

University of South Alabama

JagWorks@USA

---

Poster Presentations

Honors College

---

5-2024

## Survey of the Boletes from Fish River Nature Preserve

Noah T. Nelson

Follow this and additional works at: [https://jagworks.southalabama.edu/honors\\_college\\_posters](https://jagworks.southalabama.edu/honors_college_posters)



Part of the [Biology Commons](#), [Genetics and Genomics Commons](#), and the [Other Life Sciences Commons](#)

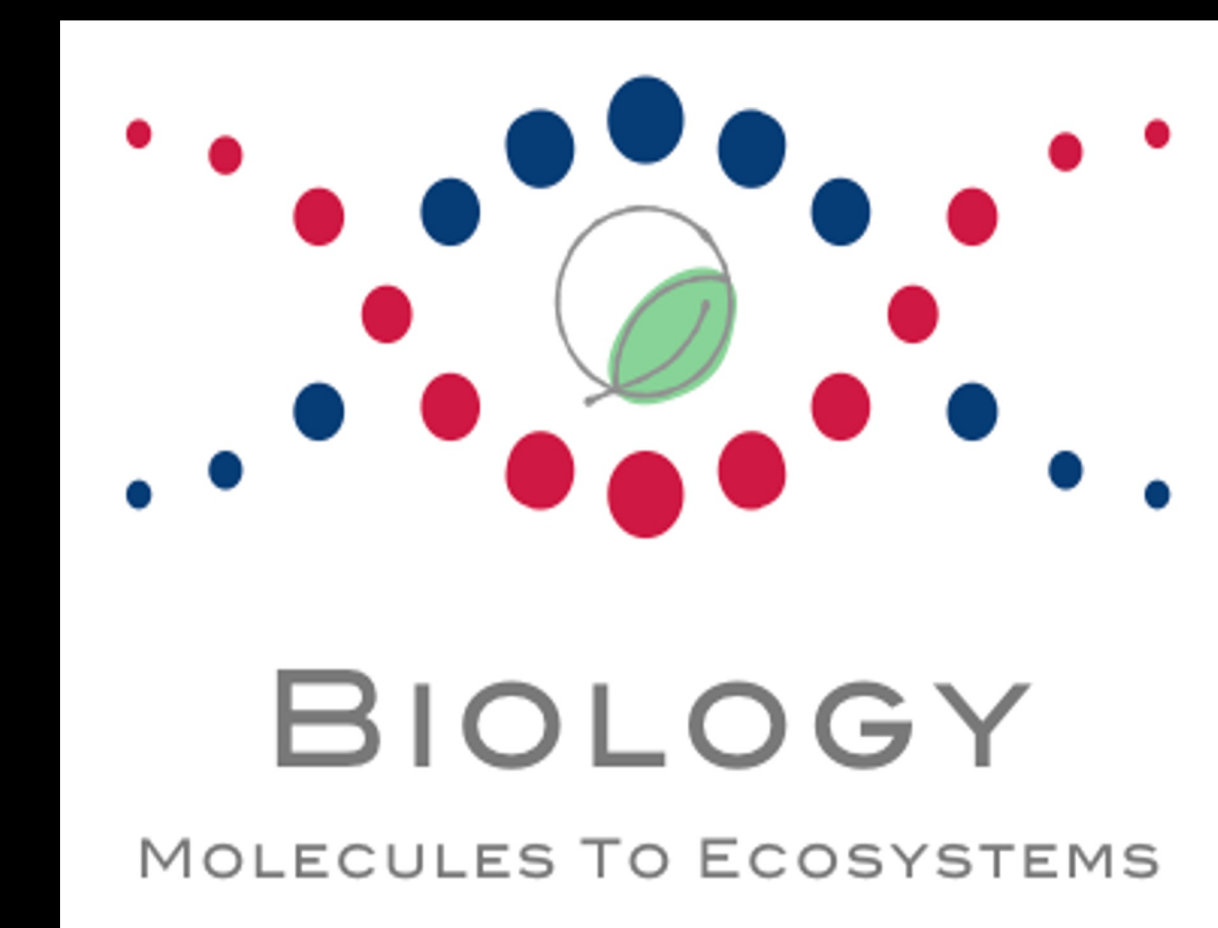
---



# A Survey of the Boletes from Fish River Nature Preserve

Noah Nelson; Mentor: Dr. Juan Luis Mata

Department of Biology, College of Arts and Sciences, University of South Alabama



## Introduction

Boletes are a special kind of mushroom inside of the Kingdom Fungi that are distinguished from other mushrooms by their uniquely pored hymenium, as opposed to having gills. Many boletes are ectomycorrhizal, meaning they are mutualists with vascular plants, and thus beneficial to the overall health of the terrestrial ecosystem. Some are well-known for their edibility and are of economic importance. In the United States 59 genus level clades comprising 290 operational taxonomic units have been described with the southeastern US and Gulf Coast region being less researched and recorded. In Alabama, only 56 species have been reported so far. The Fish River Nature Preserve (FRNP) is located south of Fairhope in Baldwin County, and was recently purchased by the South Alabama Land Trust Conservation group. This property includes an upper level section with a tract dominated by oaks and another by pines, which is fire-controlled, and one near the river dominated by palmetto palms. This project aims to answer the question: "How many bolete species can be recorded in FRNP?" By identifying the distribution and abundance of several bolete species in southern Alabama, and how it compares to the ecosystem of the FRNP.

