Uncovering the Genetic Makeup of Fungal Fruiting Bodies and Lichen Thalli Across Urban New York City

Ryan Ahmed[1], Sarah Yang[2], Sigrid Jakob[3] Craig Trester[3,4]
Bronx High School of Science[1], Brooklyn Friends School[2], New York Mycological Society[3], MYC.NYC[4]

URBAN BARCODE RESEARCH PROGRAM
Supported by the Pinkerton Foundation & Science Sandbox

NYMS New York Mycological Society

CSH Cold Spring Harbor Laboratory DNA Learning Center

The Pinkerton Foundation

SCIENCE SANDBOX

Abstract

In this study we investigated the genetic make up of fungal fruiting bodies and lichen thalli in metropolitan areas throughout two New York City boroughs: Brooklyn and Queens. This has been accomplished by DNA barcoding physical specimens of fungi and lichen collected, accompanied by the genetic sequences generated. There were 6 species collected in Prospect Park of Brooklyn Borough and 7 different species collected from the West Queens area and Forest Park. Though there was not a large abundance of species samples in the collection sites, there was successful extraction and identification of the physical specimen through studies of DNA sequences, generating primary data on urban genetic diversity through the various fungal species and their intergeneric relationships to one another. Phylogenetic trees were created in order to show the close relationship between collected samples and levels of sequence similarities. The goal was to identify mythological species that can be found in areas that are induced to higher levels of anthropogenic influence through DNA extraction and barcoding, and thus this study uncovers the genetic makeup of fungal fruiting bodies and lichen thalli in New York City. This research supports future conservation and documentation efforts to better protect fungi and ecosystems.

Introduction

Fungi are an important part of any ecosystem, acting as either decomposers of organic matter or mycorrhizal partners of trees. Within a metropolitan area there is an observable effect upon ecosystems that are more or less impacted due to human activity demonstrated by abundance or lack of fungi populations growing in the area (Newbound 2010) (Abrego, N 2020). Conservation initiatives rely upon data about the population-based distribution of the species in question. Fungal population data in natural habitats, such as old-growth forests are oftentimes heavily surveyed and documented. Conversely, there is a lack of study or reference data in metropolitan areas due to the perceived absence of fungi in urban greenspaces. Our research aims to generate novel primary data to bring attention to the importance of fungi in metropolitan ecosystems and further explore the impact that different locations have on their genetic diversity. Prospect Park, in metropolitan New York City, is a well known park and a thriving environment for different species of fungi. Consequently, we expected to find species such as Trametes, Stereum, Ganoderma, as there are existing records of these species in New York City parks. This research will support future conservation and documentation efforts to better protect fungi and ecosystems.

Acknowledgements

The authors of this study would like to formally thank the DNA Learning Center at the Cold Spring Harbor Laboratory for their organization and funding of the Urban Barcode Research Program in the turbulent times of 2020 to 2021, as well as their sponsors Sandbox Science & Pinkerton Foundation with the highest levels of gratitude. In addition, the New York Mycological Society has heavily guided this novel study through the help of our mentors Mr. Craig Trester and Sigrid Jakob, providing us with enormous amounts of guidance and resources.

