



Identifying Pathogenic and Non-pathogenic Basidiomycetes and Ascomycetes Within Central Park as Bioindicators of Biodiversity

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Abstract

Fungi are an essential part of our ecosystem, playing a vital role in decomposition, nutrient cycling, and mutualistic symbiosis with plants, algae, and cyanobacteria. The success of fungi lies in their ability to form a wide array of biological interactions. Our research focused on identifying species of fungi in the Basidiomycetes and Ascomycetes phyla within the northern- and southern-bound Central Park using DNA barcoding and checking which are pathogenic or non-pathogenic. We used two methods of extraction: Qiagen DNeasy Plant Pro Kit and Rapid DNA Isolation method and we amplified segments of the ITS region using the ITS5/ITS2 primers. Results from our BLASTN search indicated non-pathogenic fungal species as well as some pathogenic ones that are parasitic to different species of trees; some are known to be infectious to humans. Some of the non-pathogenic species have mutualistic symbiosis with plants. None of the species we identified was an ascomycete.

Introduction

Evidence suggests that fungal interactions are an ecological driving force, interconnecting different levels of biological and ecological organizations of their hosts, competitors, and antagonists, with potential to affect the macroecology and evolution of an ecosystem (Bahram & Netherway, 2021). Fungi also display high levels of endemism. A concentration of specific fungal species within a given area might provide deeper insight into the biotic and abiotic factors in an ecosystem, such as the co-localization of fungal host species, temperature, moisture, altitude, pH, and nutrient availability (Lofgren and Stajich, 2021).

Some fungi are capable of infecting plants, humans and other animals. Kohler et al. (2016) in their review of fungi that infect humans noted that most of the fungi capable of infecting healthy humans evolved to infect other hosts such as amoeba, insects, or small mammals. Ascomycetous soil dwellers that parasitize mammals and cause systemic infection mostly in tropical regions, include *Blastomyces dermatitis*, and *Coccidioides* among other species. Two species of *Cryptococcus* a. basidiomycete, *C. neoformans* and *C. gatti* are species that infect humans. Fungal pathogens of plants were reviewed by Doehlemann et al. (2016). The fungus, *Colletotrichum purpurea*, was described as capable of infecting over 400 plant species. They noted that fungal plant pathogen species are primarily in the Basidiomycota and Ascomycota. We will check to see whether any of our fungal samples are pathogenic.

The purpose of our research is to identify various species of Basidiomycetes and Ascomycetes within Central Park and observe the biodiversity of pathogenic and non-pathogenic fungal species as bioindicators within the area. Central Park, although situated within an urban enclosure, contains a vast variety of soil microbial phylotypes and communities as could be observed in that of arctic, tropical, and desert soils (Ramirez, 2014), which makes it a rich area for data.

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Materials & Methods

We visited North Woods(N) and Ramble(R), Central Park on March 9, 2024 and collected fifteen different samples from the area between our transect start at 40.1982°N, 73.9567°W and transect end at 40.7978°N, 73.9561°W. Five additional samples were collected on April 23, 2024.

DNA was extracted from fungal samples using both the DNALC's Rapid DNA Isolation method and the Qiagen DNeasy Plant Pro Kit. Extracted DNA samples were amplified following the recommended conditions for the Thermo Scientific Dreamtaq Hot Start Mix. We used the primer pairs ITS5/ITS2 and ITS1F/ITS4 to amplify the specific region of the DNA. The amplified DNA samples were analyzed using 1% agarose gel electrophoresis, following the provided steps given by DNALC and using the GeneRuler 100bp DNA Ladder to estimate the size of the bands. DNA samples, with clear bands were sent to Genewiz for Sanger sequencing.

Once we received the results back, we performed a BLAST search to match our trimmed DNA barcode with those of existing barcodes found within the databases (GenBank, European Molecular Biology Laboratory, DNA Databank of Japan). Using DNA Subway, we then created a phylogenetic tree showing the evolutionary relationships between the specimens we collected.



