An Evolutionary and Ecological Investigation into the Enigmatic Stalked Puffball Genus *Tulostoma*

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Abstract

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Tulostoma is speciose group of stalked puffballs with a high diversity in grasslands and arid environments. This cosmopolitan genus exhibits a high degree of spore morphological variation. Recent molecular analysis suggests the center of diversity of this genus occurs in European steppe habitats. In North America, taxonomic literature indicates that the center of diversity for the genus occurs in the southwestern United States. In this research, I reconstructed the phylogeny of *Tulostoma* using newly sequenced collections combined with available sequence data. This phylogeny, inferred from ITS, LSU, and Tef1α sequence data confirms the monophyly of the genus. The taxonomy of the *Tulostoma* recovered from the southwestern United States is outlined and a dichotomous key for these species is provided.

The phylogeny is then used to investigate phylogeographic patterns in the genus. The phylogeographic analysis indicates a Palearctic origin for the genus. The phylogeography also suggests both vicariance and long-distance dispersal events contributing to the diversification of the species over the evolutionary history of the genus. The evolution of spore morphology is then investigated using a phylogenetic approach. The ancestral character state for spores is medium to strong ornamentation with ornamentation being lost at least 4 times over evolutionary history. It was hypothesized that spore ornamentation and spore size are correlated with moisture availability, however comparisons between spore ornamentation and size and annual precipitation were not able to discern this pattern. Finally C:N isotopic ratios were evaluated to confirm the saprotrophic habit of *Tulostoma* species. These analyses split *Tulostoma* into 2 clusters, one associated with largely biotrophic species and the other consisting predominately of saprotrophic species. The saprotrophic status of *Tulostoma* is as expected, however the biotrophic status of *Tulostoma* as suggested by isotopic analysis warrants further investigation.

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Chapter 1: Introduction

Estimates of fungal diversity range from 1.5 million (Hawksworth and Rossman 1997) to 5.1 million species (Blackwell 2011). Despite these estimates, only about 120,000 fungal species have been described (Hawksworth and Lücking 2017). To understand fungal evolution, the impact of fungi on ecological systems, changes in species distributions, or responses to climatic factors, it is first essential to establish fungal species diversity. This research investigates the diversity, evolution, ecology, and of the fungal genus *Tulostoma* from the southwestern United States, including Arizona, New Mexico, and Colorado. Establishing the diversity of this genus will lay a foundation for understanding evolutionary pressures, biogeography, ecological roles, and responses to climate change of this genus. *Tulostoma* is a cosmopolitan fungal genus of stalked puffballs within the Basidiomycete family Agaricaceae (Figure 1.1). Species in this genus are found primarily in sandy soils, in generally arid environments. In Europe they are most commonly reported from steppe habitats (Jeppson et al. 2017).

Basidiocarps are the reproductive structures of the Basidomycetes. The production of basidiocarps increases genetic diversity and enables species dispersal through the production or spores. Tremendous variation in basidiocarp morphology exists, these forms range from finger like projections, to sticky globose spheres, to spore filled sacs, to stipitate, pileate gilled Agaricales. Gasteroid fungi like *Tulostoma* which produce their spores inside an enclosed structure (a sac or puffball). The spores are produced inside the puffball which is elevated on a stipe (Figure 1.1). The gasteroid body form has evolved multiple independent times throughout the fungal kingdom (Varga et al. 2019). Phylogenetic analyses indicate that most gasteroid genera are derived from lamellate (gilled) or poroid mushroom genera (Varga et al. 2019).

Figure 1.1. Morphological diversity of selected *Tulostoma* species. a. *T. purpusii* (MICH266163), b. *T. calcareum* (DBG21975), c. *T. punctatum* (DBG27849) d. *T. meristostoma* (MICH266306) e. T. sp. novo 10A (DBG23778). Scale bars = 10 mm.

Below I describe the biodiversity, phylogeography, spore morphology and nutritional modes of *Tulostoma.*

BIODIVERSITY OF *TULOSTOMA*

There are an unknown number of species in the genus *Tulsotoma*. Wright (1987) published a world monograph of the genus which recognized 139 species, and Kalichman et al. (2020) recognize 170 species. Many additional *Tulsotoma* species names have been published, but some of these species are not represented by type material or are known only from scant collections. To date, few phylogenetic studies on the genus *Tulostoma* have been published. Jeppson et al. (2017) published the most comprehensive phylogenetic study of the genus *Tulostoma*, which focused on European species. Their study yielded 26 species and they were able to synonymize several species names. They also discovered 19 new species through the use of molecular evidence (ITS, LSU, and TEF-α), 5 of which they were able to describe. Another phylogenetic study of *Tulostoma* focused on Macedonian species (Rusevska et al. 2019). The authors delimited 14 species in Macedonia using ITS sequence data, 4 of which are likely new to science. These studies indicate that there is great diversity in this genus, and that there are likely many undescribed species.

In the southwestern United States, there are an estimated 64 reported species of *Tulostoma* (See Table 2.1). However, no phylogenetic studies of *Tulostoma* in this region have been published to date. *Tulostoma* are infrequently collected because they occur in arid environments which are typically underexplored by mycologists. However, recent investigations into *Tulostoma* reveal high species diversity. Thus, it stands to reason that the taxonomy of the genus in southwestern United States is poorly understood.

The second chapter of this dissertation combined newly generated ITS, LSU, and Tef-α genes of *Tulostoma* from the southwestern United States with available *Tulostoma* sequences from other areas to construct a phylogeny of this genus. The biodiversity of recovered *Tulostoma* species from this region of the US is documented. The evolution of the genus is reconstructed using the most complete data set available to date.

PHYLOGEOGRAPHY

Phylogeography, the reconstruction of historical processes which gave rise to the geographic distribution of a taxonomic group, seeks to determine where species originated and the direction and potential methods or mechanisms of dispersal (Avise 2000). Fungal phylogeography is understudied (Sánchez-Ramírez et al. 2017). Most fungal phylogeographic studies have focused on mycorrhizal fungi and their plant hosts, while phylogeographic studies of non-wood rotting saprotrophic fungal species are almost non-existent. The genus *Tulostoma* provides an opportunity to investigate larger patterns of fungal dispersal since it is globally distributed. Also, it is presumed to be a saprotrophic species, thus the dispersal of this genus is likely not constrained by the distribution of a plant host.

In the third chapter, I investigated the phylogeographic history of the genus *Tulsostoma.* Using 3 loci (ITS, LSU, and Tef1α), the phylogeographic patterns of this genus were investigated using a probabilistic inference of both historical biogeography, which reconstructs the ancestral geographic ranges on a phylogeny. This method combines ancestral ranges with a comparison of different models of range evolution. The monophyly of this genus is confirmed and complex patterns of distribution emerge. There is strong support for a Palearctic origin for this genus. Multiple long-distance dispersal events are hypothesized over the evolutionary history of *Tulostoma*. The known center of biodiversity occurs in European grasslands, and diversification of this genus corresponds with historical diversification and expansion of these habitats.

Distribution patterns may suggest generalist and specialist species adapted to soil types influence species ranges, which also correspond to microhabitat differences within grasslands. This research lays the groundwork for understanding phylogeography and distribution patterns of non-wood rotting saprotrophic fungi.

SPORE MORPHOLOGY

In addition to high species diversity, *Tulostoma* also exhibits tremendous spore variation among species (Figure 1.2). The production of spores increases genetic diversity of fungal populations, which increases species fitness (Markert et al. 2010). The majority of spores are air dispersed, and spore dispersal is likely the most effiecient mechanism of increasing species ranges. Additionally, spores must land on a suitable substrate and germinate in order to complete the fungal meiotic lifecycle. Selective pressures on spore morphology are tied to species fitness, and air dispersal, which are essential to completing the meiotic lifecycle. Despite the significance of spore morphological characters, the evolutionary pressures on spore morphological diversity are largely unknown (Golan and Pringle 2017).

In the fourth chapter, the ancestral character state of *Tulostoma* spores was inferred using the phylogenetic reconstruction of *Tulostoma* from three loci (RPB2, LSU and TEF1a). Spore morphologies were analyzed using SEM imaging and published literature. Phytools was used to infer the ancestral spore morphological state. Results show ornamented spores as the ancestral character state in *Tulostoma*. Spore ornamentation was both lost and gained multiple times over the evolutionary history of *Tulostoma.* The plasticity in spore morphology indicates that selective pressures are acting upon *Tulostoma* spore morphologies and suggests that spore morphology may have reproductive advantages in certain habitats. Further investigations into selective advantages of spore morphologies are necessary to understand their evolutionary significance.

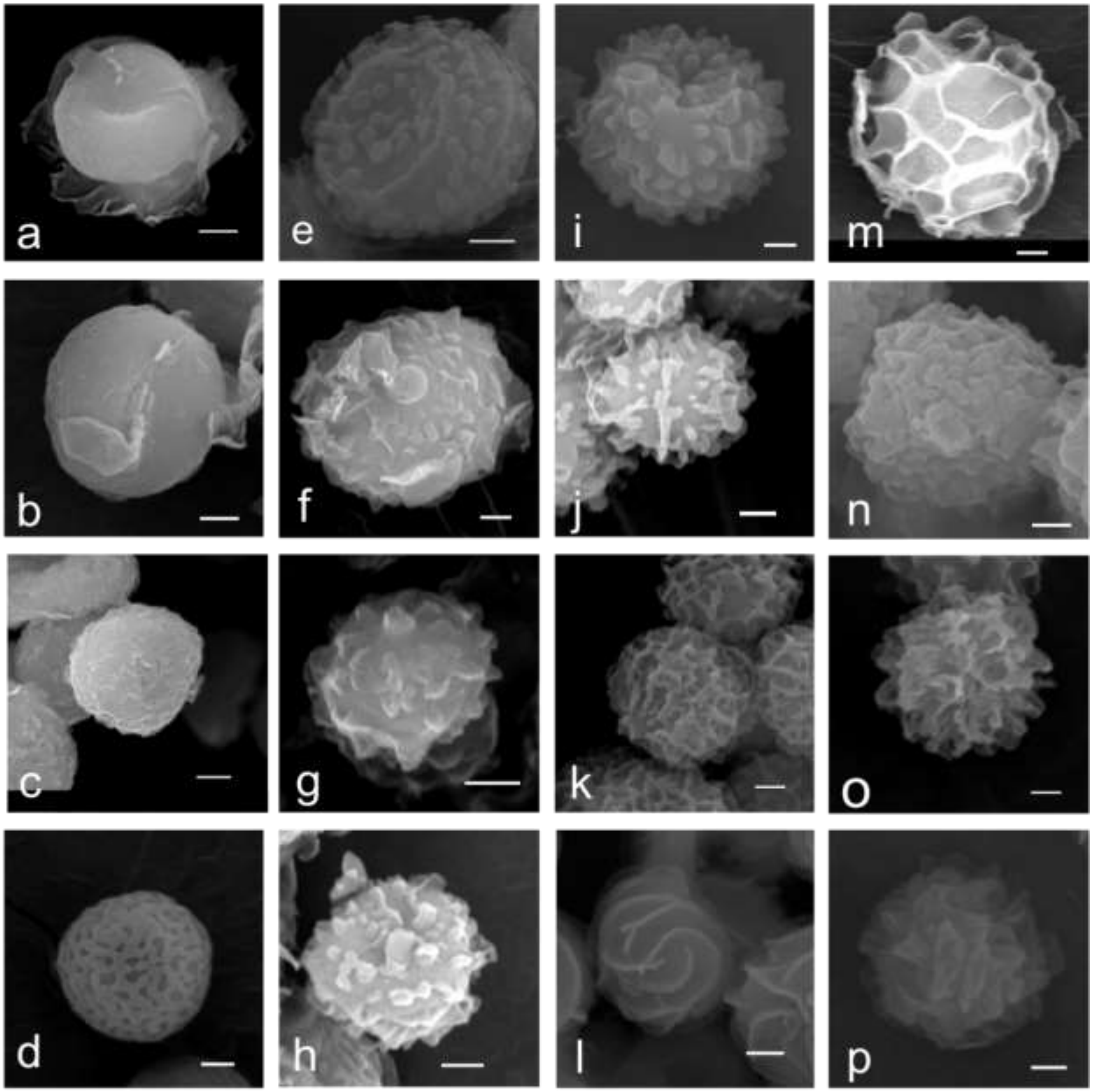


Figure 1.2. Spore morphology diversity of selected *Tulostoma* species. a. *T. meristostoma* (MICH266306), b. *T. grandisporum* (MICH 338158), c. *T. macrocephalum* (MICH28572), d. *T. obscurum* (DBG28400), e. *T. pulchellum* (MICH266205), f. *T.* sp. novo (DBG23778), g. *T. simulans* (DBG24113), h. *T. involucratum* (ILL11718), i. *T. punctilibratum* (MICH24086), j. *T. fimbriatum* (DBG24032), k. *T. purpusii* (MICH266163), l. *T. striatum* (AZ43457), m. *T. opacum* (MICH400) n. *T. punctatum* (DBG27849), o. *T. calcareum* (DBG21975), p. *T. squamosum* (MICH28574). Scale bar = 1 μm.

NUTRITIONAL MODES

Fungi are ecologically important members of all terrestrial communities, and the ecological roles of fungi are often distinguished by their nutritional modes. In terrestrial environments, fungi form mutualistic relationships with plants as mycorrhizae and decompose dead organic material as saprophytes. Despite their importance in global ecosystems, our understanding of the ecological roles of many individual fungal species and lineages remains speculative.

Studies show that gasteroid genera follow the nutritional mode of their sister mushroom taxa (Grubisha et al. 2017). *Tulostoma* are classified in the Agaricales, which contains both ectomycorrhizal lineages and saprotrophic line lineages. There is strong evidence to support the ectomycorrhizal habit (Matheny et al. 2006) and the saprotrophic habit of lignicolous lineages (Floudas et al. 2015), while the saprotrophic habit of many purely terrestrial fungi remains speculative. Within the Agaricales, species in the family Agaricaceae are thought to be largely saprotrophic. The lignicolous habit of some Agaricaceae has been investigated, but definitive evidence for the saprotrophic habit of terrestrial species is lacking.

Determining nutritive modes of fungi are essential to understanding their ecological roles. Methods in differentiating between mycorrhizal or saprotrophic nutritional modes vary. Isotopic evidence has been used to distinguish between ectomycorrhizal (EMF) and saprotrophic fungi. EMF fungi are enriched in ^{15}N and depleted in ^{13}C compared to saprotrophic fungi. In order to confirm mycorrhizal status indicated by isotopic evidence, researchers usually compare molecular analyses of the fungal ITS DNA region from tree root tips to mushroom bodies. Mycorrhizal nutritional modes are assigned to fungi that are found, through these DNA analyses, on living root tips. Saprotrophic lifestyles have historically been hypothesized based on the growth substrate. If a fungal body is found on substrates such as dead wood or litter and there is lack of mycorrhizal evidence, researchers classify that species as a saprophyte. In the absence of isotopic evidence, classification of saprotrophic fungi is often based on the lack of mycorrhizal evidence.

Tulostoma are thought to be saprotrophic (Wright 1987), however this has not been confirmed. Wright (1987) suggested that some *Tulostoma* may be mycorrhizal based on rooting stipe features. In the fifth chapter, I investigated the nutritional mode of *Tulostoma* using ^{15}N and ^{13}C isotopic evidence. The data show evidence that some species of *Tulostoma* appear saprotrophic and that the isotopic profiles of some *Tulostoma* species are non-saprotrophic, and likely biotrophic. The host of *Tulostoma* with biotrophic ^{15}N and ^{13}C signatures has not been ascertained.

OBJECTIVES

This dissertation investigates the evolution and ecology of the genus *Tulostoma* through several avenues. Using isotopic and molecular data I address the following research objectives in each of the indicated chapters:

- Chapter 2: To determine the evolutionary and phylogenetic history and taxonomic diversity of the genus, with a focus on the southwestern United States, using ITS, LSU, and TEF1α sequence data.
- Chapter 3: To reconstruct the phylogeographic history of the genus using ITS, LSU, and TEF1α sequence data

- Chapter 4: To investigate the ancestral character state of spores using the molecular data set and ancestral character state analysis. is investigated.
- Chapter 5: To infer the nutritional modes of Tulostoma species using their isotopic profiles.

Chapter 2: Biodiversity of *Tulostoma* from the Southwestern United States, a Phylogenetic and Taxonomic Investigation

INTRODUCTION

Tulostoma Pers:Pers is a cosmopolitan genus of stalked puffballs which exhibit the greatest diversity in steppe and semi-desertic habitats. The phylogenetic relationship of *Tulostoma* within Agaricaceae s.l. remains in flux, and the sister lineage to *Tulsotoma* has not been clearly established. Phylogenies by Vellinga (2004) and Matheny et al. (2006) show *Tulostoma* to be sister to Agaricaceae s.l. Velling et al. (2011) show *Tulostoma* sister to *Lepiota* and these sister to Agaricaceae s.l. (Vellinga et al. 2011). Most recently, Kalichman et al. (2020) show Tulostomataceae forms a polytomy with several lineages as part of Agaricaceae s.l.. A recent update to the tree (May 2021, https://agaric.us/a&a%202021-05-15.svg) renames Tulostomataceae as Battarraceae yet retains their original phylogenetic topology. I accept *Tulostoma* as part of Agaricaceae s.l. and suggest that further molecular evidence is necessary to better understand the phylogenetics within this group.

Species currently placed in the genus *Tulostoma* were first published as *Lycoperdon* (de Tournefort 1700, Linnaeus 1753). In 1794, Persoon erected the genus *Tulsotoma* and included two species in the genus, *T. brumale* and *T. squamosum* (Persoon 1801). The genus has also been referred to as *Tylostoma* (Sprengel 1797) and *Tulasnodea* (Fries 1849). Following the International Code of Nomenclature, *Tulostoma* is considered the correct name (Wright 1987, Jeppson et al. 2017).

Wright (1987) provides a detailed taxonomic history of the genus in a world monograph. Of greatest relevance to this work are a monograph of North American species by White (1901) and various publications of North American species by Long (1944, 1946, and 1947). Other important taxonomic contributions are those of Pouzar (1958), Kreisel (1984), Moreno et al. (1992a, 1992b), Moreno et al. (1995), Calonge (1998), and Altés et al. (1999). Jeppson (2017) published the most comprehensive phylogeny of the genus to date.

Currently, there are an unknown number of species in the genus *Tulsotoma*. Wright's (1987) world monograph of the genus recognized 139 species, and Kalichman et al. (2020) accepted 172 species in the genus. Species Fungorum (http://www.speciesfungorum.org; accessed February 16, 2021) shows 177 species names, with an additional 49 synonymized names. Mycoportal (http://mycoportal.org; accessed February 16, 2021) recognizes 152 species. Many of these names have no type material or are known only from scant collections.

The genus name *Tulostoma* is derived from the Greek words tylos meaning knob and stoma meaning mouth, which describes the stalked puffball mushrooms formed in this genus. Most species of Tulostoma occur in dry and sun-exposed situations, they are frequently found growing in moss or in places with low or patchy vegetation, either on sandy soil (sand steppes, sand dunes), in dry grasslands or steppe habitats. A few species occur among grasses in soil in wooded or at least partially shaded habitats. *Tulostoma* species are thought to be saprotrophic although several species appear to be associated with mosses (Wright 1987, Jeppson 2008). Wright (1987) suggests some members of this genus may be mycorrhizal based on the production of rhizoids formed in some species (Wright 1987).

Tulostoma are characterized by several macro- and micromorphological features. A mature *Tulostoma* basidiocarp consists of a spore sac elevated on a stem. The globose spore-sac primordium develops hypogeously, very close to the soil surface. In the early stages of development, an exoperidium surrounds the developing spore-sac, which is enclosed by an endoperidium. At maturity the stem rapidly expands, lifting the spore sac above the soil surface. During this period of development, changes in the exoperidium occur based on the morphology of the exoperidium, and are of taxonomic, but not necessarily phylogenetically, significance. The elements of the exoperidium are usually encrusted with particles from the substrate, such as sand or soil. The exoperidium is generally classified as either hyphal (appearing as a very thin layer of tissue on the endoperidium, gradually wearing away) or membranous (a thick layer of tissue, well differentiated from the endoperidium which peels or flakes off). The color of the exoperidium is usually some shade of white to grey but this is not a commonly used in taxonomic character as it can be difficult discern due to substrate adherence and because it soon disappears after maturity, particularly in the case of a hyphal exoperidium. The exoperidium generally remains at the base of the spore sac. The layer of tissue interior to the exoperidium, called the endoperidium, contains the gleba. There is some variation in endoperidium thickness between species. Endoperidial colors ranges from a reddish or orange brown to ochre to whitish or greyish. The darker endoperidial colors usually wear away with age in most species, and most species become whitish, pale ochre, or greyish in age. As such, the endoperidium color is not usually a taxonomically useful character.

A small apical stoma, referred to by most authors as a mouth, forms on the endoperidium of the spore sac, and opens to enable spore release. Mechanisms of spore release include mechanical compression by factors such as wind, rain, or trampling (Sunhede 1976). Mouth morphology is a useful taxonomic character and is distinguished as either definite, there is some type of defined opening, or indefinite, a slit or lacerate opening forms on the endoperidium to release spores. A definite mouth can be circular, where there is an even margin to the mouth, or some type of fibrillose opening, where the margin is dentate. The mouth can be planar, flat on the spore sac to projecting, where it protrudes from the spore sac. While endoperidium colors generally fade, colors around the mouth can be have taxonomic use. The mouth in some species is surrounded by a darker ring zone which does not usually fade in maturity.

The stem in some species can also be used for taxonomic identification. Most species have a tough stem with a woody texture the ranges in color from whitish to ochre to brown or dark brown. Stem surfaces can be almost smooth, longitudinally furrowed or fissured or more or less squamulose. The stem of several species produces various structures, such as a volva, bulb or rhizomorphs, that are taxonomically useful.

Inside the spore-sac, the gleba consists of sterile hyphal strands, capillitia, and basidiospores. Basidia are not observed in mature specimens. The mature gleba in most species is orange to rust brown, and thus provides little taxonomic significance. The capillitial hyphae are usually branched, usually regularly septate, usually thick walled and average 4 – 7 µm in diameter. Capillitia septum are commonly categorized as either distinctly and bilaterally widened, unilaterally widened or only slightly or not widened at all.

Tulostoma spores are globose to subglobose, the vast majority ranging from 4 – 6 µm, and sometimes with a short apiculus. The spore wall ornamentation, often only observable under SEM, varies in taxa from perfectly smooth, to minutely verrucose, to striate, to dense, prominent verrucae, to reticulate. The verrucae in some species fuse to form ridges, or anastomosis. Spore wall ornamentation is of great taxonomic significance, and the fact that

SEM imaging is necessary to provide species distinctions has likely contributed to some taxonomic uncertainty in this genus.

The genus *Tulostoma* is of interest due in part to its great, often cryptic, diversity. *Tulostoma* are infrequently collected, and herbarium collections of this genus are lacking in North America. The rarity of collections is possible due to its ecology as they grow in seemingly barren areas and could be frequently overlooked. Cunningham (1925) notes that *Tulostoma* is considered by some to be one of the most difficult genera of gasteroid fungi to delimit species. Moreno et al. (1992) also note the taxonomic complexity of this genus. The rarity is likely also, in part, attributable to habitat loss. Multiple European *Tulsotoma* species have are considered threatened, endangered, or possibly extinct (Jeppson 2008).

Additionally, *Tulostoma* display unexpectedly high diversity of spore characteristics between species (Jeppson 2017). Spores of *Tulostoma* range from completely smooth to highly ornamented. Ornamentation ranges from small bumps to prominent spines or reticulation. Often times, the ornamentation is not distinguishable with light microscopy and requires scanning electron microscopy for visualization. This adds to the taxonomic challenges of species identifications.

While *Tulostoma* species have been included in phylogenetic analyses, few phylogenetic studies on the genus have been published to date. Gube (2009) included *Tulostoma* sequence data in his investigations on the gasteromycetation process in Agaricales. Jeppson et al. (2017) published the most comprehensive phylogenetic study of the genus *Tulostoma* which focused largely on European species. Their study yielded 26 species and they were able to synonymize several species names. They also discovered 19 likely new species through the use of molecular evidence (ITS, LSU, and TEF-α), 5 of which they described. The only other phylogenetic study of *Tulostoma* focused on Macedonian species (Rusevska et al. 2019). The authors delimited 14 species within Macedonia using ITS sequence data, 4 of which are likely new to science. These studies indicate that there is great diversity in this genus, and that there are likely many undescribed species. In particular, there are likely many undescribed North American species as investigations into North American *Tulostoma* are wholly lacking.

Few phylogenetic studies of *Tulostoma* exist. Jeppson et al. (2017) published a phylogenetic study of the genus *Tulostoma*, which focused on European species. Their study yielded a total of 45 phylogenetically and morphological distinct species from Europe. In their study they synonymized several species names and report a total of 26 previously described species. They discovered 19 new species through the use of molecular and morphological evidence (ITS, LSU, and TEF-1α), 5 of which they described as new species. Another phylogenetic study of *Tulostoma* focused on Macedonian species (Rusevska et al. 2019). The authors delimited 14 species within Macedonia using ITS sequence data, 4 of which are likely new to science. Gube (2009) used *Tulostoma* ITS sequence data to study gasteromycetation events in Agaricaceae. These data show great diversity of species and resolve *Battarraea* as sister to *Tulostoma*. These studies indicate that there is great diversity in this genus, that there are likely many undescribed species, and that taxonomic redundancies likely exist. In particular, there are likely many undescribed North American species as investigations into this genus in North America are lacking.

Phylogenetic studies of North American members of this genus are necessary for several reasons. There are an unknown number of species in this genus, and many of the named species are ambiguous. Studies of this genus in Europe indicate that there is high species diversity in this genus and there are many undescribed species. Virtually no information on North American species, their distributions, or ranges exists. All information on the ecological roles of this genus is speculative. No published study exists on their nutritional modes or their role in the environment, or place in food webs. In order to understand the evolution and ecology of the species in this genus, it is necessary to first clarify the species present. With the establishment of the representative species the evolution and biogeography of the genus may be investigated. With an understanding of the evolution and biogeography inference into the selective pressures on spore morphologies can be studied. Additionally, species circumscriptions will benefit the investigations into Tulostoma nutritional modes.

In Arizona, Colorado, and New Mexico, there are an estimated 64 species of *Tulostoma* (Table 2.1), however, the scientific community lacks an in depth understanding of exact species distinctions. A phylogeny, the evolutionary history of a group of organisms, is needed to better understand the evolutionary relationships between the species of *Tulostoma.*

Pouzar (1958) first delimited infrageneric classification of *Tulostoma*. Wright (1987) expanded on these and erected infrageneric subgenera and sections of *Tulostoma* based on morphological, mainly exoperidial and mouth, characteristics. Phylogenetic analysis does not support many of the infrageneric classifications (Jeppson et al. 2017). The subgenera (*Tulostoma* and *Lacerata*) were erected based on ostiole characteristics. Within subgenus *Tulostoma*, Wright's (1987) subsections *Fimbriata* and *Poculata* have some phylogenetic significance (Jeppson et al. 2017). The remaining sections that have molecular data do not show phylogenetic congruence with the infrageneric taxonomy. Further, some taxa that were assigned to separate sections have since been synonymized. For example, *T. verrucosum* (sect. *Granulosae*) has been synonymized with *T. squamonsum* (sect. Brumalia) (Moreno et al. 1992). Jeppson et al. (2017) highlight the need for infrageneric classification of *Tulostoma* and additional genomic data and taxa are necessary for this goal.

The objective of this study was to use the ITS, LSU, and TEF-1α genes and morphology in order to construct a phylogeny of *Tulostoma* from the Southwest US. This phylogeny is necessary to first establish the species of *Tulostoma* present in this area. This information can then be used to understand biogeography, the evolution of spore morphological diversity, and ecological roles of *Tulostoma*.

METHODS

Systematic and Taxonomic Diversity of *Tulostoma*

Taxon Sampling

Fungal collections were collected from Gunnison, CO and borrowed from fungal collections from the following herbaria: DBG, MICH, ARIZ, and ILL. DNA was extracted from 58 of these collections.

Morphological Analyses

Herbarium collections were photographed and studied under a stereomicroscope and using SEM microscopy. Morphological features are named in accordance with Wright (1987). For microscopic studies, samples of mature gleba were mounted in Cotton blue + lactophenol and heated to boiling. Features of the peridium were studied in Melzer's reagent. Spores were measured at a magnification of 1000x, using the Dino-Lite 2.0 software (www.dino-lite.eu). A minimum of 20 spores were measured for each sample. Spore measurements include ornamentation.

Spores were imaged using SEM microscopy. Spores were mounted on double sided carbon tape mounted to SEM stubs. Stubs were sputter coated with gold palladium using machine for at pressure for 4 minutes. Sputter coated samples were imaged using JEOL 6450LV most commonly at 8 mm working distance at high vacuum with 50 spot size at 10K magnification with 25kV accelerating voltage. Critical point drying was performed on select samples that were collapsed on first imaging attempts.

DNA extraction, PCR, and Sequencing

To prepare fungal tissues for DNA extractions, approximately 0.5 g dried material was isolated from collections. All utensils used in the removal and weighing of fungal material were sterilized as appropriate. Metal equipment was dipped in 95% ethanol and flamed, while flammable materials were wiped with 95% ethanol and allowed to air dry. Fungal material was transferred to a 1.5 ml Eppendorf tube and ground using a sterilized micropestle and sterilized sand (Fisher Chemical S25-500). DNA was extracted from the prepared fungal tissues with the E.Z.N.A. Fungal DNA extraction kit (Omega BioTek D3390-01) used with the manufacturer's instructions.

Target DNA sequences from ITS, LSU, and TEF-1α were amplified using polymerase chain reactions (PCR). The ITS region was amplified from all collections using the fungal specific primers ITS1F and ITS4B (Gardes et al 1993). LSU regions were amplified with LR21 and LR7 primers (Hopple and Vilgalys 1999) and TEF-1α was amplified with EF983F and EF2218R primers (Rehner and Buckley 2005).

Twenty-five µl PCR reactions were performed with 5 µl template DNA (approximately 10 ng DNA/µl), 2.5 µl primer of each primer, 12.5 µl GoTaq, and 5 µl sterile H_2O. PCR amplification began with an initial denaturing at 95°C for 2 minutes. Thirty PCR cycles ran with denaturing at 94°C for 1 minute, annealing at 50°C for 30 seconds and elongation at 72°C for 1 minute. A final elongation ran at 72°C for 5 minutes. PCR products were stored at approximately 4°C.

PCR products were visualized on 3% agarose gels. Successful PCR products were determined as clean bands approximately 400-600 bp for ITS, approximately 700-900 bp for LSU and approximately 1000 bp for TEF-1α. These PCR products will be cleaned using QIAquick PCR purification kit using manufactures protocols. Clean PCR product of target regions were sent to the University of Arizona sequencing center for Sanger Sequencing.

Evolutionary Reconstruction

Individual DNA sequence base pairs were visualized and edited using Sequencher. Sequences were assembled in Excel and exported to BBEdit. Sequences from this project were combined with available sequence data (GenBank and personally loaned data from Dr. Matthias Gube (Georg-August-Universität Göttingen, Germany). Sequences were aligned in Mesquite. Aligned sequences were trimmed to the first and last informative base. Aligned sequences of each gene were concatenated using SequenceMatrix 1.8. The concatenated matrix was analyzed in CIPRES Web Portal (Miller et al. 2010). Maximum Likelihood Analyses were conducted using RaxML. The best tree from 1000 bootstrap replicates was chosen and edited in FigTree (Rambaut 2009).

RESULTS

Systematic and Taxonomic Diversity of *Tulostoma*

Under the most recent taxonomic understanding, 64 species of *Tulostoma* are reported from Arizona, Colorado, and New Mexico (Table 2.1). Collections sequenced, collection numbers, and Genbank accession numbers are listed in Appendix 2.1. Our phylogenetic and morphological analyses delimit 20 species of *Tulostoma* from the southwestern US (Figure 2.1, Table 2.1), 18 of which have been identified. A few species, such as *T. fimbriatum*, and *T. simulans* are represented by multiple sequences. The majority are represented only by one or two collections. This suggests that there is probably greater diversity of *Tulostoma* in the south western US, and that species are likely not well represented in herbaria.

Most species branches and some tentative clade circumscriptions have high levels of bootstrap support, however the backbone lacks strong support. The overall topology of the tree is largely consistent with Jeppson et al. (2017).

Clade 1 corresponds to Clade 9 in Jeppson et al. (2017). This clade is comprised of *T. striatum* and *T. pulchellum*. Both of these species are found in the southwestern US. This clade is distinguished by a distinctly membranous exoperidium and white endoperidium.

Clade 2 contains *T. submembranaceum* and an unidentified sp. (15) from Jeppson et al. (2017) Clade 2 is resolved identically in Jeppson et al. (2017) as Clade 7. Neither species in this clade are reported from the southwestern US.

Clade 3 generally corresponds to Jeppson et al. (2017) Clade 5. The majority of species in this clade are unnamed species. Collections which match sp. 10 from Jeppson et al. (2017) are shown. Currently, I am collaborating with Jeppson to identify these species using morphology and sequence data. In the phylogeny an unnamed sp. (4) from Jeppson et al. (2017) is contained within this clade while their unnamed sp. 7 is not resolved in this clade. Also absent from this clade in my phylogeny is *T. lusitanicum*, and present is *T. nanum*. The only species found in this clade that occurs in the current geographic focus is *T. lysocephalum*. None of the observed collections match the morphology or genetics of *T. lysocephalum*.

Clade 4 contains four species, three of which have mostly smooth spores*, T. meristostoma*, *T. lloydii* and *T. grandisporum*. The spores of *T. obscurum* exhibits uniquely subtle ornamented spores. This clade does not completely align with the phylogeny from Jeppson et al. (2017), and neither this phylogeny nor their corresponding clade has strong support. In their phylogeny, *T. grandisporum* and *T. lloydii* are in a clade which also contains two smooth spored species, *T. fulvellum*, and an unnamed species (16), which has *T. leiosproum* characteristics.

Clade 5 contains five species, *T. pannoncium*, *T. pseudopulchellum*, *T. creatceum*, *T. macrocephalum*, and an unnamed species (14) from Jeppson et al. (2017). This clade corresponds identically to Clade 6 from Jeppson et al. (2017). Jeppson et al. (2017) considered the species contained in T. *cretaceum* as a complex. I have added the type sequence and show that *T. cretaceum* has large sequence variation. It is perhaps a species complex, but there is no geographic signal from these sequences. There is considerable morphological variation in species in this clade. The species in this clade have similar fibrillose to fimbriate mouth morphology, with the mount in some individuals in a collection becoming lacerate. However, other morphological characters vary in this clade. Both hyphal and membranous exoperidia are exhibited in this clade as well as smooth and ornamented spores.

Clade 6 contains five named species (*T. polymorphum*, *T. cyclophorum*, *T. obesum*, *T. punctatum*, and *T. pygmaeum*). This clade corresponds to Clades 1 and 2 of Jeppson et al. (2017). The unifying characteristic of species in this clade is a hyphal exoperidium. Habitat, substrate, and mouth, spore, and endoperidial morphology varies greatly among species in this clade. The sequences of the type of *T. polymorphum* match an unnamed species (2) from Jeppson et al. (2017) suggesting a larger distribution of this species than is currently reported. Morphological data on these species are necessary to confirm these species are conspecific.

Clade 7 contains six named species (*T. winterhoffii*, *T. punctilabratum*, *T. egranulsom*, *T. calongei*, *T. tuberculatum* and *T. lusitanicium*), the *T. fimbriatum* complex, and a few unnamed species. This clade generally corresponds to Jeppson et al. (2017) Clade 3. This group has species that are characteristic of *T. fimbriatum*. These features include a hyphal exoperidium and a fibrillose-fimbriate mouth. Within the *T. fimbriatum* clade, there are three unsupported clades with some geographic signal. The clade which contains the epitype consists of European collections, another contains collections mostly from Colorado, and another clade contains collections from across the US. It is possible that these clades may represent individual, distinct species. *T. puntilabratum,* also in this clade, is shown to be closely related to an unnamed species (17) from Jeppson et al. (2017). The other species in this clade were not found in the study area.

Clade 8 consists of 11 species (*T. rufum*, *T. dominguezidae*, *T. verrucosum*, *T. squamosum*, *T. ahmadii, T. subsquamosum, T. purpusii*, *T. calcereum*, *T. melanocyclum*), and two unnamed species, one from Argentina, and a soil isolated sequence from California. This clade corresponds to Clade 11 of Jeppson et al. (2017). Species in the clade are characterized by having darker colors compared to other *Tulostoma* species, and moderately to strongly ornamented, verrucose-echinate spores.

Clade 9 contains ten named species (*T. niveum, T. exbladii*, *T. gionaellae*, *T. xerophilum*, *T. adhaerens*, *T. excentricum*, *T. simulans*, *T. brumlae*, *T. albicans*, *T. beccariacum*), as well as a few species that remain unnamed. This clade corresponds to Clade 10 from Jeppson et al. (2017). The unifying characters of species in this clade are a circular, tubular or protruding mouth and ornamented spores. Jeppson et al. (2017) first demonstrated that there is geographic signal between *T. brumale* and *T. simulans*, two morphologically similar species. The addition of my sequence data confirms that species *T. brumale* is not commonly found, at least in the southwestern US, and *T. simulans* is the correct name for species found in this area. *T. simulans* is also widely distributed in Europe. The ITS of the types of *T. albicans, T. excentricum,* and *T. xerophilum,* generated by Jeppson et al. (2017) are included. One of my newly sequenced collections matches *T. xerophilum*. In this clade, *T. excentricum* has been reported from the southwestern US, but I did not recover this species in our study. The remaining species in this clade are European and have not been reported in the southwestern US.

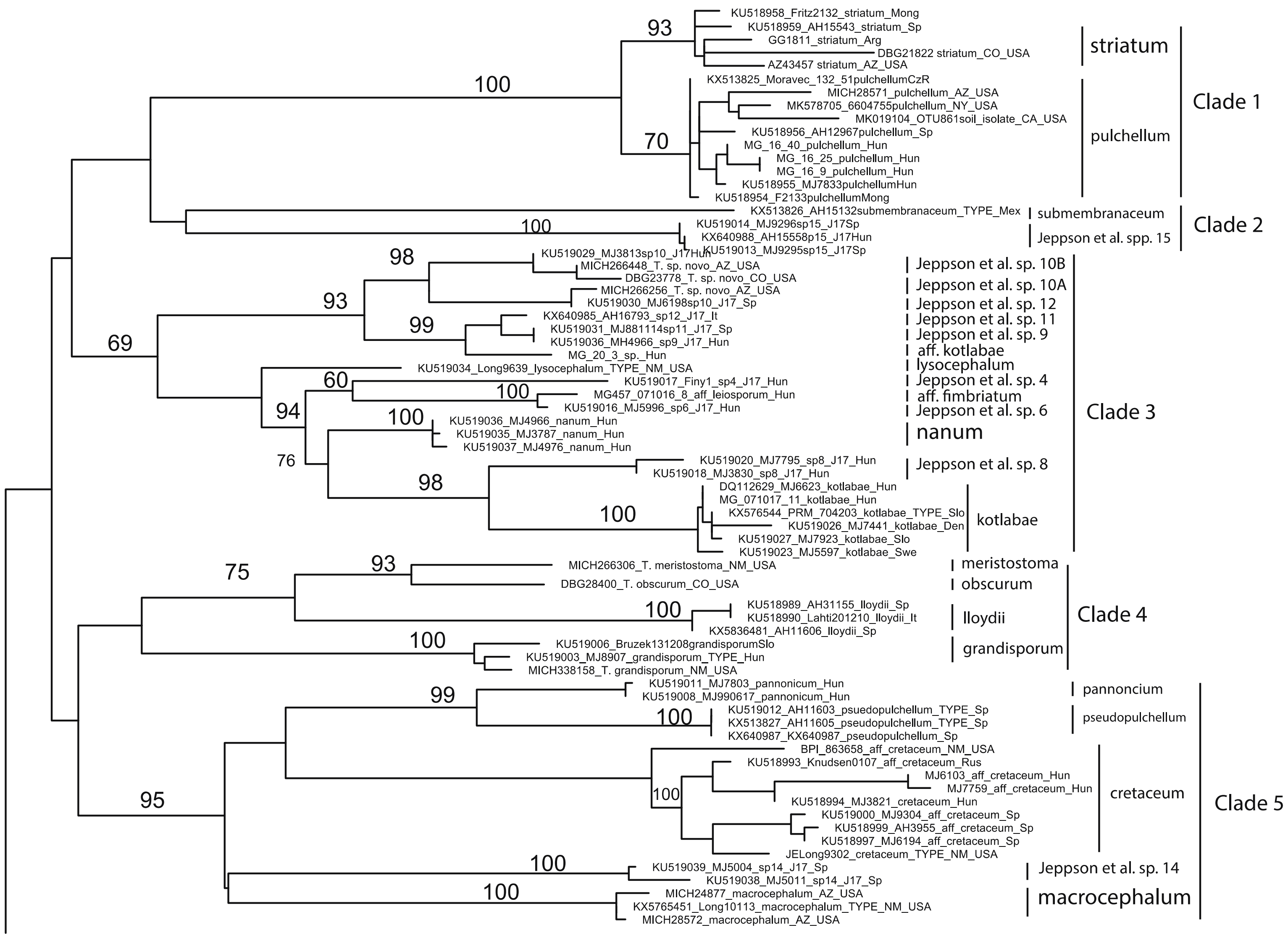


Figure 2.1. Maximum likelihood phylogeny inferred from ITS, LSU, and Tef1α sequence data. Bootstrap numbers greater than 60 are reported.

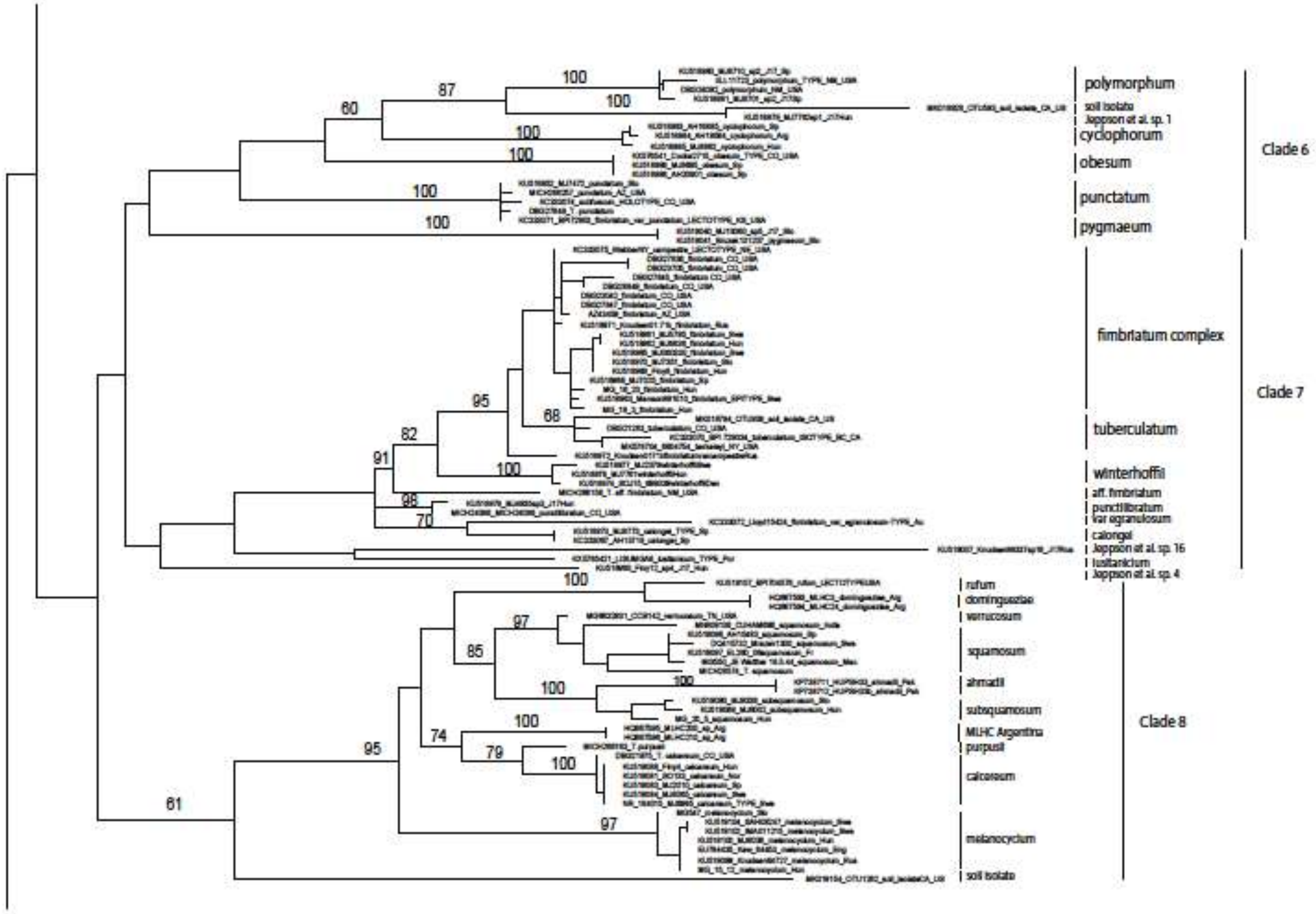


Figure 2.1. Continued.

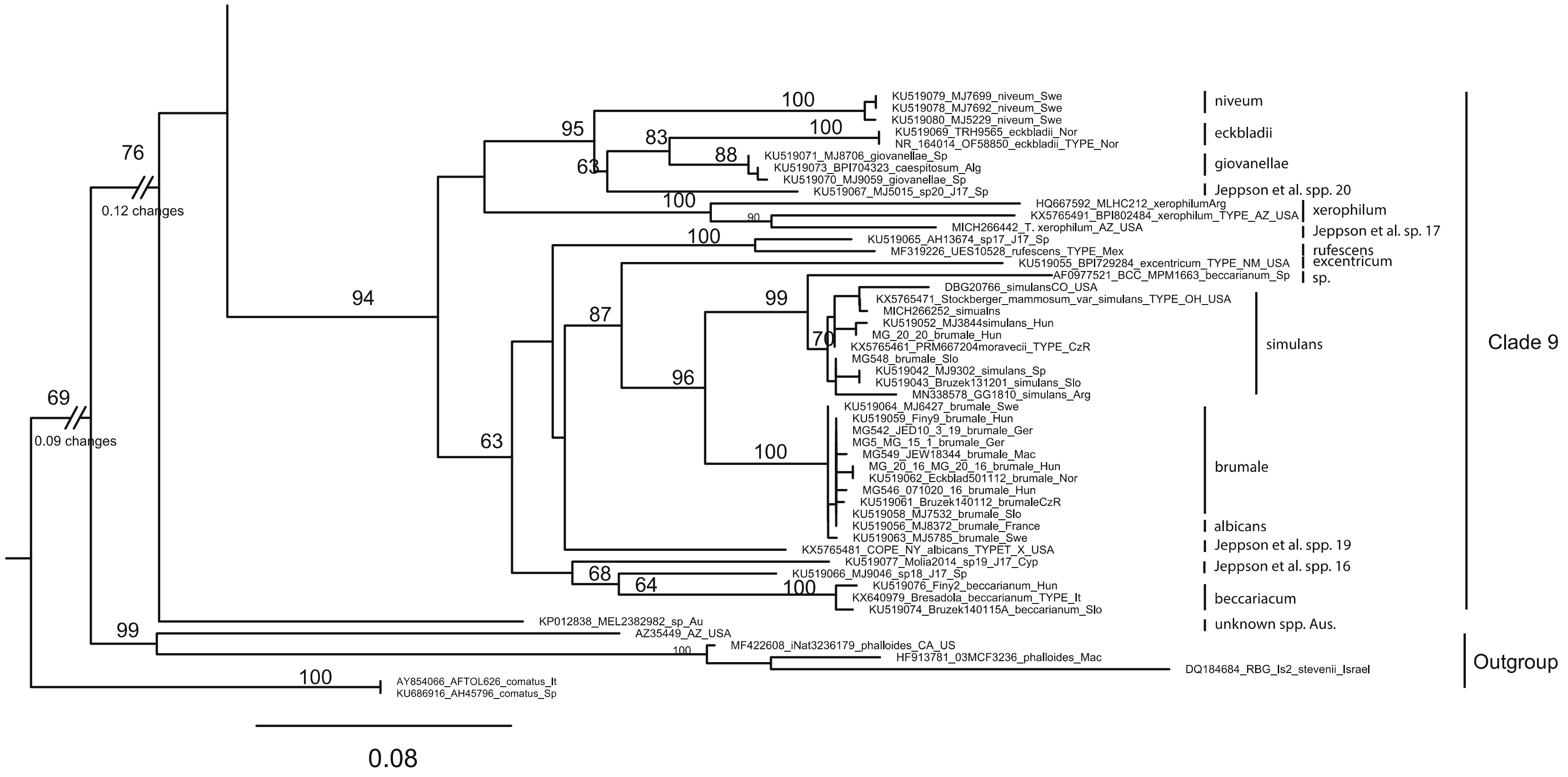


Figure 2.1. Continued.

Taxonomy

Tulostoma calcareum Jeppson, Altés, G. Moreno & E. Larsson, (Fig. 3) Mykokeys 21:33-88 2017.

See Figure 2.2

Holotype. Sweden, Södermanland, Mörkö, Egelsvik, Kalkberget, on calcareous soil under *Artemisia campestris* in rocky slope, 5 Nov. 2004, M. Jeppson 6965 (GB!, isotype AH).

Etymology. The name refers to its habitat requirement, on calcareous sandy soil or among calcareous rocks and cliffs.

Remarks: Jeppson et al. (2017) originally described this species from European collections. *T. calcareum* can be confused with *T. squamosum*. When compared to *T. squamosum*, the spores of *T. calcareum* are nearly identical in size and ornamentation, however there are differences in the exo- and endoperidial characteristics. The exoperidium of *T. calcareum* is hyphal and lacks sphaerocysts while *T. squamosum* is reported to have a membranous exoperidium containing sphaerocysts. The colors of the endoperidium differ between the species. The endoperidium of *T. calceruem* can be brownish-ochraceous, young, it fades to greyish white with age. *T. squamosum* has an ochraceous exoperidum that does not fade in age, and which is nearly concolorous with the stipe.

T. calcareum is also distinguished by a circular, projecting mouth, a basal bulb at the base of the stipe, and ornamented spores. Spore ornamentation is composed of prominent veruccae which fuse into pyramidal structures. The fusing forms some ridges on occasion.

Habitat and distribution: In Europe, *T. calcareum* occurs in dry, exposed to semi-shaded situations in calcareous, sandy habitats and on calcareous rocks and cliffs. Jeppson et al. (2017) recorded this species from Hungary, Norway, Spain, and Sweden, this is the first record from N. America, where it was reported from gravelly soil.

Material examined. UNITED STATES, **Colorado:** Pitkin County, Difficult Campground, 31 July 2009, in soil near base of clump of willows spruce and fir, soil gravelly (DBG21975).

Figure 2.2. *T. calcareum* (DBG21975) a. basidiocarps; b-d. spores. Scale bars: a = 10 mm; b-d =1 µm.

Tulostoma fimbriatum Fries Fr., Syst. mycol. (Lundae) 3(1): 43 (1829).

See Figure 2.3

=*Tulostoma fimbriatum* var. *campestre* (Morgan) G. Moreno, An. Jard. bot. Madr. 36: 18 (1980).
= *T. granulosum* Lév. forma pallida Bres. In Herb.
= *T. punctilabratum* Long, Lloydia 10: 134-135, fig. 18. 1947.
= *T. karagandense* Schwarzmann & Philimonova, Fl. Spor. Rast. Kazhastan, VI: Gasteromycetes. 216-
218, fig. 77, ab. XII, fig. 9. 1970.
= *T. kazachstanicum* Philimon. & Tart. apud Schwarzmann & Philimonova ibid. 237-238, fig. 89, ab. XIV. 1970.
= *T. granulosum* Lév. var. campestre (Morgan) J.E.Wright apud Wright, Herrera & Guzmán, Ciencia (México) 27 (4-5): 116. 1972.
= *T. costatum* Liu, Mycologia 71 (6): 1276-1277. 1979.
= *Tulostoma fibrillosum* V.S. White, Bull. Torrey Bot. Club 28: 433. 1901.
= *Tulostoma brumale* var. *fimbriatum* (Fr.) Quél., C. r. Assoc. Franç. Avancem. Sci. 18(2): 508 (1890)
= *Tulostoma campestre* Morgan, J. Cincinnati Soc. Nat. Hist. 12(4): 165 (1889)
= *Tulostoma granulosum* var. *campestre* (Morgan) J.E. Wright, in Wright, Herrera & Guzmán, Ciencia Méx. 27(4-5): 116 (1972)
= *Tulostoma fimbriatum* var. *heterosporum* J.E. Wright, Biblthca Mycol. 113: 113 (1987)

Lectotype: Nebraska, leg. Webber, det. A.P. Morgan ex Under-wood, N.A.Fgi" (NY).

Remarks: *T. fimbriatum* is distinguished by a greyish hyphal exoperidium, a white to greyish brown, commonly pitted endoperidium, a fimbriate, planar mouth, and spores with anastomosed verrucae. The hyphal exoperdium usually remains on the endoperidium throughout maturity. The spores are ornamented with low anastomosing verrucae, sometimes described as pseudo-reticulum. *T. fimibriatum* is the most commonly collected *Tulostoma* species in Colorado and is reported to be one of the most common members of the genus in North America.

Tulostoma fimbriatum has a large distribution and grows in a variety of habitats, which likely contributed to repetitive descriptions of this species. Wright (1987) synonymized multiple species with *T. fimbriatum* var. *campestre*. Jeppson et al. (2017) show that the lectotype of *T. campestre* (=*T. fimbriatum* var. *campestre*) forms a monophyletic clade with the sequences of *T. fimbriatum*. Our collections lie within the fimbriatum clade. Given this molecular and morphological evidence I accept the synonymy. *T. fimbriatum* sequences form clades with some geographic signal in our phylogeny, however they lack significant support. Sequence data of the type of *T. tuberculatum* was generated by Jeppson et al. (2017) but not included in their phylogeny. This sequence groups with other sequences from N. America with some support, and this clade is separate from other putatively N. American *T. fimbriatum* sequences. Our collection, DBG21283, is morphological indistinguishable from our other *T. fimbriatum* collections in our phylogeny. It is possible that there are distinct species within the *T. fimbriatum* complex, the addition of molecular markers may serve to resolve these clades into distinct species.

Habitat and distribution: *T. fimbriatum* as circumscribed by recent molecular evidence appears to have a nearly cosmopolitan distribution and occurs in varied habitats. It is most commonly found in dry and exposed habitats, on various soils including both calcareous and acidic soils. It has been reported from throughout Europe and North America as well as Australia, Argentina, Chile, Russia.

Material Examined: UNITED STATES, **Arizona**: Coconino County, 25 March 2015, on sandy soil with Az cypress, pine and manzanita, TA Clememts (AZ43459); **Colorado:** Adams County, Commerce City , Gary Pickett's yard, 39.870007, -104.873443, in wood mulch, 9 April 2007, Gary Pickett (DBG23705); Boulder County, Sunshine Canyon, Evenson home, 40.037426, -105.328847, VA Evenson (DBG18419); Denver County, Denver, Denver Botanic Gardens, 909 York Street, near gate to house and volunteer walk, 25 October 2005, Under low bushes in garden on east side of N-S path, 39.732207, -104.961468, VS Evenson (DBG23042); Jefferson County, Wheat Ridge Green Belt Meadow, Site 13, 25 February 2001, dead grasses and weeds, RL Brace (DBG27844); Jefferson County, Wheat Ridge Green Belt Meadow, Site 13, 25 February 2001, dead grasses and weeds, RL Brace (DBG27845).

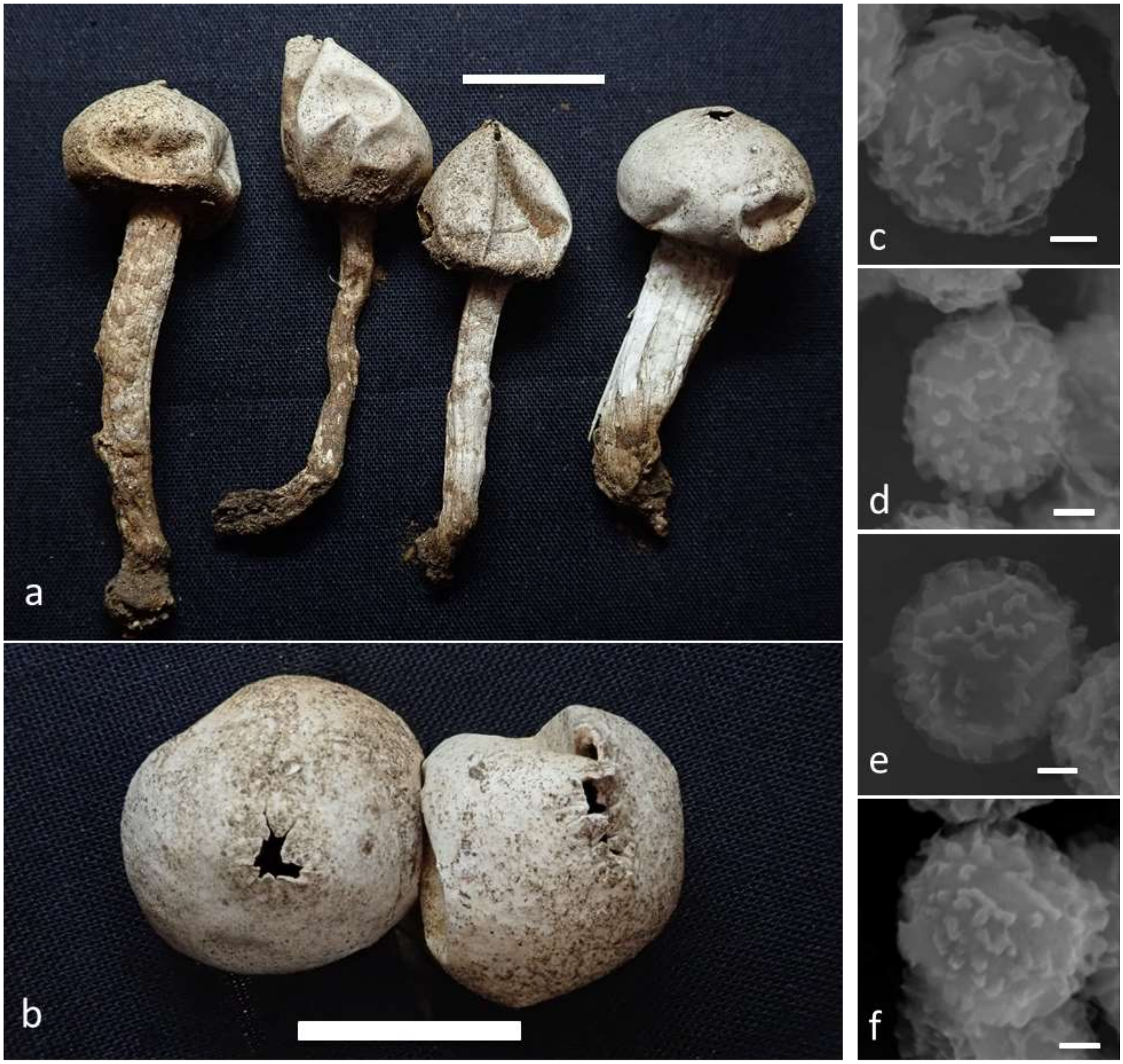


Figure 2.3. *T. fimbriatum* (DBG23042) a. basidiocarps; b. mouth; c-f. spores. Scale bars: a-b = 10 mm; c-f =1 µm.

Tulostoma grandisporum **Jeppson, Altés, G. Moreno & E. Larss. .**

See Figure 2.4

Holotype. Hungary, Pest, Csévharaszt, Csévharaszti borokás, in sand steppe vegetation: *Festucetum vaginatae*, T. Knutsson, U. Andersson, T. Gunnarsson, M. Jeppson, 11 Apr. 2011, M. Jeppson 8907 (GB! paratype AH).

Etymology. The name refers to the large spores.

Remarks: *T. grandisporum* is a recently described species. It is distinguished by a somewhat small spore-sac, a hyphal exoperidium, a whitish endoperidium, a fibrillose-fimbriate mouth, a stem which forms pseudorhiza and somewhat large (5.5–7.0 µm in our collections) completely smooth spores. Jeppson et al. (2017) distinguish this from *T. leiosporum* by spore size, *T. leiosporum* having smaller spores (4.5–5.5 µm).

Habitat and distribution: In Europe, *T. grandisporum* is found in exposed sand steppe habitats, in the US it was found under *Juniper*. Jeppson et al. (2017) report this species from Hungary and Slovakia, this is the first report from N. America, where it was found in New Mexico.

Material examined: UNITED STATES, **New Mexico**: Taos County, Taos, Ojo Caliente, under juniper 18 October 1967 K.A. Harrison (MICH338158).

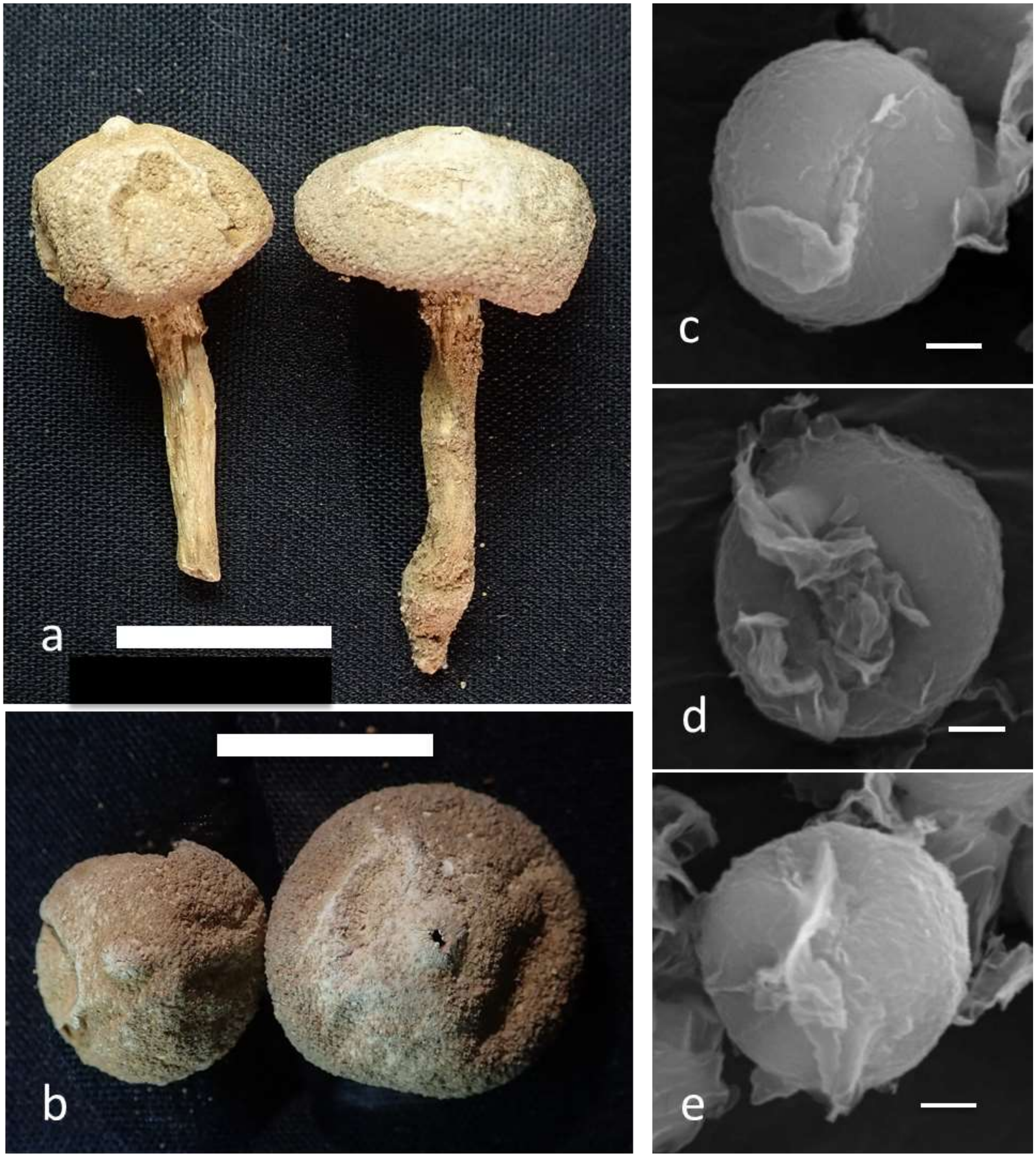


Figure 2.4. *T. grandisporum* (MICH338158) a. basidiocarps; b. mouth; c-e. spores. Scale bars: a-b = 10 mm; c-e =1 µm.

