

# Evaluation of DNA barcoding using LSU and ITS rDNA loci as a rapid diagnostic method for beech leaf disease

CSH Cold Spring Harbor Laboratory
DNA LEARNING CENTER

Funded by the Thompson Family Foundation

Oonagh Brandon<sup>1</sup>, Katherine Murtagh<sup>1</sup>, Gracyn Trudel<sup>1</sup>, Peter Popov<sup>2</sup> Mentors: Dr. Oxana Litvine<sup>1</sup>, Mrs.Stacy Unkenholz<sup>1</sup>

### Abstract

Beech leaf disease (BLD) is an ongoing threat to both the American Beech (Fagus grandifolia) and the European Beech (Fagus sylvatica) species. BLD can cause premature leaf fall and even tree mortality. The nematode (Litylenchus crenatae mccannii) has been associated with this disease, but the role of fungal microbiomes in BLD's progression is not understood. This study used DNA barcoding to evaluate the fungi living on and inside of the beech leaf specimens, both affected and unaffected by BLD. Internal transcribed spacer (ITS) and large subunit ribosomal (LSU) primers were used to amplify the DNA in Sanger sequencing and Next Generation Sequencing processes.

## Introduction

- Beech Leaf disease is a degenerative condition that affects both the American Beech (Fagus grandifolia) and the European Beech (Fagus sylvatica).
- The first occurrence of BLD was recorded by John Pogacnik in Lake County, Ohio.
- Discovered in 2012, the disease has continued to spread east to New York and beyond.
- This disease currently has no cure and can eventually lead to tree mortality.
- The nematode (Litylenchus crenatae mccannii) has been linked to BLD,
  - The cause of this disease and the potential role of fungi in its development and progression remain unclear.
- Assessing the variety of fungi living on beech tree leaves, both affected and unaffected by BLD is crucial to understanding the progression of the disease.
- DNA barcoding is used to identify fungi species and other organisms.
- Main goal of our study was identification of species on and inside of beech leaves to gain a better understanding of the microbiome and its potential relationship to BLD

## Affiliations

- 1 The Ursuline School, 1354 North Ave, New Rochelle, NY 10804
- 2 Dutchess Community College, 53 Pendell Rd, Poughkeepsie, NY 12601

## Materials & Methods

#### Sample Collection

- Beech leaves were collected between August 14–23, 2024, from six locations in Westchester County, NY.
- Each sample was placed in a ziplock bag with silica gel beads for desiccation.

#### DNA Extraction and PCR

- DNA was extracted using the DNeasy Plant Mini Kit.
- PCR was performed with Ready-To-Go beads and two nematode-targeting
- o LSU (D2A/D3B)
- Primer mixes were prepared with loading dye, and PCR followed this thermal cycling profile:
- 35 cycles of 95°C for 45 sec

- final extension at 72°C for 10 min.
- Products were stored at −20°C.

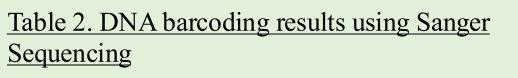
#### Gel Electrophoresis

- PCR products were separated on a 2% agarose gel in 1× TBE buffer with GelGreen dye, run at 130 V for
- ~30 min, and visualized under LED light

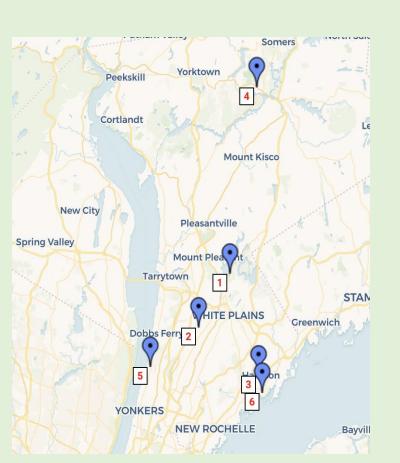
#### Sequencing and Analysis

- Samples with multiple amplicons were sequenced using Oxford Nanopore's Rapid Barcoding Kit (Flongle device).
- Raw reads were processed with Epi2me software (using Nextflow and Docker) to generate consensus sequences.
- All Sanger sequences were analyzed using the DNA Subway platform, while all Nanopore sequences were identified by direct BLAST analysis.

