Received: 19 October 2021 | Revised: 23 December 2021 | Accepted: 27 December 2021

DOI: 10.1002/edn3.281

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WILEY

ORIGINAL ARTICLE

On the origin of feces: Fungal diversity, distribution, and conservation implications from feces of small mammals

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Funding information
Natural History Museum of Utah

Abstract

Fungi are highly diverse, but only a small fraction of the total estimated species have been characterized. Often, the extent of diversity and distribution of fungal communities is difficult or near impossible to assess due to the fact that many fungi are cryptic and persist predominantly hidden within substrates such as soil or plant material. This is particularly true for hypogeous sporocarps, such as truffles and false truffles, which are extremely difficult to survey in a systematic manner. However, hypogeous fungi have evolved traits that make them highly attractive to animals, such as small mammals, which ingest and disperse fungal spores through defecation. Here, samples of feces from 138 small mammal museum vouchers collected in the western United States were assessed for total fungal diversity using a dual-index metabarcoding approach. Our findings exhibit many identifications within the mushroom-forming fungi (*Agaricomycetidae*), with 65 of the 138 samples containing sequences belonging to several species of the false truffle-forming genus *Rhizopogon*. Metadata for each collection, such as geospatial coordinates, can be used as a proxy for the presence or absence of *Rhizopogon* species identified in their feces. Utilizing these proxy data from only a few years of sampling, these records quadrupled the rate of observations of *Rhizopogon* made over the past 100 years, including some species that have only been recorded once previously. This substantial increase in datapoints has implications for how fungal distributions are interpreted, with direct impact on standard assessment tools for fungal conservation.

1 | INTRODUCTION

Kingdom *Fungi* is extremely diverse and ubiquitous across the globe, with ~148,000 currently accepted species known from the estimated 2.2–12 million species distributed on all continents and in most aquatic habitats (Hawksworth & Lücking, 2017; Wu et al., 2019). Documentation of fungal diversity has lagged far behind that of other groups of multicellular organisms, largely due to their generally cryptic habits and their unpredictable production of ephemeral macroscopic sporocarps, resulting in a severe bias in biodiversity knowledge towards plants and animals (Troudet et al., 2017). Recent technological advances such as molecular identification with high-throughput sequencing have made it possible to document fungal diversity indirectly from environmental samples, greatly accelerating the rate at which fungi can be detected and identified (Begerow et al., 2010; Chase & Fay, 2009; Nilsson et al., 2019; Schmidt et al., 2013). Despite this increase in efficiency, our knowledge of fungal distributions is still overwhelmingly incomplete. This may be due, in

part, to the inefficiency inherent in environmental sampling: small volumes over large areas require large numbers of samples, which is compounded by the generally low biomass of target organisms in each sample. Unlike plant and animal surveys, where the target organisms are more easily observed and more consistently observable over long periods of time or can be baited and trapped, comprehensive fungal surveys are much more difficult given the cryptic nature of their subjects. The resulting deficiency of information on fungal diversity has major consequences for a number of fields, such as taxonomy and conservation. Coupled with the high diversity of fungi, low sampling efficiency creates a double-edged sword that has led to the vast majority of species being undocumented, undescribed, or known only from DNA sequences (dubbed "dark matter fungi") (Ryberg & Nilsson, 2018).

Many fungi participate in specialized associations with other organisms, especially plants (Tehler et al., 2003). Therefore, accurate documentation of these fungi is important to understand how these associations function at varying scales, from one-to-one interactions up to whole ecosystems. For example, ectomycorrhizal fungi that form symbiotic relationships with plant roots can provide important benefits to their host plant, including increased uptake of micronutrients, and receive carbon via photosynthates in a mutualistic exchange (Courty et al., 2010; Smith & Read, 2010). This symbiosis is globally important, with an estimated 60% of all woody tree stems belonging to obligately ectomycorrhizal plants (Steidinger et al., 2019). Baseline documentation of both partners in this important symbiosis is fundamental to fully understanding the association and to accurately predicting its response to future change. For example, the presence/absence of host generalist or specialist symbionts, and the relative role they play in maintaining and shaping the symbiosis, is key to understanding its resilience to environmental change (Liao et al., 2016; Wilson et al., 2012).

Conserving biodiversity is a key strategy to ensuring ecosystem resilience in the face of a changing planet. The IUCN Red List Categories and Criteria provide guidelines based on aspects of a species such as population size, the extent of occurrence, fragmentation, and ongoing changes to these characteristics to place species in one of several categories indicating conservation needs (Endangered, Vulnerable, etc.) (Mace et al., 2008; Rodríguez et al., 2015). These guidelines are designed to be widely applicable to many different types of organisms, including fungi (Cannon et al., 2018). The ability to accurately assess whether an organism can be categorized as threatened or endangered is extremely important within the context of conservation. Inaccurate assessment may lead to the misallocation of resources with respect to the actual risks facing wild populations. Due to the difficulty of locating most fungi, we know relatively little about their distributions, making it challenging to assess whether a particular fungus should receive conservation focus, or if our time and resources are better spent elsewhere.

One approach to improve sampling efficiency for fungi is to use a more reliably sampled source that can function as a proxy. For instance, EJH Corner famously trained monkeys to collect fruit samples from the tops of trees at the Singapore Botanic Gardens (Mabberley, 1999). Similarly, truffle hunters utilize pigs or trained dogs to locate and excavate prized and commercially valuable culinary delicacies, such as *Tuber magnatum* Picco (the Italian white truffle) hidden belowground (Riccioni et al., 2016). Like pigs and trained dogs, many small mammals of northern temperate forests, paralleled by marsupials of Australian forests, seek out and consume belowground (hypogeous) fungi, mostly truffles and false truffles, sometimes as the majority of their diets (Lehmkuhl et al., 2004; Maser et al., 1978; Vernes et al., 2015). Hypogeous fungi have likely been selected to encourage the discovery and consumption of their sporocarps by animals as a means to spread their spores aboveground, necessary due to the loss of ballistospory leading to or during the adoption of an enclosed sporocarp (Johnson, 1996). Moreover, because identifications of fleshy macrofungi in the feces of small mammals almost certainly reflect the presence of sporocarps, due to their small home range and rapid digestions (Hawes, 1977; Langer, 2002; Padmanabhan et al., 2013), observations of fungi in feces are reliable indicators of a metabolically active species' presence at a given location at a given time. This is important, because, unlike environmental DNA, these data points represent reproductively mature individuals that are established and growing, features that are important for assessing species distributions but are inherently lacking in eDNA samples. Fungivorous animals, therefore, are potential proxies for the fungi they consume. They are attracted to scents produced by truffle-forming fungi (Splivallo et al., 2011; Stephens et al., 2017) and have the capacity to continuously survey for them. With the use of passive trapping, small mammal feces may represent a source of highly efficient sampling for some fungi, especially those with hypogeous sporocarps.

Previous studies have used DNA sequencing of feces to monitor the population of threatened and elusive animals such as snow leopards and grizzly bears, and more recently to identify populations of African elephants to aid in identifying illegal ivory trading routes (Janečka et al., 2008; Phoebus et al., 2020; Wasser et al., 2004, 2015). Fecal sampling has also been used to identify the contents of animal diets, such as in rodents, hares, and even river otters (Buglione et al., 2018; Cloutier et al., 2019; Elliott et al., 2020; Harper et al., 2020), but has focused primarily on community identification, and in the context of the host diet rather than the ecology of community members.

These studies show how powerful and informationally rich fecal sampling can be for population distribution studies, but feces can also be a valuable source of metadata for taxa associated with consumption by the host (Boyer et al., 2015). While these previous studies focused on macrofauna, this same approach could be used for consumed fungi, which comprise food sources for many metazoan taxa ranging from fungivorous arthropods to opportunistic small mammals and even great apes (Hanson et al., 2003; Pyare & Longland, 2001; Yamashita et al., 2015). By targeting fungus-consuming hosts, which are sampled in both greater number and frequency than fungi, we can harness the power of museum collections to study fungal diversity and distribution indirectly. Using information from a specimen voucher that may otherwise be nonexistent

for fungal collections, we can build a better understanding of large-scale patterns that would otherwise require a massive investment in the field collection of fungal specimens.

In this study, we present the idea that the known ecology of a host along with voucher metadata, such as the geospatial coordinates, can be used as a proxy for a rough estimation of community member distribution in the case of difficult-to-find macrofungi. To meet this objective, we set out to address four questions, (1) Can DNA metabarcoding of fecal samples from museum collections be used to identify fungi associated with small mammal consumption, such as those that produce hypogeous sporocarps? (2) What diversity of fungus species can be recovered in a diverse collection of small mammal feces? (3) Can metadata, such as the geographic location of trapping, extend our understanding of the diversity, ecology, and distribution of fungi? (4) Do these "collections" aid in making meaningful conclusions about the distribution of fungal species that can be used to inform possible conservation efforts?

2 | RESULTS

2.1 | Raw sequencing data and fungal ASVs recovered

Read and amplicon sequence variant (ASV) processing was performed with DADA2 (Callahan et al., 2016, p. 2) along with taxonomic identification based on the UNITE fungal internal transcribed spacer (ITS) database (Abarenkov et al., 2010). ASV sequences are unique variations of a sequence that are lost when clustering sequences into traditional operational taxonomic units (OTUs) (Callahan et al., 2017). Assigning ASVs as opposed to OTUs allows for greater sensitivity in identifying and documenting variation that can occur within an assignable taxon. Total ASV sequence count for samples ranged between 2 and 5209 sequences, with an average of 1624 ASV sequences per sample. After the quality processing step of DADA2, and removing all samples represented by a single ASV ($n = 2$) and ASVs only represented by a single sequence ($n = 1$), 136 of 138 samples (Table 1) and 4650 unique ASVs representing 531 genera were left for further analysis.

Amplicon sequence variants were dominated by fungi belonging to the phyla *Ascomycota* and *Basidiomycota*, with the genera *Mycosphaerella* and *Rhizopogon* being the two most abundant ASVs (Figure 1). Other fungal orders of note found in high quantity were *Mucorales* (common dung-dwelling *Mucoromycota*), *Pleosporales* (common saprobic *Ascomycota* found on decaying plants), *Agaricales* (*Basidiomycota* producing typical "mushroom" as well as hypogeous sporocarps), *Tremellales* (dimorphic *Basidiomycota* that can produce yeasts and macroscopic sporocarps), and *Pezizales* (*Ascomycota* with macroscopic sporocarps, including the economically important morels and true truffles). Due to its known false truffle morphology, generally common distribution, and large presence within our sample set, the genus *Rhizopogon* Fr. (*Rhizopogonaceae:Boletales:Agaricomycotina:Basidiomycota*) was chosen for more in-depth analysis. Our dataset includes 65 samples of *Rhizopogon*, which contained 580 ASVs. Of these, 394 were assignable to species level, representing 11 *Rhizopogon* species, while 186 could only be identified to genus. Assignable ASVs were collapsed based on species assignment and unassignable ASVs were clustered together at 99% similarity, yielding 11 assignable taxa and 11 OTUs for further analysis.

2.2 | Phylogenetic analysis of recovered *Rhizopogon* ASVs and OTUs

With 186 of our *Rhizopogon* ASVs unassignable to known species, we sought to produce a more refined taxonomy utilizing phylogenetic analysis of the *Rhizopogon* diversity we recovered. To do this we parsed the general release UNITE database (version 8.2, 04 April 2020) (Abarenkov et al., 2010) for all species hypothesis (SH) sequences from the family *Rhizopogonaceae* followed by ITSx (Bengtsson-Palme et al., 2013) to extract the ITS2 sequence for each entry to standardize our amplicons along with the UNITE SH sequences (Nilsson et al., 2014). We then generated a phylogenetic tree, containing all UNITE sequences, species level assigned *Rhizopogon* ASVs collapsed into a single representative sequence, and our genus-level ASVs clustered into OTUs at 99%. Unfortunately, non-assignable OTUs could not be assigned to known species using phylogenetic analysis. This could be representative of undescribed diversity but is most likely due to a lack of representation found within the reference database, a common problem for fungal diversity studies (Hofstetter et al., 2019).

