

# The Viability of *Prenolepis imparis* within the Fish Thicket Nature Preserve as an Indicator Species via the Analysis of Single Nucleotide Polymorphisms



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## ABSTRACT

The relatively isolated ecosystem of the Fish Thicket Nature Preserve serves as a noteworthy area to conduct environmental studies, providing both locations accessible to heavy automobile activity, as well as locations secluded from human contamination. The ant species *Prenolepis imparis* was employed as a bioindicator of its environment. In this study, differences between samples of this species were observed via the presence of single nucleotide polymorphisms (SNPs), variations in a single base pair of a DNA sequence. With the use of a Berlese Funnel apparatus and Keebler Pecan Sandies cookies, ant specimens were collected accommodating the several environmentally distinct areas. Through DNA extraction and sequencing, it was possible to identify the presence of such SNPs and correlate these genetic differences with the collection locations. The clear similarities between samples found in neighboring locations, as well as the stark contrast in DNA sequences between polluted areas and remote areas, provides compelling evidence for connections made between genetic variation and environmental factors.

# INTRODUCTION

Ants offer a variety of opportunities to study environmental factors, as they are an excellent example of indicator species that define certain traits of the environment that they reside in (What Is). Contained within the Pine Barrens



is the Fish Thicket Nature Preserve. This area is largely isolated from human activities besides the perimeter, which borders a road heavy with automobile activity. Because of this, the Fish Thicket provides a unique environment in which two distinct environments may be analyzed: one that is contaminated with various pollutants and the other which is secluded from human contact. Single Nucleotide Polymorphisms (SNPs) can be easily identified as evidence of genetic diversity. While most SNPs occur regularly without effect, some of these differences may result in health complications (What Are). Previous studies have found a significant increase in mutations inherited through the paternal germline of mice exposed to industrial steel mill air pollution (Somers, 2002). The objective of this project is to identify any correlations between environmental factors and the genetic differences shown in the nucleotide alignment data. It will be interesting to observe how *Prenolepis imparis* will serve as a bioindicator for genetic variation. Due to the vast differences throughout the Fish Thicket ecosystem, it is expected that the *Prenolepis imparis* DNA sequences will contain genetic variation through the presence of SNPs.

## METHODOLOGY

## Specimen Collection

Dozens of samples were collected across four environmentally unique locations. Keebler Pecan Sandies cookies were used as a source of bait. Ants found on the cookies were placed in small containers. The leaf litter surrounding the Keebler cookies were placed in a Berlese Funnel to collect samples.