Tulostoma involucratum Long - Mycologia 36: 330-332, fig. 13. 1947.

See Figure 2.5

Holotype: United States: New Mexico, Luna Co., 10 mi W of Dening, leg. Long & Stouffer, 13.IX.1941 (Herb. Long 9650, BPI!). Illustration: Long (op.cit.).

Etymology: The name refers to the involucre-like aspect of the exoperidium in some specimens.

Remarks: *T. involucratum* is characterized by a somewhat large sporocarp, up to 18 mm, its strongly membranous exoperidium which forms a cup at the base of the spore-sac, characteristic of the epithet, a large tubular mouth and ornamented spores, 4.6-5.7 µm in diam. The ornamentation is composed of uneven verrucae, some fused, more so at the base where some spores have short longitudinal ridges emanating from the apiculus toward the apex, running at most 1/3 the length of the spore. The stipe is reported to end in a bulb (Wright 1987, Esqueda 2004), however the base of the stipe was not present in the isotype. Capillitium subhyaline, infrequently branched and infrequently septate, slightly swollen at the infrequent septa; 3.3-6.1 µm diam.

Habitat and distribution: This species has been cited from USA, Mexico, Argentina, and South Africa (Long 1944; Wright 1987, Esqueda et al. 2004). It is most commonly reported on sandy soil in arid regions and in Mexico it was also found in microphyllous desert scrub and tropical thorn forests (Esqueda et al. 2004).

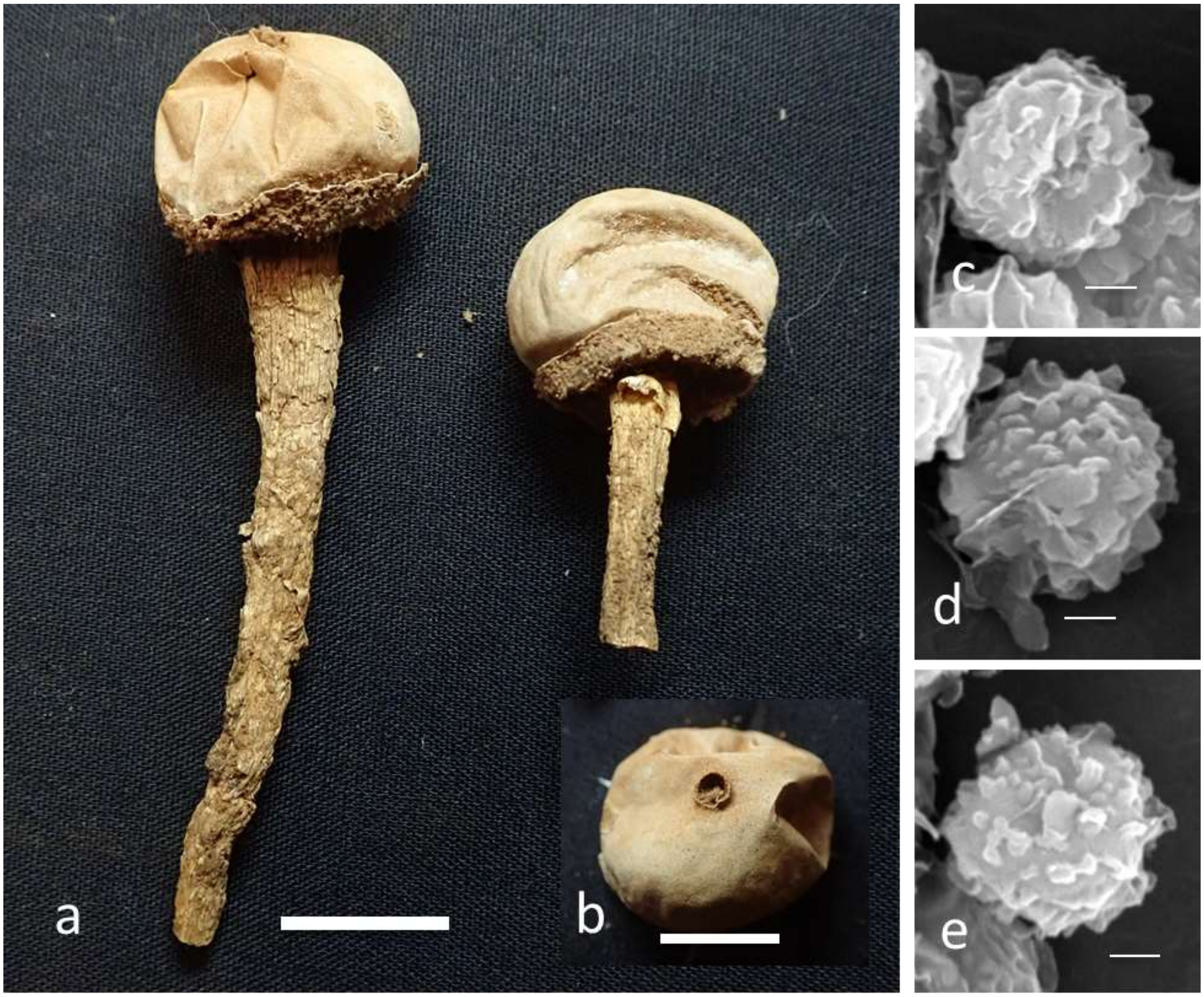


Figure 2.5. *T. involucratum* (ISOTYPE ILL11718) a. basidiocarps; b. mouth; c-e. spores. Scale bars: a-b = 10 mm; c-e =1 µm.

Tulostoma macrocephalum Long Mycologia 36: 337-338, fig. 7. 1944.

See Figure 2.6

Holotype: United States: New Mexico, White Sands Nat'l Monument, W of Administration Bldg., leg. Long, 22.IV.1942 (BPI!).

Etymology: The name refers to the large size of the spore-sac.

Remarks: Our collections of *T. macrocephlalum* agrees with Long (1944) and Moreno et al. (1995). *T. macrocephalum* is characterized by a relatively large, grayish to tannish white endoperidium, a hyphal exoperidium, a circular, sometimes slightly projected or out-rolled mouth, and subtly verrucose spores. The stem has been described as *Battarraea*-like, it is squamose concolorous with the spore-sac, tapers towards the base, and has a very conspicuous and large volvoid base. This was obvious in our smaller collections but has been dislodged in our larger specimens. *Tulostoma volvulatum* var. *elatum* Harlot & Patouillard is reportedly similar, however has smooth spores.

Habitat and distribution: This species is found in sandy soil in arid regions and is known from southwestern United States and Argentina (Wright, 1987) and southwestern Europe (Altes et al., 1992). All collections studied here were found in a similar are in Coconino County, AZ.

Material examined: UNITED STATES, **Arizona**: Coconino County, Page, site 58, 20 October 1972, Sand dune, with *Coleogyne ramosissima* J. States (MICH28572); Coconino County, Navajo Mountain, site 56 C, 19 October 1972, In sandy soil with *Pinus edulis* and *Juniperus*, J. States (MICH42461); Coconino County, 10 mi S of Page, across Glen Canyon Dam, 17 May 1987, in sandy soil, E. Trueblood (MICH24877).

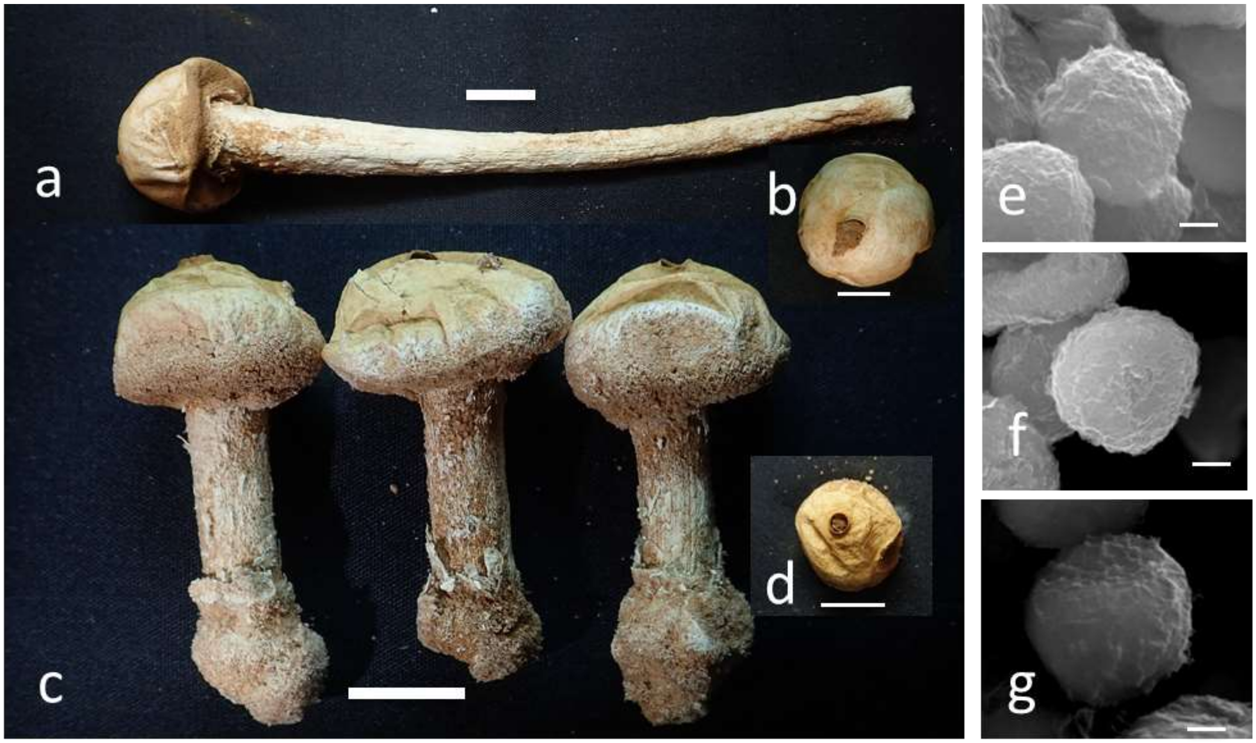


Figure 2.6. *T. macrocephalum* (MICH42461, b, d MICH28572) a,c. basidiocarps; b,d. mouth; e-g. spores. Scale bars: a-d = 10 mm; e-g =1 µm.

Tulostoma meridionale J. E. Wright in Wright, Herrera & Guzman, Ciencia (Mexico) 27 (4-5): 117. 1972.

See Figure 2.7

Holotype: United States, Texas, Denton, leg. W. H. Long 2061, XII. 1907 "ad terram arenosam in locus siccus, fimosus" (Herb. Lloyd nÂ° 53.153, BPI!).

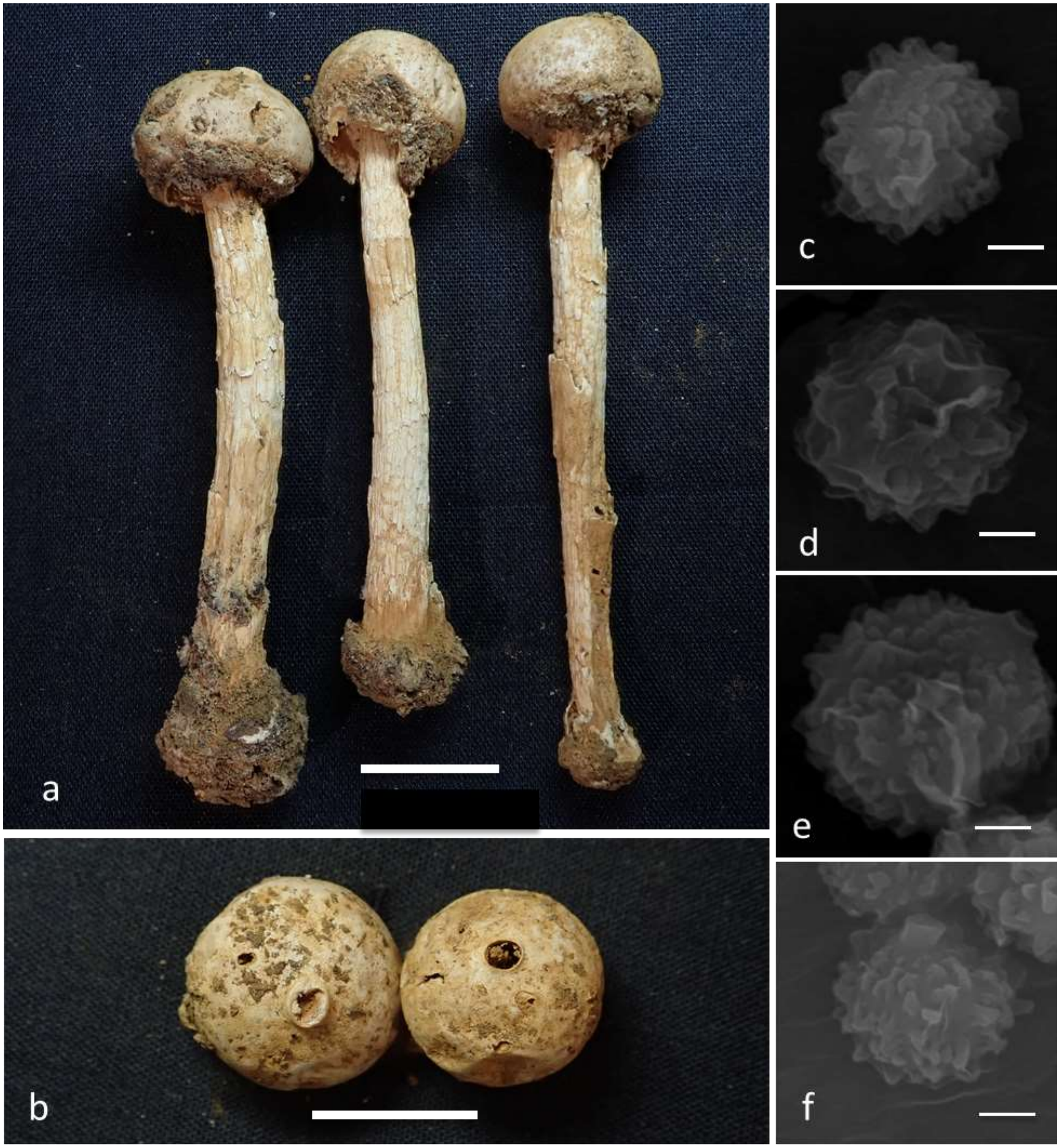
Etymology: the name refers to its southern distribution in the United States.

Remarks: *T. meridionale* is characterized by a thin, somewhat persistent, exoperidium that is considered more hyphal than membranous. The mouth is definite, circular and tubular, prominently projecting in most specimens. The stem has a conspicuous basal flare or broadening in most specimens. Spores are densely ornamented with uneven verrucae of various heights, some blunted, some pointed, oriented at different angels, and some fused.

This species may be confused with *T. utahense*, and *T. wrightii* as they are morphologically similar and found in similar habitats. The spore ornamentation is similar, between theses speices, however *T. meridonale* (6-10 µm in our collections) has larger spores compared to reported *T. wrightii* (4.0-4.8 x 4.0-6 µm) and *T. utahense* (6.6-8.4-(9.8) µm) sizes (Wright 1987, Altes et al. 1999).

Habitat and distribution: *T. meridionale* is found in rich soils in Arizona, New Mexico, Mexico and Argentina (Wright 1987, Altes et al. 1999)

Material Examined: UNITED STATES: **Colorado**, Larimer County, along sand creek, 2 July 1950, JT Reed (MICH24873); **New Mexico**, Taos County, Ojo Caliente 10 October 1967, KA Harrison, (MICH266210).



[redacted]

Figure 2.7. *T. meridionale* (MICH266210) a. basidiocarps; b. mouth; c-f. spores. Scale bars: a-b = 10 mm; c-f =1 µm.

__Tulostoma meristostoma__ Long Mycologia 36: 333-337, fig. 6. 1944.

See Figure 2.8

Holotype: United States: New Mexico, Albuquerque, Sandia Plaza (Herb. Long nÂ° 10.392, BPI!).

Etymology: Meristostoma (meristo- divided, seperable stoma- mouth) refers to the indefinite characteristic of the mouth in many basidiocarps of this species.

Remarks: The distinguishing characteristics of this species include the indefinite, slightly projecting mouth seen in most specimens and becomes lacerate in age, and the smooth spores. Under SEM, the spores have some very low irregular, minute, and scarce ornamentation, which would be mostly imperceptible under light microscopy.

Mature gastrothecia range from 8-20 mm diam and are depressed-globose in our collections. The exoperidium is hyphal. Endoperidium chalky white to whitish ochre. Mouth indefinite, not projecting, becoming lacerate with age in most specimens. Socket very distinct, separated from stem, with a ring-like membrane. Gleba dark rusty brown to tobacco brown. Stem up to 74 mm long by 5-10 mm thick, base of many specimens with enlarge bulbous structure. Our collections did not show the radicating base, these were likely broken during collection.

Spores globose to subglobose, smooth, apiculate under L.M.; under SEM they appear with some very minute, isolated warts, and the walls very minutely cracked; (3.5)4-6.5(7.4) µm diam. Capillitium branched, thin to thick-walled, branched, septa very rare, 3-9 µm diam.

Habitat and distribution: *T. meristostoma* is found in sandy soils in Arizona, New Mexico, and Argentina.

Material Examined: UNITED STATES, **Arizona:** Coconino, 10 mi S of Page, across Glen Canyon Dam, E Trueblood, 17 May 1970, in sandy soil (MICH24877); Coconino, Navajo Mountain, site 56, 20 October 1972, in sandy soil with *Pinus edulis* and *Juniperus*, J. States (MICH28575); **New Mexico:** Ojo Caliente, KA Harrison, 10 Oct 1967, (MICH266306).

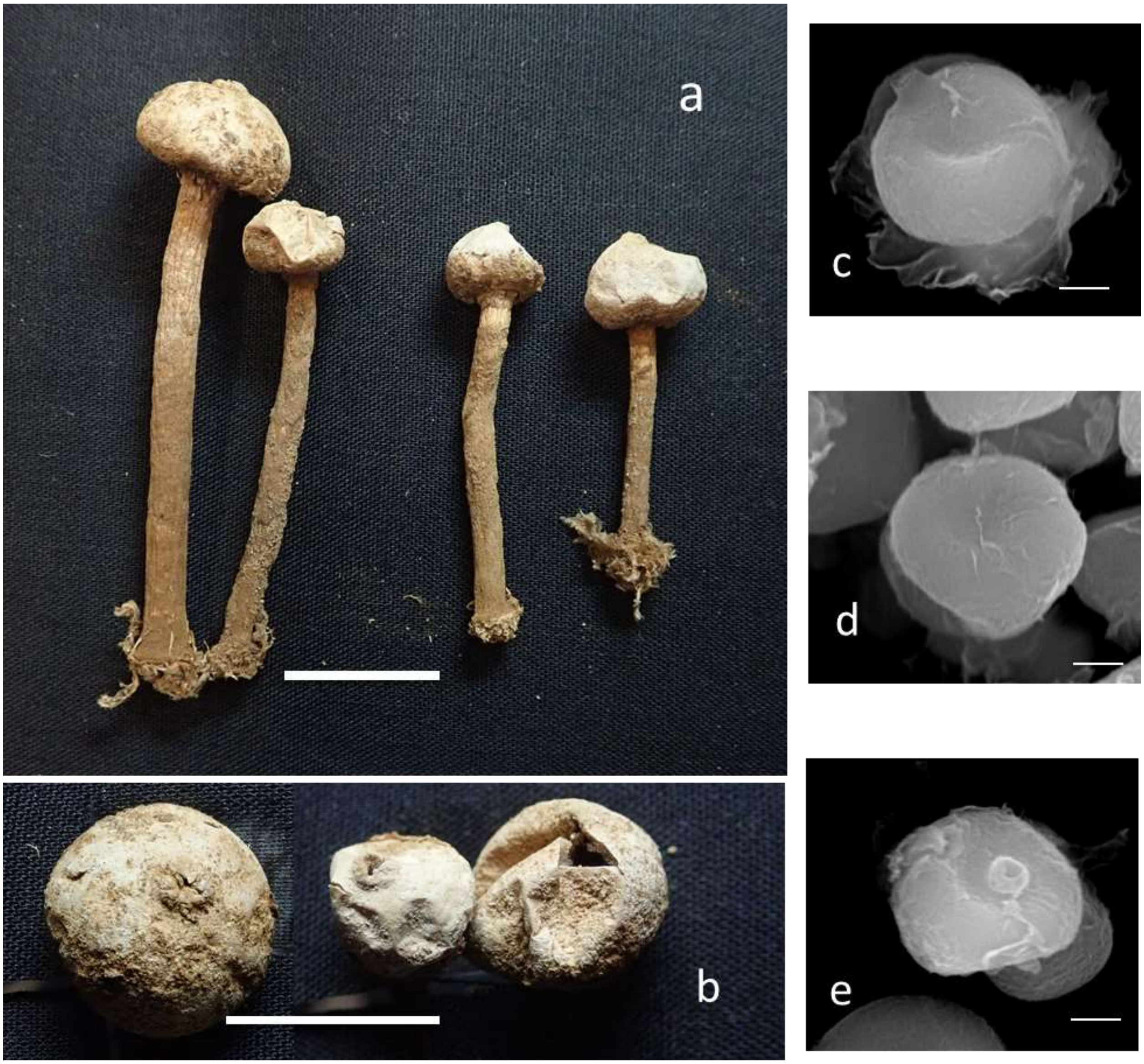


Figure 2.8. *T. meristostoma* (MICH266306) a. basidiocarps; b. mouth; c-e. spores. Scale bars: a-b = 10 mm; c-e =1 µm.

Tulostoma obscurum J.E.Wright (Fig.106) in Wright, Herrera & Guzman, Ciencia (Mexico) 27 (4-5): 118. 1972.

See Figure 2.9

Holotype: United States, Colorado, Boulder, leg. R.H. Stevens (Herb. Lloyd no 53.167, BPI!).

Etymology: The name refers to the obscure nature of the spore ornamentation.

Remarks: As the specific epithet implies, this species is distinct spore ornamentation. The spores are large, 6.7-8.7(9.8) µm, densely ornamented with very short, blunt or rounded, connected verrucae, that are nearly impossible to discern without SEM imaging. The ornamentation can be so dense and connected that individual verrucae are indiscernible. In areas on the spores with less dense verrucae, the ornamentation appears almost from reticulation. Other characters of this species include a very thin, but membranous exoperidium, and a projected, fibrillose-fimbriate, sometimes irregular in shape, mouth. The mouth morphology can vary, in some specimens it may become lacerate in age, and in other specimens a mouth may not develop at all. The base of the stem often has a mycelial bulb with brown threads. Wright (1987) reports slender rhizomorphs at the base, our collection lacked this feature.

Habitat and distribution: *T. obscurum* is found sandy soil in arid regions in the southwestern US and Mexico. North America: S and SW United States, Mexico

Material Examined: UNITED STATES, **Colorado:** Custer County, Texas Creek BLM Road 6161, 8 July 2015, B. Barzee (DBG28400).

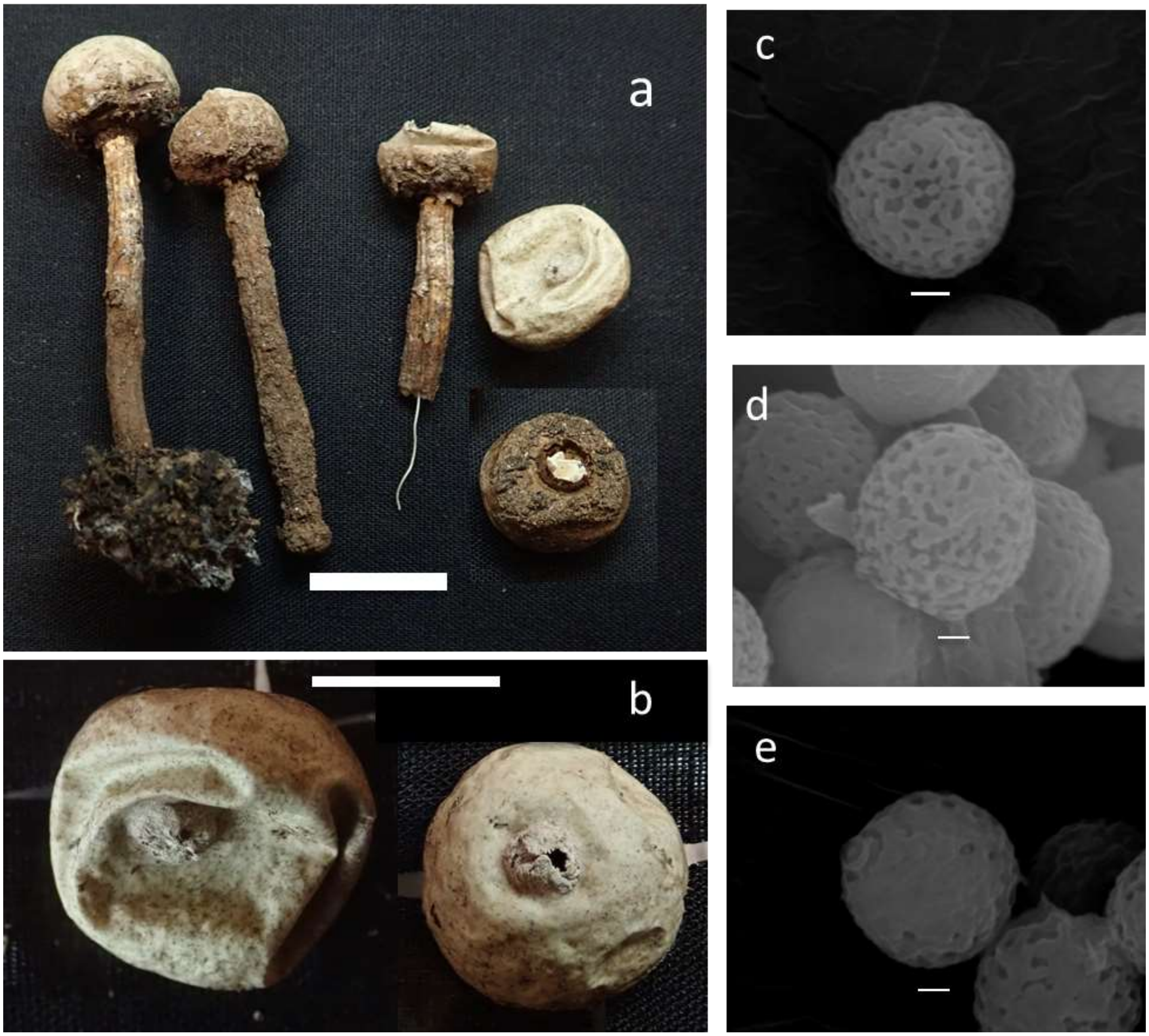


Figure 2.9. *T. obscurum* (DBG28400). a. basidiocarps; b. mouth; c-e. spores. Scale bars: a-b = 10 mm; c-e = 1 µm.

Tulostoma opacum Long - Mycologia 36: 327-329, fig. 3. 1944.

See Figure 2.10

Holotype: United States: New Mexico, Dona Ana Co., Jornada Exper. Range, 28 mi W of Las Cruces, leg. W. H. Long 8286, 12.XI.1938 (BPI!). Illustration: Long (op.cit.)

Etymology: Wright (1987) posited that the name refers to the obscure ornamentation of the spores, since it has no other features to which this adjective could be applied.

Remarks: The distinguishing characteristic of *T. opacum* is the strongly reticulate spores. Additional features of *T. opacum* include a very thinly membranous exoperidium, a whitish endoperidium, and an fibrose to indefinite or lacterate mouth. Wright (1987) reports that the mouth is of the type of *T. fimbriatum*, slightly sulcate, somewhat mammose, relatively large for the size of-the spore-sac, however out collections the mouth is ill defined and appears to be lacerate. The base of the stem flares.

T. clathrosporum Wright also has reticulate spores, but the spores are reportedly smaller (4.6-6.4 x 4.3-5.4 µm (Wright 1987) compared to *T. opacum* (7.4-11.2 µm in our collection). *T. clathrosporum* has a definite, circular mouth compared to *T. opacum* which has a fibrillose to indefinite mouth.

Habitat and distribution: *T. opacum* is found sandy soil in semiarid regions with "chaparral" vegetation and has been reported from New Mexico, Texas (Long, 1944), Mexico (Wright et al. 1972) and Africa (Moreno et al. 1995a).

Material examined: USA, Arizona, Coconino County, Lower Red Rock Rd, 3 mi from State Hwy 89A jct, W of Sedonia, 34.8167, -111.8333, 28 October 1993, On mineral soil near Pinus edulis, R. Fogel (MICH400).

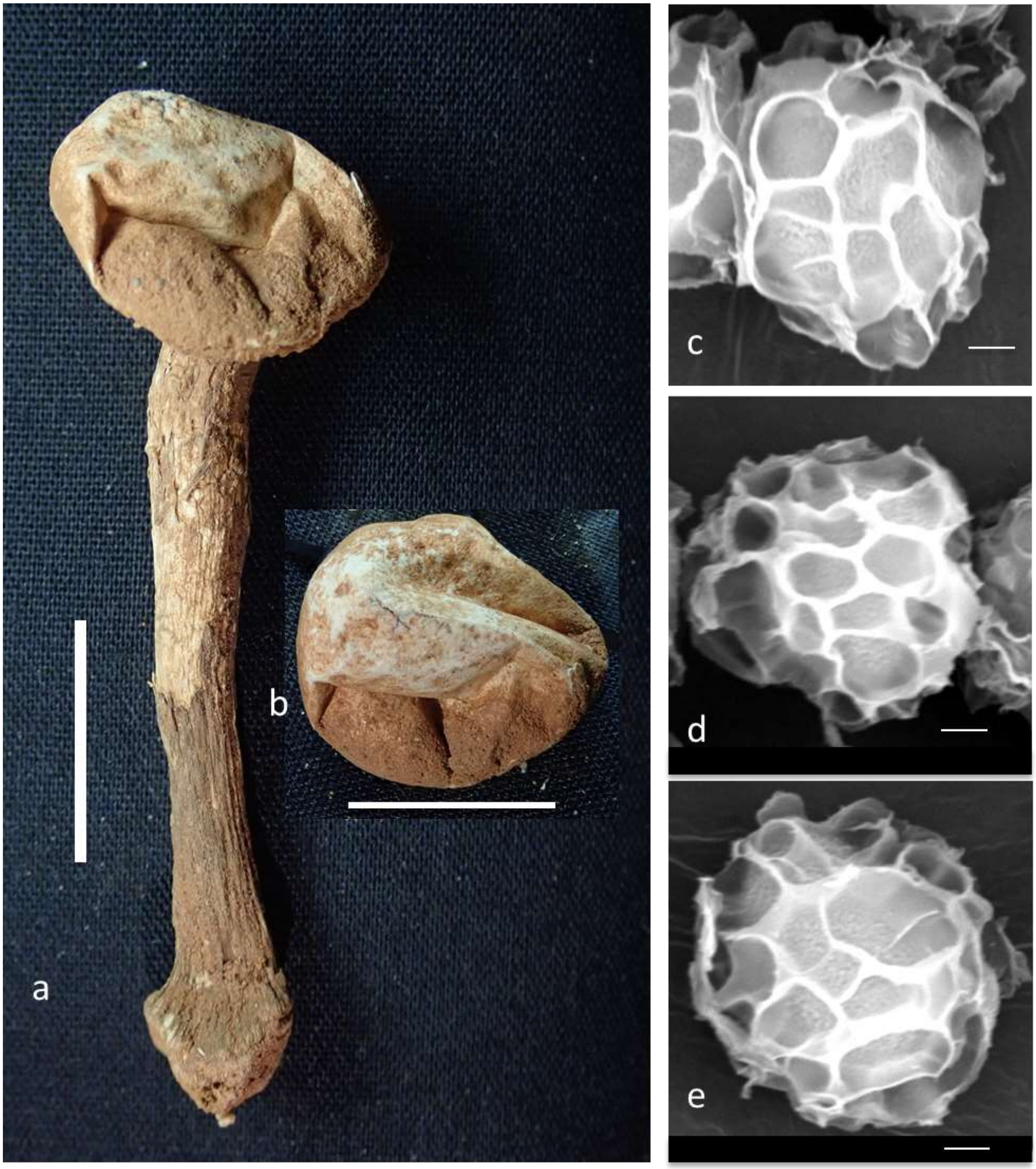


Figure 2.10. *T. opacum* (MICH400). a. basidiocarps; b. mouth; c-e. spores. Scale bars: a-b = 10 mm; c-e = 1 µm.

Tulostoma polymorphum Long Lloydia 10: 129-131, fig. 12. 1947.

See Figure 2.11

Holotype: United States, New Mexico, S of Lippetts Addition, Albuquerque, leg. W. H. Long (Herb. Long no 11.055, BPI!).
Etymology: The name refers to the varied shape of the fungus.

Remarks: *T. polymorphum* consists of a hyphal exoperidium, a white, fragile endoperidium, and a fimbriate mouth fimbriate. The mouth varies from to mammose and can become lacerate in age. The spore sac often detaches from the stem, Wright (1987) notes that in some specimens this separation can be occluded by clay. The stem is usually tapering to a bulbous base. Spores ornamentation is reminiscent of *T. fimbriatum*, with anastomosed verrucae, the spores are slightly smaller in *T. polymorphum* (4.5-6 µm) compared to *T. fimbriatum* ((4.5)5.5-6.5(8) µm). The capillitium can also be used to differentiate *T. polymorphum* from *T. fimbriatum*. In *T. polymorphum* the capillitia are hyaline, less branched, have fewer septa, are not swollen at septa, and are 1.8-6.6 µm diam. In *T. fimbriatum*, the capillitia are subhyaline, have more branching and septa, are swollen at the septa, and are slightly larger in diameter, 3.6-8.2 µm.

While some authors believed *T. polymorphum* to be a synonym of *Tulostoma fimbriatum* var. *campestre*, molecular evidence does not support this. Molecular evidence shows that *Tulostoma fimbriatum* var. *campestre* forms a clade with *T. fimbriatum* (Jeppson et al. 2017 and Figure 2.11 here). The inclusion of DNA sequence of the type of *T. polymorphum* in this study indicates that this species is genetically distinct from species contained in the *T. fimbriatum* clade. There were no additional collections of *T. polymorphum* studied other that the isotype. Collections labelled as *T. polymorphum* were identified as *T. fimbriatum*.

Habitat and distribution: *T. polymorphum* is found in sandy soil and has only been reported from the SW United States.

Material examined: UNITED STATES, **Colorado,** Adams County, Commerce City, Gary Picket's yard, in soil under shrubs, 39.87, -104.87, 12 May 2007 (DBG24092), same location 30 October 2007 (DBG24032); **New Mexico**, Bernalillo County, Albuquerque, Lippett Sandia Plaza Addition, about 4 miles from city on North 4th Street, 35.084491, -106.651137, 16 May 1941, low sand dunes. leg. W. H. Long (Herb. Long no 11.055, ISOTYPE ILL00011723).

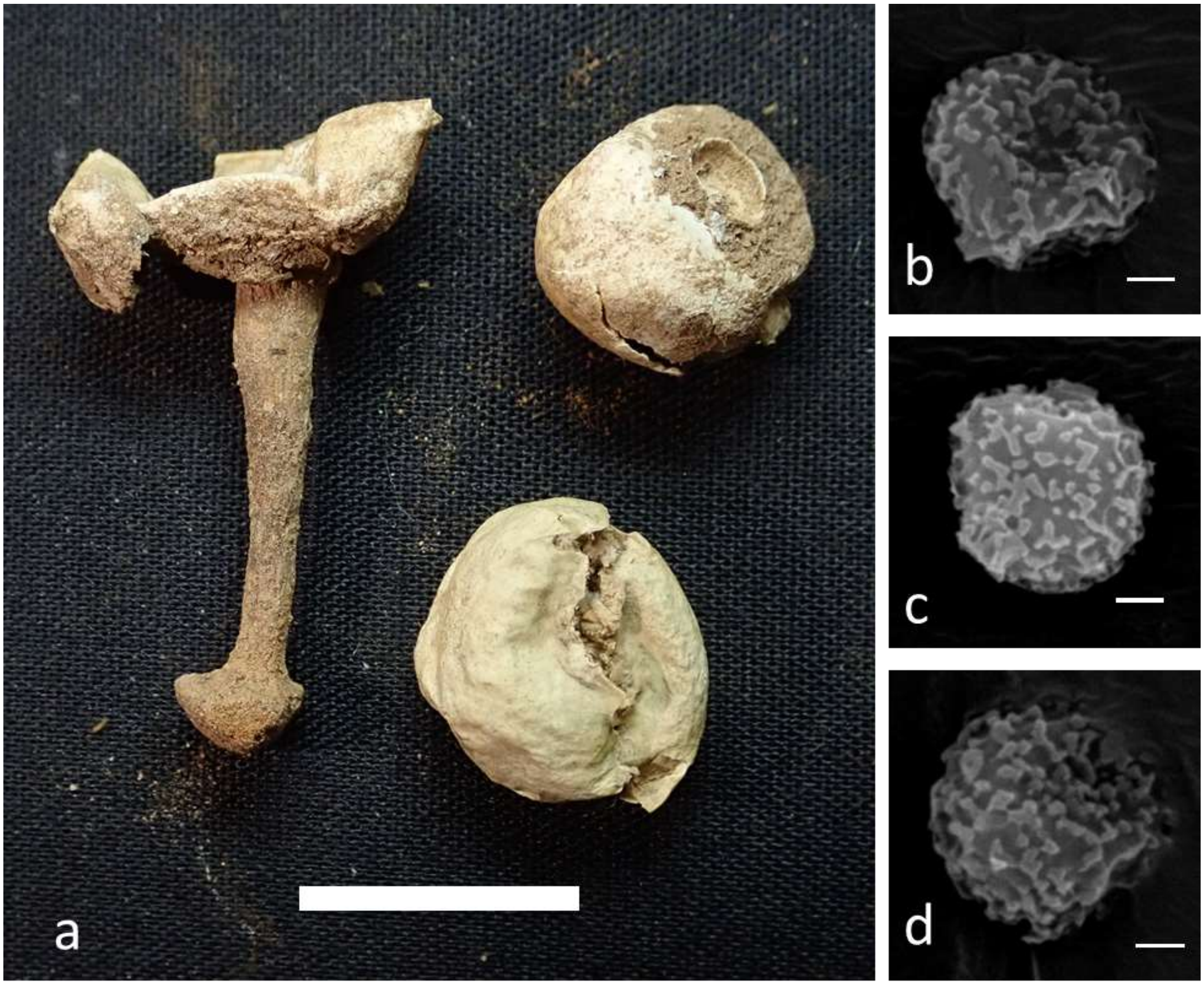


Figure 2.11. *T. polymorphum* (ILL1723-TYPE). a. basidiocarps; b-d. spores. Scale bars: a = 10 mm; b-d = 1 µm.

Tulostoma pulchellum Saccardo. - Bull. Soc. Mycol. France 5: 118, tab. XIV, fig. 4. 1889.

See Figure 2.12

= *T. poculatum* White, Bull. Torrey bot. Cl. 28 (8): 431, t. 34, figs. 4-6. 1901.
= *T. minutum* White, ibid. 28 (8): 433, tab. 31, figs. 11-13. 1901.

Holotype: Australia, leg. Tepper (PAD!).

Etymology: The name refers to the small size of the fruitbody.

Remarks: *T. pulchellum* is easily distinguished by spore morphology. Spores measure 4.5-6 (8) µm and the ornamentation is composed of short, rounded verrucae, which frequently fuse forming striations. Other characteristics include a membranous exoperidium membranous, an ochraceous endoperidium, and a fibrillose, projecting mouth.

T. pulchellum is found in sandy or clayish-sandy soils in generally in arid regions.

Habitat and distribution: Wright (1987) reports this as a very widespread species, It has been reported from throughout central and western United States as well as in Mexico, Argentina, France, Romania, Africa, Israel, Australia, and New Zealand.

Material examined: UNITED STATES, **Colorado:** Alamosa County, Blanca Wetlands Recreation Area, Bureau of Land Management, 37.539659, -105.726724, Stabilized sand dunes in *Sarcobatus vermiculatus* community, 30 July 2015, P Regensberg (DBG28388); San Miguel County, Diamond Hill, Wilson Peak was also listed on the field label, but Diamond Hill is listed as a summit in its own right in GNIS. Both in San Miguel Co., sheep pasture, 6 August 1956, AH Smith (MICH24082); **New Mexico**: Rio Arriba County, Taos County, 3 October 1967, KA Harrison, (MICH266205).

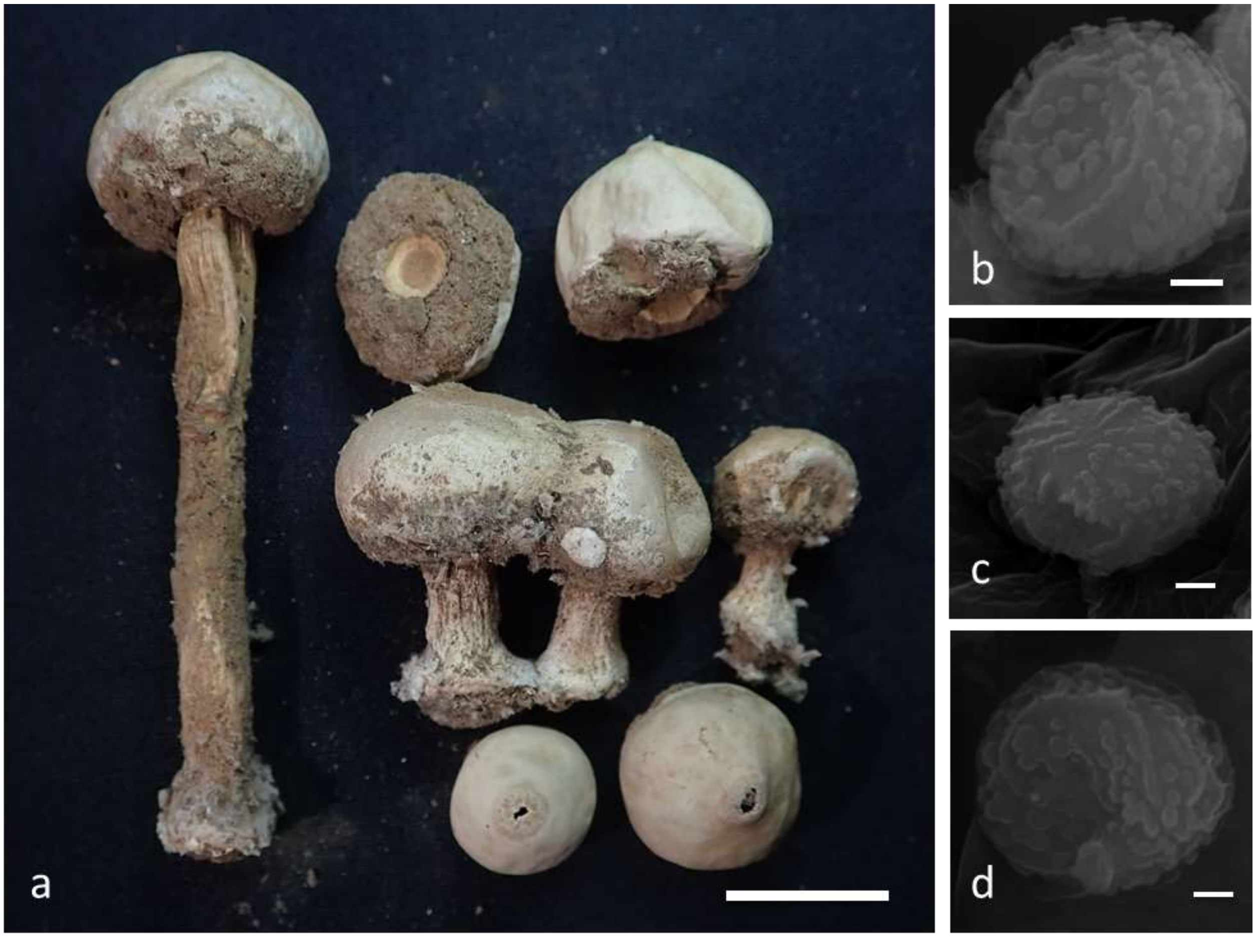


Figure 2.12. *T. pulchellum* (MICH266205). a. basidiocarps; b-d. spores. Scale bars: a = 10 mm; b-d = 1 µm.

Tulostoma punctatum Peck, Bull. Torrey Bot. Club 23(10): 419. 1896.

See Figure 2.13

≡ *Tulostoma fimbriatum* var. *punctatum* (Peck) J.E. Wright, Biblioth. Mycol. 113: 115. 1987.
≡ *Tulostoma subfuscum* V.S. White, Bull. Torrey Bot. Club 28: 433. 1901. Holotype. USA, Colorado: Denver, 16 Feb. 1896, Bethel 21 (NY!)*.**Lectotype.** USA, Kansas: Rooks Co., 23 Jul. 1896, E. Bartholomew (BPI 729033!)*.

Remarks: The specific epithet refers to the punctate or pitted appearance of the endoperidium, which Peck used as a distinguishing character for this species. It is believed that this pitted appearance arises from environmental factors (Jeppson et al. 2017). Since Tulostoma develops underground and stipe development occurs quickly at the end of maturity, granules of substrate commonly adhere to peridium during development, causing depressions. As this is occurs in many Tulostoma species, it is not unique characteristic in *T. punctatum*. Jeppson et al. (2017) used molecular and morphological to evidence synonymize *T. subfuscum* with *T. punctatum*. They were also the first to report this species outside of North America.

T. punctatum is distinguished by a hyphal exoperidium, a fimbriate planar to very slightly projected mouth, and ornamented spores. The spore ornamentation is composed of low verrucae with some anastomosis. The characteristics of T. punctatum are very similar to T. fimbriatum, but genetic analysis shows distinction between the species. The slightly smaller spores of *T. punctatum* (4-5.4 x 5.4-6.1 µm) and lack of strongly ridged ornamentation on the spores can be used to distinguish this species from *T. fimbriatum* ((4.5)5.5-6.5(8) µm).

Habitat and distribution. *T. punctatum* occurs sandy soils in pastures and exposed, anthropogeneous sites and has been reported from the US and Europe.

Material Examined: USA, **Arizona:** Yavapai County, Montezuma Well, J. States (MICH266257); **Colorado:** Las Animas County, Goat Ranch area, J.E. Canyon Rach near Branson, V.S. Evenson, sandy soil 12 June 2010 (DBG24711); Jefferson County, Wheat Ridge Green Belt Meadow, R.L. Brace 25 February 2001, Open meadow (urban) in dead grass weeds of woody debris, arid sandy soil south of clear creek. (DBG27849).

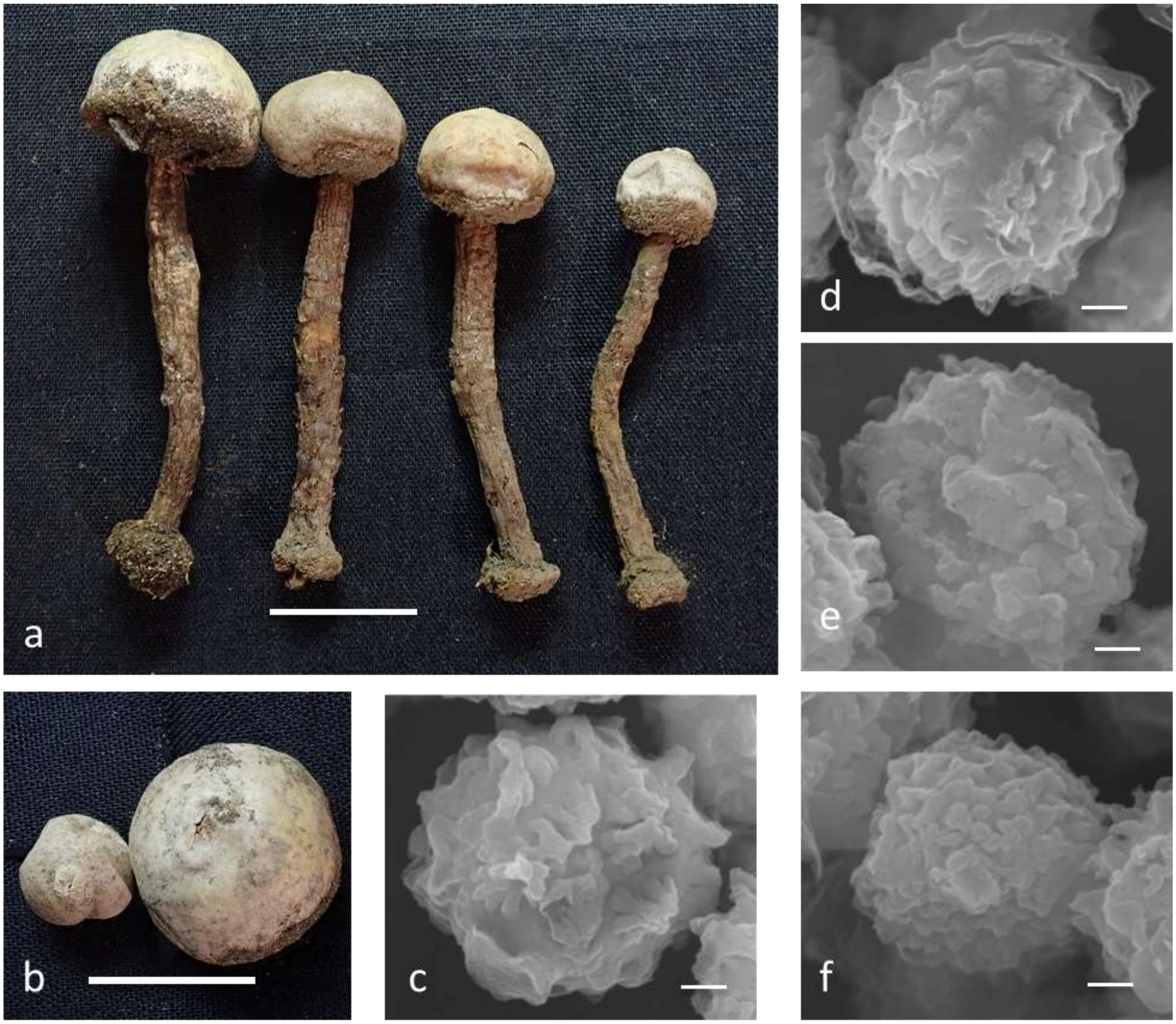


Figure 2.13. *T. punctatum* (DBG27849). a. basidiocarps; b. mouth; c-f. spores. Scale bars: a-b = 10 mm; c-f = 1 µm.

Tulostoma purpusii P. Hennings. Hedwigia 37: 274. 1898.

See Figure 2.14

= *T. patagonicum* Spegazzini var. andinum Spegazzini, An. Mus. Nac. Buenos Aires 6: 187. 1898.
= *T. orogrande* Long, Lloydia 10: 123-125, fig. 10. 1947.

Holotype: United States, W Colorado, Umrompahque Range, "auf Knollen von Sedum rhodanthum," leg. A. Purpus, 1893, ex Herb. Sydow (S!).

Etymology: The species was dedicated to the collector, A. Purpus.

Remarks: *T. purpusii* is distinguished by darker colors, a relatively large spore-sac, a more hyphal than membranous exoperidium, a projected circular mouth, with a darker peristome, and a straight stem that may be bulbous at the base. The spores are relatively large (5.4-7.2(8.4) µm), strongly ornamented, and the ornamentation forms prominent, anastomosed, sub-reticulate crests. The spore ornamentation is sufficient to distinguish this species.

Habitat and distribution: *T. purpusii* is found in sandy soil. Wright (1987) describes the distribution in southwestern US, Africa, Argentina and Australia.

Material examined: UNITED STATES, **Colorado**: Elbert County, Plains Conservation Center, West Bijou Creek property, 39.5552, -104.2987, Sandy soil near *Yucca glauca*, V Richards, J. Wingate (DBG21727); **New Mexico:** Santa Fe County, near Santa Fe, October 1958, CA Barrows(MICH266163); Santa Fe County, near Santa Fe, CA Barrows (MICH266181) (no further collection information available for the NM specimens).

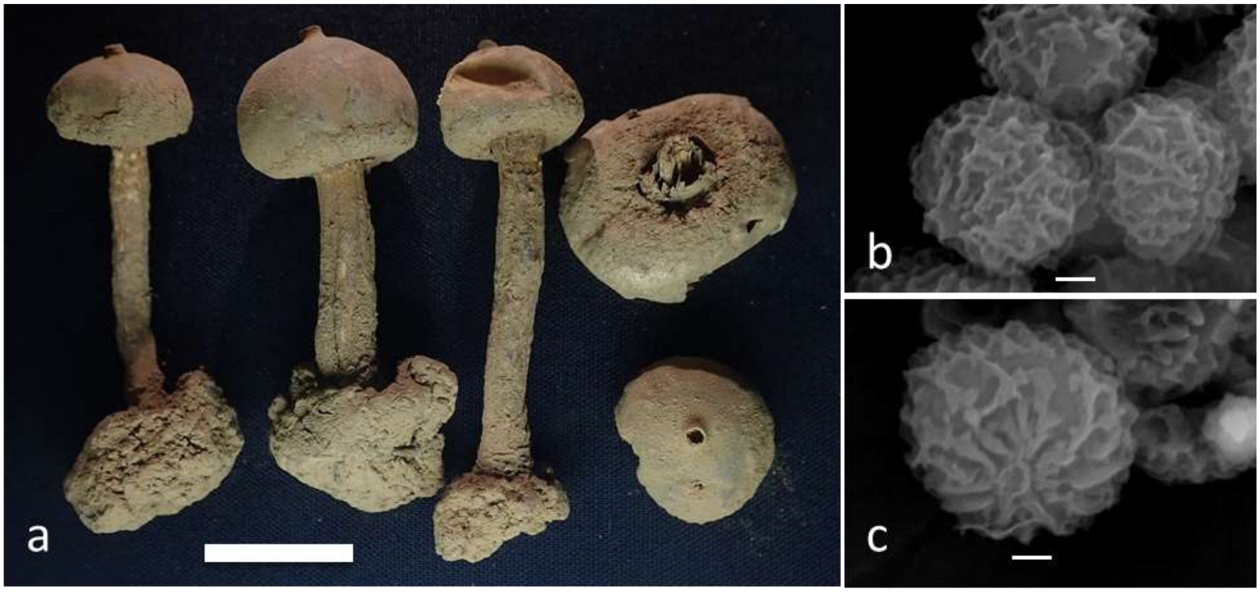


Figure 2.14. *T. purpusii* (MICH266163) a. basidiocarps; b-c. spores. Scale bars: a = 10 mm; b-c = 1 µm.

__Tulostoma simulans__ Lloyd. - The Tylostomeae. 18, pl. 79, figs. 2-3. 1906.

See Figure 2.15

=*Tulostoma mammosum var. simulans* (Lloyd) Sacc. & Trotter, Syll. fung. (Abellini) 21: 472 (1912)

Holotype: United States, Texas, Denton, leg. Long (Herb. Lloyd no 13.636, BPI!).

Etymology: *Simulans* is latin for imitating, referring to this species simulating *T. brumale*.

Remarks: The distinguishing characteristics of *T. simulans* include a thin, membranous exoperidium, a circular, projecting mouth, with a dark peristome, and strongly ornamented spores. The spores are 4-5.4 in µm diam, and ornamentation consists of verrucae that fuse to form columns.

T. simulans is morphologically very similar to *T. brumale*. Wright (1987) noted that *T. simulans* might be a North American variety of *T. brumale*. Jeppson et al. (2017) confirmed this hypothesis using molecular evidence. Based on Jeppson et al. (2017) and this study, molecular evidence indicates that *T. brumale* does not occur in N. America, while T. simulans is distributed in N. American and Europe.

Habitat and distribution: *T. simulans* is found forest soil, among litter, and in barren areas. It is thought to occur throughout North America, Argentina, Australia, New Zealand, Europe, and Russia. Wright (1987) considers the species native to North America and introduced into all other areas.

Material Examined: USA, **Colorado:** Larimer Co., Roman House. Soapstone Natural Area., 40.97176, -105.067, R.M. Hallock, 26 June 2009 (DBG24502); Jefferson Co., Golden, 1820 Zinnia St., 39.74481, -105.146, 5500 ft, Rosa-Lee Brace, barren ground where grass died, 29 October 2006 (DBG24113);
Denver Co., Denver, 4915 West 31st Ave., edge of yard, in purslain and Kochia, 5300 ft, 10 July 2001, Peter Root (DBG20766); Boulder Co. Boulder Creek Path, near Fourmile Canyon Drive, in soil near creek path, *Pinus ponderosa*, gregarious habit, Vera S. Evenson, 26 May 2009, (DBG24482); Adams Co., Rocky Mountain Arsenal, 39.8922, -104.7798, near Yucca 23 April 1975, Sam Mitchell (DBG5468).

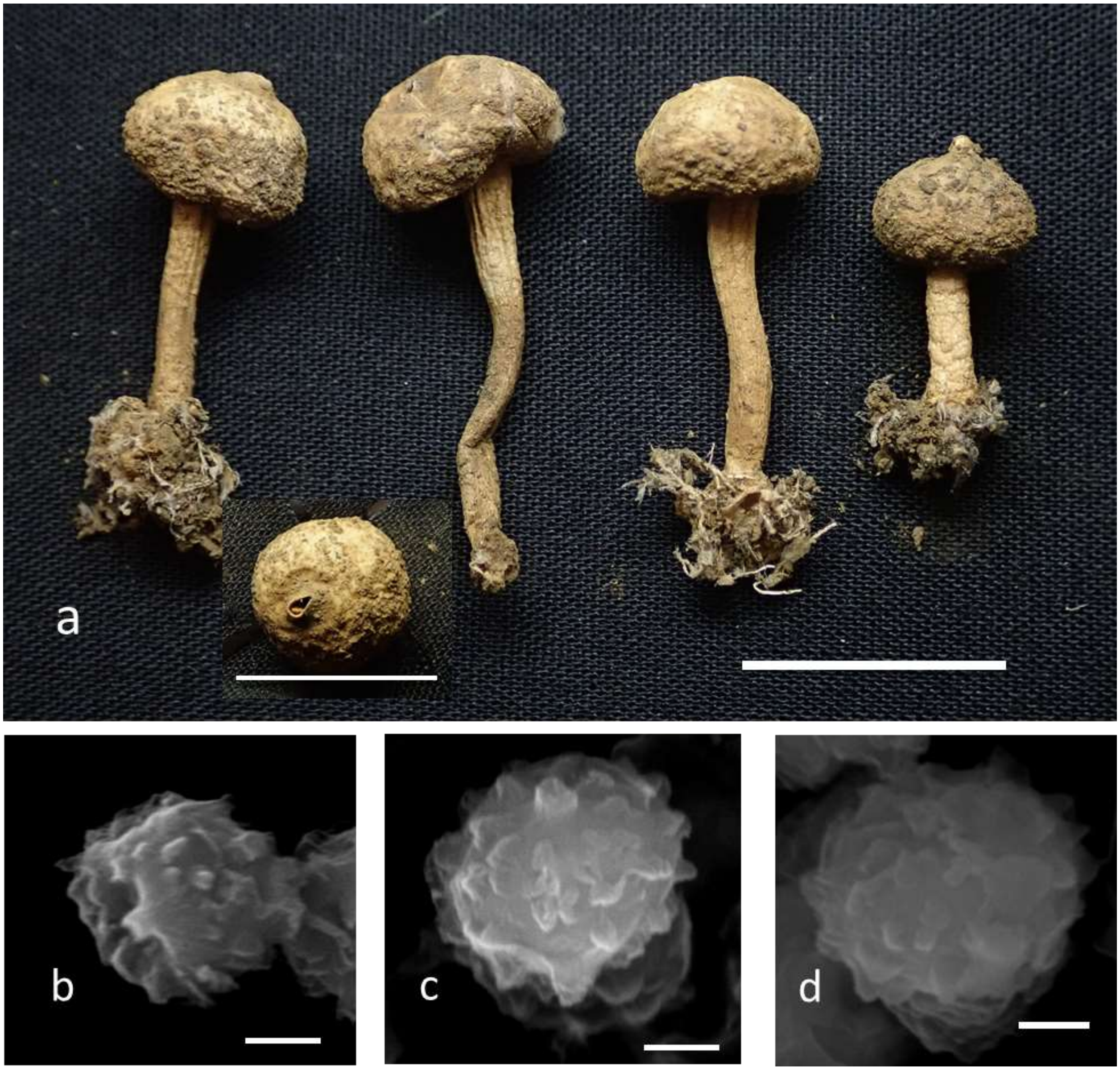


Figure 2.15. *T. simulans* (DBG24113). a. basidiocarps; b-c. spores. Scale bars: a = 10 mm; b-d = 1 µm.

Tulostoma squamosum (J.F. Gmel.) Pers., Syn. Meth. Fung. (Göttingen) 1: 139. 1801.

See Figure 2.16

= *T. squarrosum* Micheli, Nova Plantarum Genera p. 212, tab. 97, fig. 7. 1729.
= *Lycoperdon squamosum* Gmelin apud Linneo, Syst. Nat. II: 1463. 1747.
= *L. palmatum* Batsch, Elenchus Fungorum 1783-1789.
= *T. imbricatum* Pers., Tentam. Dispos. Meth. Fung. p. 6. 1797.
= *T. barlae* QuelÃ«t, Bull. Ass. France 17, tab. XII, fig. 1. 1880. = . resa olae Petri, Ann. Mycol. 2: 414. 1904.
=*Tulostoma verrucosum* Morgan, J. Cincinnati Soc. Nat. Hist. 12(4): 164. 1890. Lectotype. USA, Ohio: Cincinatti, 1883, A.P. Morgan (BPI).
= *Tulostoma mussooriense* Henn., Hedwigia 40: 337. 1901. Holotype. INDIA, Mussoorie: Arnigadh, 5500 ft alt., 9 Aug. 1900, W. Gollan (S!).

Lectotype: Italy, missit cl. Targioni, in Herb. Persoon, originally as Lycoperdon Micheli (L!).

Etymology: The name refers to the very scaly stipe.

Remarks. *T. squamosum* is one of the few species that I studied that has taxonomically distinguishing colors. The stem, exoperidium, and endoperidium are darker brown compared to most other species in the genus which I studied. Jeppson et al. (2017) report that a distinct reticulate pattern is formed by the dehiscence of the exoperidium, however this character was not readily discernable in our collection. Other distinguishing characters are a squarrose stem, brownish sphaerocysts in the exoperidium. The spore ornamentation is of prominent verrucae which often fuse, forming pyramidal groupings.

Habitat and distribution. European collections are reported to found in calcareous soils among herbaceous vegetation and among herbs in semi-shaded places, including open deciduous forests and gardens (Jeppson et al. 2017, Wright 1984). It is widespread throughout Europe and has been reported throughout N. America (Moreno et al. 1992a, Kreisel 1984, Jeppson et al. 2017, Wright 1984), it has a cosmopolitan distribution. It seems to be one of the more widespread *Tulostoma* species in Europe, reaching north to Germany (Kreisel 1984).