Having given our recovered sequences phylogenetic context, we compared *Rhizopogon* diversity across our small mammal hosts to detect any patterns of *Rhizopogon* consumption. To do this we calculated Faith's Phylogenetic Diversity (PD), a measurement of biodiversity based on phylogenetic distances between taxa, for *Rhizopogon* consumed by each mammal species (Faith, 2018). The PD of our *Rhizopogon*-consuming small mammals ranged from 1.262573 for *Peromyscus maniculatus*, to 0.213792 for *Dipodomys merriami* (Figure 2). This suggests that some small mammal species consume a wider diversity of *Rhizopogon* spp. compared to others. However, due to the small sample size and representation of hosts (some of which are singletons), it was necessary to calculate Faith's PD for individual samples. We found that our PD values fluctuated greatly across samples from a single host species (Table S3). We pursued this further using our highest small mammal PD samples (*Peromyscus maniculatus*, $n = 4$) and found that all four of them had been collected on the same day at the same geolocation. One sample, UMNH.Mamm.43034, had a high PD value of 0.775310717 while the others had values between 0.194667809 and 0.282245014 with little overlap in *Rhizopogon* species among samples. Together, these findings suggest that greater sampling of each host species is needed to determine if there is species-specific preference for some *Rhizopogon* species over others, and that species identity of the host may be less

TABLE 1 Voucher table of all specimens used in this study. Voucher information for each small mammal host including collection ID numbers, locality of collection, and time of year of each trapping

Specimen GUID	Small mammal taxa	Sex	Date collected	State	County	Latitude	Longitude
UMNH. Mamm.41281	*Zapus princeps*	Male	22-August-2016	Utah	Summit	40.6793	−110.93466
UMNH. Mamm.41283	*Zapus princeps*	Female	22-August-2016	Utah	Summit	40.6793	−110.93466
UMNH. Mamm.41598	*Sylvilagus nuttallii*	Female	21-March-2017	Utah	Salt Lake	40.57986	−111.82582
UMNH. Mamm.41635	*Tamias amoenus*	Female	17-June-2017	Idaho	Boise	44.17194	−116.1216
UMNH. Mamm.41643	*Peromyscus maniculatus*	Male	20-June-2017	Idaho	Boise	44.17194	−116.1216
UMNH. Mamm.41651	*Tamias amoenus*	Male	26-June-2017	Idaho	Boise	44.17194	−116.1216
UMNH. Mamm.41657	*Peromyscus maniculatus*	Female	26-June-2017	Idaho	Boise	44.17194	−116.1216
UMNH. Mamm.41658	*Peromyscus maniculatus*	Male	26-June-2017	Idaho	Boise	44.17194	−116.1216
UMNH. Mamm.41659	*Peromyscus maniculatus*	Male	26-June-2017	Idaho	Boise	44.17194	−116.1216
UMNH. Mamm.41748	*Sorex vagrans*	Female	15-June-2017	Utah	Box Elder	41.95416	−113.31827
UMNH. Mamm.41775	*Peromyscus truei*	Male	16-June-2017	Utah	Box Elder	41.9554	−113.32113
UMNH. Mamm.41784	*Zapus princeps*	Female	16-June-2017	Utah	Box Elder	41.95416	−113.31827
UMNH. Mamm.41785	*Zapus princeps*	Female	16-June-2017	Utah	Box Elder	41.95416	−113.31827
UMNH. Mamm.41786	*Zapus princeps*	Female	16-June-2017	Utah	Box Elder	41.95416	−113.31827
UMNH. Mamm.42078	*Callospermophilus lateralis*	Male	10-August-2017	Utah	Box Elder	41.92259	−113.42795
UMNH. Mamm.42079	*Zapus princeps*	Male	11-August-2017	Utah	Box Elder	41.92259	−113.42795
UMNH. Mamm.42172	*Sorex palustris*	Male	18-August-2017	Idaho	Custer	43.98791	−113.46612
UMNH. Mamm.42191	*Peromyscus maniculatus*	Male	18-August-2017	Idaho	Custer	43.98791	−113.46612
UMNH. Mamm.42176	*Tamias amoenus*	Female	18-August-2017	Idaho	Custer	43.98791	−113.46612
UMNH. Mamm.42178	*Tamias amoenus*	Male	18-August-2017	Idaho	Custer	43.98791	−113.46612
UMNH. Mamm.42179	*Tamias amoenus*	Male	18-August-2017	Idaho	Custer	43.98791	−113.46612
UMNH. Mamm.42244	*Tamias minimus*	Male	21-August-2017	Idaho	Custer	43.98045	−113.45559
UMNH. Mamm.42245	*Lemmiscus curatus*	Female	21-August-2017	Idaho	Custer	43.98045	−113.45559
UMNH. Mamm.42246	*Lemmiscus curatus*	Female	21-August-2017	Idaho	Custer	43.98045	−113.45559
UMNH. Mamm.42247	*Microtus montanus*	Female	21-August-2017	Idaho	Custer	43.98045	−113.45559

(Continues)

TABLE 1 (Continued)

Specimen GUID	Small mammal taxa	Sex	Date collected	State	County	Latitude	Longitude
UMNH. Mamm.42369	*Glaucomys sabrinus*	Female	29-September-2017	Utah	Summit	40.58663	−111.01935
UMNH. Mamm.42370	*Myodes gapperi*	Male	29-September-2017	Utah	Summit	40.58663	−111.01935
UMNH. Mamm.42371	*Microtus longicaudus*	Male	29-September-2017	Utah	Summit	40.58663	−111.01935
UMNH. Mamm.42383	*Myodes gapperi*	Male	29-September-2017	Utah	Summit	40.63144	−111.1756
UMNH. Mamm.42384	*Microtus longicaudus*	Female	29-September-2017	Utah	Summit	40.63144	−111.1756
UMNH. Mamm.42393	*Microtus longicaudus*	Male	29-September-2017	Utah	Summit	40.63144	−111.1756
UMNH. Mamm.42395	*Peromyscus maniculatus*	Male	29-September-2017	Utah	Summit	40.63144	−111.1756
UMNH. Mamm.42397	*Microtus longicaudus*	Male	29-September-2017	Utah	Summit	40.63144	−111.1756
UMNH. Mamm.42402	*Peromyscus maniculatus*	Male	29-September-2017	Utah	Summit	40.63144	−111.1756
UMNH. Mamm.42424	*Myodes gapperi*	Female	8-October-2017	Utah	Summit	40.6793	−110.93466
UMNH. Mamm.42428	*Otospermophilus variegatus*	Male	17-August-2017	Utah	Salt Lake	40.666	−111.806
UMNH. Mamm.42442	*Peromyscus maniculatus*	Female	17-August-2017	Oregon	Linn	44.62179	−122.04424
UMNH. Mamm.42443	*Peromyscus maniculatus*	Female	17-August-2017	Oregon	Linn	44.62179	−122.04424
UMNH. Mamm.42468	*Tamias dorsalis*	Male	12-July-2017	Nevada	White Pine	39.01477	−114.12662
UMNH. Mamm.42469	*Thomomys bottae*	Male	10-July-2016	Nevada	White Pine	39.39443	−114.77883
UMNH. Mamm.42473	*Reithrodontomys megalotis*	Female	5-July-2016	Nevada	White Pine	39.0182	−114.228825
UMNH. Mamm.42477	*Peromyscus truei*	Male	29-July-2017	Nevada	White Pine	39.0128	−114.124553
UMNH. Mamm.42510	*Peromyscus maniculatus*	Male	8-November-2017	Utah	Juab	39.82744	−113.36045
UMNH. Mamm.42511	*Peromyscus maniculatus*	Male	8-November-2017	Utah	Juab	39.82744	−113.36045
UMNH. Mamm.42512	*Dipodomys microps*	Female	8-November-2017	Utah	Juab	39.82744	−113.36045
UMNH. Mamm.41721	*Peromyscus truei*	Female	22-May-2017	Utah	Tooele	40.00957	−113.843
UMNH. Mamm.41722	*Peromyscus truei*	Male	22-May-2017	Utah	Tooele	40.00957	−113.843
UMNH. Mamm.41723	*Peromyscus truei*	Male	22-May-2017	Utah	Tooele	40.00957	−113.843
UMNH. Mamm.40479	*Thomomys bottae*	Female	28-February-2016	Utah	Juab	39.88758	−113.41237
UMNH. Mamm.42491	*Otospermophilus variegatus*	Female	21-July-2017	Utah	Salt Lake	40.76156	−111.82335
UMNH. Mamm.42492	*Otospermophilus variegatus*	Male	30-August-2017	Utah	Salt Lake	40.76156	−111.82335

TABLE 1 (Continued)

Specimen GUID	Small mammal taxa	Sex	Date collected	State	County	Latitude	Longitude
UMNH. Mamm.42504	*Thomomys bottae*	Female	10-November-2017	Utah	Juab	39.88778	−113.4128
UMNH. Mamm.42508	*Reithrodontomys megalotis*	Female	9-November-2017	Utah	Juab	39.833	−113.35395
UMNH. Mamm.42509	*Reithrodontomys megalotis*	Male	9-November-2017	Utah	Juab	39.833	−113.35395
UMNH. Mamm.42514	*Peromyscus maniculatus*	Male	9-November-2017	Utah	Juab	39.82744	−113.36045
UMNH. Mamm.42515	*Peromyscus maniculatus*	Female	8-November-2017	Utah	Juab	39.82744	−113.36045
UMNH. Mamm.42516	*Peromyscus maniculatus*	Female	8-November-2017	Utah	Juab	39.88778	−113.4128
UMNH. Mamm.42517	*Peromyscus maniculatus*	Male	8-November-2017	Utah	Juab	39.9045	−113.37015
UMNH. Mamm.42518	*Peromyscus maniculatus*	Male	8-November-2017	Utah	Juab	39.9045	−113.37015
UMNH. Mamm.42519	*Peromyscus maniculatus*	Female	8-November-2017	Utah	Juab	39.9045	−113.37015
UMNH. Mamm.42520	*Reithrodontomys megalotis*	Female	9-November-2017	Utah	Juab	39.9045	−113.37015
UMNH. Mamm.42521	*Dipodomys microps*	Female	9-November-2017	Utah	Juab	39.9045	−113.37015
UMNH. Mamm.42522	*Dipodomys microps*	Male	8-November-2017	Utah	Juab	39.9045	−113.37015
UMNH. Mamm.42523	*Dipodomys microps*	Female	9-November-2017	Utah	Juab	39.82744	−113.36045
UMNH. Mamm.42525	*Dipodomys microps*	Female	11-November-2017	Utah	Juab	39.84128	−113.39451
UMNH. Mamm.42968	*Sciurus niger*	Female	4-February-2018	Utah	Salt Lake	40.72814	−111.87483
UMNH. Mamm.42974	*Sciurus niger*	Male	3-March-2018	Utah	Salt Lake	40.72814	−111.87483
UMNH. Mamm.42975	*Sciurus niger*	Female	22-March-2018	Utah	Salt Lake	40.78208	−111.89462
UMNH. Mamm.43000	*Chaetodipus intermedius*	Male	3-April-2018	Arizona	Coconino	36.7258	−111.6366
UMNH. Mamm.43005	*Peromyscus truei*	Male	3-April-2018	Arizona	Coconino	36.6648	−111.6121
UMNH. Mamm.43006	*Peromyscus truei*	Female	3-April-2018	Arizona	Coconino	36.6648	−111.6121
UMNH. Mamm.43008	*Dipodomys ordii*	Female	3-April-2018	Arizona	Coconino	36.6648	−111.6121
UMNH. Mamm.43009	*Peromyscus crinitus*	Female	4-April-2018	Utah	San Juan	37.0507	−110.7985
UMNH. Mamm.43011	*Neotoma lepida*	Female	31-March-2018	Utah	Washington	37.121	−114.0098
UMNH. Mamm.43012	*Dipodomys merriami*	Male	31-March-2018	Utah	Washington	37.121	−114.0098
UMNH. Mamm.43013	*Dipodomys merriami*	Female	31-March-2018	Utah	Washington	37.121	−114.0098
UMNH. Mamm.43015	*Dipodomys merriami merriami*	Male	31-March-2018	Utah	Washington	37.121	−114.0098

(Continues)

TABLE 1 (Continued)

Specimen GUID	Small mammal taxa	Sex	Date collected	State	County	Latitude	Longitude
UMNH. Mamm.43017	*Dipodomys merriami*	Female	31-March-2018	Utah	Washington	37.121	−114.0098
UMNH. Mamm.43018	*Dipodomys ordii*	Female	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43019	*Dipodomys ordii*	Male	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43020	*Dipodomys ordii*	Male	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43021	*Dipodomys ordii*	Male	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43022	*Dipodomys ordii*	Male	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43023	*Dipodomys ordii*	Female	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43024	*Dipodomys ordii*	Female	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43025	*Dipodomys ordii*	Male	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43026	*Dipodomys ordii*	Male	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43027	*Dipodomys ordii*	Female	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43028	*Reithrodontomys megalotis*	Female	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43031	*Peromyscus maniculatus*	Male	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43032	*Peromyscus maniculatus*	Male	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43033	*Peromyscus maniculatus*	Male	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43034	*Peromyscus maniculatus*	Male	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43035	*Peromyscus maniculatus*	Female	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43036	*Peromyscus maniculatus*	Female	17-April-2018	Utah	Uintah	40.1854	−109.69897
UMNH. Mamm.43040	*Microtus montanus*	Male	24-April-2018	Utah	Tooele	40.753	−112.625
UMNH. Mamm.43046	*Callospermophilus lateralis*	Female	25-May-2018	Utah	Box Elder	41.9503	−113.4673
UMNH. Mamm.43046	*Tamias amoenus*	Male	25-May-2018	Utah	Box Elder	41.9496	−113.4662
UMNH. Mamm.43047	*Microtus montanus*	Male	26-May-2018	Utah	Box Elder	41.9504	−113.4656
UMNH. Mamm.43052	*Tamias amoenus*	Male	26-May-2018	Utah	Box Elder	41.9493	−113.4623
UMNH. Mamm.43054	*Zapus princeps*	Male	26-May-2018	Utah	Box Elder	41.9496	−113.4618
UMNH. Mamm.43058	*Zapus princeps*	Male	27-May-2018	Utah	Box Elder	41.9498	−113.4633
UMNH. Mamm.43060	*Zapus princeps*	Male	27-May-2018	Utah	Box Elder	41.9498	−113.4633