Figure 1. Map of the sites we collected samples from. (1 - Northwoods, 2 - Ramble)

Results

- The fungal samples identified by DNA barcoding using the ITS marker were mostly basidiomycetes (Table 1).
- Comparison of our barcode sample with the reference sequences were used to construct a phylogenetic tree (See Figure 2).

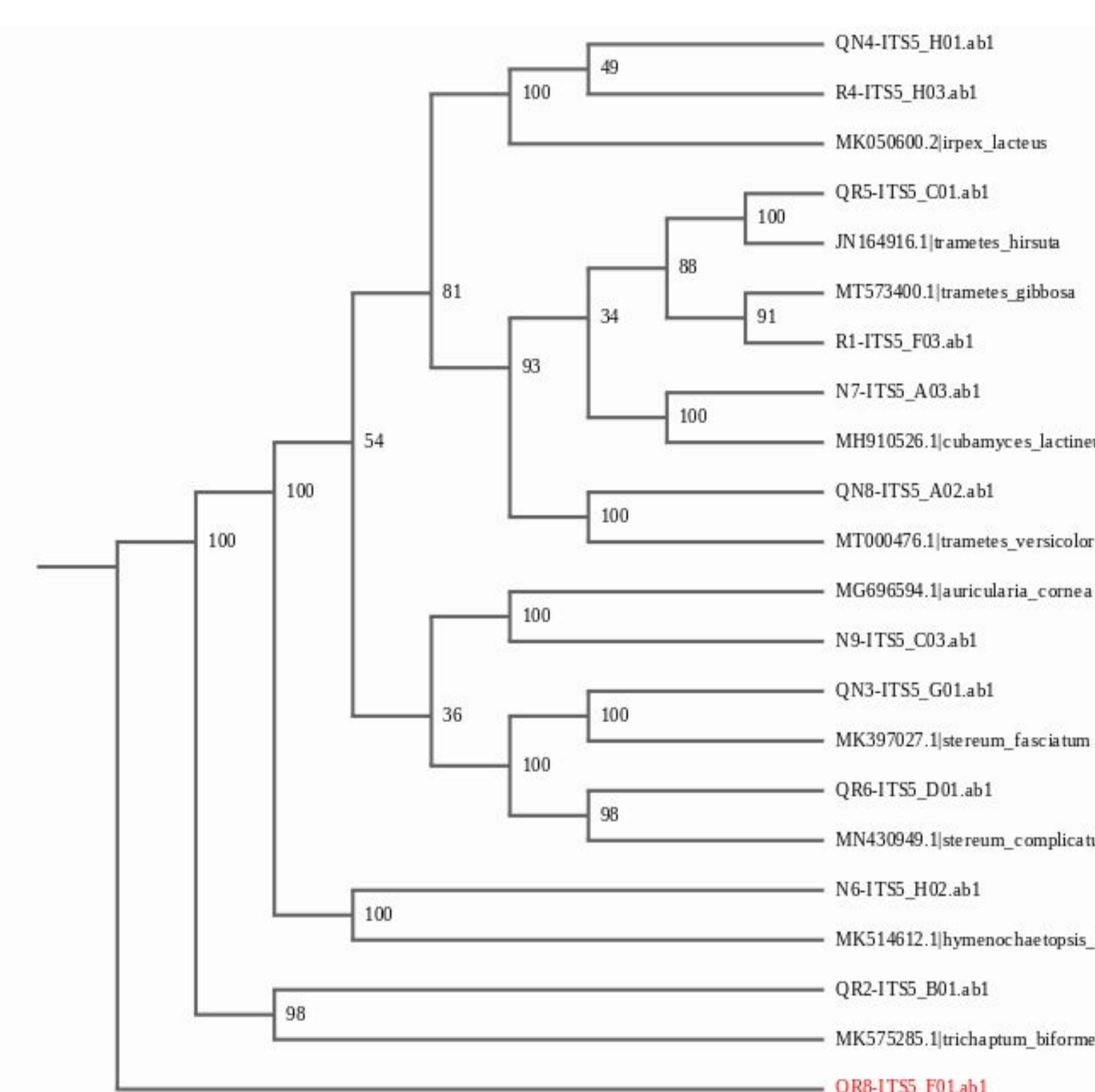


Figure 2. Phylogenetic tree of our fungal samples and BLAST results.



