Collecting and Documenting Macrofungi for Scientific Study

Los Angeles Mycological Society

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Fungi are an understudied kingdom of organisms distinguished by their absorptive mode of gaining nourishment and, in many species, filamentous growth. Despite their ubiquity in every environment, the diversity of fungal species remains poorly known. This is partly because many fungi only become visible at a macroscopic scale for short periods of time when environmental conditions are favorable for their reproduction. Fungi reproduce through spores, and their spore-bearing structures are called mushrooms. Be mindful that the term "mushroom" is not used exclusively for the gilled Basidiomycete morphology. "Mushroom" is often used to casually refer to a variety of other fungal spore-bearing structures, which include puffballs, corals, brackets, crusts, clubs, saddles, cups, and other Ascomycetes.

Knowledge of fungal species diversity and distributions improves through high-quality record-keeping. This has historically been done through fungaria and the publication of regional species lists, but online platforms - namely iNaturalist and Mushroom Observer - have provided the opportunity for public contribution to biodiversity research databases. This has been useful in the study of fungi, but failing to record key information from the fresh mushroom re-

duces the utility of an observation. This document describes steps for carefully documenting and preserving fungal specimens to maximize the scientific value of your observations and collections (Figure 1). A glossary is provided for words in bold.

Do not fear touching poisonous mushrooms. They are harmless unless ingested, with the exception of unique allergies. Breathing spores - something we do all the time - should not be a concern unless there are excessive amounts in an unventilated space; take regular precautions for those with reduced lung immunity.

Happy collecting!

Cortinarius flavofluorescens nom. prov. (C. virgatus Pk. ?) Greg Wright 2946

Yolva membrancus, light amber, appressed or the margin largely free, margin outline irregular, highest part 1.4-2.2 cm high, lowerst part 1.1-1.9 cm high. Partial veil not evident.

on yellowish "cinnamon" to "ocher" to light "ocher", with white mycelium, broadly convex to broadly concave, not unbonate, silky, 3.7-6.5 cm.

Gills yellowish where lacking mature spores, sienna where mature with spores, close, sinuate at the stem, lammellulae attenuate.

Stem cap-colored to pallid, silky, 4.8-6-7 cm x 8-11-at least 12 mm, equal or with a slightly obliquely marginate bulb, solid. Mycelium white, cottony. Flesh in the cap other-buff, moderately thick; in the stem light other-brown. Odor moderately humusy when the mushroom is whole, rank when the flesh is crushed. Taste rank-humusy, astringent.

Spore print darkish "fulvous"; the mushroom leaving greenish yellow stains on the spore print card.

KOH (10%) staining the cap fulvous, not staining the cap flesh, staining the gills dark fulvous. UV light fluorescing the cap, stem, and cap and stem flesh bright yellow.

Spores (from a deposit) football-shaped, 6.6-8.2 x 4.4-4.9 un, apex not modified, warts prominent. Basidia 4-spored. Gill cystidia absent; gill edges fertile. Caulocystidia absent.

Cap cuticle radial outside the center, narrow to broad filamentous, light greenish yellow in KCH. Cap trama radial, without oleifers. Gill trama parallel, byaline mixed_with_light_greenish_yellow_in_KCH. Clamps present.

Alvin Meadow, near the University of Southern California Idyllwild campus, San Jacinto Mtns., Riverside County, California, undrer manzanita and near <u>Quercus</u> chrysolepis and Pinus ponderosa, 2 fruitings, on dirt, March 12, 1983.

Figure 1, Description from a specimen collected in 1983 by Greg Wright, a founding member of LAMS and one of the most prolific amateur mycologists in Southern California.

1. Equipment

Hardware: Large paper bag or basket, wax paper, tackle box, field data slips or notebook, pencil, digging tool (snow tent stakes are a LAMS tradition), pocket knife, hand lens, camera, scale bar, iNaturalist app.

Chemical reagents (optional): 3-10% aqueous KOH (Potassium Hydroxide)

2. Collection and description

Regarding hunting alone versus hunting with others, recognize that this is not a scavenger hunt. Make the effort for quality notetaking, subtle analysis, and individual reflection. See Additional Resources for ready-to-print field data slips.