TABLE 1 (Continued)

Specimen GUID	Small mammal taxa	Sex	Date collected	State	County	Latitude	Longitude
UMNH. Mamm.43062	*Sciurus niger*	Male	3-June-2018	Utah	Salt Lake	40.7543	−111.871
UMNH. Mamm.43066	*Urocitellus elegans*	Male	1-July-2018	Colorado	Bent	38.11436	−102.90291
UMNH. Mamm.43067	*Sorex palustris*	Male	11-July-2018	Utah	Box Elder	41.95729	−113.45348
UMNH. Mamm.43068	*Tamias amoenus*	Female	12-July-2018	Utah	Box Elder	41.95729	−113.45348
UMNH. Mamm.43070	*Microtus montanus*	Male	13-July-2018	Utah	Box Elder	41.95729	−113.45348
UMNH. Mamm.43077	*Tamias amoenus*	Female	14-July-2018	Utah	Box Elder	41.95489	−113.45297
UMNH. Mamm.43094	*Callospermophilus lateralis*	Female	15-July-2018	Utah	Box Elder	41.95746	−113.4537
UMNH. Mamm.43120	*Zapus princeps*	Female	13-July-2018	Utah	Box Elder	41.95489	−113.45297
UMNH. Mamm.43123	*Peromyscus maniculatus*	Female	14-July-2018	Utah	Box Elder	41.95729	−113.45348
UMNH. Mamm.43124	*Peromyscus maniculatus*	Female	15-July-2018	Utah	Box Elder	41.95729	−113.45348
UMNH. Mamm.43128	*Tamias amoenus*	Female	16-July-2018	Utah	Box Elder	41.95681	−113.45214
UMNH. Mamm.43130	*Microtus montanus*	Female	17-July-2018	Utah	Box Elder	41.95681	−113.45214
UMNH. Mamm.43131	*Peromyscus truei*	Female	18-July-2018	Utah	Box Elder	41.95746	−113.4537
UMNH. Mamm.43132	*Peromyscus truei*	Male	19-July-2018	Utah	Box Elder	41.95746	−113.4537
UMNH. Mamm.43134	*Tamias amoenus*	Female	20-July-2018	Utah	Box Elder	41.95681	−113.45214
UMNH. Mamm.43137	*Microtus montanus*	Female	21-July-2018	Utah	Box Elder	41.95729	−113.45348
UMNH. Mamm.43238	*Microtus montanus*	Female	17-July-2018	Utah	Box Elder	41.9489	−113.4625
UMNH. Mamm.43290	*Tamiasciurus hudsonicus*	Female	30-June-2018	Utah	Salt Lake	40.57696	−111.81446
UMNH. Mamm.43291	*Tamiasciurus hudsonicus*	Male	24-September-2018	Utah	Salt Lake	40.57505	−111.800121
UMNH. Mamm.43293	*Thomomys bottae*	Female	September, 2018	Utah	Salt Lake	40.70417	−111.79851
UMNH. Mamm.43323	*Sciurus niger*	Female	7-December-2018	Utah	Salt Lake	40.77086	−111.871561
UMNH. Mamm.43324	*Sciurus niger*	Male	23-December-2018	Utah	Salt Lake	40.75585	−111.83236
UMNH. Mamm.43274	*Zapus princeps*	Male	18-July-2018	Utah	Box Elder	41.9496	−113.4618
UMNH. Mamm.43275	*Zapus princeps*	Male	18-July-2018	Utah	Box Elder	41.9496	−113.4618

important than geographic locality in determining the diversity of *Rhizopogon* within a host. In addition, the lack of overlap of *Rhizopogon* diversity across the *Peromyscus maniculatus* samples collected in close proximity on the same day suggests that consumption is likely generalist and opportunistic in nature, a characteristic that has been studied at great depth in small mammals and is considered to have a vital role in forest ecology (Stephens & Rowe, 2020).

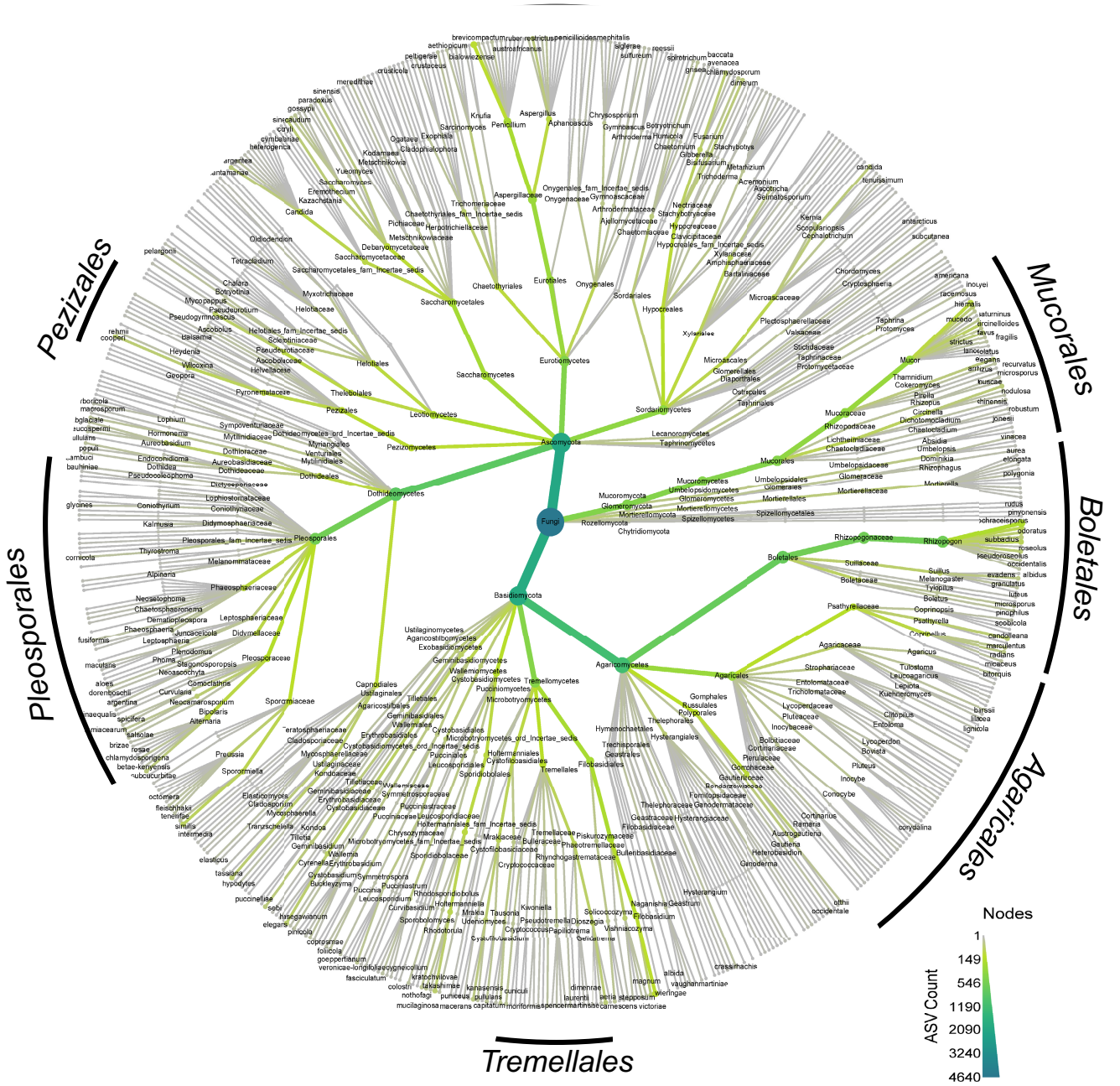

FIGURE 1 Leaflet composition of total fungal community. Leaflet distribution of the total fungal community across all samples. Amplicon sequence variant (ASV) count reports the number of ASVs that are assignable at that taxonomic level and is represented by decreasing line thickness as well as a color gradient. Taxa of interest with highly represented ASVs are indicated for quick reference

2.3 | Community diversity and environmental factors associated with *Rhizopogon* presence

Alpha diversity measurements of *Rhizopogon* in our samples were conducted using both Choa1 and Shannon indices (Figure S3). Alpha diversity across our samples did not indicate that any single species of small mammal was associated with *Rhizopogon* diversity. Beta diversity was calculated for the total fungal community across all samples using nMDS with Bray–Curtis dissimilarity distances, as well as weighted unifrac distances for samples that contained *Rhizopogon* (Figures S4 and S5). Total fungal community analysis did not uncover any clear separation of fungal consumption based on host species. In fact, many samples across multiple species overlap heavily (Figure S4). Interestingly, samples that contained *Rhizopogon* separated into three distinct groups but were not associated with any host species, or any of the metadata associated with the host vouchers, suggesting that there is structure associated with *Rhizopogon* consumption that cannot be resolved with these data (Figure S5).

However, associating WorldClim data (Fick & Hijmans, 2017) with the geospatial coordinates where each sample host voucher was collected, principal component analysis (PCA) for both qualitative and quantitative variables found structure related to the season

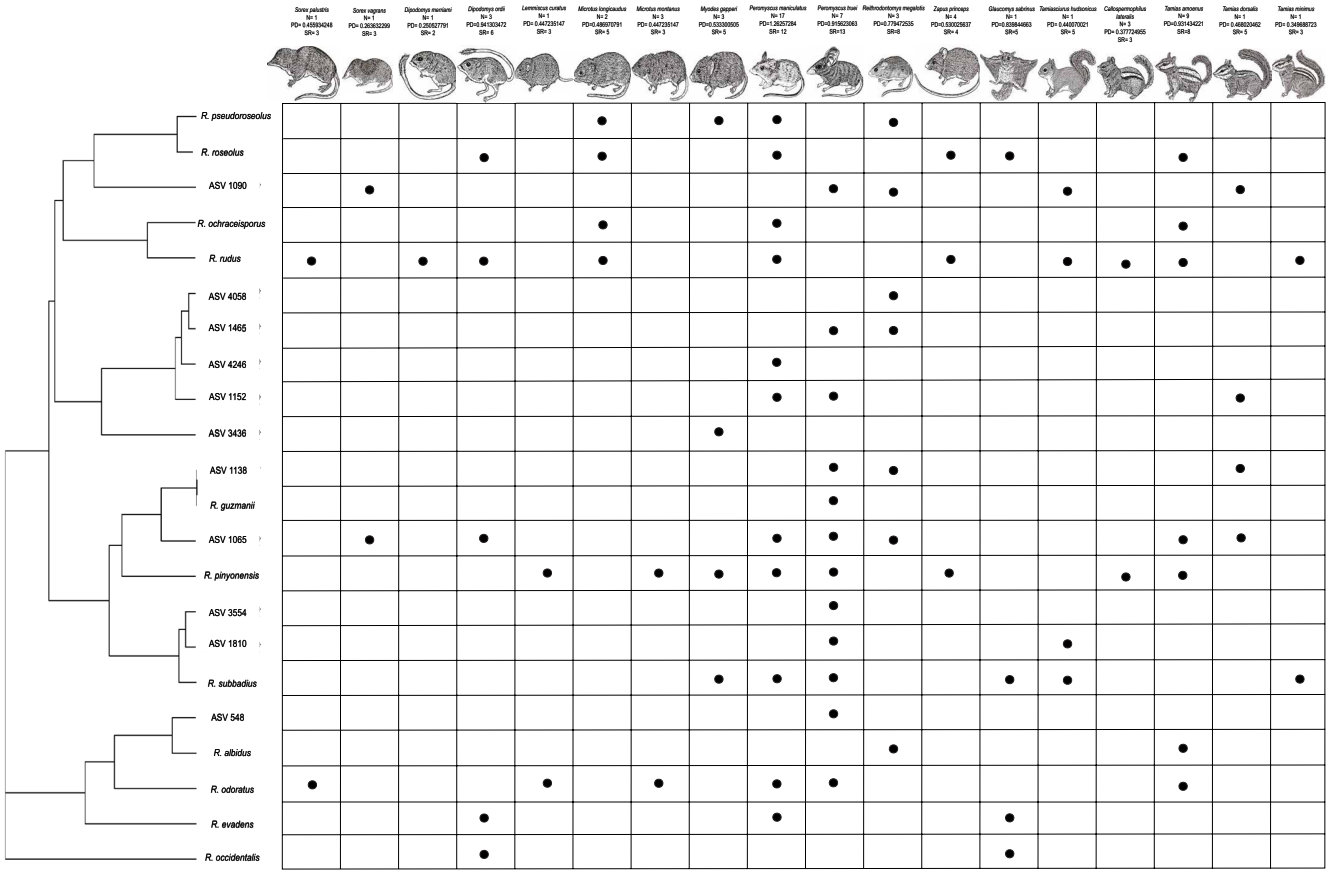

FIGURE 2 *Rhizopogon* ITS2 phylogenetic tree and presence/absence in each small mammal species along with reported Faith's phylogenetic diversity and species richness found in each host species. (Left) Phylogenetic cladogram of *Rhizopogon* amplicon sequence variants and operational taxonomic units corresponding to the presence or absence in each small mammal host. (Top) All small mammal hosts with calculated Faith's phylogenetic diversity and species richness in the dataset as well as the number of collected specimens

in which samples were collected (Figure 3a). We further dissected the bioclimatic data to see which variables contributed most to the community composition within our samples. We found that 77.38% of the variability within our *Rhizopogon* consumption set was due to two dimensions (Figure 3b). Within those dimensions we found the highest correlation to be associated with precipitation and warm temperature patterns, consistent with the individual PCA's association with seasonality, which has been studied previously in *Rhizopogon* (Hunt & Trappe, 1987; Luoma et al., 1991).

