Mushroom Biodiversity in Rockefeller State Park Preserve in Pleasantville, NY

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Abstract

Mushrooms play a vital role in an ecosystem as they break down dead plant and animal material and they return nutrients back into the food web. The aim of the study was to evaluate the biodiversity of mushrooms in Rockefeller State Park Preserve in Westchester County, NY. The use of field guides[12, 13], an online network for biodiversity information called iNaturalist, and DNA Barcoding aided us in the identification of mushroom specimens. The 26 samples we collected were first identified using iNaturalist and a mushroom field guide. Then DNA barcoding was employed to compare and clarify the preliminary identifications. We identified 10 species of fungi, 8 of them mushrooms that we collected and 2 of them microscopic

Introduction

Mushrooms are a type of fungi that are commonly found in the order of Agaricales in the phylum of Basidiomycota.[1] They come in multiple shapes and contain spores, leading mushrooms to be classified as sporophores. Unlike many other organisms, mushrooms are saprophytes which means that they obtain their nutrients from nonliving organic matter.[2] There is an abundance and wide variety of mushrooms as there are currently over 14,000 known species of mushrooms.[5]

Mushrooms play a vital role in an ecosystem. They play a key role in breaking down dead plant and animal material and returning nutrients back into the food web. Mushrooms recycle carbon from litter and dead plant material and return it to provide nutrients for the soil which allows plants to grow.[4]

It is important to research the biodiversity of mushrooms because they contribute to the ecosystem while aiding in providing nutrients for organisms and the habitat of the ecosystem. Many organisms rely on certain species of mushrooms to survive. Some mushrooms tend to absorb pollutants and as a result, become toxic and detrimental to other organisms.[6] On the other hand, other species of mushrooms can remedy contaminated soils ultimately helping the ecosystem survive, despite environmental conditions.[7]

Rockefeller State Park Preserve is a state park in Mount Pleasant, New York with a high diversity of species over the vast 1,775 acres of land.[10]

In preparation for our project, we researched a full list of mushroom species in Rockefeller State Park Preserve, and we didn't find one. Hence, the goal of our project is to evaluate the biodiversity of mushrooms in Rockefeller State Park Preserve and come up with a list of species, especially those which thrive during April. The use of field guides[12,13], iNaturalist, and DNA Barcoding will aid us in the identification of mushroom species.