## Methods

### Field Collections & Descriptions

Upon spotting Bolete mushrooms, an image is taken, and then a knife is used to dig out the specimen without damaging it. The Boletes are then wrapped in wax paper with a label to prevent mixing specimens and placed in a basket. Once in the lab, all specimens are imaged again and individually described.

### Spore Metrics

A small piece of hymenium is cut off and prepared on a semi permanent slide under a light microscope. 20 spores are measured in their length and width. Raw data are entered in a Excel worksheet and basic statistics including range, average, and spore length to width ratio are calculated.

### DNA

A small portion of the pileus is removed and wrapped in aluminum foil and stored in a -20 °C freezer. Only 0.1 – 10.0 ng of total DNA is required for this protocol. That tissue will be then subjected to the modified DNA extraction protocol. Polymerase Chain Reaction, or PCR products will be imaged using protein gel electrophoresis. The DNA extraction process is described as grounded pileus added to a centrifuge tube with a lysis buffer then after incubation, chloroform is added to the mix. The centrifuge tube is then subject to several rounds of microcentrifusion. Successfully amplified genetic products will then be sent for external sequencing and the data will be analyzed using the software Geneious to trim and align sequences and then search for matching ones in gene databases, such as GenBank.

### Taxonomic Identification

Together with morphological characteristics – field description and spore statistics – and gene sequences it will be possible to arrive at a confident conclusion regarding the taxonomic identity of each specimen. Once this is completed a list of species can be compiled to address the objectives of this work.