2.4 | Rate of *Rhizopogon* collection and the use of Extent of Occurrence and Area of Occupancy metrics for conservation assessment

The importance of the genus *Rhizopogon* to forest ecosystems lies not only in its role as a common food source for small mammals but also in its obligate ectomycorrhizal associations with *Pinaceae* (pine) trees. These factors place the genus in the precarious position of a keystone group that, if threatened with local reduction or extinction, could drastically impact a forest ecosystem. Here, we analyze the rate at which samples have been collected historically as well as the Extent of Occurrence (EOO) and Area of Occupancy (AOO), which are two main criteria commonly used by the International Union for Conservation of Nature (IUCN) to designate the risk status of an organism (Rodríguez et al., 2011, 2015; Schatz, 2009). It is thought that two out of every five fungi discovered are endangered, and many others may become extinct before they can even be documented (Nic Lughadha et al., 2020). As such, we wished to assess the utility of our data for assessing a species conservation status with commonly used tools. Geospatial collection data (latitude and longitude) associated with a host voucher specimen for which *Rhizopogon* DNA was found in the feces were used as a proxy for direct collection of a sporocarp. Coordinates of trapped small mammals were counted as a single "observation" if an ASV within that sample could be taxonomically identified to species level. In addition, geopoints for observation and collection records were imported from GBIF.org (August 2020) (GBIF Home Page. Available from: https://www.gbif.org) and Mycoportal (Miller & Bates, 2017), respectively.

We chose to compare the rate of *Rhizopogon* collection using vouchered collection information from MycoPortal against our proxy samples. Voucher collection data from MycoPortal represented 1267 specimens collected and cataloged between 1844 and 2019 (average of 7.2 *Rhizopogon* specimens a year). By comparison,

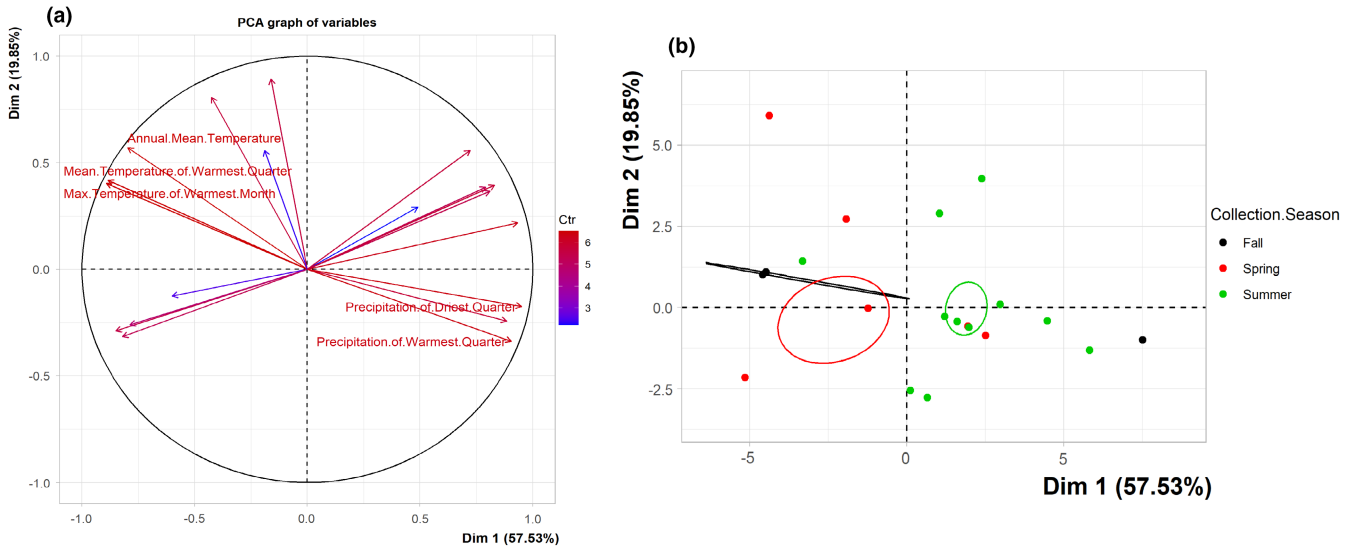

FIGURE 3 Principal component analysis of the top five Bioclim variables associated with *Rhizopogon* presence in samples and confidence ellipse in seasonality. (a) Principal component analysis of sample bioclimatic variables. More contribution to variability is shown in red with the top five variables being labeled. (b) Principal component analysis of samples based on the season of collection with confidence ellipses

we detected 65 *Rhizopogon* "samples" from feces collected in 2017 and 2018 (an average of 32.4 proxy *Rhizopogon* specimens per year). In addition to investigating the rate of collection for *Rhizopogon* specimens, we also investigated how many samples were needed before we experienced a reduction in the rate at which we recovered them. Utilizing a species accumulation curve with all 11 assignable species and 11 non-assignable species OTUs, reduction in the rate at which *Rhizopogon* richness was recovered begins to occur between 20 and 30 specimens for our dataset (Figure S6).

We wanted to know if our new records had an impact on the standard assessment procedure for fungal conservation. Geospatial data were analyzed using Geocat (Bachman et al., 2011), a tool supported by IUCN for the calculation of EOO and AOO for this purpose (Bachman et al., 2011). Geocat's mapping feature allows for graphical representation of the EOO and AOO as well as assigning an IUCN threatened category for each species. Across our assigned *Rhizopogon* species, *Rhizopogon guzmannii* Trappe & Cázares had the fewest publicly available data points ($n = 4$), while *R. roseolus* (Corda) Th. Fr. had the most data points ($n = >900$). The large amount of data for *R. roseolus* is most likely due to anthropogenic dispersal because it is used commercially in the reforestation of pine trees (Dunstan et al., 1998; Sousa et al., 2011). Based on their AOOs, 10 of the 11 species we identified would be considered Threatened under IUCN guidelines, with *R. guzmanii* considered Critically Endangered (Table S2). To determine how much impact the new, fecally derived datapoints had on EOO and AOO, measurements were calculated for each public database and the fecal samples separately, and then combined to maximize the number of datapoints that could be used for analysis. The combination of fecally derived geopoints and public data when compared to the highest EOO and AOO estimates helped to extend the EOO (0.334–74,974,251.46 km^2) and AOO (4–1056 km^2) for all *Rhizopogon* species detected, except in the case of EOO for the highly collected species *R. occidentalis* Zeller & C.W. Dodge (−2,582,942.406 km^2) and *R. roseolus* (−1,496,021.925 km^2), which decreased (Table S2). These results indicate that our method can extend EOO and AOO of undersampled species, as well as refine the distribution for species that have been heavily collected.

3 | DISCUSSION

Fungal communities and species distributions are notoriously difficult to study and map, often due to inconspicuous growth forms dominating fungal life cycles. Even though fungi with macroscopic structures (e.g., mushrooms, truffles and false truffles, lichens, etc.) have the longest history of ecological study for all fungi (Hao et al., 2020; Małgorzata & Dorota, 2013), baseline information such as known locality and species richness for many macrofungi is largely nonexistent due to difficulty in locating sporocarps, inaccurate taxonomic identification, and lack of long-term monitoring projects (van der Linde et al., 2012). Sampling from feces, or other environmental substrates such as soil, can be non-invasive and enable long-term studies that are less burdened by the difficulty of locating sporocarps in the field. Additionally, massive collections of fecal material (as well as environmental soil samples), such as those gathered by the NEON project (Dalton, 2000; Hopkin, 2006), already exist and have metadata readily available for each collection. Utilizing established collection resources could expedite the process of extensive sample collection and analysis required to answer larger-scale fungal community-level questions.

Our study began with the simple idea of using readily available fecal samples from small mammals, collected routinely by the Natural History Museum of Utah, to investigate fungal diversity by applying techniques generally used to investigate microbial

communities. Metabarcoding of environmental samples has become a popular technique to interrogate organismal communities that would otherwise be difficult to study (Al Ashhab et al., 2021; Andújar et al., 2015; Compson et al., 2020; Dieleman et al., 2015; Kesanakurti et al., 2011; McGee et al., 2019). Here we used the same strategy to target fungal communities in small mammal feces from 138 specimens and were able to identify 4650 ASVs in 531 genera of fungi. Although our methods may not have recovered the total fungal community due to technical biases such as variation in DNA extraction efficiency across taxa, sequencing depth, and primer bias during PCR amplification (Frau et al., 2019; Nilsson et al., 2019), our data represent a rich assemblage of macro- and microfungi present in the feces of small mammals collected throughout the Great Basin region in North America. While a mock community of fungi may help assess the degree of community recovery in studies like this, there is currently no mock community that would be representative of the fungi we detected in our samples. Moreover, the added complexity introduced by extracting from fecal material would be difficult to replicate in a mock community without feeding sporocarp tissue to small mammals and then collecting their feces, an activity that would be difficult to standardize. While these factors limit what conclusions can be drawn on patterns of community ecology, our method nonetheless produces empirical evidence of the existence of an organism at a particular place and time, information critical to establishing baseline information on species distributions and associations with small mammals.

Many of the species of small mammals captured in this study are known to be omnivorous foragers or herbivores (Table S1) and the presence of a large diversity of macroscopic sporocarp-producing fungi contributes to a picture of generalist and opportunistic feeding patterns among these small mammals. However, many of these fungi do not produce large, fleshy structures that are likely to be intentionally ingested by small mammals, and their presence may be explained instead by their symbiotic association with the plants consumed by the animals. For example, *Pleosporales* (specifically *Mycosphaerella*) is a ubiquitous group of plant pathogens with thousands of species (Cheewangkoon et al., 2008; Crous et al., 2007) and it is likely that any plant or grain consumed would contain members of this group.

In addition to forming relationships with plants, it is also becoming clear that there are important symbiotic fungal associations within animal hosts, comprising the gut mycobiota (van Tilburg Bernardes et al., 2020). We found 409 ASVs of *Mucorales*, a nearly cosmopolitan order of primarily saprobic soil- and dung-dwelling fungi but also diverse ecologies including animal pathogenic, plant endophytic, and coprophilous lifestyles (Benny et al., 2014; Walther et al., 2013, 2019a, 2019b). Omnivorous rodent feces are the most common source for a variety of *Mucorales* and related fungi (Benjamin, 1959). Due to the methodology we used for recovering feces (extraction from the intestinal tract during host specimen processing), there was limited opportunity for *Mucorales* fungi to colonize fecal samples from the environment. Furthermore, we also did not detect them in our negative controls, inconsistent with an environmental contamination hypothesis. This strongly suggests that these fungi were present in the feces when they were sampled. However, it is difficult to ascertain if *Mucorales* fungi may persist as symbionts within the gut of small mammals, or were consumed, possibly indirectly as symbionts of plants consumed by the animals or during coprophagy, a common diet characteristic of many small mammals (Bo et al., 2020).