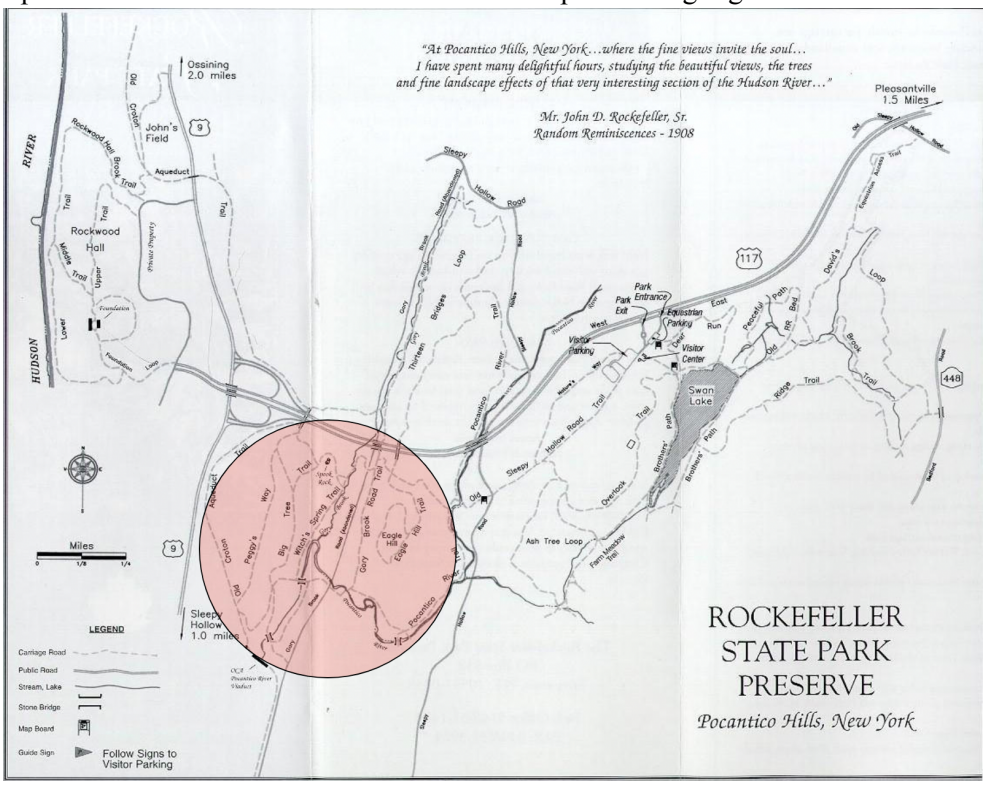
Materials and Methods

We collected 26 samples of various mushrooms from Rockefeller State Park. Mushroom samples were collected from multiple trails around the Eagle Hill area. Specifically, samples were collected from south of Highway 117 and north of the Pocantico River trail, as well as west

of Sleepy Hollow Road and east of Old Croton Aqueduct trail as shown in Figure 1.[14] We chose this area to sample because it is mostly marshland, which would most likely contain more dead matter than the rest of the preserve.

We used Rapid DNA extraction as described in UBP resource materials.[15] The isolated DNA sample was amplified using the ITS1F and ITS4 primers using the Ready-to-Go PCR Bead amplification technique.[15] After PCR, we stored the amplified DNA on ice or at -20° C until we were ready to analyze the PCR Products by gel electrophoresis. We conducted gel electrophoresis according to a standard procedure described in UBP resource materials.[15] Next, we sent our DNA samples to sequence by biotechnological company Genewitz. Upon receiving our DNA sequence results we used (1) DNA subway procedure and (2) direct BLAST analysis to identify our species.[16]

Figure 1. Map of Rockefeller State Park Preserve with sample area highlighted.



Results

Of the 26 samples of mushrooms collected from the Rockefeller State Park Preserve, 4 of the specimens didn't yield DNA sequence which could be DNA barcoded. All 26 samples were identified using iNaturalist and field guides, 25 of the samples had successful DNA extraction, and 22 of the samples were DNA barcoded with a high quality, meaning that the error rate for the sequences was less than 1%. The samples were first identified using iNaturalist and a mushroom field guide, and lastly DNA barcoding. Using the method of iNaturalist, 16 different species were found to be present in the mushroom samples. From the identification of mushrooms using a field guide 14 different species were identified, and DNA barcoding showed 8 different species in the 21 samples that were successfully DNA barcoded.

Table 1: Isabella Avila

Sample	iNaturalist	Field Guides	DNA Barcoding By DNA subway	DNA barcoding by direct BLAST analysis	Notes	Link to iNaturalist
KRE_001	*Trametes pubescens*/ Bracket Fungi	*Ganoderma applanatum*/Artist's Bracket	*Stereum fasciatum*/False Turkey-Tail	*Stereum fasciatum* (percent identity 98.65%)	All three species, *Stereum fasciatum, Ganoderma applanatum,* and *Trametes pubescens* are very similar when it comes to their shape and color. *Stereum fasciatum* are found around North America. *Stereum fasciatum* are also very commonly found in the Westchester, New York area.	https://www.inaturalist.org/observations/153712657
KRE_002	*Trametes versicolor*/ Turkey-Tail	*Trametes versicolor*/Turkey-Tail	*Phaeocalicium sp.*	*Phaeocalicium sp.* (percent identity 99.22%)	*Phaeocalicium is a genus of lichen-forming fungi in the family Mycocaliciaceae. Phaeocalicium sp.* is found on a variety of fungi but most commonly *Trametes*	https://www.inaturalist.org/observations/153713795

