Title: Final Report: Truffles of the Cascade-Siskiyou National Monument (FRF 2023-2024 cycle)
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Introduction

Fungi that fruit underground ("hypogeous") are colloquially referred to as truffles. Truffles are most famous for the few species that have culinary value; however, in some regions, such as the Pacific Northwest, there are a wealth of truffles species that contribute to regional biodiversity. Truffles rely on mycophagist animal dispersal, an evolutionary response to avoid the environmental risks aboveground fruiting ("epigeous") fungi face such as drying out or freezing. They produce myriad odors to attract potential dispersers who ingest the truffle, passing reproductive spores through the animal's gut and depositing them in a new location.

Because truffles grow underground and require specialist knowledge to find them, truffle species are often underrepresented in traditional mycological surveys. Traditionally, truffle diversity has been explored through raking, where a researcher uses a four-tine garden rake to lift the duff and explore the top layer of soil for truffles that may be there. With practice, truffle rakers can learn the signs of where truffles are most likely to be; however, even experienced rakers often disturb the forest floor without finding a truffle. In culinary truffle cultivation, truffle dogs are used to target mature truffles with minimal disturbance. Scientific researchers are beginning to use truffle dogs for researching culinary truffles (e.g., Cejka et al 2022 J Vet Behaviour, Centenaro et al 2023 Plants People Planet) or research into the classic *Tuber* genus that hosts most culinary truffle species (Berch and Bonito 2016 Mycorrhiza). In Norway, truffle dogs were used to re-describe Elaphomyces, including three new species found by the dogs (Molina et al. 2020 Fungal Systematics Evol). In this study, we used a golden retriever who has three years of experience finding a wide diversity of truffles (>45 genera to date) to explore the truffle diversity of CSNM. This allowed us to target truffles with high accuracy and low disturbance as most truffle digs indicated by the dog produced truffles. We could also locate hard-to-find truffles such as *Genea* that are small and soil-colored, and that may escape notice when raking for truffles.

In a previous grant cycle, we surveyed 11 sites and found 27 truffle species (Dawson and Dawson 2023, Final Report FRF 2022-2023). We concluded that CSNM had high truffle diversity that we were only beginning to understand. Oak dominated sites were more productive than conifer dominated sites, although conifer sites hosted greater species diversity. We demonstrated that a truffle dog trained to seek out a wide variety of truffles could be used to efficiently document truffle diversity. We obtained a second round of funding through the Friends of CSNM to continue our truffle research in 2023-24, confident that there was much more to understand about truffles of the Monument.

Methods

Sites

The Cascade-Siskiyou National Monument straddles the Oregon-California border and contains an unusually high diversity of habitats (Friends of CSNM Monument Guide). Our study focused on mixed conifer and true fir forests located centrally and in the northeast corner of the CSNM, and oak woodland and savanna on the western and southern sides of the Monument (Figure 1). Elevation ranged from 821 m to 1824 m (Table 1). True fir sites were dominated by ponderosa pine (*Pinus ponderosa*), white fir (*Abies concolor*), sugar pine (*Pinus lambertiana*), and Douglas-fir (*Pseudotsuga menziesii*), and sites near Surveyor Mountain also had Shasta red fir (*Abies magnifica* var. *shastensis*). Oak woodland sites were dominated by Oregon white oak (*Quercus garryana*) with occasional scattered California black oak (*Quercus kellogii*) and ponderosa pine. The mixed oak-conifer sites had patches dominated by Douglas-fir, ponderosa pine, Oregon white oak, and California black oak, with some mature madrone trees (*Arbutus menziesii*).

Sites were chosen in reference to our 2022-23 surveys, with the aim to expand into new habitats where possible as well as revisit existing sites at different times of the year. We revisited four sites established in the 2022-23 surveys, and added 22 new sites. The main priority during each visit was to target the areas most likely to contribute to our list of species and add the greatest diversity. Sites visited in the autumn and spring were often inaccessible when we visited in November to March, so during those visits we focused on surveying the lower elevation oak woodland areas. Rye the truffle dog works best when the air temperature is lower than 18ºC, so during spring and summer forays we focused on higher elevation areas that were cooler than the surroundings.