Of the many fungi detected, 373 ASVs were putative macrofungi potentially ingested intentionally by the animals. The presence of a diversity of *Agaricomycotina* (273 ASVs) (the main mushroom-forming fungi), as well as hypogeous fungi in *Pezizales* (100 ASVs), suggests a tendency toward the consumption of macro- and hypogeous sporocarps common to forested areas. This is evidence that many of these fungi are critical components to the ecosystem not only as symbionts of plants but also as common food sources for small mammals. Our results demonstrate that the small mammals we surveyed are consuming a variety of fungi and are likely collectively contributing to fungal dispersal, although the degree to which these mammals contribute to the dispersal of any given fungal species is unclear. However, the most common and diverse macrofungal genus was the false truffle-forming genus *Rhizopogon*, found in 65 of 138 samples (47%, Table S5).

The genus *Rhizopogon* forms hypogeous sporocarps that are otherwise difficult to survey by traditional methods, indicating a potentially large role for active foraging by a diversity of small mammals in the dispersal of these fungi. The preponderance of *Rhizopogon* spp. in our samples provided an opportunity to determine if our method, using vouchered host metadata as a proxy for individual fungi, can reasonably be used to discern fungal community members. Due to its false truffle sporocarp morphology and cosmopolitan distribution, as well as its importance to forest ecology and small mammal diet (Stephens & Rowe, 2020), we chose to focus on *Rhizopogon* to explore patterns of host diversity and ecology, and to determine how these data can contribute to establishing distributions of fungal species and how they may impact metrics used for formal conservation assessment.

Rhizopogon is a well-known, globally distributed ectomycorrhizal symbiont of the conifer tree family *Pinaceae*, with most species specializing on a genus or subgenus (Cairney & Chambers, 1999). *Rhizopogon* spp. also form dense and persistent spore banks in soil, making them a common fungal community member in many coniferous forests (Grubisha et al., 2007). Wildfires are a common disturbance in many forest ecosystems, and pine trees (*Pinus* spp.), in particular, have a fire-adapted ecology and are ecologically dominant in many fire-prone ecosystems (Badik et al., 2018). Some *Rhizopogon* species have been shown to have a competitive advantage in host root-tip colonization following conditions that mimic wildfire disturbance (Izzo et al., 2006), indicating that they are important to pine stand reestablishment following fire (Baar et al., 1999). The ectomycorrhizal habit and success in forming mycorrhizae following conditions approximating fire disturbance, in addition to a large presence within the diet of small mammals, make *Rhizopogon* a critical community member in the overall health of forest ecosystems (Grubisha et al., 2007). Therefore, establishing baseline information on their diversity and distribution is important for understanding community

assembly and environmental restoration following disturbances such as fire.

Through DADA2, we identified 11 species of *Rhizopogon* that could be assigned to known taxa. However, phylogenetic analysis was necessary to fully characterize the total *Rhizopogon* diversity. After clustering our unassigned ASVs we produced 11 additional unassignable but distinct *Rhizopogon* OTUs for analysis. Ultimately, we were unable to assign them to any known species hypothesis with phylogenetic analysis. This suggests that we may have detected undescribed *Rhizopogon* spp., or that the reference database is incomplete and inadequate for the task, an issue that is becoming increasingly problematic (Hofstetter et al., 2019). When comparing the *Rhizopogon* phylogenetic diversity between small mammal samples using Faith's PD, we found no discernible pattern of host diet specialization and even found that variation occurred between samples of the same mammal species collected in close proximity to one another (Table S3). These findings suggest that a generally opportunistic, rather than targeted, feeding pattern is being employed by these small mammals, a behavior that has been well-characterized in other small mammals (Stephens & Rowe, 2020). However, this pattern is difficult to fully support with data due to the small sample size for many of our host species. Future studies that focus on host diet would benefit from increased sampling, as well as a more targeted approach to host selection.

The small mammals we used as proxies for the fungi we detected in their feces yielded data consistent with the current understanding of *Rhizopogon* ecology and phenology. Using bioclimatic data based on the geolocation for each sample containing *Rhizopogon*, we were able to attribute over 70% of our sample variability to seasonality, precipitation, and temperature. Additionally, we found that samples that contained *Rhizopogon* tended to cluster more closely together based on the season in which they were collected. While we did not find any striking trends suggesting new insights to the ecology of *Rhizopogon*, we were able to discern trends that were consistent with those already known from decades of previous studies, providing external validation for the ecological relevance of our method. Further, we were successful in expanding our knowledge of *Rhizopogon* diversity and distribution within the arid western United States, for which few data were previously collected (Figure S1).

The use of small mammals as a proxy allows for coarse-scale identification and sampling that can be done quickly and relatively inexpensively without relying on the labor-intensive, repeated field surveys over long periods of time that characterize traditional systematic fungal community surveys. In addition to expanding our understanding of diversity and distribution of *Rhizopogon* spp. in the western United States, we were able to account for 65 new occurrences in a single year, compared to an average of 7.2 per year across the MycoPortal database, which has collections dating back to over 100 years ago (Table S4). Further refinement to reduce technical and biological issues associated with meta-amplicon sequencing of museum samples such as these could yield a massive increase in the rate at which fungal diversity and distribution is catalogued. While we were able to identify *Rhizopogon* at a much faster rate compared to individual collections, we wanted to test the practical application of our results to see if they could be impactful for the conservation assessment of fungi. The current statistical tools used to investigate an organism's vulnerability in accordance with IUCN Red List practices are the EOO and AOO. These measurements work best with a large number of data points to compare, but due to the inherent difficulties of finding and documenting fungi (especially over long periods of time), the measurements most likely underrepresent the full range of most fungi. Utilizing Geocat (Bachman et al., 2011), a tool specifically created to use geospatial information to aid in the assessment of endangered species proposals, 10 of the 11 *Rhizopogon* species assigned to known taxa could be considered Endangered according to their AOO. Generally, the addition of fecally derived geopoints helped to extend the EOO and AOO for all *Rhizopogon* species detected, except in the case of *R. roseolus* and *R. occidentalis*, which decreased.

Overall, our additional data points helped to refine these statistics, but could still be considered a small sampling for many *Rhizopogon* species. Realistically, our data do not point to *Rhizopogon* being highly endangered, but rather to an IUCN designation of Data Deficient (DD) (Rodríguez et al., 2015). Nonetheless, these designations are the tools commonly used to help determine the conservation needs of an organism, and without illustrating how poorly fungi can be analyzed within the current system, it is difficult to argue that more attention should be paid to documenting fungal diversity and distribution. Great attention has been given to the conservation of organisms such as plants and animals, to the apparent neglect of fungi (Blackwell & Vega, 2018; Griffith, 2012; Heilmann-Clausen et al., 2015). Despite this, it seems certain that fungi, in common with most groups of organisms, face increasing endangerment and risk of extinction. Fungi are generally difficult to find, ephemeral, and challenging to track year to year, necessitating a greater focus on projects dedicated to understanding populations and distribution over time before we can even begin to estimate the loss of diversity in *Rhizopogon*, or *Fungi* as a whole.

The majority of conservation effort today is placed on large, charismatic, and culturally significant organisms, which are generally plants and animals (Cao et al., 2021; Gonçalves et al., 2021). To illustrate the disparity between well-documented and poorly documented species, giant pandas, one of the world's most iconic conservation targets, are heavily studied and monitored, with 663 animals in captivity and nearly 1900 managed in the wild as of the year 2021 (https://wwf.ca/species/giant-pandas/). In contrast, many species of *Rhizopogon* have extremely small occurrence numbers within collections, such as in the most extreme case of *Rhizopogon guzmanii* ($n = 4$) (Figure S2), with many other species having fewer than 30 identifications in the last 50 years (Table S4). While this is not meant to suggest that *Rhizopogon* should be receiving this level of attention compared to flagship organisms such as pandas, it serves to illustrate that our understanding of the diversity and distribution of some fungi is so sparse that it resembles that of near-extinction conditions. Relative to more conspicuous groups, fungi are severely understudied and we run the risk

of losing species before we even know they exist or understand their ecological roles. Given the enormous ecological importance of fungi, this oversight has the potential for devastating effects on the ecological health of our planet. Our study presents one method, the use of mycophagous small mammals as a proxy, to augment the documentation of fungal diversity and reveal fungal interactions with other organisms that may be critical to ecosystem viability. By improving our knowledge of the diversity and distribution of fungal taxa, we can better focus conservation efforts toward a more comprehensive view of ecosystem health.

4 | METHODS

4.1 | Field methods and mammal specimen preparation

Fecal samples were obtained from small mammals (shrews and rodents, <500 g weight) collected during field surveys to determine patterns of local species richness and abundance across the western United States including Utah, Idaho, Nevada, and Arizona (Table 1). Small mammals were collected by removal trapping using Victor and Museum Special lethal snap-traps (Woodstream Corp.) baited with a mixture of peanut butter and rolled oats, or Sherman live traps (H. B. Sherman Traps, Inc.) baited with mixed scratch grains. At each sampling locality, traps were set in multiple discrete traplines (of 10–50 traps) across the full range of available microhabitats. Traps were spaced 3–5 m apart and placed in runways, beside fallen logs, by burrow openings, under available cover, or in other locations with probable small mammal activity. Live-trapped animals were humanely euthanized with isoflurane (MWI Animal Health). Fecal samples also were obtained from fresh road-killed rodents salvaged opportunistically. Mammal collecting was done under permit from state wildlife agencies and the US Fish and Wildlife Service. Field methods followed guidelines of the American Society of Mammalogists (Sikes & the Animal Care and Use Committee of the American Society of Mammalogists, 2016) and were approved by the Institutional Animal Care and Use Committee of the University of Utah (protocol #s 18-01007 and 18-01008).

Captured and salvaged animals were preserved as museum voucher specimens. Some specimens were prepared in the field, but most animals were frozen with dry ice in the field and moved to a freezer for later preparation in the laboratory. Fecal samples were taken directly from the intestinal tract, placed in labeled Eppendorf tubes, and frozen. Identifications of voucher specimens were verified following final preparation. Taxonomic nomenclature for mammals follows (Wilson & Reeder, 2005).

4.2 | DNA isolation and sequencing

Fecal pellets were processed using the Zymo Research DNA miniprep kit (#D4300; Zymo Research) following the protocol for fecal samples. Feces were processed by placing either a single full pellet or a subsection of a larger pellet to meet the input requirements suggested by the manufacturer (200 mg max). Pellets or pellet fragments were homogenized by placing them in 2.0 ml screw-cap tubes containing Zymo lysis solution and beads, and shaking them in a BeadBug™ microtube homogenizer (#Z763713; Sigma) for 300 s at speed setting 400.

Genomic DNA was then amplified and sequenced using a PCR-based dual index strategy to allow for samples to be pooled and sequenced using high throughput Illumina technology. To enable efficient multiplexing of each sample, we employed a two-step amplicon protocol that first amplifies the marker gene using primers that include Nextera adapter tails, then using this as a template for a second round of PCR to add unique indices and Illumina flow cell adapters (Gohl et al., 2016). First, the internal transcribed spacer 2 ("ITS2") region of the ribosomal RNA cistron was PCR-amplified using the primer pairs 5.8S-Fun Nextera and ITS4-Fun Nextera (Taylor et al., 2016) with Illumina Nextera adapter tails. Sequencing libraries were prepared from these amplicons by using a 1:99 dilution as a template for a second round of PCR with indexing primers consisting of Nextera adapter, unique 8-bp index, and Illumina flow cell adapter. The resulting DNA was then cleaned and normalized across all samples using the AxyPrep Mag™ PCR Normalizer Protocol (Axygen Biosciences). Normalized sample DNAs along with two negative controls were pooled and submitted for Illumina MiSeq™ 2 × 250 PE sequencing at the University of Utah Genomics Core facility. Of the two negative controls, which contained only reagents and water, one had faint amplified sequences determined by gel electrophoresis while the second did not. However, each control did not generate any identifiable sequence and was completely filtered out during demultiplexing and DADA2 quality filtering.

4.3 | Taxonomic assignment of sample contents and data visualization

The DADA2 pipeline (Callahan et al., 2016) was used to trim and error-correct raw sequencing reads, and to generate a table of ASVs (Callahan et al., 2017) with default parameters. Each ASV was assigned taxonomically based on its similarity to species hypothesis sequences in the UNITE general release database version 8.2 (04 April 2020) (Abarenkov et al., 2010).

After taxonomic identification by DADA2, ASV data were combined with metadata for each small mammal fecal sample and analyzed using the R package Phyloseq version 1.32.0 (McMurdie & Holmes, 2013). Pre-processing of the dataset included removal of any samples that exhibited only one ASV or fewer. Species accumulation curves were constructed using the Vegan package in R (Dixon, 2003). Phyloseq was used to analyze and visualize multiple aspects of our data such as alpha diversity and beta diversity using built-in functions for each measurement. Further, a total community analysis was done using the R package Metacoder version 0.3.4 (Foster et al., 2017) to illustrate the total community composition.

