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Think globally, barcode locally: nine years of macrofungi sampling reveals extensive biodiversity at the ordway-swisher biological station, a subtropical site in Florida

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ABSTRACT

The Ordway-Swisher Biological Station (OSBS) is a 38-km^2 reserve owned by the University of Florida and is part of the National Ecological Observatory Network (NEON). The reserve contains several iconic Florida habitats, such as sandhill, mesic hammock, and scrubby flatwoods. While plants and animals have been extensively studied at OSBS, the fungi remain poorly known. Fungal inventories are critical to increase knowledge of both fungal diversity and species ranges, and thus to provide foundational data for a wide array of applications in ecology and resource management. Here, we present the results of a nine-year effort to collect, preserve, and DNA barcode the macrofungi at OSBS. This effort generated >1200 vouchered specimens and 984 ITS rDNA sequences, representing more than 546 species. Our sampling was dominated by Basidiomycota and revealed a high diversity of symbiotic ectomycorrhizal fungi, particularly species of *Amanita, Cortinarius,* and *Russula.*

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Sampling curves and both Chao1 and Jacknife1 richness estimators suggest that our DNA barcoding efforts captured only about half of the macrofungi species and that a more complete inventory would detect 897–1177 macrofungi species at OSBS. Our sampling found more species of macrofungi at OSBS than the known number of vertebrate animal species at the reserve and our estimates also suggest that there are likely more macrofungi species than plant species at OSBS. This study is the first comprehensive macrofungi inventory within a NEON site and highlights the importance of long-term monitoring to provide novel data on fungal diversity, community structure, conservation, biogeography, and taxonomy.

1. Introduction

Globally, the number of fungi is estimated to range between 2.21 and 3.83 million species (Niskanen et al., 2025) but only approximately 150,000 fungal species have been formally described (Lücking et al., 2021). Fungi play many key roles in terrestrial ecosystems: as saprotrophs that decay organic matter and release nutrients that sustain ecosystem productivity, as mutualists that symbiotically associate with many organisms to promote growth and tolerate stress, and as parasites or pathogens that obtain their nutrients from other living organisms and serve as important regulators of host populations (Boddy, 2016; Willis, 2018).

Because of their high species richness and importance in ecosystem processes, fungi are receiving increased attention in conservation biology and systems biology (Heilmann-Clausen et al., 2015; Gonçalves et al., 2021). However, only 818 fungal species out of more than two million have been assessed for the IUCN Red List (IUCN, 2025). Knowledge gaps about basic aspects of fungal biology, such as habitat niches, ecological requirements, and species distribution patterns make studies of fungal ecology and biodiversity particularly challenging (Peay et al., 2008; Větrovský et al., 2019). Unfortunately, most long-term monitoring studies in well-documented habitats ignore fungi. For example, many of the sites of the Long-Term Ecological Research Network (LTER) across the globe and National Ecological Observatory Network (NEON) in the USA have complete or near-complete inventories of species of plants and vertebrate animals (Nagy et al., 2021), but lack extensive fungal inventories (Bruns, 2012; O'Brien et al., 2005). Even lichens, which are generally easier to assess because their thalli are visible year-round, have not been well documented from many of the NEON and LTER sites (Kaminsky and Smith, 2024).

Fungal inventories are therefore critical to increase our knowledge of fungal diversity and to elucidate the biogeographic distribution of species across regions and ecosystems. In order to generate useful fungal inventories, proper identification of fungal specimens is of the utmost importance. While many fungi can be identified based on their morphological features, the high diversity of fungal species, the large number of undescribed species, the notable morphological plasticity within species, and the common occurrence of cryptic species or species complexes can make morphological identification of fungi extremely challenging (Yahr et al., 2016; Hofstetter et al., 2019). Accordingly, the use of molecular approaches (DNA barcoding) is often the most reliable method to distinguish between closely related species. However, the use of tools such as the NCBI basic local alignment search tool (BLAST) without careful scrutiny of putative identification matches can lead to misidentification because sequence names on Genbank are often incorrect and not frequently updated (Hofstetter et al., 2019; Lücking et al., 2020). Thus, fungal inventories that are supported by vouchered specimens that also include sequence data are a valuable resource for populating public databases and improving sequence-based identification of fungi, including for environmental (eDNA) sequencing (Truong et al., 2017). Despite calls from the research community (Bruns, 2012), there have been very few long-term efforts to inventory local fungal richness based on macroscopic sporomes (i.e. macroscopic spore-producing structures of fungi, such as mushrooms, puffballs, and truffles) that are well documented, vouchered, and sequenced (Henkel et al., 2012; Vandegrift et al., 2023).

The National Ecological Observatory Network (NEON) comprises 81 sites across the United States and aims to collect long-term ecological and organismal data in terrestrial and aquatic ecosystems at a continental scale for at least 30 years (Keller et al., 2008; Loescher et al., 2017; NSF NEON, 2024). These data have been used to address ecological, taxonomic, and large-scale scientific questions across time and space (Kao et al., 2012; Loescher et al., 2017), with long-term monitoring being critical to better understanding the effects of climate change and invasive species on native communities (Kao et al., 2012). The Ordway-Swisher Biological Station (OSBS) is a 38-km^2 reserve owned and managed by the University of Florida in Putnam County, Florida and has been a core terrestrial NEON site since 2011. The OSBS is one of the southernmost sites in the NEON network and one of the only terrestrial NEON sites located in a subtropical climate. Terrestrial habitats at OSBS are representative of central Florida and North American Coastal Plain habitats including sandhill, baygall, mesic hammock and scrubby flatwoods. While the flora and fauna at OSBS have been well studied (e.g. Jantzen et al., 2019; NEON Biorepository, 2024; https://ordway-swisher.ufl.edu), the fungal communities remain poorly known.

The OSBS is located approximately 30 miles east of the University of Florida campus in Gainesville, Florida. The University of Florida is one of the oldest academic institutions in Florida, and because it is the longstanding home of the Florida Agricultural Experiment Station, it is also one of the few locations in Florida with a well-established tradition of mycology (Weber, 1961; Kimbrough, 2003). In this regard, one Florida mycologist stands out for his extensive contributions: William A. Murrill. From the 1930s to the 1950s, Murrill described approximately 700 new species of fleshy macrofungi (Weber, 1961), primarily from Gainesville and nearby sites (Halling, 1986). The type specimens of Murrill's Florida fungal species are housed primarily at the Florida Museum of Natural History (FLAS) at the University of Florida. However, his collections have been difficult to study due to their sometimes fragmented type specimens and because poor historical storage conditions and insect infestations have interfered with good preservation of their DNA (e.g. Buyck and Adamèík, 2011; Looney, 2014; Eberhardt et al., 2023). Habitat destruction due to residential and commercial construction in the Gainesville area has also hindered the re-collection of contemporary specimens from Murrill's original locations. Consequently, collections from OSBS are useful as taxonomic records that may correspond to some of the many Florida fungi that were described by Murrill and are useful for broader interpretation (and re-interpretation) of Murrill's named species (e.g. Looney, 2014; Biketova et al., 2022; Eberhardt et al., 2023).

The aim of this study is to provide a baseline survey of the macrofungal species found at OSBS supported by vouchered specimens with publicly available DNA barcode sequences. Here we provide an overview of our fungal inventory effort, provide commentary on the biogeographical patterns revealed by this sampling, and highlight the high macrofungal diversity and richness patterns based on sporome sampling at this important NEON site.

2. Material and methods

2.1. Fungal sampling

Sporomes were opportunistically collected on numerous collecting campaigns during 2013–2017, 2020 and 2022–2024 across several habitat types at OSBS, including mesic hammock, xeric hammock, sandhill, scrubby flatwood, and successional hardwood forests (https://ordway-swisher.ufl.edu). Dominant plants in these habitats consist of *Carya*, *Pinus*, *Quercus*, and other deciduous hardwoods. Two other habitats, baygall and basin swamp, were infrequently sampled because of the persistently wet soil and seasonal flooding as well as minimal coverage by ectomycorrhizal (ECM) tree hosts. During these surveys, we focused primarily on collecting fleshy macrofungi growing on soil or well decayed wood, but the surveys also included any macrofungi that were encountered. Collecting expeditions were optimized towards the summer monsoon season (May through September) and the shorter winter wet season (December and January) but also occurred at other times of the year when conditions were sufficiently wet for macrofungi production. Specimens were transported to the lab on ice and photographed within 24 h. Photographs of most of the collections during 2016–2021 were captured and stored in the Smith lab at University of Florida and/or uploaded to the Mycoportal with corresponding specimen records (Miller and Bates, 2017). Photographs of most of the collections during 2021–2024 were also uploaded to iNaturalist (https://www.inaturalist.org/) under a project entitled "Fungi of Ordway-Swisher". Several of the collections included in this study were obtained by a large group of visiting fungal biologists as part of the annual foray for the Mycological Society of America meeting on 10 July 2022. Fresh tissues from specimens were preserved in cetyltrimethylammonium bromide (CTAB) and an alkaline extraction buffer (Vandepol et al., 2020) for later DNA extraction. Specimens were dried on a food dehydrator at 40 °C and stored in plastic ziplock bags with silica gel. Dried specimens are deposited in the Fungarium of the Florida Museum of Natural History at the University of Florida (FLAS).

2.2. Fungal identification and sequencing

Macroscopic characters such as shape, size, color, odor, and habit were recorded for each specimen. Microscopic analyses were only performed on a subset of specimens where microscopic measurements were helpful to inform taxonomic decisions. To describe microscopic characters, small pieces of dried tissue were sliced with a razor blade and mounted in water, 3 % KOH, Congo Red, or Melzer's reagent and then studied by light microscopy using a Zeiss Axio Imager.A2 compound microscope (Carl Zeiss, Oberkochen, Germany). Based on a combination of macroscopic and microscopic morphological features, specimens were identified to the lowest possible taxonomic level using available dichotomous keys (Seaver, 1928; Largent and Thiers, 1977; Stuntz, 1977; Breitenbach and Kränzlin, 1986; Hanlin, 1998; Kimbrough, 2000; Beug et al., 2014; Bessette et al., 2017, 2019; Bunyard and Justice, 2020).

