20). Branches connect species with similar genetic makeup identifying closely related species. 0q551120 was used as a control in sample collection.

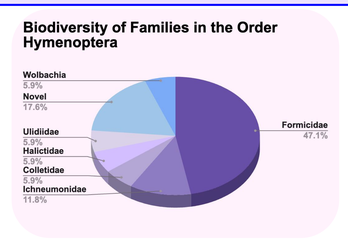


Figure 4: Shows a pie chart created to represent the biodiversity of species by family in the order Hymenoptera present at Farmingdale State College.

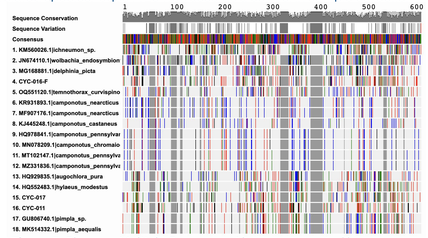


Figure 5: Shows a Multiple Sequence Alignment created by MUSCLE through DNA Subway.

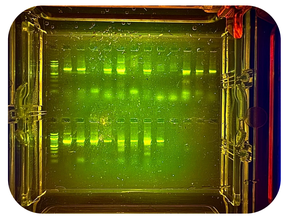


Figure 6: Displays the final electrophoresis revealing banding patterns from sampled species (N=20).

DISCUSSION

- 45% of samples were collected in the field, while 55% of samples were collected in the forest at Farmingdale State College.
- Samples CYC-011, CYC-016, and CYC-017 nucleotides were ran through the programs BLAST and BOLD and identified as novel.
- 3 of the samples were classified as novel.
- The largest percentage of Hymenoptera was from the family Formicidae and represented 47.1% of the samples.
- Sample CYC-018 DNA resulted in the identification of a Wolbachia Bacteria indicating that the insect has been infected with a parasitic bacteria.
- Simpson's diversity index was calculated to determine .79 biodiversity. This indicates a high level of biodiversity in the area because .79 is close to 1.
- The phylogenetic tree indicates a 100 score between species C & D and F & G as shown in Figure 3.
- According to the MUSCLE, species 9,10, 11 & 12 are most likely related genetically.

DATA

Sample ID	GPS Coordinate	Forest/ Field	N-Novel
CYC - 001	40.754930 -73.432509	Field Trap	
CYC - 002	40.754930 -73.432509	Field Trap	
CYC - 003	40.754930 -73.432509	Field Trap	
CYC - 004	40.754930 -73.432509	Forest Trap	
CYC - 005	40.756139 -73.433833	Forest Trap	
CYC - 006	40.756139 -73.433833	Forest Trap	
CYC - 007	40.756139 -73.433833	Forest Trap	
CYC - 008	40.756139 -73.433833	Froest Trap	
CYC - 009	40.756361 -73.433583	Field Trap	
CYC - 010	40.756361 -73.433583	Field Trap	
CYC - 011	40.755583 -73.433778	Forest Trap	N
CYC - 012	40.755583 -73.433778	Forest Trap	
CYC - 013	40.755583 - 73.433778	Forest Trap	
CYC - 014	40.755583 -73.433778	Forest Trap	
CYC - 015	40.755583 -73.433778	Forest Trap	
CYC - 016	40.755583 -73.433778	Forest Trap	N
CYC - 017	40.754930 -73.432509	Field Trap	N
CYC - 018	40.754930 -73.432509	Field Trap	
CYC - 019	40.754930 -73.432509	Field Trap	
CYC - 020	40.754930 -73.432509	Field Trap	

Figure 7: Shows GPS Coordinates of where samples were collected and if samples are novel (not previously barcoded) species.

Figure 8: Image of body and head of specimen CYC - 011.

Figure 9: Image of body and head of specimen CYC - 016.

Figure 10: Image of body and head of specimen CYC - 017.

Figure 11: Image of body and head of specimen CYC - 018.

Figure 12: Diagram of Wolbachia infection of specimen CYC - 018

SIMPSON'S DIVERSITY INDEX

$D = 1 - \left(\frac{\sum n(n-1)}{N(N-1)}\right)$

Formula

0.79

Biodiversity

n= the total number of organisms of a particular species

N= the total number of organisms of all species

The value of D ranges between 0 & 1. With this index, 1 represents infinite diversity & 0, no diversity.

ACKNOWLEDGEMENTS

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- All photos taken by researchers in a BSL-1 Lab unless otherwise cited.
- All graphs & infographics created by researchers

REFERENCES