4.4 | Phylogenetic analysis of non-assignable species and Faith's phylogenetic diversity calculations

Samples containing sequences corresponding to the *Rhizopogon* genus were subsampled from the total community for further analysis. Sequences that were not identified to the species rank by DADA2 were clustered together into OTUs based on a 99% similarity cutoff using Vsearch version 2.15.1 (Rognes et al., 2016) and then subjected to phylogenetic analysis to further clarify phylogenetic and diversity relationships.

Datasets for phylogenetic analysis were created by these OTUs, all species hypothesis reference sequences as defined by the UNITE database for the family *Rhizopogonaceae* in combination with species identified *Rhizopogon* ASVs. All sequences were run through ITSx version 1.1.2 (Bengtsson-Palme et al., 2013) to trim each sequence to only the ITS2 region, then multiple sequence alignments from these datasets were generated using the L-INS-i algorithm in MAFFTv7 (Katoh, 2002) and phylogenetic trees were inferred under maximum likelihood using IQ-TREE with the model of molecular evolution automatically determined and branch support estimated by ultrafast bootstrapping (Minh et al., 2013; Nguyen et al., 2015). Finally, community data for each tree was transformed to presence/absence reporting and combined with their respective phylogenetic tree to measure Faith's phylogenetic diversity using the R package Picante version 1.8.2 (Kembel et al., 2010).

4.5 | Principal component analysis and bioclimatic variable analysis

Bioclimatic variables were downloaded from the WorldClim database (Fick & Hijmans, 2017), based on the geolocation recorded for each small mammal specimen whose feces included sequences assigned to the *Rhizopogon* genus. Principal component analysis was performed using the PCAshiny function of the R package Factoshiny version 2.4 (Lê et al., 2008). Visualization was generated by altering the components to only display in dimensions 1 and 2 (corresponding to 77.32% of variability) and to only label the top five contributing environmental variables, of the 19 climatic variables recorded in this database. PCA was conducted on the individual samples and colored by the season collected with confidence ellipses added.

4.6 | Analysis of Extent of Occurrence and Area of Occupancy for *Rhizopogon* species

Fungi are among the primary decomposers of organic material, form critical symbiotic partnerships affecting plant health, and serve as a nutritional food source for animals. Therefore, a thorough understanding of fungal diversity and distribution is extremely important to identify targets under threat of extinction and to develop appropriate methods for their conservation. As proof of concept, we evaluated whether our data and analyses can assist in making meaningful conclusions about the distribution of *Rhizopogon*, and be used to inform possible conservation efforts. Information needed to accurately inform how conservation efforts should be focused is often difficult to ascertain and highly complex (Callmander et al., 2007; Juffe-Bignoli et al., 2016; Laity et al., 2015; Vane-Wright et al., 1991). Baseline data to establish the currently known EOO and AOO of specific *Rhizopogon* species was created by using the geospatial analysis online tool GeoCat (Bachman et al., 2011), using publicly available observation and collection data from the Global Biodiversity Informational Facility (GBIF) and MycoPortal, respectively. Geospatial coordinates were generated by subsetting our dataset for samples that had ASVs corresponding to each *Rhizopogon* species (Table S5). In each sample, all ASVs assigned to a specific *Rhizopogon* species were treated as a single occurrence and geospatial coordinates of the corresponding trapped small mammal were used as a proxy for that specific *Rhizopogon* species presence within the environment.

ACKNOWLEDGMENTS

We acknowledge the Natural History Museum of Utah for their commitment to collaborative science as well as the Genomics Core Facility, a part of the Health Sciences Cores at the University of Utah for their input and high-quality work. We are grateful to Shannen Robson for curatorial support. Further, we wish to acknowledge the dedicated and hard work performed by Allyson Jelito, Bryce Alex, and Isabelle Galland to help process many of these samples for DNA sequencing.

CONFLICT OF INTEREST

The authors report no conflicts of interest in the production of this work.

AUTHOR CONTRIBUTIONS

Alexander J. Bradshaw processed samples, generated and conducted protocols to sequence DNA, coding to process and analyze data, in addition to preparation and writing of this manuscript. Kendra C. Autumn participated in processing specimen samples as well as providing assistance in data compilation and analysis, artistic figure production, and the preparation and writing of this manuscript. Eric A. Rickart performed all fieldwork and small mammal dissection and fecal sampling as well as providing critical insight into the ecology of the specimens used in this study. Bryn T. M. Dentinger provided funding, experimental guidance, and assisted in the preparation and editing of this manuscript.

DATA AVAILABILITY STATEMENT

Raw short-read sequences for this project have been deposited in SRA through the bioproject PRJNA764247. Raw tree files, Phyloseq, and Geocat objects have been deposited in Figshare under https://doi.org/10.6084/m9.figshare.15105462.v1. Any code or specific script requests should be sent to the corresponding author.

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REFERENCES

Abarenkov, K., Henrik Nilsson, R., Larsson, K., Alexander, I. J., Eberhardt, U., Erland, S., Høiland, K., Kjøller, R., Larsson, E., Pennanen, T., Sen, R., Taylor, A. F. S., Tedersoo, L., Ursing, B. M., Vrålstad, T., Liimatainen, K., Peintner, U., & Kõljalg, U. (2010). The UNITE database for molecular identification of fungi – Recent updates and future perspectives. *New Phytologist*, *186*(2), 281–285. https://doi.org/10.1111/j.1469-8137.2009.03160.x

Al Ashhab, A., Meshner, S., Alexander-Shani, R., Dimerets, H., Brandwein, M., Bar-Lavan, Y., & Winters, G. (2021). Temporal and spatial changes in phyllosphere microbiome of *Acacia* trees growing in arid environments. *Frontiers in Microbiology*, *12*, 656269. https://doi.org/10.3389/fmicb.2021.656269

Andújar, C., Arribas, P., Ruzicka, F., Crampton-Platt, A., Timmermans, M. J. T. N., & Vogler, A. P. (2015). Phylogenetic community ecology of soil biodiversity using mitochondrial metagenomics. *Molecular Ecology*, *24*(14), 3603–3617. https://doi.org/10.1111/mec.13195

Baar, J., Horton, T. R., Kretzer, A. M., & Bruns, T. D. (1999). Mycorrhizal colonization of *Pinus muricata* from resistant propagules after a stand-replacing wildfire. *New Phytologist*, *143*(2), 409–418. https://doi.org/10.1046/j.1469-8137.1999.00452.x

Bachman, S., Moat, J., Hill, A., de la Torre, J., & Scott, B. (2011). Supporting Red List threat assessments with GeoCAT: Geospatial conservation assessment tool. *ZooKeys*, *150*, 117–126. https://doi.org/10.3897/zookeys.150.2109

Badik, K. J., Jahner, J. P., & Wilson, J. S. (2018). A biogeographic perspective on the evolution of fire syndromes in pine trees (*Pinus*: Pinaceae). *Royal Society Open Science*, *5*(3), 172412. https://doi.org/10.1098/rsos.172412

Begerow, D., Nilsson, H., Unterseher, M., & Maier, W. (2010). Current state and perspectives of fungal DNA barcoding and rapid identification procedures. *Applied Microbiology and Biotechnology*, *87*(1), 99–108. https://doi.org/10.1007/s00253-010-2585-4

Bengtsson-Palme, J., Ryberg, M., Hartmann, M., Branco, S., Wang, Z., Godhe, A., De Wit, P., Sánchez-García, M., Ebersberger, I., de Sousa, F., Amend, A. S., Jumpponen, A., Unterseher, M., Kristiansson, E., Abarenkov, K., Bertrand, Y. J. K., Sanli, K., Eriksson, K. M., Vik, U., … Nilsson, R. H. (2013). Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. *Methods in Ecology and Evolution*, *4*(10), 914–919. https://doi.org/10.1111/2041-210X.12073

Benjamin, R. K. (1959). The merosporangiferous Mucorales. *Aliso*, *4*(2), 321–433. https://doi.org/10.5642/aliso.19590402.05

Benny, G. L., Humber, R. A., & Voigt, K. (2014). 8 Zygomycetous fungi: Phylum Entomophthoromycota and subphyla Kickxellomycotina, Mortierellomycotina, Mucoromycotina, and Zoopagomycotina. In D. J. McLaughlin, & J. W. Spatafora (Eds.), *Systematics and evolution* (pp. 209–250). Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-642-55318-9_8

Blackwell, M., & Vega, F. E. (2018). Lives within lives: Hidden fungal biodiversity and the importance of conservation. *Fungal Ecology*, *35*, 127–134. https://doi.org/10.1016/j.funeco.2018.05.011

Bo, T.-B., Zhang, X.-Y., Kohl, K. D., Wen, J., Tian, S.-J., & Wang, D.-H. (2020). Coprophagy prevention alters microbiome, metabolism, neurochemistry, and cognitive behavior in a small mammal. *The ISME Journal*, *14*(10), 2625–2645. https://doi.org/10.1038/s41396-020-0711-6

Boyer, S., Cruickshank, R. H., & Wratten, S. D. (2015). Faeces of generalist predators as 'biodiversity capsules': A new tool for biodiversity assessment in remote and inaccessible habitats. *Food Webs*, *3*, 1–6. https://doi.org/10.1016/j.fooweb.2015.02.001

Buglione, M., Maselli, V., Rippa, D., de Filippo, G., Trapanese, M., & Fulgione, D. (2018). A pilot study on the application of DNA metabarcoding for non-invasive diet analysis in the Italian hare. *Mammalian Biology*, *88*, 31–42. https://doi.org/10.1016/j.mambio.2017.10.010

Cairney, J. W. G., Chambers, S. M. (Eds.) (1999). *Ectomycorrhizal fungi key genera in profile*. Springer Berlin Heidelberg. https://doi.org/10.1007/978-3-662-06827-4

Callahan, B. J., McMurdie, P. J., & Holmes, S. P. (2017). Exact sequence variants should replace operational taxonomic units in marker-gene data analysis. *The ISME Journal*, *11*(12), 2639–2643. https://doi.org/10.1038/ismej.2017.119

Callahan, B. J., McMurdie, P. J., Rosen, M. J., Han, A. W., Johnson, A. J. A., & Holmes, S. P. (2016). DADA2: High-resolution sample inference from Illumina amplicon data. *Nature Methods*, *13*(7), 581–583. https://doi.org/10.1038/nmeth.3869

Callmander, M. W., Schatz, G. E., Lowry, P. P. II, Laivao, M. O., Raharimampionona, J., Andriambololonera, S., Raminosoa, T., & Consiglio, T. K. (2007). Identification of priority areas for plant conservation in Madagascar using Red List criteria: Rare and threatened Pandanaceae indicate sites in need of protection. *Oryx*, *41*(2), 168–176. https://doi.org/10.1017/S0030605307001731

Cannon, P., Aguirre-Hudson, B., Aime, M., Ainsworth, A., Bidartondo, M., Gaya, E., Hawksworth, D., Kirk, P., Leitch, I., & Lücking, R. (2018). *State of the World's fungi 2018 1. Definition and diversity* (pp. 4–11).