You encounter a mushroom; what to do? Take a good look at it (Figure 2)! A high-quality collection is one that is morphologically-representative, carefully handled, and well-described. An individual fungal mycelium can produce many mushrooms in a given area. Collecting multiple mushrooms from the same mycelium counts as one collection. It can be difficult to determine the extent of an individual mycelium, but you can usually assume that mushrooms of the same kind that are within ten feet of each other come from the same fungal individual. If two mushrooms are of the same kind but are found much farther apart, they would count as two different collections. Keep collections wrapped separately to avoid cross-contamination and mix-ups.

It is strongly recommended that you study the technical vocabulary of mycology. Precise and consistent language enables the communication of specific scientific hypotheses. In the scientific recognition of species, technical language is linked to specific hypotheses for taxonomically-informative trait variation. When you identify a mushroom, you are putting forward a scientific hypothesis. In some cases, morphological evidence (macroscopic and microscopic) is enough to defend your hypothesis. However, some species can be indistinguishable with just their visible characters, and a confident identification cannot be made without chemical analysis or DNA sequencing. Do your best, and don't feel that you need to force a name to fit.

2.1.Describe the context for the observation.

2.1.1. Date and location: Record the month, day, and year, as well as the collectors. Describe the surroundings, starting with broader context (names of parks, trails, municipalities), then go into describing the environment and immediate habitat. Is it natural or disturbed? Along a busy trail? If in a forest, describe its composition. Are the trees mostly young or mature?

2.1.2. Ecology: What is the substrate? Is the mushroom growing from wood (may be buried), dung, vegetation, or directly from the soil? Was the substrate burned in a wildfire or burn pit? What other organisms are associated with the mushroom? What trees are nearby? List species if you can, otherwise indicate hardwood or conifer. Is it growing on another organism (an insect, another mushroom)? Are there other fungi in the vicinity?

2.2. Describe macroscopic features. It is important to examine several specimens at different stages of maturity. as the same species can look very different as it develops. It is also necessary to unearth entire mushrooms, ensuring that the base of the stipe (if there is one) is not broken off. Use a digging tool for this. As applicable, note the aspects below and how they vary across young to mature specimens.

2.2.1. Habit: Is the mushroom alone, or are there multiple? Is it common or uncommon? Are they scattered individually, or clustered?

2.2.2. Cap: Shape - Is it conic, broadly convex, plane, or funnel-shaped. Are the cap margins undulating, rolled in, uplifted,



Figure 2. Mycena haematopus photographed in situ, growing from a log.





pleated, or splitting? If there is no cap, then is this a resupinate fungus? Size – Give ranges for length, width, and thickness. Color – Use your intuition and be creative. How does color vary across the cap surface? Is it hygrophanous? Reactions – Does it stain a different color when bruised or cut? Note color changes with KOH on the interior and exterior flesh. Texture – Is it dry or slimy? Is it cracked, smooth, warty, fibrillose, pitted, velvety, or squamulose? Does it have patches (remnants of a universal veil)? Are there remnants of a partial veil on the margins? Describe their structure.

2.2.3. Stipe: Position – Is it centered, off-center, lateral, or is the mushroom sessile? Size – Record length and thickness. Texture – Is it smooth, squamulose, reticulated, dotted, fibrillose, or pruinose? How does texture vary up and down the length of the stipe? Shape and context – Is it solid, hollow, or stuffed? Brittle or flexible? Is it cylindrical, tapering, or bulbous? Is the stipe base caespitose? Is the base fuzzy, with rhizoids, or with a deep tap root? Is there a partial veil / annulus ? – Describe its position and structure. Skirt-like, upturned, double-edged, or web-like? Is there a volva? – Is it sheath-like, saccate, scaly, or with concentric rings? Reactions – Does it stain a different color or release a liquid substance if sliced? Does the liquid change color as it is exposed to the air? Note KOH color changes at different positions on the stipe, on the interior and exterior flesh.