Table 1: Species List

Collection	Species Identification	Taxonomic Level Confirmed by DNA	Location	Date Collected
NTN-16	<i>Aureoboletus russellii</i>	N/A	Environmental Study Center	08/04/2022
NTN-09	<i>Boletus alutaceus</i>	Species	FRNP	08/01/2022
NTN-21	<i>Boletus vermiculosoides</i>	Species	FRNP	08/19/2022
NTN-14	<i>Exsudoporus floridanus</i>	Species	USA Campus	08/03/2022
NTN-01	<i>Gyroporus castaneus</i>	Species	USA Campus	07/21/2022
NTN-03	<i>Gyroporus castaneus</i>	Species	USA Campus	07/21/2022
NTN-06	<i>Gyroporus castaneus</i>	Species	FRNP	07/28/2022
NTN-12	<i>Gyroporus castaneus</i>	Species	Muddy Creek	08/03/2022
NTN-17	<i>Gyroporus castaneus</i>	N/A	FRNP	08/08/2022
NTN-22	<i>Gyroporus castaneus</i>	N/A	FRNP	08/31/2022
NTN-10	<i>Hortiboletus rubellus</i>	Species	Muddy Creek	08/03/2022
NTN-13	<i>Hortiboletus rubellus</i>	N/A	Muddy Creek	08/03/2022
NTN-04	<i>Leccinellum albellum</i>	N/A	FRNP	07/28/2022
NTN-18	<i>Leccinellum albellum</i>	N/A	FRNP	08/08/2022
NTN-02	<i>Phylloporus bellus</i>	Species	USA Campus	07/21/2022
NTN-15	<i>Phylloporus rhodoxanthus</i>	Species	Environmental Study Center	08/04/2022
NTN-05	<i>Strobilomyces floccopus</i>	N/A	FRNP	07/28/2022
NTN-23	<i>Suillus decipiens</i>	Species	FRNP	08/31/2022
NTN-11	<i>Tyloporus balloui</i>	Species	Muddy Creek	08/03/2022
NTN-08	<i>Tyloporus rhoadsiae</i>	Species	FRNP	08/01/2022
NTN-20	<i>Tyloporus rubrobrunneus</i>	Species	FRNP	08/19/2022
NTN-26	<i>Tyloporus rubrobrunneus</i>	Species	FRNP	09/07/2022
NTN-24	<i>Xanthoconium affine</i>	Species	FRNP	08/31/2022
NTN-25	<i>Xanthoconium affine</i>	Species	FRNP	09/07/2022
NTN-07	<i>Xerocomus intermedius</i>	Genus	FRNP	08/01/2022
NTN-19	<i>Xerocomus subtomentosus</i>	Genus	FRNP	08/08/2022

Species are listed in descending alphabetical order.  
N/A means that there was no PCR recovered, or supportive "molecular identification" recovered for that given specimen.

Table 2: DNA Results

Collection	PCR Recovered	Query Cover	Percent Identity	Taxonomic Level Confirmed	GenBank Number	I.D.
NTN-01	Yes	99.00%	90.65%	Species	OR052614.1	<i>Gyroporus castaneus</i>
NTN-02	Yes	99%	99.47%	Species	OP749565.1	<i>Phylloporus bellus</i>
NTN-03	Yes	99%	99.47%	Species	OP643111.1	<i>G. castaneus-INO1</i>
NTN-04	No	N/A	N/A	N/A	N/A	N/A
NTN-05	No	N/A	N/A	N/A	N/A	N/A
NTN-06	Yes	99%	98.78%	Species	PP156342.1	<i>G. castaneus-INO1</i>
NTN-07	Yes	94%	99.84%	Genus	MH796996.1	<i>Xerocomus sp.</i>
NTN-08	Yes	81%	99.31%	Species	OP580287.1	<i>Tyloporus rhoadsiae</i>
NTN-09	Yes	96%	100.00%	Species	ON383386.1	<i>Boletus alutaceus</i>
NTN-10	Yes	9%	90.57%	Species	KX438318.1	<i>Hortiboletus rubellus</i>
NTN-11	Yes	98%	98.72%	Species	OP541643.1	<i>T. balloui-MS01</i>
NTN-12	Yes	99%	99.29%	Species	OP643111.1	<i>G. castaneus-INO1</i>
NTN-13	No	N/A	N/A	N/A	N/A	N/A
NTN-14	Yes	92%	79.48%	Species	MH211799.1	<i>Exsudoporus floridanus</i>
NTN-15	Yes	100%	99.71%	Species	ON134027.1	<i>Phylloporus rhodoxanthus</i>
NTN-16	Yes	97%	99.70%	N/A	Inconclusive	Inconclusive
NTN-17	Yes	93%	90.33%	N/A	Inconclusive	Inconclusive
NTN-18	Yes	94%	99.61%	N/A	Inconclusive	Inconclusive
NTN-19	Yes	94%	99.84%	Genus	MH796996.1	<i>Xerocomus sp.</i>
NTN-20	Yes	72%	85.86%	Species	OP470444.1	<i>Tyloporus rubrobrunneus</i>
NTN-21	Yes	96%	99.55%	Species	OM972363.1	<i>Boletus vermiculosoides</i>
NTN-22	Yes	63%	86.01%	N/A	Inconclusive	Inconclusive
NTN-23	Yes	100%	99.79%	Species	AF166508.1	<i>Suillus decipiens</i>
NTN-24	Yes	100%	99.65%	Species	ON412796.1	<i>Xanthoconium affine</i>
NTN-25	Yes	100%	99.47%	Species	OM716990.1	<i>Xanthoconium affine</i>
NTN-26	Yes	100%	90.84%	Species	OP470444.1	<i>Tyloporus rubrobrunneus</i>