Cao, Y., Wu, G., & Yu, D. (2021). Include macrofungi in biodiversity targets. *Science*, *372*(6547), 1160. https://doi.org/10.1126/science.abj5479

Chase, M. W., & Fay, M. F. (2009). Barcoding of plants and fungi. *Science*, *325*(5941), 682–683. https://doi.org/10.1126/science.1176906

Cheewangkoon, R., Crous, P. W., Hyde, K. D., Groenewald, J. Z., & To-anan, C. (2008). Species of *Mycosphaerella* and related anamorphs on *Eucalyptus* leaves from Thailand. *Persoonia – Molecular Phylogeny and Evolution of Fungi*, *21*(1), 77–91. https://doi.org/10.3767/003158508X370857

Cloutier, V. B., Piché, Y., Fortin, J. A., Bérubé, J. A., Glémet, H., & Desrochers, A. (2019). A novel approach for tracing mycophagous small mammals and documenting their fungal diets. *Botany-Botanique*, *97*(9), 475–785. https://doi.org/10.1139/cjb-2018-0222

Compson, Z. G., McClenaghan, B., Singer, G. A. C., Fahner, N. A., & Hajibabaei, M. (2020). Metabarcoding from microbes to mammals: Comprehensive bioassessment on a global scale. *Frontiers in Ecology and Evolution*, *8*, 581835. https://doi.org/10.3389/fevo.2020.581835

Courty, P.-E., Buée, M., Diedhiou, A. G., Frey-Klett, P., Le Tacon, F., Rineau, F., Turpault, M.-P., Uroz, S., & Garbaye, J. (2010). The role of ectomycorrhizal communities in forest ecosystem processes: New perspectives and emerging concepts. *Soil Biology and Biochemistry*, *42*(5), 679–698. https://doi.org/10.1016/j.soilbio.2009.12.006

Crous, P. W., Braun, U., & Groenewald, J. Z. (2007). *Mycosphaerella* is polyphyletic. *Studies in Mycology*, *58*, 1–32. https://doi.org/10.3114/sim.2007.58.01

Dalton, R. (2000). NEON to shed light on environment research. *Nature*, *404*(6775), 216. https://doi.org/10.1038/35005244

Dieleman, C. M., Branfireun, B. A., McLaughlin, J. W., & Lindo, Z. (2015). Climate change drives a shift in peatland ecosystem plant community: Implications for ecosystem function and stability. *Global Change Biology*, *21*(1), 388–395. https://doi.org/10.1111/gcb.12643

Dixon, P. (2003). VEGAN, a package of R functions for community ecology. *Journal of Vegetation Science*, *14*(6), 927–930. https://doi.org/10.1111/j.1654-1103.2003.tb02228.x

Dunstan, W. A., Dell, B., & Malajczuk, N. (1998). The diversity of ectomycorrhizal fungi associated with introduced *Pinus* spp. in the Southern Hemisphere, with particular reference to Western

Australia. *Mycorrhiza*, *8*(2), 71–79. https://doi.org/10.1007/s005720050215

Elliott, T. F., Georgiev, A. V., Lokasola, A. L., & Smith, M. E. (2020). Hysterangium bonobo: A newly described truffle species that is eaten by bonobos in the Democratic Republic of Congo. *Mycologia*, *112*(6), 1203–1211. https://doi.org/10.1080/00275514.2020.1790234

Faith, D. P. (2018). Phylogenetic diversity and conservation evaluation: Perspectives on multiple values, indices, and scales of application. In R. A. Scherson, & D. P. Faith (Eds.), *Phylogenetic diversity* (pp. 1–26). Springer International Publishing. https://doi.org/10.1007/978-3-319-93145-6_1

Fick, S. E., & Hijmans, R. J. (2017). WorldClim 2: New 1-km spatial resolution climate surfaces for global land areas. *International Journal of Climatology*, *37*(12), 4302–4315. https://doi.org/10.1002/joc.5086

Foster, Z. S. L., Sharpton, T. J., & Gr, N. J. (2017). Metacoder: An R package for visualization and manipulation of community taxonomic diversity data. *PLoS Computational Biology*, *15*, e1005404. https://doi.org/10.1371/journal.pcbi.1005404

Frau, A., Kenny, J. G., Lenzi, L., Campbell, B. J., Ijaz, U. Z., Duckworth, C. A., Burkitt, M. D., Hall, N., Anson, J., Darby, A. C., & Probert, C. S. J. (2019). DNA extraction and amplicon production strategies deeply influence the outcome of gut mycobiome studies. *Scientific Reports*, *9*(1), 9328. https://doi.org/10.1038/s41598-019-44974-x

Gohl, D., Gohl, D. M., MacLean, A., Hauge, A., Becker, A., Walek, D., & Beckman, K. B. (2016). An optimized protocol for high-throughput amplicon-based microbiome profiling. *Protocol Exchange*, https://doi.org/10.1038/protex.2016.030

Gonçalves, S. C., Haelewaters, D., Furci, G., & Mueller, G. M. (2021). Include all fungi in biodiversity goals. *Science*, *373*(6553), 403. https://doi.org/10.1126/science.abk1312

Griffith, G. W. (2012). Do we need a global strategy for microbial conservation? *Trends in Ecology & Evolution*, *27*(1), 1–2. https://doi.org/10.1016/j.tree.2011.10.002

Grubisha, L. C., Bergemann, S. E., & Bruns, T. D. (2007). Host islands within the California Northern Channel Islands create fine-scale genetic structure in two sympatric species of the symbiotic ectomycorrhizal fungus *Rhizopogon*: *Rhizopogon* host islands. *Molecular Ecology*, *16*(9), 1811–1822. https://doi.org/10.1111/j.1365-294X.2007.03264.x

Hanson, A. M., Hodge, K. T., & Porter, L. M. (2003). Mycophagy among primates. *Mycologist*, *17*(1), 6–10. https://doi.org/10.1017/S0269915X0300106X

Hao, T., Guillera-Arroita, G., May, T. W., Lahoz-Monfort, J. J., & Elith, J. (2020). Using species distribution models for fungi. *Fungal Biology Reviews*, *34*(2), 74–88. https://doi.org/10.1016/j.fbr.2020.01.002

Harper, L. R., Watson, H. V., Donnelly, R., Hampshire, R., Sayer, C. D., Breithaupt, T., & Hänfling, B. (2020). Using DNA metabarcoding to investigate diet and niche partitioning in the native European otter (*Lutra lutra*) and invasive American mink (*Neovison vison*). *Metabarcoding and Metagenomics*, *4*, e56087. https://doi.org/10.3897/mbmg.4.56087

Hawes, M. L. (1977). Home range, territoriality and ecological separation in sympatric shrews, *Sorex vagrans* and *Sorex obscurus*. *Journal of Mammalogy*, *58*(3), 354–367. https://doi.org/10.2307/1379334

Hawksworth, D. L., & Lücking, R. (2017). Fungal diversity revisited: 2.2 to 3.8 million species. *Microbiology Spectrum*, *5*(4). https://doi.org/10.1128/microbiolspec.FUNK-0052-2016

Heilmann-Clausen, J., Barron, E. S., Boddy, L., Dahlberg, A., Griffith, G. W., Nordén, J., Ovaskainen, O., Perini, C., Senn-Irlet, B., & Halme, P. (2015). A fungal perspective on conservation biology: Fungi and conservation biology. *Conservation Biology*, *29*(1), 61–68. https://doi.org/10.1111/cobi.12388

Hofstetter, V., Buyck, B., Eyssartier, G., Schnee, S., & Gindro, K. (2019). The unbearable lightness of sequenced-based identification. *Fungal Diversity*, *96*(1), 243–284. https://doi.org/10.1007/s13225-019-00428-3

Hopkin, M. (2006). Spying on nature. *Nature*, *444*(7118), 420–421. https://doi.org/10.1038/444420a

Hunt, G. A., & Trappe, J. M. (1987). Seasonal hypogeous sporocarp production in a western Oregon Douglas-fir stand. *Canadian Journal of Botany*, *65*(3), 438–445. https://doi.org/10.1139/b87-053

Izzo, A., Canright, M., & Bruns, T. D. (2006). The effects of heat treatments on ectomycorrhizal resistant propagules and their ability to colonize bioassay seedlings. *Mycological Research*, *110*(2), 196–202. https://doi.org/10.1016/j.mycres.2005.08.010

Janečka, J. E., Jackson, R., Yuquang, Z., Diqiang, L., Munkhtsog, B., Buckley-Beason, V., & Murphy, W. J. (2008). Population monitoring of snow leopards using noninvasive collection of scat samples: A pilot study. *Animal Conservation*, *11*(5), 401–411. https://doi.org/10.1111/j.1469-1795.2008.00195.x

Johnson, C. N. (1996). Interactions between mammals and ectomycorrhizal fungi. *Trends in Ecology & Evolution*, *11*(12), 503–507. https://doi.org/10.1016/S0169-5347(96)10053-7

Juffe-Bignoli, D., Brooks, T. M., Butchart, S. H. M., Jenkins, R. B., Boe, K., Hoffmann, M., Angulo, A., Bachman, S., Böhm, M., Brummitt, N., Carpenter, K. E., Comer, P. J., Cox, N., Cuttelod, A., Darwall, W. R. T., Di Marco, M., Fishpool, L. D. C., Goettsch, B., Heath, M., … Kingston, N. (2016). Assessing the cost of global biodiversity and conservation knowledge. *PLoS One*, *11*(8), e0160640. https://doi.org/10.1371/journal.pone.0160640

Katoh, K. (2002). MAFFT: A novel method for rapid multiple sequence alignment based on fast Fourier transform. *Nucleic Acids Research*, *30*(14), 3059–3066. https://doi.org/10.1093/nar/gkf436

Kembel, S. W., Cowan, P. D., Helmus, M. R., Cornwell, W. K., Morlon, H., Ackerly, D. D., Blomberg, S. P., & Webb, C. O. (2010). Picante: R tools for integrating phylogenies and ecology. *Bioinformatics*, *26*(11), 1463–1464. https://doi.org/10.1093/bioinformatics/btq166

Kesanakurti, P. R., Fazekas, A. J., Burgess, K. S., Percy, D. M., Newmaster, S. G., Graham, S. W., Barrett, S. C. H., Hajibabaei, M., & Husband, B. C. (2011). Spatial patterns of plant diversity below-ground as revealed by DNA barcoding: Barcoding of plant roots. *Molecular Ecology*, *20*(6), 1289–1302. https://doi.org/10.1111/j.1365-294X.2010.04989.x

Laity, T., Laffan, S. W., González-Orozco, C. E., Faith, D. P., Rosauer, D. F., Byrne, M., Miller, J. T., Crayn, D., Costion, C., Moritz, C. C., & Newport, K. (2015). Phylodiversity to inform conservation policy: An Australian example. *Science of the Total Environment*, *534*, 131–143. https://doi.org/10.1016/j.scitotenv.2015.04.113

Langer, P. (2002). The digestive tract and life history of small mammals. *Mammal Review*, *25*, 107–131. https://doi.org/10.1046/j.1365-2907.2002.00101.x

Lê, S., Josse, J., & Husson, F. (2008). FactoMineR: An *R* package for multivariate analysis. *Journal of Statistical Software*, *25*(1), 1–18. https://doi.org/10.18637/jss.v025.i01

Lehmkuhl, J. F., Gould, L. E., Cázares, E., & Hosford, D. R. (2004). Truffle abundance and mycophagy by northern flying squirrels in eastern Washington forests. *Forest Ecology and Management*, *200*(1–3), 49–65. https://doi.org/10.1016/j.foreco.2004.06.006

Liao, H.-L., Chen, Y., & Vilgalys, R. (2016). Metatranscriptomic study of common and host-specific patterns of gene expression between pines and their symbiotic ectomycorrhizal fungi in the genus *Suillus*. *PLoS Genetics*, *24*, e1006348. https://doi.org/10.1371/journal.pgen.1006348

Luoma, D. L., Frenkel, R. E., & Trappe, J. M. (1991). Fruiting of hypogeous fungi in Oregon Douglas-fir forests: Seasonal and habitat variation. *Mycologia*, *83*(3), 335–353. https://doi.org/10.1080/00275514.1991.12026018

Mabberley, D. J. (1999). Edred John Henry Corner, C.B.E. 12 January 1906 – 14 September 1996: Elected F.R.S. 1955. *Biographical Memoirs of Fellows of the Royal Society*, *45*, 77–93. https://doi.org/10.1098/rsbm.1999.0007

Mace, G. M., Collar, N. J., Gaston, K. J., Hilton-Taylor, C., Akçakaya, H. R., Leader-Williams, N., Milner-Gulland, E. J., & Stuart, S. N.