2.2.4. Hymenophore: Does the mushroom have a fertile sur-

face of gills, teeth, pores, tubes, ridges, or is it smooth? Does it

stain a different color or release a liquid substance if sliced? If it has pores, how many pores per mm? If it has gills, how are they attached to the stipe – broadly, narrowly, or not at all? What is their spacing? Are the gill margins even, serrated, wavy, or scalloped? Are the gill margins the same color as the gill faces? Note that gill color is a different feature from spore color, which can obscure the gill color as the spores mature.

2.2.5. Odor: Is it bleachy, mealy, fishy, spermatic, like anise, almonds, citrus, cinnamon, cucumbers, coal tar, chocolate, green corn, sweaty socks, or a swimming pool?

2.2.6. Taste: Tasting a mushroom is generally safe, but it's advisable to only taste a mushroom for which you already have a rough sense of the identity and are confident that tasting it will be informative. Some mushrooms are deadly poisonous when swallowed even in small amounts, with many belonging to the genera Amanita and Galerina. Learn to recognize the features of these genera before you start tasting mushrooms out in the field. Be sure to spit it out after. Some reported flavors are sweet, spicy, acrid, bitter, and mild. If the odor is unpleasant, the taste will likely also be unpleasant, so don't punish yourself.

2.2.7. Spore color: This is best observed from a deposit of mature spores. Sometimes this can be observed in situ, when a mushroom has deposited its spores onto its stipe, partial veil, or another mushroom. When this is not the case, you can take the collection to make a spore print. If the mushroom has a different hymenophore type (a puffball, stinkhorn, jelly) find different ways to observe mature spore deposits (if you can).

2.3. Describe microscopic features (optional):

If you have a microscope, report the shapes and sizes of mature spores, cystidia, and the other taxonomically-informative features for the group you are investigating. See Additional Resources.

3. Photography

If multiple mushrooms are found, arrange them to capture in a single photo all the growth stages and taxonomically important features represented in your field data. Photograph from above, from below, from the side, and zoomed out to show the original habitat and possible ecological relations (Figure 3; more details in Additional Resources). Include a scale bar or another object of standard size in at least one of the photos. You can use the FunDis field data slip for this (see Additional Resources).

4. Data upload

Open iNaturalist, and upload the photos along with the location. See Additional Resources for instructions on using iNaturalist. In the comments for the observation comments, transcribe the notes from your notebook or data slips. Once you have these down, you are ready to post it! Open MushroomObserver and post your observation there too, ensuring you add the iNaturalist accession, which is the unique nine-digit number at the end of the URL. Go back to iNaturalist and edit your observation to add the MushroomObserver accession number.

5. Specimen preservation

Food dehydrators are best for this, but any set-up for warm air flow will work. Set the drying temperature between 100° and 115° F, and mind the timing as it depends on the size of the mushroom. Small mushrooms can take two hours, larger mushrooms can take up to two days. Heavy, fleshy mushrooms should be cross-sectioned, medium-sized mushrooms may be left intact, and delicate mushrooms can be loosely wrapped in wax paper as they can get blown away. Once dried to a crisp, they will look very different from their fresh form. This is why taking photos of fresh mushrooms in situ is important. Now your specimen is desiccated enough to be stored in a tightly sealed plastic bag, ideally with a silica gel packet to absorb any additional moisture (Figure 4). Sealed specimens with even small amounts of moisture are prone to growing mold. Include the spore print, field notes, and database accession numbers with the final collection. To prevent your specimens from being pulverized by insects, freeze your dried collections for 1-2 weeks to kill pests.



Figure 4. Dried specimen sealed in a bag with a silica packet, spore print, field notes, and digital accession numbers.

Glossary

Annulus – Alternative name for partial veil. From Latin for "ring-shaped."

Ascomycota – The largest phylum in the fungal kingdom, distinguished by the production of sexual spores within cannon-like cells (asci). Species in this group are called Ascomycetes. These include morels, cups, discs, saddles, boats, flasks, truffles, earth tongues, and mildews, as well as most yeasts and lichen-forming fungi.

Basidiomycota – One of the two major phyla of macroscopic fungi (the other being Ascomycota), distinguished by their production of sexual spores on the ends of club-like cells (basidia). Species in this group are called Basidiomycetes. These include gilled mushrooms, boletes, puffballs, brackets, jellies, corals, stinkhorns, earthstars, crusts, and rusts.