DNA was extracted for the majority of specimens using an alkaline extraction buffer according to Vandepol et al. (2020) or a modified CTAB extraction protocol (Gardes and Bruns, 1993). DNA sequences were generated for most specimens using Sanger sequencing of the nuclear ribosomal DNA internal transcribed spacer region ITS1-5.8S-ITS2 (hereafter ITS) region, and in some cases part of the large ribosomal subunit (28S) as well. For Sanger sequencing, we amplified the ITS region using primers ITS1F (Gardes and Bruns, 1993) and ITS4 or Basidiomycota-specific ITS4B (White et al., 1990) following the protocols of Truong et al. (2017). For some select specimens, the 28S region was amplified with LROR and LR5 primers using a similar protocol (Vilgalys and Hester, 1990). PCR products were visualized on 1.5 % agarose gels stained with SYBR Green I (Molecular Probes, Oregon, USA) and amplicons cleaned using EXO (Exonuclease I) and AP (Antarctic Phosphatase) enzymes (New England Biolabs, Massachusetts, USA) (Werle et al., 1994). Amplicons from Sanger were sequenced using either ITS1F or ITS4 or both primers by GENEWIZ (South Plainfield, New Jersey, USA) or Eurofins Genomics (Louisville, Kentucky, USA).

For a subset of specimens collected in 2022–2024 and some older specimens where Sanger sequences could not be readily obtained during the first attempt, we sequenced the ITS and partial 28S in one reaction using PacBio sequencing. For PacBio sequencing, an approximately 1600 bp region of rDNA encompassing the full internal transcribed spacer (ITS) region and 1000 bp of the 28S was amplified using the primers ITS1catta (Tedersoo and Anslan, 2019) and LR5. A five-nucleotide padding sequence and 16-nucleotide combinatorial barcodes were added to the 5' end of both primers for dual-index sample multiplexing. Amplification was carried out using Phusion High-Fidelity PCR Master Mix with HF Buffer (Thermo Scientific, Waltham, MA, USA) and with PCR protocol as follows: initial denaturation at 98 °C for 1 min, followed by 35 cycles of 98 °C for 10 s, 52 °C for 45 s, and 72 °C for 90 s, and a final extension at 72 °C for 10 min. The DNA concentration of PCR products was coarsely quantified based on the brightness of gel electrophoresis bands when stained with SafeView Classic DNA Stain (Applied Biological Materials, Richmond, BC, Canada) and visualized under UV light (Runnel et al., 2022). Products were combined in equal concentrations and purified using Mag-Bind TotalPure NGS Kit (Omega Bio-tek Inc, Norcross, CA, USA) at a 0.8x Mag-Bind to DNA library volume ratio. SMRTbell library preparation of the purified pools was performed by the Genomics and Cell Characterization Core Facility at the University of Oregon (https://gc3f.uoregon.edu/). Libraries were prepared using the SMRTbell prep kit 3.0 with the Sequel II binding kit 3.2 (Pacific Biosciences, Menlo Park, CA, USA) and subsequently sequenced on a PacBio Sequel II HiFi system.

2.3. Bioinformatics and Statistical analyses

Sanger sequences were quality-checked and edited with Sequencher 5.0.1 (Gene Codes, Michigan, USA) and Geneious 11.1.5. (Auckland, New Zealand). For PacBio sequencing, circular consensus sequences (CCS) were quality-filtered to a minimum read quality of 40 (99.99 % mean base call accuracy per read). Reads were then demultiplexed using PacBio's Lima v. 2.7.1 software, with a minimum barcode score of 93 (Tedersoo et al., 2021). Primers were trimmed using cutadapt 3.7 (Martin, 2011) with a maximum error rate of 0.2. Sample reads from each individual specimen were de-replicated, and any reads between 100 bp and 8000 bp were retained using VSEARCH v. 2.18.0 (Rognes et al., 2016). For the purposes of this study, we selected the most abundant sequence variant for each specimen as the representative sequence and then performed BLAST searches separately on the ITS and 28S rDNA to verify the sequences and ensure that no contaminant sequences were included in our dataset.

To evaluate the diversity of macrofungi in our large dataset of macrofungi ribosomal sequences, we extracted the ITS1 region from all sequences (Sanger + PacBio) using ITSx v 1.1.2 (Bengtsson-Palme et al., 2013). We selected the ITS1 region because our dataset included more specimens with a complete ITS1 sequence than a complete ITS2 sequence. We retained all sequences longer than 100 bp. The resulting ITS1 sequences were then clustered into operational taxonomic units (OTUs) at 97 % similarity using VSEARCH v2.28.0 (Rognes et al., 2016). The 97 % cutoff is commonly used to approximate fungal species (Kõljalg et al., 2013; Blaalid et al., 2013). We then used the NCBI basic local alignment search tool (BLAST) to ensure that no sequences were obtained from contaminant fungi and to verify the taxonomic identity of our fungi. The OTUs were then assigned to functional guilds based on the FungalTraits database (Põlme et al., 2020).

Statistical analyses were performed using R version 4.1.1 (R Core Team, 2021). To assess the completeness of our sampling, we generated species accumulation curves using the "specaccum" function in the vegan version 2.6–4 package (Oksanen et al., 2019). Species richness

was estimated with the Chao2 and Jacknife1 estimators (Burkham and Overton, 1978; Chao, 1984; Colwell and Coddington, 1994) using the "spp.est" function in the fossil package (Vavrek, 2011).

2.4. Phylogenetic analysis

We used phylogenetic analyses to assess the phylogenetic diversity of *Amanita* Pers., *Cortinarius* (Pers.) Gray and *Russula* Pers., three of the most common and species-rich macrofungal genera present at OSBS. For all three phylogenetic analyses, sequences originating from OSBS specimens were supplemented with representative sequences from morphologically identified specimens of North American and European species for precise phylogenetic placement of the OSBS specimens within corresponding subgenera and sections. The *Amanita* alignment was comprised of concatenated ITS and 28S sequences generated by Sanger sequencing as well as ITS+28S reads generated by PacBio sequencing. The *Cortinarius* and *Russula* alignments were comprised of ITS sequences generated by both Sanger and PacBio. Delimitation of the *Amanita* infrageneric divisions followed Bunyard and Justice (2020), delimitation of the *Cortinarius* infrageneric division followed Landry et al. (2021), and the classification and delimitation of the *Russula* subgenera followed Buyck et al. (2018).

Sequences of *Amanita* and *Cortinarius* were aligned with Muscle v. 3.8.425 (Edgar, 2004) using the default settings in Geneious 11.1.5. (Auckland, New Zealand) and checked manually. We then performed a Maximum Likelihood (ML) analysis using RAxML 8.2.10 (Stamatakis, 2014) with a single partition and the GTR + GAMMA model and 1000 bootstrap iterations to evaluate support for nodes.

For the *Russula* phylogeny, sequences were aligned with the MAFFT online tool v. 7 (Katoh et al., 2018) using the Q-INS-i alignment strategy. The resulting alignment was manually refined in Geneious v. 10 (Kearse et al., 2012). Approximately 20 bp was trimmed from the end of ITS2 to avoid biases due to low quality sequence reads or missing data for some phylogenetically distinct specimens. The final alignment was analyzed with ML using RAxML-HPC2 on XSEDE 8.2.12 (Stamatakis, 2014) with a single partition and the GTR + GAMMA model with 1000 bootstrap

Fig. 1. Examples of macrofungal diversity from Ordway-Swisher Biological Station. a) *Amanita arkansana*, an edible *Amanita* species, b) *Lulesia alachuana*, a species described by Murrill and recently epitypified by Vizzini et al. (2023), c) *Phylloporopsis boletinoides*, originally described from a site near Gainesville, Florida as *Phylloporus boletinoides* by Smith and Thiers (1964), d) *Boletellus ananas*, a distinctive southeastern bolete species, e) *Cortinarius atrotomentosus*, a large and charismatic *Cortinarius* species described from Florida by Harrower et al. (2015), f) *Amoenoboletus weberi*, a bolete described from Gainesville, Florida by Singer (1945), g) *Amanita onusta*, a distinctive *Amanita* species, h) *Omphalotus subilludens*, a charismatic and bioluminescent mushroom species described by Murrill (1945) from Gainesville, Florida, i) *Tulostoma rufum*, j) *Tolypocladium* sp. 4, an undescribed taxon, parasitizing *Elaphomyces* cf. *muricatus,* and k) *Inocybe nucleata,* a species described by Murrill (1945) from Gainesville, Florida that is characterized by its large 'peculiar' angular spores.

iterations. The results were further edited with TreeGraph 2 (Stöver and Müller, 2010) and graphically improved in CorelDRAW X5 (Ottawa, Canada).

3. Results

3.1. Fungal richness

During the nine years of documenting the fungi of OSBS, we obtained more than 1200 vouchered macrofungi collections from diverse fungal lineages (Fig. 1, Supplemental Table S2-Supplemental Table S3). We generated DNA sequences from 1017 specimens, from which we generated ITS sequences from 984 specimens and 28S sequences from 234 specimens. From the specimens with ITS sequences, 928 were included in the richness analysis because they had ITS1 sequences that were long enough and of sufficient quality. There were 56 specimens that had partial ITS sequence data (e.g. a complete ITS2 sequence) but were not included in the ITS1 dataset because their ITS1 sequence was too short or of poor quality (Supplemental Table S2). Our clustering analysis based on 97 % similarity of ITS1 yielded 546 unique operational taxonomic units (OTUs), which we treat here as a proxy for species. (Clustering based on more stringent thresholds did not substantially alter the dataset; clustering based on 98 % similarity yielded 563 OTUs and 99 % similarity yielded 605 OTUs. Accordingly, for the main body of our analyses we used a conservative 97 % similarity threshold). From these 546 species, 488 belong to Basidiomycota and 58 to Ascomycota and these taxa represented 213 genera in 91 families and 27 orders (Table S1). The lack of an asymptote in our species accumulation curve suggests our sampling effort did not capture all the macrofungi present (Fig. 2a), and both richness estimators suggest that many additional macrofungi exist at OSBS. The Chao2 richness estimator predicts twice as many macrofungi taxa are likely present (1136 ± 41 total species)