Figure 3. QN8, *Trametes versicolor*



Figure 4. R4, *Irpex lacteus*

Collection date	Sample ID	Species name	Phylum/Family	Pathogenic or Non-Pathogenic
3/9/2024	N6-ITS5	<i>Hymenochaetopsis olivacea</i>	Basidiomycota/ Hymenochaetales	Non-pathogenic
3/9/2024	N7-ITS5	<i>Cubomyces lactineus</i>	Basidiomycota/ Polyporaceae	Non-pathogenic
3/9/2024	N9-ITS5	<i>Auricularia cornea</i>	Basidiomycota/ Auriculariales	Non-pathogenic
3/9/2024	QN3-ITS5	<i>Stereum fasciatum</i>	Basidiomycetes/ Stereaceae	Non-pathogenic
3/9/2024	QN4-ITS5	<i>Irpex lacteus</i>	Basidiomycota/ Meruliaceae	Pathogenic to tree species and humans
3/9/2024	QN8-ITS5	<i>Trametes versicolor</i>	Basidiomycota/ Polyporaceae	Opportunistically pathogenic to tree species
3/9/2024	QR2-ITS5	<i>Trichaptum biforme</i>	Basidiomycota/ Hirschioporaceae	Non-pathogenic
4/23/2024	QR5-ITS5	<i>Trametes hirsuta</i>	Basidiomycota/ Polyporaceae	Pathogenic to tree species
4/23/2024	QR6-ITS5	<i>Stereum complicatum</i>	Basidiomycota/ Stereaceae	Pathogenic to tree species
4/23/2024	QR8-ITS5	<i>Trichaptum biforme</i>	Basidiomycota/ Hirschioporaceae	Non-pathogenic
3/9/2024	R1-ITS5	<i>Trametes gibbosa</i>	Basidiomycota/ Polyporaceae	Pathogenic to tree species
4/23/2024	R4-ITS5	<i>Irpex lacteus</i>	Basidiomycota/ Meruliaceae	Pathogenic to tree species and humans

Table 1. Identification of fungal samples from the Northwoods are indicated with a prefix N and samples from the Ramble are indicated with prefix R.

Discussion

- We extracted all of our initial samples collected on March 9, 2024 using the DNALC DNA Isolation protocol and the samples collected on April 23, 2024 were extracted using the Qiagen DNeasy Plant Pro Kit to test whether the issue with our PCR was due to insufficient breaking down of the fungal cell walls.
- As we had only prime-dimer interactions in our initial PCR run of samples extracted from both extraction methods when we used the ITS1/ITS4 primer, we changed primers. The primer pair ITS5/ITS2 that we used subsequently to amplify the ITS region produced visible bands on gel electrophoresis. The extracted DNA was successfully sequenced for most samples.
- The phylogenetic tree showed the relationship between our samples and related species from the NCBI (and other databases). For instance, it showed that QN4 and R4 two separate sampling locations were likely the same species (*Irpex lacteus*).
- Something interesting that we found, is that there were various fungi from the genus *Trametes* that are regarded as being opportunistically pathogenic, meaning that while they do not normally harm their host, they can make the host more susceptible to diseases like papery bark, weak bud unions, and white rot.
- Auricularia cornea* (See Figure 3), commonly known as wood ear, have also shown to have mutualistic symbiosis with *P. mangrovi*, a certain tree species, promoting high levels of mycelial growth (Phithakrotchanakoon et al., 2022).
- Irpex lacteus* (Figure 4), a wood-decaying fungus that causes white rot and can be found on various hardwood trees, dead branches, and standing trunks is one of the species found at both locations. The species which is widely distributed in North America is also reported to cause pulmonary abscess infections in humans (Deshmukh et al., 2024).
- Based on analyses of the DNA sequences of our samples, we did not encounter any ascomycetes along the transects we sampled.

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