The Unseen Invasives Below: Investigating the DNA Barcodes of Earthworms Collected In Ethnic Neighborhoods Across New York City

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ABSTRACT

During the Pleistocene ice age, glaciers covered almost all of North America, rendering the landscape too cold for the survival or inhabitancy of native, American earthworms. When foreign travellers began colonizing the land though, they introduced earthworms from their respective countries, and as a result, except for parts of the Southeast and Pacific Northwest, nearly all earthworms found in the soil today are foreign, invasive species. In collecting and barcoding earthworm samples from Sheepshead Bay, Spanish Harlem, the Lower East Side, and Jackson Heights, we hoped to discover their origins and biodiversity, and determine if such data reflects human biodiversity in ethnic neighborhoods. We believed that while European worm species would be most common due to the prevalence of European colonization in American history, it could be possible that immigrant groups unknowingly brought earthworms with them to their ethnic neighborhoods, and thus share similar origins. In order to test such a hypothesis, we explored the city with a pair of gloves and a container, and dug up soil to collect earthworms. Of the 18 samples we collected, only 10 were successfully extracted and PCR'd, and 9 successfully sequenced. Bioinformatics analysis indicates that there are two genera and three species of earthworm among the nine samples.

INTRODUCTION

Earthworms are found in nearly all terrestrial ecosystems throughout the world, with the exception of the most extreme regions. The exact number of species, however, remains unknown to the scientific community, with overall speculation ranging between 2,000 and 7,000 species. This dearth of data is attributed to a lack of funding, low international participation, and, historically speaking, difficulty in earthworm species identification. For the most part, taxonomic procedures, such as the dissection of male earthworm genitalia, were "labor intensive, time consuming, and very difficult for non-specialists" (Huang 2007). They posed an additional problem when earthworm morphology did not vary significantly among species.

However, with the development of DNA barcoding, earthworm identification can now be performed with general ease. Proposed by Paul Herbert in 2003, DNA barcoding uses the cytochrome-c oxidase I (COI) gene present in all animals and allows for fairly simple comparisons, as insertions and deletions within the 648 base-pair region are rare. Scientists today hope to use this new technology to identify and learn more about various species of organisms. The Terrestrial Biosurveillance Working Group, for example, has researched earthworm taxonomy and biodiversity for years, and has barcoded over 3,000 specimens and identified 234 unique species for the International Barcode of Life project. In a different research group, scientists at Elsevier used DNA barcoding to study differences among Chinese earthworms. They found that "sequence divergence within species was generally less than 1%, whereas divergence between species was greater than 15% in all cases" (Huang 2007). As demonstrated by these two research groups, DNA barcoding has many applications in earthworm identification and we believe it may be particularly useful in determining the biodiversity of these organisms in New York City.

Due to the long and rich history of the city, we believe that immigrant populations may have further altered the biodiversity of worms in the soil. We will investigate the modern biodiversity of earthworms in New York City and utilize DNA barcoding to discover if worm species in ethnic neighborhoods have similar origins to the immigrants who populated, or currently populate, those areas.

ACKNOWLEDGEMENTS

A special thank you to our kind mentor, Ms. Quenzer, for her dedication, wisdom, and encouragement throughout this project. She stayed afterschool with us for countless hours, allowing us to work in the lab, and more importantly, she patiently guided us and offered earnest feedback that made us better scientists. Even when we faced great setbacks, she never lost hope in us and continued to devote her time and energy toward our success. We are truly grateful for her help, knowing that without her, this research project would not have been possible.

We would also like to acknowledge Lawrence Kwong's tremendous contribution to this project. He worked tirelessly in the lab to acquire successful results, and played an integral role in conceiving, developing, and compiling this research project.

MATERIALS AND METHODS

Selected Ethnic Neighborhoods For Sample Collections

- Sheepshead Bay, Brooklyn: large Russian population
- Spanish Harlem, Manhattan: historic Latino population
- Lower East Side, Manhattan: diverse immigrant population
- Jackson Heights, Queens: large Latino & Southeast Asian population

Collection Details

At each site, we aimed to collect four or five samples to best capture the biodiversity in the area and narrowed our collection to longer, adult earthworms, identified by the presence of a clitellum band to ensure the survival of our specimens post-cut. We began our collections as soon as the winter frost began to recede, and attempted to schedule them after light rain showers to make collection easier; however, as a result of the date and time of most of our collections (rainy weekday afternoons in mid-March and late-April), many of our desired collection sites (community gardens) were closed. Therefore, we walked through the neighborhoods searching for suitable plots of soil to dig. As soon as we found one, we used our gloved hands, and an occasional branch, to dig up the soil. We discovered that aside from collecting samples after rain showers, a particularly effective technique was pulling out deeply rooted weeds and examining the locally disturbed soil.