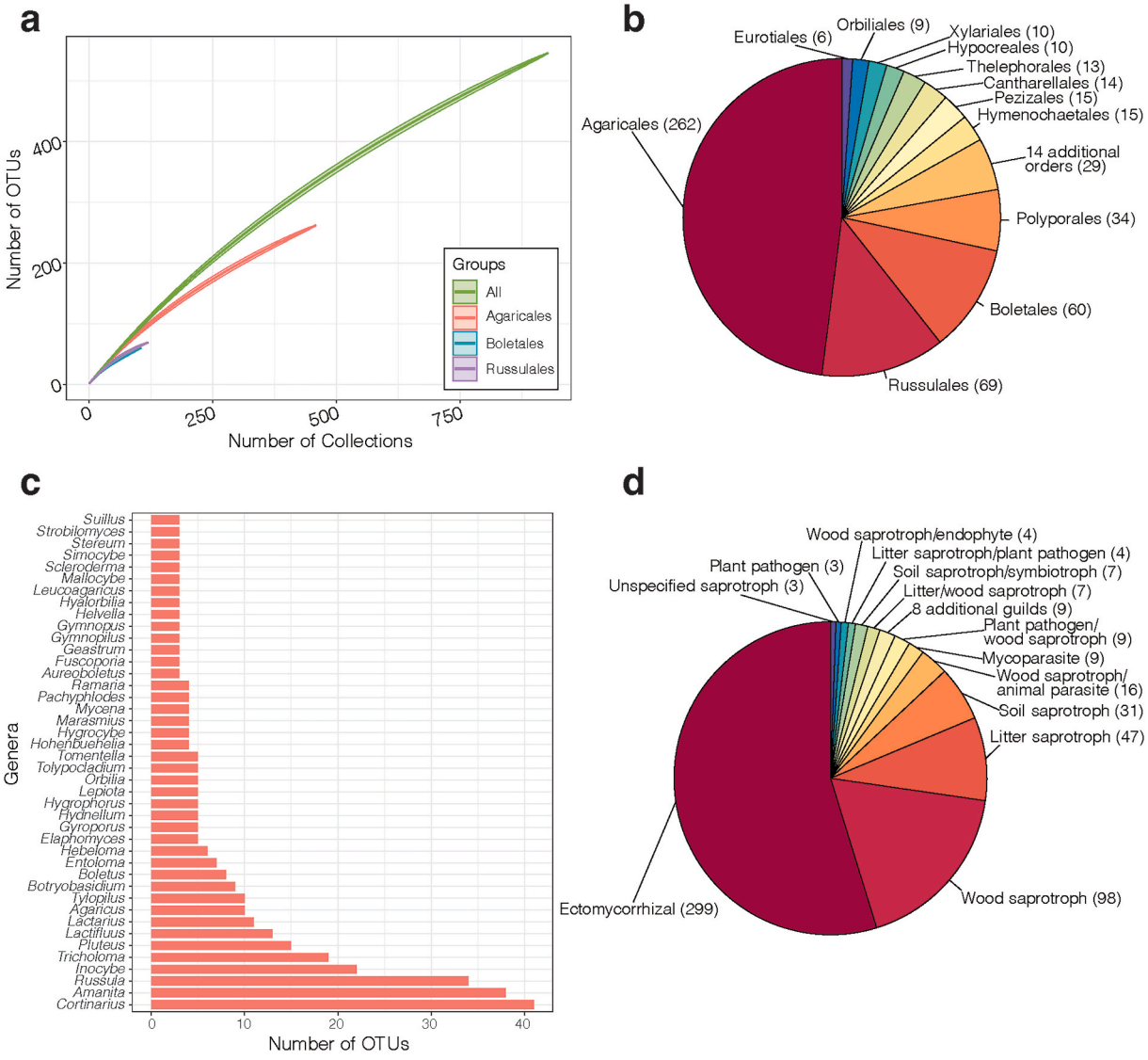

Fig. 2. Measures of Macrofungal Diversity at Ordway-Swisher Biological Station a) Operational taxonomic unit (OTU) accumulation curves for macrofungi specimens collected at Ordway-Swisher Biological Station based on ITS1 clustering, including error estimates that show the 95 % confidence intervals (shaded regions). The green curve indicates estimates based on all sampled specimens whereas the other curves indicate the sampling for the three most abundant and diverse orders of macrofungi at the site (Agaricales, Russulales and Boletales). b) Pie chart shows the order-level taxonomic placement of the 546 unique operational taxonomic units (OTUs) of macrofungi. The number of unique OTUs for each group is shown in parentheses beside the name of each order. c) Barplot shows the number of OTUs for the 42 most common macrofungi genera, 171 genera with only 1 or 2 OTUs per genus are not shown for clarity. d) Pie chart shows the functional guild assignment of the 546 unique OTUs of macrofungi. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

whereas the Jackknife 1 estimator is slightly more conservative (897 total species). Even for well-represented groups in our data, such as Agaricales, the Chao2 estimator indicates only 46–51 % sampling completeness. This is only slightly but not significantly higher than the sampling completeness for the entire dataset (46–49 %). From the specimens included in our analysis, the most species-rich orders were: Agaricales (262), Russulales (69), and Boletales (60) (Fig. 2b). The most species-rich genera collected in OSBS were *Cortinarius* (41), *Amanita* (38), and *Russula* (34) (Fig. 2c and 3b). When specimens were classified by functional guilds, most OTUs were identified as ectomycorrhizal (299), followed by wood saprotroph (98), and litter saprotroph (47) (Fig. 2d). Most specimens were collected either during summer and early fall (June to October) or during the winter months (December to February) (Fig. 3a). Among our most species-rich lineages of fungi, *Amanita* and *Russula* were collected mostly during the summer months while *Cortinarius* specimens were collected primarily during winter (Fig. 3b).

3.2. Specimens without sequences

While we attempted to generate ITS sequences for all the specimens collected at OSBS, this was not always successful. At least 40 species of charismatic macrofungi that were easily identifiable to genus based on morphological characters are included in our dataset (Supplemental Table S3) but are not included in the 546 OTUs that were calculated using ITS1 sequences. Examples of such charismatic taxa that are well characterized with ITS sequences from other sites in north Florida include *Boletellus ananas* (FLAS-F-61559) and *Schizophyllum commune* (FLAS-F-61325).

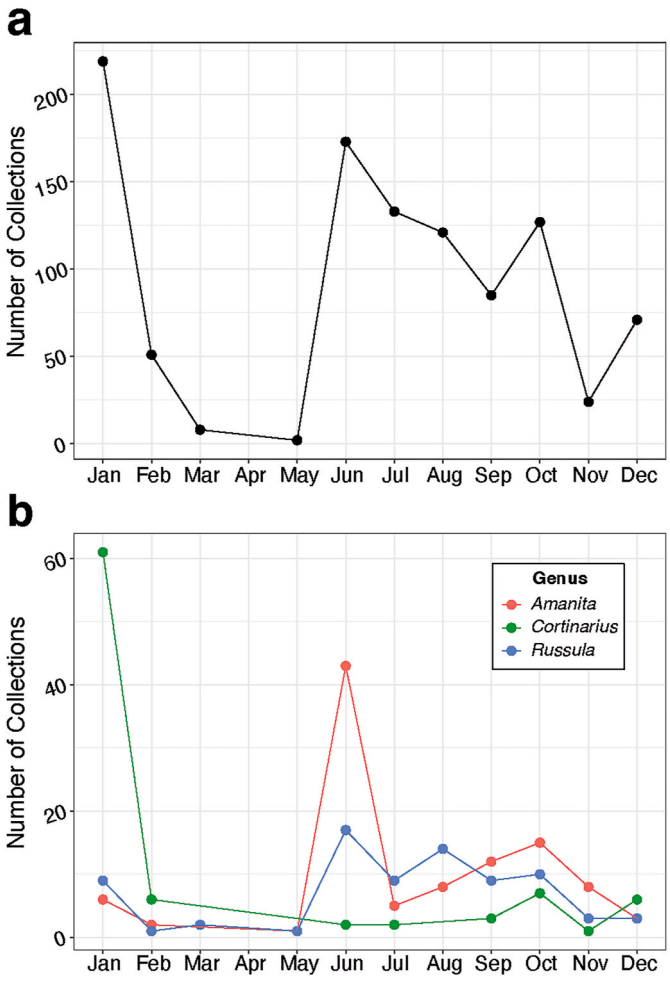

Fig. 3. Number of macrofungi collections by month. a) All macrofungi. b) Collections of the three most abundant and species-rich genera: *Amanita, Cortinarius* and *Russula*.

3.3. Sanger versus PacBio sequencing

The majority of 984 ITS sequences included in the study (782 sequences) were generated via Sanger sequencing. However, some genera such as *Amanita* (Agaricales), *Coltricia* (Hymenochaetales), *Cantharellus* (Cantharellales), *Helvella* (Pezizales), and *Strobilomyces* (Boletales), were particularly challenging to obtain ITS sequences. Starting in 2022, many specimens were sequenced with PacBio (202 sequences), including 44 specimens with previously unsuccessful attempts to generate Sanger sequences. PacBio sequencing was able to provide additional sequence data for at least some species of *Amanita, Coltricia,* and *Helvella.* However, *Cantharellus* and *Strobilomyces* had high rates of sequencing failure for both Sanger and PacBio sequencing.

3.4. Phylogenetic diversity

Based on the ITS + 28S phylogeny of the *Amanita* specimens collected at OSBS, we identified 50 species of *Amanita* and 29 of these were represented as singleton taxa that were collected and DNA barcoded only once. The OSBS species in our dataset included representatives of both major subgenera within the genus *Amanita*: subgenus *Amanita* (26 specimens/18 species) and subgenus *Lepidella* (74 specimens/33 species) (Fig. 4). Within the subgenus *Amanita*, three sections are represented at the OSBS: sect. *Amanita*, sect. *Vaginatae* and sect. *Caesareae*. Within the subgenus *Lepidella*, four sections are represented: sect. *Validae*, sect. *Phalloideae*, sect. *Amidella* and sect. *Lepidella* (Bunyard and Justice, 2020).

Similarly, we collected a phylogenetically diverse assemblage of *Cortinarius* at OSBS. We identified 43 species-level clades from which 23 were represented as singleton taxa. *Cortinarius* sensu lato at OSBS represent at least 10 different sections: Vibratiles, Delibuti, Anomali, Laeti, Dermocybe, Cortinarius, Scauri, Pseudotragani, Urbici and Firmiores (Fig. S1).