					versicolor (Turkey-Tail). The lichen picked up the primer used during DNA barcoding, allowing for it to be identified.	
KRE_003	*Trametes gibbosa*/ Lumpy Bracket	*Daedalea quercina*/Oak Mazegill	No DNA sequence is available for analysis.	No DNA sequence is available for analysis.	*Trametes gibbosa* and *Daedalea quercina* are both polypore mushrooms.	https://www.inaturalist.org/observations/153729681
KRE_004	*Cerrena unicolor*/ Mossy Maze Polypore	*Trichaptum biforme*/Violet-Toothed Polypore	*Trametes gibbosa*/Lumpy Bracket	*Trametes gibbosa* (Percent identity 94.12%)	*Cerrena unicolor, trichaptum biforme,* and *Trametes gibbosa* are very similar in shape and color, as well as the places they grow, decaying wood. *Trametes gibbosa* are found across North America. *Trametes gibbosa* can also be found in the Westchester, New York area.	https://www.inaturalist.org/observations/153729719
KRE_005	*Stereum ostrea/*	*Stereum ostrea*/False	*Trichaptum biforme/*	*Trichaptum biforme/*	*Stereum ostrea* and	https://www.inaturalist.org/observatio

	False Turkey-Tail	Turkey-Tail	Violet-Toothed Polypore	(Percent identity 99.40%)	*Trichaptum biforme* are alike in shape and size. *Trichaptum biforme* is mostly found on decaying hardwoods.	ns/153729778
KRE_006	*Fomitopsidaceae* / Bracket Polypores	*Stereum complicatum*/Crowded Parchment	*Trametes gibbosa*/Lumpy Bracket	*Trametes gibbosa* (Percent identity 99.50%)	*Fomitopsidaceae, stereum complicatum,* and *Trametes gibbosa* are all in the family of polypores. *Trametes gibbosa* is found across North America and the Westchester, New York area.	https://www.inaturalist.org/observations/153729847
KRE_007	*Cerrena unicolor*/ Mossy Maze Polypore	*Trametes betulina*/Gilled polypore	DNA subway does not give any results. Poor DNA sequence quality	BLAST does not give any results. Poor DNA sequence quality	*Cerrena unicolor* and *Trametes betulina* are found across North America. Both species are similar in size, shape, and color. They are related to each other as they are both polypores.	https://www.inaturalist.org/observations/153729889
KRE_008	*Trametes gibbosa*/ Lumpy	*Daedalea quercina*/Oak Mazegill	*Trametes versicolor*/ Turkey-Tail	*Trametes versicolor* (Percent	*Trametes gibbosa, Daedalea*	https://www.inaturalist.org/observations/153729921

	Bracket			identity 99.26%)	*quercina,* and *Trametes versicolor* are all common polypores and found across North America.	

Table 2 - Christina H-S

Sample	**iNaturalist**	**Field Guides**	**DNA Barcoding by DNA subway**	**DNA barcoding by direct BLAST analysis**	**Notes**	**Link to iNaturalist**
KRE_009	*Stereum ostrea*/ False Turkey-Tail	*Stereum ostrea*/ False Turkey-Tail	*Stereum sp.*	*Stereum lobatum* (Percent identity 96.72%)	*Stereum lobatum* is very common in the Westchester, New York area. They are found around North America. False Turkey-Tail is an imitation species that is very similar in appearance to the Turkey-Tail Fungi. This can explain why the field guides and iNaturalist incorrectly classified the sample.	False Turkey-Tail
KRE_010	*Stereum complicatum* / Crowded Parchment	*Stereum complicatum*/ Crowded Parchment	*Stereum complicatum* / Crowded Parchment	*Stereum complicatum* (Percent identity 88.60%)	*Stereum complicatum* is very common in the Westchester, New York area. They are found around North America.	Crowded Parchment
KRE_011	*Gloeoporus dichrous*/ Bicolored	*Gloeoporus dichrous*/ Bicolored	*Trametes versicolor*/ Turkey-Tail	*Trametes versicolor* (Percent	*Trametes versicolor* is very common in the Westchester,	Bicolored Bracket

