

#### **ABSTRACT:**

Biodiversity is important not only on a global scale, but also in smaller ecosystems. Species that coexist in an ecosystem can live in a mutualistic relationship in which they help each other thrive. For example, the cycle between plants, soil, and nutrients allows for plants to flourish (Shah, 2014). Prior research indicates that a greater number of plant species leads to greater stability of an ecosystem's plant production (Tilman, D., Reich, P., & Knops, J., 2006).

Two different species of earwigs were found on the inland and south shore of Long Island. This is a clear example of biodiversity amongst the earwig population of Long Island, because of the genetic variation that exists between these two species. These two collected species seemed to have diverged onto different evolutionary paths many millions of years ago, and have gone through independent evolution ever since this divergence. The supposed Maritime earwigs are a novel species, since they were not found in the Bold or NCBI genetic databases.

# RESEARCH QUESTIONS & HYPOTHESIS:

#### **Research Questions:**

To what extent does biodiversity of earwigs on Long Island exist?

Has speciation occurred among the known species?

Are there any novel species of earwigs that have yet to be discovered?

Hypothesis: There is a significant difference in the biodiversity of earwigs on Long Island due to the variation of species that are discovered.

#### REFERENCES

- 1) Shah, Anup. "Why Is Biodiversity Important? Who Cares?." Global Issues. 19 Jan. 2014. Web. 14 Mar. 2016. <a href="http://www.globalissues.org/article/170/why-is-biodiversity-important-who-cares">http://www.globalissues.org/article/170/why-is-biodiversity-important-who-cares</a>.
- 2) What Is DNA Barcoding? (n.d.). Retrieved April 17, 2015, from <a href="http://www.barcodeoflife.org/content/about/what-dna-barcoding">http://www.barcodeoflife.org/content/about/what-dna-barcoding</a>
- 3) Jacobs, S. (2013). European Earwigs (Department of Entomology). Retrieved October 15, 2015.
- 4) European Earwig Insect Information. (n.
- d.). Retrieved October 15, 2015.5) Pinol, J. (2009). Effects of the concurrent exclusion of ants and earwigs on aphid

abundance in an organic citrus grove.

- Biocontrol, 54(4), 515-527.

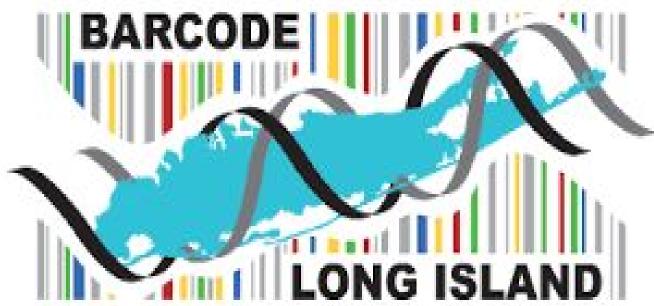
  6) Tilman, D., Reich, P., & Knops, J. (2006). Biodiversity and ecosystem stability in a decade-long grassland experiment. Nature,
- 629-632.

  7) Hill GT, Mitkowski NA, Aldrich-Wolfe L, Emele LR, Jurkonie DD (2000) Methods for assessing the composition and diversity of soil microbial communities. Appl Soil Ecol 15: 25–
- 8) Pampulha ME, Ferreira Mass, Oliveira A (2007) Effects of a phosphinothricin based herbicide on selected groups of soil microorganisms. J Basic Microbiol 47: 325–331.
- 9) Carroll, D., & Hoyt, S. (1984).
  Augmentation of European Earwigs
  (Dermaptera: Forficulidae) for Biological
  Control of Apple Aphid (Homoptera:
  Aphididae) in an Apple Orchard (pp. 738-740).

# Using DNA Barcoding to Analyze the Biodiversity of Dermaptera on Long Island

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Earwigs found on either the south shore of Long Island or more inland are important to the ecosystem, because they consume numerous plant species, garbage, dead insects, fruit, fungi, and decaying matter. Generally, they do not present a serious danger to crops. They are ingested by other insects and animals, which plays a large role in the ecosystem. For example, a large number of plant species means more variety of crops which ensures stability of all life forms. Earwigs are part of the food chain and are detritivores, which are organisms that feed on dead matter. Earwigs are active at night and hide during the day in cracks and crevices. Earwigs are considered beneficial to gardens because they feed on aphids, however, if insect prey is scarce, they may feed on roots of planted flowers (Insectidentification.org, 2016). Studies have been conducted to indicate that most likely explanation for the increase in aphid abundance in the ant-excluded trees is the absence of earwigs (Pinol, J. 2009). Earwigs have been used to control the apple aphid population in apple trees, suggesting that they are beneficial (Carroll, D., & Hoyt, S., 1984).