In the case of *Russula* species, out of 75 analyzed specimens, we identified 39 species-level clades (Fig. 5). Of these, 13 taxa were represented as singleton specimens, whereas all other specimens matched at least one other sequence from OSBS. All *Russula* species were clustered within five subgenera: *Heterophyllidia*, *Russula*, *Malodora*, *Compactae* and *Brevipes*. The subgen. *Compactae* and subgen. *Brevipes* were each represented by only one singleton specimen. The majority of specimens were clustered within subgen. *Heterophyllidia* (33 specimens/17 species) and subgen. *Russula* (31 specimens/18 species).

4. Discussion

This study provides the first comprehensive characterization of the macrofungi from Ordway-Swisher Biological Station, a subtropical long-term study site that has been part of NEON since 2011. This work resulted in more than 1200 vouchered specimens, 1017 DNA sequences added to GenBank, and the identification and DNA barcoding of more than 546 distinct fungal species, including some new and 'rediscovered' fungal taxa (Fig. 1). Our ITS1 dataset was dominated by Basidiomycota (488 taxa) with a much smaller number of Ascomycota (58 taxa). This may be due in part to the subtropical climate, sandy soils, and frequent rainfall which are often correlated with high Basidiomycota richness (e.g. Roy et al., 2016; Henkel et al., 2012) and might reduce richness of some Ascomycota (e.g. Pezizales – Tedersoo et al., 2014). However, it is impossible to rule out sampling biases in favor of the larger spore-bearing structures in Basidiomycota (e.g. mushrooms) versus the smaller and more cryptic spore-bearing structures in Ascomycota (e.g. truffles and cup fungi).

Consistent with previous studies in both temperate and tropical

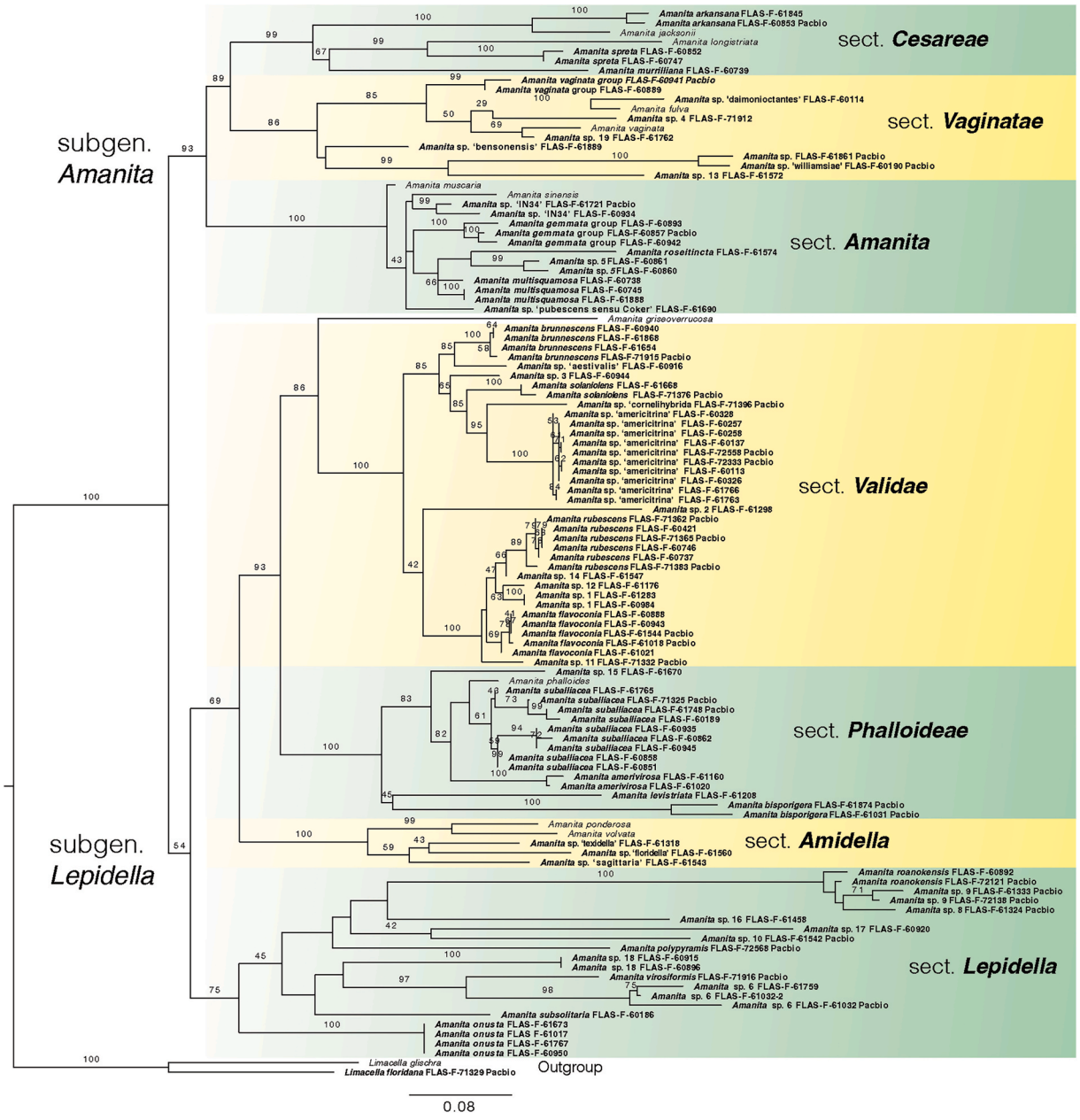

Fig. 4. Phylogenetic diversity of *Amanita* at Ordway-Swisher Biological Station. Maximum likelihood phylogeny of the genus *Amanita* based on ITS and partial 28S rDNA. All bold specimens in the phylogeny are from OSBS. *Amanita* specimens collected at OSBS represented at least 50 species from two subgenera and seven sections. Infrageneric classifications followed Bunyard and Justice (2020).

forests, Agaricales was the most diverse group of macrofungi in our dataset and represented nearly half of all specimens (Truong et al., 2017; Haelewaters et al., 2021; Vandegrift et al., 2023). We also found high species richness and phylogenetic diversity of ECM fungi at OSBS. This high diversity of ECM fungi is likely related to the high density and species richness of ECM host plants, which includes at least 25 species from five families (Cistaceae, Fagaceae, Juglandaceae, Pinaceae, Salicaceae) and a particularly strong influence of *Pinus* (4 species) and *Quercus* (13 species) (Weinstein et al., 2023; FLAS herbarium database: https://specifyportal.floridamuseum.ufl.edu/herbarium/). Results of a recent study on the ECM fungal communities associated with the short-lived, herbaceous ECM host plants in Cistaceae (*Crocanthemum* and *Lechea* species), also suggest that the four Cistaceae species from OSBS may also serve as important hosts for diverse ECM fungi (Caiafa et al., 2024).

We found marked seasonality in the macrofungi at OSBS with two distinct seasons of sporome production (Fig. 3). Although the majority of macrofungi were found during north Florida's long wet season which typically stretches from summer until early fall (June to October), we also found a distinct peak in winter fruiting during December and January. Three of the most species-rich genera of fungi in our dataset (*Amanita*, *Cortinarius*, and *Russula*) also had distinct peaks in their sporome production, with maximum diversity and production of *Amanita* and *Russula* during summer whereas *Cortinarius* were found almost exclusively in winter (Fig. 3). This pattern is consistent with collection data of these genera in Alachua County (where the FLAS collection is located, Fig. S2).

We also identified several other taxonomic groups in our dataset that produced sporomes mostly or exclusive in winter (e.g. *Hebeloma*, *Hygrophorus*, *Tricholoma*, *Tolypocladium*) and others that produced sporomes mostly or exclusively in summer (e.g. *Cantharellus*, Boletaceae). This finding suggests that the bimodal fruiting pattern at OSBS is

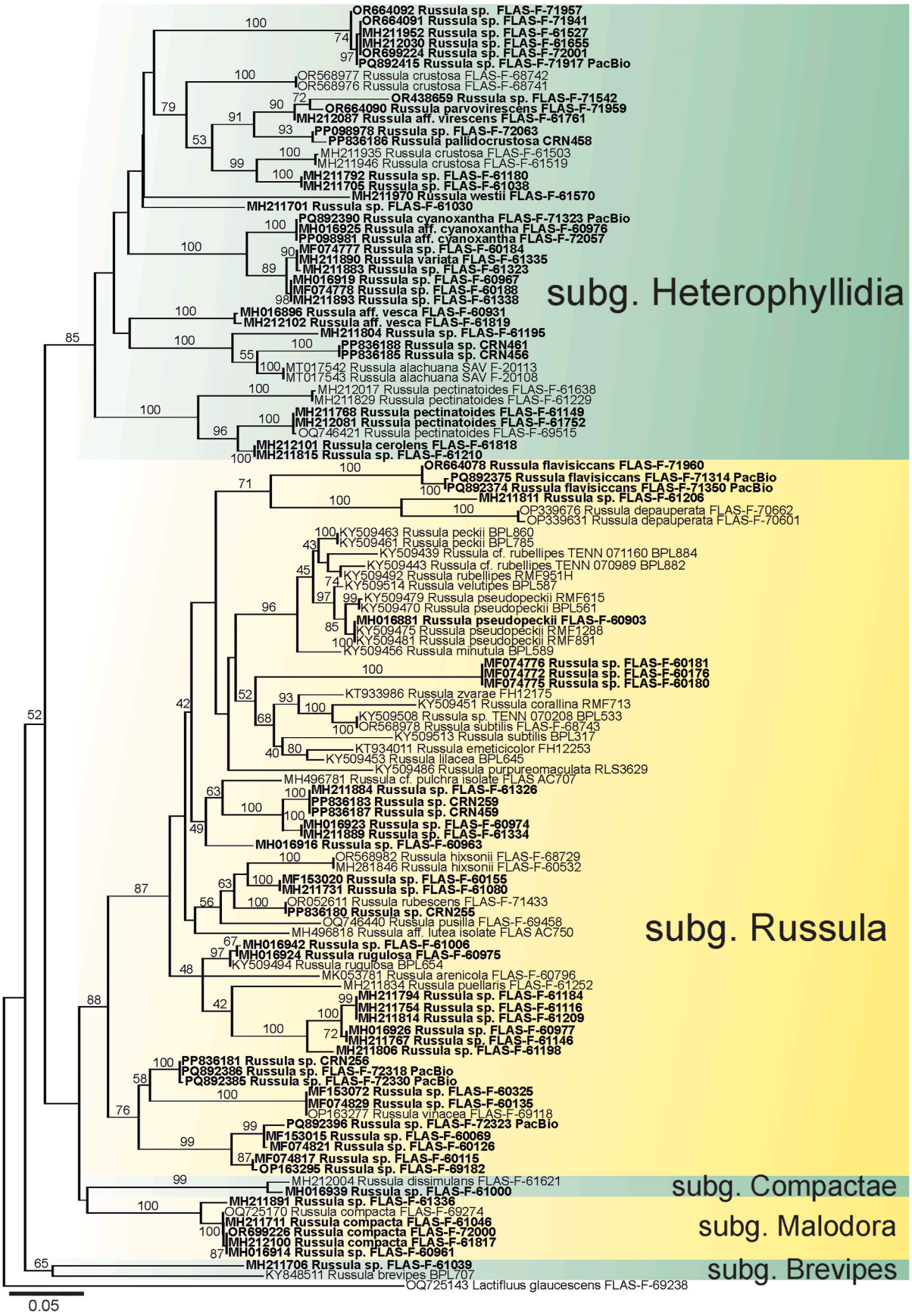

Fig. 5. Phylogenetic diversity of *Russula* at Ordway-Swisher Biological Station.
Maximum likelihood phylogeny of the genus *Russula* based on ITS rDNA. All bold specimens in the phylogeny are from OSBS. *Russula* specimens collected at OSBS represent at least 33 species from five subgenera. Subgeneric classification followed Buyck et al. (2018). *Lactifluus glaucescens* was used as the outgroup taxon.