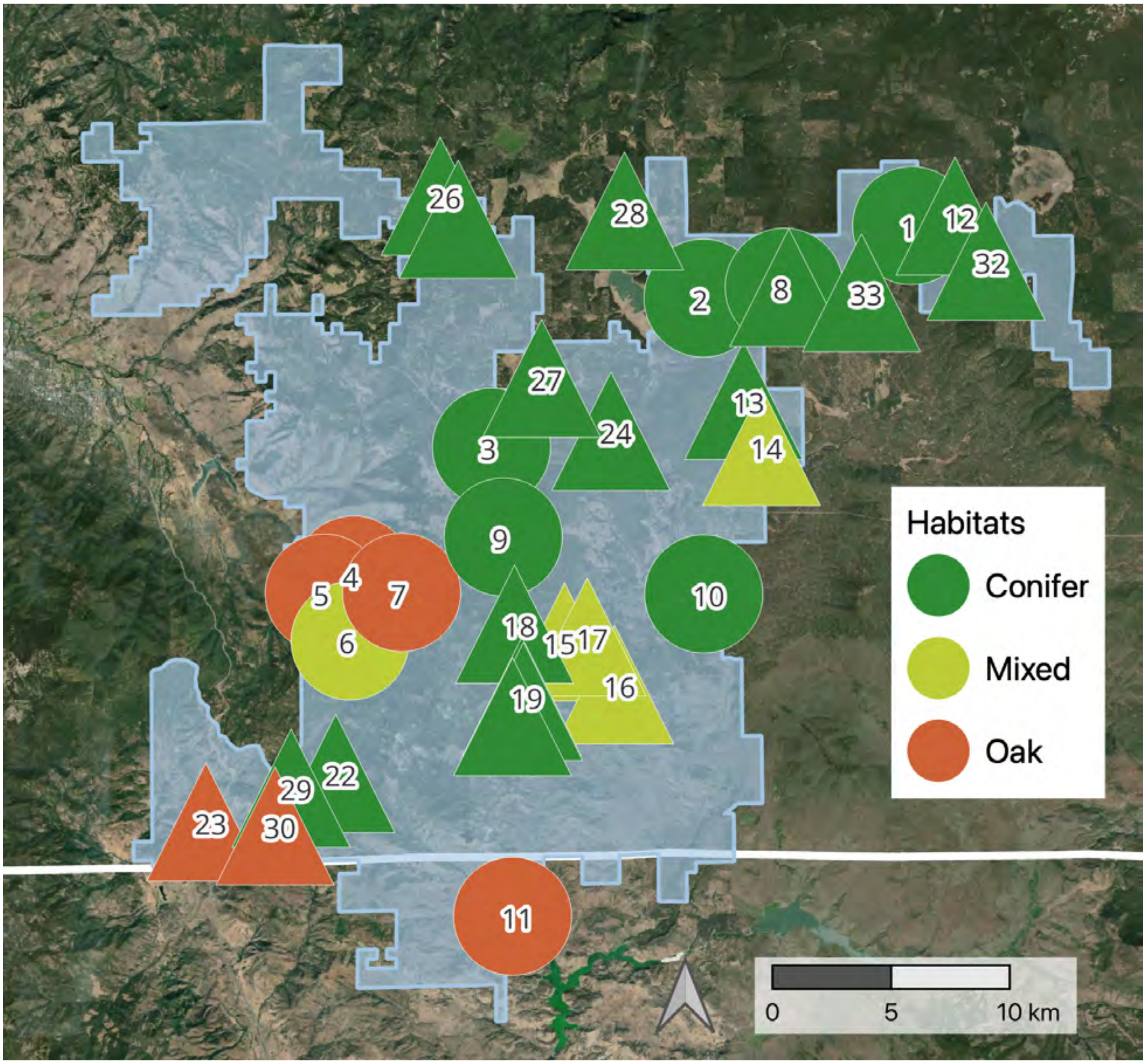

Figure 1: Map of the rough location of sites included in truffle surveys from October 2022 to May 2024. Circles are sites established in the previous grant year. Triangles are sites established in this grant year. Satellite imagery from ESRI. Site numbers correspond to Table 1.

Table 1: Site survey dates and characteristics. Elevation, precipitation, and temperature were extracted from WorldClim (worldclim.org).

Site	Survey date	Habitat	Mean annual precipitation (mm)	Mean temperature (ºC)	Elevation (m)
1	10/31/22; 06/16/23; 09/29/23	Conifer	879	6.2	1622
2	10/31/22	Conifer	832	7	1439
3	11/1/22; 5/13/23	Conifer	958	6.9	1574
4	2/4/23	Oak	568	10.5	825
5	2/4/23; 5/13/23; 1/27/24	Oak	591	10.3	900
6	10/31/22; 2/4/23	Mixed	606	9.7	975
7	2/4/23	Oak	648	9.7	1072
8	5/13/23	Conifer	825	6.8	1498
9	5/13/23	Conifer	850	7.5	1441
10	5/13/23	Conifer	580	8.8	1122
11	5/14/23; 6/17/23; 11/18/23; 1/29/24; 3/20/24; 5/5/24; 5/25/24	Oak	517	11	821
12	09/29/23	Conifer	845	6.1	1601
13	09/29/23	Conifer	712	7.7	1323
14	09/29/23	Oak, conifer	605	8.4	1154
15	09/30/23	Mixed	715	7.8	1324
16	09/30/23	Mixed	684	8	1323
17	09/30/23	Mixed	655	8.1	1222
18	09/30/23	Conifer	768	7.7	1373
19	10/02/23	Conifer	915	6.9	1603
20	10/02/23	Conifer	915	6.9	1603
21	10/02/23	Conifer	884	7	1568
22	10/13/23; 5/28/24	Oak, conifer	891	7.6	1514
23	6/18/23; 10/13/23; 1/27/24	Oak	638	9.6	1066
24	11/19/23	Conifer	879	6.9	1511
25	11/19/23	Conifer	825	6.8	1498

Site	Survey date	Habitat	Mean annual precipitation (mm)	Mean temperature (ºC)	Elevation (m)
26	1/28/24	Conifer	1043	6.8	1580
27	1/28/24	Conifer	946	6.6	1560
28	1/28/24	Conifer	885	6.9	1433
29	5/26/24	Conifer	895	7.8	1499
30	5/26/24	Oak	827	8.2	1419
31	5/27/24	Conifer	987	7	1512
32	5/27/24	Conifer	990	5.4	1824
33	5/27/24	Conifer	733	7.3	1383

