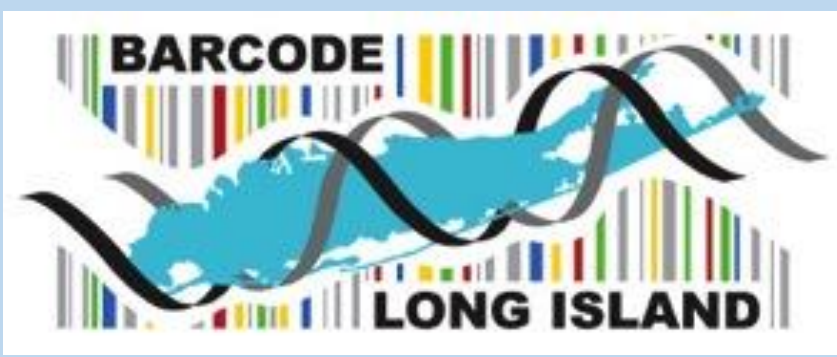


# Using DNA Barcoding to Determine Biodiversity of Insects that Inhabit Posts of Different Materials in Gardiner Park

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## Abstract

Researchers were interested in seeing what kinds of insects were inhabiting a local park on the south shore of Long Island. They were curious to research the niche of the insects collected and identify the effect of the insects on the surrounding environment. The researchers searched for insects around posts made of different materials: wood, plastic, cement, and metal. It was hypothesized that insects that are similar will be attracted to posts of similar material. It was also believed certain organisms may prefer to nest in a specific type of post. To determine the types of insects the process of DNA barcoding was used. The researchers performed DNA extraction, Polymerase Chain Reaction to isolate the CO1 gene, and gel electrophoresis to verify the presence of the gene. The DNA was sent for sequencing and results were given back revealing 5 of organism's species. Not enough data was collected from the 5 organisms to either support or refute the hypothesis; however, two of the beetles sequenced were collected from cement/stone posts which suggests that more research should be done in order to connect beetles to cement/stone posts.

## Introduction

- Insects that come attached to wood packaging materials have caused an estimated 830 million dollars decrease in property value (Aukema, Kovacs, Leung, Chivers, Britton, Englin, & McCullough, 2011). These insects are invasive, and can be harmful to residential property. Insects found with diseases may contaminate a water or food source for animals and humans and must be identified in order to address this issue.
- Researchers were interested in diverse insects that are found in Gardiner Park on the south shore of Long Island. They were curious of the effect on the surrounding environment. If invasive species were found, then it could lead to the identification of a problem which would be the first step to fixing it. Therefore, it would be beneficial to discover if other insects in the park are non-native insects.
- The purpose of this investigation was to identify the biodiversity of organisms living on posts made of different materials.
- It was hypothesized that similar insects would be found on similar posts because an article found by Malhotra (2016) about where insect nests are found and what they look like, led us to believe that organisms may nest in wood or cement or other types of posts.

## Methods and Materials

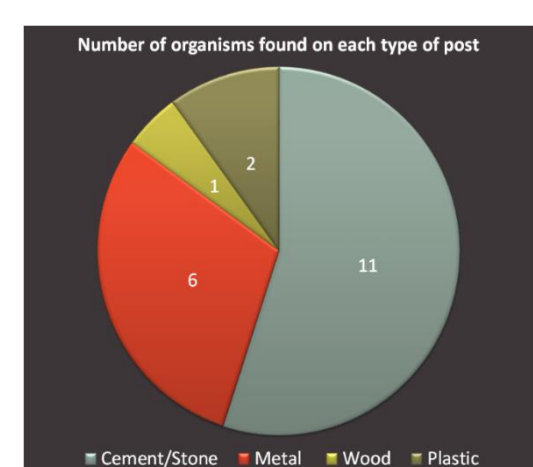
- A permit was obtained to collect samples in Gardiner Park. 4 types of posts were chosen to collect insects from: wood, metal, cement, and plastic. Samples were gathered from each post (1 organism from the wood posts, 6 from the metal, 2 from the plastic, and 11 from the cement).
- Each test tube was labeled with a number representing the location and which sample it was. DNA extraction of each sample was performed by adding a small piece of the sample tissue into a test tube. Each sample went through the DNA extraction process.
- The CO1 gene was copied by PCR. The CO1 gene was specifically searched for due to its relevance in the organisms collected. For example, an experiment conducted with mosquitoes stated, "Sequence variation was analysed in a 617-bp fragment from the 5' end of the CO1 region" (Cywinska, Hunter, & Hebert, 2006, p.413).
- Results were verified by electrophoresis, and positive samples were sent for sequencing. In DNA Subway, samples' DNA was shown, trimmed down, paired up, trimmed again, and then went through BLASTN to see what species the sample was. An alignment and a phylogenetic tree were created.