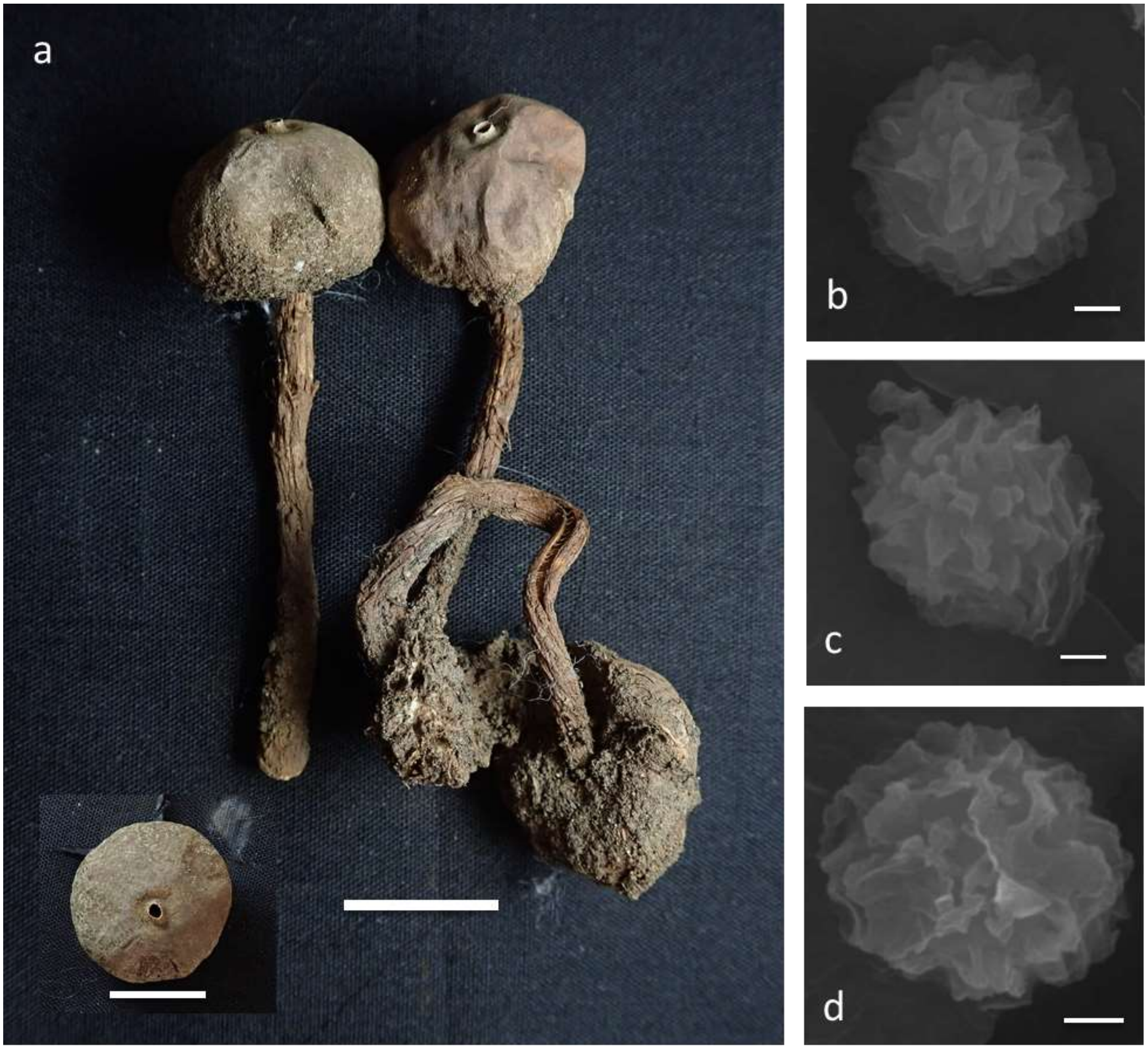


Figure 2.16. *T. squamosum* (MICH28574). a. basidiocarps; b-d. spores. Scale bars: a = 10 mm; b-d = 1 µm.

***Tulostoma striatum* G**. H. Cunningham. (Fig. 138; Pls. XXI: 1-8; XXXV: 1-2;XL:1) Proc. Linn. Soc. N. S. W. 50: 255. 1925.

See Figure 2.17

Holotype: Australia: N. S. W., Forbes, leg. J. B. Cleland, 13. VIII. 1915 (AWD 5867!).

Etymology: The epithet refers to the unique spiral ridges on the spores. This feature alone is usually enough to distinguish this species.

Remarks: This species has often been misidentified as *Tulostoma albicans* and *Tulostoma pulchellum*. The mouth features, exoperidium, endoperidium, and spore ornamentation will separate them. *T. striatum* has a membranous exoperidium, a fibrillose to mammose mouth that is usually slightly raised, and becomes lacerate in age in some specimens, and is a different color, either light or darker, than the endoperidium. The most striking feature of T. striatum is the spore ornamentation. Spores are ridged, or ribbed, and the ridges form a spiral or swirl like pattern. This pattern is

Habitat and distribution: T. striatum is typically found in psammophilous soils (Wright 1987, Calonge 1998), in exposed or shaded places or in distinctly desertic zones, at sea level or high altitudes; typically gregarious. It is widespread in the Americas, South Africa and Australia (Wright 1987). Altés and Moreno (1991) recorded it from Europe (Spain), where it to date is only known from the city of Madrid. Asai (2004) reported it from Japan Jeppson et al. (2017) show Mongolian collections.

Material examined: UNITED STATES, **Arizona:** Coconino County, on sandy soil with yucca, manzanita and juniper 6 March 2015, TA Clements (AZ43457); **Colorado**, Boulder County, Evenson property just east of cabin, 40.0374, -105.3288, sandy soil, 30 July 2004, VS Evenson (DBG21822).

Figure 2.17. *T. striatum* (AZ43457). a. basidiocarps; b-d. spores. Scale bars: a = 10 mm; b-d = 1 µm.

Tulostoma tuberculatum V.S. White, Bull. Torrey bot. Club 28: 432 (1901)

See Figure 2.18

=*Tulostoma fimbriatum* var. *tuberculatum* (V.S. White) J.E. Wright, Biblthca Mycol. 113: 117 (1987)

Holotype: Canada, British Columbia, leg. Macoun as *T. obesum* (NY; isotype at BPI).

Remarks: Jeppson et al. (2017) generated sequence data of the isotype of *T. tuberculatum*. They did not include these data in their phylogeny, but considered *T. tuberculatum* to be a distinct species based on molecular evidence. In our phylogeny, T*. tuberculatum* groups in the *T. fimbriatum* complex, but is genetically distinct. There is little distinction in the macromorphology of *T. fimbriatum* and *T. tuberculatum*. Spore morphology is distinctive. T. *fimbriatum* has low rounded verrucae which commonly form ridges, while the ornamentation on the spores of *T. tuberculatum* coalesce indistinctly and do not form distinct ridges. Better morphological data on the type of *T. tuberculatum* are necessary to definitively distinguish this from *T. fimbriatum*.

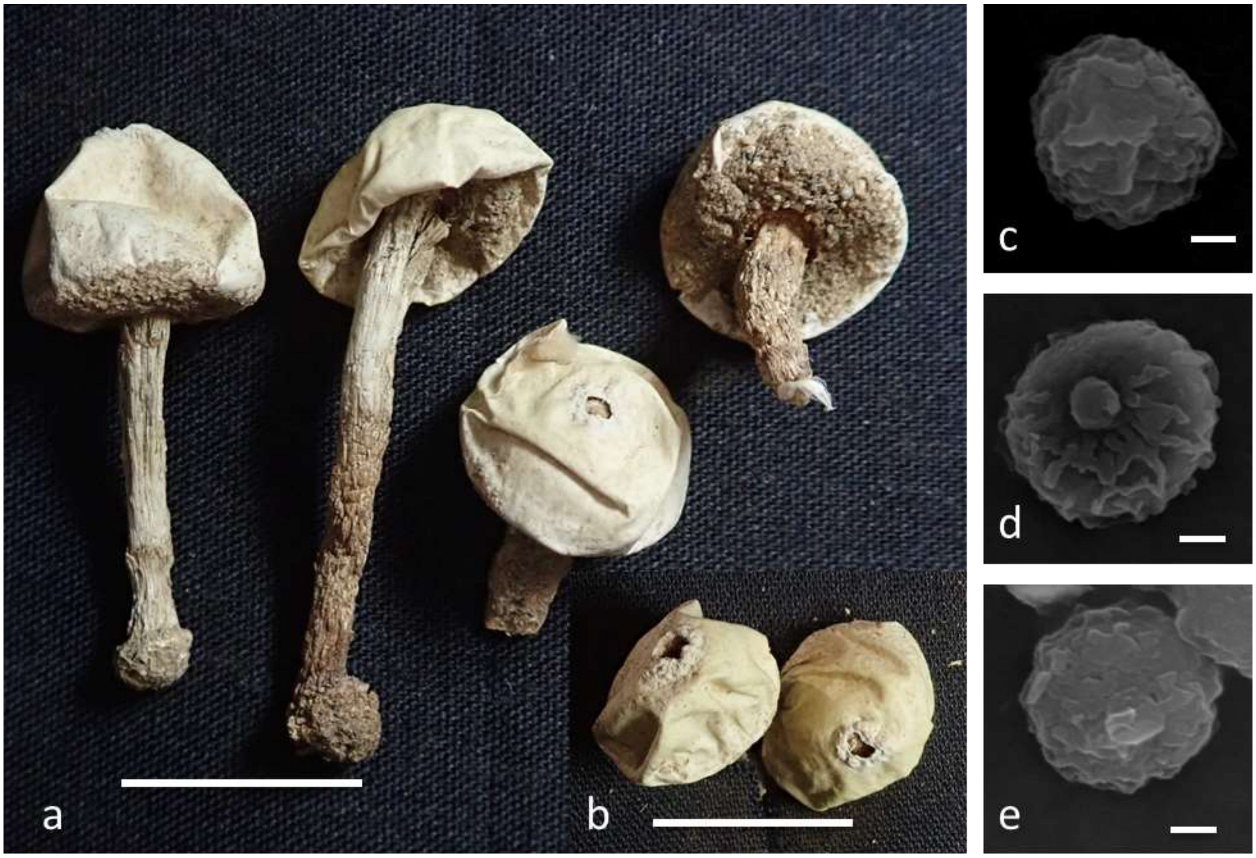


Figure 2.18. *T. tuberculatum* (AZ43457). a. basidiocarps; b-d. spores. Scale bars: a = 10 mm; b-d = 1 µm.

Tulostoma xerophilum Long Mycologia 38: 85-87, fig. 5. 1946.

See Figure 2.19

Holotype: United States: Arizona, 7 mi from Nogales, on Hway 89, leg. Long & Stouffer, 13.XI.1936 (Herb. Long 9688, BPI!).

Etymology: The name refers to the xerophilous habitat of the species.

Remarks: *T. xerophilum* is distinguished by a thick, membranous exoperidium, a tubular, circular, prominent mouth, and even stipe with no basal features, and ornamented spores. The ornamentation consists of verrucae of uneven lengths. Wright (1987) describes the spore verrucae as scant, however in our collections they are more plentiful, but not dense, and very occasionally anastomosed.

Habit and distribution: *T. xerophilum* is found in partial shade, in mesquite-catclaw flats (*Prosopis-Acacia*) in Arizona. Wright (1987) also reports collections from California, Texas and Mexico. This is the first report of *T. xerophilum* from New Mexico.

Material examined: UNITED STATES, **New Mexcio,** Socorro County, 12 mi. South to S.E. Socorro. 13 February 1973E. Trueblood and D. Holland (MICH266442); Socorro County, 12 mi. South to S.E. Socorro. 13 February 1973E. Trueblood and D. Holland (MICH266445).

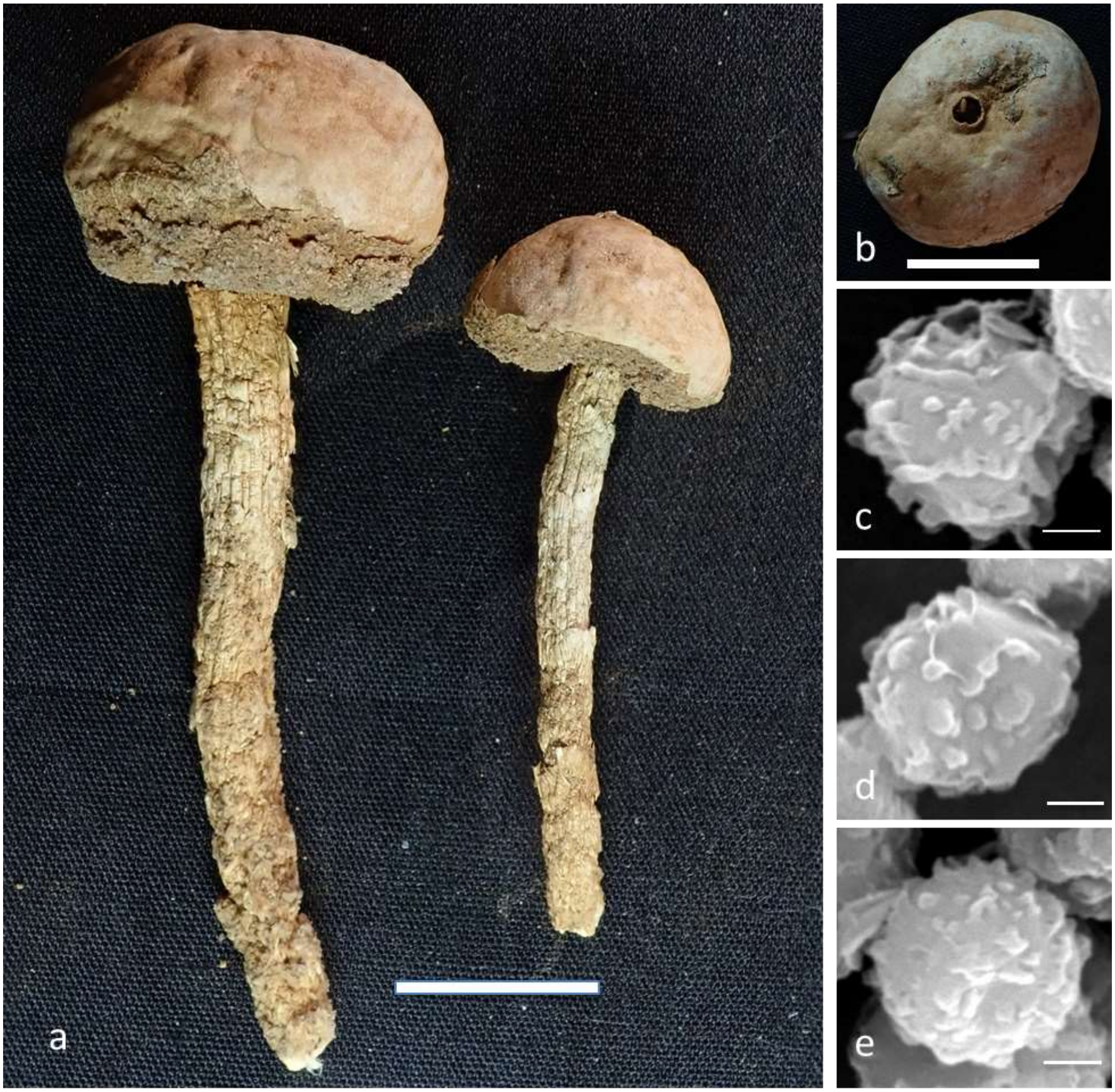


Figure 2.19. *T. xerophilum* (MICH266445). a. basidiocarps; b. mouth; c-e. spores. Scale bars: a-b = 10 mm; c-e = 1 µm.

Species Novo

The sequences of several collections aligned with 2 unnamed species published in Jeppson et al. (2017). These two species are briefly presented below. Type material of several European species has been requested by Jeppson and type material of North American species has been requested by Honan. Following type studies of these collections will either be associated with previously published species, or be presented as new species.

***Tulostoma* aff. sp. 3** (Jeppson et al. 2017)

See Figure 2.20

One collection (MICH24086) matches the molecular data identified in as sp. 3 in Jeppson et al. (2017). My collection somewhat matches the morphology of *T. punctilibratum,* however differences exist in the reported spore ornamentation and spore size. I requested the type of *T. punctilibratum* and compared my collections to the molecular and morphological data of this type collection. I am in communication with Jeppson to clarify the taxonomy of these collections.

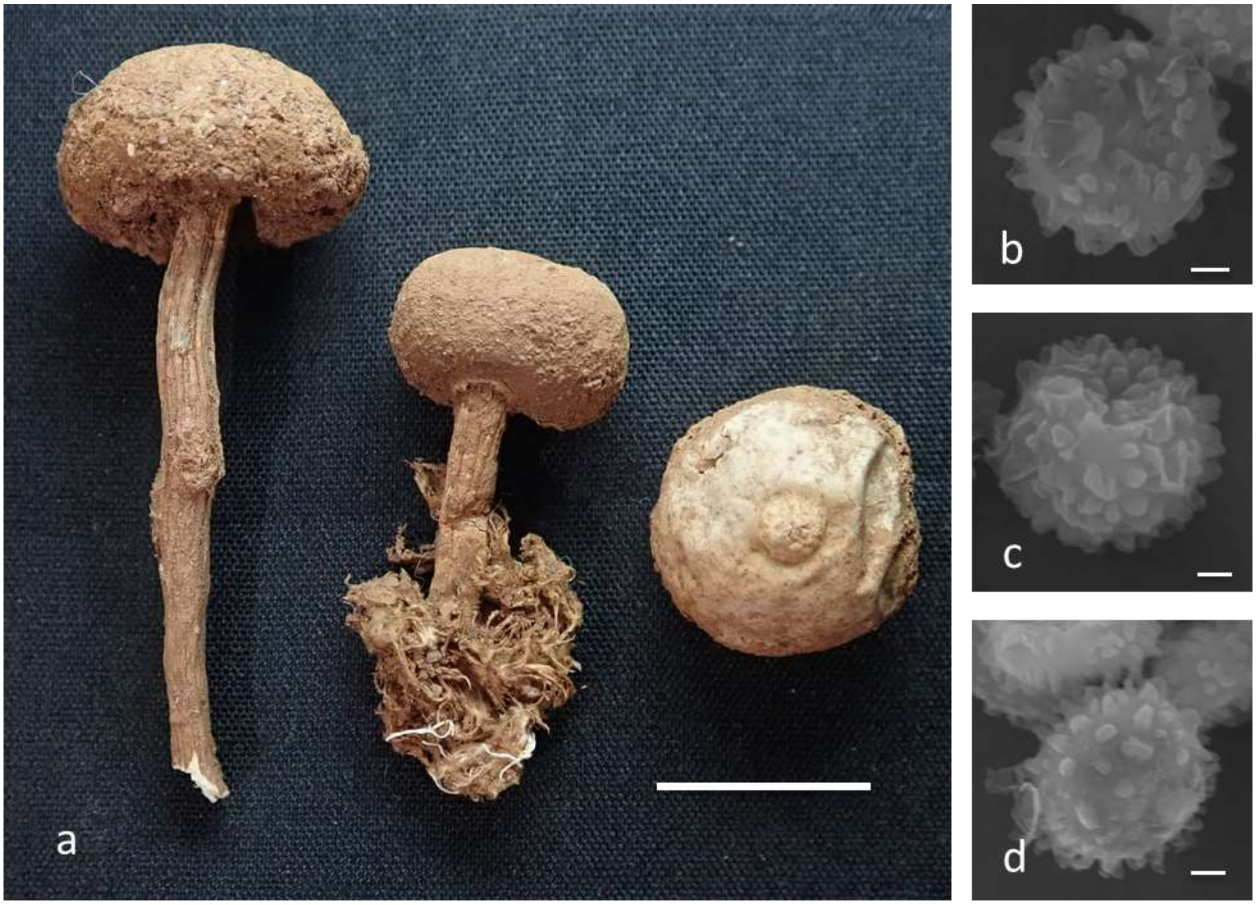


Figure 2.20. *T.* aff sp. 3 Jeppson et al. (2017) (MICH24086). a. basidiocarps; b-d. spores. Scale bars: a = 10 mm; b-d = 1 µm.

*Tulostoma* aff. sp. 10 (Jeppson et al. 2017)

See Figures 2.21 and 2.22

A few collections match the molecular data identified in as sp. 10 in Jeppson et al. (2017). These collections match the macromorphology of *T. cretaceum*, however these collections have ornamented spores, while *T. cretaceum* has smooth spores. Given morphological and sequence distinctions with collections represented in this clade, they are tentatively being considered 2 separate species, 10A and 10B. I am in communication with Jeppson to clarify the taxonomy of these collections.

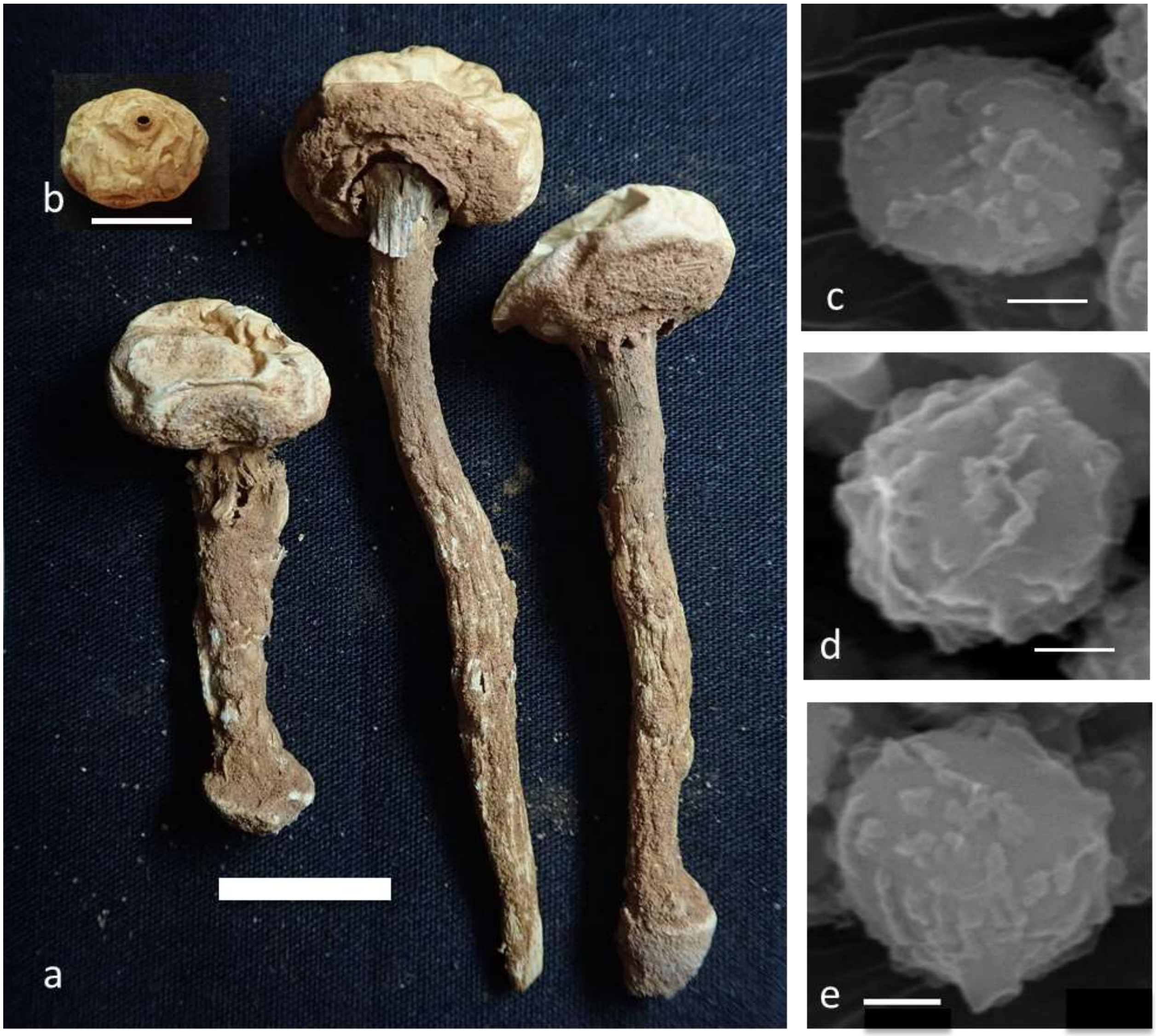


Figure 2.21. *T.* aff sp. 10A Jeppson et al. (2017) (MICH266356). a. basidiocarp; b. mouth; c-e. spores. Scale bars: a-b = 10 mm; c-e = 1 µm.

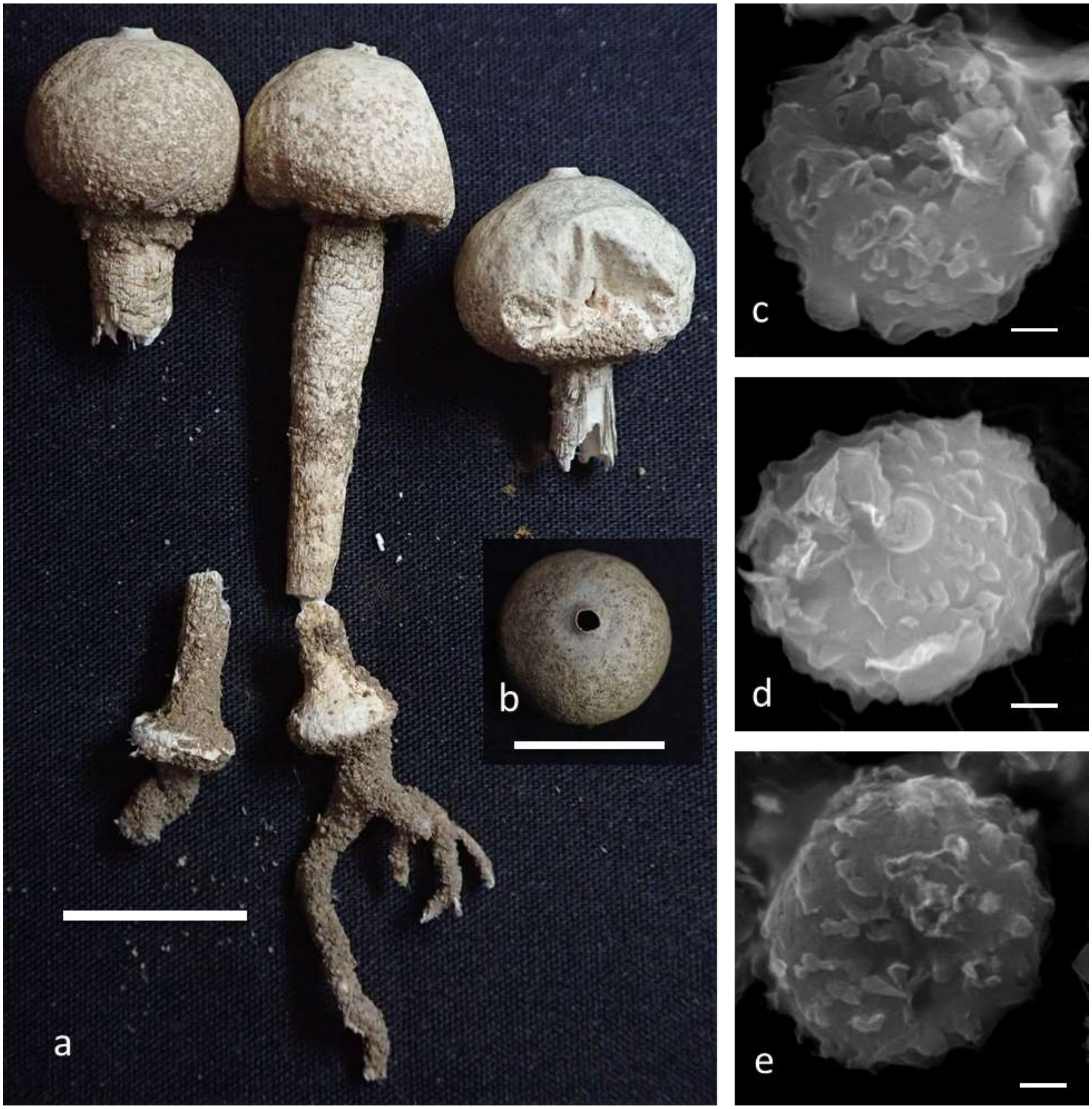


Figure 2.22. *T.* aff sp. 10B Jeppson et al. (2017) (MICH23778). a. basidiocarp; b. mouth; c-e. spores. Scale bars: a-b = 10 mm; c-e = 1 µm.

Species not recovered:

The following species have been reported from Arizona, Colorado, and New Mexico but were not recovered in this study. Sequence data from some of the types of the species were available and included in the phylogeny. The sequence data, where available, did not match any of my sequenced collections. Morphology of the following taxa did not match any of our collections.

Tulostoma albicans White, V.S. 1901. The Tylostomaceae of North America. *Bulletin of the Torrey Botanical Club* 28: 421-444.

Remarks: Jeppson et al. (2017) included type sequence of *T. albicans* in their phylogeny. None of my collections matched the morphology or the published sequence data of *T. albicans*. It is characterized by its relatively small size, white fruit-body, circular mouth, membranous exoperidium, and slightly verrucose spores. Small forms of *T. macrocephalum* may be mistaken for *T. albicans* but the former has a hyphal exoperidium and spores with well-defined warts and crests (Altes et al. 1992). Several authors discuss taxonomic problems related to *T. albicans* concept (Esqueda et al. 2004, Moreno et al. 1995, Wright 1987). *T. albicans* is an ill-defined, easily misidentified species due in large part to the indistinct ornamentation of the spores. Long (1947) considers it a synonym of *T. jourdani* Pat., but Wright (1987) did not accept this synonymy. Sequence data of *T. jourdani* are necessary to confirm synonymy of these species. Another morphologically similar species, *T. xerophilum*, has been shown to be genetically distinct from *T. albicans* (Jeppson et al. 2017). None of my collections matched the morphology or sequence data of I recovered from one collection of *T. xerophilum*, but are not able to compare with *T. albicans*. *T. albicans* has been reported from Arizona and New Mexico, Mexico, and Argentina (Wright, 1987, Moreno et al. 1995).

Tulostoma americanum Lloyd The Tylostomeae. 20, tab. 80, figs. 5-9. 1906.

Remarks: The holotype was not available for this study. It is likely that this is a synonym for *T. caespitosun* (Lloyd 1906, Wright 1987). Lloyd also states that it is identical with *T. kansense* Peck which is a synonym for *T. obesum*. See discussion in *T. obesum*.

Tulostoma berkeleyi Lloyd The Tylostomeae. 25, tab. 84, figs. 8-9. 1906.

Remarks: Wright (1987) was hesitant in the circumscription of *T. berkeleyii*. In his description, Wright (1987) included only one collection of *T. berkeleyii* from the southwestern US (NM, Herb. Long 7830, BPI, LPS). The remaining collections were from AL, CT, FL, IA, IN, ND, SC, and DC. The majority of these were from Florida. Morphologically, he noted that the stem of these collections was lighter, narrower, and the endoperidium was more pitted compared to *T. fimbriatum* and *T. fimbiratum* var. *campestre*. Molecular evidence is necessary to distinguish *T. berkeleyi* and *T. fimbiratum*.

Tulostoma brumale Pers.: Pers. Synopsis Meth. Fung., Disp. Meth. p. 6. 1801.

= ("*Fungus pulverulentus minimus*" Ray, Synop. Meth. Britt. 2 ed. 16. 1696).
= (*Lycoperdon parisiense minimum, pediculo donatum*, Tournefort, Inst. Rei Herb. 563, tab. 331, figs. E-F, 1700).
= (*Lycoperdon mammosum* Michell, Nova Plant. Gen. p. 217, nÂ° 10. 1729).
= (Lycoperdon pedunculatum Linn., Fl. Suec. nÂ° 1278. 1745).
= *T. mammosum* (Mich.) Fr., Syst. Mycol. III: 42. 1821.
= *Tulasnodea mammosa* (Mich. ex Fr.) Fr., Summa Veg. Scand. pars 2. 440. 1849.
= *T. pedunculatum* Linn.; Schroet., in Cohn's Beitr. Biol. Pflanz. 2: 65. 1877.
= *T. brevipes* Petri, Ann. Mycol. 2: 418. 1904.
= *T. mammosum* (Mich.) Fr. var. major Petri, Fl. Ital. Critt., Gasterales p. 118. 1909.
= *T. fuscoviolaceostipitatum* Schwarzm. & Philimon., Fl. Spor. Rast. Kazhastan, VI: Gasteromycetes. 227-228, fig. 83, ab. XIV, fig. 6. 1970.

Type. FRANCE, “circa Parisios” (L); several collections by Persoon available at L, but no type seems to have been formally designated (cfr. Wright 1987).

Etymology: the name applies to the season in which the species is most commonly found.

Remarks: Recent molecular evidence indicates that *T. brumale* is a European species, and that collections previously considered *T. brumale* in the US are *T. simulans*. It is possible that *T. brumale* has been introduced to parts of the US (Wright 1987), but there is not genetic evidence to support this hypothesis.

Tulostoma caespitosum Trabut apud Saccardo Sylloge Fungorum 9: 268-279. 1891.

Remarks: The defining characteristic of *Tulostoma caespitosum* is its often caespitose habitat. Despite this, Llyod’s images lack this characteristic. In Wright (1987) the basdiocarp is reported to be plate XXXII:1. The image on this plate is labeled as *T. americanum*. This could be due to Wright considering that *T. casepitosum* a distorted growth form of *T. americanum*. Nonetheless, the casepitose growth form is not demonstrated in these images, either. Further, Wright (1987) reports that this species could be considered synonymous with *T. americanum*, differing only slightly in spore morphology. I disagree with this as the spore morphology is distinct between these species. Further study of these species is necessary and neotypification warranted.

Tulostoma cretaceum Long, W.H. 1944. (Fig. 1) Studies in the Gasteromycetes X. Seven New Species of *Tylostoma. Mycologia* 36: 321-322.

Holotype: United States, New Mexico, W of Rio Grande, "2 mi below Alameda Bridge", leg. W. H. Long, 7.IV.1941 (Herb. Long 9302, BPI!). Illustration: Long (op. cit.).

Remarks: *T. cretaceum* has several easily distinguishing characteristics, a chalky white endoperidium, a rooting stipe with rhizomorphs, and smooth spores. My collections that resemble the micromorphology of *T. cretaceum*, particularly the rooting, rhizomorphic stipe, have ornamented spores. Sequence data of the type collection from NM form a clade with European species, indicating that this is likely a more widespread species than previously considered. Based on the literature, *T. cretaceum* occurs in North America in the southwestern United States, in Mexico, in South America in W. Argentina, and in Europe in Hungary and Spain and in Asia in Russia (Esqueda et al. 2004, Gube 2009, and Jeppson et al. 2017).

<u>Tulostoma excentricum</u> Long Mycologia 36: 332-333, fig. 5. 1944.

= *T. desertorum* Philimon. & Schwarzm. apud Schwarzmann & Philimonova, F1. Spor. Rast. Kazhastan, VI: Gasteromycetes. 212-213, fig. 75, tab. XIII, fig. 7. 1970.

Holotype: United States, New Mexico, E of Sandia, Vista Court, Albuquerque, leg. W. H. Long, 20.V1.1941 (Herb. Long no° 9360, BPI!).

Remarks: The type of this species was sequenced by Jeppson et al. (2017). None of my collections match the morphology or molecular sequence data of this species. Wright (1987) describes it as a relatively larger species in the genus, with a whitish to light greyish endoperidium and a prominent, projecting, tubular mouth, occasionally with a sunken peristome. The spore ornamentation is reported as uneven, sometimes anastomosed verrucae (Wright 1987). It has been reported from New Mexico in the US and Russia (Long 1944, Wright 1987).

Tulostoma giovanellae Bres., Fungi Trident. 1(1): 63. 1881.

=*Tulostoma volvulatum* Borshchov var. *volvulatum* - Materialy dla botanicseskoi geografii Aralo-Kaspiiskago kraia-Zapiski. Imp. Acad. Nauk St. Petersbourg 7: 189. 1865 (In Russian).
= *T. boissieri* Kalchbrenner, Rev. Mycol. 3: 24, tab XV, fig. 2. 1881.
= *T. barbeyanum* P. Henn., Bull. Herb. Boissier 1: 99. 1893.
= *T. ruhmerianum* P. Henn., Hedwigia 37 (6): 288. 1898.

Remarks: The taxonomy of this species is somewhat convoluted, and *T. giovanellae* has been proposed as the name for *T. vovulatum*. The original description of *T. vovulatum* lacked microscopic morphological description (Borschchov 1865). Clarification of the micromorphology was inhibited as the type was stored in Russia, and not readily available for study until 1999. Wright (1987) stated that most authors used Sorokin (1890) species concept which describes smooth spores (Sorokin 1890, plate XXV, Figure 353a). Altés et al. (1999b) studied the holotype of *T. volvulatum* and found ornamented spores, the ornamentation composed of low verrucae with fusing that forms linear ridges, somewhat similar to spores of *T. pulchellum*. Based on macro- and micromorphological characters and using Art. 56 and 57 of the Tokyo Code, Altes et al (1999) proposed *T. giovanellae* as the correct name for *T. volvatum*. Jeppson et al. (2017)

stated that a formal proposition will be put forward to the General Committee (to be published in Taxon) for *T. volvulatum* become a *nomen rejiciendum*. This process has not been completed at the time of this writing.

T. volvulatum has been applied to species found in the study area. The species with the name *T. volvulatum* were found to have smooth spores. These species align with *T. meristostoma* morphological and genetically. Calonge (1998) identified Spanish material attributed to *T. volvulatum* as *Schizostoma laceratum* (Ehrenb.: Fr.) Lév. Based on sequence data, Gube (2009) recovered *S. laceratum* as closely related to the type of *T. cretaceum*. Circumscription of *Schizostoma* is outside of the scope of this study, but further investigation on the relationship of *Schizostoma* to *Tulostoma*, and these species in particular, is warranted.

Tulostoma fusipes Hariot & Patouillard - Bull. Soc. Mycol. France 26: 207-208, pl. 9, fig. 3. 1910.

Holotype: Africa: Soudan, Timbouctu, Bon, leg. M. Chudeau, 10. VIII. 1909 (FH!).

Remarks: The morphology of this species is nearly identical to that of *T. obesum*. It is likely that these species are conspecific.

Tulostoma herteri Lohwag & Swoboda - Rev. Sudamericana bot. 7: 9, fig. 1. 1942.

Lectotype (selected by Wright 1987): Uruguay: Cerro de Montevideo, leg. Herter nÂ° 2148 (FH!).

Etymology: dedicated to the German botanist who lived most of his life in Uruguay, Dr. Guillermo Herter.

Remarks: *T. heteri* is described as having a relatively small spore-sac (up to 6 mm), a hyphal exoperidium, a light rusty brown endoperidium, a relatively large fibrillose mouth, large for the size of the peridium, with a felty dentate peristome. The spores measure 4-4.8 µm and are ornamented appears with blunt, appressed verrucae. The stem has a distinct small mycelial bulb at the base.

In the original protologue, no collection identified as a holotype, Wright (1987) established a lectotype based on morphology, habitat, and location. *T. heteri* has been found in dry sites with herbaceous vegetation in sandy soils and reported from Argentina, Bolivia and Uruguay and the southeastern and southwestern United States.

Tulostoma jourdani Patouillard [as 'jourdani'], Revue mycol., Toulouse 8(no. 31 ('30')): 143 (1886)

Isotype: ALGERIA. El Goleah, 11-1880, M. Choisy (ISOTYPE, PC).

Remarks: Type studies of *T. jourdani* by Moreno et al. (1995) and Altes et al. (1994) report the species having a hyphal exoperidium, a dirty white to creamy white endoperidium, a planar, circular mouth, and a longitudinally striate stem with a bulbous or volviform base. The most distinguishing character they describe is the rugose spores, seen under SEM, which are unique in this genus. Altes (1994) states that because *T. jourdani* can be easily mistaken for other smooth-spored species, SEM imaging is necessary for correct identification.

I was unable to recover this species in my research. Sequence data of the type species are warranted.

<u>Tulostoma lysocephalum</u> Long, Mycologia 36(4): 325 (1944)

Holotype: United States, New Mexico, 10 mi W of Deming, on Hway 70, leg. Long & Stouffer, 12.IX.1941 (Herb. Long nÂ° 9369, BPI!).

Etymology: The name applies to the fact that the spore-sacs become loose.

Remarks: Some of the distinguishing features of this species is the readily separating, relatively large spore-sac (up to 32 mm). Wright (1987) describes the exoperidium as hyphal and persisting as a thick band at the base as an acorn-cup, and Long (1944) describes it as a slowly deciduous sand case, slowly deciduous. Long (1944) recognized that heavy basal peridial sheath likely enabled the spore sac to land such that the mouth was oriented up in order to facilitate spore dispersal. The endoperidium is reported as ochraceous whitish to ochraceous pinkish, tough, and thick. The mouth is planar to raised, fibrillose, becoming lacerate in age. The stem is concolorous with the endoperidium with a volviform radicating formation at the base, slightly tapering downwards. Spores measure 5.6-6.6 µm diam, and ornamentation appears finely verruculose.

I have 2 collections that resemble the morphology of this species, however the ITS sequences differ from the type sequences and more closely match ITS from an unnamed species (referred to as species 10 in Jeppson et al. 2017). Additional DNA sequences are being gathered and the morphology of these collections are being compared to determine the correct identification of this species and ascertain whether it is a novel species.

It has only been reported from New Mexico in sand dunes partially shaded by *Prosopis* (mesquite brush) (Wright, 1987, Long 1944).

<u>Tulostoma obesum</u> Cooke & Ellis, Grevillea 6:82 (1878)

= *Tulostoma barbeyanum* Henn., Bull. Herb. Boissier 1: 99 (1893)
= *Tulostoma biostiolatum* Schwarzman & Philim., in Byzova et al., Flora Sporovykh Rasteniĭ Kazakhstana [Cryptogamic Flora of Kazakhstan], 5, Nesovershennye Griby - Fungi Imperfecti (Deuteromycetes) 3. Sphaeropsidales (Alma-Ata): 220 (1970)
= *Tulostoma giolianum* Bacc., Risultati scientifici della Missione Stefanini Paoli nella Somalia meridionale. Le Collezioni botani (Firenze): 189 (1916)
= *Tulostoma kansense* V.S. White, Bull. Torrey bot. Club 28: 430 (1901)
= *Tulostoma ruhmerianum* Henn., Hedwigia 37: 288 (1898)
= *Tulostoma volvulatum* f. *elatum* (Hariot & Pat.) Sacc. & Trotter, Syll. fung. (Abellini) 21: 476 (1912)

= *Tulostoma volvulatum* var. *elatum* Hariot & Pat., Bull. Soc. mycol. Fr. 26: 209 (1910)
= *Tulostoma volvulatum* var. *obesum* (Cooke & Ellis) J.E. Wright, Biblthca Mycol. 113: 212 (1987)

Holotype. U.S.A., **Colorado**, Coke 2715 (K 39158); Isotype: (NY!)*

Etymology: The epithet refers to the obese nature of the plant.

Remarks: Altes et al. (1999b) studied the types of *Tulostoma volvulatum* var. *obesum*, *T. kansense*, *T. volvulatum* var. *elatum* and demonstrated these are conspecific. This species is recognized by a membranous exoperidium, a whitish endoperidium, a mouth which initially develops as a circular, but rapidly becomes indefinite, and smooth spores. Jeppson et al. (2017) sequenced the type and found several collections from Spain matched the sequences of the type.

This species has been reported from Baja California, Colorado, and Spain (Ochoa & Moreno 1996). This is a psammophilous species originally described from Colorado. In Europe it is recorded from littoral sand dunes and halophytic steppe habitats in SE Spain (Jeppson et al. 2017).

Tulostoma utahense J.E. Wright, Biblthca Mycol. 113: 204 (1987)

Holotype: United States, Utah, Salt Lake City, beaches N of City Creek, leg. A. 0. Garrett 2585, III.1920 (NY!).

Etymology: The name refers to the type locality, in the state of Utah, United States.

Remarks: This species is very similar to *T. meridionale* and T. wrightii (see remarks on these species). The collections studied in this research matched T. meridionale. Sequencing of the type of T. utahense is necessary to clarify the circumscription of these species.

Tulostoma wrightii Berkeley. Grevillea 19(92): 95. 1891.

Holotype: United States, New Mexico, Rio Gande, leg. Charles Wright (K!).

T. wrightii, whose name is dedicated to the collector Charles Wright, warrants further investigation.

Remarks: Most authors distinguish *T. wrightii* by a fibrillose mouth, a membranous exoperidium, and irregularly verrucose spores. However, descriptions of mouth morphology differ, Berkeley originally described the mouth as forming a small circular stoma, while other authors refer to a fibrillose or indefinite mouth (Wright 1987, Moreno et al. 1995, Hernández-Navarro et al. 2017). *T. wrightii* resembles *T. purpusii* in macromorphological characters and habitat, but differs from *T. purpusii* in spore ornamentation. *T. purpusii* has strongly anastomosing, almost reticulate, ridges, while spores of *T. wrightii* are only weakly anastomosed. Multiple authors also note that *T. wrightii* is morphologically similar to *T.*

meridionale and *T. utahense* and that they are found in similar habitats. Spore ornamentation is also similar, the ornamentation is reported as uneven verrucae with fusing, but spore size is reportedly different. *T. wrightii* has smaller (4.0-4.8 x 4.0-6 µm) spores compared to *T. meridonale* (7-10 (-11.2) µm) and *T. utahense* (6.6-8.4-(9.8) µm). *T. wrightii* has been reported from New Mexico and Sonora, Mexico.

Wright (1987) reports that the holotype material of *T. wrightii* is in poor condition, consisting of an incomplete specimen, and fragmented stem. This species is worthy of further study to neotypify and generate DNA sequences.

Table 2.1. Using current taxonomic names, a total of 64 species have been reported from Arizona, Colorado, and New Mexico. Species investigated in this project are in bold.

Reported Species	AZ	CO	NM
Tulostoma albicans	X	X	X
Tulostoma americanum	X	X	X
Tulostoma australianum	X	X	X
Tulostoma beccarianum			X
Tulostoma caespitosum			X
Tulostoma calceruem		X	
Tulostoma chudaei	X		X
Tulostoma clathrosporum			X
Tulostoma cretaceum	X	X	X
Tulostoma egranulosum	X		
Tulostoma excentricum	X		X
Tulostoma exitum			X
Tulostoma ferrugineum			X
Tulostoma fibrillosum	X	X	X
Tulostoma fimbriatum	X	X	X
Tulostoma floridanum	X		
Tulostoma fusipes	X		X
Tulostoma giovanellae		X	X
Tulostoma gracile			X
Tulostoma grandisporum			X
Tulostoma granulosum	X		
Tulostoma herteri	X		X
Tulostoma involucratum	X	X	X
Tulostoma jourdani			X
Tulostoma juniperense			X
Tulostoma leiosporum	X		
Tulostoma lloydii			X
Tulostoma longii			X
Tulostoma lysocephalum	X		X
Tulostoma macalpinianum			X
Tulostoma macrocephalum	X	X	X
Tulostoma macrosporum			X
Tulostoma melanocyclum			X
Tulostoma meridionale	X	X	X
Tulostoma meristostoma	X		X
Tulostoma minutum		X	
Tulostoma moellerianum		X	

Tulostoma mohavei			X
Tulostoma nanum			X
Tulostoma obesum		X	X
Tulostoma obscurum		X	
Tulostoma opacum	X		X
Tulostoma perplexum			X
Tulostoma petrii	X	X	
Tulostoma polymorphum	X	X	X
Tulostoma pulchellum	X	X	X
Tulostoma punctatum	X	X	X
Tulostoma punctilabratum		X	X
Tulostoma purpusii	X	X	X
Tulostoma pygmaeum	X		X
Tulostoma rufum			X
Tulostoma simulans	X	X	X
Tulostoma squamosum	X		
Tulostoma striatum	X	X	X
Tulostoma subsquamosum			X
Tulostoma thiersii	X		
Tulostoma tuberculatum		X	X
Tulostoma utahense	X		X
Tulostoma vulgare	X		
Tulostoma wrightii	X		X
Tulostoma xerophilum	X		X

DISCUSSION

Very few of the species reported from Arizona, Colorado, and New Mexico were recovered in this study. One compelling reason for this unrecovered diversity are collection efforts and loss of habitat. *Tulostoma* are most commonly found in relatively arid areas, which are not regularly frequented by foragers. Also, they are small and inconspicuous, frequently camouflaged in their substrate. The combination of rarity of collecting in areas where the inhabit, and the ease of overlooking these species, results in few collections housed in herbaria. The quality of collections found in these herbaria can also be less than ideal. Many of these collections contain only one basidiocarp, and there are very few recent collections. The DNA in these collections can break down over time, and preservation processes such as freezing collections to combat other fungal or insect infestation can contribute to DNA degradation. This can render molecular analysis of these collections challenging.

The few number of species recovered compared to the number of described species may also be attributed to habitat loss. Widespread, generalist species, such as *T. fimbriatum* and *T. simulans*, were represented by multiple collections. Species such as *T. striatum*, which is seemingly restricted to psammophilous soils, was represented by only one collection. Other species that might be considered specialists, such as *T. lysocephalum* which has only been reported in the shade of mesquite on sand dunes, were not represented by any collections. In Argentina, *T. domingueziae* in known only from threatened woodlands (Hernández-Caffot et al. 2011). In Europe, Jeppson et al. (2017) reported that anthropogenic endeavors threaten the habitats in which many *Tulostoma* species occur. There are several Europena *Tulostoma* species on the threatened species red-list of in the countries where they occur. Throughout Europe, species of *Tulostoma*, such as *T. kotlabae* and *T. melanocyclum* are considered threatened or endangered, and some species, such as *T. pulchellum* and *T. moravecii* are potentially extinct (Jeppson 2008). Even widespread species, such as *T. fimbratum* and *T. simulans* are experiencing loss of habitat in Europe (Jeppson et al. 2017). Because many species of *Tulostoma* can be considered as indicators of valuable and threatened habitats (Jeppson et al. 2017) it is important to monitor the distribution and occurrence of these species to understand how habitat loss may impact biodiversity and ecosystem functioning.

Another hindrance to a well-developed understanding of this genus is the lack of detailed habitat and site information associated with herbarium collections. The delineation of some species using morphology can be challenging without access to SEM technology. Detailed habitat information can serve to distinguish some species in the absence of spore morphological characters. It is likely that the less encountered species are adapted to narrow niches, and recovering these species will require knowledge of the ecological parameters to determine their existences and distribution.

Future study of *Tulsotoma* is warranted. Very few collections of *Tulostoma* exist in herbaria, and collections that are present are in poor condition and not recent. This will require collection in areas that are not regularly visited by mycologists. As the diversity of *Tulostoma* is centered in mostly arid and desert like areas, these areas call for focused study. The impacts of urban and agricultural develop coupled with factors of climate change further compound the necessity of documenting species. During the summer of 2021, the western United States experienced some of the highest temperatures recorded (NOAA) and lowest precipitation levels. The climatic conditions are likely to escalate, and coupled with increases in fire severity and frequency, are likely to cause sizeable shifts in current ecosystems in which *Tulostoma* grow.

Chapter 3. Phylogeography of the Fungal Genus *Tulostoma*

INTRODUCTION

Phylogeography, the reconstruction of historical processes which gave rise to the geographic distribution of a taxonomic group, seeks to determine where species originated and the direction and potential methods or mechanisms of dispersal (Avise 2000). Fungi are one of the most diverse group of eukaryotic organisms. While only some 148,000 fungal species are described (Cheek et al. 2020), recent estimates of fungal diversity suggest between 2.2 to 3.8 million species exist (Hawksworth and Lücking 2017). Despite this tremendous biodiversity, fungal phylogeography is poorly studied (Beheregaray 2008, Sánchez-Ramírez et al. 2017). This lack of phylogeographic study is due in part to the lack of taxonomic circumscription of fungal species and the very limited fungal fossil record (Taylor et al. 2000, Han et al. 2017). Without the taxonomic knowledge of fungal groups, it is challenging to understand the full phylogeographic history of lineages. Also, establishing diversification times of fungal groups are hindered by the lack of fossil data, and can hamper phylogeographical reconstructions.

Because basidiospores (very often smaller than 10 µm) of fungi are the putative mechanism of dispersal, fungi are often considered microorganisms in terms of dispersal. The dispersal potential of microorganisms is a long-debated topic. The ubiquitous dispersal hypothesis, often described as ‘Everything is everywhere, but, the environment selects’, was long applied to patterns of microorganismal dispersal (Finlay 2002, de Wit and Bouvier 2006, Queloz et al. 2011, van der Gast 2015). Microbes are thought to be capable of long-distance dispersal, via wind or water, owing to their small size and the large number of propagules (Finlay 2002). The ubiquitous dispersal hypothesis held that many microscopic species (those less than 1 mm) were cosmopolitan and existed wherever there was suitable habitat, therefore genetic differences across their distribution would be minimal (Finlay 2002, Bass et al. 2007, Grubisha et al. 2012, van der Gast 2015). The advent of molecular methods has largely refuted this dispersal hypothesis for many microorganisms, including fungi. A few examples of cosmopolitan fungal species have been identified using molecular markers. For example, the human pathogen *Aspergillus fumigatus*, some root endophytes, and the ectomycorrhizal species *Tricholoma scalpturatum* to some extent*,* but cosmopolitan fungal species appear to be uncommon (Pringle et al. 2005, Carriconde et al. 2007, Queloz et al., 2011). More commonly, molecular studies have repeatedly resolved cryptic speciation by showing genetic differences between morphologically similar species (Taylor et al. 2006; Douhan et al. 2011, Sousa et al. 2017). The inferences resulting from phylogenetic studies using molecular data have increased our understanding of the ecology, biodiversity, and distribution of sympatric cryptic species (Grubisha et al. 2012, Accioly et al. 2019. Kobmoo et al. 2019, Sato et al. 2020).

Over the past decade, studies investigating fungal phylogeography are starting to elucidate fungal dispersal patterns (Skrede et al. 2011, Harrower et al. 2015, Koch et al. 2017, Wilson et al. 2017). These studies show a variety of phylogeographic patterns of varying complexity. Some ectomycorrhizal fungal display dispersal patterns which largely mirror their associated plant hosts (Wilson et al. 2017). The phylogeographic distributions of other ectomycorrhizal fungi, usually species with less host specificity, are much more complex (Reynolds 2011, Looney et al. 2017, Sato et al. 2017). Fungal phylogeographic studies on ectomycorrhizal, pathogenic and wood-rotting fungi show both vicariance and long-distance dispersal patterns (Reynolds 2011, Coetzee et al. 2011, Skrede 2011, Koch et al. 2017, Looney et al. 2017). Four species of saprotrophic coprinoid fungi showed varying phylogeographic signals and likely recent long-distance dispersal from Asia to Hawaii (Ko et al. 2001).

The origins of fungal lineages are also becoming resolved using phylogeographic studies. Looney et al. (2017) indicate that biogeographic origin of mycorrhizal *Russula* was in the north temperate zone. Their findings suggest *Russula* moved into and out of the tropics multiple times over their evolutionary history. The ectomycorrhizal genus *Strobilomyces* was shown to have an African origin and is hypothesized to exhibit a Boreotropical migration from Africa to Australasia and East Asia (Han et al. 2018). Pathogenic armillarioid fungi likely evolved in Eurasia (Koch et al. 2017).

Vicariance (allopatric speciation) and long-distance dispersal are used to explain intercontinental and global distributions of fungal taxa (Hosaka et al. 2008, Matheny et al. 2009, Wilson et al. 2012, Bonito et al. 2013). For example, the ectomycorrhizal group *Amanita* sect. *Phalloideae* was shown to have a Palaeotropical origin with long-distance dispersal via the Bering Land Bridge, followed by regional vicariance speciation spurred by climatic changed beginning in the middle (Cai et al. 2014). Likewise, the distribution of Serpulaceae is also explained by vicariance and long-distance dispersal events (Skrede et al. 2011). Molecular evidence also indicates that *Ganoderma applanatum* distributions throughout the Southern Hemisphere occurred as the result of long-distance dispersal (Moncalvo and Buchanan 2008). Even hypogeous and subhypogeous gasteroid fungal species demonstrate long-distance dispersal (Hosaka et al. 2008). Genetic evidence suggests a long-distance dispersal of *Pisolithus* from Australia to New Zealand (Moyersoen et al. 2003).

Distinguishing between vicariance, or allopatric speciation, and long-distance dispersal is possible in some instances. Because speciation through vicariance is thought to be ancient, it may be distinguished from more recent long-distance dispersal events by genetic differences. Meaning, more ancient vicariance events will result in greater genetic differences within species or between closely related taxa compared to more recent long-distance dispersal events. The limitation being that ancient long-distance events cannot be distinguished from vicariance because genetic differences will be greater among closely related taxa in both instances. The distinction between vicariance and long-distance dispersal can be enhanced by the use of molecular clocks. The use of molecular clocks enables the timing of diversification events to be compared to geologic events. Based on molecular dating, many phylogenetic studies have revealed striking chronological and geographical correlations between evolutionary divergence and geological events. The timing of diversification in the examples above show that divergence of *Amanita* sect. *Phalloides*, Serpulaceae, and *Ganoderma applanatum* occurred long after the breakup of Gondwana. Thus, long-distance dispersal is the most likely explanation for the large disjunct distributions seen in these, and other, fungal taxa.

Fungal phylogeography is relatively poorly understood, and fungi are one of the least studied groups of organisms for phylogeography (Grubisha et al. 2012). Beheregaray (2008) found that of the 3,049 articles published on phylogeography between 1987 and 2006 only 13 explored fungi species. In the years since, there has been increased study on biogeographic patterns, particularly in mycorrhizal fungi, however investigations into non-wood rotting saprobes, and puffballs in particular, are lacking (Ko et al. 2001). Fungi are vastly understudied with respect to phylogeography and taxonomic diversity.

Tulostoma Pers.:Pers.(Agaricaceae) is a cosmopolitan fungal genus of stalked puffballs (Figure 3.1). Species in this genus are found primarily in sandy soils, with steppe habitats harboring great biodiversity of this genus (Jeppson et al. 2017). Members of *Tulostoma* are thought to be mostly saprotrophic and consume dead or decaying matter and function in nutrient cycling and soil formation (Wright 1987). Within the Agaricales, *Tulostoma* is one of 2 speciose gasteroid genera. The actual number species in this genus is yet to be determined. Wright (1987) published a world monograph of the genus which recognized 139 species. Most recently, Kalichman et al. (2020) recognized 172 accepted species names in *Tulostoma*. In addition to the species diversity in this group, members of this genus also display a great diversity of spore morphology and some size variation relative to other mushroom forming genera. Spores morphology ranges from completely smooth to highly ornamented and sizes range from under 3 µm to over 8 µm, averaging about 4.5-6.5 µm (See Chapter 4).

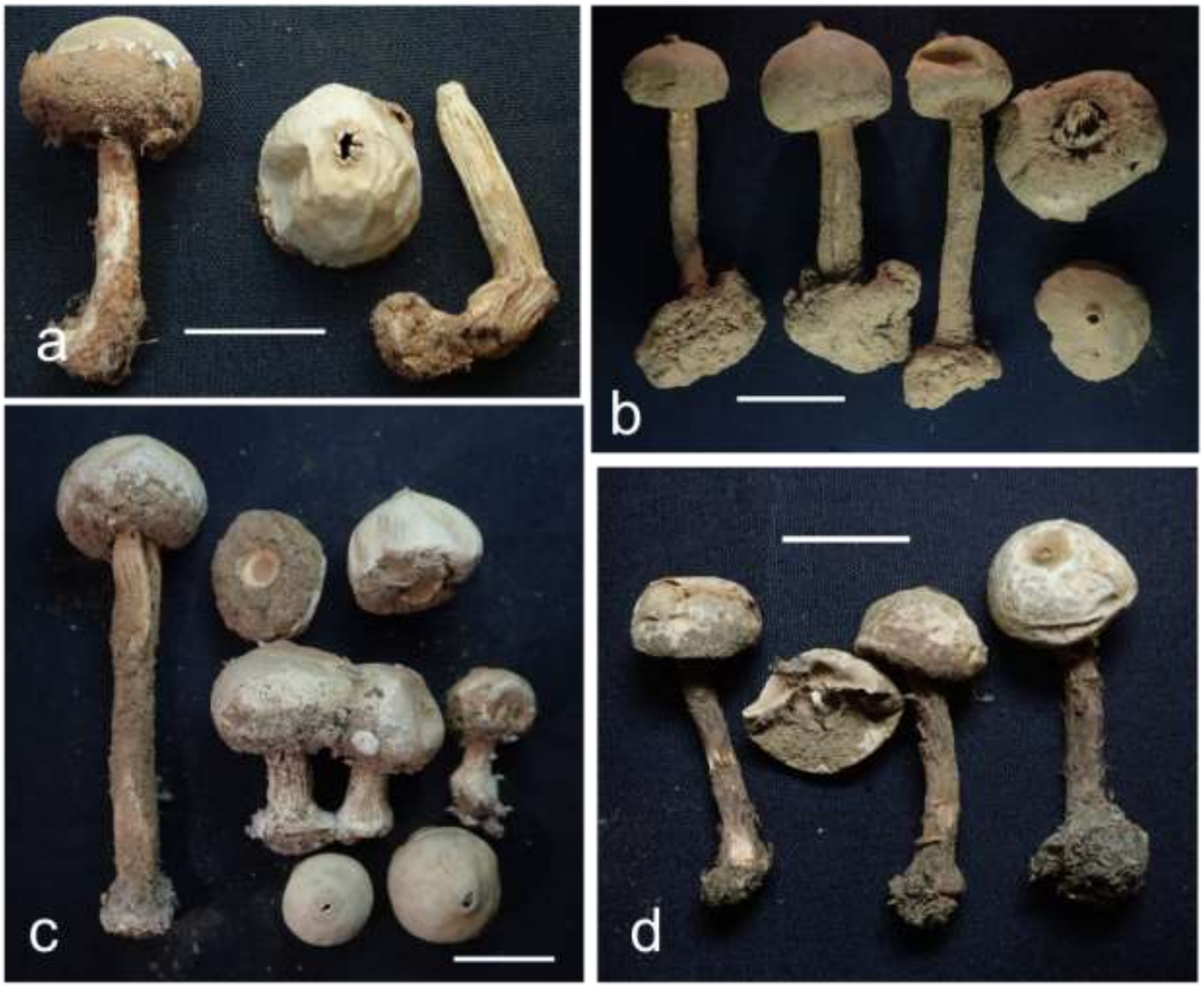


Figure 3.1. Morphological variation of selected *Tulostoma* basidiocarp collections. a. *T. striatum* (MICH266163), b. *T. purpusii* (AZ43457), c. *T. pulchellum* (MICH266205), d. *T. calcareum* (DBG21975).

Because puffballs produce their spores in enclosed structures, they have lost forcible discharge of spores as seen in other epigeous, non-gasteroid Agaricales (Wilson et al. 2011). Puffballs are thought to rely on the impact of raindrops to disperse their spores or wind currents (Pady and Kramer 1967, Hassett et al. 2015). Dispersal of puffball fungi is not well researched. Given the great diversity in spore morphology and ranges of *Tulostoma* species, it is possible that differences in spore morphological characters may contribute dispersal of species in this genus. The global distribution of this group warrants investigation.

The objective of this study was to reconstruct the phylogeographical history of *Tulostoma* using ITS, LSU, and TEF1α genetic data. From the phylogeographic reconstruction, the geographic location where the species originated was inferred. This phylogeographic reconstruction can also infer patterns of dispersal and speciation within this diverse genus.