(2008). Quantification of extinction risk: IUCN's system for classifying threatened species. *Conservation Biology*, *22*(6), 1424–1442. https://doi.org/10.1111/j.1523-1739.2008.01044.x

Małgorzata, G., & Dorota, H. (2013). *Research on truffles: Scientific journals analysis* (Vol. *8*, pp. 1837–1847). Academic Journals. https://doi.org/10.5281/ZENODO.3266787

Maser, C., Trappe, J. M., & Nussbaum, R. A. (1978). Fungal-small mammal interrelationships with emphasis on Oregon Coniferous forests. *Ecology*, *59*(4), 799–809. https://doi.org/10.2307/1938784

McGee, K. M., Eaton, W. D., Shokralla, S., & Hajibabaei, M. (2019). Determinants of soil bacterial and fungal community composition toward carbon-use efficiency across primary and secondary forests in a Costa Rican conservation area. *Microbial Ecology*, *77*(1), 148–167. https://doi.org/10.1007/s00248-018-1206-0

McMurdie, P. J., & Holmes, S. (2013). phyloseq: An R package for reproducible interactive analysis and graphics of microbiome census data. *PLoS One*, *8*(4), e61217. https://doi.org/10.1371/journal.pone.0061217

Miller, A. N., & Bates, S. T. (2017). The mycology collections portal (MyCoPortal). *IMA Fungus*, *8*(2), A65–A66. https://doi.org/10.1007/BF03449464

Minh, B. Q., Nguyen, M. A. T., & von Haeseler, A. (2013). Ultrafast approximation for phylogenetic bootstrap. *Molecular Biology and Evolution*, *30*(5), 1188–1195. https://doi.org/10.1093/molbev/mst024

Nguyen, L.-T., Schmidt, H. A., von Haeseler, A., & Minh, B. Q. (2015). IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Molecular Biology and Evolution*, *32*(1), 268–274. https://doi.org/10.1093/molbev/msu300

Nic Lughadha, E., Bachman, S. P., Leão, T. C. C., Forest, F., Halley, J. M., Moat, J., Acedo, C., Bacon, K. L., Brewer, R. F. A., Gâteblé, G., Gonçalves, S. C., Govaerts, R., Hollingsworth, P. M., Krisai-Greilhuber, I., Lirio, E. J., Moore, P. G. P., Negrão, R., Onana, J. M., Rajaovelona, L. R., … Walker, B. E. (2020). Extinction risk and threats to plants and fungi. *Plants, People, Planet*, *2*(5), 389–408. https://doi.org/10.1002/ppp3.10146

Nilsson, R. H., Anslan, S., Bahram, M., Wurzbacher, C., Baldrian, P., & Tedersoo, L. (2019). Mycobiome diversity: High-throughput sequencing and identification of fungi. *Nature Reviews Microbiology*, *17*(2), 95–109. https://doi.org/10.1038/s41579-018-0116-y

Nilsson, R. H., Hyde, K. D., Pawłowska, J., Ryberg, M., Tedersoo, L., Aas, A. B., Alias, S. A., Alves, A., Anderson, C. L., Antonelli, A., Arnold, A. E., Bahnmann, B., Bahram, M., Bengtsson-Palme, J., Berlin, A., Branco, S., Chomnunti, P., Dissanayake, A., Drenkhan, R., … Abarenkov, K. (2014). Improving ITS sequence data for identification of plant pathogenic fungi. *Fungal Diversity*, *67*(1), 11–19. https://doi.org/10.1007/s13225-014-0291-8

Padmanabhan, P., Grosse, J., Asad, A. B. M. A., Radda, G. K., & Golay, X. (2013). Gastrointestinal transit measurements in mice with 99mTc-DTPA-labeled activated charcoal using NanoSPECT-CT. *EJNMMI Research*, *3*(1), 60. https://doi.org/10.1186/2191-219X-3-60

Phoebus, I., Boulanger, J., Eiken, H. G., Fløystad, I., Graham, K., Hagen, S. B., Sorensen, A., & Stenhouse, G. (2020). Comparison of grizzly bear hair-snag and scat sampling along roads to inform wildlife population monitoring. *Wildlife Biology*, *2020*(3). https://doi.org/10.2981/wlb.00697

Pyare, S., & Longland, W. S. (2001). Patterns of ectomycorrhizal-fungi consumption by small mammals in remnant old-growth forests of the Sierra Nevada. *Journal of Mammalogy*, *82*(3), 681–689. https://doi.org/10.1644/1545-1542(2001)082<0681:POEFCB>2.0.CO;2

Riccioni, C., Rubini, A., Belfiori, B., Gregori, G., & Paolocci, F. (2016). *Tuber magnatum*: The special one. What makes it so different from the other *Tuber* spp.? In A. Zambonelli, M. Iotti, & C. Murat (Eds.), *True truffle* (Tuber *spp.) in the World* (Vol. *47*, pp. 87–103). Springer International Publishing. https://doi.org/10.1007/978-3-319-31436-5_6

Rodríguez, J. P., Keith, D. A., Rodríguez-Clark, K. M., Murray, N. J., Nicholson, E., Regan, T. J., Miller, R. M., Barrow, E. G., Bland, L. M., Boe, K., Brooks, T. M., Oliveira-Miranda, M. A., Spalding, M., & Wit, P. (2015). A practical guide to the application of the IUCN Red List of ecosystems criteria. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *370*(1662), 20140003. https://doi.org/10.1098/rstb.2014.0003

Rodríguez, J. P., Rodríguez-clark, K. M., Baillie, J. E. M., Ash, N., Benson, J., Boucher, T., Brown, C., Burgess, N. D., Collen, B., Jennings, M., Keith, D. A., Nicholson, E., Revenga, C., Reyers, B., Rouget, M., Smith, T., Spalding, M., Taber, A., Walpole, M., … Zamin, T. (2011). Establishing IUCN Red List criteria for threatened ecosystems: IUCN Red List criteria for ecosystems. *Conservation Biology*, *25*(1), 21–29. https://doi.org/10.1111/j.1523-1739.2010.01598.x

Rognes, T., Flouri, T., Nichols, B., Quince, C., & Mahé, F. (2016). VSEARCH: A versatile open source tool for metagenomics. *PeerJ*, *4*, e2584. https://doi.org/10.7717/peerj.2584

Ryberg, M., & Nilsson, R. H. (2018). New light on names and naming of dark taxa. *MycoKeys*, *30*, 31–39. https://doi.org/10.3897/mycokeys.30.24376

Schatz, G. E. (2009). Plants on the IUCN Red List: Setting priorities to inform conservation. *Trends in Plant Science*, *14*(11), 638–642. https://doi.org/10.1016/j.tplants.2009.08.012

Schmidt, P.-A., Bálint, M., Greshake, B., Bandow, C., Römbke, J., & Schmitt, I. (2013). Illumina metabarcoding of a soil fungal community. *Soil Biology and Biochemistry*, *65*, 128–132. https://doi.org/10.1016/j.soilbio.2013.05.014

Sikes, R. S., & the Animal Care and Use Committee of the American Society of Mammalogists. (2016). 2016 Guidelines of the American Society of Mammalogists for the use of wild mammals in research and education. *Journal of Mammalogy*, *97*(3), 663–688. https://doi.org/10.1093/jmammal/gyw078

Smith, S. E., & Read, D. J. (2010). *Mycorrhizal symbiosis*. Academic Press.

Sousa, N. R., Franco, A. R., Ramos, M. A., Oliveira, R. S., & Castro, P. M. L. (2011). Reforestation of burned stands: The effect of ectomycorrhizal fungi on *Pinus pinaster* establishment. *Soil Biology and Biochemistry*, *43*(10), 2115–2120. https://doi.org/10.1016/j.soilbio.2011.06.013

Splivallo, R., Ottonello, S., Mello, A., & Karlovsky, P. (2011). Truffle volatiles: From chemical ecology to aroma biosynthesis. *New Phytologist*, *189*(3), 688–699. https://doi.org/10.1111/j.1469-8137.2010.03523.x

Steidinger, B. S., Crowther, T. W., Liang, J., Van Nuland, M. E., Werner, G. D. A., Reich, P. B., Nabuurs, G. J., de-Miguel, S., Zhou, M., Picard, N., Herault, B., Zhao, X., Zhang, C., Routh, D., & Peay, K. G. (2019). Climatic controls of decomposition drive the global biogeography of forest-tree symbioses. *Nature*, *569*(7756), 404–408. https://doi.org/10.1038/s41586-019-1128-0

Stephens, R. B., Remick, T. J., Ducey, M. J., & Rowe, R. J. (2017). Drivers of truffle biomass, community composition, and richness among forest types in the northeastern US. *Fungal Ecology*, *29*, 30–41. https://doi.org/10.1016/j.funeco.2017.05.004

Stephens, R. B., & Rowe, R. J. (2020). The underappreciated role of rodent generalists in fungal spore dispersal networks. *Ecology*, *101*(4), e02972. https://doi.org/10.1002/ecy.2972

Taylor, D. L., Walters, W. A., Lennon, N. J., Bochicchio, J., Krohn, A., Caporaso, J. G., & Pennanen, T. (2016). Accurate estimation of fungal diversity and abundance through improved lineage-specific primers optimized for Illumina Amplicon sequencing. *Applied and Environmental Microbiology*, *82*(24), 7217–7226. https://doi.org/10.1128/AEM.02576-16

Tehler, A., Little, D. P., & Farris, J. S. (2003). The full-length phylogenetic tree from 1551 ribosomal sequences of chitinous fungi, Fungi. *Mycological Research*, *107*(8), 901–916. https://doi.org/10.1017/S0953756203008128

Troudet, J., Grandcolas, P., Blin, A., Vignes-Lebbe, R., & Legendre, F. (2017). Taxonomic bias in biodiversity data and societal preferences.

Scientific Reports, *7*(1), 9132. https://doi.org/10.1038/s41598-017-09084-6

van der Linde, S., Holden, E., Parkin, P. I., Alexander, I. J., & Anderson, I. C. (2012). Now you see it, now you don't: The challenge of detecting, monitoring and conserving ectomycorrhizal fungi. *Fungal Ecology*, *5*(5), 633–640. https://doi.org/10.1016/j.funeco.2012.04.002

van Tilburg Bernardes, E., Pettersen, V. K., Gutierrez, M. W., Laforest-Lapointe, I., Jendzjowsky, N. G., Cavin, J.-B., Vicentini, F. A., Keenan, C. M., Ramay, H. R., Samara, J., MacNaughton, W. K., Wilson, R. J. A., Kelly, M. M., McCoy, K. D., Sharkey, K. A., & Arrieta, M.-C. (2020). Intestinal fungi are causally implicated in microbiome assembly and immune development in mice. *Nature Communications*, *11*(1), 2577. https://doi.org/10.1038/s41467-020-16431-1

Vane-Wright, R. I., Humphries, C. J., & Williams, P. H. (1991). What to protect?—Systematics and the agony of choice. *Biological Conservation*, *55*(3), 235–254. https://doi.org/10.1016/0006-3207(91)90030-D

Vernes, K., Cooper, T., & Green, S. (2015). Seasonal fungal diets of small mammals in an Australian temperate forest ecosystem. *Fungal Ecology*, *18*, 107–114. https://doi.org/10.1016/j.funeco.2015.09.015

Walther, G., Pawłowska, J., Alastruey-Izquierdo, A., Wrzosek, M., Rodriguez-Tudela, J. L., Dolatabadi, S., Chakrabarti, A., & de Hoog, G. S. (2013). DNA barcoding in Mucorales: An inventory of biodiversity. *Persoonia – Molecular Phylogeny and Evolution of Fungi*, *30*(1), 11–47. https://doi.org/10.3767/003158513X665070

Walther, G., Wagner, L., & Kurzai, O. (2019a). Outbreaks of Mucorales and the species involved. *Mycopathologia*, *185*, 765–781. https://doi.org/10.1007/s11046-019-00403-1

Walther, G., Wagner, L., & Kurzai, O. (2019b). Updates on the taxonomy of Mucorales with an emphasis on clinically important taxa. *Journal of Fungi*, *5*(4), 106. https://doi.org/10.3390/jof5040106

Wasser, S. K., Brown, L., Mailand, C., Mondol, S., Clark, W., Laurie, C., & Weir, B. S. (2015). Genetic assignment of large seizures of elephant ivory reveals Africa's major poaching hotspots. *Science*, *349*(6243), 84–87. https://doi.org/10.1126/science.aaa2457

Wasser, S. K., Shedlock, A. M., Comstock, K., Ostrander, E. A., Mutayoba, B., & Stephens, M. (2004). Assigning African elephant DNA to geographic region of origin: Applications to the ivory trade. *Proceedings of the National Academy of Sciences USA*, *101*(41), 14847–14852. https://doi.org/10.1073/pnas.0403170101

Wilson, D. E., & Reeder, D. M. (Eds.). (2005). *Mammal species of the world: A taxonomic and geographic reference* (3rd ed.). Johns Hopkins University Press.

Wilson, J. S., Forister, M. L., Dyer, L. A., O'Connor, J. M., Burls, K., Feldman, C. R., Jaramillo, M. A., Miller, J. S., Rodríguez-Castañeda, G., Tepe, E. J., Whitfield, J. B., & Young, B. (2012). Host conservatism, host shifts and diversification across three trophic levels in two Neotropical forests: Diversification across three trophic levels. *Journal of Evolutionary Biology*, *25*(3), 532–546. https://doi.org/10.1111/j.1420-9101.2011.02446.x

Wu, B., Hussain, M., Zhang, W., Stadler, M., Liu, X., & Xiang, M. (2019). Current insights into fungal species diversity and perspective on naming the environmental DNA sequences of fungi. *Mycology*, *10*(3), 127–140. https://doi.org/10.1080/21501203.2019.1614106

Yamashita, S., Ando, K., Hoshina, H., Ito, N., Katayama, Y., Kawanabe, M., Maruyama, M., & Itioka, T. (2015). Food web structure of the fungivorous insect community on bracket fungi in a Bornean tropical rain forest: Bornean fungivorous insect food webs. *Ecological Entomology*, *40*(4), 390–400. https://doi.org/10.1111/een.12200

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How to cite this article: Bradshaw, A. J., Autumn, K. C., Rickart, E. A., & Dentinger, B. T. M. (2022). On the origin of feces: Fungal diversity, distribution, and conservation implications from feces of small mammals. *Environmental DNA*, 4, 608–626. https://doi.org/10.1002/edn3.281