There are conservation concerns in the Long Island Pine Barrens due to an imbalance in the natural process of the landscape. Experiments indicate that the soil in the Pine Barrens Forests presents a harsh environment for the microorganisms to grow and survive. Widespread use of herbicides present negative effects on microorganisms and overall soil quality (Pampulha, 2007). All microorganisms play an important role in the soil geology which influence quality of soil and the health of the ecosystem (Hill, 2000). Earwigs play a significance in the ecosystem because of their diet. By ingesting decaying matter, garbage and fungi, they return nutrients back into the ecosystem when they are later eaten by an animal or other insects. Without decomposers, carcasses would pile up and soil would lack the plants needed to grow. Without plants, omnivores would have no food source, there would be additional drastic changes to the ecosystem that could seriously damage it (Batema, 2001). The Pine Barrens is very diverse in animals, plants and habitats (The Nature Conservancy, 2016).

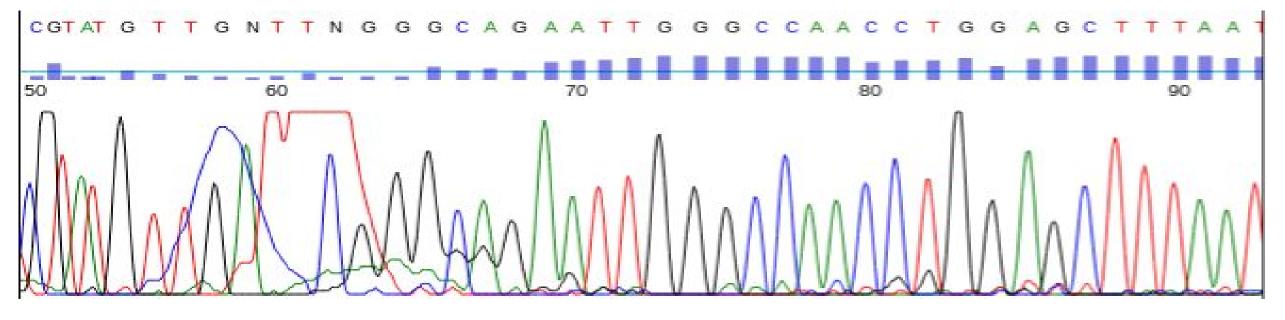
DNA barcoding was used to determine if there is a significant difference in the biodiversity of earwigs between the South Shore of Long Island and inland regions. It uses short genetic sequences that come from the genome, by analyzing tiny amounts of tissue samples. DNA barcoding makes species identification more accurate (CSHL, 2010). This technique determined if there were genetic variations among earwigs and also helped to determine if there is diversity among the genepool of earwigs of different species that were found.

The specific gene that was analyzed was the CO1 gene, which is a part of the mitochondrial DNA, it has enough variation between organisms of the same species. If there is enough variation in this gene between two organisms of the same species, then there will be a novel species shown through genetic analysis.

#### **MATERIALS & METHODOLOGY**

INTRODUCTION

DNA was extracted from tissue sample using lysis solution and grinding techniques. The primer CO1 was the primer used during PCR analyses and silica resin was the reagent used to bind DNA. After, gel electrophoresis determined the quality of the samples before being sent to genewiz. Then the sequence of nucleotides were trimmed and analyzed, utilizing the DNALC's bioinformatics tool, DNA Subway. After the samples were trimmed, they were compared to determine if there was a significant difference in the nitrogenous base sequence. The new sequence is then represented in letters of CATG showing nucleic acids.