Caespitose – Describes mushrooms growing in clusters of two or more, with the stipes fused at the base.

Cystidium – pl. cystidia. Large sterile cells with diverse forms that can be found in the hymenophores of some Basidiomycetes.

Fungarium – pl. fungaria. A scientific collection of preserved and documented fungal specimens held by institutions or individuals for the purpose of research.

Hygrophanous – Describes a mushroom cap with zonate coloration that results from variable rate of water loss. From Greek for "shown by water."

Hymenophore – The specific structural arrangement for a surface of spore-bearing cells on a mushroom, including gills, pores, teeth, wrinkles, and ridges.

Mycelium – pl. mycelia. The vegetative structure ("body") in most mushroom-forming fungi. Mycelia move in the environment through the branching growth of filamentous cells (hyphae).

Partial veil – A lasting or evanescent layer of tissue that covers the early-developing hymenophore in some stipitate Basidiomycetes (mostly gilled mushrooms); i.e., partially covers the surface of the mushroom. As the cap expands, the partial veil is torn from the margins and can remain on the stipe as a ring (annulus).

Resupinate – Describes a mushroom with a hymenophore that lacks a cap, growing instead as a crust-like sheet, often on the underside of logs. From Latin for "lying back."

Rhizoids – Also rhizomorphs. Thick, root-like aggregations of hyphae.

Sessile – Describes a mushroom without a stipe (e.g., Crepidotus spp.). From Latin for "seated."

Spore print – The deposit of spores left by a mushroom. This can be observed in the field or made at home by placing a cap on top of a chosen surface, usually white or black paper, but ideally on a glass microscope slide or aluminum foil for preservation. Fully encase the cap with a solid covering - such as a drinking glass or bowl - to prevent external airflow so that the spores drop down and are not blown away. Ensure there is no obstruction of the hymenophore - this could be a stipe, or veil (which indicates immaturity). Be mindful that spores can be dark or light, so the visibility of a spore deposit is affected by the background color of the surface you choose (glass gives you more options).

Squamulose – Describes a surface "with small scales" (from Latin).

 ${\bf Stipe-} The \ stalk \ of \ a \ mushroom. \ See \ sessile.$

Stipitate - Describes a mushroom as having a stipe.

Substrate – The specific medium in which a mycelium grows and derives nourishment.

Taxonomically-informative – Describes a biological feature that has been scientifically investigated for its utility in distinguishing species.

Universal veil – A lasting or evanescent layer of tissue that covers an entire early-developing mushroom, such that it resembles an egg in its youngest stages; i.e., universally covers the surface of the mushroom. As the mushroom expands, the egg-like universal veil is torn at the margins and can remain at the base of the stipe as a cup-shaped remnant tissue (volva) and on the cap as patches or warts.

Volva – Cup-shaped remnant tissue of a universal veil that remains at the stipe base in some mushrooms, namely Amanita and Vovariella spp.

ADDITIONAL RESOURCES

Recording field data:

Field data slips from Fungal Diversity Survey: https://fundis.org/sequence/collect-dry/field-data-slips

Using biodiversity databases: https://fundis.org/protocol/choose-a-platform

Photography best practices: https://fundis.org/get-started/photograph

Fungal morphology:

Pictorial key to major groups. "The Wheels" from Fungi of Temperate Europe (Laessoe and Petersen, 2019): <u>http://www.mycokey.com/Downloads/FungiOfTemperateEurope_Wheels.pdf</u>

Kellerman's Mycological Glossary (1905): https://www.mykoweb.com/systematics/literature/Mycological%20Glossary%20Kellerman.pdf

Fungal microscopy:

Pictorial key to major groups. "The Wheels" from Fungi of Temperate Europe (Laessoe and Petersen, 2019): <u>http://www.mycokey.com/Downloads/FungiOfTemperateEurope_Wheels.pdf</u>

Collecting and documenting procedures:

Details on drying from Fungal Diversity Survey: <u>https://fundis.org/sequence/collect-dry/</u><u>dry-your-specimens</u>

"Collecting and Describing Macrofungi" (D. Lodge, et al., 2004): <u>https://www.fs.usda.gov/research/tree-search/7111</u>