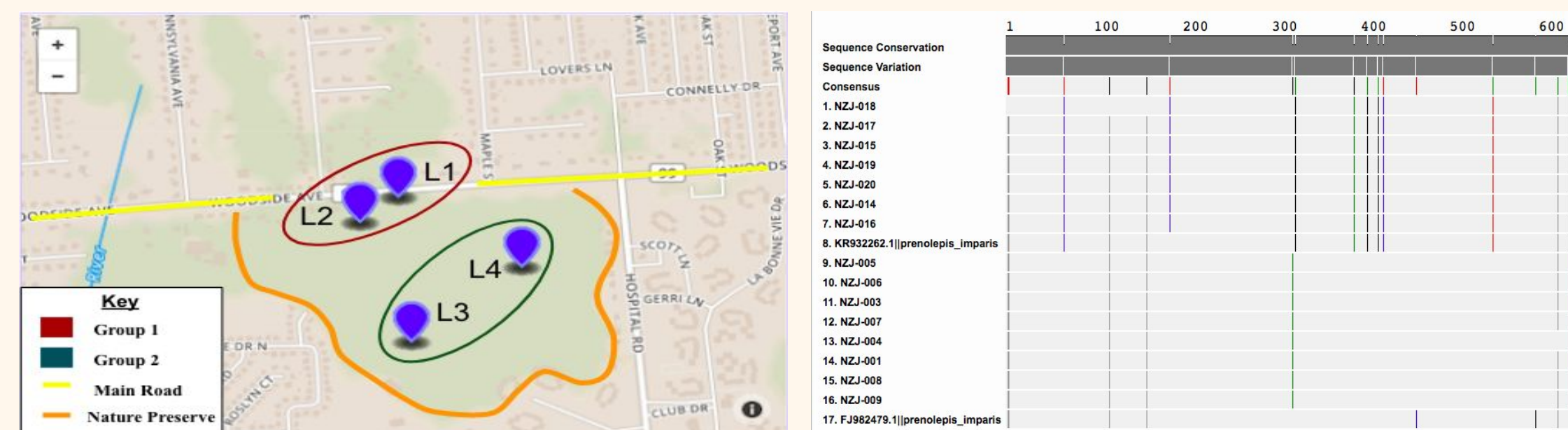
## DNA Extraction and Amplification

5 samples from each of the locations were selected. Using protocol, the DNA was extracted and amplified. The DNA of each sample was amplified through PCR with the COI primer, and the amplified DNA was subsequently analyzed through gel electrophoresis and sent to be sequenced.

## Genetic Analysis

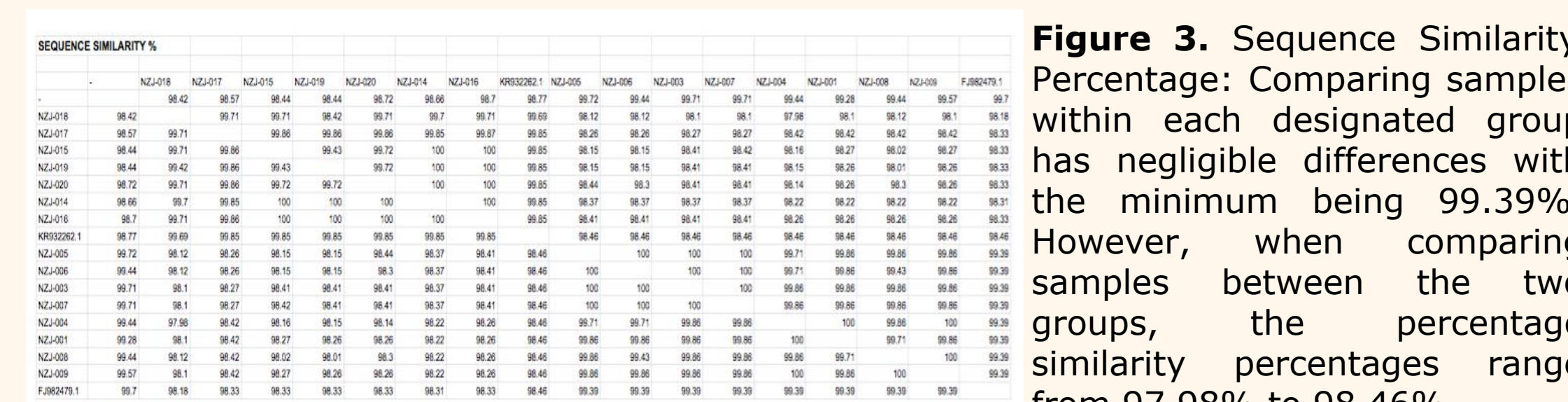
DNA Subway genome analysis online program that enabled the analysis of sequences. With BLAST, 15 samples were a high-confidence match with *Prenolepis imparis*. The MUSCLE Alignment Viewer and Sequence Similarity Percentage were utilized to identify specific base pair differences and compare how genetically similar samples of the same group were.

## RESULTS



**Figure 1.** Map of the Fish Thicket Nature Preserve: Ant species *Prenolepis imparis* samples were collected in four designated locations. L1 and L2 were within a road heavy with automobile activity. L3 and L4 consisted of healthy woodlands relatively isolated from human activity. Two distinct groups emerged and genetic variation appears to have a correlation with distance from areas of human activity.