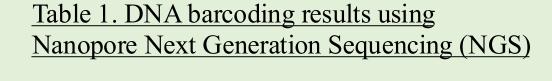
## Tables & Figures



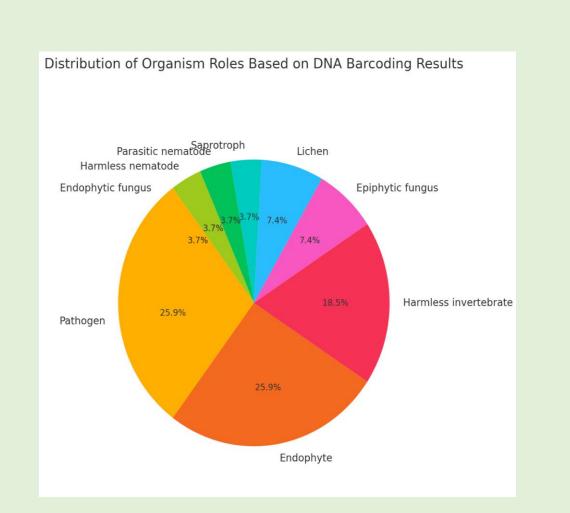
BLD Signs?	Tree Species	Primers	Organism. identified as a result of barcoding (Sanger sequencing)
No	Fagus grandifolia	LSU	Fagus sylvatica
No	Fagus grandifolia	LSU	No significant Blast match
Yes	Fagus grandifolia	LSU	Fagus sylvatica
No	Fagus grandifolia	LSU	No significant Blast match
Yes	Fagus grandifolia	LSU	Fagus sylvatica
Yes	Fagus grandifolia	LSU	Fagus sylvatica
No	Fagus grandifolia	LSU	Parthenocissus heptaphyll
Yes	Fagus grandifolia	LSU	Fagus sylvatica
No	Fagus grandifolia	LSU	Fagus sylvatica
No	Fagus sylvatica	LSU	Fagus sylvatica
Yes	Fagus sylvatica	LSU	No data from both lines
No	Fagus sylvatica	LSU	No data from both lines
No	Fagus sylvatica	LSU	Fagus sylvatica
No	Fagus sylvatica	LSU	No significant Blast match
No	Fagus sylvatica	LSU	No significant Blast match
Yes	Fagus grandifolia	LSU	Paraconiothyrium   The state of
No	Fagus grandifolia	ITS	No data from both lines
No	Fagus grandifolia	ITS	No data from both lines
Yes	Fagus grandifolia	ITS	Cladosporium cladosporioides isolate
Yes	Fagus grandifolia	ITS	Dothideomycetes
No	Fagus grandifolia	ITS	Ramularia unterseheri
Yes	Fagus grandifolia	ITS	Uncultured Phaeosphaeria
No	Fagus grandifolia	ITS	Pseudomicrostroma glucosiphilum
Yes	Fagus grandifolia	ITS	Pseudomicrostroma glucosiphilum
Yes	Fagus grandifolia	ITS	No significant Blast match
Yes	Fagus grandifolia	ITS	Embryophyte environmental
No	Fagus grandifolia	ITS	Dothideomycetes
Yes	Fagus grandifolia	ITS	Ramularia unterseheri isolate
No	Fagus grandifolia	ITS	Rhynchopelta concentrica
No	Fagus sylvatica	ITS	Erysiphe wadae genes

