RECOMMENDATIONS FOR COLLECTING MUSHROOMS FOR SCIENTIFIC STUDY[1]

Introduction

Mushrooms (agarics, boletes, and other macrofungi) and other fleshy fungi are an important and conspicuous part of most terrestrial ecosystems. Although the collection techniques employed for collecting mushrooms are not complex, they differ considerably from those used to collect plants. Pressing mushrooms will destroy their value as scientific specimens and often render a taxonomic determination impossible. In the following paragraphs, a summary of techniques for gathering, documenting and preserving mushroom specimens is given. Although these may seem at times idiosyncratic and tedious, they are essential for providing useful specimens for later study.

Collecting Specimens

Adequate notes are essential to make complete and valuable specimens. When collecting a mushroom, take note of its habitat and substrate. Common substrates for mushrooms include wood, soil or leaf litter. If growing on wood, note whether the wood is dead or living. In the latter cases, is the mushroom growing on the bark, or has the bark disappeared exposing bare wood? The habitat description should include the kind of trees growing in the area, because many fleshy fungi will associate with particular types of tree roots, or they may be substrate dependent.

When collecting a specimen from soil, be sure to dig down deep enough to remove the whole specimen. Otherwise, remove part of the substrate with the specimen still attached. Try to collect young as well as mature individuals; several stages of development may be necessary for identification purposes. Furthermore, as many individuals as possible of each "taxon" should be collected.

Once collected, the mushroom must be handled carefully. Specimens should be wrapped in aluminum foil or waxed paper (never plastic!) for protection and moisture retention. A sheet of foil or paper is torn so that a collection can be rolled up inside with the ends twisted closed. The wrapped mushroom can then be placed in a sturdy basket, box, or bag, and carried to the laboratory or base camp.

Never pile mushrooms so high atop one another that delicate structures are broken. Small tin boxes, rigid plastic boxes, or fishing tackle boxes are useful for collecting and transporting small or fragile specimens.

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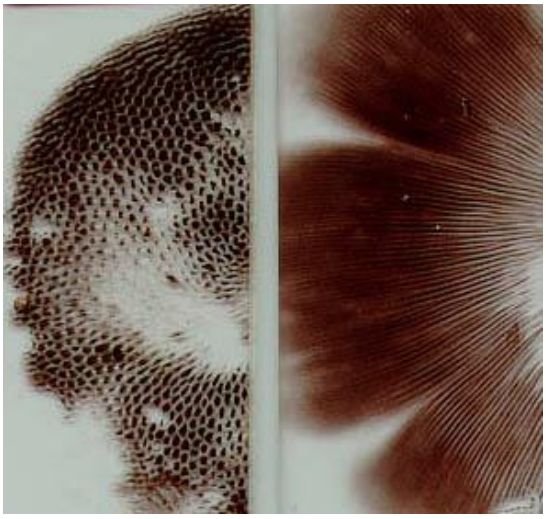
[1] Adapted from: Halling, R. E. 1996. Recommendations for collecting mushrooms for scientific study. Pp. 135–141. In: Alexiades, M. N. and J. W. Sheldon (eds.), *Selected Guidelines for Ethnobotanical Research: A Field Manual*. The New York Botanical Garden Press, Bronx.

Preparing Specimens

You should begin working on your collections as soon as possible after returning from the field. Many agarics shrivel or fade within a few hours of collection, even when wrapped in foil or paper. Prevention from overheating and waterlogging during transport to the lab will aid in maintaining the specimens in as fresh a state as possible. It is best if collecting can be done in the morning with the remaining daylight or afternoon allocated to work on collections. Extensive sampling protocols are documented in Mueller et al. (2004) for major inventory work.



1. Making Spore Prints

The first thing to do is prepare spore prints. As the name indicates, spore prints are pieces of paper covered with mushroom spore deposits. These are invaluable for identifying many specimens. To make a spore print, first remove the stipe, if present, and place the gill or pore surface down on a white (**never** black or colored) piece of paper. Cover the whole mushroom with some type of enclosed, moisture resistant container (e.g., a drinking glass or jar or plastic sandwich bag; even wrapping in wax paper can be sufficient). If there are only one or two mushrooms of a given collection, it is better to cut a hole in the paper for the stipe rather than removing it.