METHODS

Taxon Sampling

A total of 82 sequences were used in this analysis. Information on taxa used in this study, including GenBank accession numbers and geographic locations is given in Appendix 2.1.

DNA extraction, PCR, and Sequencing

DNA extractions were performed on approximately 0.5 g of dried material from each collection. All utensils used in the removal and weighing of fungal material were sterilized as appropriate. Metal equipment was dipped in 95% ethanol and flamed, while flammable materials were wiped with 95% ethanol and allowed to air dry. Fungal material was transferred to a 1.5 ml Eppendorf tube and ground using a sterilized micropestle and sterilized sand (Fisher Chemical S25-500). DNA was extracted from the prepared fungal tissues using the E.Z.N.A. Fungal DNA extraction kit (Omega BioTek D3390-01) using the manufacturer's instructions.

Target DNA sequences from ITS, LSU, and TEF-1α were amplified using polymerase chain reactions (PCR). The ITS region was amplified from all collections using the fungal specific primers ITS1F and ITS4B (Gardes et al 1993). LSU regions were amplified with LR21 and LR7 primers (Hopple and Vilgalys 1999). TEF-1α was amplified using EF983F and EF2218R primers (Rehner and Buckley 2005).

Twenty-five µl PCR reactions were performed with 5µl template DNA (approximately 10 ng DNA/µl), 2.5 µl primer of each primer, 12.5 µl GoTaq, and 5 µl sterile H_2O. PCR amplification began with an initial denaturing at 95°C for 2 minutes. Thirty PCR cycles ran with denaturing at 94°C for 1 minute, annealing at 50°C for 30 seconds and elongation at 72°C for 1 minute. A final elongation ran at 72°C for 5 minutes. PCR products were stored at approximately 4°C.

PCR products were visualized on 3% agarose gels. Successful PCR products were determined as clean bands approximately 400-600 bp for ITS, approximately 700-900 bp for LSU and approximately 1000 bp for TEF-1α. These PCR products were cleaned using QIAquick PCR purification kit using the manufacturer's protocols. Clean PCR product of target regions were sent to the University of Arizona sequencing center for Sanger Sequencing.

Evolutionary Reconstruction

Individual DNA sequence base pairs were visualized and edited, and forward and reverse contigs assembled using Sequencher (Gene Codes Corporation, Ann Arbor, MI). Sequences were assembled in Excel and exported to BBEdit. Sequences from this project were combined with available sequence data (GenBank and personally loaned from Dr. Matthias Gube (Georg-August-Universität Göttingen, Germany)). Sequences were aligned using Muscle in Mesquite. Aligned sequences were trimmed to the first and last informative base. Aligned sequences of each gene were concatenated using SequenceMatrix 1.8. The concatenated matrix was analyzed in CIPRES Web Portal (Miller et al. 2010). Maximum Likelihood Analyses was conducted using RaxML. The best tree from 1000 bootstrap replicates was chosen and edited in FigTree (Rambaut 2009).

Phylogeography

Biogeographic History

Biogeographic analysis was conducted using the R package BioGeoBEARS v.1.1.2 (Matzke 2013) in R v.4.0.2 (R Core Team 2020). Species geographic ranges were determined from published genetic data and Wright (1987) monograph. Geographic ranges were delineated using 6 of the 8 biogeographic provinces established by Udvarydy (1975). The regions are defined as Nearctic, Palearctic, Afrotropical, Indomalayan, Australasian, and Neotropical. Oceania and Antarctic were excluded in this analysis as no *Tulostoma* collections have been reported from these regions. Geographic centroids for all samples were measured using the Haversine distance between centroids using the R package geosphere v.1.5-10 (Hijmans et al. 2019). Those distances (Appendix 3.2) were included in the BioGeoBEARS distance file. I used the phylogeny estimated from Chapter 1 as input data, and a tree with one representative per species (or monophyletic group, for the species exhibiting cryptic lineage diversity). I then used the chronos function in ape applying the correlated rate model (Sanderson 2002) to convert the pruned tree to an ultrametric chronogram. I then used the chronogram to test the following biogeographic models: DEC, a dispersal-extinction-cladogenesis biogeographic model (Ree and Smith 2008; Matzke 2014, Massana et al. 2015); DIVALIKE, a maximum likelihood-based biogeographic model similar to DIVA (Ronquist 1997); BAYAREALIKE, a maximum likelihood-based biogeographic model similar to BayArea (Landis et al. 2013); and all of these models with an additional parameter (+J) that accounts for founder event speciation via 233 jump dispersal (i.e., DEC+J, DIVALIKE+J, and BAYAREALIKE+J). Note that the DEC+J model has been critiqued for not adequately modelling founder event speciation (Ree and Sanmartín 2018).

RESULTS

Phylogenetic Reconstruction

The phylogenetic tree generated by RaxML using the concatenated data set is show in Figure 3.2. Eighty-two *Tulostoma* taxa were included in the final phylogenetic analysis with *Battarrea* selected as the outgroup. The aligned data set included 2564 nucleotides; Genbank numbers are shown in Appendix 3.1. My phylogeny corroborates the monophyly of *Tulostoma* (Gube 2009, Jeppson et al. 2017). I also show infrageneric clades similar to Jeppson et al. (2017) with strong bootstrap support. Relationships between clades remain ambiguous with low or no bootstrap support.

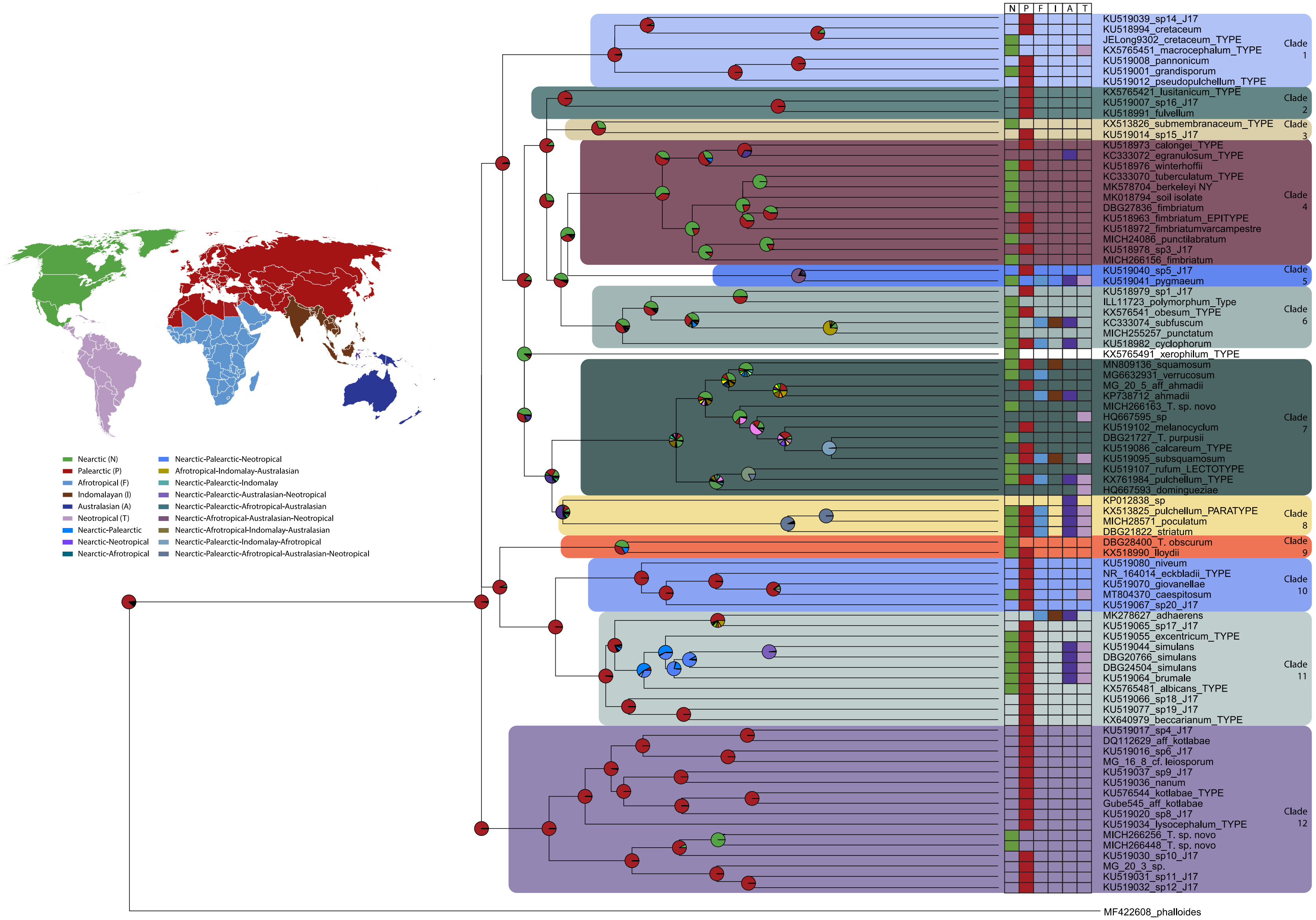

Nearctic (N)
Palearctic (P)
Afrotropical (F)
Indomalayan (I)
Australasian (A)
Neotropical (T)
Nearctic-Palearctic
Nearctic-Neotropical
Nearctic-Afrotropical
Nearctic-Palearctic-Neotropical
Afrotropical-Indomalay-Australasian
Nearctic-Palearctic-Indomalay
Nearctic-Palearctic-Australasian-Neotropical
Nearctic-Palearctic-Afrotropical-Australasian
Nearctic-Afrotropical-Australasian-Neotropical
Nearctic-Afrotropical-Indomalay-Australasian
Nearctic-Palearctic-Indomalay-Afrotropical
Nearctic-Palearctic-Afrotropical-Australasian-Neotropical
N
P
F
I
A
T
KU519039_sp14_J17
KU518994_cretaceum
JELong9302_cretaceum_TYPE
KX5765451_macrocephalum_TYPE
KU519008_pannonicum
KU519001_grandisporum
KU519012_pseudopulchellum_TYPE
Clade 1
KX5765421_lusitanicum_TYPE
KU519007_sp16_J17
KU518991_fulvellum
Clade 2
KX513826_submembranaceum_TYPE
KU519014_sp15_J17
Clade 3
KU518973_calongei_TYPE
KC333072_egranulosum_TYPE
KU518976_winterhoffii
KC333070_tuberculatum_TYPE
MK578704_berkeleyi NY
MK018794_soil isolate
DBG27836_fimbriatum
KU518963_fimbriatum_EPITYPE
KU518972_fimbriatumvarcampestre
MICH24086_punctilabratum
KU518978_sp3_J17
MICH266156_fimbriatum
Clade 4
KU519040_sp5_J17
KU519041_pygmaeum
Clade 5
KU518979_sp1_J17
ILL11723_polymorphum_Type
KX576541_obesum_TYPE
KC333074_subfuscum
MICH255257_punctatum
KU518982_cyclophorum
Clade 6
KX5765491_xerophilum_TYPE
MN809136_squamosum
MG6632931_verrucosum
MG_20_5_aff_ahmadii
KP738712_ahmadii
MICH266163_T. sp. novo
HQ667595_sp
KU519102_melanocyclum
DBG21727_T. purpusii
KU519086_calcareum_TYPE
KU519095_subsquamosum
KU519107_rufum_LECTOTYPE
KX761984_pulchellum_TYPE
HQ667593_domingueziae
Clade 7
KP012838_sp
KX513825_pulchellum_PARATYPE
MICH28571_poculatum
DBG21822_striatum
Clade 8
DBG28400_T. obscurum
KX518990_lloydii
Clade 9
KU519080_niveum
NR_164014_eckbladii_TYPE
KU519070_giovanellae
MT804370_caespitosum
KU519067_sp20_J17
Clade 10
MK278627_adhaerens
KU519065_sp17_J17
KU519055_excentricum_TYPE
KU519044_simulans
DBG20766_simulans
DBG24504_simulans
KU519064_brumale
KX5765481_albicans_TYPE
KU519066_sp18_J17
KU519077_sp19_J17
KX640979_beccarianum_TYPE
Clade 11
KU519017_sp4_J17
DQ112629_aff_kotlabae
KU519016_sp6_J17
MG_16_8_cf. leiosporum
KU519037_sp9_J17
KU519036_nanum
KU576544_kotlabae_TYPE
Gube545_aff_kotlabae
KU519020_sp8_J17
KU519034_lysocephalum_TYPE
MICH266256_T. sp. novo
MICH266448_T. sp. novo
KU519030_sp10_J17
MG_20_3_sp.
KU519031_sp11_J17
KU519032_sp12_J17
Clade 12
MF422608_phalloides

Figure 3.2. Phylogenetic tree generated from 3 loci (ITS, LSU and Tef1α). Each clade is color-coded but does not represent biogeographic distributions. Pies represent probability of geographic origin for each clade. Table at branch tips represents extant species distributions.

Table 3.1 Results from BioGeoBEARS analysis. Each biogeographic model is displayed along with its log-likelihood score, the number of parameters included in each model, parameter values for dispersal (d), extinction (e), and founder (j), Akaike Information Criterion scores corrected for small sample sizes (AICc), and weighted Akaike Information Criterion scores (AICc_wt). The three models that account for jump dispersal (+J) all had the highest likelihood scores, Iselected the model with the best score that accurately models founder event speciation. Because DEC+J is a poor model of founder event speciation (Ree and Sanmartin 2018), the most appropriate model for these data is BAYAREALIKE+J, displayed in bold font.

Model	LnL	# parameters	d	e	j	AICc	AICc_wt
DEC	-315	2	3.89	1.45	0	633.9	$7.3\text{x}10^{-27}$
DEC+J	-253.8	3	2.71	$1.0\text{x}10^{-12}$	0.025	513.5	1.00
DIVALIKE	-315	2	3.89	1.45	0	633.9	$7.3\text{x}10^{-27}$
DIVALIKE+J	-308.5	3	3.61	0.71	0.017	622.9	$1.8\text{x}10^{-24}$
BAYAREALIKE	-350.3	2	1.9	5	0	704.7	$3.1\text{x}10^{-42}$
BAYAREALIKE+J	**-281.6**	**3**	**1.88**	**0.68**	**0.072**	**569.3**	**$7.9\text{x}10^{-13}$**

Biogeographic History

BioGeoBEARS results show that DEC+J model followed by the BAYAREALIKE+J have the best likelihood score (Table 3.1). Because the DEC+J does not model jump dispersal well (Ree and Sanmartín 2018), I chose the BAYAREALIKE+J model for my analysis. Likelihood scores of both models were similar. The BAYAREALIKE+J biogeographic model accounts for large geographic ranges and a large number of geographic ranges range expansion or contraction. The model also accounts for vicariance, distance dependence, and has correction for extinction events. The model employs Bayesian sampling of biogeographic histories which allows analyses on large numbers of areas, and founder event speciation via jump dispersal (Landis et al. 2013).

My analyses indicate a Palearctic origin of *Tulostoma*. Twelve clades were distinguished.

Clade 1 shows a Palearctic origin with dispersal of *T. cretaceum* and *T. grandisporum* into Nearctic and *T. macrocephalum* into Nearctic and Neotropical.

Clade 2 consists of solely Palearctic species distributions of *T. lusitanisum*, a species only genetically circumscribes as sp. 16 by Jeppson et al. (2017) and *T. fulvellum*.

Clade 3 shows a Palearctic origin with a species only genetically circumscribes as sp. 15 by Jeppson et al. (2017) and a jump dispersal into the Nearctic with *T. submembranaceum*.

Clade 4 consists of the apparent cryptic species within and close relatives of *T. fimbriatum*. This clade shows a higher likelihood of a Nearctic origin with multiple dispersals into the Palearctic, and one Australasian dispersal. Within Clade 4, *T. winterhoffii* has a Nearctic and Palearctic distribution, *T. fimbriatum var. egranulosum* has an Australasian distribution, and *T. calongei* has a Palearctic distribution. The sister to this group in Clade 4 appears to have a Nearctic origin with two dispersals into the Palearctic. This group includes the Nearctic species *T. fimbriatum* var. *tuberculatum*, several genetically distinct species whose morphologies closely resemble *T. fimbriatum*, *T. punctilabratum*, and *T. campestre*. *T. fimbriatum*, *T. fimbriatum* var. *campestre*, and an unnamed species identified as sp. 3 from Jeppson et al. (2017) show Palearctic ranges.

Clade 5 consists of an unnamed species identified as sp. 5 in Jeppson et al. (2017) with a Palearctic distribution and *T. pygmaeum* with a Nearctic, Afrotropical, Neotropical, and Australasian distribution.

Clade 6 has equal probability of a Nearctic or Palearctic origin. *T. cyclophorum* is sister to this clade and has a Nearctic, Palearctic, Afrotropical and Australasian distribution. An unnamed species 1 published in Jeppson et al. (2017) has a Palearctic distribution and is sister to *T. polymorphum* with a Nearctic distribution. The other lineage in this clade consists of *T. obesum* with a Nearctic and Palearctic distribution, *T. subfuscum* with a Nearctic, Palearctic, Afrotropical and Australasian distribution, and *T. punctatum* which is restricted to the Nearctic.

T. xerophilum, which is restricted to the Nearctic, did not resolve to show close relationships with any other taxa in this analysis.

In Clade 7, there are 3 major lineages. One lineage consists of genetically distinct but morphologically similar species which resemble *T. ahmadii*. Work is ongoing to determine if these represent distinct species or are conspecific. Another lineage contains one unnamed species in Clade 7 is known only from the Palearctic, another undescribed species from Neotropics, *T. melanocyclum* and *T. calcareum* from the Palearctic, *T.* purpusii from the Nearctic, while *T. subsquamosum* is widely distributed and found in all regions except Australasia. The last lineage consists of 2 species with limited distribution and one widely distributed. *T. rufum* is known only from the Nearctic with *T. pulchellum* is found globally except in Indomalaya. Sister to these species, *T. domingueziae* is restricted to the Neotropics.

Clade 8 consists of mostly largely distributed species, *T. pulchellum*, *T. poculatum*, and *T. striatum*, which are all found globally except in Indomalaya. An unidentified species found only in Australasia is sister to this clade.

Clade 9 is comprised of two species, *T. obscurum* from the Nearctic and *T. lloydii* from the Nearctic and Palearctic.

Clade 10 consists largely of species from the Palearctic. *T. niveum*, *T. eckbladii*, *T. giovanellae*, and undescribed species labelled species 10 from Jeppson et al. 2017. Within this group, *T. caespitosum* is found in the Palearctic as well as the Nearctic and Neotropics.

Clade 11 has three lineages with somewhat distinct distribution patterns. One lineage is comprised of *T. adhaerens* which has an Afrotropical, Indomalayan, and Australasian distribution. *T. adhaerens* is sister to an unnamed species, Species 17 from Jeppson et al. (2017). Another lineage contains *T. brumale* and *T. simulans* with global distributions except Afrotropical and Indomalaya. The other species in this clade, *T. excentricum* and *T. albicans* are found in the Neoarctic and Palearctic. Species in Clade 11C are distributed solely in the Palearctic. Two of these species are undescribed species from Jeppson et al. (2017) and the third is *T. beccarianum*.

Clade 12 is comprised of 16 lineages representing 14 species. Thirteen of these species are known only from the Palearctic and one, an undescribed species, is known from the Nearctic.

DISCUSSION

This is the first phylogeographic analysis of a gasteroid member of Agaricaceae s.l. of which I am aware. The results support the monophyly of *Tulostoma* and indicate that the genus has a Palearctic origin. The biogeographic analysis indicates multiple dispersal events throughout evolutionary history. It is possible the Palearctic origin is skewed by the sampling effort as the majority of available sequences are from Palearctic collections. However, given the biodiversity within steppe habitats, there are additional lines of support for a Palearctic origin

My analysis represents approximately 47% of the current estimated diversity of *Tulostoma* (Wright 1987, Jeppson et al. 2017, Kalichman et al. 2020). This lack of taxonomic sampling limits the understanding of the true biogeographic patterns in this genus. Nonetheless, some inferences about the distribution of this genus can be made. Some clades show strong biogeographic signals while the geographic origin in other clades are less discernable. Clades 1, 2, 3, 10, and 12 all have strong support for a Palearctic origin. Within these clades are several long-distance dispersal events into the Nearctic and two into the Neotropical are shown. The biogeographic patterns are less resolved in other clades and will be discussed below.

<u>Biogeography</u>

According to the most recent phylogenetic analysis, *Tulostoma* is contained within Agaricaceae s.l. (Kalichman et al. 2020). The most current molecular clock analysis suggests that Agaricaceae s.l. evolved more recently than 50 mya (He et al. 2019), and most gasteroid lineages are considered young, having evolved between 4-50 mya (Sánchez-García et al. 2020). Jeppson et al. (2017) noted that European steppe habitats were hot spots for *Tulostoma* species diversity. Steppe habitats are a particular type of grassland. Grasslands are likely the world's largest biomes, covering around 3.5 billion hectares globally, representing 26-40% of Earth's total terrestrial area (Henwood 2010). In the Paleartic, grasslands are referred to as steppes, in the Nearctic, grasslands are termed prairies, in the Neotropics, these biomes are pampas, and in the Afrotropics, velds. The Palaearctic steppe is the largest grassland worldwide, is likely the largest continuous terrestrial natural habitat, and comprises 10.5 million km^2, or 22% of the total area of the palearctic, (Török and Dengler 2018, Hurka et al. 2019, Török et al. 2020). Prior to human encroachment, Nearctic grasslands comprised 8 million km^2 (Strömberg 2011). Grasslands are the characteristic climax formation of semiarid temperate regions and have high biodiversity at multiple scales (Hurka et al. 2019, Török et al. 2020). The climates of these temperate grasslands are characterized by cold winters, hot summers with a drought period, and summer rain (Strömberg 2011, Török and Dengler 2018, Hurka et al. 2019 Török et al. 2020). Grassland soils exhibit nutrient diversity on microhabitats but are known to store large amounts of carbon (Lal 2004; Li et al. 2009).

Divergence of Agaricaceae s.l. began around 48 mya, in the Eocene epoch (56-34 mya). The early Eocene epoch was characterized by higher CO_2 levels (~1000-2000 ppm) and higher temperatures (+ 9-14 °C) compared to current global conditions (Pearson and Palmer 2000, Lowenstein and Demicco 2006). Carbon dioxide concentrations decreased in the later Eocene, and corresponded with lower temperatures and increased aridity. Eocene temperatures peaked approximately 49 mya, followed by a cooling period until around 41-42 mya (Bohaty and Zachos 2003). This warming event, the Middle Eocene Climatic Optimum, ended approximately 40 mya, and the climate began cooling again (Pagani et al. 2005).

In the early Eocene the high temperatures spurred forest expansion, and forest covered most terrestrial environments (Lopston et al. 2014, Willard et al. 2019). There was a nearly worldwide distribution of grasses in the early Eocene, however they were not dominant (Strömberg 2011).

When the cooling period of the middle Eocene progressed, continental interiors began to dry, seasonality increased, and grasses began to diversify (Tiffney 1985, Eberle and Greenwood 2012, Sun et al. 2014). Grass dominated ecosystems occupied continental North America and Europe by the late Oligocene (Jacobs et al. 1999, Strömberg 2011, Bond and Midgley 2012). At the end of the Oligocene, global temperatures began to rise and open grass-dominated habitats developed, becoming dominant biomes during the Miocene and Pliocene (Jacobs et al. 1999, Bredekamp et al. 2002, Strömberg 2011). The warm climate continued to the middle Miocene climate optimum followed by a cooling trend and increased seasonality culminating in the Pleistocene (Jacobs et al. 1999; Retallack 2001; Strömberg, 2011). As aridity in intercontinental areas increased, grasslands with scattered trees gave to diversified grasslands, including steppes, short-, mixed-, and tallgrass prairies (Jacobs et al. 1999, Bredekamp et al. 2002, Strömberg 2011).

The timing and development of Palearctic steppe habitats is consistent with the center of *Tulostoma* biodiversity. *Tulostoma* biodiversity has been shown to be greatest in steppe habits, and that the largest steppe habitats occur within the Palearctic (Jeppson et al. 2017, Török et al. 2020). *Tulostoma* diverged sometime more recently than 50 mya, which coincides with early evolution of grassland habitats. To summarize the historical phylogeography of the grassland biome beginning in the Eocene (~50 mya) continuing to current day, temperature increases and precipitation decreases resulted in reduced tree cover and increased herbaceous flora. Grasses then became dominant, and biodiversity in these areas was driven by adaptive radiation of species from within grasslands as well as immigration of desert species from adjacent areas. Biodiversity was further driven by expansions and contractions created by regional climatic changes from the Miocene onward (Gleason 1922, Axelrod 1985). Investigations into the timeline of diversification in *Tulostoma* can serve to support the Palearctic grassland origin of this genus.

Long Distance Dispersal

This phylogeography suggests long distance dispersal occurred several times throughout the phylogenetic history of *Tulostoma*. Basidiospores are thought to be the primary mechanism of dispersal for puffballs. Research on fungal dispersal distances via spores indicates that long distance dispersal is possible, but perhaps uncommon. Modeling and theory suggest strong potential for long distance spore dispersal (Golan and Pringle 2017). Spore morphological characters, such as thick walls and melanin provide protection for putative long-distance dispersal challenges, and spore shape and ornamentation contribute to wind dispersal dynamics. Experimental and observational data suggest both short and long-distance dispersal potential. Peay et al. (2010) found that despite the development of numerous sporocarps producing millions of spores, dispersal is greatly limited in ectomycorrhizal fungi. Li (2005) reported only 5% of *Amanita muscaria* var. *alba* spores dispersed beyond 5.2 m from their sporocarps. Several reports suggest that rust fungi are capable of long-distance dispersal. Rusts of white pine, coffee leaf, peanut, and soybean have all been reported to be transported long, sometimes oceanic, distances (Frank et al. 2008). Spores have been found in air samples throughout the world. Biogenic aerosols collections revealed basidiospores from all sampled areas, including continental, coastal, marine air samples (Frohlich-Nowoisk et al. 2012). Further, the timing of spore dispersal contributes to the distance of spore travel. The time of day and season of spore release is important in dispersing spores and is dependent on location because air turbulence differs between geographic areas (Oneto et al. 2020). The timing of *Tulostoma* spore release has not been established, however *Tulostoma* have small, pigmented basidiospores.

Phylogeographic studies have repeatedly demonstrated multiple examples of long-distance dispersal in fungal lineages. Phylogenetic studies show long-distance dispersal events in ectomycorrhizal, mutualistic, pathogenic, and wood-rotting and non-wood-rotting saprotrophic fungi (Reynolds 2011, Coetzee et al. 2011, Skrede 2011, Looney et al. 2017). The species which have demonstrated long-distance dispersal are not only ecologically diverse, they are morphologically diverse. Species which demonstrate long distance dispersal include lamellate, coprinoid, effuse, poroid, and hypogeous gasteroid forms (Ko et al. 2001, Hosaka et al. 2008, Moncalvo and Buchanan 2008, Skrede et al. 2011, Cai et al. 2014). This demonstrates that spores from many fungal fruiting body forms are capable of long-distance dispersal, however there are likely different mechanisms of dispersal associated with these morphologies. Because the breakup of Gondwana predates the divergence of *Tulostoma* within the Agaricaceae, the majority of dispersals in the phylogeographic history of this genus are likely long-distance.

In addition to the body forms described above, the stalked puffball *Battarrea phalloides* has reported from Australia, New Zealand, Europe, Africa, the Middle East, North and South

America, and India (Shepherd and Cooper 2018, Kantharaja and Krishnappa 2020). Molecular evidence indicates that there were likely some long-distance dispersal events during the evolution of *B. phalloides*, however greater sampling is necessary to better support this hypothesis. As a stalked puffball, *Tulostoma* is similar to *Battarrea,* which also occurs most commonly in sandy soils in mostly arid areas. However, the endoperidium erodes in *Battarrea* at maturity, leaving the gleba exposed. In *Tulostoma,* spores are released through an opening of the endoperidium, or mouth. Mouth morphology in *Tulostoma* varies from circular or tubular, to fimbriate, to lacerate. Mouth morphology combined with spore shape and ornamentation likely contributes to dispersal potential. The apparent long-distance dispersal of species in *Tulostoma* suggest and efficient dispersal mechanism from the gasterothecium.

To my knowledge, *Tulostoma* has not been detected within animals or from plant tissue. It is possible that ornamented spores of *Tulostoma* can attach to and then be dispersed by animals, but this has not been shown as a dispersal vector. Additionally, while *Tulostoma* sequences have been recovered from soil samples, no *Tulostoma* sequences have been identified from fungal targeted sequencing from plant tissues. While it is possible that *Tulostoma* is using other vectors, this has yet to be detected, and basidiospores should be considered the predominant mechanism of dispersal for species in the genus.

Holarctic Distributions

The area encompassing the Nearctic (predominately North America) and the Palearcitc (including Eurasia and North Africa) is considered the Holarctic. Many *Tulostoma* species appear to have a Holarctic distribution. This could attributable to sampling effort, as most taxa included in this study are from Europe and North America, and taxa from the Southern Hemisphere are minimally represented. However, the Palearctic origination of this taxa is plausible, and multiple dispersal events into North America are demonstrated. Holarctic distributions have been demonstrated in several other fungal taxa, and some dispersal of fungi between the Nearctic and Palearctic is also documented. Multiple species in *Pluteus* section *Pluteus* are found in both the Nearctic and Palearctic (Justo et al. 2014). Oda et al. (2004) proposed that *Amanita muscaria* and *Amanita pantherina* migrated across the North American-Eurasian land bridge during the Pleistocene. The wood decomposer *Artomyces pyxidatus* is distributed throughout Eurasian and North American, and forms 3 phylogenetic clades corresponding to Eurasia, northeastern North America, and southeastern North America (Lickey et al. 2002). It is thought that in the absence of land bridges, biotic exchange betweenacross the North Atlantic Ocean could have been aided by prevailing westerly winds below 60N (Tiffney and Manchester 2001).

Additionally, dispersal of plants between North America and Europe during the Tertiary period is well documented. There are a large number of shared plant genera between Europe and North America originating during the Oligocene, which may indicate that migration between these continents was possible at some time during or prior to the Oligocene (~33.5 mya) (Axelrod, 1975, Habley et al. 2000). Cornelain cherries (*Cornus* spp.) evolved in Europe likely dispersed into North America in the late Paleocene and early Eocene. *Juniperus* probably originated in Eurasia, and likely dispersed into North American once in the Oligocene, once in the Miocene and once more recently (Mao et al. 2010). Again, because the taxa of Europe are perhaps the most completely known compared to the rest of the world, our concept of dispersal may be biased due to species circumscriptions and genetic sampling.

Substrate Specificity

The variation in species specificity may explain the phylogeographic patterns seen in several clades throughout the tree. There has been speculation that fungi in high diversity systems likely exhibit generalist behaviors due to the plentitude of resources, and host specificity increases as biodiversity decreases (Beaver 1979, May 1991). Because grasslands are biodiverse ecosystems, species that evolved in these areas are likely generalists, and once dispersed, derived species became adapted to specialized niches. Because *Tulostoma* is terrestrial and not found attached to organic material, it more probably that specificity, or recurrence, of these fungi is correlated with soil substrate characteristics rather than a plant host. *T. calcerum* is found on most commonly on calcareous soils. It has only been reported from the Palearctic. Calcareous soils are high in calcium carbonate, and generally low in organic matter, micronutrients, nitrogen, and phosphorous (Strom et al. 2004). This is also seen with *T. calongei*, which is restricted to sandy acidic soils and has only been found in central Spain. Species such as *T. subsquamosum*, *T. pulchellum, T. striatum*, *T. simulans*, and *T. brumale* have broad distributions. The first three of these species are reported from sandy or sandy-clay soils, which have a variety of nutrient profiles. These species are also found in a variety of vegetative types, indicating that soil chemistry in these areas is also varied. *T. brumale* and *T. simulans* are reported from an even greater diversity of soil types and habitats. This serves as some indication that distribution may be tied to substrate specificity. Substrate preferences was also shown to contribute to the phylogeographic history of *Pluteus* species (Justo et al. 2014). This is consistent with grassland soils. Soil conditions in steppe habitats depend heavily on the microscale abiotic and biotic environment (Wesche et al. 2016). The addition of *Tulostoma* taxa and their distributions to the phylogenetic analysis may change this hypothesis.

The broad distribution of some taxa compared to the narrow distribution of other *Tulostoma* species may be explained by substrate specificity. It bears to reason that species with broad distributions, such as *T. subsquamosum*, *T. cyclophorum*, and *T. pulchellum*, are generalists. They likely are able to decompose various organic material and are adapted to various soil characteristics. Conversely species with narrow distributions, such as *T. polymorphum*, *T. pannonocium*, and *T. domingueziae*, are assumed to be specialists, utilizing a particular organic substrate and are adapted to certain soil parameters.

It is rather well documented that *Aspergillus fumigatus* has a global distribution. It has been proposed that this global distribution can be attributed to several factors, including air dispersal, small spores (2-3 µm), and being a generalist soil decomposer. Widespread *Tulostoma* species have similar ecological characteristics; their spores are putatively air dispersed, small (~4-6 µm), and appear to be soil saprotrophs. This adds to traits of fungal species that are widely distributed.

CONCLUSIONS

This study indicates a Palearctic origin for *Tulostoma.* While this inference may be biased due to a preponderance of Palearctic *Tulostoma* sequences, diversity can be somewhat correlated with habitat expansion during the time of divergence in this genus.

This study highlights the importance of metadata when collecting fungi. Detailed soil chemistry may serve to better understand the biology of saprotrophic fungi not associated with organic material. My study points to substrate specificity driving patterns of dispersal and phylogeography. More precise information on collection habitat will strengthen this hypothesis.

Chapter 4. Evolution of Spore Morphological Variation in *Tulostoma*

INTRODUCTION

Agaricomycetes produce basidiospores as the result of sexual reproduction, or meiosis. These meiotically produced basidiospores increase genetic diversity in fungal species, and fungi rely on the dispersal of fungal spores for multiple reasons. Dispersal enables species to find additional substrates, escape predation or toxicity, and to colonize new areas. Basidiospores are thought to enable farther distance dispersal than vegetative hyphae (Nara 2009, Halbwachs et al. 2015). Vašutová et al. (2019) suggest that spores are the most important propagules of fungi. Despite the importance of basidiospores in fungal dispersal, our understanding of the evolution of spore morphological traits is in its infancy (Pringle et al. 2015, Halbwachs et al. 2017).

All aspects of basidiospore morphology, including ornamentation, shape, size, spore wall, and color, exhibit considerable variation. These traits, and trait combinations, have some functional or ecological significance (Kauserud et al. 2011, Hussein et al. 2013, Aguilar-Trigueros et al. 2015, Halbwachs and Bässler 2015, Calhim et al. 2018, Purhonen et al. 2020). Researchers suggest that spore characteristics influence many aspects of dispersal including aerodynamics, distance, longevity, resistance to desiccation, as well as substrate deposition and penetration, moisture availability upon germination, and resistance to abiotic and biotic degradation (Norros et al. 2014, Golan and Pringle 2017, Halbwachs et al. 2017). Spore characteristics may also be tied to particular fungal trophic states, substrates, habitats, or species prevalence (Halbwachs et al. 2015, Gange et al. 2017, Calhim et al. 2018, Crandall et al. 2020). The potential implications of spore ornamentation, shape, and size are discussed below.

Spores ornamentation can range from smooth to highly ornamented, with ornamentation such as bumps, nodules, ridges, or spines. In some cases, spore ornamentation facilitates dispersal by adhering to animals, particularly terrestrial invertebrates, which not only transport fungal spores, but can deposit them on suitable substrates (Lilleskov and Bruns 2005). Ornamentation may also provide resistance to predation, or resistance to digestion when passing through the digestive system of animals (Lilleskov and Bruns 2005, Vašutová et al. 2019). Ornamented spores are more common in ectomycorrhizal basidiomycetes than saprotrophic species, and within saprotrophs, ornamented spores were shown more often on litter specialists (Halbwachs et al. 2015, Calhim et al. 2018). Smooth spores are more common in arid areas, while ornamented spores are found in areas with greater moisture availability (Kreisel and Al-Fatimi 2008, Jeppson et al. 2017). Ornamentation is thought to drive water condensation and likely also influences spore aerodynamics and dispersal ability (Halbwachs and Bässler 2015, Golan and Pringle 2017). Ornamented spores generally repel water which may lead to extended dormancy, because it takes longer to absorb water needed for germination (Wösten and Wessels 1997, Halbwachs and Bässler 2015). The effect of ornamentation on aerodynamics is likely dependent on the size and shape of the ornamentation. Some small ornamentation or irregular spore shape may contribute to wind dispersal, particularly in low air movement, because of an increased drag, however warts, spines, or ridges do not strongly contribute to drag (Roper et al. 2008, Gube and Dörfelt 2011, Halbwachs and Bässler 2015).

Spore shapes can include globose, elongate, allantoid (curved), or even tetrahedral. Spore sizes commonly range from about 5-10 µm, yet larger and smaller spores exist. Size has been shown to influence dispersal potential; smaller spores spread farther than larger ones. However, longer distance dispersed spores may experience greater desiccation depending on their location (Kauserud et al. 2010, Norros et al. 2014). Larger and elongated spores were more common in chaparral (more arid and open) habitats compared to the smaller and more globose spores which are more commonly found in forests (wetter and more closed) (Crandall et al. 2020). Larger spores have also been found in arid areas compared to moister environments in gasteroid species (Kreisel and Al-Fatimi 2008, Jeppson et al. 2017). In Norway, along coastal areas with greater moisture availability, fungal species had smaller spores compared to larger spored species found in interior regions in the country, where there is less available moisture (Kauserud et al. 2010). Elongated spores were more common in saprotrophic fungi compared to mycorrhizal species, and allantoid spores more common in above ground substrates, while uncurved spores were more common in hypogeous substrates (Calhim et al. 2018). In wood-inhabiting fungi, broadleaved trees had fungal species with larger, elongated spores compared to conifer inhabiting fungal species, which had smaller, more globose spores (Purhonen et al. 2020). The same study showed larger and more spherical spores in larger pieces of dead wood compared to smaller, elongate spores in smaller portions of wood. Additionally, spore wall characteristics such as thickness and melanin concentration can increase spore viability through resistance to desiccation and exposure to UV radiation (Norros et al. 2015). Differences in spore sizes may be attributable in part to water evaporation during air dispersal. Presumably larger spores may be able to counteract factors of desiccation they experience as they disperse through dryer environments. Smaller spores may be able to carry sufficient moisture during dispersal in higher humidity areas and do not experience the impacts of desiccation as species found in more arid conditions.

One study suggests that fungal species prevalence may be influenced by spore morphological characteristics. Gange et al. (2017) found that fungal species with smooth spores became more common in records from 1970-2014 in Britain compared to species with ornamented spores. This could be due to reductions in ectomycorrhizal species (more likely to have ornamented spores) due to habitat loss, however there was very limited change in the forest types during the 45-year study period. It is also possible that mycorrhizal species decreased due to anthropogenic fertilization, a possibility that was not addressed in their study. Gange et al. (2017) suggest that the decrease in frequency of species with ornamented spores indicates that selective pressures from factors of climate change may also act differentially upon species with smooth spores compared to those with ornamented spores.

Given the tremendous variation in spore morphology, ecological and functional significance, and because they are essential for dispersal, basidiospore morphological characters may be driven by selective evolutionary processes (Halbwachs et al. 2014, Halbwachs and Bässler 2015, Pringle et al. 2015, Calhim et al. 2018, Purhonen et al. 2020). Despite the likelihood that morphological characteristics of spores, particularly spore ornamentation, are under selective pressures, only a few studies have investigated the evolution of spore morphology. Golan and Pringle (2017) point out that scant research investigating the evolutionary history of spore morphologies exists. Co-David et al. (2009) used phylogenetic methods to investigate the ancestral character state of spore morphology in Entolomotaceae. Their research suggests that the ancestral spore state in Entolomataceae was either rhodocyboid (ornamented with bumps, undulating ridges, or weakly angular) or entalolomatoid (ornamentation of interconnecting ridges which result in an angular spore appearance) spores. Their phylogeny was unable to resolve which of these ornamented spores was the true ancestral characteristic.

Gasteroid fungi, some of which are referred to as puffballs, are those which produce their reproductive structures, spores, within a sac like structure (gasterothecium). Within Agaricomycetes (Basdiomycetes), the transition to a gasteroid form has occurred multiple times over evolutionary history (Gube and Dörfelt 2011). Within Agaricales, most non-gasteroid species exhibit forcible spore discharge, where hydrostatic pressure ejects the basidiospore from the sterigmata of a basidium (Buller 1922). This forcible discharge of basidiospores from basidia is the ancestral character state of Agaricales (Agaricomycetes) and gasteroid Agaricales have lost forcible spore discharge basidia at some point during the evolutionary development of the gasteroid form (Miller and Miller 1988, Wilson et al. 2011). Because spores develop inside an enclosed structure, forcible discharge of spores would not provide any advantage as spores would simply be expelled inside their gastrothecium. As a result of the loss of forcible discharge, spores of gasteroid fungi generally rely on mechanical dispersion factors, such as the force exerted from a rain drop, to release spores from their gasterothecium. Once released, spores have dispersal potential, and as discussed, basidiospore morphological characters likely influence spore dispersal (Galante et al. 2011, Norros et al. 2012, Norros et al. 2014).

The stalked puffball genus *Tulostoma* (Pers:Pers) offers a unique opportunity to study the evolution of spore morphology, particularly spore ornamentation in Agaricomycetes. *Tulostoma* is a cosmopolitan and speciose genus, with an estimated 172 accepted species

(Kalichman et al. 2020, Wright 1987). As a gasteroid fungus, *Tulostoma* has lost forcible spore discharge seen in other Agaricomycetes, and thus relies on derived methods of spore dispersal compared to non-gasteroid Agaricomycetes. Spores in this genus are mostly globose to sub-globose, are most commonly 4-6 µm in diameter and range from smooth to highly ornamented. Spore ornamentation in *Tulostoma* species includes low bumps, ridges, spirals, and dense and prominent spines (Figure 4.1). The global distribution and high number of species indicate successful dispersal strategies in this genus. The diversity of spore ornamentation enables reconstruction of the evolutionary history of spore morphology. The ancestral state reconstruction of the evolutionary history can provide a framework from which to understand how spore morphology evolved in this group. This framework can provide a foundation for future study on the selective pressures on spore morphology.

The objectives of this study were to determine: (1) the ancestral character state of *Tulostoma*, and (2) whether moisture may predict spore ornamentation or size of *Tulostoma* spores. I hypothesized that the ancestral character state of *Tulostoma* spores is moderately to strongly ornamented. I hypothesized that spore ornamentation losses and size increases would occur in species in more arid environments, while spore ornamentation increases and size decreases would be detected for species in higher moisture environments. I tested these hypotheses by constructing a 3 gene phylogeny and using the phytools R package for ancestral state reconstruction. Spore ornamentation and size was compared to annual precipitation in species habitats and a linear regression was used to evaluate correlation between ornamentation or size and available moisture.

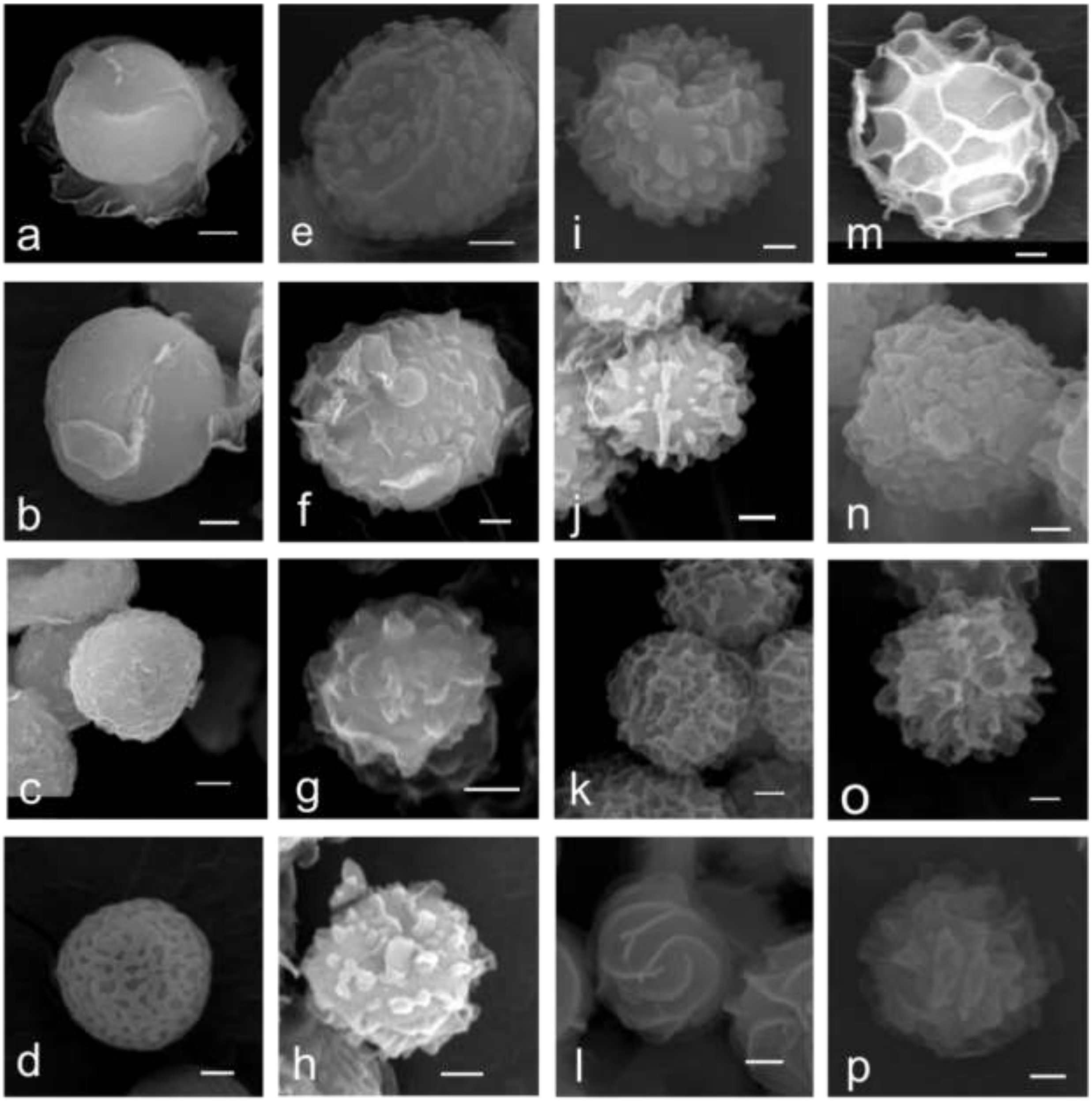


Figure 4.1. Spore morphology diversity of selected *Tulostoma* species. a. *T. meristostoma* (MICH266306), b. *T. grandisporum* (MICH 338158), c. *T. macrocephalum* (MICH28572), d. *T. obscurum* (DBG28400), e. *T. pulchellum* (MICH266205), f. *T.* sp. novo (DBG23778), g. *T. simulans* (DBG24113), h. *T. involucratum* (ILL11718), i. *T. punctilibratum* (MICH24086), j. *T. fimbriatum* (DBG24032), k. *T. purpusii* (MICH266163), l. *T. striatum* (AZ43457), m. *T. opacum* (MICH400) n. *T. punctatum* (DBG27849), o. *T. calcareum* (DBG21975), p. *T. squamosum* (MICH28574). Scale bar = 1 μm.

METHODS

Molecular Phylogeny of *Tulostoma*

Spore Morphology Analyses

Spore morphological categories were assigned based on scanning electron microscopy (SEM). For collections that were imaged, spores were mounted on double sided carbon tape mounted on SEM stubs. Stubs were sputter coated with gold palladium using (what machine? machine at pressure for 4 minutes. Sputter coated samples were imaged using JEOL 6450LV most commonly at 8 mm working distance at high vacuum with 50 spot size at 10K magnification with 25kV accelerating voltage. Published resources were used to categorize spore morphology of unavailable *Tulostoma* species.

DNA extraction, PCR, and Sequencing

To prepare fungal tissues for DNA extractions, approximately 0.5 g dried material was extracted from collections. All utensils used in the removal and weighing of fungal material were sterilized as appropriate. Metal equipment was dipped in 95% ethanol and flamed, while flammable materials were wiped with 95% ethanol and allowed to air dry. Fungal material was transferred to a 1.5 ml Eppendorf tube and ground using a sterilized micropestle and sterilized sand (Fisher Chemical S25-500). DNA was extracted from the prepared fungal tissues using the E.Z.N.A. Fungal DNA extraction kit (Omega BioTek D3390-01) using the manufacturer's instructions.

Target DNA sequences from ITS, LSU, and TEF-1α were amplified using polymerase chain reactions (PCR). The ITS region was amplified from all collections using the fungal specific primers ITS1F and ITS4B (Gardes et al. 1993). LSU regions was amplified with LR21 and LR7 primers (Hopple and Vilgalys 1999) TEF-1α was amplified EF983F and EF2218R (Rehner and Buckley 2005).

Twenty-five µl PCR reactions were performed with 5 µl template DNA (approximately 10 ng DNA/µl), 2.5 µl primer of each primer, 12.5 µl GoTaq, and 2.5 µl sterile H_2O. PCR amplification began with an initial denaturing at 95°C for 2 minutes. Thirty PCR cycles ran with denaturing at 94°C for 1 minute, annealing at 50°C for 30 seconds and elongation at 72°C for 1 minute. A final elongation ran at 72°C for 5 minutes. PCR products were stored at approximately 4°C.

PCR product were visualized on 3% agarose gels. Successful PCR products were determined as clean bands approximately 400-600 bp for ITS, approximately 700-900 bp for LSU and approximately 1000 bp for TEF-1α. These PCR products were cleaned using a QIAquick PCR purification kit following manufacturer's protocols. Clean PCR product of target regions were sent to the University of Arizona Sequencing Center for Sanger Sequencing.

Evolutionary Reconstruction

Individual DNA sequence base pairs were visualized and edited using Sequencher. Sequences were assembled in Excel and exported to BBEdit. Sequences from this project were combined with available sequence data (GenBank and personally loaned data from Dr. Matthias Gube (Georg-August-Universität, Göttingen, Germany). Sequences were aligned in Mesquite. Aligned sequences were trimmed to the first and last informative base. Aligned sequences of each gene were concatenated using SequenceMatrix 1.8. The concatenated matrix was analyzed in CIPRES Web Portal (Miller et al. 2010). Maximum Likelihood Analyses was conducted using RAxML, the best tree from 1000 bootstrap replicates was chosen and edited in

FigTree (Rambaut 2009). Includied sequences and GenBank Accession numbers are shown in Appendix 4.1.

Evolution of Spore Morphology

Spores were imaged using scanning electron microscopy. Methodology for imaging is described in Chapter 2 Methods, Morphological Analysis. Spore character states were based on their ornamentation; 10 categories were assigned ranging from completely smooth to dense ornamentation. Representative spore categories are shown in Figure 4.2. Based on Gube (2009), it was hypothesized that *Battarrea* is the ancestral genus of *Tulostoma*. *Battarrea* species have ornamented spores which range from finely verrucose to anastomosing truncate ridges, however *Battarrea* spore morphology was left uncoded to account for the possibility that another group is sister to *Tulostoma*.

In order to study spore morphology evolution, ancestral state reconstruction analysis was investigated following Pagel et al. (2004) and Pagel and Meade (2006). Character reconstruction was carried out in R using the package 'phytools' (Revell 2012). Discrete characters were reconstructed under a continuous-time Markov chain model (Lewis 2001) using the "ace" function from the 'ape' package (Paradis et al. 2004) and the phytools function 'lik.anc'; continuous traits were reconstructed using the phytools functions 'fastAnc' and 'contMap'. For all reconstructions, the BI majority rule consensus tree was used; for visualization purposes, the tree was ultrametricized using the 'compute.brlen' function in 'ape'.

Average annual rainfall was compared to spore surface area and spore size. Average annual rainfall in mm was determined using the AQUASTAT Climate Information Tool developed by the Food and Agriculture Organization of the United Nations (https://aquastat.fao.org/climate-information-tool/) on type specimen locality when available. Spore size was recorded as the median of average spore size from studied species or published literature. Spore surface areas were categorized on a 0-10 scale. Completely smooth spores were rated as 0 and the value increase with increased ornamentation. Strongly ornamented spores were classified as a 10. Scatter plots were constructed to compare spore ornamentation to average rainfall. Linear regression was used to determine correlation significance (p=0.05) in R (R Core Team 2015).

RESULTS

Seventy-four *Tulostoma* species were included in the final phylogenetic analysis with *Battarrea* selected as the outgroup. The aligned data set included 2274 nucleotides; Genbank numbers are shown in Appendix 3.1 The phylogeny with the ancestral state reconstruction is show in Figure 4.2.

The ancestral state of spore morphology is shown to be ornamented (red indicators, Figure 4.2). The ancestral character state of *Tulostoma* is moderately to strongly ornamented, as exemplified by *T. berkelyii*. The phylogenetic analysis shows complete loss of ornamentation occurred at least four times (Figure 4.2, black and grey boxes at branch tips). Increases in ornamentation occurred at least five times in the phylogeny (Figure 4.2, orange and yellow boxes at branch tips).

Clade 1 include 15 taxa. Most species in this clade have moderately dense ornamentation of rounded spines, some that coalesce, and some that form rudimentary ridges occasionally. This spore morphology is exemplified by *T. berkeleyii*. There is strong support for the ancestral character state of this clade having this ornamentation, which is also shown to be the most likely ancestral character state of the genus. Changes in spore ornamentation can be seen three times in this clade. The formation of ridges from the ornamentation appears once on the tree in *T. excentricum*. Ornamentation increased to sharp, dense, prominent spines in *T. adhaerens*. Ridges are formed once in the sister taxa, *T. caespitosum* and *T. giovanellae*. Low ornamentation forming longitudinal striations are characteristic of *T. giovanellae*, while *T. caespitosum* has low ornamentation forming irregular ridge patterns.

Clade 2 includes 12 taxa and displays great variation in spore morphology. There is high support for the overall genus ancestral character state of this clade. Multiple changes in spore morphology occur in this clade. The formation of irregular ridges appears three times. Low ridges and irregularly low bumps are present in *T. pulchellum*. More prominent ridges evolved in *T. subsquamosum* and an as yet unnamed species, MICH266163. Dense prominent spines evolved three times, in *T. rufum*, *T. calcereum*, and sister taxa, *T. ahmadii*, and a morphologically similar, but genetically distinct taxa from Hungary. Very pronounced ornamentation appears to have evolved independently twice, in *T. domingueziae* and *T. melanocyclum.*

Clade 3 includes 14 taxa. The majority of the species are thought to be undescribed species discussed in Jeppson et al. (2107). These unnamed taxa have ornamented spores. However, detailed spore morphological information is unavailable. It does appear that the formation of ridges characteristic of *T. fimbriatum* evolved three times, low irregular ornamentation characteristic of *T. pulchellum* evolved twice, and prominent spines evolved once in *T*. *lyscocephalum*. Additional spore morphological information is needed to understand evolution of spore morphology in this clade.

Clade 4 consists of two taxa, *T. obscurum* and *T. lloydii*. Loss of ornamentation is apparent in this clade, and there is little support for the ancestral character state of this clade.

Clade 5 consists of six species. There is great spore diversity in this clade and the ancestral character state of this clade is not resolved. This clade consists of two species with completely smooth spores, *T. cretaceum* and *T. grandisporum*. *T. pannoncium* has very low ornamentation that is only visible using SEM imaging. On the other spectrum, *T. macrocephalum* has pronounced, dense spines. *T. pseudopulchellum* has low ornamented spores with some ornamentation forming ridges.

Clade 6 consists of five species. Ornamentation was lost once in this clade in two species. The smooth spored species in this clade are *T. fulvellum* and unnamed species from Jeppson et al. (2017). These smooth spored species are sister to a group that displays the characteristic ancestral state (*T. lusitanicum, T. pygmaeum*, and an unnamed species from Jeppson et al (2017)).

Clade 7 consists of nine species and the ancestral character state of this group is irregular ridges characteristic of *T. fimbriatum*. This clade suggests that there was a return to the generic ancestral character state twice in *T. winterhoffii* and *T. berkeleyi*. Prominent spines evolved once in this group in *T. calongei*.

Clade 8 consists of three species with the ancestral character state in this clade being low ornamentation occasionally forming ridges. In this lineage, spiral ridges evolved once in *T. striatum*.

Clade 9 consists of two species whose ornamentation forms low, sparse ridges. Clade 10 consists of two species with ornamented species. *T. cyclophorum* is sister to Clades 1-10. Spores *of T. cyclophorum* have irregular ridges and less ornamentation when compared to *T. fimbriatum*.

Clade 11 consists of three species and is sister to the genus. This clade consists of two species with the generic ancestral character state, *T. subfuscum* and *T. punctatum*. *T. obesum* is sister to these species and has smooth spores.

Figure 4.3 compares spore ornamentation to annual rainfall. In this analysis, correlation of spore ornamentation and annual rainfall was not significant (r^2=0.023, p=0.36, n=40, Figure 4.3). Figure 4.4 compares spore size to annual rainfall. In this analysis, correlation of spore ornamentation and annual rainfall was not significant (r^2=0.036, p=0.45, n=40, Figure 4.4).

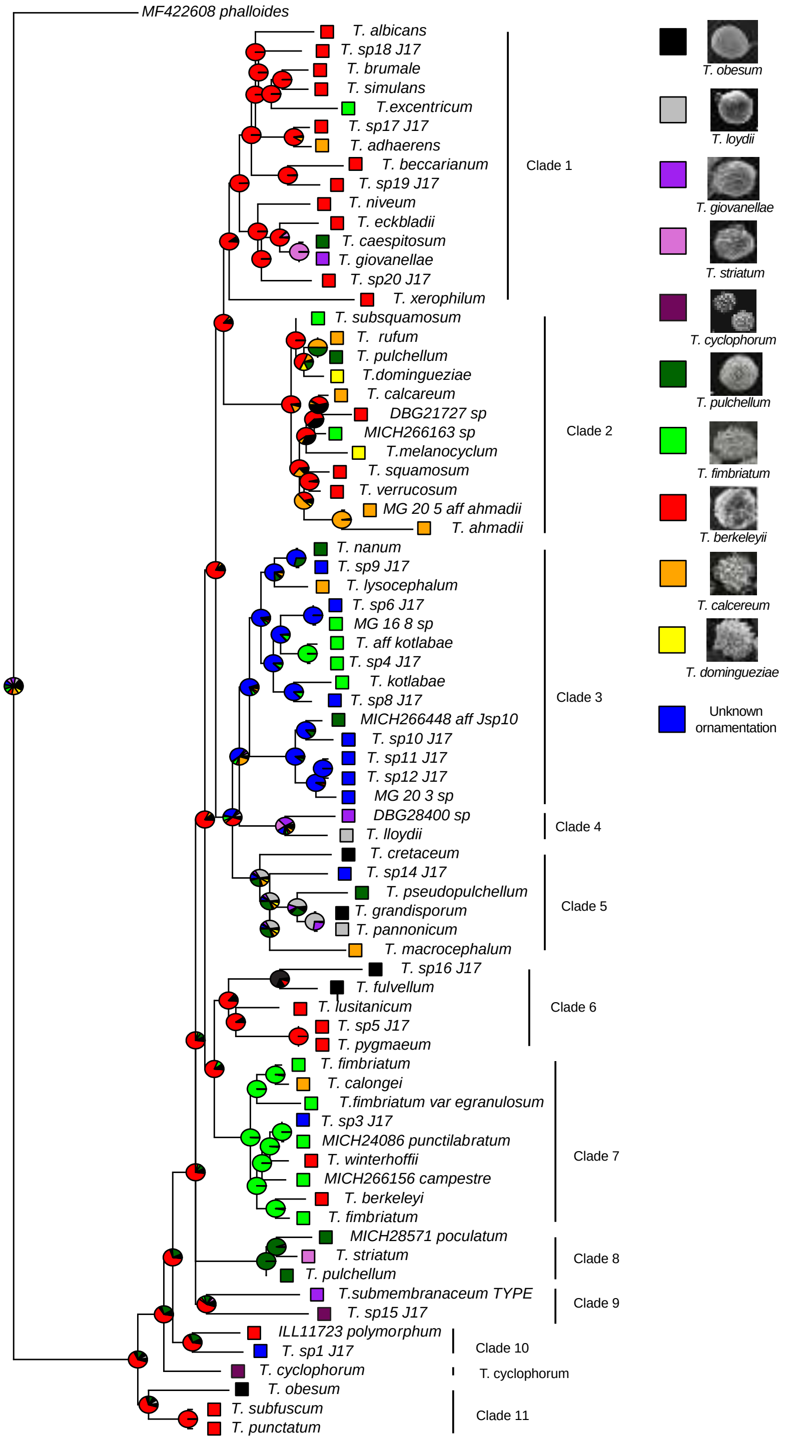


Figure 4.2. Ancestral state reconstruction of *Tulostoma* based on ITS, LSU, and Tef1a sequence data. Ancestral state probabilities are given at each branch in pie charts. Spore ornamentation was lost at least four times (black and grey boxes at branch tips). Spore ornamentation was gained at least five times (orange and yellow boxes at branch tips). Representative spores for each character state are shown in the legend.

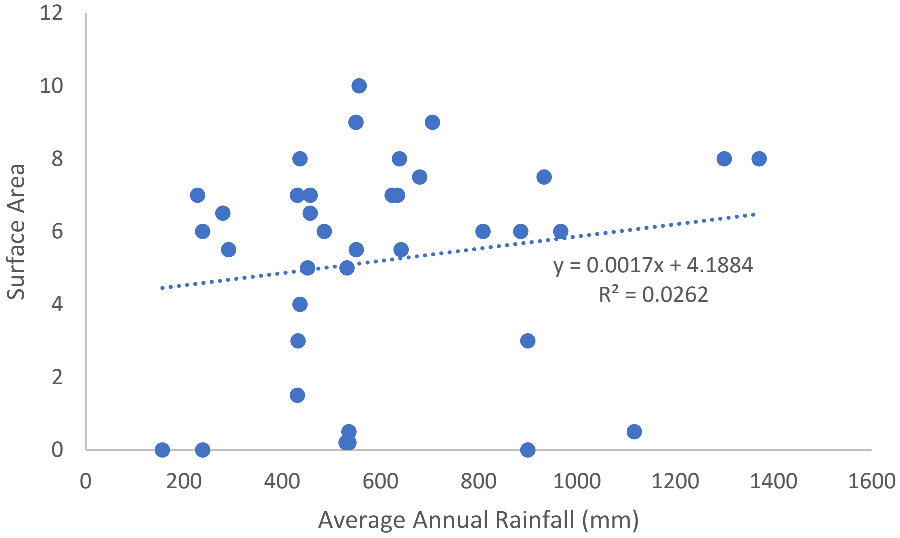


Figure 4.3. Spore surface area ranking compared to the average annual rainfall (mm) of collection locale. The correlation was not significant (r^2=0.026, p=0.36, n=40), but there was a slight positive trend.