Truffle hunting

All truffles were located by trained truffle diversity dog "Rye", a golden retriever with three years experience in diversity truffling (Figure 2). While most truffle dogs focus on culinary truffles (*Leucangium carthusianum*, *Tuber oregonense*, and *T. gibbosum*), Rye will indicate any type of hypogeous sporocarp that produces mature aroma. At each site, he was given the command to search and allowed to roam free to follow his nose. Mature truffles were indicated by digging until the truffle was excavated. We collected each truffle that was presumed to be a unique species and documented its fresh characteristics with photos and field notes. Any duplicate specimens were noted and placed back in the soil where they were found. We continued each search until the dog expressed no further interest (a general indication that he has found a majority of the truffles). We carried a Garmin eMap 67i or Garmin eTrex 30 hand-held GPS unit that tracked our path while truffling and marked each truffle found.

A small amount of fresh tissue was taken from each collected truffle and placed in Extract 'n Amp extraction solution for future DNA analysis. Truffles were dried at the end of each day in a dehydrator at 35ºC. Each collected truffle has an associated iNaturalist observation (with obscured coordinates) with color images of fresh characteristics. A list of iNaturalist truffle observations is included in Appendix 1.

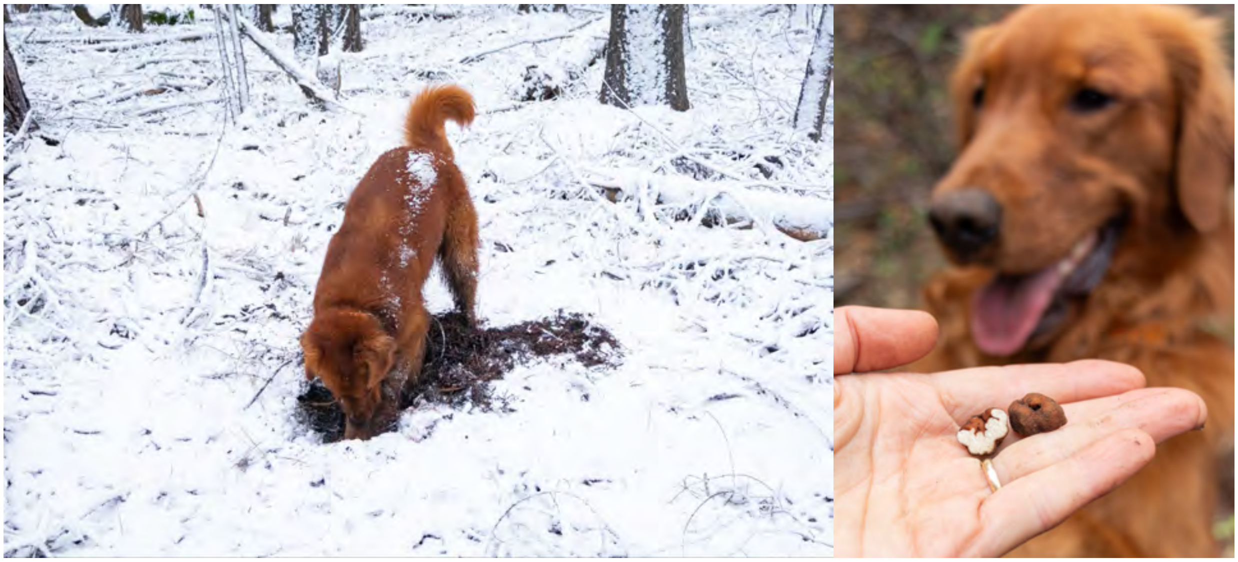

Figure 2: Rye digging for truffles in the snow (left); Rye with a Balsamia truffle that he found (right).

DNA analyses

Sporocarp DNA extraction was performed using the Extract ‘n Amp method (Truffle Smith lab protocol) by placing a fragment of fresh tissue in 50 µL of the Extraction Solution. Samples were kept frozen at -20C until ready for processing, then incubated at 95ºC for 10 minutes and diluted 1:1 with 3% Bovine Serum Albumin (BSA). Sporocarp collections were prepared for PacBio SMRT sequencing with rDNA target regions including the Internal Transcribed Spacer (ITS) region into the Large Sub-unit (LSU), using primers ITS1catta and LR5 (TW14) with attached PacBio barcodes. PCR amplification was performed in 25 µL reactions containing 12.5 µL GoTaq® Green Master Mix, 0.4 µL 2% BSA, 1 µL unique primer/barcode pairs, 0.8 µL DNA template, and 10.3 µL nuclease-free water. Reaction conditions were set to an initial 2 min denaturation at 95ºC, followed by 30 cycles of 30 s at 95ºC, 30 s at 51ºC, and 90 s at 72ºC, then a final 7 min at 72ºC. Gel electrophoresis was performed with 5 µL of the PCR product, and gel band brightness was scored from 1-5 (faint/no band to brightest band) for quantification. Pools were purified using Omega Mag-Bind® TotalPure NGS Beads at 0.85X to remove small DNA fragments, and were sent to the UO Genomics and Cell Characterization Core facility for final preparation and sequencing. Reads were demultiplexed and dereplicated using a custom bioinformatics pipeline (C. Delevich, unpublished).

Resulting DNA sequences were compared with similar sequences in the GenBank nucleotide database using Megablast. Along with morphological characteristics, identifications were based on percent similarity to vouchers in the GenBank database, with >99% generally being considered a species match and <99% as inconclusive or potentially undescribed (depending on the amount of variation expected in a lineage).