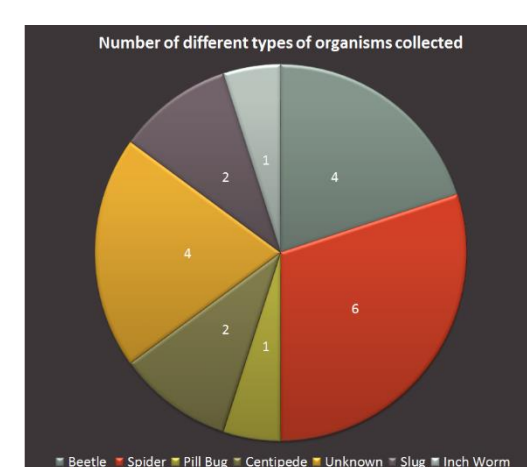
**Figure 1:** Aerial view of Gardiner Park. The red line indicates the main path and the red boxed number indicate areas where samples were collected.

## Results

Researchers collected 20 organisms from 4 different types of posts. The results of DNA electrophoresis showed that 5 of the organisms contained the CO1 gene. Three of the organisms collected are believed to be of the same species and were collected at stone/cement posts.



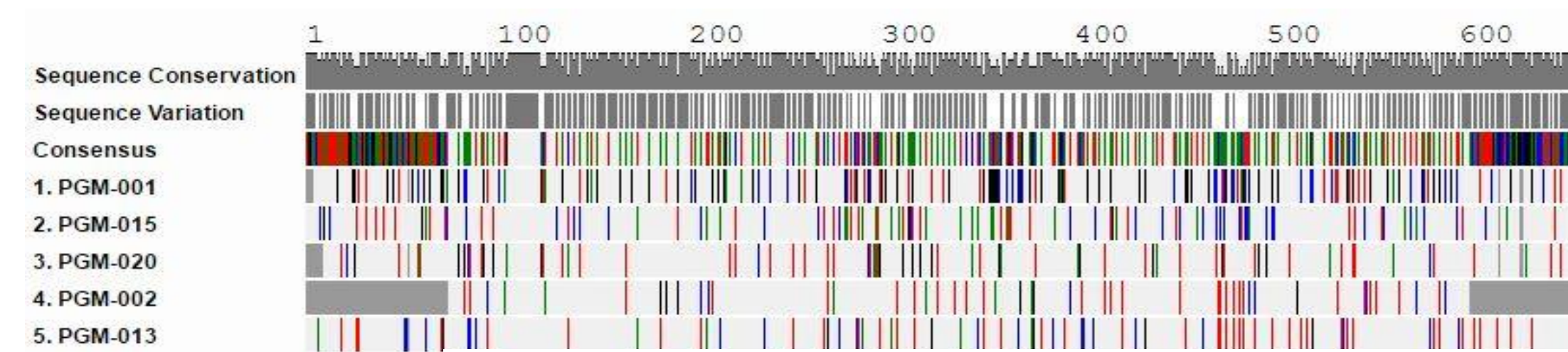
**Figure 2:** A majority of organisms were collected from cement/stone posts.