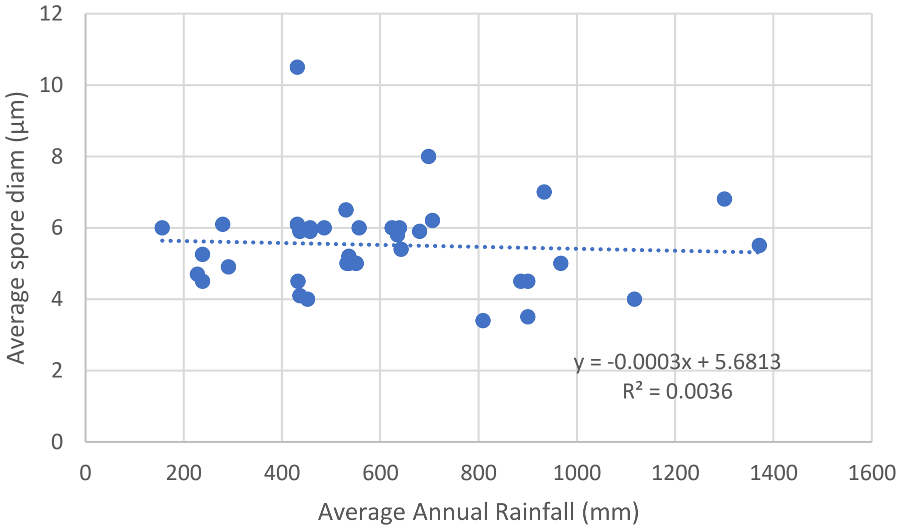


Figure 4.4. Average spore diameter compared to the average annual rainfall (mm) of collection locale. The correlation was not significant (r^2=0.036, p=0.36, n=40), but there was slight negative trend.

DISCUSSION

The character analysis indicates that ancestral state of *Tulostoma* was ornamented spores (72%). The ancestral ornamentation is suggested to be moderate to prominent spines with some fusing of spines. The character analysis and the *Tulsotoma* phylogeny show that spore ornamentation was lost at least 4 times, once in *T. obesum* in Clade 11, once in two species in Clade 6, once in *T. lloydii* in Clade 4, and at least once in Clade 5 (Figure 1). Additionally, spore ornamentation appears to have increased in Clade 2, and possible in *T. macrocephalum* in Clade 5 and *T. calongei* in Clade 7. The multiple losses and gains of spore ornamentation suggests repeated convergence in the evolution of spore morphology in *Tulostoma*. There is strong support for the ancestral character state of ornamented spores. The ancestral ornamentation is suggested to be moderate to prominent spines with some fusing of spines.

Most *Tulostoma* species appear to be saprotrophic and have ornamented spores. This is counter to previous research that shows that the probability of spore ornamentation is statistically significantly higher in ectomycorrhizal fungi compared to saprotrophic fungi (Calhim et al. 2019). While isotopic data indicate that some species of *Tulostoma* are biotrophic, the biotrophic status has not been confirmed. Thus, *Tulostoma* should be considered saprotrophic. Calhim et al. (2019) show the majority of saprotrophic fungi with ornamented spores are litter specialists. *Tulostoma* species generally develop hypogeously, and once mature, their stipe elongates quickly, bringing the gastrothecium above ground. As such, they are not reported as litter specialists, and their substrate is hypogeous.

While there is a very slight positive trend showing that spore ornamentation decreases with average annual precipitation, and a very slight negative trend showing spore size increases as average annual precipitation decreases, the r^2 values are not significant. Occasionally, species with smaller more ornamented spores are found in areas of higher annual precipitation, and larger, smoother spored species are found in drier climates. Annual precipitation in the type locations of *Tulostoma* species ranges from approximately 156 mm to about 1300 mm and the average spore size of *Tulostoma* species range is approximately 4-6 µm diam (Jeppson et al. 2017). *T. beccarianum* averages about 4.5 µm, has strong ornamentation, and is found in places with higher annual precipitation. *T. obscurm* and *T. obesum* have relatively large spores (averaging ~10 µm and ~6 µm, respectively) compared to the average spore diameter in *Tulostoma* species, which have very inconspicuous or no ornamentation, and isare found in areas with very low annual precipitation.

Kauserud et al. (2010) discuss the selective pressures on spores resulting from the interplay between seasonal temperature and moisture and how these seasonal climate factors might also contribute to spore sizes. In most habitats along mid-northern latitudes (~55-65°N), spring (April, May) has markedly higher temperatures and lower precipitation relative to annual averages. In these mid-northern latitudes, the majority of described species complete the meiotic portion of their lifecycles during non-winter seasons, and many species are delineated as early or late seasonal. Early seasonal species are considered those which complete their meiotic lifecycle stages in spring months (April-May), while late seasonal species complete those lifecycle stage predominately during the fall (Andrew et al. 2018). Kauserud et al. (2010) hypothesized that the spores of early seasonal fungal species were large to combat the desiccation experienced during the spring compared to the fall in these mid-northern latitudes. Despite moisture being a likely a selective pressure on spore size and morphology (Kauserud et al. 2010, Halbwachs et al. 2017), these data do not support this hypothesis. It is likely that climatic date used to evaluate this correlation should be measured on a finer scale. Microhabitat characteristics such as relative humidity, potential evaporation, and timing, or seasonality, of basidiocarp production are probably important factors necessary to better understand the dynamics which impact spore morphological evolution.

Few studies investigating the evolution of spore morphology in Agaricomycetes exist. A previous study examined the evolution of spore morphology in *Entolomataceae*. Co-David et al. (2009) showed that the ancestral character state In *Entolomataceae* is either entolomatoid (spores having ridged facets) or rhodocyboid (spores having nodules or bumps). They show that species with clitopiloid spores (having longitudinal ridges) are derived and shared a common ancestor with species that exhibit rhodocyboid spores. They believed that angular spores in *Entolomataceae* likely arose from smooth Tricholomatoid spores, but this has not been confirmed. In fact, more recent molecular studies show that Tricholomataceae consists of 7 genera with a diversity of spore morphologies, ranging from smooth to ornamented (Sánchez-García et al. 2014). Co-David et al. (2009) suggest that evolution of ornamentation evolved independently several times between *Tricholomataceae* and *Entolmotaceae*, using phylogenetic and spore ultrastructure evidence. The phylogenetics of *Tulostoma* show multiple evolutionary changes in spore ornamentation, but in gain or changes in spore ornamentation, and loss of ornamentation. I cannot, however, establish if these differences are due to homology or homoplasy *Tulosotoma.*

Independent evolution, such as seen in smooth spores evolving multiple times in *Tulostoma*, can result from common adaptive responses to selection pressures (Wake et al. 2011). Because these species are closely related, this could be an example of parallel evolution rather than convergent evolution. Due to numerous constraints, such as space or resource availability, an organism is not capable of expressing all potential morphological states. This inherent limitation on form increases the likelihood of homoplasy (Wake and Larson 1987, Wake et al. 2011). To truly understand apparent homoplasy of smooth spores, an understanding of the spore ultrastructure and genetics is necessary. As shown by Co-David et al. (2009) the ultrastructure indicated homoplasy of spore ornamentation in *Tricholomataceae* and *Entolmotaceae*. Evidence of homoplasy through convergence and not parallelism points to adaptive evolution. Due to numerous constraints, such as space or resource availability, an organism is not capable of expressing all potential morphological states. This inherent limitation on form increases the likelihood of homoplasy (Wake and Larson 1987, Wake et al. 2011). While loss of spore ornamentation may be explained by a lack of developmental stimuli, this does not explain increases in spore ornamentation. I posit that the homoplasy of both ornamentation losses and increases strongly indicate selective pressures on spore morphology.

There are few phylogenetic studies on the loss of spore ornamentation over evolutionary history. Pringle et al. (2015) state that loss of spore ornamentation has not been previously documented. It is thought that most gasteroid fungi rely on raindrops, or wind, to dislodge spores from the gasterothecium and that ornamentation may provide water repellence during rain instigated dispersal (Kreisel and Al-Fatimi 2008, Halbwach and Bässler 2015). Kreisel and Al-Fatimi (2008) suggest that gasteromycetes have ornamented spores to promote wind dispersal after being dislodged by rain. The authors suggest that taxa of gasteroid fungi with ornamented spores are found in areas with regular rainfall, while gasteroid taxa with smooth spores, such as *T. obesum*, are found in arid areas with sparse rainfall. My comparison of average rainfall from locations where type species were collected does not fully support this hypothesis (Figure 2) (which figure is this?). However, this hypothesis is worthy of further investigation. Reliable rainfall data for many locations are lacking. Also, the number of annual rain events, and the timing of these events may provide better evidence to support this idea.

It is also important to consider the spore morphology of Agaricaceae s.l. While most lamellate species in the group have elongated, smooth spores, the majority of gasteroid species in this group have ornamented spores. Within Agaricaceae s. l. the majority of gasteroid taxa have ornamented spores. The spores of lamellate species, such as Agaricaceae s. str., Coprinaceae, and *Lepiota,* generally lack ornamentation and are oblong, not globose (Vellinga et al. 2003, Vellinga et al. 2011). Gasteroid taxonomic groups in Agaricaceae s.l. include species closely related to *Tulostoma* such as *Battarrea*, and genera whose monophyly is not well established, such as *Queletia*, *Chlamydopus*, and *Schizostoma* as well as more distantly related groups, Lycoperdaceae, which is thought to consists of 22 genera (Kalichman et al. 2020). The species contained within these gasteroid groups predominately produce globose, or sub-globose, ornamented spores, with a few exceptions. Within Lycoperdaceae, there are some smooth spored species, such as *Calvatia pachyderma* and *Bryoperdon acuminatum*, but smooth spores in this group are apparently uncommon (Bates et al. 2009, Vizzini and Ercole 2017).

Investigations into these smooth spored species can support the hypothesis that selective pressures are acting on fungal spore morphology.

Selective pressures leading to homoplastic spore morphology warrant further investigation. Because gasteroid fungi likely disperse their spores via raindrops and wind, relationships between moisture and spore ornamentation deserve greater study. More detailed microhabitat information from locations where *Tulostoma* species are collected may serve to clarify the relationship between spore ornamentation and moisture. It is also likely that other spore characteristics are involved. There may be tradeoffs with ornamentation and spore wall pigmentation, chemical components, and thickness. Spores that lose ornamentation may have thicker cell walls or increased melanin to increase longevity or their viability. It is also likely that loss of ornamentation may be seen in other fungi under similar selective pressures.

CONCLUSIONS

The ancestral character state analysis demonstrate that ancestral state of spores is ornamented. The character state analysis shows loss of spore ornamentation occurred 4 separate times over the evolutionary history of this genus. Spore ornamentation was not shown to be correlated with climatic, environmental, or dispersal patterns. Further evidence is necessary to understand the selective pressures on spore ornamentation in this genus.

Chapter 5. Isotope Analysis Reveals Novel Nutritional Modes in *Tulostoma*

INTRODUCTION

Fungi are integral to ecosystem functioning in all terrestrial communities. Ecological roles of fungi in terrestrial environments are varied and fungi are often distinguished by these ecological roles. Through their ecological roles fungi are critical for global carbon (C) and nitrogen (N) cycling, and plant community composition and succession (van der Wal et al. 2013). The ecological roles of terrestrial fungi in the Agaricales (Basidiomycete) traditionally been distinguished by their nutritional modesectomycorrhizal (EcM), parasitic, or saprotrophic. From this traditional viewpoint biotrophic fungi, such as EcM fungi and parasitic fungi, obtain C from living hosts, while saprotrophic fungi obtain nutrients through the decomposition of dead organic material (van der Heijden et al. 2015, Brundrett and Tedersoo 2018). Fungi were generally classified as parasitic when basidiocarps were found on host tissue. Mycorrhizal status was most commonly confirmed by comparing molecular sequence data of tree root tips to those from mushroom. When fungal DNA found on plant root tips matched a fungal sporocarp that species was classified as mycorrhizal. And in the absence of evidence suggesting parasitism or EcM, fungi were considered saprotrophic. Saprotrophic substrates were classified using the material that the basidiocarp was found on, such as soil, leaf litter, or wood. Advances in technology have enabled a greater understanding of the complexity of these nutritional modes, and our understanding of the continuum between saprotrophic and biotrophic fungi is developing.

More recently, this traditional viewpoint has begun shifting. Research has found that species of EcM fungi can act as decomposers, and that saprotrophic fungi interact with living hosts, and in the absence of EcM structures, are considered biotrophs. The evolution of fungal lineages is helpful in understanding the complexity of fungal nutritional modes. Current evidence suggests that the ectomycorrhizal lifestyle in fungi has evolved independently 78 times (Tedersoo and Smith 2013). In each case, ectomycorrhizal fungi evolved from a saprotrophic ancestor (Sánchez-García and Matheny 2017, Smith et al 2017). While EcM fungi have fewer genes that code for plant cell wall degrading enzymes (PCWDEs) compared to their ancestral saprotrophic fungi, EcM fungi still retain some of these genes (Kohler et al. 2014). These genes have been termed the decomposition “toolbox” and have diverged somewhat in EcM fungi but are capable of oxidizing organic material (Lindahl and Tunlid 2015, Shah et al. 2015).

Conversely, putatively saprotrophic species can interact with living hosts (Smith et al. 2017, Peng et al. 2021). In many of these relatively newly discovered nutritional modes, the relationship between the fungi and the host is not clearly defined, and these fungi are considered biotrophic. These biotrophic associations are distinguished from EcM by the lack of EcM morphological structures, such as a Hartig-net, however some saprotrophic species have been shown to develop a Hartig-net like structure (Smith et al. 2017) Biotrophic ectomycorrhizal relationships between Agaricales and vascular plants is well documented (Ryberg and Matheny 2012). In these EcM relationships, EcM fungi provide nutrients, such as N and phosphorus (P), to plants in exchange for photosynthates, such as glucose.

In addition to EcM, fungi can be biotrophic in many other capacities, living inside of the tissues of plants and even other fungi. Fungi facilitated the colonization of land by plants (Read et al. 2000, Heckman et al. 2001). As such, it’s not surprising that like vascular plants, nonvascular plants also have fungal associations, and more recently study of these associations has begun. Three genes have been identified in plants that are required for mycorrhizal formation, and these three genes have been documented in almost all major plant lineages (Wang et al. 2010). Bryophytes have shown to harbor multiple species of filamentous Basidiomycete fungi, and this relationship between bryophytes and fungi appears mutualistic (Bidartaondo and Duckett 2009, Pressel et al. 2021). Basidiomycetes are found as endophytes throughout plant tissues (Martin et al. 2015, Vaz et al. 2020), and are also plant pathogens (Matheny et al. 2006, Qhanya et al. 2015, Niveiro et al. 2015). Species of Agaricales can be biotrophic on other fungi (fungicolous). These fungicolous biotrophic fungi can be commensal or mycoparasitic (Barnett 1963, Koch and Herr 2021).

As saprobes, fungi decompose dead organic material, facilitating the recycling of nutrients by other organisms. The ecological role of recycling organic matter occurs as saprobes excrete hydrolytic enzymes such as cellulases and proteinases. These hydrolases are crucial in N cycling and the breakdown of organic material (Marschner and Dell 1994). These enzymes initialize the breakdown of plant cell walls and the mineralization of complex compounds. This mineralization process enables the assimilation of these smaller compounds by plants and microbes (Crowther et al. 2012). Despite their importance in global ecosystems, our understanding of the ecological roles of many individual fungal species and lineages remains speculative.

Over the past two decades, C and N isotopic evidence has been used to delimit saprotrophic fungi from mycorrhizal fungi. In EcM relationships EcM fungi provide nutrients, such as N and P, to plants in exchange for photosynthates, such as glucose. Because there is some antagonism in the exchange of nutrients between living plants and their fungal associates, EcM fungi are depleted in ^{13}C compared to saprotrophic fungi, whose C source is not mitigated by a living host (Hobbie et al. 1999, Kohzu et al. 1999, Hobbie et al. 2001). Conversely, EcM fungi are enriched in ^{13}N compared to saprotrophic fungi. This is also thought to be due to the interplay between the living hosts. It is believed that mycorrhizal fungi preferentially transfer isotopically depleted compounds to plants and as a result, mycorrhizal fungi become enriched with ^{15}N when compared to plants or saprotrophic fungi (Gebauer and Taylor 1999, Hobbie et al. 1999, Seitzman et al. 2017).

Because evidence is accumulating that the diversity of biotrophic relationships between fungi and plants is greater than previously expected (Halbwachs et al. 2018) isotopic analyses are being employed to delimit biotrophic fungi, not just EcM fungi, from saprotrophs. This has given new insights on the diversity of fungal nutrition and led to some novel discoveries on nutritional modes. Seitzman et al. (2011) sampled Hygrophoraceae taxa and found evidence of biotrophic lineages in the family based on isotopic evidence and suggested that these biotrophic taxa were likely symbionts of bryophytes and other understory plants. Halbwachs et al. (2018) focused on members of Hygrophoraceae that were long considered saprotrophic as they did not grow in association with known mycorrhizal plant species. They also concluded that previously putative saprotrophic species of Hygrophoraceae are biotrophic, and possible mycorrhizal. Additionally, recent evidence shows that *Mycena*, long considered a saprotrophic genus, is frequently found in plant roots, and that some sporocarps of *Mycena* exhibit isotope profiles of biotrophic species (Thoen et al. 2020, Harder et al. 2021).

Tulostoma is a cosmopolitan gasteroid fungal genus, which produces stalked puffballs, is in the order Agaricales, in the family Agaricaceae s.l. (Vellinga 2004, Matheny et al. 2006, Kalichman et al. 2020). Current phylogenetic analysis of Agaricaea s.l. includes the groups Agaricaceae s.st., Coprinaceae, "Lepiotaceae", Lycoperdaceae, and Tulostomaceae (or Battaraceae), however the phylogenetic relationships within Agaricaceae s.l. remain unresolved (Vellinga 2004, Matheny et al. 2006, Kalichman et al. 2020). The order Agaricales contain both ectomycorrhizal lineages and saprotrophic lineages (Matheny et al. 2006, Tedersoo and Smith 2013, Sánchez-García and Mathey 2017), while Agaricaceae s.l is thought to contain only saprotrophic species (Vellinga 2004, Matheny et al. 2006). While a few reports speculate that some species in the genus *Calvatia*, in the Lycoperdaceae, may be ectomycorrhizal (Trappe 1962, Riffle 1968, Rimóczi 1987), this has yet to be demonstrated using molecular techniques.

The gasteroid body form has evolved multiple independent times throughout the fungal kingdom (Varga et al. 2019). Phylogenetic analyses indicate that most gasteroid genera are derived from lamellate (gilled) or poroid mushroom genera (Varga et al. 2019). Studies also show that gasteroid genera follow the nutritional mode of their sister mushroom taxa (Grubisha et al. 2017). Because the intergeneric relationships within Agaricaceae s.l. are unresolved, it difficult to determine which lineages share most common ancestry with *Tulostoma*. In the absence of evidence which indicates otherwise, the lineages within Agaricaeae are considered saprotrophic, including lignicolous species, as is *Tulostoma* (Wright 1987, Jeppson et al. 2017). In light of recent findings that show fungal nutritional modes should not be assigned without evidence.

The objective of this study was to confirm the hypothesized saprotrophic nutritional mode of the genus *Tulsotoma*. Isotopic carbon and nitrogen ratios will be compared to published profiles of fungal species with established nutritional roles to determine whether *Tulostoma* are saprotophic. The determination of the nutritional mode of fungal species is necessary to understand their ecological roles.

METHODS

Taxa Selection

Ten Colorado *Tulostoma* collections were selected for study. I isolated C and N isotopes from these 10 collections and compared their isotopic signatures to two known saprotrophic and two known mycorrhizal species from the Western Colorado University (WCU) fungal collection and published isotope data. The saprotrophic species used were *Ganoderma applanatum* and *Mycena overholtzii*. The mycorrhizal species were *Cortinarius* sect. *Colymbadini* and *Cantharellus cibarius*. In total, I analyzed 20 samples: eight individual *Tulostoma* species, four replicates of *Tulostoma fimbriatum*, and two from each of *Ganoderma applanatum*, *Mycena overholtzii*, *Cortinarius* sect. *Colymbadini*, and *Cantharellus cibarius.* I analyzed replicates of the selected species to determine how much variation in isotope concentrations exists within species.

Isotope Extraction

Approximately 1 g of dried fungal tissue was extracted from each sporocarp. The outer layer of stem tissue was removed, and internal tissue was excised. The internal tissue was used to minimize any contamination from non-target isotopic sources. Fungal tissue was placed in glass Petri dishes and washed in 10 ml 2:1 chloroform:methanol solution using a glass transfer pipette. Each sample was gently agitated for 30 seconds using metal forceps to remove lipids and other surface contaminants (e.g., dirt and pollen) according to previously published methods (Wassenaar and Hobson 1998). The wash solution was removed using a pipette. The washing and agitation steps were repeated twice. Samples were air dried in glass Petri dishes in a fume hood for three days.

Dried samples were transferred to an incubator and further dried for 24-48 hours at 50-60°C then transferred to 1-dram glass vials and shipped to the Stable Isotope Paleo Environments Research Group (SIPERG) Laboratory at Iowa State University (ISU) for isotopic analysis.

ISU SIPERG Laboratory Analysis

At the SIPERG facility, samples were placed in tin capsules and immediately sealed. Carbon ($d^{13}C$) and N ($d^{15}N$) stable isotopes analyses was conducted on a Costech EA attached to a Finnigan MAT Delta Plus SL. $d^{13}C$ was corrected to a Vienna Pee Dee Belemnite (VPDB) standard, and $d^{15}N$ was corrected to an air standard.

Reference standards (Caffeine [IAEA-600], Caffeine [USGS 62], Cellulose [IAEA-CH-3] and Acetanilide [laboratory standard]) were used for isotopic corrections, and to assign the data to the appropriate isotopic scale. Corrections were done using a regression method and isotope results are reported in parts per thousand (per mil, ‰). Percent concentration (%) was calculated using the peak area of the sample. The combined uncertainty (analytical uncertainty and average correction factor) for ($d^{13}C$ is ± 0.13‰ (VPDB) and ($d^{15}N$ is ± 0.32‰ (Air), respectively.

Statistical Analysis

To examine trophic states of *Tulostoma* samples, two sets of published data were assembled (Appendix 5.1). Data set 1 included my $\delta^{15}N$ and $\delta^{13}C$ data and published data from Hart et al. (2006). Data set 2 included data from Hart et al. (2006), as well as Mayor et al. (2009), Hou et al. 2012, Birkebak et al. (2013), Sánchez-García and Matheny (2017), and Korotkin et al. (2018). Previous authors established the nutritive mode (listed as habit in

Appendix 5.1) as saprotrophic (SAP), ectomycorrhizal (ECM), neither saprotrophic nor ectomycorrhizal (NS-NE), or unknown (UNK). The species used, δ^{15}N and δ^{13}C data, and nutritional mode are shown in Appendix A.

Statistical analyses and graphing were conducted in R v3.2.2 (R Core Team 2015) using packages ggplot2 v.2.1.0 (Wickham 2009) and mgcv v.1.8 (Wood 2006). I used the NBClust package in R version 3.3.1 to determine the optimal number of my data points to use (R Development Core Team; Charrad et al. 2014). The EClust function in R was used for visualizing the cluster analysis. Cluster Analyses of variance (ANOVA) and Tukey-Kramer post-hoc tests (Tukey 1949) were performed to test null hypotheses of no differences between C and N isotope values of each cluster and *Tulostoma* in the same trophic cluster. Determination of saprotrophic and mycorrhizal habits was based on C:N ratios produced from ggplot and mgcv graphs and the assigned cluster.

RESULTS

Cluster Analysis

Cluster analysis results are shown in Figures 5.1-5.3. The optimal number of clusters for all analyses was two (Figure 5.1). The majority of taxa that group in cluster 1 are EMF, and the majority of taxa that group in cluster 2 are saprotrophic (Tables 5.1 and 5.2). Taxa included in these analyses are reported in Appendix A, and isotopic results of individual *Tulostoma* collections and their corresponding clusters are shown in Table 5.1. Figure 5.2 shows *Tulostoma* species and Colorado collections are included with published data from Hart et al. (2006). The published data were selected as the ecology of Snowbowl, AZ most resembles that of Colorado when considering all published isotope data. Figures 5.2 and 5.3 shows *Tulostoma* clustering into both clusters, which indicates some *Tulostoma* isotopic profiles more closely align with mycorrhizal species (grey triangles) and others align with saprotrophic species (blue circles).

Cluster 1 contained 597 taxa and had an average -0.07 ± 2.74 δ^{15}N‰ and -23.06 ± 1.49 δ^{13}C‰ (Appendix 5.1, Table 5.2). Cluster 2 contained 447 taxa 5.88 ± 4.56 δ^{15}N‰ and -23.06 ± 1.49 δ^{13}C‰ (Appendix 5.1, Table 5.2). Six *Tulostoma* collections, two duplicates, are in cluster 1 and had an average 11.22 ± 4.38 δ^{15}N‰ and -23.43 ± 1.03 δ^{13}C‰ Tables 5.1 and 5.2). Five *Tulostoma* species, two duplicates, are included in cluster 2 and had an average 3.05 ± 1.62 δ^{15}N‰ and -19.95 ± 1.82 δ^{13}C‰ (Tables 5.1 and54.2). Our replicated samples showed no statistical variation.

Isotope data were compared betweenclusters in Figures 5.4 and 5.5. The statistical analysis comparing these data are presented in Table 5.3. There were statistically significant differences between clusters 1 and 2 and between *Tulostoma* within clusters 1 and 2 or both d^{15}N and d^{13}C. While there was a significant difference in d^{15}N between *Tulostoma* in cluster 1 and cluster 2, there was no significant difference in d^{13}C between these groups. Conversely, there was nosignificant difference in d^{15}N between Tulostoma in cluster 2 and cluster 1, there was a significant difference in d^{13}C between these groups. There was no significant difference in d^{15}N or d^{13}C between *Tulostoma* in cluster 2 and cluster 1.

Figures 5.6 and 5.7 show the putative nutritive mode, or habit, of the collections in each cluster. Of the 597 collections in Cluster 1, 46 (7.7%) were classified as NS-NE, 22 (3.7%) were classified as SAP, 480 (80.4%) were classified as ECM, and 49 (8.2%) were classified as UNK. Of the 447 collections in Cluster 2, 0 were classified as NS-NE, 329 (73.6%) were classified as SAP, 85 (19%) were classified as ECM, and 33% (7.4%) were classified as unknown. Using NBClust analysis the best number of clusters is 2 (Figure 5.1).

Table 5.1. Collection numbers, species, habitat, isotope, and cluster data from analyzed samples.

Collection	Species	Habitat	δ^{15}N‰	δ^{13}C‰	Cluster
DBG24092	*T. polymorphum*	Lawn in urban yard	11.97	-23.59	1
DBG27840	*T. fimbriatum*	No data	11.84	-22.31	1
DBG27840-1	*T. fimbriatum*	No data	12.47	-22.19	1
DBG27844	*T. fimbriatum*	Dead grasses & weeds	12.40	-23.30	1
DBG27849	*T. punctatum*	Open meadow (urban) in dead grass weeds of woody debris, arid sandy soil south of clear creek.	12.97	-24.15	1
DBG27849-1	*T. punctatum*	Open meadow (urban) in dead grass weeds of woody debris, arid sandy soil south of clear creek.	14.61	-23.64	1
DBG28849	*T. fimbriatum*	meadow	12.90	-22.88	1
DBG23778	T. sp. novo	Grassland adjacent to oil well and gas tank area. Sandy soils of *Hesperostipa comata*. Gregarious	0.98	-17.71	2
DBG23778-1	T. sp. novo	Grassland adjacent to oil well and gas tank area. Sandy soils of *Hesperostipa comata*. Gregarious	1.29	-17.55	2
DBG24482	*T. simulans*	in soild near creek path, Pinus ponderosa; gregarious habit	0.60	-21.36	2
DBG24482-1	*T. simulans*	in soild near creek path, Pinus ponderosa; gregarious habit	5.22	-21.02	2
DBG28400-1	*T. obscurum*	Sandy soil	3.37	-20.47	2
DBG21081	*T. meristostoma*	Rabbitbrush community on loose sand, partially stabilized by vegetation.	4.52	-20.93	2
DBG28400	*T. obscurum*	No data	3.84	-19.46	2

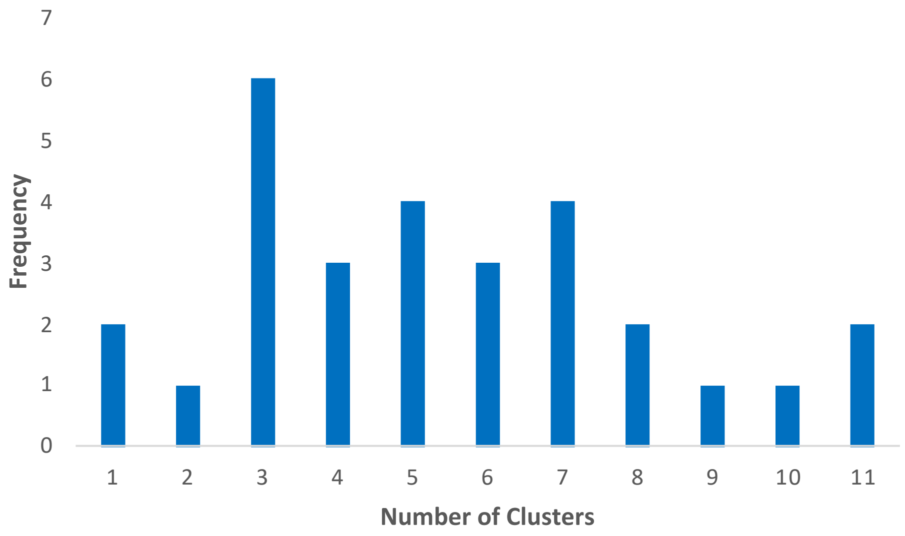


Figure 5.1. Result of **NbClust** analysis. The optimal number of clusters is shown to be 2.

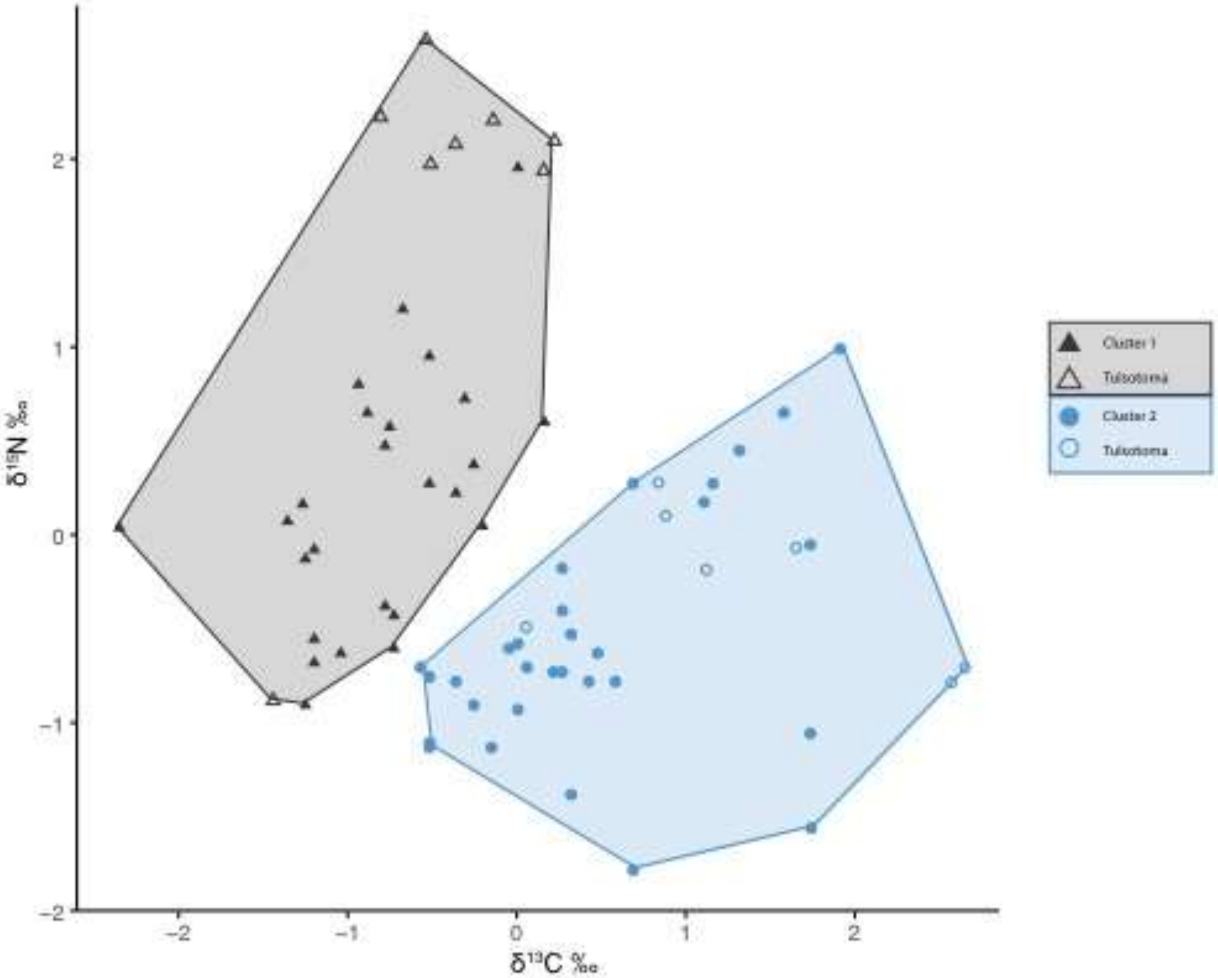


Figure 5.2. Cluster analysis of *Tulostoma* collections and Hart (2006) isotope data. Solid grey triangles= Cluster 1, predominately mycorrhizal species; open grey triangles= putatively biotrophic *Tulostoma*. Solid blue circle=Cluster 2, predominately saprotrophic species; open blue circle= putatively saprotrophic *Tulostoma*.

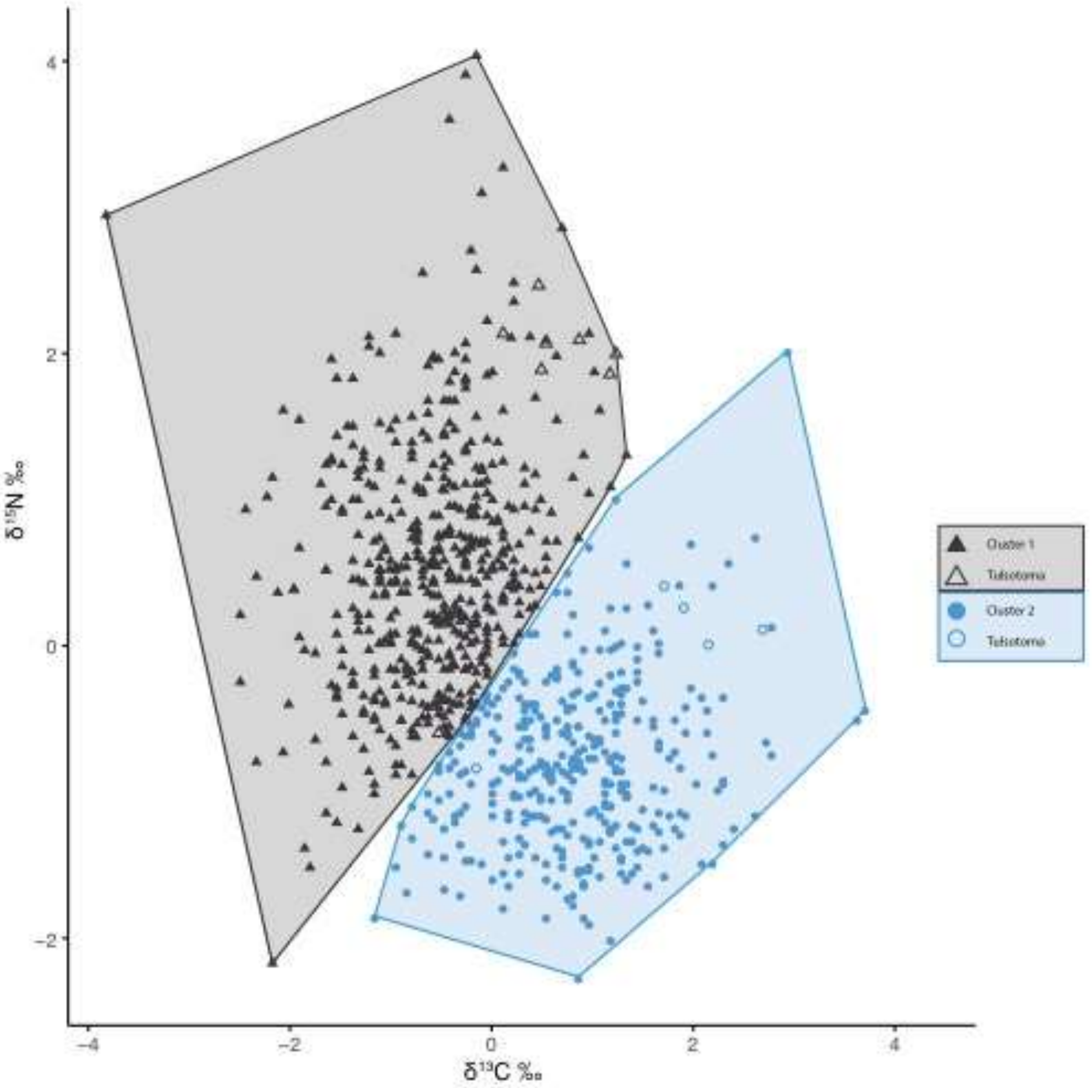


Figure 5.3. Cluster analysis of d^{13}C and d^{15}N for *Tulostoma* collections and published isotope data. Solid grey triangles= Cluster 1, predominately mycorrhizal species; open grey triangles= putatively biotrophic *Tulostoma*. Solid blue circle=Cluster 2, predominately saprotrophic species; open blue circle putatively saprotrophic *Tulostoma*.

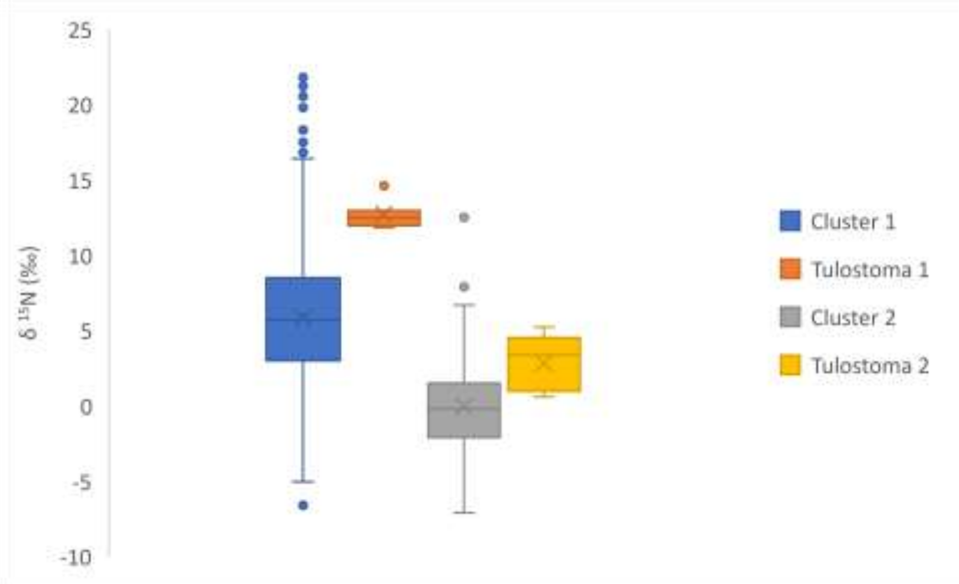


Figure 5.4. Box and whisker plot of δ ^{15}N (‰).

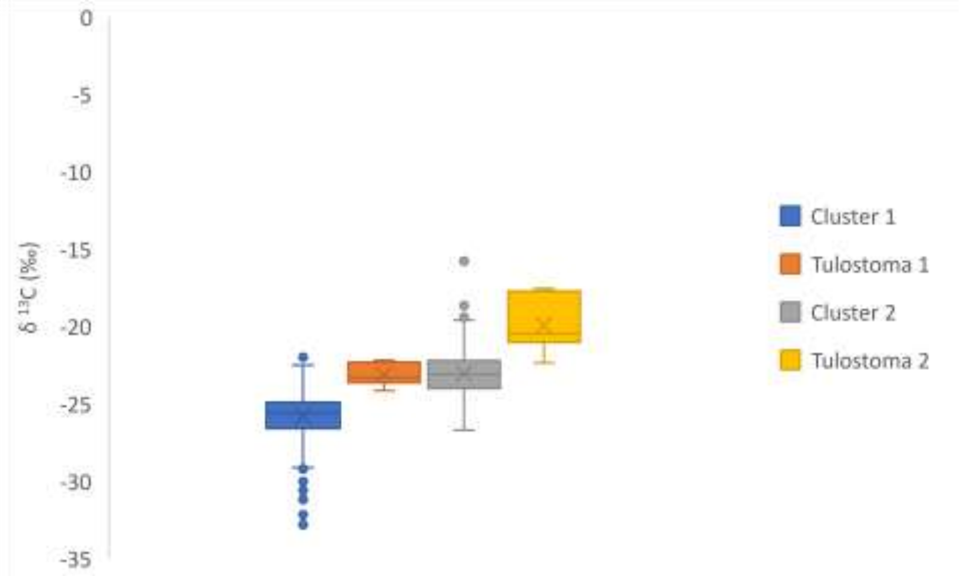


Figure 5.5. Box and whisker plot of δ ^{13}C (‰).

Table 5.2. Average and standard error $\delta^{15}N$‰ and $\delta^{13}C$‰ in Clusters 1 and 2.

Cluster	**$\delta^{15}N$ ‰**	**$\delta^{13}C$ ‰**
1	5.13 ± 0.23	-24.88 ± 0.18
Tulostoma (1)	12.74 ± 1.46	-23.15 ± 0.57
2	-0.58 ± 0.27	-22.57 ± 0.22
Tulostoma (2)	2.83 ± 1.46	-19.93 ± 0.06

Table 5.3. Tukey-Kramer statistical analysis on $d^{15}N$ and $d^{13}C$ isotope data. There were statistically significant differences between clusters 1 and 2 and between Tulostoma within clusters 1 and 2 or both $d^{15}N$ and $d^{13}C$. While there was a significant difference in $d^{15}N$ between Tulostoma in cluster 1 and cluster 2, there was no significant difference in $d^{13}C$ between these groups. Conversely, there was not significant difference in $d^{15}N$ between Tulostoma in cluster 2 and cluster 1, there was a significant difference in $d^{13}C$ between these groups. There was not significant difference in $d^{15}N$ or $d^{13}C$ between Tulostoma in cluster 2 and cluster 2.

Group 1	**Group 2**	**$\delta^{15}N$ ‰ p-value**	**$\delta^{15}C$ ‰ p-value**
Cluster 1	*Tulostoma* (1)	0.003	0.75
Cluster 1	Cluster 2	1.2×10^{-13}	2.0×10^{-13}
Cluster 1	*Tulostoma* (2)	0.49	0.01
Tulostoma (1)	Cluster 2	1.6×10^{-8}	0.997
Tulostoma (1)	*Tulostoma* (2)	0.002	0.0004
Cluster 2	*Tulostoma* (2)	0.54	0.29

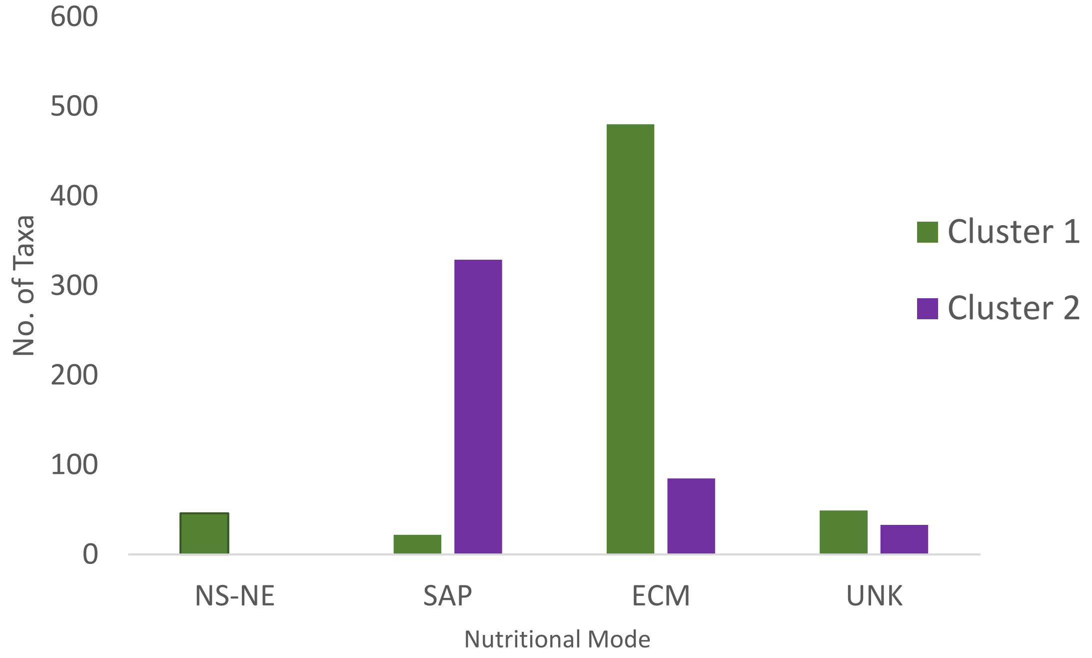


Figure 5.6. Number of taxa from each nutritional mode in each cluster. NS-NE = neither saprotrophic nor ectomycorrhizal, SAP = saprotrophic, ECM = ectomycorrhizal, and UNK = unknown.

DISCUSSION

The cluster analysis resulted in *Tulostoma* collections falling into 2 distinct clusters. One group of *Tulostoma* falls within Cluster 1 which contains predominately mycorrhizal or biotrophic species, and the other group of *Tulostoma* lies within Cluster 2, which contains predominately saprotrophic fungi (Figs 4.2, 4.3, 4.6, 4.7, and Appendix 1). These analyses infer, based on published data sets (Kohzu et al.,1999; Zeller et al. 2007, Hou et al. 2012), that some *Tulostoma* collections present as biotrophic while others saprotrophic.

The d^{13}C or d^{15}N values compared individually between *Tulostoma* within clusters and clusters presents a more convoluted, inconsistent picture. Clusters 1 and 2 were statistically significantly different in both d^{15}N and d^{13}C (Figures 5.4 and 5.5). However, when d^{15}N ‰ and d^{13}C ‰ of *Tulostoma* were compared within clusters and between clusters, the results were not as expected. The lack of statistical significance between d^{13}C ‰ Tulostoma in Cluster 1 and the rest of Cluster 1 and the rest of Cluster 2 is surprising. The comparison of d^{15}N ‰ within and between Clusters is as expected save for *Tulostoma* in Cluster 2 compared to Cluster 1, where there is no statistically significant difference. This could be attributable both to low sample size, and a lack of isotopic data from nearby locations. Despite this lack of statistical significance in these individual comparisons, *Tulostoma* species show strong affinity for separate groupings in the cluster analysis, which strongly suggests variation in nutritional modes.

T. obscurum, *T. meristostoma*, and an undescribed *Tulostoma* species are associated with known saprotrophic species (Table 5.1, Figures 5.2 and 5.3). The one anomaly in comparing the isotope data individually, is that the d^{15}N ‰ *Tulostoma* in Cluster 2 is lower, but not statistically significantly, compared to Cluster 1. As *Tulostoma* is reported to be a saprotrophic species, these results are as expected.

The putatively biotrophic species of *Tulostoma* include 3 psammophilous species: *T. fimbriatum*, *T. polymorphum*, and *T. punctatum*. *T. fimbriatum* is widely distributed throughout N. America and has a cosmopolitan distribution. Throughout its distribution it is commonly reported from calcareous soils, both acidic and alkaline (Olsson et al. 2010, Jeppson et al. 2017). In North America, it is most commonly associated with *Artemisia tridentata* and other related sage brush species. Other common plants associates are grasses, including *Bromus tectorum*, *Salix*, *Populus*, *Juniperus*, and occasionally conifers such as *Pseudotsuga menziesii*. In Europe, *T. fimbriatum* has been used as an indicator species for xerothermic grasslands, and has been reported from many habitats: pine, Greek juniper, and cedar forests, oak-hornbeam, degraded oak forests, pine plantings, *Salix* associations or meadows (Jaworska et al. 2012, Doğan et al., 2012, Rusevska et al. 2018). *T. polymorphum*, which is known only from the southwestern United States, is reported from sandy soil and occasionally near *Cupressus* species. *T. punctatum* is distributed in North America and Europe (Jeppson et al. 2017). In North America, *T. punctatum* is reported from sandy areas or meadows, but plant associations are not documented (Wright 1987). In Europe *T. punctatum* is reported from sandy soil on anthropogenic sites such as lawns, parking lots, and abandoned orchards (Jeppson et al. 2017).

Given the distribution and plant associates of *T. fimbriatum*, the most likely biotrophic plant associate would likely be either grass species or species of *Artemisia. Artemisia* spp. and many grass species are known arbuscular mycorrhizal fungal (AMF) associates (Carter et al. 2014, Brundrett et al. 2017, Serpe et al. 2020). Most AMF belong to the fungal phyla

Glomeromycota and Mucoromycotina (Orchard et al. 2017). Lab studies show that *Artemisia* species increase AMF colonization in the absence of nutrients and in the presence of other plants, such as grasses, which is thought to be a response to competition (Montesinos-Navarro et al. 2019, Serpe et al. 2020). The majority of the molecular studies on the mycorrhizal associates of plant species assumed to be AMF associates focus solely on AMF and use DNA primers specific for those taxonomic groups (Busby et al. 2013, Carter et al. 2014). Given the increase in root fungal colonization in plants under competition and stress, predominately AMF associated plants may associate with other fungal lineages. Other lineages of fungi may easily be overlooked because molecular studies preclude their detection through primer selection.

It has been shown that AMF plant associates can interact with EcM fungi. The ectomycorrhizal fungal species *Tricholoma matsutake* and *Tricholoma fulvocastaceum*, are able to form root associations with the arbuscular mycorrhizal plants *Cederela odorata* and *Prunus speciosa* (Murata et al. 2013, Murata et al. 2014). The fungal hyphae growing into the plant root, however do not form Hartig-net like structures as they do when associated with EcM plant associates. This ability of plants to form root associations with different ecological groups of fungi, coupled with the ecological diversity shown in the putative saprotrophic species, such as *Mycena*, it is possible that there is plasticity in the nutritional roles of other fungal species and plant associates. More careful examination of AMF plant root tips from areas where the putatively biotrophic *Tulostoma* occur are necessary to confirm or reject this possibility.

When considering the individual comparison of $d^{13}C$ or $d^{15}N$ isotopes between groups, the $d^{13}C$ does not strongly support the delineation of biotrophic *Tulostoma* species. The differences in $d^{15}N$ between clusters warrants consideration. Biotic processes are frequently limited by N availability (Hyvönen et al. 2008). In the absence of anthropogenic inputs, most biodegradable N is found in living and dead organic residues (Nehls and Plassard 2018). Biodegradable N sources include nitrate, ammonium, free amino acids, oligonucleotides, proteins and nucleic acids. These sources are largely released by saprotrophic decomposers (Talbot et al. 2013). Saprotrophic fungi are efficient at scavenging N from decomposing wood (Rayner and Boddy 1988). In response to decreases in water availability, fungal N increases (Shi et al. 2002). It is possible that these species high in d^{15}N are incredibly efficient in accessing N.

Without sequence data of *Tulostoma* on from plant tissue, the biotrophic status of *Tulostoma* cannot be determined definitively. However, this evidence does indicate that there are non-saprotrophic members of *Tulostoma*, and that the *Tulostoma* in cluster 1 are likely biotrophic. Potential hosts plants co occurring with *T. fimbiratum* should be first screened for *Tulostoma.* Genetic sequences matching *Tulostoma* will provide evidence that these species are biotrophic. If *Tulostoma* sequence data are not recovered from plant tissues, other explanations of the different isotopic ratios, d^{15}N in particular should be explored.

Cluster analysis consistently found 2 clusters based on isotopic evidence, while a similar data set from (Korotkin et al. 2018) showed 3 clusters. In their analysis they chose 957 taxa, both fungal and plant (autotrophic). One cluster (1) contained ECM fungi and all fungi that they classified as non-saprotrophic and non-mycorrhizal. Another cluster (2) contained most of saprotrophic fungi included in the analysis, and the last cluster (3) contained autotrophs as well as a few ECM and saprotrophic taxa. Further, they show broad variation in bryophilous fungal isotopes, these taxa resolved in all 3 of the clusters. Can you rewrite this sentence a little.

Cluster 1 contains 80% ECM fungi, while almost 74% of the collections in cluster 2 are saprotrophic.

There are many explanations as to why putatively mycorrhizal signatures occur within the predominately saprotrophic cluster and vice-versa. Decomposition of organic material by saprotrophic fungi and nutrient exchange in mycorrhizal fungi are both dynamic and complex. Understanding of these dynamics and complexities is just starting to be studied. Research shows that fungi within each nutritive group may distinguish among isotopes. Brearley et al. (2005) found that ECM in tropical mineral soils utilize N from mineral soils preferentially to N from organic sources. They also found that fungi show $d^{15}N$ discrimination differentially based on substrate, and that some species alter $d^{15}N$ discrimination when grown on different substrates. Hobbie et al. (2020) showed how selectivity in saprotrophic fungi would potentially impact $\delta^{13}C$ fractions. They provide a model describing how white wood rot fungi selective to hemicellulose would show higher $\delta^{13}C$‰ compared to less selective saprotrophic fungi. Similarly, they suggest that selectivity for heartwood would show decreased $\delta^{15}N$‰ compared to sapwood selective saprotrophs.

The decrease of $\delta^{13}C$ in atmospheric CO_2 over time (the Suess effect) may also serve to explain some of the nutritive variation within clusters (Hobbie et al. 2020). Age of substrates and age of collections will result in differences in available $\delta^{13}C$. Partitioning of ^{13}C within compounds of substrates can also lead to variations in $\delta^{13}C$. This is also true for composition within sporocarps. Proteins and carbohydrates differ in their $\delta^{15}N$ and $\delta^{13}C$ compositions, so sporocarps will be influenced by these chemical compositions.

Other research has shown that the enzymatic activity of saprotrophic fungi alters in response to environmental factors. Macroinvertebrate grazing on saprotrophic hyphae resulted in physiological responses (Crowther et al 2012). Lignocellulolytic, α-amylase, and protease enzyme production increased in response to macroinvertebrate grazing. These enzymatic increases changed nutrient production during decomposition (Osono 2007). Responses to grazing also differ based on fungal growth rates; fast growing species increase enzyme production in response to grazing while slow growing species decrease enzyme production (Crowther et al. 2011). These studies highlight that enzymatic activity varies and may result in variation in isotopic concentrations. It is possible that enzymatic decomposition plasticity could lead to variations in isotopic compositions. However, given the large data sets of isotopic values, this seems unlikely.

Soil organic matter (SOM) composition will also lend itself to variations in $\delta^{13}C$ and $\delta^{15}N$ in both saprotrophic and mycorrhizal fungi. SOM refers to all organic matter in soils and can contain plant, animal, and microbial tissues as well as organic substances such as enzymes. SOM is critical in the global climate cycle (Ohno et al. 2017). Residence time, defined as how long material exists in a system, varies based on the chemical structure and environmental factors. Residence times of organic material in soils varies greatly, from 1 to 1000 years (Brantley 2008). The residence time of organic compounds in SOM can be regulated by saprotrophic fungi. Radiocarbon dating of soil has shown that fungal taxa differentially degrade C and N based on age of material (Lindahl et al. 2006, Newsham et al. 2018, Hobbie et al. 2020). A better understanding of the decomposition processes of saprotrophic fungi is integral to understanding soil and decomposition processes. A deeper understanding of soil processes is essential to both understanding how saprotrophic fungi influence carbon dynamics of global carbon cycles, the role of climate change in altering these processes and how fungi may be utilized to promote C sinks to alleviate the effects of climate change.

Further, based on genetics, enzymatic assays, transcriptome analysis, and isotopic evidence, there is plasticity in nutritive modes in some taxa. Korotkin et al. (2018) show that some moss-inhabiting species of the fungal species *Rickenella* exhibit multiple trophic modes. Based on the isotopic signatures, they suggest that *Rickenella* sp. are ECM-like or possibly parasitic or even endophytic (Bresinsky and Schötz, 2006; Chen et al., 2018). There is also growing evidence that mycorrhizal fungi may act as saprotrophs. Genomic evidence shows that while some genes involved in decomposition have been lost, other genes associated with decomposition remain active in mycorrhizal fungi (Lindahl and Tunlid 2014, Shah et al. 2016). Martin et al. (2008) showed that *Laccaria bicolor* can be both saprotrophic and biotrophic. Smith et al. (2017) showed that the ECM lifestyle has evolved independently at least 78 times from saprotrophic fungal lineages. They showed that almost 17% of wood-decay saprotrophic fungi that they tested colonized tree root tips. Kariman et al. (2018) argue that root-associated fungi are potentially overlooked due to the absence of definitive visible structures, such as a Hartig net. Most researchers do not perform fungal molecular analysis on plant roots without a clear fungal structure, as such interactions of fungal taxa with plant roots in the absence of mycorrhizal structures could be unknown.

My data add to studies which show C:N isotopes can differentiate nutritional modes of fungi. Research continues to show δ^{13}C values are lower in ECM than in saprotrophic sporocarps. Generally, δ^{15}N values are higher in EcM fungi, or biotrophic fungi, than in saprotrophic sporocarps (Hobbie et al. 1999, Kohzu et al. 1999, Taylor et al. 2003, Trudell et al. 2004), but not always significantly (Hart et al. 2005). The isotopic signatures of almost half of the sampled *Tulostoma* collections suggest that there is some biotrophy involved in their nutritive modes. This may make sense considering that the ancestral character of many fungal lineages is saprotrophic (Martin et al. 2008, Wolfe et al. 2012). Given that the saprotrophic lifestyle is putatively ancestral, it is not unreasonable that derived lineages of fungi may diversify their nutritive modes. Further study is necessary to determine what the source of biotrophy is in *Tulostoma.*

The advent of molecular tools has enabled an understanding of the biochemical machinery behind mechanisms of biotrophy. Cycochrome P450 monoocygenases are believed to be integral in lignin degradation, an important component of plant cell walls. Pathogenic Agaricomycotina have diversified and increased their cycochrome P450 monoocygenase gene families compared to saprotrophic fungi (Qhanya et al. 2015). Oxalic acid increases can increase the virulence of plant pathogens (Kim et al. 2008). The mycoparasite *Entomola abortivum* produces enzymes that degrade oxalic acid in order to parasitize the root pathogen *Armillaria mellea* Koch and Herr (2021). Future studies into the biochemistry in closely related fungal taxa which demonstrate a range of nutritive modes may elucidate the gradient from saprotrophy to biotrophy and provide a greater understanding of the evolution of these nutritive modes.

CONCLUSIONS

The nutritional modes of this genus do not appear to be strictly saprotrophic. Isotopic data analysis aligns several species in this genus with biotrophic fungi. However, the biotrophic partner of these species was not identified in this study.

Chapter 6: Conclusions

The overaching objective of this dissertation was to investigate the evolution and ecology of the genus *Tulostoma* using molecular and isotopic data.

Using ITS, LSU, and TEF1α sequence data:

- The monophyly of this genus was supported. Sequence data from 58 collections and 23 species were combined with available sequences. A dichotomous key of the recovered southwestern US taxa was generated. The taxonomy of recovered species was delineated.
- The phylogeographic history of the genus was reconstructed. The phylogeographic analysis indicates a Palearctic origin of *Tulostoma*. The Palearctic origin indicated may be due to the sampling effort as the majority of species in the phylogeny occur in the Palearctic. The emergence and diversification of steppe habitats in the Palearctic, where the majority of *Tulsotoma* species occur, coincide with the time of divergence of the genus, it is possible that sampling effort is not skewing these results. Phylogenetic analysis with additional taxa representing the global diversity of this genus is necessary to support this hypothesis.
- The ancestral character state analysis demonstrate that ancestral state of spores is ornamented. The character state analysis shows loss of spore ornamentation occurred 4 separate times over the evolutionary history of this genus. Spore ornamentation was not shown to be correlated with climatic, environmental, or dispersal patterns. Further evidence is necessary to understand the selective pressures on spore ornamentation in this genus.

Using isotopic evidence:

- The nutritional modes of this genus do not appear to be strictly saprotrophic. Isotopic data analysis aligns several species in this genus with biotrophic fungi. However, the biotrophic partner of these species was not identified in this study.

Literature Cited

Accioly, T., Sousa, J.O., Moreau. A., Lécuru, C., Silva, B.D., Roy, M., Gardes, M., Baseia, I.G. and Martín, M.P., 2019. Hidden fungal diversity from the Neotropics: *Geastrum hirsutum, G. schweinitzii* (Basidiomycota, Geastrales) and their allies. *Plos One 14*(2):e0211388.

Aguilar-Trigueros, C.A., Hempel, S., Powell, J.R., Anderson, I.C., Antonovics, J., Bergmann, J., Cavagnaro, T.R., Chen, B., Hart, M.M., Klironomos, J. and Petermann, J.S., 2015. Branching out: towards a trait-based understanding of fungal ecology. *Fungal Biology Reviews 29*(1):34-41

Altes AL, Moreno GA., and Hausknecht, A. 1994. Two interesting species of *Tulostoma* from the Mediterranean Basin. *Ost Zeitsch fPilzk 3*:87-93.

Altés, A. and Moreno, G. 1999a. Notes on type materials of *Tulostoma* (Tulostomataceae*) T. macrosporum, T. meridionale* and *T. utahense*. *Persoonia-Molecular Phylogeny and Evolution of Fungi 17*(2):259-264.

Altés, A., Moreno, G. and Wright, J.E., 1999b. Notes on *Tulostoma volvulatum* and *T. giovanellae*. *Mycological Research 103*(1):91-98.

Andersson, O., 1950. Larger fungi on sandy grass heaths and sand-dunes in Scandinavia. *Gleerup 2*:1-89.

Asplund, J., Kauserud, H., Ohlson, M. and Nybakken, L., 2019. Spruce and beech as local determinants of forest fungal community structure in litter, humus and mineral soil. *FEMS Microbiology Ecology 95*(2):fiy232.

Avise, J.C., Arnold, J., Ball, R.M., Bermingham, E., Lamb, T., Neigel, J.E., Reeb, C.A. and Saunders, N.C., 1987. Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics 18*(1):489-522.

Avise, J.C., 2000. *Phylogeography: the history and formation of species*. Harvard University Press. 447.

Axelrod, D.I., 1975. Evolution and biogeography of Madrean-Tethyan sclerophyll vegetation. *Annals of the Missouri Botanical Garden*280-334.

Bates, S.T., Roberson, R.W. and Desjardin, D.E., 2009. Arizona gasteroid fungi I: Lycoperdaceae (Agaricales, Basidiomycota). *Fungal Diversity 37*:153.

Barbolini, N., Woutersen, A., Dupont-Nivet, G., Silvestro, D., Tardif, D., Meijer, N., Chang, C., Zhang, H.X., Licht, A., Rydin, C. and Koutsodendris, A., 2020. Cenozoic evolution of the steppe-desert biome in Central Asia. *Science Advances 6*(41):eabb8227.

Barnett, H.L., 1963. The nature of mycoparasitism by fungi. *Annual Reviews in Microbiology 17*(1):1-14.

Bates, S.T., Roberson, R.W. and Desjardin, D.E., 2009. Arizona gasteroid fungi I: Lycoperdaceae (Agaricales, Basidiomycota). *Fungal Diversity 37*:1-153.

Bass, D., Richards, T.A., Matthai, L., Marsh, V. and Cavalier-Smith, T., 2007. DNA evidence for global dispersal and probable endemicity of protozoa. *BMC Evolutionary Biology 7*(1):1-13.

Beaver, R.A., 1979. Host specificity of temperate and tropical animals. *Nature 281*(5727):139-141.

Berggren, W.A. and Prothero, D.R., 2014. *Eocene-Oligocene climatic and biotic evolution: an overview*. Princeton University Press.

Bidartondo, M.I. and Duckett, J.G., 2010. Conservative ecological and evolutionary patterns in liverwort–fungal symbioses. *Proceedings of the Royal Society B: Biological Sciences 277*(1680):485-492.