Findings

Truffle diversity

We found 74 truffle species in 29 genera at 25 sites across the Cascade-Siskiyou National Monument from June 2023 to March 2024 (Table 2). Of these, 39 species were taxonomically unresolved using the global genetic database GenBank, suggesting that they may be new species. Twenty-four of the species we found this year were not found in our previous year's survey. From June 2023 to May 2024, we located 1,093 individual truffles and collected 130 of these for DNA analysis. A few of these collections are displayed in Figure 3.

Table 2: List of species found in the Cascade-Siskiyou National Monument between June 2023 and March 2024 based on DNA matching with GenBank. Specific epithets beginning with 'CS' indicate a species that has no described matches in GenBank. The number following 'CS' indicates the field collection number assigned to this truffle. Abbreviations 'aff.' = *species affinis* (within the group), 'cf.' = *conferre* (compare to), 'sp.' = *species* (unnamed/unresolved species. **Not yet sequenced, identification based on morphology.*

Species name	Habitat
Agaricus inapertus	Conifers
Albatrellaceae sp. CS113	Mixed oak/conifer
Balsamia alba	Oaks
Balsamia aff. *latispora*	Conifers
Balsamia quercicola	Oaks
Balsamia cf. *setchellii*	Oaks
Balsamia sp. CS101	Conifers
Balsamia sp. CS105	Conifers
Balsamia sp. CS166	Conifers
Choiromyces sp. CS5	Conifers

Species name	Habitat
Choiromyces sp. CS61	Conifers
Coniophora sp. CS143	Oaks
Cortinarius pinguis	Conifers
Elaphomyces decipiens	Conifers
Elaphomyces sp. CS64	Conifers
Elaphomyces sp. CS72	Oaks
Endogonaceae sp. CS155	Conifers
Endogonaceae sp. CS168	Conifers
Gautieria cf. *pterosperma*	Conifers
Gautieria sp. CS56	Conifers
Gautieria sp. CS82	Oaks
Gautieria sp. CS137	Conifers

Species name	Habitat
Gautieria sp. CS169	Conifers
Geastraceae sp. CS131	Conifers
Geastraceae sp. CS140	Conifers
Genea arenaria	Oaks
Genea sp. CS79	Oaks
Genea sp. CS99	Oaks
Genea sp. CS112	Oaks
Genea sp. CS115	Mixed oak/conifer
Genea sp. CS163	Oaks
Genea sp. CS171	Conifers
Genea sp. CS196	Oaks
Geopora aff. *cooperi*	Conifers
Geopora sp. CS150	Conifers
Geopora sp. CS189	Oaks
Glomerales spp.	Oaks; Conifers
Glomus sp. CS195	Oaks
Hydnotryopsis cf. *setchellii*	Oaks
Hymenogaster parksii	Oaks; Conifers
Hymenogaster cf. *raphanodorus*	Oaks
Hysterangium cf. *setchellii*	Conifers
Hysterangium sp. CS38	Oaks
Hysterangium sp. CS63	Conifers
Hysterangium sp. CS76	Oaks
Hysterangium sp. CS97	Conifers
Lactarius sp. CS40	Oaks

Species name	Habitat
Lactarius sp. CS123	Conifers
Lepiota viridigleba	Conifers
Leucangium carthusianum	Conifers
Leucogaster cf. *rubescens*	Conifers
Melanogaster euryspermus	Oaks
Paragalactinia infossa	Oaks
Phlegmacium sublilacinum	Conifers
Rhizopogon arctostaphyli	Conifers
Rhizopogon cf. *hawkerae*	Conifers
Rhizopogon cf. *roseolus*	Conifers
Rhizopogon sp. CS174	Conifers
Russula cf. *similaris*	Conifers
Russula sp. CS77	Oaks
Russula sp. CS81	Oaks
Schenella pityophila	Mixed oak/conifer
Sedecula pulvinata	Conifers
Trappea sp. CS127	Conifers
Tuber aff. *anniae*	Conifers
Tuber beyerlei	Conifers
Tuber candidum	Oaks
Tuber luomae	Conifers
Tuber quercicola	Oaks
Tuber whetstonense	Conifers
Tuber sp. CS114	Mixed oak/conifer
Xerocomellus cf. *mcmurphyi*	Conifers

Figure 3 (next page): Some truffle biodiversity of the Cascade-Siskiyou National Monument. A. *Agaricus inapertus* B. *Melanogaster euryspermus* C. *Tuber* sp. CS114 D. *Russula* sp. CS81 E. *Balsamia* sp. CS166 F. *Genea* sp. CS99 G. *Genea* sp. CS112 H. *Cazia flexiascus* I. *Lactarius* sp. CS123 J. *Schenella pityophila* K. *Geopora* aff. *cooperi* L. *Balsamia* cf. *setchellii* M. *Balsamia* cf. *latispora*

Rare and noteworthy finds

Our overwhelming number of intriguing and unusual finds means we have only covered a handful here in detail, but anticipate multiple future publications that explore the identity and ecology of the other truffles that are equally deserving of attention. For example, while we collected many distinctly different *Hysterangium* and *Gautieria,* species from both of these genera are under-represented on GenBank and identification to species for our collections may be possible through microscopic examinations. However, nearly all of our undetermined collections of *Genea* truffles are almost certainly undescribed species, as very few North American *Genea* are described. The genetic data from a few of our collections were so distant from any GenBank submissions that we were uncertain which genus they belonged to, as was the case with two Geastraceae truffles that morphologically resemble *Schenella* but are genetically closer to the earthstar genus *Geastrum.* A truffle in poor condition that we did obtain a quality sequence from is a very distant match to a *Leucophleps,* but is far enough that we can only be certain it belongs in the family Albatrellaceae. Our wealth of collections and sequence data means there is plenty of work to be done, and we are excited to see what our fieldwork efforts at CSNM can contribute to the field of mycology. The following section highlights a few of the finds that we found most noteworthy.