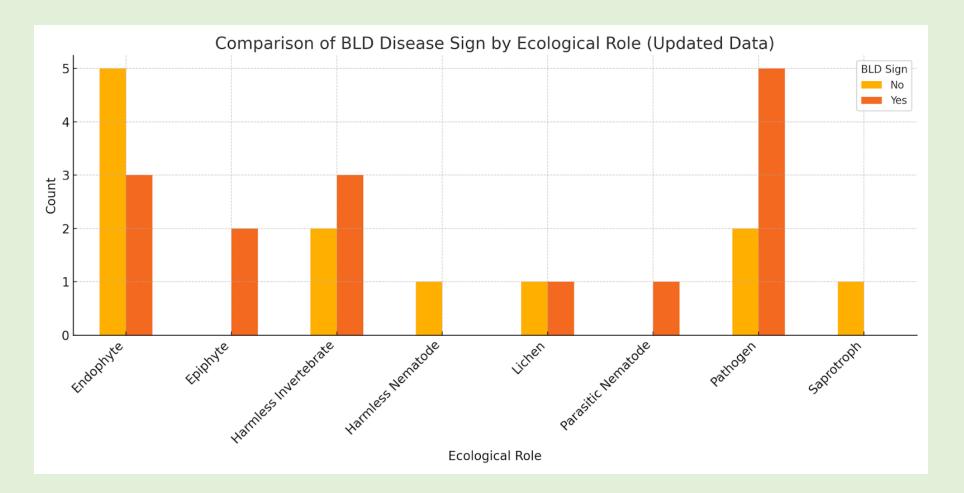






BLD signs?	Tree Species	Primers	Microorganism identified as a result of barcoding (NGS)
yes	Fagus grandifolia	LSU	Pseudofusicoccum sp
yes	Fagus grandifolia	LSU	Fagus grandifolia
yes	Fagus grandifolia	LSU	Exobasidium noetherae
yes	Fagus grandifolia	LSU	Pseudofusicoccum sp
no	Fagus grandifolia	LSU	Filobasidium sp.
no	Fagus grandifolia	LSU	Fagus grandifolia
yes	Fagus grandifolia	LSU	Could not complete
yes	Fagus grandifolia	LSU	Fagus grandifolia
yes	Fagus grandifolia	LSU	Litylenchus crenatae
yes	Fagus grandifolia	LSU	Lestodiplosis juniperina
no	Fagus grandifolia	LSU	Litylenchus crenatae
no	Fagus grandifolia	LSU	Fagus grandifolia
yes	Fagus grandifolia	LSU	Fagus grandifolia
yes	Fagus grandifolia	LSU	Acaphyllisa populus
no	Fagus grandifolia	LSU	Caenorhabditis elegans
no	Fagus grandifolia	LSU	Fagus grandifolia
no	Fagus sylvatica	LSU	Adineta vaga
no	Fagus sylvatica	LSU	Fagus grandifolia
yes	Fagus grandifolia	ITS	Erysiphe erineophila
yes	Fagus grandifolia	ITS	Litylenchus crenatae
no	Fagus grandifolia	ITS	Litylenchus crenatae
no	Fagus grandifolia	ITS	Teratosphaericola ellenclarkiae
yes	Fagus grandifolia	ITS	Erysiphe <u>wadae</u>
yes	Fagus grandifolia	ITS	Meira sp. IK-2015
no	Fagus grandifolia	ITS	Litylenchus crenatae mccannii
no	Fagus grandifolia	ITS	Meira sp. IZ-1189
yes	Fagus grandifolia	ITS	Litylenchus crenatae mccannii
no	Fagus grandifolia	ITS	Litylenchus crenatae mccannii
no	Fagus grandifolia	ITS	Erysiphe ostryae
no	Fagus grandifolia	ITS	Erysiphe wadae
no	Fagus sylvatica	ITS	Erysiphe wadae
no	Fagus sylvatica	ITS	Uncultured Ramariopsis
no	Fagus sylvatica	ITS	Uncultured Taphrin
no	Fagus sylvatica	ITS	Ceramothyrium linnaeae
yes	Fagus sylvatica	ITS	Erysiphe wadae
yes	Fagus sylvatica	ITS	Colpoda steini
no	Fagus sylvatica	ITS	Xanthoria montana
no	Fagus sylvatica	ITS	Uncultured fungus
no	Fagus sylvatica	ITS	Ramularia stellenboschensis
no	Fagus sylvatica	ITS	Uncultured fungus
yes	Fagus sylvatica	ITS	Pseudofusicoccum s
yes	Fagus sylvatica	ITS	Dothideomycetes sp