Binder, M., Hibbett, D.S., Wang, Z. and Farnham, W.F., 2006. Evolutionary relationships of Mycaureola dilseae (Agaricales), a basidiomycete pathogen of a subtidal rhodophyte. *American Journal of Botany 93*(4):547-556

Blackwell, M. 2011. The Fungi: 1, 2, 3 … 5.1 million species? *American Journal of Botany* 98(3): 426-438.

Bohaty, S.M. and Zachos, J.C., 2003. Significant Southern Ocean warming event in the late middle Eocene. *Geology 31*(11):1017-1020.

Bond, W.J. and Midgley, G.F., 2012. Carbon dioxide and the uneasy interactions of trees and savannah grasses. *Philosophical Transactions of the Royal Society B: Biological Sciences 367*(1588):601-612.

Brantley, S.L., 2008. Understanding soil time. *Science 321*(5895):1454-1455

Brearley, F.Q., Scholes, J.D. and See, L.S., 2005. Nitrogen nutrition and isotopic discrimination in tropical ectomycorrhizal fungi. *Research in Microbiology 156*(2):184-190.

Brundrett, M.C., 2017. Global diversity and importance of mycorrhizal and nonmycorrhizal plants. In *Biogeography of Mycorrhizal Symbiosis* 533-556. Springer, Cham.

Brundrett MC, Tedersoo L. 2018. Evolutionary history of mycorrhizal symbioses and global host plant diversity. *New Phytologist* 220:1108–1115.

Bruns, T.D., Bidartondo, M.I. and Taylor, D.L., 2002. Host specificity in ectomycorrhizal communities: what do the exceptions tell us?. *Integrative and Comparative Biology 42*(2):352-359.

Busby, R.R., Stromberger, M.E., Rodriguez, G., Gebhart, D.L. and Paschke, M.W., 2013. Arbuscular mycorrhizal fungal community differs between a coexisting native shrub and introduced annual grass. *Mycorrhiza 23*(2):129-141

Caffot, M.L.H., Pereyra, L.C., Robledo, G.L. and Domínguez, L.S., 2018. Ecology of gasteroid fungi (Agaricomycetidae and Phallomycetidae) affected by different successional stages of Polylepis australis Bitt. woodlands of Córdoba, central Argentina. *Nova Hedwigia 105*(3-4):3-4.

Calhim, S., Halme., Petersen, J.H., Læssøe, T., Bässler, C. and Heilmann-Clausen, J., 2018. Fungal spore diversity reflects substrate-specific deposition challenges. *Scientific Reports 8*(1): 1-9.

Carter, K.A., Smith, J.F., White, M.M. and Serpe, M.D., 2014. Assessing the diversity of arbuscular mycorrhizal fungi in semiarid shrublands dominated by Artemisia tridentata ssp. wyomingensis. *Mycorrhiza 24*(4):301-314.

Charrad, M., Ghazzali, N., Boiteau, V., Niknafs, A. and Charrad, M.M., 2014. Package ‘nbclust’. *Journal of statistical software 61*(6):1-36.

Cheek, M., Nic Lughadha, E., Kirk, Lindon, H., Carretero, J., Looney, B., Douglas, B., Haelewaters, D., Gaya, E., Llewellyn, T. and Ainsworth, A.M. 2020. New scientific discoveries: Plants and fungi. *Plants, People, Planet 2*(5):371-388.

Clemmensen, K.E., Bahr, A., Ovaskainen, O., Dahlberg, A., Ekblad, A., Wallander, H., Stenlid, J., Finlay, R.D., Wardle, D.A. and Lindahl, B.D., 2013. Roots and associated fungi drive long-term carbon sequestration in boreal forest. *Science 339*(6127):1615-1618.

Co-David, D., Langeveld, D. and Noordeloos, M.E., 2009. Molecular phylogeny and spore evolution of Entolomataceae. *Persoonia 23*:147.

Coetzee, M.P., Bloomer, Wingfield, M.J. and Wingfield, B.D. 2011. Paleogene radiation of a plant pathogenic mushroom. *Plos ONE 6*(12):p.e28545.

Coxall, H.K., Wilson. A., Pälike, H., Lear, C.H. and Backman, J., 2005. Rapid stepwise onset of Antarctic glaciation and deeper calcite compensation in the Pacific Ocean. *Nature 433*(7021):53-57.

Crowther, T.W., Boddy, L. and Jones, T.H., 2011. Outcomes of fungal interactions are determined by soil invertebrate grazers. *Ecology Letters 14*(11):1134-1142.

Crowther, T.W., Boddy, L. and Jones, T.H., 2012. Functional and ecological consequences of saprotrophic fungus–grazer interactions. *The ISME journal 6*(11):1992-2001.

de Wit, R., and Bouvier, T. 2006 'Everything is everywhere, but, the environment selects'; what did Baas Becking and Beijerinck really say? *Environmental Microbiology* 8:755–758.

Den Bakker, H.C., Zuccarello, G.C., Kuyper, T.W. and Noordeloos, M.E., 2004. Evolution and host specificity in the ectomycorrhizal genus Leccinum. *New Phytologist 163*(1):201-215.

Drummond AJ, Rambaut A. 2007. BEAST: Bayesian evolutionary analysis by sampling trees. *BMC Evolutionary Biology 7*:214.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUTi and the BEAST 1.7. *Molecular Biology and Evolution 29*:1969–1973.

Dupont-Nivet, G., Krijgsman, W., Langereis, C.G., Abels, H.A., Dai, S. and Fang, X., 2007. Tibetan plateau aridification linked to global cooling at the Eocene–Oligocene transition. *Nature 445*(7128):635-638.

Eberle, J.J. and Greenwood, D.R., 2012. Life at the top of the greenhouse Eocene world—A review of the Eocene flora and vertebrate fauna from Canada's High Arctic. *Bulletin 124*(1-2):3-23.

Ehleringer, J.R., Cerling, T.E. and Helliker, B.R., 1997. C 4 photosynthesis, atmospheric CO 2, and climate. *Oecologia 112*(3):285-299.

Eldrett, J.S., Greenwood, D.R., Harding, I.C. and Huber, M., 2009. Increased seasonality through the Eocene to Oligocene transition in northern high latitudes. *Nature 459*(7249):969-973.

Ekman, S., Andersen, H.L. and Wedin, M., 2008. The limitations of ancestral state reconstruction and the evolution of the ascus in the Lecanorales (lichenized Ascomycota). *Systematic Biology 57*(1):141-156.

Esqueda, M., Moreno, G., Perez-Silva, E., Sanchez, A. and Altes, A., 2004. The genus Tulostoma in Sonora, Mexico. *Mycotaxon 90*(2):409-422.

Esqueda, M., Coronado, M., Sánchez, A., Perez-Silva, E. and Herrera, T., 2006. Macromycetes of pinacate and great altar desert biosphere reserve, Sonora, Mexico. *Mycotaxon 95*(1):81-90.

Evans, S., Henrici, A. and Ing, B., 2006. Red data list of threatened British fungi. *Report by the British Mycological Society (BMS), Working with the Joint Nature Conservation Committee*.

Evans, R.D., 2001. Physiological mechanisms influencing plant nitrogen isotope composition. *Trends in Plant Science 6*(3):121-126.

Finlay, BJ. 2002. Global dispersal of free-living microbial eukaryote species. *Science 296*(5570):1061-1063.

Finlay, B.J. and Fenchel, T., 2004. Cosmopolitan metapopulations of free-living microbial eukaryotes. *Protist 155*(2):237-244.

Floudas, D., B.W. Held, R. Riley, and L.G. Nagy. 2015. Evolution of novel wood decay mechanisms in Agaricales revealed by the genome sequences of *Fistulina hepatica* and *Cylindrobasidium torrendii*. Fungal Genetics Biology 76: 78-92.

Frank, K.L., Geils, B.W., Kalkstein, L.S. and Thistle, H.W.2008. Synoptic climatology of the long-distance dispersal of white pine blister rust II. Combination of surface and upper-level conditions. *International Journal of Biometeorology 52*(7):653-666.

Fröhlich-Nowoisky, J., Burrows, S.M., Xie, Z., Engling, G., Solomon. A., Fraser, M.P., Mayol-Bracero, O.L., Artaxo. , Begerow, D., Conrad, R. and Andreae, M.O., 2012. Biogeography in the air: fungal diversity over land and oceans. *Biogeosciences 9*(3):1125-1136.

Galante, T.E., Horton, T.R. and Swaney, D.P., 2011. 95% of basidiospores fall within 1 m of the cap: a field-and modeling-based study. *Mycologia 103*(6):1175-1183.

Gange, A.C., Heegaard, E., Boddy, L., Andrew, C., Kirk. , Halvorsen, R., Kuyper, T.W., Bässler, C., Diez, J., Heilman-Clausen, J. and Høiland, K., 2018. Trait-dependent distributional shifts in fruiting of common British fungi. *Ecography 41*(1):51-61.

Gilbert, G.S., Gorospe, J. and Ryvarden, L. 2008. Host and habitat preferences of polypore fungi in Micronesian tropical flooded forests. *Mycological Research 112*(6):674-680.

Golan, J.J. and Pringle, A. 2017. Long-distance dispersal of fungi. *The Fungal Kingdom*:309-333.

Grubisha, L.C., Levsen, N., Olson, M.S. and Lee Taylor, D., 2012. Intercontinental divergence in the Populus-associated ectomycorrhizal fungus, *Tricholoma populinum*. *New Phytologist 194*(2):548-560.

Gube, M., 2009. *Ontogeny and phylogeny of gasteroid members of Agaricaceae (Basidiomycetes).* Doctoral dissertation. Friedrich-Schiller-Universität Jena, Germany. 145 pp.

Gube, M. and Dörfelt, H., 2011. Gasteromycetation in Agaricaceae s.l. (Basidiomycota): Morphological and ecological implementations. *Feddes Repertorium122*(5-6):367-390

Hably, L., Kvacek, Z. and Manchester, S.R., 2000. Shared taxa of land plants in the Oligocene of Europe and North America in context of Holarctic phytogeography. *Acta-Universitatis Carolinae Geologica (1):*59-74.

Halbwachs, H. and Bässler, C., 2015. Gone with the wind–a review on basidiospores of lamellate agarics. *Mycosphere 6*(1):78-112.

Halbwachs, H., Easton, G.L., Bol, R., Hobbie, E.A., Garnett, M.H., Peršoh, D., Dixon, L., Ostle, N., Karasch. and Griffith, G.W., 2018. Isotopic evidence of biotrophy and unusual nitrogen nutrition in soil-dwelling Hygrophoraceae. *Environmental Microbiology 20*(10):3573-3588.

Halbwachs, H., Heilmann-Clausen, J. and Bässler, C., 2017. Mean spore size and shape in ectomycorrhizal and saprotrophic assemblages show strong responses under resource constraints. *Fungal Ecology 26*:59-64.

Han, L.H., Feng, B., Wu, G., Halling, R.E., Buyck, B., Yorou, N.S., Ebika, S.T. and Yang, Z.L., 2018. African origin and global distribution patterns: Evidence inferred from phylogenetic and biogeographical analyses of ectomycorrhizal fungal genus *Strobilomyces*. *Journal of Biogeography 45*(1):201-212.

Hanson, S., 2008. An ecological study of the Tulostoma species in sandy habitats in SE Sweden. *Svensk Mykologisk Tidskrift 29*:93-109.

Hart S.C., Gehring C.A., Selmants P.C., and Deckert R.J., 2006. Carbon and nitrogen elemental and isotopic patterns in macrofungal sporocarps and trees in semiarid forests of the south-western USA. *Functional Ecology 20*(1):42-51.

Harrower, E., Bougher, N.L., Henkel, T.W., Horak, E. and Matheny. B., 2015. Long-distance dispersal and speciation of Australasian and American species of *Cortinarius* sect. *Cortinarius*. *Mycologia 107*(4):697-709.

Harder, C.B., Hesling, E., Botnen, S.S., Dima, B., von Bonsdorf-Salminen, T.K., Niskanen, T., Jarvis, S., Lorberau, K., Ouimette, A.P., Hester, A. and Hobbie, E.A., 2021. Mycena species can be opportunist-generalist plant root invaders. *bioRxiv*.

Hart, S.C., Gehring, C.A., Selmants. C. and Deckert, R.J., 2006. Carbon and nitrogen elemental and isotopic patterns in macrofungal sporocarps and trees in semiarid forests of the south-western USA. *Functional Ecology 20*(1):42-51.

Heckman, D.S., Geiser, D.M., Eidell, B.R., Stauffer, R.L., Kardos, N.L. and Hedges, S.B., 2001. Molecular evidence for the early colonization of land by fungi and plants. *Science 293*(5532):1129-1133.

Hassett, M.O., Fischer, M.W. and Money, N.P. 2015. Mushrooms as rainmakers: how spores act as nuclei for raindrops. *PloS One 10*(10):e0140407.

Hawksworth, D.L. and Lücking, R., 2017. Fungal diversity revisited: 2.2 to 3.8 million species. *The Fungal Kingdom* 79-95.

Hawksworth D.L., and Rossman A.Y., 1997. Where are all the undescribed fungi? *Phytopathology* 87(9):888-891.

He, M.Q., Zhao, R.L., Hyde, K.D., Begerow, D., Kemler, M., Yurkov, A., McKenzie, E.H., Raspe, O., Kakishima, M., Sanchez-Ramirez, S. and Vellinga, E.C., 2019. Notes, outline and divergence times of Basidiomycota. *Fungal Diversity 99*(1):105-367.

Herbert, H., Nikolai, F., Karl-Georg, B., Barbara, N., Sergej, S., Alexander, S. and Frank, B., 2019. The Eurasian steppe belt: Status quo, origin and evolutionary history. *Turczaninowia 22*(3).

Hosaka, K., Castellano, M.A. and Spatafora, J.W., 2008. Biogeography of Hysterangiales (Phallomycetidae, Basidiomycota). *Mycological Research 112*(4):448-462.

Hibbett, D. D., R. Bauer, M. Binder, A.J. Giachini, K. Hosaka, A. Justo, E. Larsson, K.H. Larsson, J.D. Lawrey, O. Miettinen, L. Nagy, R.H. Nilsson, M. Weiss, and R.G. Thorn. 2014. Agaricomycetes. Pp. 373-429 In: The Mycota, vol. VII, Second Ed., Part A. *Systematics and Evolution* (D. J. McLaughlin and J. W. Spatafora, eds.). Springer Verlag.

Hobbie, E.A., Grandy, A.S. and Harmon, M.E., 2020. Isotopic and compositional evidence for carbon and nitrogen dynamics during wood decomposition by saprotrophic fungi. *Fungal Ecology 45*:100915.

Hobbie E.A., Hobeber N.S., and Trappe J.M., 2001. Mycorrhizal vs saprotrophic status of fungi: the isotopic evidence. *New Phytologist* 1:601-10

Hobbie, E.A., Macko, S.A. and Shugart, H.H., 1999. Insights into nitrogen and carbon dynamics of ectomycorrhizal and saprotrophic fungi from isotopic evidence. *Oecologia 118*(3):353-360.

Hobbie, E.A., Grandy, A.S. and Harmon, M.E., 2020. Isotopic and compositional evidence for carbon and nitrogen dynamics during wood decomposition by saprotrophic fungi. *Fungal Ecology 45*:100915.

Hobbie, E.A., Weber, N.S. and Trappe, J.M., 2001. Mycorrhizal vs saprotrophic status of fungi: the isotopic evidence. *New Phytologist 150*(3):601-610.

He, M.Q., Zhao, R.L., Hyde, K.D., Begerow, D., Kemler, M., Yurkov, A., McKenzie, E.H., Raspe, O., Kakishima, M., Sanchez-Ramirez, S. and Vellinga, E.C. 2019. Notes, outline and divergence times of Basidiomycota. *Fungal Diversity 99*(1):105-367.

Herbert, H., Nikolai, F., Karl-Georg, B., Barbara, N., Sergej, S., Alexander, S. and Frank, B., 2019. The Eurasian steppe belt: Status quo, origin and evolutionary history. *Turczaninowia 22*(3).

Högberg. , Högberg, M.N., Quist, M.E., Ekblad, A.L.F. and Näsholm, T., 1999. Nitrogen isotope fractionation during nitrogen uptake by ectomycorrhizal and non-mycorrhizal Pinus sylvestris. *New Phytologist 142*(3):569-576.

Hosaka, K., Castellano, M.A. and Spatafora, J.W., 2008. Biogeography of Hysterangiales (Phallomycetidae, Basidiomycota). *Mycological Research 112*(4):448-462.

Hou, W., B. Lian, H. Dong, H. Jiang, and X. Wu. 2012. Distinguishing ectomycorrhizal and saprotrophic fungi using carbon and nitrogen isotopic conditions. *Geoscience Frontiers* 3(3):351-356.

Huang, W.Y., Cai, Y., Surveswaran, S., Hyde, K.D., Corke, H. and Sun, M., 2009. Molecular phylogenetic identification of endophytic fungi isolated from three *Artemisia* species. *Fungal diversity*.

Hussein, T., Norros, V., Hakala, J., Petäjä, T., Aalto. P., Rannik, Ü., Vesala, T. and Ovaskainen, O., 2013. Species traits and inertial deposition of fungal spores. *Journal of Aerosol Science 61*:81-98.

Hyvönen, R., Persson, T., Andersson, S., Olsson, B., Ågren, G.I. and Linder, S., 2008. Impact of long-term nitrogen addition on carbon stocks in trees and soils in northern Europe. *Biogeochemistry 89*(1):121-137.

Ivany, L.C., Patterson, W.P. and Lohmann, K.C., 2000. Cooler winters as a possible cause of mass extinctions at the Eocene/Oligocene boundary. *Nature 407*(6806):887-890.

Jacobs, B.F., Kingston, J.D. and Jacobs, L.L., 1999. The origin of grass-dominated ecosystems. *Annals of the Missouri Botanical Garden* 1:590-643.

Jeppson, M., 2008. The genus Tulostoma in Slovakia. *Catathelasma 10*:5-19.

Jeppson M., Altés A., Moreno G., Nilsson R., Loarce Y., de Bustos A., and Larsson E., 2017. Unexpected high species diversity among European stalked puffballs – a contribution to the phylogeny and taxonomy of the genus *Tulostoma* (Agaricales). *MycoKeys* 21:33-88.

Ju, Y.M., Rogers, J.D. and Hsieh, H.M. 2018. *Xylaria* species associated with fallen fruits and seeds. *Mycologia 110*(4):726-749.

Justo, A., Malysheva, E., Bulyonkova, T., Vellinga, E.C., Cobian, G., Nguyen, N., Minnis, A.M. and Hibbett, D.S., 2014. Molecular phylogeny and phylogeography of Holarctic species of *Pluteus* section *Pluteus* (Agaricales: Pluteaceae), with description of twelve new species. *Phytotaxa 180*(1):1-85.

Kalichman, J., Kirk. M., and Matheny. B., 2020. A compendium of generic names of agarics and Agaricales. *Taxon 69*(3):425-447.

Kantharaja, R. and Krishnappa, M., 2020. Morphological and molecular phylogenetic studies on *Battarrea phalloides* (Agaricales): a new report to Indian mycobiota. *Journal of Threatened Taxa*, *12*(8):15881-15888.

Kass R.E., and Raftery A.E., 1995. Bayes factors. Journal of the American Statistics Association. 90(430):773-95.

Kariman, K., Barker, S.J. and Tibbett, M., 2018. Structural plasticity in root-fungal symbioses: diverse interactions lead to improved plant fitness. *PeerJ*, *6*:e6030

Kim, K.S., Min, J.Y. and Dickman, M.B., 2008. Oxalic acid is an elicitor of plant programmed cell death during Sclerotinia sclerotiorum disease development. *Molecular Plant-Microbe Interactions 21*(5):605-612.

Kobmoo, N., Mongkolsamrit, S., Arnamnart, N., Luangsa-Ard, J.J. and Giraud, T., 2019. Population genomics revealed cryptic species within host-specific zombie-ant fungi (Ophiocordyceps unilateralis). *Molecular Phylogenetics and Evolution 140*:106580.

Ko, K.S., Lim, Y.W., Kim, Y.H. and Jung, H.S., 2001. Phylogeographic divergences of nuclear ITS sequences in Coprinus species sensu lato. *Mycological Research 105*(12):1519-1526.

Koch, R.A., Wilson, A.W., Séné, O., Henkel, T.W. and Aime, M.C. 2017. Resolved phylogeny and biogeography of the root pathogen *Armillaria* and its gasteroid relative, Guyanagaster. *BMC Evolutionary Biology17*(1):1-16.

Koch, R.A. and Herr, J.R., 2021. Transcriptomics reveals the mycoparasitic strategy of the mushroom *Entoloma abortivum* on species of the mushroom Armillaria. *bioRxiv*.

Kreisel, H. and Al-Fatimi, M., 2008. Further basidiomycetes from Yemen. *Feddes Repertorium 119*(5-6):463-483.

Laessøe, T. and Lodge, D.J., 1994. Three host-specific *Xylaria* species. *Mycologia 86*(3):436-446.

Landis, M.J., Matzke, N.J., Moore, B.R. and Huelsenbeck, J.P. 2013. Bayesian analysis of biogeography when the number of areas is large. *Systematic Biolog*y *62*(6):789-804.

Lawless. J., Baskin, J.M. and Baskin, C.C., 2006. Xeric limestone prairies of eastern United States: review and synthesis. *The Botanical Review 72*(3):235-272.

Lear, C.H., Bailey, T.R., Pearson. N., Coxall, H.K. and Rosenthal, Y., 2008. Cooling and ice growth across the Eocene-Oligocene transition. *Geology 36*(3):251-254.

Lewis. O., 2001. A likelihood approach to estimating phylogeny from discrete morphological character data. *Systematic Biology 50*(6):913-925.

Li, D.W. 2005. Release and dispersal of basidiospores from *Amanita muscaria* var. *alba* and their infiltration into a residence. *Mycological Research 109*(11):1235-1242.

Lickey, E.B., Hughes, K.W. and Petersen, R.H., 2002. Biogeographical patterns in *Artomyces pyxidatus*. *Mycologia 94*(3):461-471.

Lilleskov, E.A. and Bruns, T.D., 2005. Spore dispersal of a resupinate ectomycorrhizal fungus, *Tomentella sublilacina*, via soil food webs. *Mycologia 97*(4):762-769.

Lindahl, B.D., Ihrmark, K., Boberg, J., Trumbore, S.E., Högberg, Stenlid, J. and Finlay, R.D., 2007. Spatial separation of litter decomposition and mycorrhizal nitrogen uptake in a boreal forest. *New Phytologist 173*(3):611-620.

Lindahl, B.D. and Tunlid, A., 2015. Ectomycorrhizal fungi–potential organic matter decomposers, yet not saprotrophs. *New Phytologist 205*(4):1443-1447.

Linnaeus, C., 1753. Species plantarum–Holmiae: Laurentii Salvii.

Looney, B.P., Ryberg, M., Hampe, F., Sánchez-García, M. and Matheny. B. 2016. Into and out of the tropics: global diversification patterns in a hyperdiverse clade of ectomycorrhizal fungi. *Molecular Ecology 25*(2):630-647.

Long, W.H., 1944. Studies in the Gasteromycetes X. Seven New Species of *Tylostoma*. *Mycologia 36*(4):318-339.

Long, W.H., 1946a. Studies in the Gasteromycetes XII. Five Species of *Tylostoma* with Membranous Exoperidia. *Mycologia 38*(1):77-90.

Long, W.H., 1946b. Studies in the Gasteromycetes XIII. The Types of Miss White's Species of *Tylostoma*. *Mycologia 38*(2):171-179.

Long, W.H., 1947. Studies in the Gasteromycetes: XV. Notes on new or rare species of *Tylostoma*. *Lloydia 10*:115-135

Loptson, C.A., Lunt, D.J. and Francis, J.E., 2014. Investigating vegetation–climate feedbacks during the early Eocene. *Climate of the Past 10*(2):419-436.

Lowenstein, T.K. and Demicco, R.V., 2006. Elevated Eocene atmospheric CO2 and its subsequent decline. *Science 313*(5795):1928-1928.

Mao, K., Hao, G., Liu, J., Adams, R.P. and Milne, R.I., 2010. Diversification and biogeography of Juniperus (Cupressaceae): variable diversification rates and multiple intercontinental dispersals. *New Phytologist 188*(1):254-272.

Markert, J.A., Champlin, D.M., Gutjahr-Gobell, R., Grear, J.S., Kuhn, A., McGreevy, T.J., Roth, A., Bagley, M.J. and Nacci, D.E., 2010. Population genetic diversity and fitness in multiple environments. *BMC Evolutionary Biology 10*(1):1-13.

May, R.M., 1991. A fondness for fungi. *Nature 352*(6335):475-476.

Martin, F., Aerts, A., Ahrén, D., Brun, A., Danchin, E.G.J., Duchaussoy, F., Gibon, J., Kohler, A., Lindquist, E., Pereda, V. and Salamov, A., 2008. The genome of *Laccaria bicolor* provides insights into mycorrhizal symbiosis. *Nature 452*(7183):88-92.

Martin, R., Gazis, R., Skaltsas, D., Chaverri. and Hibbett, D., 2015. Unexpected diversity of basidiomycetous endophytes in sapwood and leaves of Hevea. *Mycologia 107*(2):284-297.

Matheny. B., Curtis, J.M., Hofstetter, V., Aime, M.C., Moncalvo, J.M., Ge, Z.W., Yang, Z.L., Slot, J.C., Ammirati, J.F., Baroni, T.J. and Bougher, N.L., 2006. Major clades of Agaricales: a multilocus phylogenetic overview. *Mycologia 98*(6):982-995.

Mayor J.R., Schuur E.A. andHenkel T.W., 2009. Elucidating the nutritional dynamics of fungi using stable isotopes. *Ecological Letters 20*(2):171-83.

Miller, M.A., Pfeiffer, W. and Schwartz, T., 2011, July. The CIPRES science gateway: a community resource for phylogenetic analyses. In Towns, J. (ed.) pp. 1-8 in *Proceedings of the 2011*

TeraGrid Conference: Extreme Digital Discovery. Association for Computing Machinery, 256 pp.

Miller, S.L. and Miller Jr, O.K., 1988. Spore release in hypogeous, gasteroid and agaricoid Russulales. *Transactions of the British Mycological Society*, *90*(4):513-526.

Moncalvo, J.M. and Buchanan. K., 2008. Molecular evidence for long distance dispersal across the Southern Hemisphere in the *Ganoderma applanatum-australe* species complex (Basidiomycota). *Mycological Research*. *112*(4):425-436.

Montesinos-Navarro, A., Valiente-Banuet, A. and Verdú, M., 2019. Mycorrhizal symbiosis increases the benefits of plant facilitative interactions. *Ecography 42*(3):447-455.

Moreno, G., Altés, A. and Wright, J.E., 1992. *Tulostoma squamosum, T. verrucosum* and *T. mussooriense* are the same species. *Mycotaxon 43*:61-68.

Moreno, G., Altés, A. and Hausknecht, A., 1995a. *Tulostoma opacum* Long in northern Africa. *Mycotaxon 46:* 68.

Moreno, G., Altés, A., Ochoa, C. and Wright, J.E., 1995b. Contribution to the study of the Tulostomataceae in Baja California, Mexico. I. *Mycologia 87*(1):96-120.

Moyersoen, B., Beever, R.E. and Martin, F. 2003. Genetic diversity of *Pisolithus* in New Zealand indicates multiple long-distance dispersal from Australia. *New Phytologist160*(3):569-579.

Morgan, A.P. 1890. North American fungi: third paper. *Journal of the Cincinnati Society of Natural History* 12:165.

Murata, H., Yamada, A., Maruyama, T., Endo, N., Yamamoto, K., Ohira, T. and Shimokawa, T., 2013. Root endophyte interaction between ectomycorrhizal basidiomycete *Tricholoma matsutake* and arbuscular mycorrhizal tree Cedrela odorata, allowing in vitro synthesis of rhizospheric “shiro”. *Mycorrhiza 23*(3):235-242.

Murata, H., Yamada, A., Yokota, S., Maruyama, T., Endo, N., Yamamoto, K., Ohira, T. and Neda, H., 2014. Root endophyte symbiosis in vitro between the ectomycorrhizal basidiomycete *Tricholoma matsutake* and the arbuscular mycorrhizal plant Prunus speciosa. *Mycorrhiza 24*(4):315-321.

Nara, K., 2009. Spores of ectomycorrhizal fungi: ecological strategies for germination and dormancy. *New Phytologist 181*(2):245-248.

Nehls, U. and Plassard, C., 2018. Nitrogen and phosphate metabolism in ectomycorrhizas. *New Phytologist 220*(4):1047-1058.

Newsham, K.K., Garnett, M.H., Robinson, C.H. and Cox, F., 2018. Discrete taxa of saprotrophic fungi respire different ages of carbon from Antarctic soils. *Scientific Reports 8*(1):1-10.

Niveiro, N., Ramírez, N.A., Michlig, A., Lodge, D.J. and Aime, M.C., 2020. Studies of Neotropical tree pathogens in *Moniliophthora*: a new species, *M. mayarum*, and new combinations for *Crinipellis ticoi* and *C. brasiliensis*. *MycoKeys 66*:39.

Norros, V., Penttilä, R., Suominen, M. and Ovaskainen, O., 2012. Dispersal may limit the occurrence of specialist wood decay fungi already at small spatial scales. *Oikos 121*(6):961-974.

Norros, V., Rannik, Ü., Hussein, T., Petäjä, T., Vesala, T. and Ovaskainen, O., 2014. Do small spores disperse further than large spores? *Ecology 95*(6):1612-1621.

Norros, V., Karhu, E., Nordén, J., Vähätalo, A.V. and Ovaskainen, O., 2015. Spore sensitivity to sunlight and freezing can restrict dispersal in wood-decay fungi. *Ecology and Evolution 5*(16):3312-3326.

Oda, T., Tanaka, C. and Tsuda, M., 2004. Molecular phylogeny and biogeography of the widely distributed *Amanita* species, *A. muscaria and A. pant henna*. *Mycological Research 108*(8):885-896.

Olsson. A., Mårtensson, L.M. and Bruun, H.H., 2009. Acidification of sandy grasslands–consequences for plant diversity. *Applied Vegetation Science 12*(3):350-361.

Olsson. A., Schnoor, T.K. and Hanson, S.Å., 2010. pH preferences of red-listed gasteromycetes in calcareous sandy grasslands: Implications for conservation and restoration. *Fungal Ecology 3*(4):357-365.

Olsson. A., Mårtensson, L.M. and Bruun, H.H., 2009. Acidification of sandy grasslands–consequences for plant diversity. *Applied Vegetation Science 12*(3):350-361.

Olsson. A., Schnoor, T.K. and Hanson, S.Å., 2010. pH preferences of red-listed gasteromycetes in calcareous sandy grasslands: Implications for conservation and restoration. *Fungal Ecology 3*(4):357-365.

Oneto, D.L., Golan, J., Mazzino, A., Pringle, A. and Seminara, A., 2020. Timing of fungal spore release dictates survival during atmospheric transport. *Proceedings of the National Academy of Sciences 117*(10):5134-5143.

Olsson. A., Mårtensson, L.M. and Bruun, H.H., 2009. Acidification of sandy grasslands–consequences for plant diversity. *Applied Vegetation Science 12*(3):350-361.

Olsson. A., Schnoor, T.K. and Hanson, S.Å., 2010. pH preferences of red-listed gasteromycetes in calcareous sandy grasslands: Implications for conservation and restoration. *Fungal Ecology 3*(4):357-365.

Orchard, S., Hilton, S., Bending, G.D., Dickie, I.A., Standish, R.J., Gleeson, D.B., Jeffery, R.P., Powell, J.R., Walker, C., Bass, D. and Monk, J., 2017. Fine endophytes (*Glomus tenue*) are related to Mucoromycotina, not Glomeromycota. *New Phytologist 213*(2):481-486.

Osono, T., 2007. Ecology of ligninolytic fungi associated with leaf litter decomposition. *Ecological Research 22*(6):955-974.

Pagani, M., Zachos, J.C., Freeman, K.H., Tipple, B. and Bohaty, S., 2005. Marked decline in atmospheric carbon dioxide concentrations during the Paleogene. *Science 309*(5734):600-603.

Pagel, M., 1999. Inferring the historical patterns of biological evolution. *Nature 401*(6756):877-884.

Pagel, M. and Meade, A., 2006. Bayesian analysis of correlated evolution of discrete characters by reversible-jump Markov chain Monte Carlo. *The American Naturalist 167*(6):808-825.

Pagel, M., Meade, A. and Barker, D., 2004. Bayesian estimation of ancestral character states on phylogenies. *Systematic Biology 53*(5):673-684.

Paradis, E., Claude, J. and Strimmer, K., 2004. APE: analyses of phylogenetics and evolution in R language. *Bioinformatics 20*(2):289-290.

Persoon C.H., 1794. Neuer Versuch einer systematischen Eintheilung der Schwämme. Neues Magazin für die Botanik 1: 63–128.

Persoon C.H., 1801. Synopsis Methodica Fungorum. Gottingae.

Pearson. N. and Palmer, M.R., 2000. Atmospheric carbon dioxide concentrations over the past 60 million years. *Nature 406*(6797):695-699.

Peay, K.G., Garbelotto, M. and Bruns, T.D. 2010. Evidence of dispersal limitation in soil microorganisms: isolation reduces species richness on mycorrhizal tree islands. *Ecology 91*(12):3631-3640.

Peng, L., Shan, X., Yang, Y., Wang, Y., Druzhinina, I.S., Pan, X., Jin, W., He, X., Wang, X., Zhang, X. and Martin, F.M., 2021. Facultative symbiosis with a saprotrophic soil fungus promotes potassium uptake in American sweetgum trees. *Plant, Cell and Environment*.

Pound, M.J., Haywood, A.M., Salzmann, U. and Riding, J.B., 2012. Global vegetation dynamics and latitudinal temperature gradients during the Mid to Late Miocene (15.97–5.33 Ma). *Earth-Science Reviews 112*(1-2):1-22.

Pressel, S., Bidartondo, M.I., Field, K.J. and Duckett, J.G., 2021. Advances in understanding of mycorrhizal-like associations in bryophytes. *Bryophyte Diversity and Evolution 43*(1):284-306.

Pringle, A., Vellinga, E. and Peay, K., 2015. The shape of fungal ecology: does spore morphology give clues to a species' niche? *Fungal Ecology 100*(17):213-216.

Purhonen, J., Ovaskainen, O., Halme. O., Komonen, A., Huhtinen, S., Kotiranta, H., Læssøe, T. and Abrego, N., 2020. Morphological traits predict host-tree specialization in wood-inhabiting fungal communities. *Fungal Ecology 46*: 100863.

Qhanya, L.B., Matowane, G., Chen, W., Sun, Y., Letsimo, E.M., Parvez, M., Yu, J.H., Mashele, S.S. and Syed, K., 2015. Genome-wide annotation and comparative analysis of cytochrome P450 monooxygenases in Basidiomycete biotrophic plant pathogens. *PLoS One 10*(11):e0142100.

Queloz, V., Sieber, T.N., Holdenrieder, O., McDonald, B.A. and Grünig, C.R., 2011. No biogeographical pattern for a root-associated fungal species complex. *Global Ecology and Biogeography 20*(1):160-169.

R. Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Rambaut, A., 2009. FigTree v1. 3.1. *http://tree. bio. ed. ac. uk/software/figtree/*.

Rayner, A.D. and Boddy, L., 1988. *Fungal decomposition of wood. Its biology and ecology*. John Wiley & Sons Ltd.

Read, D.J., Duckett, J.G., Francis, R., Ligrone, R. and Russell, A., 2000. Symbiotic fungal associations in 'lower 'land plants. *Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences 355*(1398):815-831.

Ree R.H. and Smith S.E., 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology* 57:4–14.

Ree, R.H. and Sanmartín, I., 2018. Conceptual and statistical problems with the DEC+ J model of founder-event speciation and its comparison with DEC via model selection. *Journal of Biogeography 45*(4):741-749.

Ree, R.H. and Smith, S.A. 2008. Maximum likelihood inference of geographic range evolution by dispersal, local extinction, and cladogenesis. *Systematic Biology 57*(1):4-14.

Revell, L.J., 2012. phytools: An R package for phylogenetic comparative biology (and other things). *Methods in Ecology and Evolution 3*(2):217-223.

Reynolds, H.T., 2011. *Systematics, phylogeography, and ecology of Elaphomycetaceae* (Doctoral dissertation, Department of Biology, Duke University).

Riffle, J.W., 1968. Effect of an Aphelenchoides species on growth of mycorrhizal fungi. *Nematologica* 14 1. 14.

Rimóczi, I., 1987. Ecology, cenology, and distribution of the giant puff-ball (*Langermannia gigantea* (Batsch ex Pers.) Rostk.) in Hungary. *Acta Botanica Hungarica 33*(3):279-294.

Roper, M., Pepper, R.E., Brenner, M.P. and Pringle, A., 2008. Explosively launched spores of ascomycete fungi have drag-minimizing shapes. *Proceedings of the National Academy of Sciences 105*(52):20583-20588.

Rozema, J., Laan. ?, Broekman, R., Ernst, W.H.O. and Appelo, C.A.J., 1985. On the lime transition and decalcification in the coastal dunes of the province of North Holland and the island of Schiermonnikoog. *Acta Botanica Neerlandica 34*(4):393-411.

Rusevska, K., de Diego Calonge, F., Karadelev, M. and MartÍn, M.P., 2019. Fungal DNA barcode (ITS nrDNA) reveals more diversity than expected in *Tulostoma* from Macedonia. *Turkish Journal of Botany 43*(1):102-115.

Ryberg, M. and Matheny. B., 2012. Asynchronous origins of ectomycorrhizal clades of Agaricales. *Proceedings of the Royal Society B: Biological Sciences 279*(1735):2003-2011.

Saitta, A., Anslan, S., Bahram, M., Brocca, L. and Tedersoo, L. 2018. Tree species identity and diversity drive fungal richness and community composition along an elevational gradient in a Mediterranean ecosystem. *Mycorrhiza 28*(1):39-47.

Salzmann, U., Dolan, A.M., Haywood, A.M., Chan, W.L., Voss, J., Hill, D.J., Abe-Ouchi, A., Otto-Bliesner, B., Bragg, F.J., Chandler, M.A. and Contoux, C., 2013. Challenges in quantifying Pliocene terrestrial warming revealed by data–model discord. *Nature Climate Change 3*(11):969-974.

Sánchez-García, M. and Matheny. B., 2017. Is the switch to an ectomycorrhizal state an evolutionary key innovation in mushroom-forming fungi? A case study in the Tricholomatineae (Agaricales). *Evolution 71*(1):51-65.

Sánchez-García, M., Matheny. B., Palfner, G. and Lodge, D.J., 2014. Deconstructing the Tricholomataceae (Agaricales) and introduction of the new genera Albomagister, Corneriella, Pogonoloma and Pseudotricholoma. *Taxon 63*(5):993-1007.

Sánchez-Ramírez, S., Wilson, A.W. and Ryberg, M. 2017. Overview of phylogenetic approaches to mycorrhizal biogeography, diversity and evolution. *Biogeography of Mycorrhizal Symbiosis*. 1-37.

Sánchez-García, M., Ryberg, M., Khan, F.K., Varga, T., Nagy, L.G. and Hibbett, D.S., 2020. Fruiting body form, not nutritional mode, is the major driver of diversification in mushroom-forming fungi. *Proceedings of the National Academy of Sciences 117*(51):32528-32534.

Sato, H., Ohta, R. and Murakami, N., 2020. Molecular prospecting for cryptic species of the *Hypholoma fasciculare* complex: toward the effective and practical delimitation of cryptic macrofungal species. *Scientific Reports 10*(1):1-13.

Seitzman biotrophy in Hygrophoraceae inferred from, B.H., Ouimette, A., Mixon, R.L., Hobbie, E.A. and Hibbett, D.S., 2011. Conservation of combined stable isotope and phylogenetic analyses. *Mycologia 103*(2):280-290.

Serpe, M.D., Thompson, A. and Petzinger, E., 2020. Effects of a companion plant on the formation of mycorrhizal propagules in Artemisia tridentata seedlings. *Rangeland Ecology and Management 73*(1):138-146.

Shepherd, L.D. and Cooper, J.A., 2018. First record of the fungus *Battarrea phalloides* (Agariacaceae) in New Zealand. *New Zealand Journal of Botany 56*(1):109-114.

Skrede, I., Engh, I.B., Binder, M., Carlsen, T., Kauserud, H. and Bendiksby, M., 2011. Evolutionary history of Serpulaceae (Basidiomycota): molecular phylogeny, historical biogeography and evidence for a single transition of nutritional mode. *BMC Evolutionary Biology 11*(1):1-13.

Shi, L., Guttenberger, M., Kottke, I. and Hampp, R., 2002. The effect of drought on mycorrhizas of beech (*Fagus sylvatica* L.): changes in community structure, and the content of carbohydrates and nitrogen storage bodies of the fungi. *Mycorrhiza 12*(6):303-311.

Smith, G.R., Finlay, R.D., Stenlid, J., Vasaitis, R. and Menkis, A., 2017. Growing evidence for facultative biotrophy in saprotrophic fungi: data from microcosm tests with 201 species of wood-decay basidiomycetes. *New Phytologist 215*(2):747-755

Sousa, J.O., Suz, L.M., García, M.A., Alfredo, D.S., Conrado, L.M., Marinho, Ainsworth, A.M., Baseia, I.G. and Martín, M.P., 2017. More than one fungus in the pepper pot: Integrative taxonomy unmasks hidden species within *Myriostoma coliforme* (Geastraceae, Basidiomycota). *Plos One 12*(6):e0177873.

Sun, J., Ni, X., Bi, S., Wu, W., Ye, J., Meng, J. and Windley, B.F., 2014. Synchronous turnover of flora, fauna and climate at the Eocene–Oligocene Boundary in Asia. *Scientific Reports 4*(1):1-6.

Sunhede, S. 1976. Spore liberation in *Tulostoma* (Gasteromycetes). Windahlia 5-6: 68-70.

R. Core Team. 2013. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.

Talbot, J.M., Bruns, T.D., Smith, D.P., Branco, S., Glassman, S.I., Erlandson, S., Vilgalys, R. and Peay, K.G., 2013. Independent roles of ectomycorrhizal and saprotrophic communities in soil organic matter decomposition. *Soil Biology and Biochemistry 57*:282-291.

Tedersoo, L., Bahram, M., Ryberg, M., Otsing, E., Kõljalg, U. and Abarenkov, K. 2014. Global biogeography of the ectomycorrhizal/sebacina lineage (Fungi, Sebacinales) as revealed from comparative phylogenetic analyses. *Molecular Ecology 23*(16):4168-4183.

Tedersoo, L. and Smith, M.E., 2013. Lineages of ectomycorrhizal fungi revisited: foraging strategies and novel lineages revealed by sequences from belowground. *Fungal BiologyReviews 27*(3-4):83-99.

Team R.C. 2015. R: A language and environment for statistical computing.

Thoen, E., Harder, C.B., Kauserud, H., Botnen, S.S., Vik, U., Taylor, A.F., Menkis, A. and Skrede, I., 2020. In vitro evidence of root colonization suggests ecological versatility in the genus *Mycena*. *New Phytologist 227*(2):601-612.

Tiffney, B.H. and Manchester, S.R., 2001. The use of geological and paleontological evidence in evaluating plant phylogeographic hypotheses in the Northern Hemisphere Tertiary. *International Journal of Plant Sciences 162*(S6):S3-S17.

Tomaszewska, A., Luszczynski, J., Luszczynska, B. and Jaworska, J., 2012. The contribution and importance of rare macrofungi in selected plots of endangered xerothermic grasslands in the Nida Basin. *Acta Agrobotanica 65*(2).

Török. T., Neuffer, B., Heilmeier, H., Bernhardt, K.G. and Wesche, K., 2020. Climate, landscape history and management drive Eurasian steppe biodiversity. *Flora* 271:15685.

de Tournefort, J.P., 1700. *Institutiones rei herbariae* (Vol. 1). e Typographia regia.

Trappe, J.M., 1962. Fungus associates of ectotrophic mycorrhizae. *The Botanical Review 28*(4):538-606.

Tyler, T., 2005. The Bryophyte flora of Scanian sand-steppe vegetation and its relation to soil pH and phosphate availability. *Lindbergia* 1:11-20.

Udvardy, M.D. and Udvardy, M.D.F., 1975. *A classification of the biogeographical provinces of the world* (Vol. 8). Morges: International Union for Conservation of Nature and Natural Resources.

Ulrich, B., 1991. An ecosystem approach to soil acidification. In *Soil Acidity* (pp. 28-79). Springer, Berlin, Heidelberg.

van der Heijden, M.G., Martin, F.M., Selosse, M.A. and Sanders, I.R., 2015. Mycorrhizal ecology and evolution: the past, the present, and the future. *New Phytologist 205*(4):1406-1423.

van der Gast, C.J., 2015. Microbial biogeography: the end of the ubiquitous dispersal hypothesis? *Environmental Microbiology 17*(3):544-546.

Vanderpoorten A. and Goffinet B., 2006. Mapping uncertainty and phylogenetic uncertainty in ancestral character state reconstruction: an example in the moss genus *Brachytheciastrum*. *Systematic Biology* 55(6):957-71.

van der Wal, A., Geydan, T.D., Kuyper, T.W. and De Boer, W., 2013. A thready affair: linking fungal diversity and community dynamics to terrestrial decomposition processes. *FEMS Microbiology Reviews 37*(4):477-494.

Vašutová, M., Mleczko. V, López-García, A., Maček, I., Boros, G., Ševčík, J., Fujii, S., Hackenberger, D., Tuf, I.H., Hornung, E. and Páll-Gergely, B., 2019. Taxi drivers: the role of animals in transporting mycorrhizal fungi. *Mycorrhiza 29*(5):413-434.

Vaz, A.B.M., Fonseca. L.C., Silva, F.F., Quintanilha-Peixoto, G., Sampedro, I., Siles, J.A., Carmo, A., Kato, R.B., Azevedo, V., Badotti, F. and Ocampo, J.A., 2020. Foliar mycoendophytome of an endemic plant of the Mediterranean biome (*Myrtus communis*) reveals the dominance of basidiomycete woody saprotrophs. *PeerJ*, *8*. e10487.

Vellinga, E.C., 2004. Ecology and distribution of lepiotaceous fungi (Agaricaceae)-A review. *Nova Hedwigia 78*(3):273-300.

Vellinga, E.C. 2004. Genera in the family Agaricaceae: evidence from nrITS and nrLSU sequences. *Mycological Research 108*(4):354-377.

Větrovský, T., Kohout S., Kopecký, M., Machac, A., Man, M., Bahnmann, B.D., Brabcová, V., Choi, J., Meszárošová, L., Human, Z.R. and Lepinay, C., 2019. A meta-analysis of global fungal distribution reveals climate-driven patterns. *Nature Communications 10*(1):1-9.

Vizzini, A. and Ercole, E., 2017. Detecting the phylogenetic position of *Bovista acuminata* (Agaricales, Basidiomycota) by an ITS-LSU combined analysis: the new genus *Bryoperdon* and revisitation of *Lycoperdo*n subgen. *Apioperdon*. *Phytotaxa 299*(1):77-86.

Udvardy, M.D. and Udvardy, M.D.F., 1975. *A classification of the biogeographical provinces of the world* (Vol. 8). Morges: International Union for Conservation of Nature and Natural Resources.

Wake, D.B. and Larson, A., 1987. Multidimensional analysis of an evolving lineage. *Science 238*(4823):42-48.

Wake, D.B., 1991. Homoplasy: the result of natural selection, or evidence of design limitations? *The American Naturalist 138*(3):543-567.

Wang, B., Yeun, L.H., Xue, J.Y., Liu, Y., Ané, J.M. and Qiu, Y.L., 2010. Presence of three mycorrhizal genes in the common ancestor of land plants suggests a key role of mycorrhizas in the colonization of land by plants. *New Phytologist 186*(2):514-525

Wassenaar, L. I., and K. A. Hobson. 1998. Natal Origins of Migratory Monarch Butterflies At Wintering Colonies in Mexico : New Isotopic Evidence. PNAS 95: 4.

Wesche, K., Ambarlı, D., Kamp, J., Török T., Treiber, J. and Dengler, J., 2016. The Palaearctic steppe biome: a new synthesis. *Biodiversity and Conservation 25*(12):2197-2231.

Wickham, H. 2009. ggplot2: elegant graphics for data analysis. New York, NY: Springer-Verlag.

Willard, D.A., Donders, T.H., Reichgelt, T., Greenwood, D.R., Sangiorgi, F., Peterse, F., Nierop, K.G., Frieling, J., Schouten, S. and Sluijs, A., 2019. Arctic vegetation, temperature, and hydrology during Early Eocene transient global warming events. *Global and Planetary Change 178*:139-152.

Willis, K. and McElwain, J., 2014. *The Evolution of Plants*. Oxford University Press.

Wilson, A.W., Binder, M. and Hibbett, D.S., 2011. Effects of gasteroid fruiting body morphology on diversification rates in three independent clades of fungi estimated using binary state speciation and extinction analysis. *Evolution: International Journal of Organic Evolution 65*(5):1305-1322.

Wilson, A.W., Hosaka, K. and Mueller, G.M., 2017. Evolution of ectomycorrhizas as a driver of diversification and biogeographic patterns in the model mycorrhizal mushroom genus Laccaria. *New Phytologist 213*(4):1862-1873.

Wilson, A.W., Hosaka, K. and Mueller, G.M., 2017. Evolution of ectomycorrhizas as a driver of diversification and biogeographic patterns in the model mycorrhizal mushroom genus Laccaria. *New Phytologist 213*(4):1862-1873.

Wolfe, B.E., Tulloss, R.E. and Pringle, A., 2012. The irreversible loss of a decomposition pathway marks the single origin of an ectomycorrhizal symbiosis. *PloS one 7*(7):e39597.

Wood, S. N., 2006. Generalized Additive Models: An Introduction with R, C. and Hall [ed.]. CRC Press.

Wösten, H.A. and Wessels, J.G., 1997. Hydrophobins, from molecular structure to multiple functions in fungal development. *Mycoscience 38*(3):363-374.

Wright J. E., 1987. The Genus *Tulostoma* (Gasteromycetes): A World Monograph. *Bibliotheca Mycologia* 113 J. Cramer, Berlin, Germany. 338 pp.

Wright, J.E., 1987. Two new species of *Tulostoma* (Gasteromycetes) from Texas. *Mycologia*, *79*(1):155-157.

Yu Y, Harris AJ, Blair C, and He X. 2015. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Molecular Phylogenetics and Evolution* 87:46–9.

Xiang, Q.Y., Manchester, S.R., Thomas, D.T., Zhang, W. and Fan, C., 2005. Phylogeny, biogeography, and molecular dating of cornelian cherries (*Cornus*, Cornaceae): tracking Tertiary plant migration. *Evolution 59*(8):1685-1700.

Zanazzi, A., Kohn, M.J., MacFadden, B.J. and Terry, D.O., 2007. Large temperature drop across the Eocene–Oligocene transition in central North America. *Nature 445*(7128):639-642.

Zhou, D. and Hyde, K.D., 2001. Host-specificity, host-exclusivity, and host-recurrence in saprobic fungi. *Mycological Research 105*(12):1449-1457.

Appendices

Appendix 2.1. Collection and sequence information

Taxon	Voucher	locality/year	ITS -- LSU	Tef-1α	Source
soil isolate	OTU861	USA, CA 2016	MK019104		Gao 2018 Unpublished
soil isolate	OTU593	USA, CA 2016	MK018926		Gao 2018 Unpublished
soil isolate	OTU938	USA, CA 2016	MK018794		Gao 2018 Unpublished
soil isolate	**OTU1282**	USA, CA 2016	MK019154		Gao 2018 Unpublished
T. aff. *cretaceum*	BPI 863658	USA_NM	X		Gube 2009
T. aff. *cretaceum*	Knudsen 01.79n (C)	Russia, 2001	KU518993	KU843988	Jeppson et al. 2017
T. aff. *cretaceum*	Jeppson 6103 (GB)	Hungary, 2001	KU518995	KU843994	Jeppson et al. 2017
T. aff. cretaceum	Jeppson 7759 (GB)	Hungary, 2006	KU518996	KU843995	Jeppson et al. 2017
T. aff. *cretaceum*	Jeppson 3821 (GB)	Hungary, 1997	KU518994	KU843993	Jeppson et al. 2017
T. aff. *cretaceum*	Jeppson 9304 (GB)	Spain, 2011	KU519000	KU843990	Jeppson et al. 2017
T. aff. *cretaceum*	Barrasa (AH 3995 -- GB)	Spain, 1983	KU518999	KU843992	Jeppson et al. 2017
T. aff. *cretaceum*	Jeppson 6194 (GB)	Spain, 2003	KU518997	KU843989	Jeppson et al. 2017
T. aff. Leiosporum	MG 071016_8	Hungary	X		This study
T. ahmadii	HUPSH33	Pakistan 2013	KP738711		Hussain et al. 2016
T. ahmadii	HUPSH33b	Pakistan 2014	KP738712		Hussain et al. 2016
T. albicans	Cope (NY) -- holotype	USA, Texas, 1893	KX576548		Jeppson et al. 2017
T. beccarianum	BCC-MPM 1663		AF097752		Martin and Johannesson 2000
T. beccarianum	Finy 2 (GB)	Hungary, 2014	KU519076	KU843959	Jeppson et al. 2017
T. beccarianum	Beccari (S) -- holotype	Italy	KX640979		Jeppson et al. 2017
T. beccarianum	Brůžek 140115--1 (GB)	Slovakia, 2014	KU519074	KU843958	Jeppson et al. 2017
T. berkeleyi	JLH MyCoPortal 6604754	USA, NY 2017	MK578704		Russel and Horman NAMycoflora Project
T. brumale	Jeppson 6427 (GB)	Sweden, 1998	KU519064	KU843947	Jeppson et al. 2017

T. brumale	Finy 9 (GB)	Hungary, 2011	KU519059	KU843944	Jeppson et al. 2017
T. brumale	JE Diedicke ex Henkel 1.-10.3.19	Germany	X		Gube 2009
T. brumale	MG 061115_1	Germany	X		Gube 2009
T. brumale	MG549 JE Walther 18.3.44	Macedonia	X		Gube 2009
T. brumale	MG456 MG 071020_16	Hungary	X		Gube 2009
T. brumale	Eckblad (O--58849)	Norway, 1950	KU519062	KU853949	Jeppson et al. 2017
T. brumale	Brůžek 140112 (GB)	Czech Republic, 2014	KU519061	KU843948	Jeppson et al. 2017
T. brumale	Jeppson 8372 (GB)	France 2007	KU519056		Jeppson et al. 2017
T. brumale	Jeppson 7532 (GB)	Slovakia 2005	KU519058		Jeppson et al. 2017
T. brumale	Jeppson 5785 (GB)	Sweden, 2001	KU519063	KU843946	Jeppson et al. 2017
T. calcareum	DBG21975	USA, CO 2009	X	X	This study
T. calcareum	Finy 4 (GB)	Hungary, 2013	KU519088	KU843895	Jeppson et al. 2017
T. calcareum	Breili S0133 (GB)	Norway, 2010	KU519081	KU843876	Jeppson et al. 2017
T. calcareum	Aarnaes 2010--1 (GB)	Spain, 2010	KU519083	KU843877	Jeppson et al. 2017
T. calcareum	Aarnaes 2010--1 (GB)	Spain, 2010	KU519083	KU843877	Jeppson et al. 2017
T. calcareum	Jeppson 6965 (GB) -- holotype	Sweden, 2004	KU519086	KU843881	Jeppson et al. 2017
T. calongei	Jeppson 8773 (GB) -- holotype	Spain, 1989	KU518973	KU844000	Jeppson et al. 2017
T. calongei	Álvarez (AH 13718)	Spain, 1978	KC333067		Jeppson et al. 2017
T. cf.*pygmaeum*	Bruzek131207	Slovakia	KU51904	KU843931	Jeppson et al. 2017
T. cretaceum	JE Long #9302 7.5.41 TYPE	USA, NM 1941	X		Gube 2009
T. cyclophorum	Silverio--Reyes (AH 19564 -- GB)	Argentina, 1998	KU518984	KU843962	Jeppson et al. 2017
T. cyclophorum	Jeppson 8862 (GB)	Hungary, 2009	KU518985	KU843963	Jeppson et al. 2017
T. cyclophorum	Gonzáles (AH 16885, GB)	Spain, 1994	KU518983	KU843961	Jeppson et al. 2017
T. domingueziae	MLHC3 (CORD)	Argentina, 2003	HQ667593		Hernández Caffot et al. 2011
T. domingueziae	MLHC 24 (CORD) – holotype	Argentina, 2003	HQ667594		Hernández Caffot et al. 2011
T. eckbladii	Jonsell (TRH 9565)	Norway, 1993	KU519069	KU843952	Jeppson et al. 2017
T. eckbladii	Jørstad (O F--58850) -- holotype	Norway, 1957	KU519068	KU843951	Jeppson et al. 2017
T. egranuslosum	Lloyd15424, BPI 704420-holotype	Australia, Mel	KC333072		Jeppson et al. 2017
T. excentricum	Lloyd 15424 (BPI) -- holotype	USA, New Mexico	KU519055		Jeppson et al. 2017

T. fimbriatum	Webber (NY) -- lectotype T. campestre	USA, Nebraska	KC333075		Jeppson et al. 2017
T. fimbriatum	DBG27836	USA, CO 2001	X	X	This study
T. fimbriatum	DBG23705	USA, CO 2007	X	X	This study
T. fimbriatum	DBG27845	USA, CO 2001	X	X	This study
T. fimbriatum	DBG23042	USA, CO 2005	X	X	This study
T. fimbriatum	DBG28849	USA, CO 2013	X	X	This study
T. fimbriatum	DBG27847	USA, CO 2001	X	X	This study
T. fimbriatum	Knudsen00.190e ©	Russia, 2001	KU518971	KU843911	Jeppson et al. 2017
T. fimbriatum	Jeppson 5795 (GB)	Sweden, 2001	KU518961	KU843903	Jeppson et al. 2017
T. fimbriatum	Jeppson 6636 (GB)	Hungary, 2004	KU518962	KU843906	Jeppson et al. 2017
T. fimbriatum	Jeppson 060330 (GB)	Sweden, 2006	KU518965	KU843908	Jeppson et al. 2017
T. fimbriatum	Jeppson 7351 (GB)	Slovakia, 2014	KU518970	KU843907	Jeppson et al. 2017
T. fimbriatum	Finy 8 (GB)	Hungary, 2012	KU518968	KU843912	Jeppson et al. 2017
T. fimbriatum	Jeppson 7025 (GB)	Spain, 2004	KU518966	KU843910	Jeppson et al. 2017
T. fimbriatum	MG 071016_20	Hungary	X		Gube 2009
T. fimbriatum	Månsson 991010 (GB) -- epitype	Sweden, 1999	KU518963	KU843904	Jeppson et al. 2017
T. fimbriatum	MG 071019_3	Hungary	X		Gube 2009
T. fimbriatum	Knudsen 01.71k	Russia, 2000	KU518972	KU843914	Jeppson et al. 2017
T. giovanellae	Patouillard 4693 (BPI)	Africa	KU519073		Jeppson et al. 2017
T. giovanellae	Jeppson 8706 (GB)	Spain, 2009	KU519071	KU843954	Jeppson et al. 2017
T. giovanellae	Jeppson 9059 (GB)	Spain 2009	KU519070	KU843953	Jeppson et al. 2017
T. grandisporum	Jeppson 8907 (GB) -- holotype	Hungary, 2011	KU519003	KU843924	Jeppson et al. 2017
T. grandisporum	Brůžek 131208 (GB)	Slovakia, 2013	KU519006	KU843923	Jeppson et al. 2017
T. grandisporum	MICH338158	USA, NM 1967	X	X	This study
T. kotlabae	MJ6623	Hungary	DQ112629		Larsson and Jeppson 2005
T. kotlabae	MG 071017_11	Hungary	X		Gube 2009
T. kotlabae	Kotlaba (PRM 704203) -- holotype	Slovakia, 1954	KX576544		Jeppson et al. 2017
T. kotlabae	Jeppson 7441 (GB)	Denmark, 2005	KU519026	KU843975	Jeppson et al. 2017
T. kotlabae	Jeppson 7923 (GB)	Slovakia, 2006	KU519027	KU843973	Jeppson et al. 2017

T. kotlabae	Jeppson 5597 (GB)	Sweden, 2005	KU519023	KU843972	Jeppson et al. 2017
T. lloydii	Lahti 201210 (GB)	Italy, 2012	KU518990	KU843965	Jeppson et al. 2017
T. lloydii	Gómez (AH 11606)	Spain, 1988	KX583648		Jeppson et al. 2017
T. lloydii	González (AH 31156 -- GB)	Spain, 2003	KU518989		Jeppson et al. 2017
T. lusitanicum	Almeida et al. (LISU--MAG 8) -- holotype	Portugal, 1991	KX576542		Jeppson et al. 2017
T. lysocephalum	Long 9639 (BPI) -- holotype	USA, New Mexico, 194	KU519034		Jeppson et al. 2017
T. macrocephalum	MICH24877	USA, AZ 1970	X	X	This study
T. macrocephalum	Long 10113 (BPI)-TYPE	USA, NM 1942	KX576545		Jeppson et al. 2017
T. macrocephalum	MICH28572	USA, AZ 1972	X	X	This study
T. melanocyclum	JE Schnittle and Günther 3.4.89	Slovakia 1989	X		Gube 2009
T. melanocyclum	Månsson 011215 (GB)	Sweden, 2001	KU519102	KU843885	Jeppson et al. 2017
T. melanocyclum	Hanson 2008--247 (GB)	Sweden, 2008	KU519104	KU843887	Jeppson et al. 2017
T. melanocyclum	Jeppson 6036 (GB)	Hungary, 2002	KU519100	KU843884	Jeppson et al. 2017
T. melanocyclum	Knudsen 00.314 (C)	Russia, 2000	KU519099	KU843883	Jeppson et al. 2017
T. melanocyclum	**RBG Kew K(M)64453**	England	EU78443		Brock et al. 2009
T. melanocyclum	MG 071015_12	Hungary	X		Gube 2009
T. meristostoma	MICH266306	USA, NM 1967	X	X	This study
T. moravecii	**PRM 667204 – holotype**	Czech Republic 1952	KX576546		Jeppson et al. 2017
T. nanum	MJ4966	Hungary	KU519036	KU843968	Jeppson et al. 2017
T. nanum	MJ3787	Hungary	KU519035		Jeppson et al. 2017
T. nanum	MJ4976	Hungary	KU519037	KU843969	Jeppson et al. 2017
T. niveum	Jeppson 7692 (GB)	Sweden, 2005	KU519078	KU843932	Jeppson et al. 2017
T. niveum	Jeppson 7699 (GB)	Sweden, 2005	KU519079	KU843933	Jeppson et al. 2017
T. niveum	Jeppson 5229 (GB)	Sweden, 1999	KU519080	KU843934	Jeppson et al. 2017
T. obesum	Jeppson 8695 (GB)	Spain, 2008	KU518986	KU843985	Jeppson et al. 2017
T. obesum	Ayllón (AH 20901 -- GB)	Spain, 1995	KU518988	KU843987	Jeppson et al. 2017
T. obesum	Cooke 2715 (NY) -- isotype	USA, Colorado	KX576541		Jeppson et al. 2017
T. obscurum	DBG28400	USA, CO 2015	X	X	This study
T. pannonicum	Jeppson 990617a (GB)	Hungary, 1999	KU519008		Jeppson et al. 2017