<u>Coniophoraceae truffles</u>

The family Coniophoraceae is a group of brown rot fungi generally characterized by a resupinate growth form (also called fungal crusts). The widespread genus *Coniophora* is one of the leading culprits in structure decay of man-made wooden buildings. Recent molecular evidence placed a previously phylogenetically ambiguous truffle species into the Coniophoraceae, representing a highly unusual case of an evolutionary transition from a crust into a truffle form (Trappe et al., 2015, *Mycologia*). *Sedecula pulvinata* is a puffball-like fungus that superficially resembles a *Scleroderma* with a firm exterior and a dark interior that becomes powdery upon maturity (Figure 4). This species is typically found in dry, high elevation habitat under conifers, and is considered to be rare. We found *S. pulvinata* at two sites in CSNM in September 2023.

During a November 2023 survey in oak savanna, we found an unusual truffle that DNA analysis revealed to be a *Coniophora* truffle previously unknown to science (provisional name *Coniophora* sp. CS143) (Figure 4). Unlike *S. pulvinata*, it had a dense, marbled interior with holes inhabited by termites. While invertebrates are common visitors and consumers of truffles, we have never seen a truffle with an established termite population. We have speculated on the possibility that this particular species may attract termites as a spore dispersal method, considering the wood-decomposing family history of Coniophoraceae. The truffle had a fairly strong, paint-like aroma with nutty undertones. We are currently in the process of formally describing this unusual new species.

Figure 4: ***Sedecula pulvinata*** **(left) and** ***Coniophora*** **sp. CS143 (right).**

<u>*Hydnotryopsis* cf. *setchellii*</u>

Hydnotryopsis setchellii is a rare and poorly known species, sparsely collected from few sites in California and Oregon. Several of the California sites represent only historic collections (late 1800s, early 1900s), despite continued efforts to recollect at these sites. In June 2023, we collected two *Hydnotryopsis* fruiting bodies from white oak savanna, ca. 2 miles south of the Oregon border. While there is some variation in the DNA barcoding ITS region of these collections compared with pre-existing *H. setchellii* sequences, ours are very likely to be *Hydnotryopsis setchellii*, representing a new location for this rare species. *Hynotryopsis* is a genus of truffle fungi in the family Pezizaceae, which includes many of the so-called "desert truffles", including *Terfezia*, *Tirmania*, *Kalaharituber*, and *Delastria.* While unremarkable at first glance, this truffle had a uniquely rich and complex aroma (Figure 5).

Figure 5: *Hydnotryopsis* cf. *setchellii* from white oak savanna in June, 2023. Mature fruiting body on the left, and spores in ascus on the right.

<u>*Lepiota viridigleba*</u>

During a June 2023 foray in old growth conifer forest, Rye unearthed several small but uniquely colored basidiomycete truffles. Varying from blue to green, this truffle initially appeared to resemble some kind of *Rhizopogon* based on the spongy nature and intricate hymenial folds. Microscopy revealed non-*Rhizopogon* spores, and DNA sequencing provided a near perfect match for *Lepiota viridigleba*, a sequestrate species from the typically epigeous genus *Lepiota* (Figure 6). Previously called *Amogaster viridigleba*, this truffle is only known from two sites in California, and efforts to rediscover additional fruiting bodies at these sites have not been successful despite yearly attempts during a mycology course. NaturServe lists this species as Critically Imperiled (S1) in Oregon, and this species has also been assessed by the IUCN Red List but was deemed data deficient. Hopefully, our new records of this rare truffle will be beneficial in determining the range and current level of threat to this unusual truffle.