During our first two collections, we cut the samples directly at the dig sites; however, for the last two collections, we decided to store the earthworms in a container and cut them in the lab instead. All of our collected, cut samples were placed in the freezer as soon as possible.

DNA Extraction Procedure Overview

1) Lyse, grind, and incubate samples at 65°C
2) Add silica resin to removed supernatant, mix, and incubate for 10 minutes at 57°C
3) Use ethanol-based wash buffer to remove potential impurities
4) Remove all wash buffer with micropipette, and leave tubes open for 15-20 minutes to verify no residual traces of alcohol remain among the silica resin bead
5) Add distilled water, incubate at 57°C, and remove supernatant for PCR

Polymerase Chain Reaction Set-Up and Protocol

1) Combine 2 µl of extracted DNA with 12.5 µl of NEB Taq 2X Master Mix and 10.5 µl of invertebrate COI primer mix or Ready-To-Go PCR bead and 23 µl invertebrate COI primer mix
2) Amplify DNA using invertebrate-specific PCR program* on thermal cycler

a) Initial step: 94°C	(60 seconds)	*We attempted amplification using both the general and the invertebrate-specific PCR program, finding the latter more effective. We also tried amplification on both the miniPCR and the school mastercycler, although these results did not indicate any clear sign of causation.
35 cycles of the following profile:		
b) Denaturing step: 95°C	(30 seconds)	
c) Annealing step: 50°C	(30 seconds)	
d) Extending step: 72°C	(45 seconds)	

Electrophoresis Set-up and Protocol

We cast 2% agarose gels and loaded a mix of 3 µl of PCR product and 2 µl of SYBR green dye, before running them at 91 to 94 Volts for increased resolution. After electrophoresis, we placed the gel on a UV transilluminator to check for successful PCR product.

Bioinformatics Analysis

1) Assemble sequences by trimming and pairing
2) Add sequences through BLASTN
3) Analyze sequences by selecting data, aligning through MUSCLE
4) Create phlyogenetic trees (PHYLIP NJ & ML)

RESULTS

After performing eight sets of DNA extractions, and running a total of seven gels, we obtained electrophoresis bands indicating successful DNA amplification for 10 of our 18 samples (#9-18, KFD-010 to -019). Figure 1 shows the two gels with their appropriate annotations; the samples in the middle wells produced the most distinct bands.

With this confirmation, we submitted our PCR product to the lab for sequencing. Of the ten samples, nine yielded satisfactory sequences (#10-18, KFD-011 to -019). Bioinformatics analysis from DNA Subway indicates that there are two genera and three species among the nine samples. Figure 2 is a "PHYLIP NJ" phylogenetic tree that demonstrates the evolutionary relationship between the samples, and Figure 3 is a photo of the sequence alignment viewer, showing the alignments between their sequences.

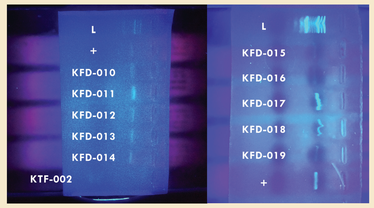

Figure 1: An electrophoresis gel under UV light with bands indicating successful PCR product for (left) the positive control and samples #9-13, and (right) the positive control and samples #14-18

Figure 2: "PHYLIP NJ" Phylogenetic Tree

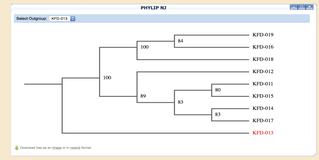

Figure 3: Sequence Alignment Viewer

DISCUSSION

The bioinformatics analysis indicated that our worms ranged from Aporrectodea caliginosa and Aporrectodea longa, to Aporrectodea tuberculata and Octolasium sp., all of which are fairly unknown genera and species of earthworm. The caliginosa species was most prevalent in the Lower East Side, while the longa species were most prevalent in Jackson Heights. More research is required in order to determine any relationship between earthworm and human biodiversity in our select ethnic neighborhoods.

In regards to our PCR protocol, we also observed that when closing the miniPCR lid and tightening the screw, the cap buckled slightly in the middle and produced a tighter fit there, than by the edges. Ultimately, these observations led us to believe that the middle gel bands were brightest because these samples received the most even distribution of heating from the snug fit in the miniPCR, therefore resulting in optimal PCR conditions that allowed for the greatest amplification of DNA. In contrast, the samples located in the outer wells of the heating block, in which the lid was not as tightly pressed, did not have optimal conditions for PCR, leading to lower amplification and fainter bands on the gel.

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