T. pannonicum	Jeppson 7803 (GB)	Hungary, 2006	KU519011	KU843996	Jeppson et al. 2017
T. polymorphum	ILL00011723-TYPE	USA, NM 1941	X	X	This study
T. polymorphum	DBG24092	USA, CO 2007	X	X	This study
T. pseudopulchellum	Illana, Moreno, Altés (AH 11605) -- holotype	Spain, 1989	KX513827		Jeppson et al. 2017
T. pseudopulchellum	Illana, Moreno, Altés (AH 11603) -- paratype	Spain, 1989	KU519012	KU843997	Jeppson et al. 2017
T. pseudopulchellum	Moreno, Manjón, Altés (AH 11699)	Spain, 1989	KX640987	KX513825	Jeppson et al. 2017
T. pulchellum	Moravec 132/51 (UPS) -- paratype T. hollosii	Czech Republic, 1951	KX513825	KU843926	Jeppson et al. 2017
T. pulchellum	MICH28571	USA, AZ 1972	X	X	This study
T. pulchellum	JLH MyCoPortal 6604755	USA, NY 2017	X	X	Russel and Horman NAMycoflora Project
T. pulchellum	Ochoa, Altés (AH 12967--GB)	Mexico, 1987	KU518956	KU843928	Jeppson et al. 2017
T. pulchellum	MG 071016_25	Hungary	X		Gube 2009
T. pulchellum	MG 071016_40	Hungary	X		Gube 2009
T. pulchellum	MG 071016_9	Hungary	X		Gube 2009
T. pulchellum	Jeppson 6647 (GB)	Hungary, 2004	KU518955	KU843926	Jeppson et al. 2017
T. pulchellum	Fritz 2010--1 (GB)	Mongolia, 2010	KU518954	KU843925	Jeppson et al. 2017
T. pulchellum	PAD-holotype	Australia	KX761984		Altes et al. 2016 unpublished
T. punctatum	Jeppson 7472 (GB)	Slovakia, 2005	KU518952	KU843875	Jeppson et al. 2017
T. punctatum	MICH266257	USA, AZ	X	X	This study
T. punctatum	Bethel 21 (NY) -- holotype T. subfuscum	USA, CO 1896	KC333074		Jeppson et al. 2017
T. punctatum	DBG27849	USA, CO 2001	X	X	This study
T. punctatum	BPI 729033 (BPI) -- lectotype	USA, Kansas 1896	KC333071		Jeppson et al. 2017
T. punctilabratum	MICH24086	USA, CO 1956	X	X	This study
T. purpusii	MICH266212	USA, AZ 1973	X	X	This study
T. rufescens	UES 10528-holotype	Mexico, Sonora 2010	MF319226		Hernandez-Navarro et al. 2018
T. rufum	Lloyd 15542 (BPI)-- holotype	USA, Alabama	KU519107		Jeppson et al. 2017
T. simulans	DBG20766	USA, CO 2001	X	X	This study
T. simulans	Stockberger (PC) -- paratype T. mammosum var. simulant	USA, Ohio	KX576547		Jeppson et al. 2017

T. simulans	MICH266252	USA, CO 1956	X	X	This study
T. simulans	MG 071020_20	Hungary	X		Gube 2009
T. simulans	JE Schnittler&Günther 3.4.89 coll. mix.	Slovakia	X		Gube 2009
T. simulans	Jeppson 9302 (GB)	Spain, 2011	KU519042	KU843935	Jeppson et al. 2017
T. simulans	PRM667204-TYPE	Czech Republic, 2013	KX5765461	KU843936	Jeppson et al. 2017
T. simulans	GG1810	Argentina	MN338578		Gonzalez et al. 2019 unpublished
T. sp.	MG 071020_3	Hungary	X		Gube 2009
T. sp.	**MLHC200 (CORD)**	Argentina 2007	HQ667595		Hernández Caffot et al. 2011
T. sp.	**MLHC210 (CORD)**	Argentina 2007	HQ667596		Hernández Caffot et al. 2011
T. sp. 1	Jeppson 7762 (GB)	Hungary, 2006	KU518979	KU843981	Jeppson et al. 2017
T. sp. 10A	Jeppson 6198 (GB)	Spain, 2003	KU519030		Jeppson et al. 2017
T. sp. 10B	Jeppson 3813 (GB)	Hungary, 1997	KU519029		Jeppson et al. 2017
T. sp. 11	Jeppson 881114 (GB)	Spain, 1988	KU519031	KU843978	Jeppson et al. 2017
T. sp. 12	Hausknecht AH 16793 (AH)	Italy, 1992	KX640985		Jeppson et al. 2017
T. sp. 14	Jeppson 5004 (GB)	Spain, 1999	KU519039	KU843999	Jeppson et al. 2017
T. sp. 14	Jeppson 5011 (GB)	Spain, 1999	KU519038	KU843998	Jeppson et al. 2017
T. sp. 15	Jeppson 990617b (GB)	Hungary, 1999	KU519015		Jeppson et al. 2017
T. sp. 15	Jeppson 9295 (GB)	Spain, 2011	KU519013		Jeppson et al. 2017
T. sp. 15	Jeppson 9296 (GB)	Spain, 2011	KU519014	KU843984	Jeppson et al. 2017
T. sp. 16	Knudsen 99.337 (C)	Russia, 1999	KU519007	KU843980	Jeppson et al. 2017
T. sp. 17	Wequipo mycolgía UAH (AH 13674 - GB)	Spain, 1990	KU519065		Jeppson et al. 2017
T. sp. 18	Jeppson 9046 (GB)	Spain, 2010	KU519066	KU843956	Jeppson et al. 2017
T. sp. 19	Molia 140115-2 (GB)	Cyprus, 2014	KU519077	KU843957	Jeppson et al. 2017
T. sp. 2	Jeppson 8710 (GB)	Spain, 2008	KU518980	KU843982	Jeppson et al. 2017
T. sp. 2	Jeppson 8701 (GB)	Spain, 2008	KU518981	KU843983	Jeppson et al. 2017
T. sp. 20	Jeppson 5015 (GB)	Spain, 1999	KU519067	KU843950	Jeppson et al. 2017
T. sp. 3	Jeppson 4935 (GB)	Hungary, 1999	KU518978	KU843918	Jeppson et al. 2017
T. sp. 4	Finy 12 (GB)	Hungary, 2011	KU518960	KU843902	Jeppson et al. 2017
T. sp. 4	Finy 12 (GB)	Hungary, 2011	KU518960	KU843902	Jeppson et al. 2017

T. sp. 5	Jeppson 10060 (GB)	Slovakia, 2014	KU519040		Jeppson et al. 2017
T. sp. 6	Jeppson 5996 (GB)	Hungary, 2002	KU519016	KU843966	Jeppson et al. 2017
T. sp. 8	Jeppson 7795 (GB)	Hungary, 2006	KU519020	KU843970	Jeppson et al. 2017
T. sp. 8	Jeppson 3830 (GB)	Hungary, 1997	KU519018		Jeppson et al. 2017
T. sp. 9	Jeppson 4966 (GB)	Hungary, 1999	KU519036	KU843968	Jeppson et al. 2017
T. sp. novo	MICH266156	USA, NM 1969	X	X	This study
T. sp. novo aff 10A	MICH	266256	1972	USA	Arizona
T. sp. novo aff. 10A	MICH266256	USA, AZ, 1972	X	X	This study
T. sp. novo aff. 10B	MICH266448	USA,, NM	X	X	This study
T. sp. novo aff. 10B	DBG23778	USA, CO 2007	X	X	This study
T. squamosum	CUH AM696	India 2019	MN809136		Dutta and Paloi 2019
T. squamosum	Honrubia (AH 15483 -- GB)	Spain, 1992	KU519096	KU843891	Jeppson et al. 2017
T. squamosum	Mrazek 1300 (GB)	Austria, 1989	DQ415732	KU843894	Jeppson et al. 2017
T. squamosum	Larsson 260--06 (GB)	France, 2006	KU519097	KU843892	Jeppson et al. 2017
T. squamosum	JE Walther 18.5.44	Macedonia	X		Gube 2009
T. squamosum	MICH28574	USA, AZ 1988	X	X	This study
T. striatum	Fritz 2010--2 (GB)	Mongolia, 2010	KU518958	KU843929	Jeppson et al. 2017
T. striatum	González (AH 15543 -- GB)	Spain, 1993	KU518959	KU843930	Jeppson et al. 2017
T. striatum	GG1811	Argentina,	MN338574		Gonzalez et al. 2019 unpublished
T. striatum	ARIZ AN 043457	USA, AZ 2015	X	X	This study
T. striatum	DBG-F-021822	USA, CO 2004	X	X	This study
T. submembranaceum	Ayala et al. (AH 15132) -- holotype	Mexico, 1991	KX513826		Jeppson et al. 2017
T. subsquamosum	Jeppson 9336 (GB)	Slovakia, 2011	KU519090	KU843897	Jeppson et al. 2017
T. subsquamosum	Jeppson 6002 (GB)	Hungary, 2001	KU519089	KU843896	Jeppson et al. 2017
T. subsquamosum	MG468 MG 071020_5	Hungary	X		Gube 2009
T. tuberculatum	DBG21283	USA, CO 2002	X	X	This study
T. tuberculatum	BPI 729034-ISOTYPE	Canada, BC	KC333070		Jeppson et al. 2017
T. verrucosum	CCB142	USA, TN 2013	MG663293		Matheny et al. unpublished
T. winterhoffii	Jensen SOJ2105--699039 (C)	Denmark, 2015	KU518974	KU843915	Jeppson et al. 2017

T. winterhoffii	Jeppson 7761 (GB)	Hungary, 2006	KU518976	KU843916	Jeppson et al. 2017
T. winterhoffii	Jeppson 2379 (GB)	Sweden, 1987	KU518977		Jeppson et al. 2017
T. xerophilum	MLHC212 (CORD)	Argentina	HQ667592		Hernández Caffot et al. 2011
T. xerophilum	Long 9688 (BPI) -- holotype	USA, Arizona, 1936	KX576549		Jeppson et al. 2017
T. xerophilum	MICH266442	USA, NM 1973	X	X	This study
Unknown	MEL 2382982	Australia	KP012838		Bonito et al. 2014 Unpublished
Unknown	AZ35549	USA, AZ 1972	X		This study
OUTGROUP					
Battarrea phalloides	iNaturalist # 3236179	USA, CA 2016	MF422608		Rockefeller 2017 Unpublished
B. phalloides	03MCF3236	Macedonia 2003	HF913781		Martin et al. 2013
B. stevenii	RBG Is2	Israel	DQ184684		Jefferies and McLain 2005 Unpublished
Coprinus comatus	AH:45796	Spain 2014	KU686916		Moreno et al. 2016
C. comatus	AFTOL-ID 626		AY854066	AY881026	Matheny et al. 2004

Appendix 2.2. Dichotomous Key to Sampled *Tulostoma* Taxa

Dichotomous Key to the Genus Tulostoma found in the study:

1a. Mouth indefinite 2
1b. Mouth definite 3

<u>Mouth Indefinite</u>
2a. Spores smooth .... *Tulostoma meristostoma*
2b. Spores ornamented .... *T. opacum*

<u>Mouth Definite</u>
3a. Mouth circular or tubular 4
3b. Mouth fimbriose, fibriullose, not circular or tubular 11

<u>Mouth Circular</u>
4a. Spores smooth or very nearly smooth, ornamentation only visible under SEM............
.... *T. macrocephalum*
4b. Spores ornamented 5

<u>Mouth Circular, Spores Ornamented</u>
5a. Expoeridium hyphal 6
5b. Exoperidium membranous 8

<u>Mouth Circular, Spores Ornamented, Exoperidium Hyphal</u>
6a. Expoeridium with sphaerocysts .... *T. squamosum*
6b. Exoperidium lacking sphaerocysts 7

7a. Spores strongly ornamented, verrucae frequently fused forming crests and ridges, stipe with reddish tones, peristome differently colored than endoperidium ....
.... *T. calcereum*
7b. Spore ornamentation of low, uneven verrucae, rarely fused, not forming crests, stipe not reddish, with bulbous base, peristome concolorous with endoperidium ....
.... *T. meridionale*

<u>Mouth Circular, Spores Ornamented, Exoperidium Membranous</u>
8a. Exoperidium thickly membranous 9
8b. Exoperidium membranous 10

<u>Mouth Circular, Spores Ornamented, Exoperidium Thickly Membranous</u>
9a. Exoperidium not forming involucre, spore verrucae very rarely fusing, ridges very rarely formed on spores, basidiocarps associated with *Prosopis-Acacia* vegetation ....
.... *T. xerophilum*

9b. Exoperidium forming an almost involucre structure at base of spore-sac, spore verrucase commonly fused forming short ridges at base of apiculus, basidiocarps not commonly associated with *Prosopis-Acacia* vegetation .... *T. involucratum*

Mouth Circular, Spores Ornamented, Exoperidium Thinly Membranous

10a. Spores strongly ornamented, ornamentation sub-reticulate, mouth strongly projecting, basidocarp of darker colors .... *T. purpusii*

10b. Spore ornamentation of uneven individual verrucae, rarely fusing, if fused, not reticulate, peristome often not concolorous with endoperidium .... *T. simulans*

Mouth Fimbriate-Fibrillose

11a. Spores smooth or very nearly smooth, ornamentation only visible under SEM 12

11b. Spores ornamented 13

Mouth Fimbriate-Fibrillose, Spores Smooth

12a. Spores smooth under SEM, 5.5–7.0 µm, exoperidium hyphal, base of stem forming pseudorhiza.. *T. grandisporum*

12b. Spores 6.7-8.7(9.8) µm, ornamented with low, dense, strongly anastomosed verrucae, almost reticulate, exoperidium thinly membranous, mouth becoming lacerate in age, base of stipe often with mycelial bulb or rhizomorphs .... *T. obscurum*

Mouth Fimbriate-Fibrillose, Spores ornamented

13a. Exoperidium membranous.. 14

13b. Exoperidium hyphal 15

Mouth Fimbriate-Fibrillose, Spores ornamented, Exoperidium Membranous

14a. Spore ornamentation striate, forming spiraling ridges .... *T. striatum*

14b. Spore ornamentation low blunt verrucae, with some fused into lines... *T. pulchellum*

Mouth Fimbriate-Fibrillose, Spores ornamented, Exoperidium Hyphal

15a. Spore densely verrucose, many verrucae fused into pyrmidial structures, but not strongly anastomosed.. *T. punctatum*

15b. Spore ornamentation of low, anastomosing verrucae... 16

16a. Spores 4.5-6 µm, capillitium sparsely branched and septate, not swollen at septa, 1.8-6.6 µm .... *T. polymorphum*

16b. Spores 5.5-6.5 µm, capillitium prominently branched and septate, swollen at septa, 3.6-8.2 µm .... *T. fimbriatum*

Appendix 3.1. Taxa and sequence data used in phylogeography analysis.

Taxon	**Voucher**	**locality/year**	**ITS -- LSU**	**Tef-1α**	**Source**
Tulostoma albicans	Cope (NY) -- holotype	USA, Texas 1893	KX576548		Jeppson et al. 2017
T. adhaerens	B2087	Australia	MK278627		Varga et al. 2019
T. aff. *ahmadii*	MG_20_5	Hungary, Fülöpháza	X		Gube 2009
T. aff. *cretaceum*	Jeppson 3821 (GB)	Hungary 1997	KU518994	KU843993	Jeppson et al. 2017
T. aff. *kotlabae*	MJ6623	Hungary	DQ112629		Larrson and Jeppson 2008
T. aff. *kotlabae*	Gube545	Germany 1978	X		Gube 2009
T. aff. *leiosporum*	MG 071016_8	Hungary	X		Gube 2009
T. ahmadii	HUP SH-33b	Pakistan 2014	KP738712		Hussain et al. unpublished
T. beccarianum	Beccari (S) -- holotype	Italy	KX640979	KU843959	Jeppson et al. 2017
T. berkeleyi	JLH MyCoPortal 6604754	USA, NY 2017	MK578704		Russel and Horman NAMycoflora Project
T. brumale	Jeppson 6427 (GB)	Sweden 1998	KU519064	KU843947	Jeppson et al. 2017
T. caespitosum	ANK Akata 6329	Turkey 2015	MT804370		Akata et ak 2020 unpublished
T. calcareum	Jeppson 6965 (GB) -- holotype	Sweden 2004	KU519086	KU843881	Jeppson et al. 2017
T. calongei	Jeppson 8773 (GB) -- holotype	Spain 1989	KU518973	KU844000	Jeppson et al. 2017
T. cretaceum-TYPE	JELong9302-TYPE	USA, NM 1941	X		Gube 2009
T. cyclophorum	Lowen 99 (GB)	Spain 1984	KU518982	KU843960	Jeppson et al. 2017
T. domingueziae	MLHC24 (CORD)	Argentina	HQ667593	HQ667597	Hernandez Caffot, M.L et al. 2011
T. eckbladii	Jørstad (O F--58850) -- holotype	Norway 1957	KU519068	KU843951	Jeppson et al. 2017
T. egranulosum	Lloyd 15424, BPI 704420	Australia, Vic 1906	KC333072		Jeppson et al. 2017
T. excentricum	Lloyd 15424 (BPI) -- holotype	USA, New Mexico	KU519055		Jeppson et al. 2017
T. fimbriatum	DBG27836	USA, CO 2001	X	X	This study
T. fimbriatum	Månsson 991010 (GB) -- epitype	Sweden 1999	KU518963	KU843904	Jeppson et al. 2017
T. fimbriatum	Knudsen 00.190e (C)	Russia 2000	KU518972	KU843914	Jeppson et al. 2017
T. fimbriatum	MICH266156	USA, NM 1969	X	X	This study
T. fulvellum	Kabát 970428 (BRA)	Slovakia 1997	KU518991	KU844001	Jeppson et al. 2017
T. giovanellae	Jeppson 9059 (GB)	Spain 2009	KU519070	KU843953	Jeppson et al. 2017
T. grandisporum	Jeppson 6633 (GB)	Hungary 2004	KU519001	KU843919	Jeppson et al. 2017

T. kotlabae	Kotlaba (PRM 704203) -- holotype	Slovakia, 1954	KX576544	KU843973	Jeppson et al. 2017
T. lloydii	Lahti 201210 (GB)	Italy 2012	KU518990	KU843965	Jeppson et al. 2017
T. lusitanicum	Almeida et al. (LISU--MAG 8) -- holotype	Portugal 1991	KX576542		Jeppson et al. 2017
T. lysocephalum	Long 9639 (BPI) -- holotype	USA, New Mexico, 194	KU519034		Jeppson et al. 2017
T. macrocephalum-TYPE	JELong10113, BPI 729448	USA, NM	KX5765451		Jeppson et al. 2017
T. melanocyclum	Månsson 011215 (GB)	Sweden 2001	KU519102	KU843885	Jeppson et al. 2017
T. nanum	MJ4966	Hungary	KU519036		Larrson and Jeppson 2008
T. niveum	Jeppson 5229 (GB)	Sweden 1999	KU519080	KU843934	Jeppson et al. 2017
T. obesum	Cooke 2715 (NY) -- isotype	USA, CO	KX576541		Jeppson et al. 2017
T. obscurum	DBG28400	USA, CO 2015	X	X	This study
T. pannonicum	Jeppson 990617a (GB)	Hungary 1999	KU519008		Jeppson et al. 2017
T. poculatum	MICH28571	USA, AZ 1995	X	X	This study
T. polymorphum-TYPE	ILL11723	USA, NM 1941	X	X	This study
T. pseudopulchellum	Illana, Moreno, Altés (AH 11603) -- paratype	Spain 1989	KU519012	KU843997	Jeppson et al. 2017
T. pulchellum	Moravec 132/51 (UPS) -- paratype T. hollosii	Czech Republic 1951	KX513825	KU843926	Jeppson et al. 2017
T. pulchellum	Moravec 132/51 (UPS) -- paratype T. hollosii	Czech Republic 1951	KX513825	KU843926	This study
T. punctatum	MICH255257	USA, AZ	X	X	This study
T. punctilibratum	MICH24086	USA, CO 1956	X	X	This study
T. purpusii	DBG21727	USA, CO 2004	X	X	This study
T. pygmaeum	Bruzek131207	Slovakia	KU519041		Varga et al. 2019
T. simulans	Brůžek 140214 (GB)	Czech Republic 2014	KU519044	KU843937	Jeppson et al. 2017
T. simulans	DBG20766	USA, CO	X	X	This study
T. sp.	MLHC200 (CORD)	Argentina	HQ667595		Hernandez Caffot, M.L et al. 2011
T. sp.	MEL:2382982	Australia, NT 2014	KP012838		Bonito et al. unpublished
T. sp.	MG 071020_3	Hungary	X		Gube 2009
T. sp. 1	Jeppson 7762 (GB)	Hungary 2006	KU518979	KU843981	Jeppson et al. 2017
T. sp. 10	Jeppson 3813 (GB)	Hungary, 1997	KU519029		Jeppson et al. 2017
T. sp. 11	Jeppson 881114 (GB)	Spain, 1988	KU519031	KU843978	Jeppson et al. 2017
T. sp. 11	Jeppson 881114 (GB)	Spain, 1988	KU519031	KU843978	Jeppson et al. 2017
T. sp. 13	Long 11431 (BPI)	USA, New Mexico	KU519039		Jeppson et al. 2017

T. sp. 15	Jeppson 9296 (GB)	Spain 2011	KU519014	KU843984	Jeppson et al. 2017
T. sp. 16	Knudsen 99.337 (C)	Russia 1999	KU519007	KU843980	Jeppson et al. 2017
T. sp. 17	Wequipo mycolgía UAH (AH 13674 - GB)	Spain 1990	KU519065		Jeppson et al. 2017
T. sp. 18	Jeppson 9046 (GB)	Spain 2010	KU519066	KU843956	Jeppson et al. 2017
T. sp. 19	Molia 140115-2 (GB)	Cyprus 2014	KU519077	KU843957	Jeppson et al. 2017
T. sp. 20	Jeppson 5015 (GB)	Spain 1999	KU519067	KU843950	Jeppson et al. 2017
T. sp. 3	Jeppson 4935 (GB)	Hungary 1999	KU518978	KU843918	Jeppson et al. 2017
T. sp. 4	Finy 12 (GB)	Hungary 2011	KU518960	KU843902	Jeppson et al. 2017
T. sp. 5	Brůžek 131207 (GB)	Slovakia 2013	KU519041	KU843931	Jeppson et al. 2017
T. sp. 6	Jeppson 5996 (GB)	Hungary 2002	KU519016	KU843966	Jeppson et al. 2017
T. sp. 8	Jeppson 7795 (GB)	Hungary, 2006	KU519020	KU843970	Jeppson et al. 2017
T. sp. 9	Jeppson 4976 (GB)	Hungary 1999	KU519037	KU843969	Jeppson et al. 2017
T. sp. novo	MICH266163	USA, NM 1958	X	X	This study
T. sp. novo	MICH266256	USA, AZ 1972	X	X	This study
T. sp. novo	MICH266448	USA, NM	X	X	This study
T. squamosum	CUH AM696	India	MN809136		Dutta et al. 2020
T. striatum	DBG21822	USA, CO 2004	X	X	This study
T. subfuscum	Bethel21	USA, CO 1896	KC333074		Jeppson et al. 2017
T. submembranaceum	Ayala et al. (AH 15132) -- holotype	Mexico 1991	KX513826		Jeppson et al. 2017
T. subsquamosum	Jeppson 4956 (GB)	Hungary 1999	KU519095	KU843898	Jeppson et al. 2017
T. tuberculatum -TYPE	Long, BPI 729034	Canada, BC	KC333070		Jeppson et al. 2017
T. verrucosum	TENN:067762	USA, TN 2013	MG66329		Matheny et al. 2017 unpublished
T. winterhoffii	Jeppson 7761 (GB)	Hungary 2006	KU518976	KU843916	Jeppson et al. 2017
T. xerophilum	Long 9688 (BPI) -- holotype	USA, AZ 1936	KX576549		Jeppson et al. 2017
soil isolate	OTU938	USA, CA 2019	MK018794		Gao 2018 Unpublished

Appendix 3.2. Distance matrix for phylogeographic analysis.

	Nearctic	Palearctic	Afrotropical	Indo-Malay	Australasian	Neotropical
Nearctic	0	8129	13142	12586	14525	8513
Palearctic	8129	0	7608	3500	10269	13888
Afrotropical	13142	7608	0	7480	12330	9700
Indo-Malay	12586	3500	7480	0	7250	16421
Australasian	14525	10269	12330	7250	0	15980
Neotropical	8513	13888	9700	16421	15980	0

Appendix 4.1. Sequence data used in ancestral state reconstruction of spore morphology.

Taxon	**Voucher**	**locality/year**	**ITS -- LSU**	**Tef-1α**	**Source**
B. phalloides	03MCF3236	Macedonia 2003	HF913781		Martin et al. 2013
T. adhaerens	B2087	Australia	MK278627		Varga et al. 2019
T. aff. leiosporum	MG457_MG 071016_8	Hungary	X		Gube 2009
T. aff. subsquamosum	MG468 MG 071020_5	Hungary	X		Gube 2009
T. ahmadii	HUPSH33	Pakistan 2013	KP738711		Hussain et al. 2016
T. albicans	Cope (NY) -- holotype	USA, Texas, 1893	KX576548		Jeppson et al. 2017
T. beccarianum	Beccari (S) -- holotype	Italy	KX640979	KU843958	Jeppson et al. 2017
T. berkeleyi	JLH MyCoPortal 6604754	USA, NY 2017	MK578704		Russel and Horman NAMycoflora Project
T. brumale	Jeppson 6427 (GB)	Sweden, 1998	KU519064	KU843947	Jeppson et al. 2017
T. caespitosum	Patouillard 4693 (BPI)	Africa	KU519073		Jeppson et al. 2017
T. calcareum	Jeppson 6965 (GB) -- holotype	Sweden, 2004	KU519086	KU843881	Jeppson et al. 2017
T. calongei	Jeppson 8773 (GB) -- holotype	Spain, 1989	KU518973	KU844000	Jeppson et al. 2017
T. calongei	Álvarez (AH 13718)	Spain, 1978	KC333067		Jeppson et al. 2017
T. cretaceum	JE Long #9302 7.5.41 TYPE	USA, NM 1941	X		Gube 2009
T. cyclophorum	Gonzáles (AH 16885, GB)	Spain, 1994	KU518983	KU843961	Jeppson et al. 2017
T. domingueziae	MLHC 24 (CORD) – holotype	Argentina, 2003	HQ667594		Hernández Caffot et al. 2011
T. eckbladii	Jørstad (O F--58850) -- holotype	Norway, 1957	KU519068	KU843951	Jeppson et al. 2017
T. egranuslosum	Lloyd15424, BPI 704420-holotype	Australia, Mel	KC333072		Jeppson et al. 2017
T. excentricum	Lloyd 15424 (BPI) -- holotype	USA, New Mexico	KU519055		Jeppson et al. 2017
T. fimbriatum	Månsson 991010 (GB) -- epitype	Sweden, 1999	KU518963	KU843904	Jeppson et al. 2017
T. fulvellum	Röllin 74--5 (pers. herb. Specht)	Switzerland, 1974	KU518992		Jeppson et al. 2017
T. giovanellae	Jeppson 8706 (GB)	Spain, 2009	KU519071	KU843954	Jeppson et al. 2017
T. grandisporum	Jeppson 8907 (GB) -- holotype	Hungary, 2011	KU519003	KU843924	Jeppson et al. 2017
T. kotlabae	MJ6623	Hungary	DQ112629		Larsson and Jeppson 2005

T. kotlabae	Kotlaba (PRM 704203) -- holotype	Slovakia, 1954	KX576544	KU843975	Jeppson et al. 2017
T. lloydii	Lahti 201210 (GB)	Italy, 2012	KU518990	KU843965	Jeppson et al. 2017
T. lusitanicum	Almeida et al. (LISU--MAG 8) -- holotype	Portugal, 1991	KX576542		Jeppson et al. 2017
T. lysocephalum	Long 9639 (BPI) -- holotype	USA, New Mexico, 194	KU519034		Jeppson et al. 2017
T. macrocephalum	MICH24877	USA, AZ 1970	X	X	This study
T. melanocyclum	Månsson 011215 (GB)	Sweden, 2001	KU519102	KU843885	Jeppson et al. 2017
T. nanum	MJ4966	Hungary	KU519036	KU843968	Jeppson et al. 2017
T. niveum	Jeppson 7692 (GB)	Sweden, 2005	KU519078	KU843932	Jeppson et al. 2017
T. obesum	Jeppson 8695 (GB)	Spain, 2008	KU518986	KU843985	Jeppson et al. 2017
T. obscurum	DBG28400	USA, CO 2015	X	X	This study
T. pannonicum	Jeppson 7803 (GB)	Hungary, 2006	KU519011	KU843996	Jeppson et al. 2017
T. poculatum	MICH28564	USA, AZ 1995	X	X	This study
T. polymorphum	ILL00011723-TYPE	USA, NM 1941	X	X	This study
T. pseudopulchellum	Illana, Moreno, Altés (AH 11603) -- paratype	Spain, 1989	KU519012	KU843997	Jeppson et al. 2017
T. pulchellum	Fritz 2010--1 (GB)	Mongolia, 2010	KU518954	KU843925	Jeppson et al. 2017
T. pulchellum	PAD-holotype	Australia	KX761984		Altes et al. 2016 unpublished
T. punctatum	DBG27849	USA, CO 2001	X	X	This study
T. punctatum	Bethel 21 (NY) -- holotype T. subfuscum	USA, CO 1896	KC333074		Jeppson et al. 2017
T. punctilabratum	MICH24086	USA, CO 1956	X	X	This study
T. purpusii	DBG21727	USA, CO 2004	X	X	This study
T. purpusii	MICH266163	USA, NM 1958	X	X	This study
T. rufum	Lloyd 15542 (BPI)-- holotype	USA, Alabama	KU519107		Jeppson et al. 2017
T. simulans	MICH266252	USA, CO 1956	X	X	This study
T. sp.	MG461 MG 071020_3	Hungary	X		Gube 2009
T. sp. 1	Jeppson 7762 (GB)	Hungary, 2006	KU518979	KU843981	Jeppson et al. 2017
T. sp. 10A	Jeppson 6198 (GB)	Spain, 2003	KU519030		Jeppson et al. 2017
T. sp. 11	Jeppson 881114 (GB)	Spain, 1988	KU519031	KU843978	Jeppson et al. 2017
T. sp. 12	Hausknecht AH 16793 (AH)	Italy, 1992	KX640985		Jeppson et al. 2017
T. sp. 14	Jeppson 5004 (GB)	Spain, 1999	KU519039	KU843999	Jeppson et al. 2017

T. sp. 15	Jeppson 9296 (GB)	Spain, 2011	KU519014	KU843984	Jeppson et al. 2017
T. sp. 16	Knudsen 99.337 (C)	Russia, 1999	KU519007	KU843980	Jeppson et al. 2017
T. sp. 17	Wequipo mycolgía UAH (AH 13674 - GB)	Spain, 1990	KU519065		Jeppson et al. 2017
T. sp. 18	Jeppson 9046 (GB)	Spain, 2010	KU519066	KU843956	Jeppson et al. 2017
T. sp. 19	Molia 140115-2 (GB)	Cyprus, 2014	KU519077	KU843957	Jeppson et al. 2017
T. sp. 20	Jeppson 5015 (GB)	Spain, 1999	KU519067	KU843950	Jeppson et al. 2017
T. sp. 3	Jeppson 4935 (GB)	Hungary, 1999	KU518978	KU843918	Jeppson et al. 2017
T. sp. 4	Finy 12 (GB)	Hungary, 2011	KU518960	KU843902	Jeppson et al. 2017
T. sp. 5	Brůžek 131207 (GB)	Slovakia, 2013	KU519041	KU843931	Jeppson et al. 2017
T. sp. 5	Jeppson 10060 (GB)	Slovakia, 2014	KU519040		Jeppson et al. 2017
T. sp. 6	Jeppson 5996 (GB)	Hungary, 2002	KU519016	KU843966	Jeppson et al. 2017
T. sp. 8	Jeppson 7795 (GB)	Hungary, 2006	KU519020	KU843970	Jeppson et al. 2017
T. sp. 9	Jeppson 4966 (GB)	Hungary, 1999	KU519036	KU843968	Jeppson et al. 2017
T. sp. novo	MICH266156	USA, NM 1969	X	X	This study
T. sp. novo aff. 10B	MICH266448	USA, NM	X	X	This study
T. squamosum	MICH28574	USA, AZ 1988	X	X	This study
T. striatum	DBG-F-021822	USA, CO 2004	X	X	This study
T. submembranaceum	Ayala et al. (AH 15132) -- holotype	Mexico, 1991	KX513826		Jeppson et al. 2017
T. subsquamosum	Jeppson 6002 (GB)	Hungary, 2001	KU519089	KU843896	Jeppson et al. 2017
T. verrucosum	CCB142	USA, TN 2013	MG663293		Matheny et al. unpublished
T. winterhoffii	Jeppson 7761 (GB)	Hungary, 2006	KU518976	KU843916	Jeppson et al. 2017
T. xerophilum	MICH266442	USA, NM 1973	X	X	This study

Appendix 5.1. Isotope data. NS-NE = neither saprotrophic nor ectomycorrhizal, SAP = saprotrophic, ECM = ectomycorrhizal, and UNK = unknown.

Taxon	δ^{15}N (Air)	δ^{13}C (VPDB)	cluster	Reference	Habit
Camarophyllopsis atropuncta	11.18	-25.64	1	Birkebak et al. (2013)	NS-NE
Camarophyllopsis foetens	5.93	-23.77	1	Birkebak et al. (2013)	NS-NE
Clavaria acuta	9.22	-22.57	1	Birkebak et al. (2013)	NS-NE
Clavaria aff. Argillacea	12.35	-23.14	1	Birkebak et al. (2013)	NS-NE
Clavaria fragilis	13.16	-23.79	1	Birkebak et al. (2013)	NS-NE
Clavaria fragilis	15.24	-26.42	1	Birkebak et al. (2013)	NS-NE
Clavaria fragilis	14.08	-23.34	1	Birkebak et al. (2013)	NS-NE
Clavaria Zollinger	12.73	-26.37	1	Birkebak et al. (2013)	NS-NE
Clavaria Zollinger	12.32	-25.44	1	Birkebak et al. (2013)	NS-NE
Clavaria Zollinger	13.25	-25.67	1	Birkebak et al. (2013)	NS-NE
Clavulinopsis sulcate	15.72	-29.8	1	Birkebak et al. (2013)	NS-NE
Clavuniopsis aurantiocinnabarina	16.82	-28.09	1	Birkebak et al. (2013)	NS-NE
Ramariopsis corniculate	13.82	-25.98	1	Birkebak et al. (2013)	NS-NE
Ramariopsis fusiformis	16.02	-26.32	1	Birkebak et al. (2013)	NS-NE
Ramariopsis fusiformis	20.54	-27.38	1	Birkebak et al. (2013)	NS-NE
Ramariopsis fusiformis	14.3	-24.51	1	Birkebak et al. (2013)	NS-NE
Ramariopsis kunzei	13.86	-24.89	1	Birkebak et al. (2013)	NS-NE
Ramariopsis kunzei	14.61	-24.19	1	Birkebak et al. (2013)	NS-NE
Ramariopsis kunzei	13	-25.94	1	Birkebak et al. (2013)	NS-NE
Ramariopsis laeticolor	11.45	-26.16	1	Birkebak et al. (2013)	NS-NE
Amanita pantherine	7.3	-24.4	1	Hart et al. 2006	ECM
Bovista spp.	7	-23.2	1	Hart et al. 2006	SAP
Gomphidius smithii	7.9	-23.6	1	Hart et al. 2006	ECM
Inocybe fastigiate	3.6	-25	1	Hart et al. 2006	ECM
Pholiota squarrosa	5.2	-23.6	1	Hart et al. 2006	SAP
Russula emetica	1.9	-24.9	1	Hart et al. 2006	ECM
Russula integra	3.8	-24.9	1	Hart et al. 2006	ECM
Russula sororia	4.4	-25.2	1	Hart et al. 2006	ECM
Suillus lakei	8.9	-23.9	1	Hart et al. 2006	ECM
Suillus lakei	11.9	-22.6	1	Hart et al. 2006	ECM
Suillus pseudobrevipes	6.7	-24.3	1	Hart et al. 2006	ECM
Suillus sibiricus	6	-24.1	1	Hart et al. 2006	ECM
Russula crustosa	-25.03	4.54	1	Hou et al. 2012	ECM

Russula virescens	-24.76	4.78	1	Hou et al. 2012	ECM
Russula vinosa	-25.44	2.78	1	Hou et al. 2012	ECM
Russula sp.	-26.01	2.9	1	Hou et al. 2012	ECM
Tylopilus ballouii	-24.22	4.07	1	Hou et al. 2012	ECM
Boletinus pinetorum	-24.51	4.24	1	Hou et al. 2012	ECM
Strobilomyces confuses	-25.13	3.71	1	Hou et al. 2012	ECM
Xerocomus badius	-25.07	2.34	1	Hou et al. 2012	ECM
Boletus ravenelii	-25.26	3.89	1	Hou et al. 2012	ECM
Scleroderma lycoperdoides	-25.28	3.22	1	Hou et al. 2012	ECM
Scleroderma cepa	-25.34	3.46	1	Hou et al. 2012	ECM
Ramaria mairei	-24.24	4.49	1	Hou et al. 2012	ECM
Cantharellus cibarius	-25.79	3.88	1	Hou et al. 2012	ECM
Strobilomyces floccopus	-24.99	4.35	1	Hou et al. 2012	ECM
Ramaria rufescens	-25.33	3.53	1	Hou et al. 2012	ECM
Clavulina cristata	-24.85	3.99	1	Hou et al. 2012	ECM
Cantharellus minor	-26.41	4.21	1	Hou et al. 2012	ECM
Alloclavaria purpurea	4.92	-24.28	1	Korotkin et al. 2018	UNK
Alloclavaria purpurea	3.61	-24.46	1	Korotkin et al. 2018	UNK
Alloclavaria purpurea	4.01	-26.51	1	Korotkin et al. 2018	UNK
Amanita multisquamosa	4.11	-27.04	1	Korotkin et al. 2018	ECM
Blasiphalia pseudogrisella	3.41	-26.18	1	Korotkin et al. 2018	UNK
Cantharellopsis prescotii	0.42	-27.79	1	Korotkin et al. 2018	UNK
Cantharellopsis prescotii	-0.7	-32.82	1	Korotkin et al. 2018	UNK
Cantharellopsis prescotii	0.39	-26.66	1	Korotkin et al. 2018	UNK
Cantharellopsis prescotii	-0.58	-28.85	1	Korotkin et al. 2018	UNK
Cantharellopsis prescotii (as Gerronema albidum)	-1.86	-28.79	1	Korotkin et al. 2018	UNK
Cantharellopsis prescotii (as Gerronema albidum)	-1.56	-29.12	1	Korotkin et al. 2018	UNK
Coltricia cinnamomea	6.64	-26.91	1	Korotkin et al. 2018	ECM
Coltricia cinnamomea	5.65	-26.79	1	Korotkin et al. 2018	ECM
Coltricia montagnei	8.19	-25.24	1	Korotkin et al. 2018	ECM
Coltricia montagnei	8.28	-25.21	1	Korotkin et al. 2018	ECM
Coltricia perennis	4.45	-24.94	1	Korotkin et al. 2018	ECM
Coltricia perennis	11.86	-24.62	1	Korotkin et al. 2018	ECM
Coltricia perennis	8.49	-25.66	1	Korotkin et al. 2018	ECM
Coltricia perennis	8.56	-24.76	1	Korotkin et al. 2018	ECM
Contumyces rosellus	4.82	-27.62	1	Korotkin et al. 2018	UNK
Contumyces vesuvianus	-1.75	-27.83	1	Korotkin et al. 2018	UNK
Cortinarius corrugatus	12.04	-26.65	1	Korotkin et al. 2018	ECM
Cotylidia pannosa	8.32	-24.06	1	Korotkin et al. 2018	UNK

Loreleia marchantiae	1.51	-26.07	1	Korotkin et al. 2018	UNK
Loreleia marchantiae	1.89	-25.58	1	Korotkin et al. 2018	UNK
Muscinupta laevis	-3.55	-27.24	1	Korotkin et al. 2018	UNK
Muscinupta laevis	1.15	-25.93	1	Korotkin et al. 2018	UNK
Rickenella fibula	-3.79	-28.44	1	Korotkin et al. 2018	UNK
Rickenella fibula	1.87	-28.23	1	Korotkin et al. 2018	UNK
Rickenella fibula	2.08	-25.42	1	Korotkin et al. 2018	UNK
Rickenella fibula	-4.96	-28.71	1	Korotkin et al. 2018	UNK
Rickenella fibula	-4.52	-27.37	1	Korotkin et al. 2018	UNK
Rickenella fibula	0.73	-28.54	1	Korotkin et al. 2018	UNK
Rickenella fibula	-3.25	-29.67	1	Korotkin et al. 2018	UNK
Rickenella fibula	-2.09	-29.22	1	Korotkin et al. 2018	UNK
Rickenella fibula	-2.09	-28.67	1	Korotkin et al. 2018	UNK
Rickenella fibula	0.32	-25.95	1	Korotkin et al. 2018	UNK
Rickenella fibula	-4.87	-28.63	1	Korotkin et al. 2018	UNK
Rickenella fibula	-2.82	-28.36	1	Korotkin et al. 2018	UNK
Rickenella fibula	-2.93	-30.03	1	Korotkin et al. 2018	UNK
Rickenella fibula	-5.04	-28.89	1	Korotkin et al. 2018	UNK
Rickenella minuta	6.45	-25.43	1	Korotkin et al. 2018	ECM
Rickenella minuta	2.37	-25.28	1	Korotkin et al. 2018	ECM
Rickenella minuta	0.72	-25.36	1	Korotkin et al. 2018	ECM
Rickenella minuta	-1.65	-26.44	1	Korotkin et al. 2018	ECM
Rickenella minuta	0.43	-26.14	1	Korotkin et al. 2018	ECM
Rickenella minuta	1.41	-26.64	1	Korotkin et al. 2018	ECM
Rickenella setipes	-0.6	-27.22	1	Korotkin et al. 2018	UNK
Rickenella setipes	-1.84	-26.38	1	Korotkin et al. 2018	UNK
Rickenella swartzii	-3.22	-29.59	1	Korotkin et al. 2018	UNK
Rickenella swartzii	-0.18	-26.68	1	Korotkin et al. 2018	UNK
Rickenella swartzii	0.49	-26.3	1	Korotkin et al. 2018	UNK
Rickinella minuta	5.74	-26.45	1	Korotkin et al. 2018	ECM
Rickinella minuta	8.14	-25.3	1	Korotkin et al. 2018	ECM
Rickinella minuta	3.28	-25.12	1	Korotkin et al. 2018	ECM
Ricknella minuta	2.96	-26.35	1	Korotkin et al. 2018	ECM
Agaricus subrutilescens	11.4	-25	1	Mayor et al. (2009)	SAP
Albatrellus avellaneus	4.3	-25.1	1	Mayor et al. (2009)	ECM
Albatrellus ovinus	6.1	-25.9	1	Mayor et al. (2009)	ECM
Amanita aff. Ovalispora	1.5	-28.3	1	Mayor et al. (2009)	ECM
Amanita angustilamellata	3.2	-26.8	1	Mayor et al. (2009)	ECM
Amanita angustilamellata	6.6	-24.8	1	Mayor et al. (2009)	ECM
Amanita citrina	1.7	-26.7	1	Mayor et al. (2009)	ECM

Amanita constricta	1.8	-25.5	1	Mayor et al. (2009)	ECM
Amanita franchetii	1.1	-25.4	1	Mayor et al. (2009)	ECM
Amanita fulva	2.1	-26.3	1	Mayor et al. (2009)	ECM
Amanita fulva	1.5	-25.6	1	Mayor et al. (2009)	ECM
Amanita longistriata	6.3	-25	1	Mayor et al. (2009)	ECM
Amanita muscaria	5.5	-26.6	1	Mayor et al. (2009)	ECM
Amanita muscaria	7.9	-26.3	1	Mayor et al. (2009)	ECM
Amanita muscaria	1.2	-25.5	1	Mayor et al. (2009)	ECM
Amanita muscaria	3.5	-24.6	1	Mayor et al. (2009)	ECM
Amanita neo-ovoidea	5.2	-24	1	Mayor et al. (2009)	ECM
Amanita pachycolea	1.5	-25.8	1	Mayor et al. (2009)	ECM
Amanita pantherine	5.6	-23.7	1	Mayor et al. (2009)	ECM
Amanita pantherine	7.3	-24.4	1	Mayor et al. (2009)	ECM
Amanita porphyria	2.1	-24.8	1	Mayor et al. (2009)	ECM
Amanita rubescens	4.3	-25.3	1	Mayor et al. (2009)	ECM
Amanita rubescens	1	-26.2	1	Mayor et al. (2009)	ECM
Amanita rubrovolvata	3.6	-24.3	1	Mayor et al. (2009)	ECM
Amanita sp.	1.7	-25.4	1	Mayor et al. (2009)	ECM
Amanita sp.	6.8	-25.4	1	Mayor et al. (2009)	ECM
Amanita sp.	10.2	-27.2	1	Mayor et al. (2009)	ECM
Amanita vaginata	-2.4	-27	1	Mayor et al. (2009)	ECM
Amanita vaginata	0.4	-27.8	1	Mayor et al. (2009)	ECM
Amanita virosa	4.1	-25.1	1	Mayor et al. (2009)	ECM
Aureoboletus subvirens	5.4	-25.6	1	Mayor et al. (2009)	ECM
Aureoboletus thibetanus	2.1	-27.5	1	Mayor et al. (2009)	ECM
Boletaceae sp.	4.3	-25.3	1	Mayor et al. (2009)	ECM
Boletaceae sp.	5.7	-25.8	1	Mayor et al. (2009)	ECM
Boletellus exiguous	4.3	-25.3	1	Mayor et al. (2009)	ECM
Boletellus russellii	2.7	-25.2	1	Mayor et al. (2009)	ECM
Boletopsis leucomelaena	12.3	-25.5	1	Mayor et al. (2009)	ECM
Boletus aestivalis	5.7	-23.9	1	Mayor et al. (2009)	ECM
Boletus chrysenteron	4.9	-25.6	1	Mayor et al. (2009)	ECM
Boletus coniferarum	11	-25.3	1	Mayor et al. (2009)	ECM
Boletus edulis	8.5	-24.6	1	Mayor et al. (2009)	ECM
Boletus edulis	3.8	-25.9	1	Mayor et al. (2009)	ECM
Boletus edulis	3.8	-27	1	Mayor et al. (2009)	ECM
Boletus mirabilis	3.7	-26.4	1	Mayor et al. (2009)	ECM
Boletus pinophilus	17.5	-24.7	1	Mayor et al. (2009)	ECM
Boletus subvelutipes	4.7	-24	1	Mayor et al. (2009)	ECM
Boletus truncates	9.4	-25	1	Mayor et al. (2009)	ECM

Boletus zelleri	5.9	-26.8	1	Mayor et al. (2009)	ECM
Boletus zelleri	4.9	-24.6	1	Mayor et al. (2009)	ECM
Boletus zoelleri	2.6	-25	1	Mayor et al. (2009)	ECM
Camarophyllopsis atropuncta	11.18	-25.64	1	Mayor et al. (2009)	NS-NE
Camarophyllopsis foetens	5.93	-23.77	1	Mayor et al. (2009)	NS-NE
Camarophyllopsis foetens	6.14	-25.59	1	Mayor et al. (2009)	NS-NE
Cantharellus atratus	5.7	-26.3	1	Mayor et al. (2009)	ECM
Cantharellus cibarius	4	-25.5	1	Mayor et al. (2009)	ECM
Cantharellus cibarius	5.3	-26.4	1	Mayor et al. (2009)	ECM
Cantharellus cibarius	5.3	-26.3	1	Mayor et al. (2009)	ECM
Cantharellus cibarius	0.7	-26.6	1	Mayor et al. (2009)	ECM
Cantharellus cibarius1	7.7	-25.2	1	Mayor et al. (2009)	ECM
Cantharellus formosus	5	-26.5	1	Mayor et al. (2009)	ECM
Cantharellus formosus	3.2	-25.8	1	Mayor et al. (2009)	ECM
Cantharellus lutescens	2.6	-25	1	Mayor et al. (2009)	ECM
Cantharellus tubaeformis	1.5	-25.2	1	Mayor et al. (2009)	ECM
Cantharellus tubiformis	0.9	-25.9	1	Mayor et al. (2009)	ECM
Cantherellus guianensis	6.7	-26	1	Mayor et al. (2009)	ECM
Cantherellus pleurotoides	2.8	-25.24	1	Mayor et al. (2009)	ECM
Cantherellus pleurotoides	0.48	-25.919	1	Mayor et al. (2009)	ECM
Catathelasma ventricosum	12.9	-23.5	1	Mayor et al. (2009)	ECM
Chalciporus piperatus	9.3	-22	1	Mayor et al. (2009)	ECM
Chroogomphus rutilus	3.8	-26	1	Mayor et al. (2009)	ECM
Chroogomphus rutilus	4.2	-25.3	1	Mayor et al. (2009)	ECM
Chroogomphus tomentosus	4.1	-24.8	1	Mayor et al. (2009)	ECM
Chroogomphus tomentosus	4.6	-25.4	1	Mayor et al. (2009)	ECM
Chroogomphus tomentosus	-0.7	-26	1	Mayor et al. (2009)	ECM
Clavaria acuta	9.22	-22.57	1	Mayor et al. (2009)	NS-NE
Clavaria aff. Argillacea	12.35	-23.14	1	Mayor et al. (2009)	NS-NE
Clavaria fragilis	13.16	-23.79	1	Mayor et al. (2009)	NS-NE
Clavaria fragilis	15.24	-26.42	1	Mayor et al. (2009)	NS-NE
Clavaria fragilis	14.08	-23.34	1	Mayor et al. (2009)	NS-NE
Clavaria Zollinger	12.73	-26.37	1	Mayor et al. (2009)	NS-NE
Clavaria Zollinger	12.32	-25.44	1	Mayor et al. (2009)	NS-NE
Clavaria Zollinger	12.32	-26.09	1	Mayor et al. (2009)	NS-NE
Clavaria Zollinger	12.39	-23.73	1	Mayor et al. (2009)	NS-NE
Clavaria Zollinger	13.25	-25.67	1	Mayor et al. (2009)	NS-NE
Clavulina ,coral tiny wood	3	-24.7	1	Mayor et al. (2009)	ECM
Clavulina , grey	9.7	-24.9	1	Mayor et al. (2009)	ECM
Clavulina caespitosa	5.8	-26.2	1	Mayor et al. (2009)	ECM

Clavulina caespitosa	7.4	-24.8	1	Mayor et al. (2009)	ECM
Clavulina caespitosa	3.3	-25.9	1	Mayor et al. (2009)	ECM
Clavulina cristata	1.6	-25.5	1	Mayor et al. (2009)	ECM
Clavulina cristata	3.5	-26.4	1	Mayor et al. (2009)	ECM
Clavulina humicola	4.8	-25.1	1	Mayor et al. (2009)	ECM
Clavulina rugosa	3.4	-26.4	1	Mayor et al. (2009)	ECM
Clavulinopsis sulcate	15.72	-29.8	1	Mayor et al. (2009)	NS-NE
Clavuniopsis aurantiocinnabarina	16.82	-28.09	1	Mayor et al. (2009)	NS-NE
Collybia laccata	1.77	-26.9	1	Mayor et al. (2009)	SAP
Cortinariaceae sp.	8.9	-24.8	1	Mayor et al. (2009)	UNK
Cortinariaceae sp.	6.8	-27	1	Mayor et al. (2009)	UNK
Cortinarius acutus	4	-25.5	1	Mayor et al. (2009)	ECM
Cortinarius aff. Violaceous	7.9	-27	1	Mayor et al. (2009)	ECM
Cortinarius albo-violaceus	10.7	-28.4	1	Mayor et al. (2009)	ECM
Cortinarius alboviolaceus	10.4	-28.1	1	Mayor et al. (2009)	ECM
Cortinarius anomalus	7.4	-26.8	1	Mayor et al. (2009)	ECM
Cortinarius armeniacus	7.2	-24.5	1	Mayor et al. (2009)	ECM
Cortinarius armeniacus	5.4	-25.1	1	Mayor et al. (2009)	ECM
Cortinarius armillatus	9.2	-26.9	1	Mayor et al. (2009)	ECM
Cortinarius armillatus	9.1	-27.5	1	Mayor et al. (2009)	ECM
Cortinarius bataillei	3.1	-25.3	1	Mayor et al. (2009)	ECM
Cortinarius biformis	8.3	-25.2	1	Mayor et al. (2009)	ECM
Cortinarius bolaris	8.8	-27	1	Mayor et al. (2009)	ECM
Cortinarius bolaris	6.3	-25.9	1	Mayor et al. (2009)	ECM
Cortinarius brunneus	5.8	-26.1	1	Mayor et al. (2009)	ECM
Cortinarius brunneus	5.6	-25.3	1	Mayor et al. (2009)	ECM
Cortinarius cacao-color	5.9	-25	1	Mayor et al. (2009)	ECM
Cortinarius cacao-color	6.7	-25	1	Mayor et al. (2009)	ECM
Cortinarius calochrous	5.3	-24.8	1	Mayor et al. (2009)	ECM
Cortinarius calopus	5.8	-25	1	Mayor et al. (2009)	ECM
Cortinarius camphoratus	12.3	-25.6	1	Mayor et al. (2009)	ECM
Cortinarius camphoratus	7.6	-23.7	1	Mayor et al. (2009)	ECM
Cortinarius cinnamomeoluteus	5.3	-26.9	1	Mayor et al. (2009)	ECM
Cortinarius clandestinus	8.1	-24.6	1	Mayor et al. (2009)	ECM
Cortinarius croceus	4.3	-29.2	1	Mayor et al. (2009)	ECM
Cortinarius delibutus	7.5	-26.2	1	Mayor et al. (2009)	ECM
Cortinarius evernuis	7.7	-24.8	1	Mayor et al. (2009)	ECM
Cortinarius fennoscandicus	-0.3	-27.6	1	Mayor et al. (2009)	ECM
Cortinarius gentilis	5.3	-25.6	1	Mayor et al. (2009)	ECM
Cortinarius gentilis	5.3	-26.6	1	Mayor et al. (2009)	ECM

Cortinarius gentilis	4.4	-25.1	1	Mayor et al. (2009)	ECM
Cortinarius hemitrichus	3.1	-27.8	1	Mayor et al. (2009)	ECM
Cortinarius laniger	10.1	-26.4	1	Mayor et al. (2009)	ECM
Cortinarius laniger	8.3	-25.9	1	Mayor et al. (2009)	ECM
Cortinarius laniger	8.2	-25.4	1	Mayor et al. (2009)	ECM
Cortinarius limonius	6.6	-25.9	1	Mayor et al. (2009)	ECM
Cortinarius malachius	10.5	-24.8	1	Mayor et al. (2009)	ECM
Cortinarius malachius	6.3	-25.5	1	Mayor et al. (2009)	ECM
Cortinarius malachius	4.9	-25.5	1	Mayor et al. (2009)	ECM
Cortinarius malicorius	9.4	-26	1	Mayor et al. (2009)	ECM
Cortinarius montanus	6.3	-26.5	1	Mayor et al. (2009)	ECM
Cortinarius montanus	5	-26.2	1	Mayor et al. (2009)	ECM
Cortinarius muscigenus	5	-26.1	1	Mayor et al. (2009)	ECM
Cortinarius muscigenus	6.7	-26.6	1	Mayor et al. (2009)	ECM
Cortinarius obtusus	4.9	-26.5	1	Mayor et al. (2009)	ECM
Cortinarius obtusus	6.9	-25.9	1	Mayor et al. (2009)	ECM
Cortinarius olympianus	6	-24.7	1	Mayor et al. (2009)	ECM
Cortinarius paleaceus	3.5	-25.7	1	Mayor et al. (2009)	ECM
Cortinarius paleaceus	2.6	-25	1	Mayor et al. (2009)	ECM
Cortinarius paragaudis ssp. paragaudis	15	-25.8	1	Mayor et al. (2009)	ECM
Cortinarius pholideus	5.7	-27.6	1	Mayor et al. (2009)	ECM
Cortinarius pholideus	5.4	-26.3	1	Mayor et al. (2009)	ECM
Cortinarius sanguineus	5.8	-26.3	1	Mayor et al. (2009)	ECM
Cortinarius saturninus	4.1	-25.4	1	Mayor et al. (2009)	ECM
Cortinarius scaurus	5.4	-25.2	1	Mayor et al. (2009)	ECM
Cortinarius semisanguineus	7.4	-24.9	1	Mayor et al. (2009)	ECM
Cortinarius semisanguineus	6.4	-25.2	1	Mayor et al. (2009)	ECM
Cortinarius semisanguineus	10.4	-26.3	1	Mayor et al. (2009)	ECM
Cortinarius septentrionalis	2.7	-27.3	1	Mayor et al. (2009)	ECM
Cortinarius sp.	5.1	-23.8	1	Mayor et al. (2009)	ECM
Cortinarius sp.	4.3	-25.6	1	Mayor et al. (2009)	ECM
Cortinarius sp.	0.1	-26.9	1	Mayor et al. (2009)	ECM
Cortinarius sp.	0	-28.4	1	Mayor et al. (2009)	ECM
Cortinarius sp.	12.7	-26.8	1	Mayor et al. (2009)	ECM
Cortinarius sp.	3.4	-26.6	1	Mayor et al. (2009)	ECM
Cortinarius sp.	7.5	-23.4	1	Mayor et al. (2009)	ECM
Cortinarius sp.	9.7	-24.4	1	Mayor et al. (2009)	ECM
Cortinarius spadiceus	5.7	-24.5	1	Mayor et al. (2009)	ECM
Cortinarius speciosissimus	6	-25.8	1	Mayor et al. (2009)	ECM
Cortinarius stillatitius	6.4	-24.2	1	Mayor et al. (2009)	ECM

Cortinarius strobilaceus	8.6	-27	1	Mayor et al. (2009)	ECM
Cortinarius strobilaceus	4.2	-25.5	1	Mayor et al. (2009)	ECM
Cortinarius subtortus	10.6	-25.7	1	Mayor et al. (2009)	ECM
Cortinarius talus	7	-25.9	1	Mayor et al. (2009)	ECM
Cortinarius torvus	5.8	-24.3	1	Mayor et al. (2009)	ECM
Cortinarius traganus	11.9	-25.2	1	Mayor et al. (2009)	ECM
Cortinarius traganus	9	-25.6	1	Mayor et al. (2009)	ECM
Cortinarius uliginosus	3.2	-28	1	Mayor et al. (2009)	ECM
Cortinarius vanduzerensis	8.9	-24.7	1	Mayor et al. (2009)	ECM
Cortinarius vibratilis	6.2	-25.1	1	Mayor et al. (2009)	ECM
Cortinarius vibratilis	6.1	-26.1	1	Mayor et al. (2009)	ECM
Craterellus sp.	6.3	-25.6	1	Mayor et al. (2009)	ECM
Craterellus tubaeformis	5	-25.7	1	Mayor et al. (2009)	ECM
Entoloma sp.	9.3	-22.8	1	Mayor et al. (2009)	ECM
Entoloma sp.	11.1	-23.7	1	Mayor et al. (2009)	ECM
Galerina heterocystis	-1.9	-27.6	1	Mayor et al. (2009)	SAP
Ganoderma austral	1.8	-27.4	1	Mayor et al. (2009)	SAP
Ganoderma stipitatum	3.74	-24.6	1	Mayor et al. (2009)	SAP
Gomphidius glutinosus	6	-26.4	1	Mayor et al. (2009)	ECM
Gomphidius oregonensis	6.1	-26.6	1	Mayor et al. (2009)	ECM
Gomphidius roseus	6.7	-24.9	1	Mayor et al. (2009)	ECM
Gomphidius smithii	7.9	-23.6	1	Mayor et al. (2009)	ECM
Gomphidius subroseus	1.6	-26.7	1	Mayor et al. (2009)	ECM
Gomphidus glutinosus	5.5	-24.6	1	Mayor et al. (2009)	ECM
Gomphus clavatus	7.6	-24.1	1	Mayor et al. (2009)	ECM
Gomphus fujisanensis	9.8	-24.6	1	Mayor et al. (2009)	ECM
Gymnopilus junonius	4.3	-24	1	Mayor et al. (2009)	SAP
Hebeloma crustuliniforme	2.1	-27.1	1	Mayor et al. (2009)	ECM
Hebeloma mesophaeum	8.4	-26.8	1	Mayor et al. (2009)	ECM
Hebeloma mesophaeum	4.6	-24.6	1	Mayor et al. (2009)	ECM
Hebeloma radicosoides	21.2	-25	1	Mayor et al. (2009)	ECM
Hebeloma velutipes	1.9	-26.6	1	Mayor et al. (2009)	ECM
Hebeloma vinosophyllum	21.8	-24.8	1	Mayor et al. (2009)	ECM
Heimiella sp.	7.4	-25.6	1	Mayor et al. (2009)	ECM
Hydnellum aurantiacum	7.6	-25.1	1	Mayor et al. (2009)	ECM
Hydnellum caeruleum	13.1	-22.7	1	Mayor et al. (2009)	ECM
Hydnellum caeruleum	8.4	-25.3	1	Mayor et al. (2009)	ECM
Hydnellum ferrugineum	10.7	-22.5	1	Mayor et al. (2009)	ECM
Hydnellum peckii	10.4	-23.3	1	Mayor et al. (2009)	ECM
Hydnellum peckii	6.3	-25.5	1	Mayor et al. (2009)	ECM

Hydnum repandum	9.2	-26	1	Mayor et al. (2009)	ECM
Hydnum repandum	8.6	-28.6	1	Mayor et al. (2009)	ECM
Hydnum repandum	8.6	-25.4	1	Mayor et al. (2009)	ECM
Hydnum rufescens	8.9	-25.4	1	Mayor et al. (2009)	ECM
Hydnum rufescens	7	-26	1	Mayor et al. (2009)	ECM
Hydnum umbilicatum	6.3	-25	1	Mayor et al. (2009)	ECM
Hygrocybe ,Äútoe-head,Äù	7.994	-28.7	1	Mayor et al. (2009)	UNK
Hygrophorus agathosmus	6.4	-28.1	1	Mayor et al. (2009)	ECM
Hygrophorus bakerensis	3.5	-25.5	1	Mayor et al. (2009)	ECM
Hygrophorus camarophyllus	3.1	-24.6	1	Mayor et al. (2009)	ECM
Hygrophorus camarophyllus	4.1	-25.5	1	Mayor et al. (2009)	ECM
Hygrophorus chrysodon	1.1	-25.7	1	Mayor et al. (2009)	ECM
Hygrophorus eburneus	3.1	-25.7	1	Mayor et al. (2009)	ECM
Hygrophorus lindtneri	-6.6	-28.6	1	Mayor et al. (2009)	ECM
Hygrophorus olivaceoalbus	2.7	-24.9	1	Mayor et al. (2009)	ECM
Hygrophorus purpurascens	7.6	-24.9	1	Mayor et al. (2009)	ECM
Hygrophorus sp.	3	-25.3	1	Mayor et al. (2009)	ECM
Hymenogasterales sp.	13.1	-26.3	1	Mayor et al. (2009)	ECM
Hypholoma udum	6.9	-25.3	1	Mayor et al. (2009)	UNK
Inocybe acuta	2.9	-26.2	1	Mayor et al. (2009)	ECM
Inocybe assimilata	2.8	-24.7	1	Mayor et al. (2009)	ECM
Inocybe ayangannae	7.4	-25.5	1	Mayor et al. (2009)	ECM
Inocybe cincinnata	3.5	-26.5	1	Mayor et al. (2009)	ECM
Inocybe fastigiata	3.6	-25	1	Mayor et al. (2009)	ECM
Inocybe friesii	4.1	-27	1	Mayor et al. (2009)	ECM
Inocybe geophylla	1.2	-26.9	1	Mayor et al. (2009)	ECM
Inocybe geophylla	4.2	-25	1	Mayor et al. (2009)	ECM
Inocybe hirsuta var. maxima	3.3	-24.6	1	Mayor et al. (2009)	ECM
Inocybe leiocephala	3.8	-25.9	1	Mayor et al. (2009)	ECM
Inocybe pseudodestricta	1.5	-26.5	1	Mayor et al. (2009)	ECM
Inocybe sp.	-0.7	-26.3	1	Mayor et al. (2009)	ECM
Inocybe tigrina	1.3	-26.9	1	Mayor et al. (2009)	ECM
Inocybe xanthomelas	1	-26.2	1	Mayor et al. (2009)	ECM
Laccaria amethystea	-3.6	-27.9	1	Mayor et al. (2009)	ECM
Laccaria amethystina	1.7	-27.4	1	Mayor et al. (2009)	ECM
Laccaria bicolor	1.3	-25.3	1	Mayor et al. (2009)	ECM
Laccaria laccata	1.6	-25.3	1	Mayor et al. (2009)	ECM
Lactarius badiosanguinea	8.5	-26.2	1	Mayor et al. (2009)	ECM
Lactarius camphoratus	3.8	-26.8	1	Mayor et al. (2009)	ECM
Lactarius deliciosus	6.5	-24.9	1	Mayor et al. (2009)	ECM