Query Cover is the percentage of the "query sequence" (uploaded nucleotide sequence) that aligns with a "hit sequence" in the GenBank database.

Percent Identity is the percentage of identical nucleotides or amino acids between two sequences.

N/A means that there was no PCR recovered for that given specimen.

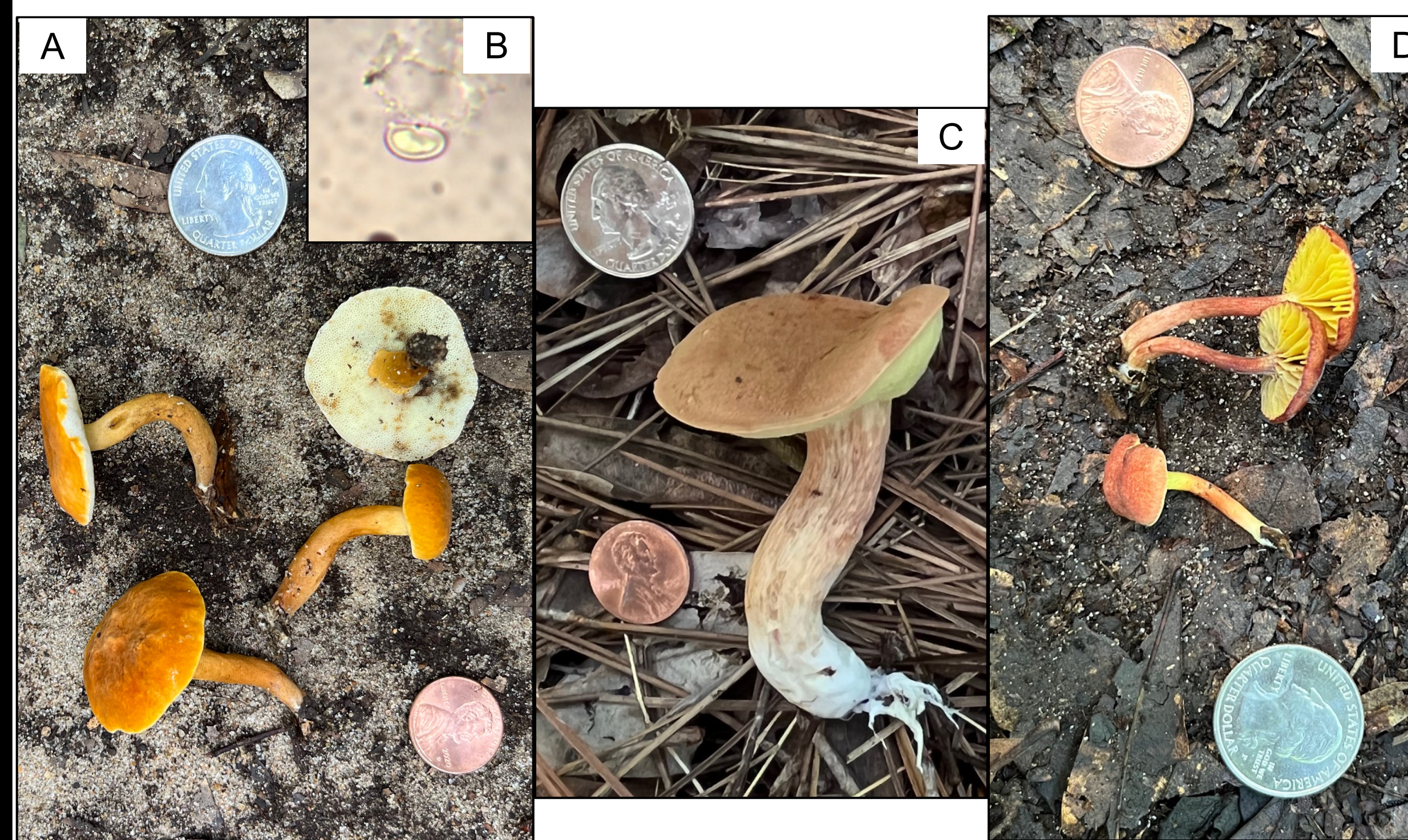
Inconclusive means Query Cover, Percent Identity, or "molecular identification" were not supportive.