	Bracket	Bracket		identity 90.46%)	New York area. They are found around North America. *Gloeoporus dichrous* and *Trametes versicolor* are very similar in color and appearance, which may have been attributed to the incorrect classification on iNaturalist and by field guides.	
KRE_012	Genus *Sarcophsyca* Elf Cups	*Sarcophsyca austriaca*/ Scarlet Elf Cups	*Sarcophsyca austriaca*/ Scarlet Elf Cups	*Sarcophsyca austriaca* (Percent identity 99.82%)	Scarlet Elf Cups are local to Westchester, New York area. They are found around North America.	Elf Cups
KRE_013	*Trametes betulina*/ Gilled Polypore	*Trametes betulina*/ Gilled Polypore	*Lenzites betulinus*/ Gilled Polypore	*Lenzites betulinus* (Percent identity 100.00%)	*Lenzites betulinus* is the former name of *Trametes betulina. Lenzites betulinus* is local to Westchester, New York area. They are found around North America.	Gilled Polypore
KRE_014	*Trametes versicolor*/ Turkey-Tail	*Trametes versicolor*/Turkey-Tail	*Stereum lobatum*/ False Turkey-Tail	*Stereum lobatum* (Percent identity 95.69%)	False Turkey-Tail is an imitation species that is very similar in appearance to the Turkey-Tail Fungi. This can explain why the field guides and iNaturalist incorrectly classified the sample. Stereum lobatum is local to Westchester, New York area.	Trametes Versicolor

					They are found around North America.	
KRE_015	*Ischnoderma resinosum*/ Resinous Polypore	*Ischnoderma resinosum*/ Resinuous Polypore	*Trametes versicolor*/ Turkey-Tail	*Trametes versicolor* (Percent identity 99.01%)	*Ischnoderma resinosum* and *Trametes versicolor* are very similar in color and appearance, which may have contributed to the incorrect classification on iNaturalist and by field guides. *Trametes versicolor* is one of the most common mushrooms in North America and grows all over the world.	Resinous Polypore
KRE_016	*Punctularia strigosozonata/* White-rot Fungus	*Punctularia strigosozonata/* White-rot Fungus	*Stereum ostrea*/ False Turkey-Tail	*Stereum ostrea* (Percent identity 99.49%)	*Punctularia strigosozonata* and *Stereum ostrea share* similar colors which may make them appear like each other. *Stereum ostrea* is local to Westchester, New York area. They are found around North America.	White-Rot Fungus
KRE_017	*Stereum ostrea*/ False Turkey-Tail	*Stereum ostrea*/ False Turkey-Tail	*Stereum lobatum*/ False Turkey-Tail	*Stereum lobatum* (Percent identity 97.11%)	*Stereum lobatum* and *Stereum ostrea* are two different names for the same species. They are both False Turkey-Tail. *Stereum lobatum* is local to Westchester,	False Turkey-Tail

					New York area. They are found around North America.	
KRE_018	*Punctularia strigosozonata*/ White-rot Fungus	*Punctularia strigosozonata*/ White-rot Fungus	*Stereum ostrea*/ False Turkey-Tail	*Stereum ostrea* (Percent identity 99.17%)	*Punctularia strigosozonata* and *Stereum ostrea share* similar colors which may make them appear like each other. *Stereum ostrea* is local to Westchester, New York area. They are found around North America.	White-Rot Fungus

Table 3: Anjali Shafiee

Sample	**iNaturalist**	**Field Guides**	**DNA Barcoding by DNA subway**	**DNA barcoding by direct BLAST analysis**	**Notes**	**Link to iNaturalist**
KRE-019	*Xylobolus subpileatus*/ Bacon of the Woods	*Xylobolus Frustulatus*/ Ceramic fungus	*Stereum Ostrea*/ False turkey-tail	Inconclusive	*Stereum Ostrea* is found all over North America. *Xylobolus Frustulatus* and *Stereum Ostrea* they have similar color and appearance with a similar stripe pattern.	https://www.inaturalist.org/observations/156403870
KRE-020	*Trametes Versicolor*/ Turkey Tail	*Trichaptum biforme*/ Violet-toothed polypore	Inconclusive	Inconclusive	*Trametes versicolor* and *Trichaptum biforme* are alike in size and shape, but different in color.	https://www.inaturalist.org/observations/156403448
KRE-021	*Stereum Ostrea*/	*Trichaptum biforme*/	Inconclusive	*Trichaptum biforme*	*Stereum ostrea* and *Trichaptum* are	https://www.inaturalist.org/obser