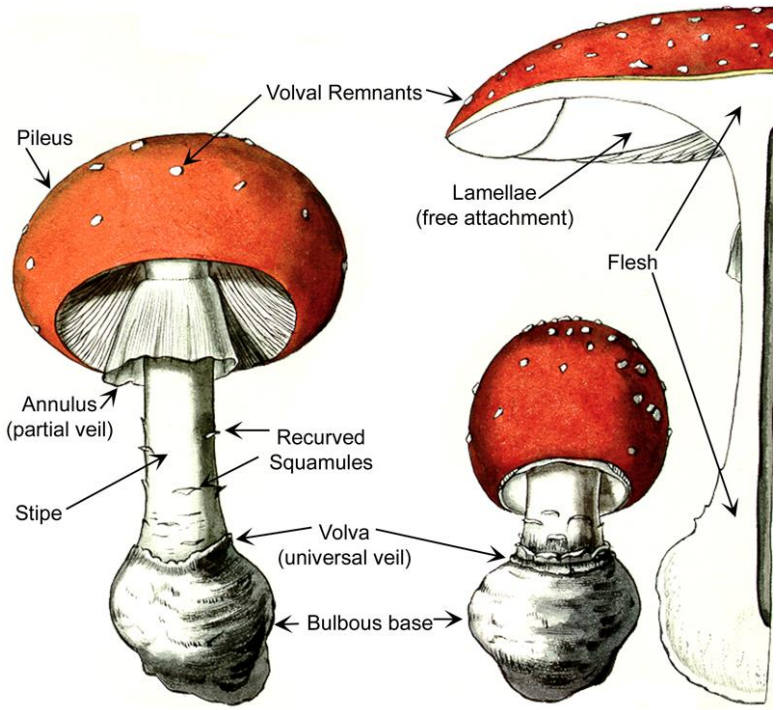
After one to several hours (sometimes overnight), a white or colored spore print should result (old or immature mushrooms may not give a spore print). You should note the color of the fresh spore print, then fold and dry it with the specimen. In cases where return to the laboratory necessitates travel from one extreme elevation to another, it is better to attempt the spore deposits in the field. In our experience, agarics collected at high elevations and returned to low elevations will not sporulate. In these instances, however, one can facilitate spore deposits by placing the enclosed preparations in the bottom of the basket or box, even while still collecting, with an attendant note explaining to which collection the preparation belongs.

2. Taking Photographs

There is much to be said for taking photographs of freshly collected material. Benefits include showing the specimens in their natural habitat in natural daylight. In addition, a "natural" scene can be arranged artificially from native plant materials on return to the laboratory or base camp. Another alternative is to arrange a flat or upright, neutral background against which the specimens can be photographed. Whichever alternative is employed, the photographer should ensure that all possible salient features of the mushrooms be observed. (two images below courtesy G. Mueller, Field Museum)

3. Making Notes

While the spore prints are going on, you can begin to take notes on your collections. Describing the fresh characteristics of macrofungi is of paramount importance to preparing a valuable specimen because many important and diagnostic features will disappear when the specimens are dry. Most importantly, color, shape, and size will change after drying, and the odor and/or taste if present will no longer be evident. Notes on the fresh appearance should include any descriptive information that will not be evident after drying. The quality of light for color interpretation is important. Natural daylight is best, but lacking that, there are lamps which feature full spectrum bulbs, LED's or tubes that approximate daylight. Standard fluorescent tubes are notorious for lacking red wave lengths of light.



Agaric (or bolete) sporocarps (basidiomata) can be divided into 3 parts: the **pileus** or **cap**, including the interior flesh, the **hymenophore** (lamellae or tubes/pores), and the **stipe** (including the interior flesh). Other features may, or may not, be present. These might include a **universal veil** or **partial veil**; both are discussed in more detail below. A source for more in depth information on this topic is available from Largent (1977). Below is a summary of important features to note.

The ***Glossary***, defines terms appropriate to use in writing notes.

Pileus:
* Size: range of diameter
* Shape: viewed and described as if sectioned longitudinally: Shapes include convex, concave, bell-shaped, mammillate.
* Color: center vs. margin; surface ornamentation vs. background; does it change or discolor with age or when bruised and handled?
* Texture and ornamentation: e.g. hairy, smooth, scaly, fibrous, fragile and membranous, etc.; slimy, dry, moist, sticky, etc. Is the margin (outer edge) of pileus different or not?
* Flesh: overall thickness, color; does it discolor when exposed to the air? Sometimes discoloration is rapid (a quick, cut and look is useful) and localized. Is a latex or juice produced?
* Odor and taste: Never swallow a mushroom, masticate briefly, spit out, and describe if distinctive or not. Aromas can be deceiving and culturally idiosyncratic. Squeezing or rubbing a specimen is sometimes helpful to release or accentuate an odor.