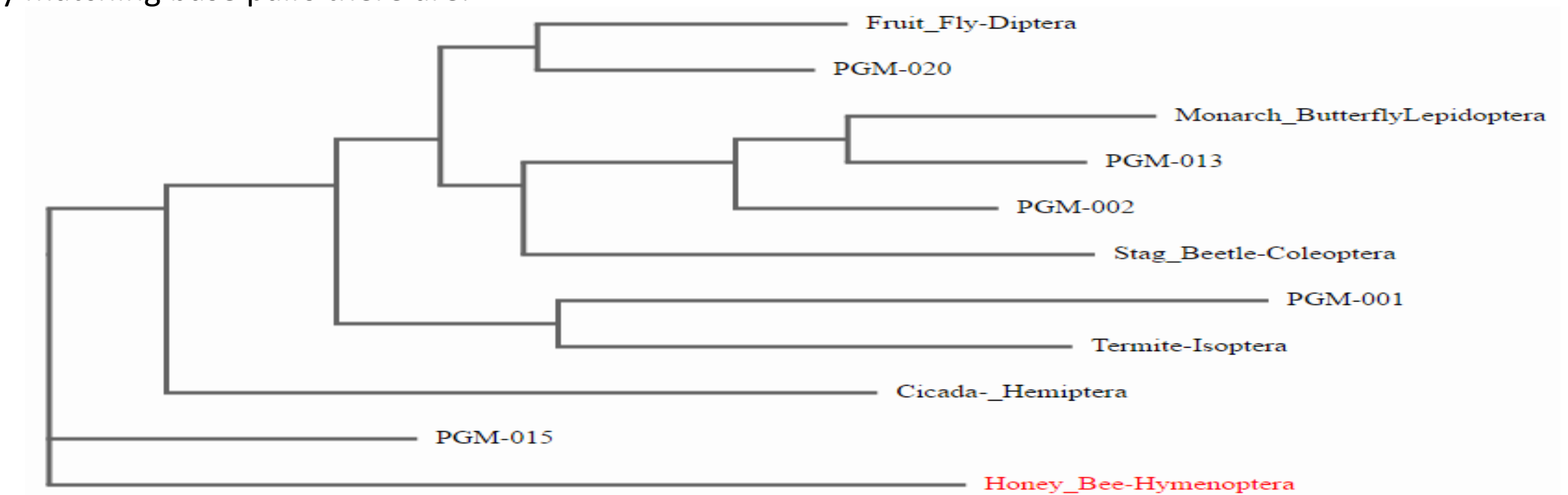
**Figure 3:** The most common collected organism was a spider.

Table: Metadata and DNA Subway data for each sequenced sample

Sample ID	Name	Direction post is facing	Post Type	Scientific Name	Bit Score	E Value	Mis-matches
PGM-001	Beetle	S	Plastic Fence	Forficula aff. auricularia	1186	0	0
PGM-002	Moth	N	Wood	Anania tertialis	957	0	0
PGM-013	Moth	SW	Cement Stone	Agriphila ruricolellus	1171	0	0
PGM-015	Beetle	SW	Cement Stone	Eremocoris ferus or Eremocoris borealis	1166	0	0
PGM-020	Beetle	NE	Cement Stone	Harpalus pensylvanicus or Carabidae sp.	1173	0	0



**Figure 4:** DNA alignment showing the similarity of the organisms to one another. The similarities are determined by how many matching base pairs there are.



**Figure 5:** Phylogenetic tree showing how closely each sample is related to one another. It shows that there is diversity among insects in Gardiner Park.

## Discussion

- The data table shows that the organisms barcoded had high bit scores. This means that there was a strong alignment between the sample and its closest match in GenBank.
- The data table also shows that all organisms barcoded have an e-value score of 0. This means that the results received were accurate and not by chance.
- Since there were no mismatches amongst the sequenced organisms, it means that the samples are already known based on their closest matches in GenBank.
- Researchers are still unaware if post composition completely impacts where organisms live.
- The samples collected are not believed to be harmful to humans and their health. As seen in the phylogenetic tree pictured above, the organisms sequenced show that there is biodiversity amongst organisms living in Gardiner Park.
- The samples as of now do not refute or support the hypothesis, as it has not been possible to find out if the organisms are new or invasive.
- In future research it will be possible to determine how the species react with the surrounding area. It was not possible to control the weather on the day that the organisms were collected, so it may have been possible to collect different organisms that may not have been present due to the weather conditions unsuited for them.
- For future experiments researchers have to completely crush the samples with a pestle, and concentrate more on the technique of DNA extraction. Also, in the future it would be beneficial to narrow the focus of the organisms collected.

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