Table 3: Initial vs Reevaluated Identifications

Collection	Initial Identification	Reevaluated Identification	Identity Change
NTN-01	<i>Gyroporus castaneus</i>	<i>Gyroporus castaneus</i>	No
NTN-02	<i>Phylloporus sp.</i>	<i>Phylloporus bellus</i>	No
NTN-03	<i>Gyroporus castaneus</i>	<i>Gyroporus castaneus</i>	No
NTN-04	<i>Leccinellum albellum</i>	<i>Leccinellum albellum</i>	No
NTN-05	<i>Strobilomyces floccopus</i>	<i>Strobilomyces floccopus</i>	No
NTN-06	<i>Gyroporus castaneus</i>	<i>Gyroporus castaneus</i>	No
NTN-07	<i>Boletus rubropunctus</i>	<i>Xerocomus intermedius</i>	Yes
NTN-08	<i>Tyloporus rhoadsiae</i>	<i>Tyloporus rhoadsiae</i>	No
NTN-09	<i>Boletus rubellus</i>	<i>Boletus alutaceus</i>	Yes
NTN-10	<i>Boletus patrioticus</i>	<i>Hortiboletus rubellus</i>	Yes
NTN-11	<i>Tyloporus balloui</i>	<i>Tyloporus balloui</i>	No
NTN-12	<i>Gyroporus castaneus</i>	<i>Gyroporus castaneus</i>	No
NTN-13	<i>Hortiboletus rubellus</i>	<i>Hortiboletus rubellus</i>	No
NTN-14	<i>Boletus sp.</i>	<i>Exsudoporus floridanus</i>	Yes
NTN-15	<i>Phylloporus sp.</i>	<i>Phylloporus rhodoxanthus</i>	No
NTN-16	<i>Aureoboletus russellii</i>	<i>Aureoboletus russellii</i>	No
NTN-17	<i>Gyroporus castaneus</i>	<i>Gyroporus castaneus</i>	No
NTN-18	<i>Leccinellum albellum</i>	<i>Leccinellum albellum</i>	No
NTN-19	<i>Tyloporus peralbidus</i>	<i>Xerocomus subtomentosus</i>	Yes
NTN-20	<i>Leccinellum chalybaeum</i>	<i>Tyloporus rubrobrunneus</i>	Yes
NTN-21	<i>Suillus cothurnatus</i>	<i>Boletus vermiculosoides</i>	Yes
NTN-22	<i>Gyroporus castaneus</i>	<i>Gyroporus castaneus</i>	No
NTN-23	<i>Leccinellum albellum</i>	<i>Suillus decipiens</i>	Yes
NTN-24	<i>Boletus sp.</i>	<i>Xanthoconium affine</i>	Yes
NTN-25	<i>Leccinellum albellum</i>	<i>Xanthoconium affine</i>	Yes
NTN-26	<i>Leccinellum chalybaeum</i>	<i>Tyloporus rubrobrunneus</i>	Yes

Initial Identification given to all collections based on morphological analysis  
Reevaluated Identification based on molecular analysis

Figure 1: Mushroom Field Collections



- A) (NTN-17) *Gyroporus castaneus* "Chestnut Bolete" field collection from FRNP, AL (Baldwin County); Oak Dominated Forest under drier conditions; 08/08/2022
- B) (NTN-17) *Gyroporus castaneus* spore image: spore range is 7.4-10.0 X 4.2- 5.8 μm with an average of 8.8 X 5.0 μm; The Q is 1.5 - 2.1 and has a Qavg of 1.8.
- C) (NTN-09) *Boletus alutaceus* "Leather Colored Bolete" field collection from FRNP, AL (Baldwin County); Oak and pine dominated forest; 08/01/2022.
- D) (NTN-02) *Phylloporus bellus* field collection from University of South Alabama: Bike Trails; Scattered on soils in the mixed pine conifer forest; 07/21/2022.