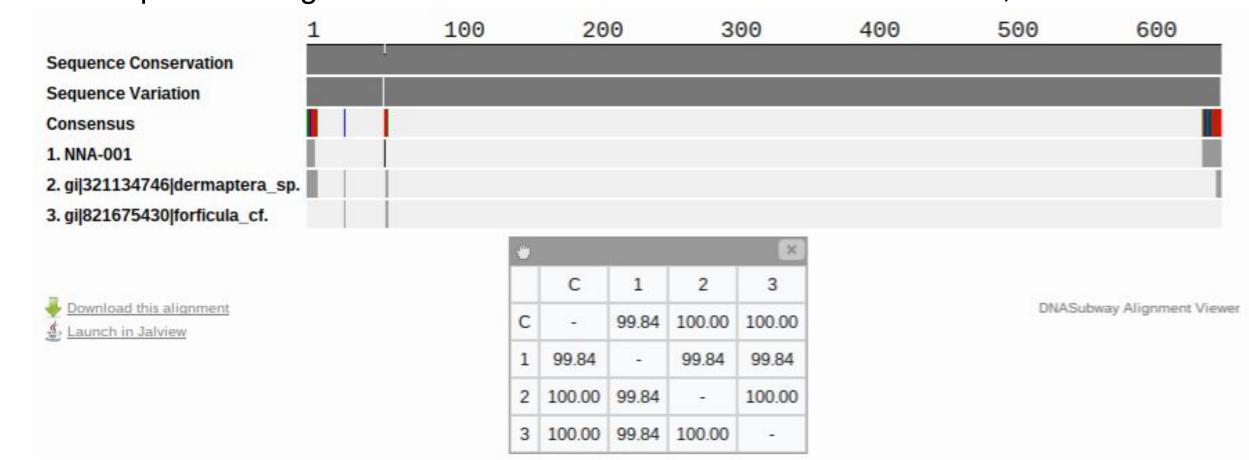


**Figure 1**: The phred score determines how sure the computer is of the nucleotide in the sequence of DNA. If it is above a phred score of 20, which is indicated by the blue line, then it can be accepted that the given nitrogenous base is correct.

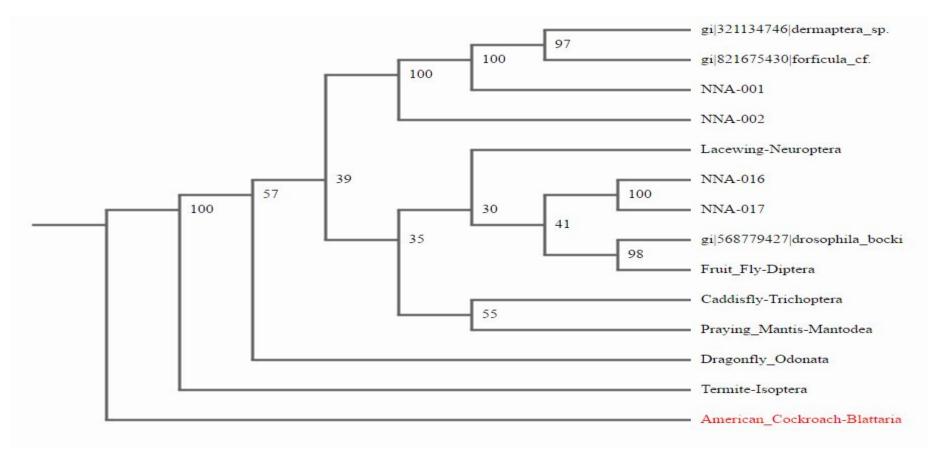
### **RESULTS**

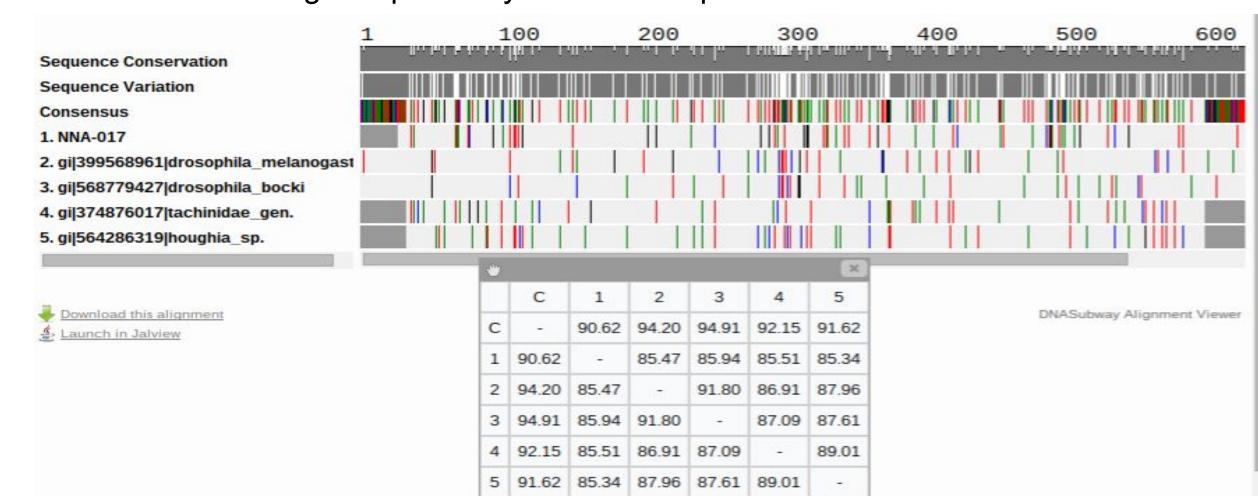
Through the processes of PCR (polymerase chain reaction), and gel electrophoresis, DNA was obtained from 14 of the 20 samples included in the genetical analysis. Of these 14 earwig samples, 7 were European Earwigs (*Forficula auricularia*), and 7 of what were thought to be Maritime Earwigs (*Anisolabis maritima*). The European Earwigs all exhibited signs of being very common to organisms already discovered and documented in various databases, such as BOLD, and NCBI Genbank. All 7 European Earwigs previously referenced had either 0 or 1 mismatch between the CO1 gene of European Earwigs already in the databases, and many were either 100% or 99% similar in their genetic sequence of this gene. Furthermore, the e scores were all 0 and the bit scores were all above 1100, indicating that the DNA sequence in the database is very accurate.