due at least in part to temporal partitioning among different taxonomic lineages which typically exhibit some adaptation to produce sporomes in only one of the two main fruiting periods (Tables S1–S2 but see notable exceptions of taxonomic groups with sporome production almost equally divided between winter and summer, such as Inocybaceae). The macrofungi production pattern at OSBS is distinctly subtropical and therefore different from most other sites in the temperate zones, where macrofungi production is primarily in summer and fall but where fruiting typically ceases due to freezing temperatures at the end of fall (Halbwachs and Simmel, 2018; Krah et al., 2023). Although OSBS experiences freezing temperatures during most years and occasionally experiences 'hard freeze' events down to −12 °C (Osland et al., 2021), the macrofungi production is in some ways more similar to tropical sites that have peak macrofungi production that tends to track periods of highest precipitation (Henkel et al., 2012; Krah et al., 2023).

Despite documenting high macrofungi species richness, sampling curves suggest that our current database of DNA-barcoded taxa represents only about half of the true macrofungi richness at OSBS (Fig. 2). The number of macrofungi species that we found (>546) was more than the combined species richness of all known vertebrates from OSBS [n = 284, birds (149 spp.), reptiles (45 spp.) mammals (37 spp.), amphibians (27 spp.), and fishes (26 spp.)] and was also more than the documented species richness of Lepidoptera (488 species) (Kaminsky and Smith, 2024). Our richness estimates based on two methods of extrapolation suggest that macrofungi are among the most diverse groups of eukaryotes at OSBS, are probably more diverse than plants (700 species), and might even be as diverse as insects (Kaminsky and Smith, 2024). However, even with the significant efforts of our study, some groups of fungi such as wood decay fungi in Polyporales and Hymenochaetales, are likely undersampled from OSBS and probably have much higher richness than is reported here (Smith and Dreschler-Santos, personal observations). Our study also highlights the benefits of high-throughput sequencing, such as PacBio, to obtain DNA barcode sequences from material that has proved challenging for Sanger sequencing. While Sanger sequencing is usually faster to process and relatively inexpensive, PacBio sequencing can be cost effective for large datasets and may help to recover high-quality sequences for taxa that are challenging to amplify and sequence (Runnel et al., 2022; Swenie et al., 2024). Although we used Sanger sequencing for the vast majority of our samples, we found PacBio sequencing yielded high-quality ITS sequences for many specimens of *Amanita* and *Helvella* that were previously unsuccessful with Sanger sequencing, in some cases after multiple attempts.

4.1. Biogeography of fungi at OSBS

Along with high species-level richness, we also identified interesting and unique biogeographical patterns among the species of fungi that were documented. Because of its geographic location, Florida also has floristic connections with both Nearctic and Neotropical elements (Neill, 1957; Myers and Ewel, 1990), providing a distinctive combination of plant hosts for mycorrhizal and plant parasitic fungi. This unique combination of climate and biogeography make Florida a key location for fungal diversity studies, capturing multiple ecological intersections and transition zones. Some taxa present at the OSBS have a broad distribution across eastern North America, with species ranges stretching into Atlantic Canada, whereas other taxa have a neotropical or pantropical distribution, with species distributions across the Neotropics or into tropical zones across the globe. For example, *Tricholoma subsejunctum* (FLAS-F-60079, FLAS-F-69155, FLAS-F-60122) was described by Charles H. Peck from New York and appears to have a Nearctic distribution, with most known records from the northern USA and Canada (Peck, 1912). Similarly, the recently described *Hygrophorus aesculeticola* (FLAS-F-72315, FLAS-F-72314, FLAS-F-72304 and FLAS-F-62822) has a Nearctic distribution, extending from Florida and nearby states north to Ontario, Canada (Crous et al., 2023). Some other species appear to be restricted only to the Gulf Coast region or the Atlantic Coast of eastern North America. For example, *Amanita suballiacea* (FLAS-F-71325, FLAS-F-60945, FLAS-F-60935, and others), an ECM taxon described by Murrill from Florida, is distributed from Florida northward to New Brunswick, Canada (Tulloss et al., 2021). On the other hand, *Amoenoboletus weberi* is one of the species present at OSBS that appears to have a more limited distribution along the Gulf of Mexico and the Atlantic coast of eastern North America (Biketova et al., 2022). Still other taxa, such as *Mallocybe praevillosa*, are known only from a handful of specimens and are putatively restricted only to Florida (Matheny et al., 2023).

Ectomycorrhizal fungi are associated with particular plant symbionts, so their distributions are influenced by the distributions of their hosts (Tedersoo et al., 2010; Van Der Linde et al., 2018). In contrast, many generalist saprotrophs and wood decay fungi have less stringent substrate needs so it is possible that they may have larger ranges (Sato et al., 2012; but see Bazzicalupo et al., 2019). For example, the wood decay fungus *Cubamyces lactineus* (= *Trametes lactinea*), which was collected several times at OSBS (FLAS-F-72061, FLAS-F-61796, FLAS-F-61001, and others), was originally described from Sri Lanka by Berkeley (Ryvarden, 1976). *C. lactineus* is known to have an almost cosmopolitan distribution, with well documented specimens from North America, South America and the Caribbean (Ryvarden, 1976; Welti et al., 2012; Lücking et al., 2020). Similarly, *Microporellus obovatus* (FLAS-F-61795, FLAS-F-61517, FLAS-F-60948), originally described as *Polyporus obovatus* from Java by Junghuhn, is known to have a pantropical distribution (Junghuhn, 1839; Ryvarden, 1974). Other pantropical species from OSBS include *Fuscoporia callimorpha* (FLAS-F-61706) and *Earliella scabrosa* (FLAS-F-61025) (Vlasák et al., 2011; Olou et al., 2023).

4.2. Notable species from the OSBS

Our work resulted in the recollection of several fungal species originally described or collected by Murrill, and several of these taxa have been re-characterized in recent studies using specimens from OSBS (e.g. Farid et al., 2018; Biketova et al., 2022). Examples of OSBS specimens that have been used to re-characterize Murrill's historical taxa include *Exsudoporus floridanus* (FLAS-F-61008) (Biketova et al., 2022), *A. weberi* (FLAS-F-61525) (Biketova et al., 2022) and *Lulesia alachuana* (FLAS-F-61088) (Vizzini et al., 2023) (Fig. 1). Another example of an important historical Gulf Coast species is *Phylloporopsis boletinoides*, for which specimens from OSBS were used to show that *Phylloporopsis* is distinct from *Phylloporus* (Farid et al., 2018). *Mallocybe praevillosa* (FLAS-F-61523) is a rare and morphologically unusual species, originally described as *Lepista praevillosa* by Murrill and recently combined into *Mallocybe* (Inocybaceae) based in part on OSBS collections (Matheny et al., 2023). Similarly, OSBS specimens of *Laetiporus persicinus* (FLAS-F-60416) were useful to recombine this species into the genus *Kusaghiporia* as *K. persicinus* (Paez et al., 2023).

Given the high richness of macrofungi from the OSBS, along with the poor condition of some type specimens in the FLAS fungarium, the collections reported here can also serve as a reference for additional taxonomic and ecological studies. In some cases, our recent collections have identified species complexes that will need to be resolved. For example, in the case of *Exsudoporus frostii* (FLAS-F-60742), specimens from OSBS helped to reveal four distinct North American species in this complex (Biketova et al., 2022). In addition, recent studies have identified a number of new taxa based on material from OSBS, such as *Pulchroboletus sclerotiorum* (FLAS-F-60908) (Crous et al., 2019), *Tuber mujicii* (FLAS-F-60274) (Lemmond et al., 2022), *H. aesculeticola* (FLAS-F-62822) (Crous et al., 2023), *Cortinarius flaureifolius* (K-M 001434094) (Liu et al., 2024), *Tricholoma pallidogriseum* (FLAS-F-69157) (Lebeuf et al., 2024) and *Mallocybe leucothrix* (TENN-F-075564) (Matheny et al., 2023). In the future, we hope that advances in sequencing technology will also allow for more molecular data to be obtained from Murrill's collections and that they can then be directly connected via DNA sequences to the vouchers from OSBS.

4.3. Diversity of Amanita, Cortinarius, and Russula

We identified three genera of fleshy macrofungi at OSBS that were exceptionally diverse based on the morphological features of fruiting bodies and DNA sequencing from ribosomal DNA. To more completely assess the species-level diversity of these lineages and to determine which infrageneric lineages were represented, we placed them into a phylogenetic context. Phylogenies of *Amanita, Cortinarius* and *Russula* confirmed the high species richness that can be found for these three genera in the relatively small area of OSBS. We suspect the high richness of the three genera is likely due to a combination of high habitat heterogeneity at OSBS, which includes variation in soil types, vegetation types, fire return intervals, and the diversity of ECM hosts (Weinstein et al., 2023).