	False turkey-tail	Violet-toothed polypore		(Percent identity 88.47%)	similar in both size and shape, but differ in color.	vations/156403401
KRE-022	*Stereum Ostrea*/ False turkey-tail	*Trichaptum biforme*/ Violet-toothed polypore	*Stereum Ostrea*/ False turkey-tail	*Stereum ostrea* (Percent identity 99.49%)	*Stereum Ostrea* is found all over North America. *Stereum ostrea* and *Trichaptum* are similar in both size and shape, but differ in color.	https://www.inaturalist.org/observations/156403362
KRE-023	*Stereum ostrea*/ False turkey-tail	*Trichaptum biforme*/ Violet-toothed polypore	*Stereum ostrea*/ False turkey-tail	*Stereum ostrea* (Percent identity 99.83%)	*Stereum ostrea* is found all over North America. *Stereum ostrea* and *Trichaptum* are similar in both size and shape, but differ in color.	https://www.inaturalist.org/observations/156403320
KRE-024	*Trametopsis cervina*/ Deer-colored Trametes	*Xylobolus frustulatus*/ Ceramic fungus	No DNA sequence is available	No DNA sequence is available	*Trametopsis cervina* and *Xylobolus frustulatus* are very similar in color, shape, and size. They both are local to the Westchester, New York area.	https://www.inaturalist.org/observations/158606039
KRE-025	*Fomitopsis betulina*/ Birch polypore	*Fomes fomentarius*/ Hoof fungus	*Trametes versicolor*/ Turkey Tail	*Trametes versicolor*/ (Percent identity 100.00%)	The *Fomitopsis Betulina* and *Fomes Fomentarius* exist on alive trees and look almost identical in appearance, which may be why they were used to classify the sample, which looks similar to both of the fungi.	https://www.inaturalist.org/observations/156403288
KRE-026	*Lycoperdon*/ Puffballs	*Lycoperdon pyriforme*/ *Pear shaped*	*Suhomyces vadensis*/ Budding	*Forward sequence Lycoperdon*	*Suhomyces vadensis* is a yeast fungus. The DNA subway	https://www.inaturalist.org/observations/1566162

		Puffball	yeasts	*pyriforme* (Percent identity 98.93%) Apioperdon - Wikipedia Reverse sequence *Suhomyces vadensis* (Percent identity 97.14%)	results yielded two different species of fungus. *Lycoperdon pyriforme* is a mushroom in the puffball family. Previously to collecting the sample the mushroom had died and started to decay, causing *Suhomyces vadensis* to grow. *Suhomyces vadensis* picked up the fungi primer while DNA barcoding, allowing for both species to be identified.	76

Table 4: List of species of mushrooms identified by DNA barcoding

Species 1	*Stereum fasciatum/Stereum lobatum/Stereum ostrea*
Species 2	*Trametes gibbosa*
Species 3	*Trichaptum biforme*
Species 4	*Trametes versicolor*
Species 5	*Stereum complicatum*
Species 6	*Sarcophsyca austriaca*
Species 7	*Lenzites betulinus/Trametes betulina*
Species 8	*Lycoperdon pyriforme*

Table 5: List of microscopes species of fungi, identified inadvertently

Genus 1	*Phaeocalicium sp*
Species 1	*Suhomyces vadensis*

Discussion

The research goal of this study was to determine the biodiversity of mushrooms in Rockefeller State Park. We collected 26 specimens of mushrooms. We successfully isolated

DNA for 24 specimens. Out of these 24 specimens we were able to analyze DNA sequence by DNA subway and/or directly by BLAST in 22 cases. We identified 8 species of mushrooms, and also 2 species of microscopic fungi, which are not mushrooms, and which got into our samples inadvertently.

From the results obtained, we can conclude that our use of DNA barcoding was successful in finding the biodiversity in Rockefeller State Park, as we have identified and compiled a list of various fungi that exist in the area. We found that many of the species of our samples that we identified using iNaturalist and field guides did not match the species of the mushroom samples once DNA barcoding was complete. This suggests that iNaturalist and Field Guides may not always have the most accurate or reliable results.

This study can be improved in the future by potentially examining the biodiversity of fungi in the Rockefeller State Park in different seasons, not just in April strictly. Several fungi may exist in the Rockefeller that were not in season, and therefore could not be sampled. To make a more comprehensive list, we could improve this by collecting year-round.

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