Materials & Methods

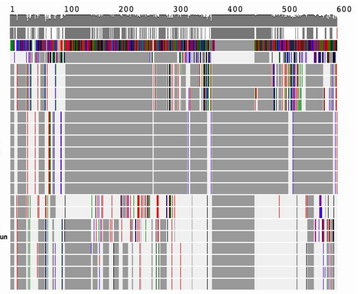
We will collect multiple physical specimens from parks in the metropolitan New York City area within a narrow time frame in order to reduce inconsistencies in the data collected from seasonal variation and sample degradation. From these samples we will extract DNA from specimen tissue with extraction buffers. The samples were then processed through the rapid DNA isolation method We will then amplify fungal and algal rDNA barcode regions (ITS1, ITS2, 5.8S, SSU, LSU) using PCR, an assortment of specific primers, and utilize Genewiz's Sanger sequencing service to produce sequences for analysis. (Bokulich, et al., 2013). We will then compare our results to database reference sequences through Benchling and DNA Subway's bioinformatics tools. Samples were collected based upon the phenotype of different types of fungi. Samples were collected from Prospect Park on two separate occasions. The first set of samples (S3, S6) were collected on April 30th, 2021 and the second set of samples (S1, S2, S3, S4, S5) were collected on May 14th, 2021. This limited time frame was used to prevent variability among the samples. All samples were collected using hands, and then placed into a brown paper bag to ensure the specimens were kept fresh, and to prevent the degradation of the sample. Sample 1 was obtained on the ground, surrounded by dead foliage, next to a brick wall. The area was chosen, notably for its decomposing matter. Sample 2 was obtained from a pile of wood chips and bark. Under the guidance of our mentor, the pestles used to grind the samples in the DNA isolation protocols were reused. In order to reduce contamination between specimens, the pestles were rinsed with tap water, soaked in rubbing alcohol for 5 minutes, and consequently air dried.

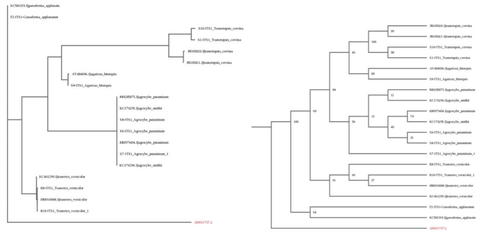
Results

We found a total of 10 samples throughout Kissena and Prospect Parks. We have compiled tentative phylogenetic trees based on sequences obtained from GenBank of the ITS1 and ITS2 regions of the species collected as identified by iNaturalist. Below are the species identified by inaturalist and their corresponding accession number and version from GenBank, which is how they are identified on the phylogenetic trees, as well as one neighbor-joined and one maximum-likelihood phylogenetic tree derived from the GenBank sequences. There is also the multiple-sequence alignment.

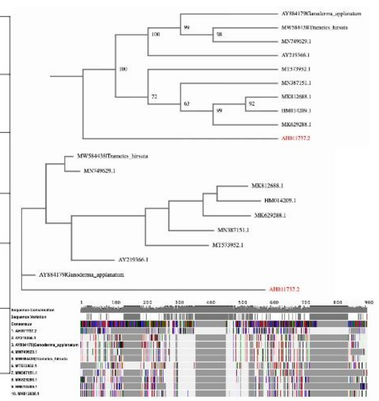
Tables & Figures

Proposed Name	Photo		
Camillea asp.		Pluteus sp.	
Camillea tinctor		Trametes cinnabarinus	
Candelaria concolor		Trichaptum biforme	
Calendelaria concolor		Xanthoparmelia	
Physcia		Trichaptum biforme	





Number	Tentative ID	Location	Location Description	Observed	Photos
D1	Phlyctis argena	Kissena Park, East Flushing, Queens, NY, USA	Found on bark on ground near tree, possibly Acer platanoides.	Apr 26, 2021 - 6:02 PM EDT	
D2	Trametes conchifer	Kissena Park, East Flushing, Queens, NY, USA	On a tree trunk and roots, probably Salix babylonica. Near a moderately traveled path.	Apr 26, 2021 - 5:24 PM EDT	
D3	Phlebia radiata	Kissena Park, East Flushing, Queens, NY, USA	On tree trunk and roots, probably Salix babylonica. Near moderately traveled path. Also present with Trametes Conchifer.	Apr 26, 2021 - 5:33 PM EDT	
D4	Kretzschmaria deusta	Kissena Park	Out of the way, on a tree, maybe Acer saccharum.	May 8, 2021 - 3:35 PM UTC	
D5	Unknown	Kissena Park	Found on a tree, maybe Liquidambar styraciflua, near a path.	May 8, 2021 - 4:07 PM UTC	
D6	Flavoparmelia caperata	Kissena Park	Found near a path, on a tree, maybe Quercus shumardii.	May 8, 2021 - 4:18 PM UTC	
D7	Melanelixia subaurifera	Kissena Park	Near path. On tree, maybe Platanus occidentalis.	May 8, 2021 - 4:38 PM UTC	
D8	Lepraria finkii	Kissena Park	Near a path, on a tree, maybe Quercus palustris.	May 8, 2021 - 4:58 PM UTC	
F1	Trametes hirsuta	Prospect Park	Growing on a dead fallen down tree trunk.	May 2, 2021 - 5:27 PM EDT	
F2	Ganoderma megaloma, also Ganoderma applanatum	Prospect Park	Larger, growing on a tree.	May 2, 2021 - 5:30 PM EDT	