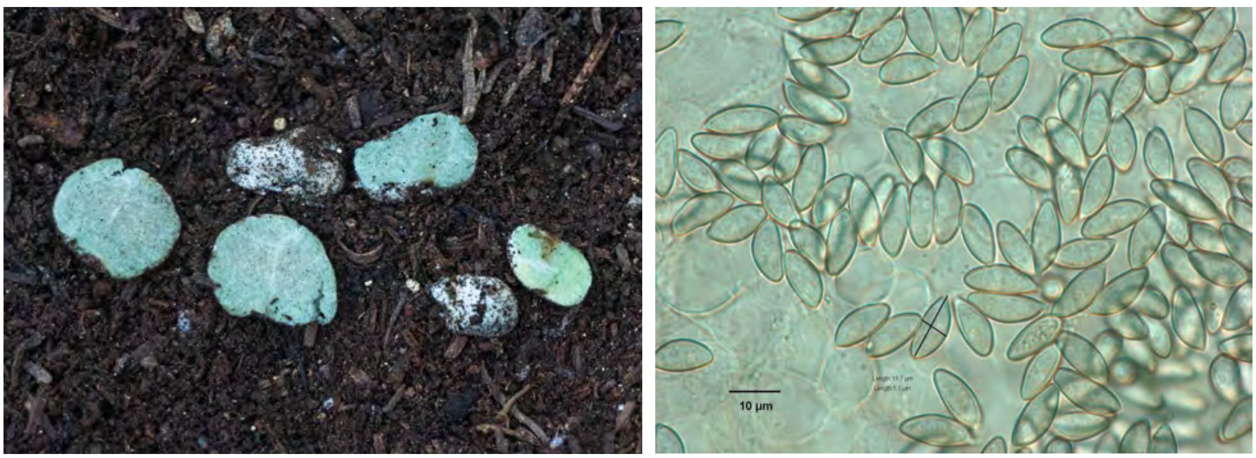

Figure 6: *Lepiota viridigleba* fruiting bodies (left) and spores (right).

<u>*Xerocomellus*</u> cf. <u>*mcmurphyi*</u>

While most truffles are easily identified to the genus level based on a suite of traits including color, texture, density, gleba patterns, and aroma, the genus *Hymenogaster* has a notoriously polyphyletic history due to similarity in both macro- and microscopic characteristics across species from multiple separate lineages. The increased accessibility of DNA barcoding has made it possible to determine the true phylogeny of the *Hymenogaster*-like truffles and rename those that actually belong to separate lineages. As *Hymenogaster* truffles tend to be common, we have become fairly confident in identifying truffles of this genus in the field, but the sequence data from a recent collection from high elevation fir forest surprised us and challenged our assumption of what characterizes a *Hymenogaster* in the field. While superficially resembling other *Hymenogaster* that we have seen, this new collection had a bright yellow peridium and gleba, and did not have the typical weed-y or radish-y aroma that we expect from

Hymenogaster **(Figure 7)**. DNA placed this truffle in the bolete family, as a sequestrate *Xerocomellus.* This collection is potentially a match for the rarely collected species *Xerocomellus mcmurphyi* (previously *Hymenogaster mcmurphy*i), but a large amount of variation in the ITS region leaves its placement uncertain. *X. mcmurphyi* has previously only been collected under oaks; our collections were from pure conifer forest, but this forest was adjacent to an extensive stand of oaks on the opposite side of the ridge.

Figure 7: *Xerocomellus* cf. *mcmurphyi* fruiting bodies collected under fir in early October 2023.

Culinary truffles

The Oregon black truffle (*Leucangium carthusianum*) is generally associated with Douglas-fir plantations west of the Cascades in central Oregon to Washington state. Although it has been found through northern California, we were surprised to find it in CSNM old growth forest because occurrences this far south are unusual. However, we found five specimens in four sites, indicating that this truffle, prized for its culinary value, is present in limited quantities. One of these individuals weighed in excess of 5 ounces (Figure 8). Given that we found it only five times out of [~900] truffles located over two years, black truffle hunting is unlikely to be a productive activity in this region, though they may be more productive in homogenous stands of Douglas-fir; our collections were all from high elevation (~5000’) mixed conifer forest. We have not found any Oregon white truffles (*Tuber gibbosum*, *T. oregonense*) or Oregon brown truffles (*Kalapuya brunnea*) in CSNM. These species are typically only found in lower elevation Douglas-fir forest.

Figure 8: *Leucangium carthusianum* truffle shown with truffle dog Rye.

Parasitized truffles

We found six *Choiromyces* truffles that had black-speckled gleba, one in June 2023 and five in May 2024. Microscopy revealed that alongside the translucent round spores of *Choiromyces* were dark, lemon-shaped spores of another fungus (Figure 9). This fungus is likely *Melanospora* (Ascomycota), a known mycoparasite that has been found in *Tuber* truffles (Fan et al. 2012 Mycologia). Both the *Choiromyces* and parasite spores appeared healthy, so it is unlikely that this parasite is affecting the truffle's ability to reproduce. Although we were unsuccessful in extracting DNA of the parasite in our first round, we recollected a parasitized *Choiromyces* truffle May and plan to make a second attempt at identifying the parasite with DNA analysis. This particular species of *Choiromyces* (temporary name "CS5"), is near *C. alveolatus* but not a good match. It was fruiting extraordinarily abundantly in high elevation conifer forest near snowmelt during our May 2024 survey, and we recorded a total of 38 finds in a single day. Most of these truffle fruiting bodies were normal to our eye, but 15% obviously carried the *Melanospora* parasite based on the distinctly blackened gleba.

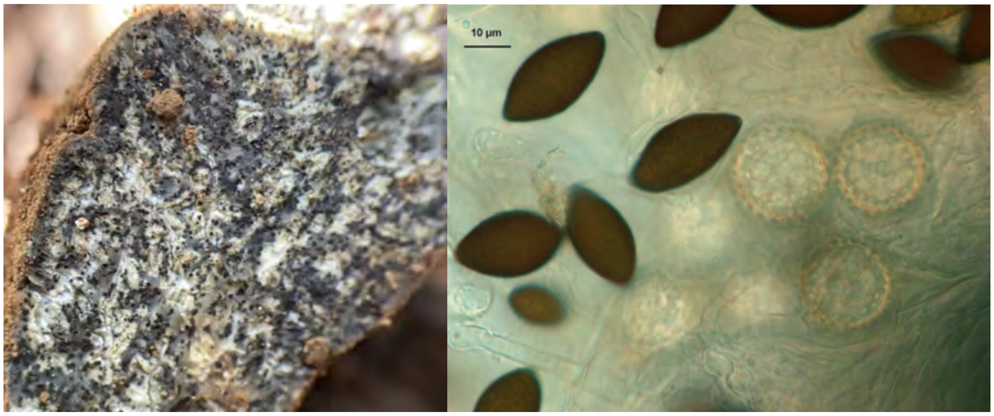

Figure 9: ***Choiromyces*** **truffle and parasite (likely** ***Melanospora*****).**