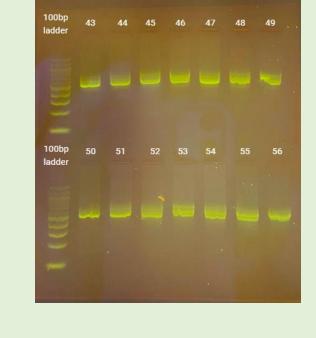


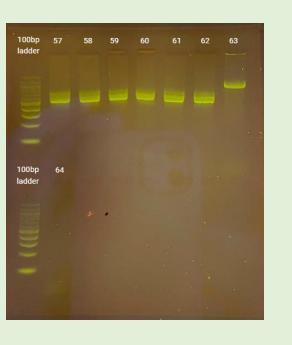
## Results











## Discussion

The results show that primer choice greatly influences the species detected during DNA barcoding. In Sanger sequencing, the LSU primers identified plant species such as the American beech itself. The ITS primer pairs yielded fungal results. The NGS results also reflected differences between the two primer sets. LSU sequencing detected the American beech itself and less specific fungal identification. In contrast, ITS identified a broad range of fungi and even the nematode associated with BLD, Litylenchus crenatae mccannii. Interestingly, NGS sequencing picked up Litylenchus crenatae mccannii on both samples: affected and not visibly affected by BLD. These findings have meaningful implications for understanding the complex causes of BLD. The abundance of pathogenic fungi on beech leaves damaged by BLD shows that the fungal community likely has a role in BLD.

## References

EAB Network -. (2020). Emeraldashborer.info. https://www.emeraldashborer.info/other-species/BLD

Beech Leaf Disease. (n.d.). Invasive Species Centre. Retrieved from

https://www.invasivespeciescentre.ca/invasive-species/meet-the-species/invasivepathogens/beech-leaf-disease/

New Research into Potential Impacts of Beech Leaf Disease (BLD) to Begin at UNH. (2023, April 10).

UNH Today. Retrieved From

https://www.unh.edu/unhtoday/2023/04/new-research-examines-potentialimpacts-beech-leaf-disease Gosling, N. (2023, April 18). New research into potential impacts of beech leaf disease (BLD) to begin at

UNH. UNH Today. Retrieved from

https://www.unh.edu/unhtoday/2023/04/new-research-examines-potentialimpacts-beech-leaf-disease

New York State Department of Environmental Conservation. Beech Leaf Disease (n.d.). Dec.ny.gov.

Retrieved from <a href="https://dec.ny.gov/nature/forests-trees/forest-health/forest-">https://dec.ny.gov/nature/forests-trees/forest-health/forest-</a> health-beech-leaf-disease

North Carolina Extension Gardener Plant Toolbox. Fagus sylvatica (Common Beech, European Beech)

(n.d.). Plants.ces.ncsu.edu. <a href="https://plants.ces.ncsu.edu/plants/fagus-sylvatica/">https://plants.ces.ncsu.edu/plants/fagus-sylvatica/</a> DNA Learning Center Barcoding 101. (n.d.). Dnabarcoding101.org. Retrieved

https://dnabarcoding101.org/lab/index.html

National Audubon Society. (1998). Familiar trees of North America. Eastern region.

# Acknowledgements

We would like to thank our mentors and teachers for their valuable guidance throughout this project. Additionally, special thanks the Thompson Family Foundation for making this research possible.