Discussion

Our proposal aimed to extract eDNA from soil samples to determine biodiversity, but due to Covid-19 restrictions, we were unable to complete the wet lab procedures necessary to work with those samples, and instead took fungal samples from parks. We found a variety of species in a variety of locations, indicating the presence of a reasonable amount of fungal species in the park ecosystems. Given the tentative phylogenetic trees, we can assume a reasonable level of biodiversity among the fungal population. Some of the fungal species would be considered parasitic or invasive, however, indicating that the actual health of the ecosystem is less than ideal.

Kretzschmaria deusta is a pathogenic fungus that causes a soft rot in the lower stem or root of trees in the temperate Northern Hemisphere ("Brittle Cinder (Kretzschmaria deusta)"). *Lepraria finkii* is a common type of lichen in North America north of Mexico. It is remarkably able to withstand pollution and disturbance, and therefore survives well in urban environments (Lendemer, James). *Melanelixia subaruifera* is a lichen native to Canada with seasonal spikes in May and August ("Abraded Camouflage Lichen (Melanelixia Subaurifera)."). *Phlebia radiata* is a common species of crust fungus in the Northern Hemisphere. It causes a white rot in fallen logs and branches of various kinds of trees ("Wrinkled Crust (Phlebia Radiata)."). *Phlyctis argena* is a lichen native to Canada and the US. It has a seasonal spike in January and possibly April ("Whitewash Lichen (Phlyctis Argena)."). *Trametes conchifer* is found in North America on trees ("Little Nest Polypore (Trametes Conchifer)."). *Flavoparmelia caperata* is a common lichen that grows on tree bark and occasionally rocks in a variety of regions, the US included. It has seasonal spikes in February, April, and September, but is relatively common year-round ("Common Greenshield Lichen (Flavoparmelia caperata)"). *Trametes hirsuta* is a pathogenic fungus that grows on the dead wood of deciduous trees. It is not native to the US ("Hairy Bracket (Trametes Hirsuta)."). *Ganoderma applanatum* is a parasitic fungal species native to the US that grows on living and dead wood. It has slight spikes around April and August, but is relatively common year-round ("Artist's Bracket (Ganoderma Applanatum)").