During a November 2023 survey in mixed conifers, we collected a gray, marbled truffle that superficially seemed to be a *Tuber* species at the time of collection. PacBio sequencing revealed two different fungi present in similar quantities in the DNA extraction, one being *Balsamia latispora* and one an unknown *Micoascus* species. *Balsamia latispora* without any visual evidence of the parasite was fairly abundant in this forest, and comparatively the two forms look distinctly different (Figure 10). The genus *Microascus* (Ascomycota) is more broadly known as a saprotroph and occasional tissue pathogen in humans, and does not form macrofungal fruiting bodies. We did not obtain spore images from this truffle so we are uncertain if both the host *B. latispora* and parasite *Microascus* are reproductively viable.

Figure 10: ***Balsamia latispora*** **with a gray gleba caused by a** ***Microascus*** **fungal infection (left) and an uninfected** ***Balsamia latispora*** **(right).** Image on the left was taken at 20x.

Another instance of parasitism, though less visually distinctive, was a *Tuber candidum* truffle with a white *Entoloma* "mold" that also discolored the typically brown peridium into a strong yellow color (Figure 11). The *T. candidum* was immature, but had begun producing an odor, likely triggered by the infection. Most *Entoloma* are saprotrophic fungi that produce classic "mushroom" sporocarps; however, there are a few known *Entoloma* truffles that are ectomycorrhizal and a handful of mycoparasitic *Entoloma* fungi. *E. parasiticum* parasitizes *Cantharellus* mushrooms, and *E. abortivum* parasitizes mushrooms in the genus *Armillaria*, producing a desirable edible called "shrimp of the woods". However, we are not aware of any known *Entoloma* species that targets *Tuber* truffles, and the DNA sequence of this species did not match any described *Entoloma* collections in GenBank.

Figure 11: Immature *Tuber candidum* that likely started producing aroma due to the co-occurring *Entoloma* parasite which is visible as white patches on the peridium.

Overall diversity across years

We found 84 truffle species (as determined by DNA analysis) between October 2022 and Mar 2024. These were part of 1,236 truffles found in 29 genera (as determined by morphology and DNA analysis) from October 2022 to May 2024. This represents 24% of the estimated truffle species diversity and 56% of the truffle genera abundance in the Pacific Northwest (Trappe et al. 2009). We will continue DNA analysis on truffles collected in May 2024, and we expect to add several new species to our list. Nearly all forays at all sites in all seasons uncovered truffles, a fact indicative of how wide-spread truffles are in CSNM. *Genea, Tuber,* and *Balsamia* were the most abundant truffle genera, accounting for more than half of the truffles we found (Figure 12). Other species were more rare and some were limited to single or double finds. Both conifers and oaks supported a wide diversity of truffle species (28 and 43 species respectively); however there was little overlap between the two habitats and only two species were confirmed to exist in both.