## Results

- ❖ A total 26 collections were made throughout this project, 16 of which were collected from the FRNP (61.5% of collected specimens).
- ❖ In this project a total of 17 individual species across 12 genera were identified (Tab 01).
- ❖ After joint morphology, spore, and molecular analysis were completed, it was concluded that 11 unique species across 8 genera have been identified at the FRNP; 64.7% of all mushrooms species encountered and classified throughout this project (Tab 01).
- ❖ Of the 26 specimens, 23 yielded an extracted PCR product (Tab 02).
- ❖ Of the 23 products, 19 yielded conclusive results after amplification, where 4 yielded inconclusive results (Tab 02).
- ❖ Of the 19 conclusive results, 17 specimens were successfully confirmed to the genus and species level, where the other 2 specimens were confirmed only to the genus level; The remaining 7 specimens that did not yield a DNA confirmed identity were identified morphologically to the assumed species level (Tab 02).

## Conclusions

- ❑ The genus *Boletus* consisted of two collections of individual species (*alutaceus* and *vermiculosoides*) with *Boletus alutaceus* being native to New England, making it a truly unique and rare collection (Fig 01-C).
- ❑ The genus *Gyroporus* was the most collected genera with 6 total collections (all of the same species *castaneus*) accounting for 23% of all collections made. It ties with the genus *Tyloporus* as the most frequent genera found on the FRNP - both having 3 collections each.
- ❑ The species *Gyroporus castaneus* however was the most frequently encountered species found on the FRNP accounting for 18.75% of all collections made on the property (Fig 01-A).
- ❑ The genus *Tyloporus* was the second most collected genera in this project, accounting for 15% of the total collection.
- ❑ Two species of the genus *Phylloporus* were identified (*rhodoxanthus* and *bellus*) with *Phylloporus bellus* having no previous reported sightings on iNaturalist for Mobile or Baldwin Counties, AL, making this a novel collection (Fig 01-D).
- ❑ By morphological analysis alone, species identification was successful up to only 57.6% of the time; 15 out of 26 species identified correctly initially (Tab 03).

## Literature Cited

- Bessette, A.E., Roody, W.C., Bessette, A.R., (2016). Boletes of Eastern North America. Syracuse University Press
- Hibbett, D. S., Binder, M., Bischoff, J. F., Blackwell, M., Cannon, P. F., Eriksson, O. E., ... & Zhang, N. (2007). A higher-level phylogenetic classification of the Fungi. *Mycological Research*, 111(5), 509-547. <https://doi.org/10.1016/j.mycres.2007.03.004>
- Lee, S.B., Taylor, J.W. (1990). Isolation of DNA from fungal mycelia and single spores. *Mitchell, J. I., Roberts, P. J., & Moss, S. T.* (1995). Sequence or structure?: A short review on the application of nucleic acid sequence information to fungal taxonomy. *Mycologist*, 9(2), 67-75. ISSN 0269-915X. doi: 10.1016/S0269-915X(09)80212-7.
- O'Reilly, P., *First Nature*. (2023, June 13). Boletus subtomentosus, Suede Bolete mushroom. Retrieved from <https://www.firstnature.com/boletus-subtomentosus>
- White, T & Bruns, Tom & Lee, Steven & Taylor, John & Innis, M & Gelfand, D & Sninsky, John. (1990). Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics.

### Acknowledgements

We are thankful to the staff of FRNP for allowing collections in their property. Thank you also to the USA Foundation.