References

"Abraded Camouflage Lichen (Melanelixia Subaurifera)." *INaturalist*, www.inaturalist.org/taxa/194686-Melanelixia-subaurifera.
Abrego, N., Crosier, B., Somervuo, P., Ivanova, N., Abrahamyan, A., Abdi, A., Hämäläinen, K., Junninen, K., Maunula, M., Purhonen, J. & Ovaskainen, O. (2020). Fungal communities decline with urbanization - more in air than in soil. *The ISME Journal,* 14, 2806–2815. https://doi.org/10.1038/s41396-020-0732-1
"Artist's Bracket (Ganoderma Applanatum)." *INaturalist*, www.inaturalist.org/taxa/48473-Ganoderma-applanatum.
"Brittle Cinder (Kretzschmaria Deusta)." *INaturalist*, www.inaturalist.org/taxa/128051-Kretzschmaria-deusta.
"Common Greenshield Lichen (Flavoparmelia Caperata)." *INaturalist*, www.inaturalist.org/taxa/117943-Flavoparmelia-caperata.
Eichmiller, J. J., Miller, L. M., & Sorensen, P. W. (2016). Optimizing techniques to capture and extract environmental DNA for detection and quantification of fish. *Molecular Ecology Resources,* 16, 56-68. https://www.maisrc.umn.edu/sites/maisrc.umn.edu/files/optimizing_techniques_0.pdf
"Hairy Bracket (Trametes Hirsuta)." *INaturalist*, www.inaturalist.org/taxa/116695-Trametes-hirsuta.
Helgason, T., & Fitter, A. H. (2009). Natural Selection and the evolutionary ecology of the arbuscular mycorrhizal fungi (Phylum Glomeromycota). Journal of Experimental Botany, 60(9), 2465-2480. https://doi.org/10.1093/jxb/erp144
Henslee, B. (2001). New York Combined Checklist. *New York Mycological Society*. http://newyorkmyc.org/nyc-combined-checklist/
Ivashchenko, K., Ananyeva, N., Vasenev, V., Sushko, S., Seleznyova, S., & Kudeyarov, V. (2019). Microbial C-Availability and Organic Matter Decomposition in Urban Soils of Megalopolis Depend on Functional Zoning. *Soil Environ.,* 38(1), 31-41. https://doi.org/10.25252/SE/19/61524
Jemo, M., Dhiba, D., Hashem, A., Abd'Allah E. F., Alqarawi, A. A., & Phan Tran L.-S. (2018). Mycorrhizal fungal community structure in tropical humid soils under fallow and cropping conditions. *Scientific Reports*, 8(17061). https://doi.org/10.1038/s41598-018-34736-6
Ji, B., Gehring, C. A., Wilson, G. W. T., Miller, R. M., Flores-Rentería, L., & Johnson, N. C. (2013). Patterns of diversity and adaptation in Glomeromycota from three prairie grasslands. *Molecular Ecology*, 22, 2573-2587. https://doi.org/10.1111/mec.12268
Lendemer, James. (2013). A monograph of the crustose members of the genus Lepraria Ach. s. str. (Stereoculaceae, Lichenized Ascomycetes) in North America north of Mexico. Opuscula Philolichenum. 13. 36-141.
"Little Nest Polypore (Trametes Conchifer)." *INaturalist*, www.inaturalist.org/taxa/880970-Trametes-conchifer.
McGuire, K. L., Payne, S. G., Palmer, M. I., Gillikin, C. M., Keefe, D., Kim, S. J., Gedallovich, S. M., Discenza, J., Rangamannar, R., Koshner, J. A., Massmann, A. L., Orazi, G., Essene, A., Leff, J. W., & Fierer, N. (2013). Diggin the New York City Skyline: Soil Fungal Communities in Green Roofs and City Parks. *PLOS ONE*. https://doi.org/10.1371/journal.pone.0058020
Tan, X., Kan, L., Su, Z., Liu, X., & Zhang, L. (2019). The Composition and Diversity of Soil Bacterial and Fungal Communities Along an Urban-To-Rural Gradient in South China. *Forest*. https://doi.org/10.3390/f10090797
Tonn, N. (2016). The Effect of Urbanization On the Mycorrhizal Associations and Survival of Three Species of Eastern Hardwoods. *University of Michigan*. https://deepblue.lib.umich.edu/bitstream/handle/2027.42/122695/NTONNthesisFull2016.pdf?sequence=1&isAllowed=y
Veysi, H., Heidari, G. R., & Sohrabi, Y. (2017). The Effect of Mycorrhizal Fungi and Humic Acid on Yield and Yield Components of Sunflower. Journal of Agroecology, 8(4), 567-582. https://doi.org/10.22067/JAG.V8I4.47568