Based on our analyses *Amanita* was the most species-rich genus at OSBS. Although *Amanita* did not have the highest richness in our ITS1 clustering, this was due to the fact that many *Amanita* specimens failed ITS sequencing (with both the Sanger or Pacbio sequencing methods) but were nonetheless resolved as unique taxa based on the ITS+28S phylogeny. We were able to confidently place most *Amanita* species within their respective subgenera and sections with moderate to strong support (Fig. 4). We also reliably identified 31 out of 50 *Amanita* specimens to the species level. Our phylogeny shows particularly high diversity in subgenus *Lepidella*, especially within section *Validae.* This is consistent with the high diversity of section *Validae* reported for eastern North America by other recent studies (Quintero-Corrales et al., 2024). Also consistent with previous studies, our phylogeny reveals high intraspecific variation among specimens identified as *A. suballiacea* and also high variation among the taxa of section Phalloideae (Cai et al., 2014). We also generated sequences for many *Amanita* specimens that did not match well with any other sequences in GenBank, highlighting the fact that more taxonomic work is still needed for this genus in the eastern USA.

For *Cortinarius* sensu lato, our ML analysis (Fig. S1) highlights the high phylogenetic diversity of the group, with a least 10 different sections represented at OSBS. However, it is important to note that the topology has some deviations from recently published, multi-locus phylogenies (Liimatainen et al., 2022) and many identified clades are not supported due to the reliance only on ITS sequences. Given the high diversity of *Cortinarius* at OSBS, a deeper phylogenetic treatment of the genus would require additional loci to be sequenced.

For *Russula*, all species were confidently placed at the subgenus level and all of the subgenera were moderately to strongly supported in our ML analysis (Fig. 5). The overall topology of the tree deviates slightly from some recently published phylogenies (e.g. Adamčík et al., 2019; Looney et al., 2022) due to the fact that we relied only on ITS rDNA. Nonetheless, our phylogeny was useful for identifying some taxa and determining relationships among the taxa from OSBS. Due to the high diversity of *Russula* and the fact that some sequences we generated were distinct when compared to current GenBank accessions, we only reliably identified seven of the 33 taxa to the species level. Our phylogenetic analysis also revealed cryptic taxa in the dataset. For example, we noted at least two clades within specimens that were morphologically identified as *R. pseudopeckii* and also *R. pectinatoides* (Fig. 5). We also identified significant diversity within the Roseinae lineage of subgenus *Russula*, and our analysis suggests a distinct assembly of Gulf Coast species at the OSBS that is different from related taxa from other areas of the eastern USA (Looney et al., 2020, 2022).

4.4. Conclusions and future directions

While our nine-year study at the OSBS represents a significant contribution to the knowledge of the fungi in Florida, we estimate that roughly half of the macrofungal richness at the OSBS remains unknown (Fig. 1a). This highlights the importance of long-term monitoring to identify both common and rare species that occur at a particular site and also to provide specimens for conducting taxonomic work to adequately document these organisms. This is especially useful for long-term study sites (such as those in the NEON and LTER networks) that are intensively studied and referenced with a wide array of biological and climatic data. While several species have been described from OSBS, many of the species collected during this project remain undescribed or have not yet been linked to an older taxonomic name. Long-term studies such as the one presented here can serve as a starting point for the taxonomic work that is needed for several groups, such as the hyperdiverse taxa in *Amanita*, *Cortinarius* and *Russula* (Figs. 4 and 5, Fig. S1). We also anticipate that new sequencing technologies and lowering costs of DNA sequencing will facilitate the generation of molecular data for specimens that have already been collected but never subjected to DNA sequencing. Beyond improving taxonomic knowledge for diverse groups of fungi, having a more complete knowledge of fungal diversity at local sites can also provide novel insights into broader macroecological and biogeographical patterns. Improving our knowledge of fungal communities is imperative for including fungi in future conservation efforts. For example, only one species (*Hericium erinaceus*) of the more than 500 collected at the OSBS has been assessed for the IUCN Red List (Kałucka and Olariaga Ibarguren, 2019). *Amanita westii,* which is a species likely to be in the OSBS but not collected in this survey, is considered as Near Threatened (Lewis, 2019). Thus, monitoring and describing fungal diversity is crucial to assess the conservation status of fungal species and characterize global extinction risk for fungi. Species that are not formally described cannot be evaluated under the IUCN framework (Mueller et al., 2022; van Galen et al., 2025). Furthermore, long-term monitoring of fungal diversity is crucial to understand the response of fungal communities to disturbances that are increasing in frequency or amplitude, such as land use changes, nutrient pollution, climate change, and biological invasions.

CRediT authorship contribution statement

Marcos V. Caiafa: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Laurel Kaminsky:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Rosanne Healy:** Writing – review & editing, Resources, Project administration, Investigation, Funding acquisition, Data curation. **Leanne P. Sheffer:** Methodology, Investigation, Data curation. **C. Benton Willis:** Writing – review & editing, Methodology, Investigation, Data curation. **Katy Deitz:** Writing – review & editing, Investigation. **Brantlee S. Richter:** Writing – review & editing, Resources, Investigation, Funding acquisition. **Benjamin R. Lemmond:** Writing – review & editing, Validation, Methodology, Investigation. **David Borland:** Investigation. **Bitty A. Roy:** Writing – review & editing, Resources, Investigation, Funding acquisition, Data curation. **Heather A. Dawson:** Writing – review & editing, Methodology, Investigation. **Carolyn A. Delevich:** Software, Methodology. **John S. Conery:** Resources, Funding acquisition. **Dylan Warner:** Investigation, Data curation. **Miroslav Caboň:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Formal analysis. **Elena Karlsen-Ayala:** Writing – review & editing, Methodology, Investigation. **Arthur C. Grupe:** Writing – review & editing, Validation, Methodology, Investigation. **Nattapol Kraisitudomsook:** Writing – review & editing, Methodology, Investigation. **Nicole K. Reynolds:** Writing – review & editing, Methodology, Investigation, Data curation. **Elisandro Ricardo Drechsler-Santos:** Writing – review & editing, Validation, Methodology, Investigation. **Camille Truong:** Writing – review & editing, Methodology, Investigation. **Adriana Corrales:** Writing – review & editing, Methodology, Investigation. **Alija B. Mujic:** Writing – review & editing, Validation, Methodology, Investigation, Data curation. **Peter G. Kennedy:** Writing – review & editing, Resources, Funding acquisition. **Michelle A. Jusino:** Writing – review & editing, Methodology, Investigation. **Rachel A. Swenie:** Writing – review & editing, Validation,

Methodology, Investigation. **Chance R. Noffsinger:** Validation, Methodology, Investigation. **Django Grootmyers:** Writing – review & editing, Validation, Methodology, Investigation. **P. Brandon Matheny:** Writing – review & editing, Validation, Resources, Methodology, Investigation, Funding acquisition, Data curation. **Andrew W. Wilson:** Writing – review & editing, Resources, Investigation, Funding acquisition. **Matthew E. Smith:** Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2025.101643.

References

Adamčík, S., Looney, B., Caboň, M., et al., 2019. The quest for a globally comprehensible *Russula* language. Fungal Divers. 99, 369–449. https://doi.org/10.1007/s13225-019-00437-2.

Bazzicalupo, A.L., Whitton, J., Berbee, M.L., 2019. Over the hills, but how far away? Estimates of mushroom geographic range extents. J. Biogeogr. 46, 1547–1557. https://doi.org/10.1111/jbi.13617.

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., et al., 2013. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol. Evol. 4, 914–919. https://doi.org/10.1111/2041-210X.12073.

Bessette, A.E., Bessette, A.F., Lewis, D.P., 2019. Mushrooms of the Gulf Coast States: a Field Guide to Texas. University of Texas Press, Louisiana, Mississippi, Alabama, and Florida.

Bessette, A.E., Roody, W.C., Bessette, A.R., 2017. Boletes of Eastern North America. Syracuse University Press.

Beug, M., Bessette, A.E., Bessette, A.R., 2014. Ascomycete Fungi of North America: a Mushroom Reference Guide. University of Texas Press.

Biketova, A.Y., Gelardi, M., Smith, M.E., et al., 2022. Reappraisal of the Genus *Exsudoporus* (Boletaceae) worldwide based on multi-gene phylogeny, morphology and biogeography, and insights on amoenoboletus. Journal of Fungi 8. https://doi.org/10.3390/jof8020101.

Blaalid, R., Kumar, S., Nilsson, R.H., et al., 2013. ITS1 versus ITS2 as DNA metabarcodes for fungi. Mol Ecol Resour 13, 218–224. https://doi.org/10.1111/1755-0998.12065.

Boddy, L., 2016. Chapter 11 - fungi, ecosystems, and global change. In: Watkinson, S.C., Boddy, L., Money, N.P. (Eds.), The Fungi, third ed. Academic Press, Boston, pp. 361–400.

Breitenbach, J., Kränzlin, F., 1986. Fungi of Switzerland, vol. 2. Non gilled fungi-Heterobasidiomycetes, Aphyllophorales, Gasteromycetes.

Bruns, T.D., 2012. The North American Mycoflora project–the first steps on a long journey. New Phytol. 196, 972–974.

Bunyard, B.A., Justice, J., 2020. Amanitas of North America. FUNGI Press, Batavia, IL, USA.

Burkham, K.P.U.K.P., Overton, W.S., 1978. Estimation of the size of a closed population when capture probabilities vary among animals. Biometrika 65, 625–633. https://doi.org/10.1093/biomet/65.3.625.

Buyck, B., Adamèík, S., 2011. Type studies of *Russula* species described by W.A. Murrill, 1. R. roseiisabellina, R. sericella, and R. obscuriformis. Mycotaxon 115, 131–144. https://doi.org/10.5248/115.131.

Buyck, B., Zoller, S., Hofstetter, V., 2018. Walking the thin line… ten years later: the dilemma of above- versus below-ground features to support phylogenies in the Russulaceae (Basidiomycota). Fungal Divers. 89, 267–292. https://doi.org/10.1007/s13225-018-0397-5.

Cai, Q., Tulloss, R.E., Tang, L.P., et al., 2014. Multi-locus phylogeny of lethal amanitas: implications for species diversity and historical biogeography. BMC Evol. Biol. 14, 143.

Caiafa, M.V., Grazziotti, P.H., Karlsen-Ayala, E., et al., 2024. Ectomycorrhizal fungal communities associated with *Crocanthemum* and *Lechea* (Cistaceae) in subtropical Florida sandhill habitats. Mycorrhiza 34, 391–401. https://doi.org/10.1007/s00572-024-01172-6.