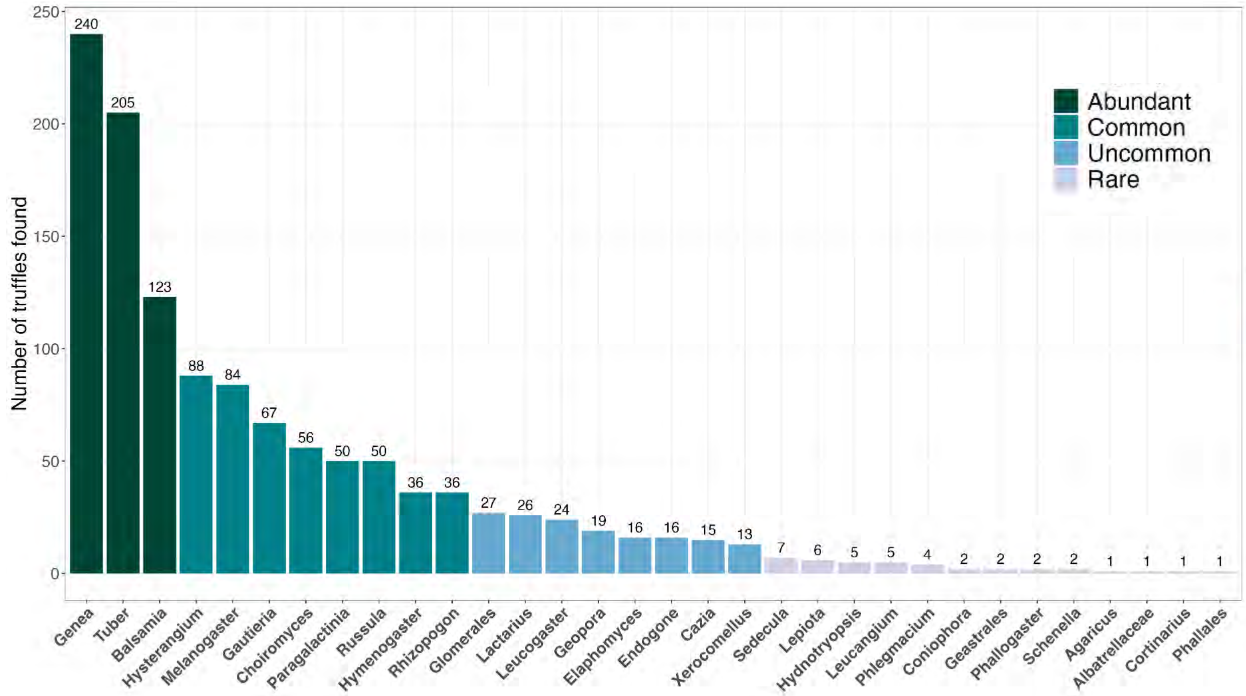

Figure 12: Number of truffles found by genus in two years of sampling. Graph includes truffles found between October 2022 and May 2024. Numbers above each bar indicate the number of truffles found.

A rarefaction curve reveals that we have only begun to uncover the predicted diversity at CSNM. Based on the species accumulation curve, there are an estimated 175 truffle species to be found in CSNM (Fig. 13a). We have found half of the potential truffle diversity in 23 trip-days spread across all seasons (up until March 2024). However, tripling our effort would cover only approximately 75% of the expected truffle diversity (Fig. 13b).

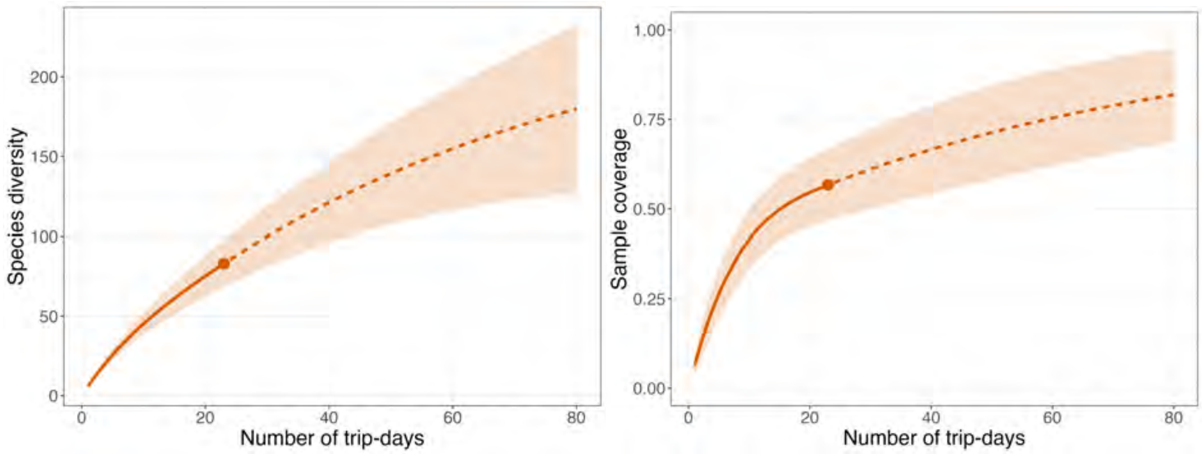

Figure 13: Rarefaction curve illustrating a) extrapolated species diversity and b) sampling completeness. Solid line indicates species accumulation curve to date across both years of sampling. Dotted line indicates extrapolated accumulation. Solid shape indicates error.

Truffle data availability

Fresh material photographs and DNA data for all truffles collected as part of this project are publicly viewable on iNaturalist (see Appendix 1 for list of URLs). Location data have been obscured to within a 500 km^2 cell and observation dates are stated as months rather than days. DNA data will be deposited in GenBank (NCBI) once all sequences from this project have been processed. Herbarium specimens will be deposited at the SOU Herbarium once DNA and morphological analyses are complete.

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About the authors

Hilary Rose Dawson

Hilary Rose is a Ph.D. candidate in the Soil Plant Atmosphere Lab at the University of Oregon. During the tenure of this grant, she also became a postdoctoral research fellow in the Nicotra Lab at the Australian National University. Her research focuses on plant-soil-fungi interactions under climate change. She became interested in truffles through her sister Heather Dawson's work training truffle dogs, including Rye the golden retriever who diversified on his own (with Heather's encouragement) and sparked the sisters into wondering what other truffles were out there. Once Hilary Rose defends and graduates with her Ph.D. in June 2024, she will return to her postdoc in Australia where she studies the carbon and water effects of snow gum (*Eucalyptus pauciflora*) dieback in the Australian Alps. Hilary Rose developed this grant proposal, participated in the autumn and spring fieldwork (when not in the Southern Hemisphere for winter 2023-24), presented research findings, and co-authored the final report.

Heather Dawson

Heather is a Ph.D. student in the Roy and Bohannan Labs at the University of Oregon. During the tenure of this grant she began her degree and was awarded the NSF Graduate Research Fellowship (GRFP) for her work studying the biodiversity of Willamette Valley oak savanna-associated truffle biodiversity. She competes in the dog training trials of Rally and Obedience. Training dogs for truffle research combines her two passions of fungi and dog training. Heather helped develop this grant proposal, led all fieldwork, processed DNA analyses, and co-authored the final report.