Lactarius deterrimus	7.5	-26.6	1	Mayor et al. (2009)	ECM
Lactarius fuliginosus	6.4	-24.9	1	Mayor et al. (2009)	ECM
Lactarius glyciosmus	4.3	-27	1	Mayor et al. (2009)	ECM
Lactarius hatsudake	2.5	-24.8	1	Mayor et al. (2009)	ECM
Lactarius helvus	6.9	-23.8	1	Mayor et al. (2009)	ECM
Lactarius kauffmanii var. kauffmanii	5.3	-25.6	1	Mayor et al. (2009)	ECM
Lactarius mitissimus	5.4	-25.1	1	Mayor et al. (2009)	ECM
Lactarius necator	2.5	-26	1	Mayor et al. (2009)	ECM
Lactarius obscuratus	6	-27.1	1	Mayor et al. (2009)	ECM
Lactarius olivaceo-umbrinus	1.8	-25.1	1	Mayor et al. (2009)	ECM
Lactarius olympianus	5.3	-24.8	1	Mayor et al. (2009)	ECM
Lactarius olympianus	5.4	-24.9	1	Mayor et al. (2009)	ECM
Lactarius pallescens var. pallescens	3.3	-25.1	1	Mayor et al. (2009)	ECM
Lactarius pseudomucidus	2.9	-25.5	1	Mayor et al. (2009)	ECM
Lactarius pseudomucidus	1.5	-25.1	1	Mayor et al. (2009)	ECM
Lactarius quietus	3.4	-24.3	1	Mayor et al. (2009)	ECM
Lactarius repraesentaneus	6.4	-26.5	1	Mayor et al. (2009)	ECM
Lactarius rubrilacteus	4.7	-24.3	1	Mayor et al. (2009)	ECM
Lactarius rufus	4	-24.9	1	Mayor et al. (2009)	ECM
Lactarius rufus	2	-25.3	1	Mayor et al. (2009)	ECM
Lactarius scrobiculatus	7.6	-27.3	1	Mayor et al. (2009)	ECM
Lactarius sp.	3.7	-25.9	1	Mayor et al. (2009)	ECM
Lactarius sp.	6.7	-25.5	1	Mayor et al. (2009)	ECM
Lactarius sp.	8.7	-23.7	1	Mayor et al. (2009)	ECM
Lactarius sp.	10.3	-26.6	1	Mayor et al. (2009)	ECM
Lactarius subcircellatus	2.6	-26.7	1	Mayor et al. (2009)	ECM
Lactarius subdulcis	-1	-26.7	1	Mayor et al. (2009)	ECM
Lactarius subflammeus	5.2	-25.8	1	Mayor et al. (2009)	ECM
Lactarius theiogalus	2.5	-27.1	1	Mayor et al. (2009)	ECM
Lactarius theiogalus	2.6	-24.6	1	Mayor et al. (2009)	ECM
Lactarius torminosus	5.5	-25.8	1	Mayor et al. (2009)	ECM
Lactarius trivialis	6.6	-23.5	1	Mayor et al. (2009)	ECM
Lactarius uvidus	3.4	-26	1	Mayor et al. (2009)	ECM
Lactarius uvidus	3.5	-26	1	Mayor et al. (2009)	ECM
Lactarius vietus	1.7	-26.2	1	Mayor et al. (2009)	ECM
Leccinum aurantiacum	9	-27.3	1	Mayor et al. (2009)	ECM
Leccinum holopus	2.2	-29.2	1	Mayor et al. (2009)	ECM
Leccinum molle	9	-27.6	1	Mayor et al. (2009)	ECM
Leccinum rotundifoliae	8.3	-26.7	1	Mayor et al. (2009)	ECM
Leccinum scabrum	4.5	-24.6	1	Mayor et al. (2009)	ECM

Leccinum scabrum	9.2	-27.5	1	Mayor et al. (2009)	ECM
Leccinum scabrum	7.3	-26.4	1	Mayor et al. (2009)	ECM
Leccinum scabrum	4.3	-27.4	1	Mayor et al. (2009)	ECM
Leccinum variicolor	7.7	-27.6	1	Mayor et al. (2009)	ECM
Leccinum variicolor	5.3	-26.1	1	Mayor et al. (2009)	ECM
Leccinum versipelle	12.3	-27.5	1	Mayor et al. (2009)	ECM
Leccinum versipelle	11.7	-27.1	1	Mayor et al. (2009)	ECM
Leccinum vulpinum	12.8	-25	1	Mayor et al. (2009)	ECM
Leotia lubrica	3	-25.7	1	Mayor et al. (2009)	ECM
Lyophyllum fumosum	16.4	-23.2	1	Mayor et al. (2009)	UNK
Lyophyllum semitale	11.7	-25	1	Mayor et al. (2009)	UNK
Marasmius maximus	8.6	-23	1	Mayor et al. (2009)	SAP
Microporus vernicipes	-0.3	-28.9	1	Mayor et al. (2009)	SAP
Mycena sp.	-3	-28	1	Mayor et al. (2009)	SAP
Mycena sp.	6.1	-23.5	1	Mayor et al. (2009)	SAP
Naucoria escharoides	4.3	-25.7	1	Mayor et al. (2009)	ECM
Paxillus involutus	9.1	-26.6	1	Mayor et al. (2009)	ECM
Paxillus involutus	6.6	-24.6	1	Mayor et al. (2009)	ECM
Perenniporia inflexibilis	2.57	-26.3	1	Mayor et al. (2009)	SAP
Perenniporia inflexibilis	2.8286	-25.823	1	Mayor et al. (2009)	SAP
Perenniporia inflexibilis	3.1919	-25.7969	1	Mayor et al. (2009)	SAP
Phaeocollybia attenuata	9.1	-24.3	1	Mayor et al. (2009)	ECM
Phaeocollybia benzokauffmanii	12.8	-23.5	1	Mayor et al. (2009)	ECM
Phaeocollybia fallax	6.9	-24.3	1	Mayor et al. (2009)	ECM
Phaeocollybia gregaria	13	-23.8	1	Mayor et al. (2009)	ECM
Phaeocollybia kauffmanii	11	-25.4	1	Mayor et al. (2009)	ECM
Phaeocollybia lilacifolia	10.5	-26	1	Mayor et al. (2009)	ECM
Phaeocollybia piceae	7	-25.3	1	Mayor et al. (2009)	ECM
Phellodon niger	13.5	-24.6	1	Mayor et al. (2009)	ECM
Phellodon niger	8.1	-22.7	1	Mayor et al. (2009)	ECM
Phellodon tomentosa	14.1	-24.1	1	Mayor et al. (2009)	ECM
Pholiota squarrosa	5.2	-23.6	1	Mayor et al. (2009)	SAP
Pisolithus tinctorius	8.8	-24.7	1	Mayor et al. (2009)	ECM
Pleurotus ostreatus	1.4	-25.7	1	Mayor et al. (2009)	SAP
Pseudotulostoma volvata	3.7	-27.4	1	Mayor et al. (2009)	ECM
Pseudotulostoma volvata	8.9	-26.6	1	Mayor et al. (2009)	Ecm
Pseudotulostoma volvata	5.721	-26.42	1	Mayor et al. (2009)	ECM
Ramaria sp.	11.7	-27.4	1	Mayor et al. (2009)	ECM
Ramaria testaceoflava	6	-25.4	1	Mayor et al. (2009)	ECM
Ramariopsis corniculata	13.82	-25.98	1	Mayor et al. (2009)	NS-NE

Ramariopsis fusiformis	16.02	-26.32	1	Mayor et al. (2009)	NS-NE
Ramariopsis fusiformis	20.54	-27.38	1	Mayor et al. (2009)	NS-NE
Ramariopsis fusiformis	14.3	-24.51	1	Mayor et al. (2009)	NS-NE
Ramariopsis kunzei	13	-25.94	1	Mayor et al. (2009)	NS-NE
Ramariopsis kunzei	13.86	-24.89	1	Mayor et al. (2009)	NS-NE
Ramariopsis kunzei	13.8	-25.94	1	Mayor et al. (2009)	NS-NE
Ramariopsis kunzei	13.71	-27.18	1	Mayor et al. (2009)	NS-NE
Ramariopsis kunzei	14.61	-24.19	1	Mayor et al. (2009)	NS-NE
Ramariopsis laeticolor	11.45	-26.16	1	Mayor et al. (2009)	NS-NE
Rhizopogon obtextus	9.5	-25	1	Mayor et al. (2009)	ECM
Rhizopogon sp.	9.3	-25.4	1	Mayor et al. (2009)	ECM
Rozites caperata	4	-25.3	1	Mayor et al. (2009)	ECM
Rozites caperata	8.1	-25.8	1	Mayor et al. (2009)	ECM
Rozites caperata	6.6	-24.7	1	Mayor et al. (2009)	ECM
Rozites caperatus	3.4	-26.6	1	Mayor et al. (2009)	ECM
Russula adusta	4.3	-26.8	1	Mayor et al. (2009)	ECM
Russula aeruginea	2.2	-26	1	Mayor et al. (2009)	ECM
Russula aff. mariae	6.2	-24.6	1	Mayor et al. (2009)	ECM
Russula alboareolata	2	-25.3	1	Mayor et al. (2009)	ECM
Russula albonigra	5.4	-27	1	Mayor et al. (2009)	ECM
Russula atrorubens	1.4	-25.5	1	Mayor et al. (2009)	ECM
Russula betularum	0.6	-25.5	1	Mayor et al. (2009)	ECM
Russula bicolor	-1.3	-26.7	1	Mayor et al. (2009)	ECM
Russula brevipes	3.5	-24.8	1	Mayor et al. (2009)	ECM
Russula brevipes	6	-24.4	1	Mayor et al. (2009)	ECM
Russula coerulea	5.1	-24.1	1	Mayor et al. (2009)	ECM
Russula crassotunicata	0.4	-26.4	1	Mayor et al. (2009)	ECM
Russula cremericolor	1.2	-27.3	1	Mayor et al. (2009)	ECM
Russula decolorans	0.8	-25.4	1	Mayor et al. (2009)	ECM
Russula delica	7.9	-24.5	1	Mayor et al. (2009)	ECM
Russula emetica	3.4	-24.9	1	Mayor et al. (2009)	ECM
Russula emetica	1.9	-24.9	1	Mayor et al. (2009)	ECM
Russula fellea	-0.4	-26.2	1	Mayor et al. (2009)	ECM
Russula foetens	8.4	-24.8	1	Mayor et al. (2009)	ECM
Russula fragilis	1.6	-26	1	Mayor et al. (2009)	ECM
Russula fragilis	0.9	-25.7	1	Mayor et al. (2009)	ECM
Russula gr. fragilis	0.5	-27.1	1	Mayor et al. (2009)	ECM
Russula gracillima	8.4	-27.7	1	Mayor et al. (2009)	ECM
Russula griseascens	2.6	-26.4	1	Mayor et al. (2009)	ECM
Russula integra	4.4	-25.4	1	Mayor et al. (2009)	ECM

Russula integra	3.8	-24.9	1	Mayor et al. (2009)	ECM
Russula krombholzii	3.6	-28.1	1	Mayor et al. (2009)	ECM
Russula laurocerasi	4.7	-24.9	1	Mayor et al. (2009)	ECM
Russula mairei	-2.2	-27.4	1	Mayor et al. (2009)	ECM
Russula nigricans	4.6	-25.2	1	Mayor et al. (2009)	ECM
Russula ochroleuca	2.4	-26.7	1	Mayor et al. (2009)	ECM
Russula ochroleuca	5.2	-24.2	1	Mayor et al. (2009)	ECM
Russula paludosa	6.3	-25	1	Mayor et al. (2009)	ECM
Russula paludosa	3.8	-25.2	1	Mayor et al. (2009)	ECM
Russula puellaris	1.8	-25.5	1	Mayor et al. (2009)	ECM
Russula puellaris	1	-26.7	1	Mayor et al. (2009)	ECM
Russula queletii	7.9	-27.5	1	Mayor et al. (2009)	ECM
Russula raoultii	-1.1	-27.3	1	Mayor et al. (2009)	ECM
Russula rhodopoda	0.5	-26	1	Mayor et al. (2009)	ECM
Russula sanguinea	9	-24.5	1	Mayor et al. (2009)	ECM
Russula sardonia	4.4	-26.3	1	Mayor et al. (2009)	ECM
Russula sororia	4.4	-25.2	1	Mayor et al. (2009)	ECM
Russula sp.	2.6	-25.8	1	Mayor et al. (2009)	ECM
Russula sp.	6.3	-24.3	1	Mayor et al. (2009)	ECM
Russula sp.	4.9	-24.3	1	Mayor et al. (2009)	ECM
Russula sp.	6.8	-25.1	1	Mayor et al. (2009)	ECM
Russula sp.	7.2	-24.4	1	Mayor et al. (2009)	ECM
Russula sp.	8.4	-25	1	Mayor et al. (2009)	ECM
Russula sp.	4.4	-24.5	1	Mayor et al. (2009)	ECM
Russula xerampelina	-0.4	-26.3	1	Mayor et al. (2009)	ECM
Russula xerampelina	4	-24.8	1	Mayor et al. (2009)	ECM
Sarcodon fuscoindicum	9.1	-25.3	1	Mayor et al. (2009)	ECM
Sarcodon scabrosum	7.6	-24.9	1	Mayor et al. (2009)	ECM
Scleroderma citrinum	2.6	-25.4	1	Mayor et al. (2009)	ECM
Scleroderma citrinum	5.7	-24.7	1	Mayor et al. (2009)	ECM
Scleroderma sp.	5.5	-28.9	1	Mayor et al. (2009)	ECM
Scleroderma verrucosum	2	-27.4	1	Mayor et al. (2009)	ECM
Stipitochaete damaechornis	-0.6	-26.8	1	Mayor et al. (2009)	SAP
Stipitochaete damaechornis	6.5	-24.9	1	Mayor et al. (2009)	ECM
Strobilomyces polypyramis	4.5	-27.1	1	Mayor et al. (2009)	ECM
Strobilomyces sp.	8.6	-25.7	1	Mayor et al. (2009)	ECM
Suillus bovinus	10.1	-25.2	1	Mayor et al. (2009)	ECM
Suillus bovinus	12.5	-25.2	1	Mayor et al. (2009)	ECM
Suillus granulatus	5.6	-24.1	1	Mayor et al. (2009)	ECM
Suillus lakei	5.8	-24.5	1	Mayor et al. (2009)	ECM

Suillus lakei	8.9	-23.9	1	Mayor et al. (2009)	ECM
Suillus lakei	11.9	-22.6	1	Mayor et al. (2009)	ECM
Suillus luteus	11.8	-24.6	1	Mayor et al. (2009)	ECM
Suillus pseudobrevipes	6.7	-24.3	1	Mayor et al. (2009)	ECM
Suillus punctatipes	11.6	-25	1	Mayor et al. (2009)	ECM
Suillus sibiricus	6	-24.1	1	Mayor et al. (2009)	ECM
Suillus variegatus	6	-25	1	Mayor et al. (2009)	ECM
Suillus variegatus	4.8	-24.3	1	Mayor et al. (2009)	ECM
Thelephora sp.	9.9	-27.4	1	Mayor et al. (2009)	ECM
Thelephoraceae sp.	5.8	-24.3	1	Mayor et al. (2009)	ECM
Thelephoraceae sp.	5.7	-27.6	1	Mayor et al. (2009)	ECM
Tomentella sp.	5	-28.5	1	Mayor et al. (2009)	ECM
Trametes scopulosa	1.6	-26.5	1	Mayor et al. (2009)	SAP
Tricholoma "hygrophorous lg. orange"	5.35	-26.6	1	Mayor et al. (2009)	ECM
Tricholoma atroviolaceum	7.5	-27.3	1	Mayor et al. (2009)	ECM
Tricholoma atroviolaceum	11	-25.7	1	Mayor et al. (2009)	ECM
Tricholoma columbetta	10.2	-27.1	1	Mayor et al. (2009)	ECM
Tricholoma flavovirens	10	-25.4	1	Mayor et al. (2009)	ECM
Tricholoma flavovirens	2.7	-26.1	1	Mayor et al. (2009)	ECM
Tricholoma flavovirens	7.7	-25.3	1	Mayor et al. (2009)	ECM
Tricholoma focale	9.7	-26	1	Mayor et al. (2009)	ECM
Tricholoma fracticum	13.1	-24.3	1	Mayor et al. (2009)	ECM
Tricholoma fucatum	8.2	-25.8	1	Mayor et al. (2009)	ECM
Tricholoma fulvum	12.5	-26.6	1	Mayor et al. (2009)	ECM
Tricholoma imbricatum	6.6	-26.7	1	Mayor et al. (2009)	ECM
Tricholoma inamoenum	5.4	-25.3	1	Mayor et al. (2009)	ECM
Tricholoma magnivelare	12.1	-25.7	1	Mayor et al. (2009)	ECM
Tricholoma myomyces	6.4	-25.5	1	Mayor et al. (2009)	ECM
Tricholoma nigrum	9.7	-26.3	1	Mayor et al. (2009)	ECM
Tricholoma pardinum	7.6	-24.9	1	Mayor et al. (2009)	ECM
Tricholoma saponaceum	6.5	-24	1	Mayor et al. (2009)	ECM
Tricholoma saponaceum	7.7	-24.2	1	Mayor et al. (2009)	ECM
Tricholoma saponaceum	10.3	-25.3	1	Mayor et al. (2009)	ECM
Tricholoma sciodes	8.9	-26.9	1	Mayor et al. (2009)	ECM
Tricholoma sejunctum	6.8	-25.5	1	Mayor et al. (2009)	ECM
Tricholoma sp.	8	-24.8	1	Mayor et al. (2009)	ECM
Tricholoma sp.	18.3	-24.3	1	Mayor et al. (2009)	ECM
Tricholoma sp.	19.8	-25.3	1	Mayor et al. (2009)	ECM
Tricholoma ustale	9.6	-27.1	1	Mayor et al. (2009)	ECM
Tricholoma vaccinum	9.4	-26.9	1	Mayor et al. (2009)	ECM

Tricholoma vaccinum	6.8	-25.3	1	Mayor et al. (2009)	ECM
Tricholoma virgatum	11	-25.2	1	Mayor et al. (2009)	ECM
Tricholoma virgatum	10.7	-24.3	1	Mayor et al. (2009)	ECM
Tricholoma virgatum	9.8	-25.7	1	Mayor et al. (2009)	ECM
Tricholoma virgatum	8.4	-23.9	1	Mayor et al. (2009)	ECM
Tylopilus castaneiceps	7.4	-24.6	1	Mayor et al. (2009)	ECM
Tylopilus exiguus	5.4	-27.1	1	Mayor et al. (2009)	ECM
Tylopilus exiguus	4.1	-25.6	1	Mayor et al. (2009)	ECM
Tylopilus exiguus	5.659	-25.3	1	Mayor et al. (2009)	ECM
Tylopilus felleus	3	-25	1	Mayor et al. (2009)	ECM
Tylopilus porphyrosporus	3.9	-24.4	1	Mayor et al. (2009)	ECM
Tylopilus potamogeton var. irengensis	4.3	-24.8	1	Mayor et al. (2009)	ECM
Tylopilus sp.	6.6	-23.3	1	Mayor et al. (2009)	ECM
Tylopilus sp.	5.7	-23.5	1	Mayor et al. (2009)	ECM
Tylopilus sp.	7.7	-23.9	1	Mayor et al. (2009)	ECM
Tylopilus sp.	8.4	-24.7	1	Mayor et al. (2009)	ECM
Xanthoconium affine	5.4	-24.1	1	Mayor et al. (2009)	ECM
Xerocomus badius	5.6	-25.3	1	Mayor et al. (2009)	ECM
Xerocomus badius	2.1	-25.8	1	Mayor et al. (2009)	ECM
Xerocomus chrysenteron	4.2	-26.4	1	Mayor et al. (2009)	ECM
Xerocomus subtomentosus	6.7	-25.9	1	Mayor et al. (2009)	ECM
Xylaria sp.	0.2	-26.3	1	Mayor et al. (2009)	SAP
Xylaria sp. "on D. cor. Pod"	3.7	-26.3	1	Mayor et al. (2009)	SAP
Xylaria sp. "on D. cor. Pod"	3.7	-25.1	1	Mayor et al. (2009)	SAP
Albomagister sp.	1.7	-27.05	1	Sanchez-Garcia et al. (2017)	ECM
Albomagister subaustralis	9.08	-25.86	1	Sanchez-Garcia et al. (2017)	ECM
Albomagister subaustralis	7.33	-24.53	1	Sanchez-Garcia et al. (2017)	ECM
Albomagister subaustralis	10.28	-25.7	1	Sanchez-Garcia et al. (2017)	ECM
Leucopaxillae gracillimus	13.1	-24.03	1	Sanchez-Garcia et al. (2017)	ECM
Lyophyllum aff. decastes	14.64	-25.24	1	Sanchez-Garcia et al. (2017)	ECM
Porpoloma portentosum	8.02	-26.99	1	Sanchez-Garcia et al. (2017)	ECM
Porpoloma sejunctum	9.84	-21.82	1	Sanchez-Garcia et al. (2017)	ECM
Porpoloma sp.	6.53	-30	1	Sanchez-Garcia et al. (2017)	ECM
Porpoloma sp.	7.78	-23.25	1	Sanchez-Garcia et al. (2017)	ECM
Porpoloma terreum	6.69	-24.35	1	Sanchez-Garcia et al. (2017)	ECM
Cortinarius sp. AHH158	4.26	-27.10	1	This study	ECM

Hygrocybe erubescens AHH296	6.40	-24.05	1	This study	ECM
Leccinum insigne AHH100	4.76	-25.03	1	This study	ECM
Tulostoma sp. DBG24092	11.97	-23.59	1	This study	UNK
Tulostoma sp. DBG27840	11.84	-22.31	1	This study	UNK
Tulostoma sp. DBG27840-1	12.47	-22.19	1	This study	UNK
Tulostoma sp. DBG27844	12.40	-23.30	1	This study	UNK
Tulostoma sp. DBG27849	12.97	-24.15	1	This study	UNK
Tulostoma sp. DBG27849-1	14.61	-23.64	1	This study	UNK
Tulostoma sp. DBG28849	12.90	-22.88	1	This study	UNK
Agaricus praeclaresquamous	6.7	-19.6	2	Hart et al. 2006	SAP
Agaricus silvicola	1.1	-23.6	2	Hart et al. 2006	SAP
Agaricus silvicola	5.2	-20.4	2	Hart et al. 2006	SAP
Armillaria albolanaripes	1	-21.5	2	Hart et al. 2006	SAP
Armillaria straminea var. americana	3.4	-22.1	2	Hart et al. 2006	SAP
Clavicorona pyxidata	5.2	-21.3	2	Hart et al. 2006	SAP
Claviocorona pyxidata	2	-22	2	Hart et al. 2006	SAP
Clitocybe dealbata	1.2	-22.2	2	Hart et al. 2006	SAP
Clitocybe subconnexa	1	-21.8	2	Hart et al. 2006	SAP
Coprinus atramentarius	0.4	-22.6	2	Hart et al. 2006	SAP
Coprinus comatus	-0.3	-23.6	2	Hart et al. 2006	SAP
Crucibulum laeve	-3	-21.3	2	Hart et al. 2006	SAP
Cyptotrama asprata	-0.1	-19.3	2	Hart et al. 2006	SAP
Cyptotrama chrysopeplum	0.5	-23.1	2	Hart et al. 2006	SAP
Gymnopilus bellulus	5	-23.3	2	Hart et al. 2006	SAP
Hygrophorus camarophyllus	-0.4	-23.6	2	Hart et al. 2006	ECM
Inocybe fastigiata	0.5	-25	2	Hart et al. 2006	ECM
Inocybe geophylla	1.7	-22.7	2	Hart et al. 2006	ECM
Inocybe geophylla	1.6	-24.6	2	Hart et al. 2006	ECM
Inocybe lacera	1.3	-22.5	2	Hart et al. 2006	ECM
Laccaria bicolor	2.4	-24	2	Hart et al. 2006	ECM
Lepiota cristata	1.8	-22.6	2	Hart et al. 2006	SAP
Lepiota cristata	1.6	-21.7	2	Hart et al. 2006	SAP
Leucopaxillis amarus	5.9	-20.1	2	Hart et al. 2006	SAP
Leucopaxillus amarus	3.9	-19.3	2	Hart et al. 2006	SAP
Lycoperdon perlatum	6.5	-22.3	2	Hart et al. 2006	SAP
Lycoperdon perlatum	4.8	-20.5	2	Hart et al. 2006	SAP
Megacollybia fallax	1.2	-22.1	2	Hart et al. 2006	SAP
Omphalotus olearius	-0.4	-22.9	2	Hart et al. 2006	SAP
Peziza badoconfusa	2.5	-22.1	2	Hart et al. 2006	SAP
Pholiota squarrosa	5.6	-23.1	2	Hart et al. 2006	SAP

Pluteus lutescens	1.7	-24	2	Hart et al. 2006	SAP
Russula claroflava	2.6	-24.1	2	Hart et al. 2006	ECM
Russula decolorans	1	-23.3	2	Hart et al. 2006	ECM
Russula integra	1.4	-24.9	2	Hart et al. 2006	ECM
Russula rosacea	1.3	-23.7	2	Hart et al. 2006	ECM
Russula sororia	4.3	-23	2	Hart et al. 2006	ECM
Xeromphalina campanella	-1.4	-22	2	Hart et al. 2006	SAP
Clavulina kunzei	-20.18	-0.83	2	Hou et al. 2012	SAP
Ganoderma applanatum	-20.56	-1.24	2	Hou et al. 2012	SAP
Pleurotus ostreatus	-22.68	-0.48	2	Hou et al. 2012	SAP
Ganoderma lucidum	-22.9	-1.31	2	Hou et al. 2012	SAP
Trametes versicolor	-21.7	-2.02	2	Hou et al. 2012	SAP
Clavicorona pyxidata	-20.99	-0.5	2	Hou et al. 2012	SAP
Calvatia craniiformis	-22.5	0.21	2	Hou et al. 2012	SAP
Cyathus striatus	-23.66	-0.32	2	Hou et al. 2012	SAP
Copyinds comatus	-23.19	-0.46	2	Hou et al. 2012	SAP
Lyophyllum decastes	-22.97	-0.87	2	Hou et al. 2012	SAP
Alloclavaria purpurea	3.1	-22.18	2	Korotkin et al. 2018	UNK
Alloclavaria purpurea	1.41	-24.86	2	Korotkin et al. 2018	UNK
Alloclavaria purpurea	2.8	-23.26	2	Korotkin et al. 2018	UNK
Bjerkandera adusta	2.53	-23.59	2	Korotkin et al. 2018	SAP
Blasiphalia pseudogrisella	1.07	-23.54	2	Korotkin et al. 2018	UNK
Blasiphalia pseudogrisella	1.68	-23.76	2	Korotkin et al. 2018	UNK
Blasiphalia pseudogrisella	2.04	-24.66	2	Korotkin et al. 2018	UNK
Cotylidia diaphana	2.42	-23.2	2	Korotkin et al. 2018	UNK
Cotylidia pannosa	-1.87	-24.04	2	Korotkin et al. 2018	UNK
Cotylidia undulata	-4.41	-24.29	2	Korotkin et al. 2018	UNK
Cotylidia undulata	-3.97	-24.34	2	Korotkin et al. 2018	UNK
Cotylidia undulata	-2.04	-23.29	2	Korotkin et al. 2018	UNK
Galerina marginata	0.42	-23.33	2	Korotkin et al. 2018	SAP
Gerronema cinctum (Omphalina brevibasidiata)	-1.51	-23.48	2	Korotkin et al. 2018	UNK
Inocybe subochracea	-3.06	-24.84	2	Korotkin et al. 2018	ECM
Loreleia postii	-0.66	-23.41	2	Korotkin et al. 2018	UNK
Loreleia postii	-2.56	-25.71	2	Korotkin et al. 2018	UNK
Muscinupta laevis	-0.9	-25.81	2	Korotkin et al. 2018	UNK
Odonticium romellii	-2.9	-22.32	2	Korotkin et al. 2018	SAP
Onnia tomentosa	-1.43	-21.37	2	Korotkin et al. 2018	SAP
Onnia tomentosa	-4.28	-22.66	2	Korotkin et al. 2018	SAP
Phellinus nigricans	0.44	-23.44	2	Korotkin et al. 2018	SAP
Phellinus nigricans	-4.48	-23.56	2	Korotkin et al. 2018	SAP

Rickenella fibula	-3.25	-26.04	2	Korotkin et al. 2018	UNK
Rickenella fibula	-2.92	-23.4	2	Korotkin et al. 2018	UNK
Rickenella fibula	-2.7	-25.54	2	Korotkin et al. 2018	UNK
Rickenella fibula	-0.16	-24.87	2	Korotkin et al. 2018	UNK
Rickenella fibula	-2.49	-25.81	2	Korotkin et al. 2018	UNK
Rickenella fibula	-0.25	-25.26	2	Korotkin et al. 2018	UNK
Rickenella fibula	-1.59	-24.89	2	Korotkin et al. 2018	UNK
Rickenella fibula	-0.13	-25.09	2	Korotkin et al. 2018	UNK
Rickenella fibula	-1.21	-22.95	2	Korotkin et al. 2018	UNK
Rickenella minuta	-3.77	-25.98	2	Korotkin et al. 2018	ECM
Rickenella minuta	0.78	-24.83	2	Korotkin et al. 2018	ECM
Rickenella minuta	2.36	-24.46	2	Korotkin et al. 2018	ECM
Rickenella minuta	-2.13	-25.11	2	Korotkin et al. 2018	ECM
Rickenella swartzii	0.73	-24.23	2	Korotkin et al. 2018	UNK
Rigidoporus populinus	-3.64	-23.39	2	Korotkin et al. 2018	SAP
Rigidoporus populinus	-0.09	-22.11	2	Korotkin et al. 2018	SAP
Trametes ochracea	-3.5	-22.42	2	Korotkin et al. 2018	SAP
Trichaptum fuscoviolaceum	-4.15	-22.12	2	Korotkin et al. 2018	SAP
Trichaptum fuscoviolaceum	-2.14	-23.11	2	Korotkin et al. 2018	SAP
Agaricales sp.	-2.4	-20	2	Mayor et al. (2009)	UNK
Agaricus praeclaresquamosus	-0.1	-23.2	2	Mayor et al. (2009)	SAP
Agaricus praeclaresquamous	6.7	-19.6	2	Mayor et al. (2009)	SAP
Agaricus silvaticus	5.9	-22	2	Mayor et al. (2009)	SAP
Agaricus silvicola	1.1	-23.6	2	Mayor et al. (2009)	SAP
Agaricus silvicola	5.2	-20.4	2	Mayor et al. (2009)	SAP
Agaricus sp.	3.2	-22.8	2	Mayor et al. (2009)	SAP
Agaricus sp.	3.1	-21.4	2	Mayor et al. (2009)	SAP
Agaricus subrutilescens	-0.4	-23.5	2	Mayor et al. (2009)	SAP
Amanita abrupta	-1.7	-25	2	Mayor et al. (2009)	ECM
Amanita citrina	1.3	-23.1	2	Mayor et al. (2009)	ECM
Amanita esculenta	2.4	-23.4	2	Mayor et al. (2009)	ECM
Amanita pantherina	-1.3	-24.5	2	Mayor et al. (2009)	ECM
Amanita porphyria	2.3	-23.3	2	Mayor et al. (2009)	ECM
Amanita rubescens	2.5	-22.9	2	Mayor et al. (2009)	ECM
Amanita sp.	1.7	-24.4	2	Mayor et al. (2009)	ECM
Amauroderma gusmanianum	0.6	-24.5	2	Mayor et al. (2009)	SAP
Amauroderma gusmanianum	0.4	-24.4	2	Mayor et al. (2009)	SAP
Amauroderma gusmanianum	1	-23.9	2	Mayor et al. (2009)	SAP
Aphyllophorales sp.	6.5	-20.8	2	Mayor et al. (2009)	UNK
Aphyllophorales sp.	-2.6	-21	2	Mayor et al. (2009)	UNK

Armillaria albolanaripes	1	-21.5	2	Mayor et al. (2009)	SAP
Armillaria borealis	-2	-23.8	2	Mayor et al. (2009)	SAP
Armillaria gallica	-0.9	-20.2	2	Mayor et al. (2009)	SAP
Armillaria gallica	0.6	-20.9	2	Mayor et al. (2009)	SAP
Armillaria nabsnona	0.6	-23.2	2	Mayor et al. (2009)	SAP
Armillaria ostoyae	-0.6	-22.2	2	Mayor et al. (2009)	SAP
Armillaria straminea var. americana	3.4	-22.1	2	Mayor et al. (2009)	SAP
Armillariella mellea	-0.1	-22.2	2	Mayor et al. (2009)	SAP
Ascocoryne sarcoides	-5.2	-22.8	2	Mayor et al. (2009)	SAP
Auriscalpium vulgare	-3	-22.2	2	Mayor et al. (2009)	SAP
Baeospora myosura	-1.9	-21	2	Mayor et al. (2009)	SAP
Boletaceae sp.	0	-24.5	2	Mayor et al. (2009)	ECM
Boletus edulis	3.7	-23.8	2	Mayor et al. (2009)	ECM
Boletus pseudocalopus	-0.3	-24	2	Mayor et al. (2009)	SAP
Boletus pseudocalopus	1.5	-23.7	2	Mayor et al. (2009)	ECM
Bondarzewia montana	1.8	-22.1	2	Mayor et al. (2009)	SAP
Calocera viscosa	-2.3	-21.5	2	Mayor et al. (2009)	SAP
Calvatia craniiformis	2.4	-23.3	2	Mayor et al. (2009)	SAP
Calvatia craniiformis	-1.5	-23.4	2	Mayor et al. (2009)	SAP
Cantherellus pleurotoides	1	-25	2	Mayor et al. (2009)	ECM
Chalciporus piperatus	8.3	-22.3	2	Mayor et al. (2009)	ECM
Chalciporus piperatus	7.9	-22.2	2	Mayor et al. (2009)	ECM
Chroogomphus tomentosus	-3.5	-24.7	2	Mayor et al. (2009)	ECM
Chroogomphus tomentosus	-4.4	-26.1	2	Mayor et al. (2009)	ECM
Chrysomphalina aurantiaca	-1.2	-23.7	2	Mayor et al. (2009)	SAP
Clavicorona pyxidata	0	-22.4	2	Mayor et al. (2009)	SAP
Clavicorona pyxidata	5.2	-21.3	2	Mayor et al. (2009)	SAP
Claviocorona pyxidata	2	-22	2	Mayor et al. (2009)	SAP
Clavulina cristata	2	-24.3	2	Mayor et al. (2009)	ECM
Clitocybe clavipes	-2	-23.6	2	Mayor et al. (2009)	SAP
Clitocybe clavipes	-2.1	-25.6	2	Mayor et al. (2009)	SAP
Clitocybe clavipes	0.3	-23	2	Mayor et al. (2009)	SAP
Clitocybe dealbata	1.2	-22.2	2	Mayor et al. (2009)	SAP
Clitocybe gibba	-0.6	-23.8	2	Mayor et al. (2009)	SAP
Clitocybe subconnexa	1	-21.8	2	Mayor et al. (2009)	SAP
Clitocybe subditopoda	0.5	-23.5	2	Mayor et al. (2009)	SAP
Clitocybe vibecina	0.8	-23.1	2	Mayor et al. (2009)	SAP
Clitopilus prunulus	6.7	-22.9	2	Mayor et al. (2009)	SAP
Clitopilus prunulus	3.8	-22.4	2	Mayor et al. (2009)	SAP
Coliatia sp.	-1.4	-24	2	Mayor et al. (2009)	UNK

Collybia butyracea	-0.5	-22.9	2	Mayor et al. (2009)	SAP
Collybia dryophila	-2.2	-23.9	2	Mayor et al. (2009)	SAP
Collybia dryophila	-0.2	-22.3	2	Mayor et al. (2009)	SAP
Collybia sp.	1	-22.4	2	Mayor et al. (2009)	SAP
Collybia sp.	-3.3	-24.2	2	Mayor et al. (2009)	SAP
Cookiena ,Äúon-wood,Äù	0.6	-24.9	2	Mayor et al. (2009)	SAP
Coprinus atramentarius	0.4	-22.6	2	Mayor et al. (2009)	SAP
Coprinus comatus	-0.3	-23.6	2	Mayor et al. (2009)	SAP
Coprinus disseminatus	0.8	-23.5	2	Mayor et al. (2009)	SAP
Coprinus radians	4.5	-22	2	Mayor et al. (2009)	SAP
Coprinus sp.	0.6	-23.1	2	Mayor et al. (2009)	SAP
Coprinus sp.	4.5	-23.8	2	Mayor et al. (2009)	SAP
Cortinarius sp.	0.2	-22.6	2	Mayor et al. (2009)	ECM
Cortinarius variosimilis	-1.5	-23.1	2	Mayor et al. (2009)	ECM
Crepidotus cesatii var. cesatii	-5.4	-22.7	2	Mayor et al. (2009)	SAP
Crepidotus sp.	1.5	-22.1	2	Mayor et al. (2009)	SAP
Crucibulum leave	-3	-21.3	2	Mayor et al. (2009)	SAP
Cyptotrama chrysopeplum	0.5	-23.1	2	Mayor et al. (2009)	SAP
Cyptotrma chrysopeplum	-0.1	-19.3	2	Mayor et al. (2009)	SAP
Cystoderma amianthinum	-2.3	-26.2	2	Mayor et al. (2009)	ECM
Cystoderma amianthinum	-2.8	-24.3	2	Mayor et al. (2009)	SAP
Cystoderma amiantinum	0.6	-22.2	2	Mayor et al. (2009)	SAP
Cystoderma caracharias	1	-23.8	2	Mayor et al. (2009)	SAP
Cystoderma granulosum	-0.3	-25.2	2	Mayor et al. (2009)	ECM
Cystoderma granulosum	-2.1	-24.2	2	Mayor et al. (2009)	SAP
Daedalea dickinsii	1.7	-20.2	2	Mayor et al. (2009)	SAP
Dermocybe cinnamomea	-5.2	-26.7	2	Mayor et al. (2009)	ECM
Dermocybe sp.	-0.7	-23.1	2	Mayor et al. (2009)	ECM
Earliella scabrosa	-0.5	-23.5	2	Mayor et al. (2009)	SAP
Entoloma clypeatus	-0.6	-23.4	2	Mayor et al. (2009)	SAP
Entoloma nitidum	2.2	-23.5	2	Mayor et al. (2009)	SAP
Favolaschia sp. "bubble-wrap"	-0.8	-23.9	2	Mayor et al. (2009)	SAP
Flammulina velutipes	-0.8	-24	2	Mayor et al. (2009)	SAP
Fomes fomentarius	-0.2	-22.7	2	Mayor et al. (2009)	SAP
Fomes fomentarius	-3.7	-22.9	2	Mayor et al. (2009)	SAP
Fomitopis cajanderi	-2.5	-23.4	2	Mayor et al. (2009)	SAP
Fomitopsis feei	-0.4	-25.5	2	Mayor et al. (2009)	SAP
Fomitopsis pinicola	-1	-20.7	2	Mayor et al. (2009)	SAP
Fomitopsis pinicola	-2.5	-22.3	2	Mayor et al. (2009)	SAP
Fomitopsis pinicola	-3.6	-21.8	2	Mayor et al. (2009)	SAP

Fomitopsis pinicola	-3.9	-22.5	2	Mayor et al. (2009)	SAP
Fomitopsis pinicola	-4.1	-22.8	2	Mayor et al. (2009)	SAP
Fomitopsis pinicola	-3.1	-21.6	2	Mayor et al. (2009)	SAP
Fomitopsis sp.	-5.2	-23.5	2	Mayor et al. (2009)	SAP
Ganoderma applanatum	3.3	-21.8	2	Mayor et al. (2009)	SAP
Ganoderma applanatum	-4.2	-22.8	2	Mayor et al. (2009)	SAP
Ganoderma oregonense	-2	-21.2	2	Mayor et al. (2009)	SAP
Grifola frondosa	2.5	-21.8	2	Mayor et al. (2009)	SAP
Gymnopilus bellulus	5	-23.3	2	Mayor et al. (2009)	SAP
Gymnopilus penetrans	-1.7	-22.4	2	Mayor et al. (2009)	SAP
Gymnopilus penetrans	-1.5	-23.6	2	Mayor et al. (2009)	SAP
Gymnopus acervatus	0.1	-22.8	2	Mayor et al. (2009)	SAP
Gymnopus acervatus	1	-22.2	2	Mayor et al. (2009)	SAP
Hebeloma incarnatulum	1.8	-24.6	2	Mayor et al. (2009)	ECM
Heterobasidion annosum	-1.6	-24.5	2	Mayor et al. (2009)	SAP
Heterobasidion annosum	-4.5	-25.1	2	Mayor et al. (2009)	SAP
Hexagonia tennis	-2.7	-26	2	Mayor et al. (2009)	SAP
Hirschioparus sp.	-3.8	-22.1	2	Mayor et al. (2009)	SAP
Hygrophoropsis aurantiaca	-0.1	-20.4	2	Mayor et al. (2009)	SAP
Hygrophoropsis aurantiaca	0.3	-19.4	2	Mayor et al. (2009)	SAP
Hygrophoropsis aurantiaca	-1	-20.2	2	Mayor et al. (2009)	SAP
Hygrophoropsis aurantiaca	4.6	-21.6	2	Mayor et al. (2009)	SAP
Hygrophoropsis aurantiaca	-2.8	-22	2	Mayor et al. (2009)	SAP
Hygrophoropsis aurantiaca	-1.2	-20.3	2	Mayor et al. (2009)	SAP
Hygrophoropsis auriantiaceae	-2	-19.6	2	Mayor et al. (2009)	SAP
Hygrophorus camarophyllus	-0.4	-23.6	2	Mayor et al. (2009)	SAP
Hygrophorus olivaceoalbus	-1.7	-26	2	Mayor et al. (2009)	ECM
Hypholoma capnoides	-1.3	-24.1	2	Mayor et al. (2009)	SAP
Hypholoma capnoides	-3.8	-21.9	2	Mayor et al. (2009)	SAP
Hypholoma fasciculare	-3.8	-24.3	2	Mayor et al. (2009)	SAP
Hypholoma fasciculare	0.1	-21.4	2	Mayor et al. (2009)	SAP
Hypholoma fasciculare	-0.5	-22.2	2	Mayor et al. (2009)	SAP
Hypholoma fasciculare	-0.9	-23.4	2	Mayor et al. (2009)	SAP
Hypholoma fasciculare	0.4	-23.8	2	Mayor et al. (2009)	SAP
Hypholoma fasiculare	-0.6	-22.8	2	Mayor et al. (2009)	SAP
Hypholoma marginatum	-2.8	-22.5	2	Mayor et al. (2009)	SAP
Inocybe fastigiata	0.5	-25	2	Mayor et al. (2009)	ECM
Inocybe geophylla	1.5	-24.5	2	Mayor et al. (2009)	ECM
Inocybe geophylla	1.7	-22.7	2	Mayor et al. (2009)	ECM
Inocybe geophylla	1.6	-24.6	2	Mayor et al. (2009)	ECM

Inocybe lacera	1.3	-22.5	2	Mayor et al. (2009)	ECM
Inocybe olympiana	0.5	-24.9	2	Mayor et al. (2009)	ECM
Inocybe rimosa	1.4	-24.7	2	Mayor et al. (2009)	ECM
Inocybe sp.	5	-23.1	2	Mayor et al. (2009)	ECM
Inocybe sp.	3.7	-24	2	Mayor et al. (2009)	ECM
Inocybe vaccina	1.2	-24.6	2	Mayor et al. (2009)	ECM
Inonotus tomentosus	-2.1	-23.5	2	Mayor et al. (2009)	SAP
Inonotus tomentosus	-3.9	-21.3	2	Mayor et al. (2009)	SAP
Ischnoderma resinosum	-2.4	-21.1	2	Mayor et al. (2009)	SAP
Kobayasia nipponica	-3.6	-23.2	2	Mayor et al. (2009)	SAP
Laccaria amethstina	-3.6	-26.3	2	Mayor et al. (2009)	ECM
Laccaria amethystina	-0.5	-24.1	2	Mayor et al. (2009)	ECM
Laccaria bicolor	0.2	-25.1	2	Mayor et al. (2009)	ECM
Laccaria bicolor	-0.5	-25.3	2	Mayor et al. (2009)	ECM
Laccaria bicolor	2.4	-24	2	Mayor et al. (2009)	ECM
Laccaria laccata	-3.3	-25.4	2	Mayor et al. (2009)	ECM
Laccaria sp.	1.4	-23	2	Mayor et al. (2009)	ECM
Lactarius chrysorrheus	1.3	-23.9	2	Mayor et al. (2009)	ECM
Lactarius chrysorrheus	2.6	-23.3	2	Mayor et al. (2009)	ECM
Lactarius chrysorrheus	-0.5	-24.3	2	Mayor et al. (2009)	ECM
Lactarius fallax var. concolor	1.1	-24.4	2	Mayor et al. (2009)	ECM
Lactarius musteus	1.6	-24.9	2	Mayor et al. (2009)	ECM
Lactarius theiogalus	-0.6	-25	2	Mayor et al. (2009)	ECM
Laetiporus conifericola	-2.4	-21.8	2	Mayor et al. (2009)	SAP
Laetiporus sp.	0.4	-22.5	2	Mayor et al. (2009)	SAP
Laetiporus sulphureus	-0.2	-22.6	2	Mayor et al. (2009)	SAP
Laetiporus sulphureus	1	-21.1	2	Mayor et al. (2009)	SAP
Lentinula edodes	0.9	-24.8	2	Mayor et al. (2009)	SAP
Lentinus sp.	-1.9	-24.2	2	Mayor et al. (2009)	SAP
Lepiota acutesquamosa	0.5	-22.7	2	Mayor et al. (2009)	SAP
Lepiota clypeolaria	3.8	-21.5	2	Mayor et al. (2009)	SAP
Lepiota cristata	1.8	-22.6	2	Mayor et al. (2009)	SAP
Lepiota cristata	1.6	-21.7	2	Mayor et al. (2009)	SAP
Lepiota magnispora	-0.8	-23.4	2	Mayor et al. (2009)	SAP
Lepiota sp.	-0.7	-23.1	2	Mayor et al. (2009)	SAP
Leucopaxillis amarus	5.9	-20.1	2	Mayor et al. (2009)	SAP
Leucopaxillus amarus	3.9	-19.3	2	Mayor et al. (2009)	SAP
Leucopaxillus giganteus	-2.2	-23	2	Mayor et al. (2009)	SAP
Lycoperdon foetidum	-2	-24.5	2	Mayor et al. (2009)	SAP
Lycoperdon perlatum	2.4	-23.3	2	Mayor et al. (2009)	SAP

Lycoperdon perlatum	1.9	-22.9	2	Mayor et al. (2009)	SAP
Lycoperdon sp.	-4.9	-24.3	2	Mayor et al. (2009)	SAP
Lycoperdon sp.	-1	-24.9	2	Mayor et al. (2009)	SAP
Lyophyllum connatum	1.4	-20.9	2	Mayor et al. (2009)	SAP
Macrolepiota sp.	0.8	-23.5	2	Mayor et al. (2009)	SAP
Marasmius androsaceus	-3.2	-25.7	2	Mayor et al. (2009)	SAP
Marasmius sp.	2.9	-21.8	2	Mayor et al. (2009)	SAP
Marasmius sp.	-0.9	-22.4	2	Mayor et al. (2009)	SAP
Marasmius sp.	-2.2	-25.2	2	Mayor et al. (2009)	SAP
Megacollybia platyphylla	-0.9	-23	2	Mayor et al. (2009)	SAP
Micromphale perforans	-3.4	-25	2	Mayor et al. (2009)	SAP
Microporus vernicipes	2.5	-24	2	Mayor et al. (2009)	SAP
Mycena aurantiidisca	-4.3	-25.4	2	Mayor et al. (2009)	SAP
Mycena aurantiidisca	-3.4	-24.9	2	Mayor et al. (2009)	SAP
Mycena clavicularis	-4	-24.5	2	Mayor et al. (2009)	SAP
Mycena clavicularis	-2	-25.2	2	Mayor et al. (2009)	SAP
Mycena epipterygia	-3.7	-22.8	2	Mayor et al. (2009)	SAP
Mycena galericulata	-3.3	-22.1	2	Mayor et al. (2009)	SAP
Mycena galopus	-3.6	-22.7	2	Mayor et al. (2009)	SAP
Mycena gr. murina	-3.8	-24	2	Mayor et al. (2009)	SAP
Mycena haematopoda	-2.2	-22.4	2	Mayor et al. (2009)	SAP
Mycena haematopoda	-2.9	-20.2	2	Mayor et al. (2009)	SAP
Mycena haematopus	-2.9	-22.7	2	Mayor et al. (2009)	SAP
Mycena inclinata	-1.7	-22.3	2	Mayor et al. (2009)	SAP
Mycena leptophylla	-0.4	-23.3	2	Mayor et al. (2009)	SAP
Mycena maculata	-1	-22.1	2	Mayor et al. (2009)	SAP
Mycena pura	6.4	-22.7	2	Mayor et al. (2009)	SAP
Mycena pura	0.8	-22.9	2	Mayor et al. (2009)	SAP
Mycena pura	0.4	-23.9	2	Mayor et al. (2009)	SAP
Mycena rosella	-3	-23.3	2	Mayor et al. (2009)	SAP
Mycena rosella	-2.9	-23.9	2	Mayor et al. (2009)	SAP
Mycena simia	4.6	-23.7	2	Mayor et al. (2009)	SAP
Mycena sp.	-1	-22.3	2	Mayor et al. (2009)	SAP
Mycena sp.	-0.2	-21.2	2	Mayor et al. (2009)	SAP
Mycena sp.	0.8	-21.5	2	Mayor et al. (2009)	SAP
Mycena sp.	0.6	-25.1	2	Mayor et al. (2009)	SAP
Mycena strobilinoides	-3.3	-23.2	2	Mayor et al. (2009)	SAP
Mycoleptodonoides aitchisonii	-0.2	-22.1	2	Mayor et al. (2009)	SAP
Naematoloma fasciculare	-0.8	-24	2	Mayor et al. (2009)	SAP
Oligoporus perdelicatus	-3.5	-20.4	2	Mayor et al. (2009)	SAP

Omphalotus olearis	-0.4	-22.9	2	Mayor et al. (2009)	SAP
Onnia vallata	-4.2	-24.2	2	Mayor et al. (2009)	SAP
Oudemansiella mucida	-7.1	-22.9	2	Mayor et al. (2009)	SAP
Oudemansiella mucida	-3.4	-22.1	2	Mayor et al. (2009)	SAP
Oudemansiella radicata	-0.6	-23.2	2	Mayor et al. (2009)	SAP
Oudemansiella venusolamellata	0.7	-23.5	2	Mayor et al. (2009)	SAP
Oxyporus cuneatus	-0.7	-23.5	2	Mayor et al. (2009)	SAP
Paxillus atromentarius	2.8	-23	2	Mayor et al. (2009)	SAP
Paxillus atrotomentosus	-1.3	-22.4	2	Mayor et al. (2009)	SAP
Perenniporia sp.	-1	-24.5	2	Mayor et al. (2009)	SAP
Peziza badoconfusa	2.5	-22.1	2	Mayor et al. (2009)	SAP
Phaeolepiota aurea	3.4	-21.4	2	Mayor et al. (2009)	SAP
Phaeolus schweinitzii	-0.2	-23	2	Mayor et al. (2009)	SAP
Phaeolus schweinitzii	0	-22.9	2	Mayor et al. (2009)	SAP
Phallus impudicus	-4.5	-23	2	Mayor et al. (2009)	SAP
Phallus impudicus	-2.8	-22.7	2	Mayor et al. (2009)	SAP
Phellinus pini	-3.1	-21.8	2	Mayor et al. (2009)	SAP
Phellinus robustus	-0.7	-24	2	Mayor et al. (2009)	SAP
Phellinus xeranticus	-0.9	-23.4	2	Mayor et al. (2009)	SAP
Pholiota astragalina	-1.2	-22.1	2	Mayor et al. (2009)	SAP
Pholiota astragalina	-3.3	-22.6	2	Mayor et al. (2009)	SAP
Pholiota decorata	-2	-23.7	2	Mayor et al. (2009)	SAP
Pholiota decorata	-2.6	-23.1	2	Mayor et al. (2009)	SAP
Pholiota flammans	-2.4	-23.2	2	Mayor et al. (2009)	SAP
Pholiota flammans	-3.7	-22.7	2	Mayor et al. (2009)	SAP
Pholiota flavida	-1	-22	2	Mayor et al. (2009)	SAP
Pholiota lenta	-1.5	-25.5	2	Mayor et al. (2009)	SAP
Pholiota lenta	-4	-23.5	2	Mayor et al. (2009)	SAP
Pholiota lubrica	-1.7	-22.3	2	Mayor et al. (2009)	SAP
Pholiota spumosa	-1.9	-21.4	2	Mayor et al. (2009)	SAP
Pholiota squarrosa	5.6	-23.1	2	Mayor et al. (2009)	SAP
Piptoporus betulinus	-4.6	-23.1	2	Mayor et al. (2009)	SAP
Pleurocybe porrigens	-1.2	-21.3	2	Mayor et al. (2009)	SAP
Pleurocybella porrigens	-1.1	-21.5	2	Mayor et al. (2009)	SAP
Pleurotus sp.	-2.3	-22.2	2	Mayor et al. (2009)	SAP
Pluteus cervinus	-1.8	-22.6	2	Mayor et al. (2009)	SAP
Pluteus cervinus	-2	-23.8	2	Mayor et al. (2009)	SAP
Pluteus lutescens	1.7	-24	2	Mayor et al. (2009)	SAP
Podoscypha nitidula	0.8	-23.9	2	Mayor et al. (2009)	SAP
Polyporaceae sp.	-3.5	-20.6	2	Mayor et al. (2009)	SAP

Polyporus alveolarius	-2.4	-23.3	2	Mayor et al. (2009)	SAP
Polyporus arcularius	-1.6	-22.4	2	Mayor et al. (2009)	SAP
Polyporus badius	-5.9	-22.3	2	Mayor et al. (2009)	SAP
Polyporus melanopus	0.1	-23.8	2	Mayor et al. (2009)	SAP
Polyporus melanopus	-2.9	-25.1	2	Mayor et al. (2009)	SAP
Polyporus sp.	-2	-22.5	2	Mayor et al. (2009)	SAP
Polyporus tenuiculus	-1.9	-23.9	2	Mayor et al. (2009)	SAP
Psathyrella gr. tephrophylla	2.4	-22.3	2	Mayor et al. (2009)	SAP
Psathyrella piluliformis	-3.3	-23.7	2	Mayor et al. (2009)	SAP
Psathyrella sp.	0.3	-22.5	2	Mayor et al. (2009)	SAP
Psathyrella sp.	-0.5	-24.8	2	Mayor et al. (2009)	SAP
Psathyrella sp.	1.1	-25	2	Mayor et al. (2009)	SAP
Psathyrella sp.	2.2	-23.9	2	Mayor et al. (2009)	SAP
Pseudocolus schellenbergiae	1.5	-23.9	2	Mayor et al. (2009)	SAP
Pseudohydnum gelatinosum	-2.7	-24	2	Mayor et al. (2009)	SAP
Pseudoplectania melaena	0.8	-24.9	2	Mayor et al. (2009)	UNK
Pseudoplectania melaena	-2.4	-25.4	2	Mayor et al. (2009)	UNK
Psilocybe sp.	12.5	-19	2	Mayor et al. (2009)	SAP
Ramaria abietina	-0.5	-23	2	Mayor et al. (2009)	ECM
Ramariaceae sp.	-1.3	-25.3	2	Mayor et al. (2009)	ECM
Rhodocollybia extuberans	2.2	-21.8	2	Mayor et al. (2009)	SAP
Rhodocollybia extuberans	-1.4	-23.6	2	Mayor et al. (2009)	SAP
Rhodocollybia maculata	-0.5	-21	2	Mayor et al. (2009)	SAP
Rhodocybe nitellina	2	-20.8	2	Mayor et al. (2009)	SAP
Roseoformes subflexibilis	-2.5	-22.3	2	Mayor et al. (2009)	UNK
Russula betularum	-0.7	-25.1	2	Mayor et al. (2009)	ECM
Russula claroflava	2.6	-24.1	2	Mayor et al. (2009)	ECM
Russula decolorans	1	-23.3	2	Mayor et al. (2009)	ECM
Russula fellea	2.4	-24.6	2	Mayor et al. (2009)	ECM
Russula fragilis	-0.6	-25.5	2	Mayor et al. (2009)	ECM
Russula integra	1.4	-24.9	2	Mayor et al. (2009)	ECM
Russula occidentalis	0.5	-25.3	2	Mayor et al. (2009)	ECM
Russula puellaris	3.1	-24.1	2	Mayor et al. (2009)	ECM
Russula queletii	1	-24.8	2	Mayor et al. (2009)	ECM
Russula rosacea	1.3	-23.7	2	Mayor et al. (2009)	ECM
Russula sororia	4.3	-23	2	Mayor et al. (2009)	ECM
Russula sp.	-0.6	-24.2	2	Mayor et al. (2009)	ECM
Russula sp.	-1.9	-25.1	2	Mayor et al. (2009)	ECM
Russula sp.	0.8	-24	2	Mayor et al. (2009)	ECM
Russula sp.	0.8	-22.9	2	Mayor et al. (2009)	ECM

Russula vinosa	0	-25.3	2	Mayor et al. (2009)	ECM
Stereum red-sta avg	-2.5	-21.4	2	Mayor et al. (2009)	SAP
Stereum-o hym	-1.2	-23.2	2	Mayor et al. (2009)	SAP
Stipitochaete damaechornis	2.7	-23	2	Mayor et al. (2009)	ECM
Strobilurus esculentus	-2.3	-21.9	2	Mayor et al. (2009)	SAP
Stropharia ambigua	-4.2	-23.3	2	Mayor et al. (2009)	SAP
Stropharia hornemanii	1.4	-23.1	2	Mayor et al. (2009)	SAP
Trametes aff. brevis	1.5	-23.8	2	Mayor et al. (2009)	ECM
Trametes hirsuta	0.2	-24	2	Mayor et al. (2009)	SAP
Trametes versicolor	2.2	-24.2	2	Mayor et al. (2009)	SAP
Trametes versicolor	-3.1	-23.2	2	Mayor et al. (2009)	SAP
Trametes versicolor	-1.3	-23.1	2	Mayor et al. (2009)	SAP
Trichaptum biforme	-2.5	-22.3	2	Mayor et al. (2009)	SAP
Trichholomopsis platyphylla	1.2	-22.1	2	Mayor et al. (2009)	SAP
Tricholomataceae sp.	3.7	-23.1	2	Mayor et al. (2009)	UNK
Tricholomopsis decora	-1.9	-22.7	2	Mayor et al. (2009)	SAP
Tricholomopsis decora	-3.8	-22.9	2	Mayor et al. (2009)	SAP
Tricholomopsis sulfureoides	-1.3	-22	2	Mayor et al. (2009)	SAP
Xerocomus badius	3.7	-23.7	2	Mayor et al. (2009)	ECM
Xerocomus subtomentosus	3.4	-24.1	2	Mayor et al. (2009)	ECM
Xeromphalina campanella	-3	-21.7	2	Mayor et al. (2009)	SAP
Xeromphalina campanella	-1.4	-22	2	Mayor et al. (2009)	SAP
Xeromphalina cornui	-3.5	-23.5	2	Mayor et al. (2009)	SAP
Xeromphalina cornui	-4.8	-23	2	Mayor et al. (2009)	SAP
Aspropaxillus septentrionalis	4.34	-20.85	2	Sanchez-Garcia et al. (2017)	SAP
Callistosporium luteo-olivaceum	0.45	-21.92	2	Sanchez-Garcia et al. (2017)	SAP
Calocybe naucoria	-2.6	-23.44	2	Sanchez-Garcia et al. (2017)	SAP
Cleistocybe vernalis	0.84	-18.65	2	Sanchez-Garcia et al. (2017)	SAP
Clitocybe candicans	1.01	-22.08	2	Sanchez-Garcia et al. (2017)	SAP
Clitocybe dealbata	2.67	-21.79	2	Sanchez-Garcia et al. (2017)	SAP
Clitocybe nebularis	1.56	-22.96	2	Sanchez-Garcia et al. (2017)	SAP
Clitocybe sp.	-1.08	-23.31	2	Sanchez-Garcia et al. (2017)	SAP
Clitopilus prunulus	2.42	-20.97	2	Sanchez-Garcia et al. (2017)	SAP
Clitopilus scyphoides	1.05	-24.02	2	Sanchez-Garcia et al. (2017)	SAP
Dennisiomyces sp.	0.95	-24.52	2	Sanchez-Garcia et al. (2017)	SAP
Infundibulicybe gibba	0.1	-21.4	2	Sanchez-Garcia et al. (2017)	SAP

Lepista irina	4.98	-23.03	2	Sanchez-Garcia et al. (2017)	SAP
Lepista personata	6.64	-21.91	2	Sanchez-Garcia et al. (2017)	SAP
Lepista sordida	4.53	-15.79	2	Sanchez-Garcia et al. (2017)	SAP
Notholepista subzonalis	-0.41	-23.98	2	Sanchez-Garcia et al. (2017)	SAP
Pseudoclitocybe sp.	1.76	-23.84	2	Sanchez-Garcia et al. (2017)	SAP
Pseudoomphalina kalchbrenneri	-0.94	-22.24	2	Sanchez-Garcia et al. (2017)	SAP
Pseudoomphalina pachyphylla	-0.41	-23.06	2	Sanchez-Garcia et al. (2017)	SAP
Rhodocybe mundula	1.88	-22.63	2	Sanchez-Garcia et al. (2017)	SAP
Singerocybe adirondackensis	1.19	-22.7	2	Sanchez-Garcia et al. (2017)	SAP
Termitomyces microcarpus	1.86	-23.67	2	Sanchez-Garcia et al. (2017)	SAP
Tulostoma sp. DBG28413	2.15	-22.51	2	This study	SAP
Agaricus sp. AHH154	8.06	-18.97	2	This study	SAP
Tubaria sp. AHH259	-2.11	-19.29	2	This study	SAP
Tulostoma sp. DBG23778	0.98	-17.71	2	This study	SAP
Tulostoma sp. DBG23778-1	1.29	-17.55	2	This study	SAP
Tulostoma sp. DBG24482	0.60	-21.36	2	This study	SAP
Tulostoma sp. DBG24482-1	5.22	-21.02	2	This study	SAP
Tulostoma sp. DBG28400-1	3.37	-20.47	2	This study	SAP
Tulostoma sp. DBG29564	4.52	-20.93	2	This study	SAP
*Tulostoma sp.*DBG28400	3.84	-19.46	2	This study	SAP