Chao, A., 1984. Nonparametric estimation of the number of classes in a population. Scand. J. Stat. 11, 265–270.

Colwell, R.K., Coddington, J.A., 1994. Estimating terrestrial biodiversity through extrapolation. Philos. Trans. R. Soc. Lond. B Biol. Sci. 345, 101–118.

Crous, P.W., Costa, M.M., Kandemir, H., et al., 2023. Fungal planet description sheets: 1550-1613. Persoonia: Molecular Phylogeny and Evolution of Fungi 51, 280–417. https://doi.org/10.3767/persoonia.2023.51.08.

Crous, P.W., Wingfield, M.J., Lombard, L., et al., 2019. Fungal Planet Description Sheets, pp. 223–425, 951 – 1041.

Eberhardt, U., Grilli, E., Schütz, N., et al., 2023. Old but not obsolete: a new life for some of Murrill's (Hebeloma) names. Mycologia 115, 375–426. https://doi.org/10.1080/00275514.2023.2188387.

Edgar, R.C., 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Res. 32, 1792–1797.

Farid, A., Gelardi, M., Angelini, C., et al., 2018. *Phylloporus* and *Phylloboletellus* are no longer alone: *phylloporopsis* gen. nov. (Boletaceae), a new smooth-spored lamellate genus to accommodate the American species *Phylloporus boletinoides*. Fungal Syst Evol 2, 341–359. https://doi.org/10.3114/fuse.2018.02.10.

Gardes, M., Bruns, T.D., 1993. ITS primers with enhanced specificity for basidiomycetes - application to the identification of mycorrhizae and rusts. Mol. Ecol. 2, 113–118. https://doi.org/10.1111/j.1365-294X.1993.tb00005.x.

Gonçalves, S.C., Haelewaters, D., Furci, G., Mueller, G.M., 2021. Include all fungi in biodiversity goals. Science 373, 403, 1979.

Haelewaters, D., Schoutteten, N., Medina-Van Berkum, P., et al., 2021. Pioneering a fungal inventory at Cusuco National Park, Honduras. Journal of Mesoamerican Biology 1, 111–131.

Halbwachs, H., Simmel, J., 2018. Some like it hot, some not – tropical and arctic mushrooms. Fungal Biol Rev 32, 143–155.

Halling, R.E., 1986. An Annotated Index to Species and Infraspecific Taxa of Agaricales and Boletales Described by William A. Murrill. New York Botanical Garden.

Hanlin, R.T., 1998. Combined keys to illustrated genera of ascomycetes. APS Press 1 & 2.

Harrower, E., Bougher, N.L., Winterbottom, C., et al., 2015. New species in *Cortinarius* section *Cortinarius* (Agaricales) from the Americas and Australasia. MycoKeys 11, 1–21. https://doi.org/10.3897/mycokeys.11.5409.

Heilmann-Clausen, J., Barron, E.S., Boddy, L., et al., 2015. A fungal perspective on conservation biology. Conserv. Biol. 29, 61–68. https://doi.org/10.1111/cobi.12388.

Henkel, T.W., Aime, M.C., Chin, M.M.L., et al., 2012. Ectomycorrhizal fungal sporocarp diversity and discovery of new taxa in *Dicymbe* monodominant forests of the Guiana Shield. Biodivers. Conserv. 21, 2195–2220. https://doi.org/10.1007/s10531-011-0166-1.

Hofstetter, V., Buyck, B., Eyssartier, G., et al., 2019. The unbearable lightness of sequenced-based identification. Fungal Divers. 96, 243–284. https://doi.org/10.1007/s13225-019-00428-3.

IUCN, 2025. The IUCN red list of threatened species. https://www.iucnredlist.org.

Jantzen, J.R., Whitten, W.M., Neubig, K.M., et al., 2019. Effects of taxon sampling and tree reconstruction methods on phylodiversity metrics. Ecol. Evol. 9, 9479–9499. https://doi.org/10.1002/ece3.5425.

Junghuhn, F.W., 1839. Praemissa in floram cryptogamican Javae insulae. Bataviaasch Genootschap van Kunsten en Wetenschappen.

Kałucka, I.L., Olariaga Ibarguren, I., 2019. *Hericium erinaceus*. The IUCN red list of threatened species 2019: e.T70401627A70401637. https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T70401627A70401637.en. (Accessed 7 March 2025).

Kaminsky, L., Smith, M.E., 2024. Assessment of macrolichen diversity at Ordway-Swisher biological station in Northern Florida contributes to the scientific Mission of NEON. Castanea 89, 1–11. https://doi.org/10.2179/0008-7475.89.1.1.

Kao, R.H., Gibson, C.M., Gallery, R.E., et al., 2012. NEON terrestrial field observations: designing continental-scale, standardized sampling. Ecosphere 3, 1–17. https://doi.org/10.1890/es12-00196.1.

Katoh, K., Rozewicki, J., Yamada, K.D., 2018. MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. Briefings Bioinf. 20, 1160–1166. https://doi.org/10.1093/bib/bbx108.