Additionally, the 7 that were believed to be Maritime Earwigs were demonstrating signs of not being previously documented in any databases. All of these samples showed upwards of 70 mismatches on the CO1 gene between organisms of the closest match shown in the databases. Some of the organisms in the database that came up as the closest match to the Maritime Earwigs were *Tachinidae gen.*, *Houghia sp.*, *and Chetogena sp.* The samples used in this experiment were shown as either 86% or 87% similar to their closest match in the database. Furthermore, although all of the e scores were 0, the bit scores were not as high as that of the European Earwigs. The bit scores were only approximately 700, as opposed to the European Earwigs which had bit scores of about 1100. Therefore, this information suggests that the Maritime Earwig samples may be a novel species.



**Figure 2:** This is a barcode to a sample of a European earwig. As portrayed in the image, the sample (NNA-001) is very similar in its DNA sequence to the organisms already in the database. This is based on the fact that the barcodes between the sample and the other species are lined up. Furthermore, as shown in the table, the European Earwigs that were collected were either 99% or 100% similar in their genetic sequence to organisms that were already found in BOLD and Genbank databases.





**Figure 3:** This is a barcode to a sample of what was believed to be a Maritime earwig. As portrayed in the image, the sample (NNA-017) is not as similar in its DNA sequence to the organisms already in the database, since Maritime earwigs were not in the database. These species were the closest match entered in the database. The maritime earwigs were approximately 80-90% similar to the samples already in the database.

**Figure 4:** The cladogram that is shown portrays the ancestral lineage of the samples of earwigs compared to that of organisms found in the various databases. NNA-016 and NNA-017, the supposed Maritime samples, are shown as being closer in ancestry to Drosophila and Diptera. This is due to the fact that sample NNA-016 and NNA-017 have more similar genetic sequences to the previously referenced organisms than to the European Earwigs found in the databases. These analyses are based on the CO1 gene. Similarly, sample NNA-001 and NNA-002 which are *Forficula auricularia* samples, are related to Dermaptera.

# DISCUSSION & CONCLUSION

Approximately, 27 earwigs in total were found, 10 of which were from the south shore of Long Island, and 17 of which were inland. The species that were found were *Forficula auricularia* (European Earwigs), and what we believed to be *Anisolabis maritima* (Maritime Earwigs). Since only these supposed maritime earwigs were found southshore and only European earwigs were found inland, this furthers our hypothesis because it suggests that their evolutionary history diverged due to geographic differences. This is because phenotypically, the samples of the same species may look the same, but Barcode Long Island was used as a tool to determine if there are genetic differences in their nucleotide sequences. Furthermore, both maritime and european earwigs are indigenous to New York, which indicates that they play a major role in the ecosystems in which they exist.

Since there was a high number of mismatches in the DNA sequences of the CO1 gene in the supposed maritime earwigs, this suggests that they are a novel species because it means that no other documented organisms have shown the same degree of variation as this population of earwigs. Therefore, this implies that speciation did occur, because of the potential for a novel species. However, it is assumed that this speciation occurred because of environmental factors. The apparent Maritime Earwigs, based on DNA analysis, were actually not earwigs at all, and are more genetically similar to a species of fly. The DNA sequence of the CO1 gene shows more similarities between the apparent Maritime Earwigs and the Tachinidae fly species than between the Maritime Earwigs and the European Earwigs. This shows that the Maritime samples are more closely related to the fly species indicated on the above cladogram, such as Diptera and Tachinidae. They are in fact not earwigs at all, and are not as closely related to the European Earwigs or Dermaptera. Further research needs to be conducted and more data needs to be collected to determine what caused this speciation. Nonetheless, it is definite that speciation did occur, which means that in the future more signs of biodiversity among earwig populations will begin to show themselves, and ecosystems with these populations will thrive. This emphasizes the importance of DNA Barcoding, because looking at the samples taxonomically, in this case, the apparent Maritime samples compared to the Europeans, was simply not enough to determine that the Maritime earwigs were not earwigs at all. Analyzing the DNA will definitively determine what species is actually present.

# **FUTURE IMPLICATIONS**

research.

To get a deeper analysis of the general population of earwigs on Long Island, there needs to be future research and a larger sample size to examine. More samples need to be collected in order to determine population density of earwigs on Long Island holistically. Furthermore, this will give a better understanding of the biodiversity of these earwigs on the Island. Moreover, the discovery of any significant difference in genetic biodiversity will promote further

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