**Figure 2.** Data received from MUSCLE: MUSCLE was utilized to pair similar DNA sequences together, resulting in two distinct groups of *Prenolepis imparis* correlating with the locations in which they were found NZJ-001 and NZJ-003 to NZJ-009 are genetically similar to the known sequence FJ982479.1. The other distinct group is between the known sequence KR932262.1 and samples NZJ-014 to NZJ-020 .



**Figure 3.** Sequence Similarity Percentage: Comparing samples within each designated group has negligible differences with the minimum being 99.39%. However, when comparing samples between the two groups, the percentage similarity percentages range from 97.98% to 98.46%.

## DISCUSSION

Our results displayed a significant basis for the distinguishing ant species *Prenolepis imparis*. Locations 1 and 2 came into contact with various pollutants, while the presence of 3 and 4 were indicative of a healthy ecosystem. Specimens were placed in groups based on percentage genetic similarity. The clear divergence between the two ant groups is indicative of the potential effects of harmful pollutants on the diversity of bioindicator species. It is likely that there are two distinct populations, or perhaps one is a subspecies. Since only a small region of DNA less than a 1000 base pairs was sequenced, it would not be possible to attribute the level of pollution found at these locations to the mutations displayed by the Single Nucleotide Polymorphisms. Another explanation could be the fact that the ant populations are physically isolated and hence genetically isolated. The specimen collection sites were several hundred yards apart, a distance that is unlikely to have been traveled by ants. While previous studies have researched the harmful effects of industrial air pollution on wildlife, studying the less complex arthropods in a protected, isolated ecosystem like the Fish Thicket may provide more substantial evidence. Future steps we can take from this project would be to expand our sample size by collecting variations of *Prenolepis imparis* across Long Island, as well as increasing the size of the region being sequenced. In this way, it would be possible to garner further evidence to corroborate the effect of harmful pollutants on the biodiversity within species, which may provide a basis on studies regarding human health.

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## REFERENCES

Andras, Tiffany. "How Does Pollution Affect Animal Genetics?" Sciencing, <http://sciencing.com/pollution-affect-animal-genetics-23866.html>. 24 April 2017.

Berlese Funnels - Collecting Methods - Mississippi Entomological Museum Home, [mississippientomologicalmuseum.org.msstate.edu/collecting.preparation.methods/Berlesefunnel.htm#.WsUn7tPwYdU](http://mississippientomologicalmuseum.org.msstate.edu/collecting.preparation.methods/Berlesefunnel.htm#.WsUn7tPwYdU).

*Long Island Pine Barrens*, [nctc.fws.gov/pubs5/web\\_link/text/li\\_pine.htm](http://nctc.fws.gov/pubs5/web_link/text/li_pine.htm).

"Rookies Use Cookies in Science Alliance to Track Ants' Advance." *Entomology Today*, 7 July 2014, [entomologytoday.org/2014/07/07/rookies-use-cookies-in-science-alliance/](http://entomologytoday.org/2014/07/07/rookies-use-cookies-in-science-alliance/).

Somers, Christopher M. et al. "Air Pollution Induces Heritable DNA Mutations." *Proceedings of the National Academy of Sciences of the United States of America* 99.25 (2002): 15904–15907. *PMC*. Web. 14 Apr. 2018.

"What Are Single Nucleotide Polymorphisms (SNPs)? - Genetics Home Reference." *U.S. National Library of Medicine*, National Institutes of Health, [ghr.nlm.nih.gov/primer/genomicrosearch/snp](http://ghr.nlm.nih.gov/primer/genomicrosearch/snp).

"What Is an Indicator Species?" *Encyclopedia of Life*, [eol.org/info/465](http://eol.org/info/465).

Winter Ant - *Prenolepis Imparis*, [entnemdept.ufl.edu/creatures/misc/ants/Prenolepis\\_imparis.htm](http://entnemdept.ufl.edu/creatures/misc/ants/Prenolepis_imparis.htm).