Kearse, M., Moir, R., Wilson, A., et al., 2012. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28, 1647–1649. https://doi.org/10.1093/bioinformatics/bts199.
Keller, M., Schimel, D.S., Hargrove, W.W., Hoffman, F.M., 2008. A continental strategy for the national ecological observatory network. The Ecological Society of America 282–284.
Kimbrough, J.W., 2003. The twilight years of William Alphonso Murrill. Mushroom 21, 22–47.
Kimbrough, J.W., 2000. Common Florida Mushrooms. University of Florida. Extension Institute of Food and Agricultural Sciences.
Kõljalg, U., Nilsson, R.H., Abarenkov, K., et al., 2013. Towards a unified paradigm for sequence-based identification of fungi. Mol. Ecol. 22, 5271–5277. https://doi.org/10.1111/mec.12481.
Krah, F.S., Büntgen, U., Bässler, C., 2023. Temperature affects the timing and duration of fungal fruiting patterns across major terrestrial biomes. Ecol. Lett. 26, 1572–1583.
Landry, J., Lamoureux, Y., Lebeuf, R., Paul, A., Lambert, H., Labbé, R., 2021. Répertoire des cortinaires du Québec Première édition. Mycoquébec.
Largent, D.L., Thiers, H.D., 1977. How to Identify Mushrooms to Genus II: Field Identification of Genera. Mad River Press Inc.
Lebeuf, R., Landry, J., Saar, I., et al., 2024. New species of *Tricholoma* in sections *Genuina* and *Fucata* from eastern North America. Botany 102, 395–420. https://doi.org/10.1139/cjb-2024-0057.
Lemmond, B.R., Healy, R.A., Bonito, G., Smith, M.E., 2022. *Tuber eburneum* and *Tuber mujicii*: new pine-associated *Tuber* species from eastern North America. Mycologia 114, 575–586. https://doi.org/10.1080/00275514.2022.2037338.
Lewis, D.P., 2019. *Amanita westii*. The IUCN red list of threatened species 2019: e. T95383225A95385374. https://dx.doi.org/10.2305/IUCN.UK.2019-1.RLTS.T95383225A95385374.en. (Accessed 7 March 2025).
Liimatainen, K., Kim, J.T., Pokorny, L., Kirk, P.M., Dentinger, B., Niskanen, T., 2022. Taming the beast: a revised classification of Cortinariaceae based on genomic data. Fungal Divers 112, 89–170. https://doi.org/10.1007/s13225-022-00499-9.
Liu, S.L., Wang, X.W., Li, G.J., et al., 2024. Fungal diversity notes 1717–1817: taxonomic and phylogenetic contributions on genera and species of fungal taxa. Fungal Divers. 124, 1–216. https://doi.org/10.1007/s13225-023-00529-0.
Loescher, H.W., Kelly, E.F., Lea, R., 2017. National ecological observatory network: beginnings, programmatic and scientific challenges, and ecological forecasting. In: Terrestrial Ecosystem Research Infrastructures. CRC Press, pp. 27–52.
Looney, B.P., 2014. Molecular annotation of type specimens of *Russula* species described by W.A. Murrill from the Southeast United States. Mycotaxon 129, 255–268. https://doi.org/10.5248/129.255.
Looney, B.P., Adamčík, S., Matheny, P.B., 2020. Coalescent-based delimitation and species-tree estimations reveal Appalachian origin and Neogene diversification in *Russula* subsection roseinae. Mol. Phylogenet. Evol. 147. https://doi.org/10.1016/j.ympev.2020.106787.
Looney, B.P., Manz, C., Matheny, P.B., Adamčík, S., 2022. Systematic revision of the Roseinae clade of *Russula*, with a focus on eastern North American taxa. Mycologia 114, 270–302. https://doi.org/10.1080/00275514.2021.2018881.
Lücking, R., Aime, M.C., Robbertse, B., et al., 2021. Fungal taxonomy and sequence-based nomenclature. Nat. Microbiol. 6, 540–548.
Lücking, R., Truong, B.V., Huong, D.T.T., et al., 2020. Caveats of fungal barcoding: a case study in *Trametes* s. In: Lat. (Basidiomycota: Polyporales) in Vietnam Reveals Multiple Issues with Mislabelled Reference Sequences and Calls for third-party Annotations, vol. 50. Willdenowia, pp. 383–403. https://doi.org/10.3372/wi.50.50302.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet journal 17, 10–12.
Matheny, P.B., Kudzma, L.V., Graddy, M.G., et al., 2023. A phylogeny for North American *Mallocybe* (Inocybaceae) and taxonomic revision of eastern North American taxa. Fungal Syst Evol 12, 153–201. https://doi.org/10.3114/fuse.2023.12.09.
Miller, A.N., Bates, S.T., 2017. The mycology collections portal (MyCoPortal). IMA Fungus 8 (2), A65–A66.
Mueller, G.M., Cunha, K.M., May, T.W., et al., 2022. What do the first 597 global fungal Red list assessments tell Us about the threat status of fungi? Diversity 14. https://doi.org/10.3390/d14090736.
Murrill, W.A., 1945. New florida fungi. Q. J. Fla. Acad. Sci. 8, 175–198.
Myers, R.L., Ewel, J.J., 1990. Ecosystems of Florida. University of Central Florida Press, Orlando (FL).
Nagy, R.C., Balch, J.K., Bissell, E.K., et al., 2021. Harnessing the NEON data revolution to advance open environmental science with a diverse and data-capable community. Ecosphere 12. https://doi.org/10.1002/ecs2.3833.
Neill, W.T., 1957. Historical biogeography of present-day Florida. Bulletin of the Florida State Museum Biological Sciences 2, 175.
NEON Biorepository, 2024. Arizona State University Biodiversity Knowledge Integration Center (accessed through the NEON Biorepository Data Portal) https://biorepo.neonscience.org (Accessed 17 February 2025).
Niskanen, T., Lücking, R., Dahlberg, A., Gaya, E., Suz, L.M., Mikryukov, V., Liimatainen, K., Druzhinina, I., Westrip, J.R.S., Mueller, G.M., Martins-Cunha, K., Kirk, P., Tedersoo, L., Antonelli, A., 2025. Pushing the Frontiers of Biodiversity Research: Unveiling the Global Diversity, Distribution, and Conservation of Fungi. Annu Rev Environ Resour 48, 149–176. https://doi.org/10.1146/annurev-environ-112621.
NSF NEON, 2024. Neon. https://www.neonscience.org/about. (Accessed 18 November 2024).
O'Brien, H.E., Parrent, J.L., Jackson, J.A., et al., 2005. Fungal community analysis by large-scale sequencing of environmental samples. Appl. Environ. Microbiol. 71, 5544–5550. https://doi.org/10.1128/AEM.71.9.5544-5550.2005.
Oksanen, J., Blanchet, F.G., Friendly, M., et al., 2019. v. Egan: Community Ecology Package.
Olou, B.A., Hègbè, A.D.M.T., Piepenbring, M., et al., 2023. Genetic diversity and population differentiation in Earliella scabrosa, a pantropical species of Polyporales. Sci. Rep. 13, 23020. https://doi.org/10.1038/s41598-023-50398-5.
Osland, M.J., Stevens, P.W., Lamont, M.M., Brusca, R.C., Hart, K.M., Waddle, J.H., Langtimm, C.A., Williams, C.M., Keim, B.D., Terando, A.J., Reyier, E.A., Marshall, K.E., Loik, M.E., Boucek, R.E., Lewis, A.B., Seminoff, J.A., 2021. Tropicalization of temperate ecosystems in North America: The northward range expansion of tropical organisms in response to warming winter temperatures. Glob Chang Biol 27, 3009–3034. https://doi.org/10.1111/gcb.15563.
Paez, C.A., Kraisitudomsook, N., Smith, J.A., et al., 2023. Revising the taxonomic placement of *Laetiporus persicinus* within the Laetiporaceae. Mycologia 115, 107–121. https://doi.org/10.1080/00275514.2022.2139144.
Peay, K.G., Kennedy, P.G., Bruns, T.D., 2008. Fungal community ecology : a hybrid beast with a molecular master. Bioscience 58, 799–810.
Peck, H.C., 1912. Report of the state botanist 1911. N. Y. State Mus. Bull. 157, 5–135.
Põlme, S., Abarenkov, K., Henrik Nilsson, R., et al., 2020. FungalTraits: a user-friendly traits database of fungi and fungus-like stramenopiles. Fungal Divers. 105. https://doi.org/10.1007/s13225-020-00466-2.
Quintero-Corrales, C.A., Vega, M., Ramírez-Terrazo, A., et al., 2024. Downfall of an empire: unmasking the hidden diversity and distribution of the *Amanita rubescens* species complex. Mycologia 116, 642–649. https://doi.org/10.1080/00275514.2024.2355276.
R Core Team, 2021. R: a Language and Environment for Statistical Computing.
Rognes, T., Flouri, T., Nichols, B., et al., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ. https://doi.org/10.7717/peerj.2584, 2016:1–22.
Roy, M., Schimann, H., Braga-Neto, R., et al., 2016. Diversity and distribution of ectomycorrhizal fungi from Amazonian Lowland white-sand forests in Brazil and French Guiana. Biotropica 48, 90–100. https://doi.org/10.1111/btp.12297.
Runnel, K., Abarenkov, K., Copoț, O., et al., 2022. DNA barcoding of fungal specimens using PacBio long-read high-throughput sequencing. Mol Ecol Resour 22, 2871–2879. https://doi.org/10.1111/1755-0998.13663.
Ryvarden, L., 1974. Type-Studies in the Polyporaceae 2 Species Described by M. Beeli. Bull. Jard. Bot. Natl. Belg. 44, 65–76.
Ryvarden, L., 1976. Type studies in the Polyporaceae. Kew Bull. 31, 81–103.
Sato, H., Tsujino, R., Kurita, K., et al., 2012. Modelling the global distribution of fungal species: new insights into microbial cosmopolitanism. Mol. Ecol. 21, 5599–5612. https://doi.org/10.1111/mec.12053.
Seaver, F.J., 1928. The North American cup-fungi (Operculates). The author, New York.
Singer, R., 1945. New bolataceae from Florida (A preliminary communication). Mycologia 37, 797–799.
Smith, A.H., Thiers, H.D., 1964. Contributions Toward a Monograph of North American Species. Lubrecht & Cramer.
Stamatakis, A., 2014. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30, 1312–1313.
Stöver, B.C., Müller, K.F., 2010. TreeGraph 2: combining and visualizing evidence from different phylogenetic analyses. BMC Bioinf. 11, 1–9.
Stuntz, D.E., 1977. How to Identify Mushrooms to Genus IV: Keys to Families and Genera. Mad River Press Inc., Eureka, CA, USA.
Swenie, R.A., Looney, B.P., Ke, Y.H., et al., 2024. PacBio high-throughput multi-locus sequencing reveals high genetic diversity in mushroom-forming fungi. Mol Ecol Resour 24. https://doi.org/10.1111/1755-0998.13885.
Tedersoo, L., Anslan, S., 2019. Towards PacBio-based pan-eukaryote metabarcoding using full-length ITS sequences. Environ Microbiol Rep 11, 659–668. https://doi.org/10.1111/1758-2229.12776.
Tedersoo, L., Bahram, M., Põlme, S., et al., 2014. Global diversity and geography of soil fungi. Science 346. https://doi.org/10.1126/science.1256688, 1979.
Tedersoo, L., May, T.W., Smith, M.E., 2010. Ectomycorrhizal lifestyle in fungi: global diversity, distribution, and evolution of phylogenetic lineages. Mycorrhiza 20, 217–263. https://doi.org/10.1007/s00572-009-0274-x.
Tedersoo, L., Mikryukov, V., Anslan, S., et al., 2021. The Global Soil Mycobiome consortium dataset for boosting fungal diversity research. Fungal Divers. 111, 573–588. https://doi.org/10.1007/s13225-021-00493-7.
Truong, C., Mujic, A.B., Healy, R., et al., 2017. How to know the fungi: combining field inventories and DNA-barcoding to document fungal diversity. New Phytol. 214, 913–919. https://doi.org/10.1111/nph.14509.
Tulloss, R.E., Kudzma, V., Tulloss, M.K., Rockefeller, A., 2021. *Amanita amerivirosa*-a New Toxic North American Species of Amanita Section Phalloideae, vol. 1, pp. 1–15.
Vandegrift, R., Newman, D.S., Dentinger, B.T.M., et al., 2023. Richer than gold: the fungal biodiversity of Reserva Los Cedros, a threatened Andean cloud forest. Bot. Stud. 64. https://doi.org/10.1186/s40529-023-00390-z.
van Galen, L.G., Corrales, A., Truong, C., et al., 2025. The biogeography and conservation of Earth's 'dark' ectomycorrhizal fungi. Curr. Biol. 35, R563–R574.
Van Der Linde, S., Suz, L.M., Orme, C.D.L., et al., 2018. Environment and host as large-scale controls of ectomycorrhizal fungi. Nature 558, 243–248. https://doi.org/10.1038/s41586-018-0189-9.
Vandepol, N., Liber, J., Desirò, A., et al., 2020. Resolving the Mortierellaceae phylogeny through synthesis of multi-gene phylogenetics and phylogenomics. Fungal Divers. 104, 267–289. https://doi.org/10.1007/s13225-020-00455-5.
Vavrek, M.J., 2011. Fossil: palaeoecological and palaeogeographical analysis tools. Palaeontol. Electron. 14, 16.

Větrovský, T., Kohout, P., Kopecký, M., et al., 2019. A meta-analysis of global fungal distribution reveals climate-driven patterns. Nat. Commun. 10. https://doi.org/10.1038/s41467-019-13164-8.
Vilgalys, R., Hester, M., 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J. Bacteriol. 172, 4238–4246. https://doi.org/10.1128/jb.172.8.4238-4246.1990.
Vizzini, A., Alvarado, P., Consiglio, G., et al., 2023. Lulesia Singer (1970), an older name for Clitocella Kluting. In: TJ Baroni & Bergemann (2014, Entolomataceae), vol. 3. Rivista Micologica Romana, Bollettino dell'Associazione Micologica Ecologica Romana. https://doi.org/10.57624/AMER.2023.18, 3–23.
Vlasák, J., Kout, J., Vlasák, J., Ryvarden, L., 2011. New records of polypores from southern Florida. Mycotaxon 118, 159–176. https://doi.org/10.5248/118.159.
Weber, G.F., 1961. William Alphonso Murrill. Mycologia 53, 543–557.
Weinstein, B.G., Marconi, S., Graves, S.J., et al., 2023. Capturing long-tailed individual tree diversity using an airborne imaging and a multi-temporal hierarchical model. Remote Sens Ecol Conserv 9, 656–670. https://doi.org/10.1002/rse2.335.
Welti, S., Moreau, P.A., Favel, A., et al., 2012. Molecular phylogeny of Trametes and related genera, and description of a new genus Leiotrametes. Fungal Divers. 55, 47–64. https://doi.org/10.1007/s13225-011-0149-2.
Werle, E., Schneider, C., Renner, M., et al., 1994. Convenient single-step, one tube purification of PCR products for direct sequencing. Nucleic Acids Res. 22, 4354.
White, T., Bruns, T.D., Lee, S., Taylor, J., 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR protocols: a guide to methods and applications 18, 315–332.
Willis, K.J., 2018. State of the World's Fungi.
Yahr, R., Schoch, C.L., Dentinger, B.T.M., 2016. Scaling up discovery of hidden diversity in fungi: impacts of barcoding approaches. Phil. Trans. Biol. Sci. 371, 20150336. https://doi.org/10.1098/rstb.2015.0336.