Hymenophore:
* Type: lamellae or tubes/pores.
* Color: Note changes between young and old or as a result of injuries and bruises. If injured, is there a juice or latex exuded, is it colored, to what color does it change slowly or rapidly, does it stain surrounding tissues some other color?
* Attachment to stipe (when viewed in a longitudinal section from pileus down through stipe): free, adnexed, adnate, decurrent; again ranges or intermediates may exist—use ranges not absolutes.
* Edge: Note color, if different from the sides, and whether it is smooth or uneven in some manner (wavy, serrate, fimbriate).

Stipe:
* Size: Include range of length and width.
* Shape: These include, equal, clavate, bulbous, tapering downward.
* Attachment to pileus: e.g. central, eccentric, lateral, absent.
* Color: when young and old, above and below, when handled or bruised.
* Texture and ornamentation (as in pileus): when young and old, above and below. Note basal mycelium and its color, abundance.
* Flesh: note same as mentioned above in pileus. Upper portion may behave differently than lower portion.

Universal veil:
The universal veil is formed of tissue that completely surrounds the immature button stage of an agaric or bolete. It ruptures with stipe elongation and may leave remnants on pileus surface and/or margin, stipe base and/or surface; it may be persistent or ephemeral; it may be represented by warts on pileus and warts/concentric rings around stipe base and on stipe surface or flap-like patches on pileus and a cup-like structure around the stipe base. This structure is referred to as a volva. As with other features, note colors and color changes.

Partial veil:
The partial veil is formed of tissue that extends from the pileus margin to the stipe and thus covers the hymenophore before maturity. It ruptures to form a ring around stipe or a fringe of tissue at the pileus margin; intermediates may occur. This ring is referred to as an annulus. Note persistence, location, attached or movable, as well as color/color changes, surface ornamentation, etc.

You can expect to spend about 15–30 minutes per collection preparing spore prints and notes; a bit more time is required if photographs are taken.

Material for DNA analyses
Material collected as part of an inventory should be preserved as potential sources of DNA for lab-based studies. While this can be sometimes accomplished directly from dried material (see below), it can be best done by submerging carefully excised pieces of a mushroom in a fixative solution that has been dispensed in cryo-vials or microcentrifuge tubes. There are several commonly used buffer solutions that prevent degradation of DNA during long-term storage of pieces of sporocarps. The two most commonly used solutions are (1) 2× CTAB buffer [100mM Tris-HCl pH 8, 1.4M NaCl, 20mM EDTA, 2% CTAB (hexadecyltrimethylammonium bromide)], and (2) a supersaturated solution of DMSO [20% DMSO, 250 mM EDTA, and saturated NaCl]. Also, small pieces of fresh tissue can be placed in sealed vials of activated silica gel. Dried, and chemically untreated herbarium specimens can be used as well.

Drying Specimens (two images below courtesy G. Mueller, Field Museum)

When you have finished preparing spore prints and have taken notes and/or photographs, the specimens can be dried. This is a very critical step and can make the difference between a valuable scientific specimen and a useless one. Dryers used for vascular plant specimens are usually too hot for drying mushrooms. Methods for drying mushrooms vary from collector to collector and place to place. The feature they all have in common is that they utilize some kind of fine screen shelving, for suspending the mushrooms over a dry heat source. The heat source can be an electric heater, a tent heater, hot plate, light bulbs, kerosene stove (←) or lantern, etc. In any case, heat should be directed from the source upward via a chimney effect circulating around the specimens with moist air escaping above. Commercially available fruit & vegetable dehydrators work very well. Also, it is critical that the specimens are dried slowly (not cooked) at a temperature not to exceed 65ºC. If DNA extraction is to be attempted from the dry material, a lower drying temperature of 42–55ºC is recommended. Specimens confined in an oven-like space will bake and be useless. They must remain on the dryer at the appropriate temperature until they are crisp and brittle (but not baked or burned). NEVER, NEVER place mushroom specimens between newspaper sheets and in a plant press.

Once dry, specimens must be kept dry or they will re-hydrate and then there is the possibility that they will become moldy and worthless. Removal of freshly dried specimens directly from the dryer to a plastic bag (below↓ image courtesy G. Mueller, Field Museum) large enough to accommodate the specimens when sealed will help insure that the specimens remain dry. In extremely humid environments, a small amount of desiccant can be added to safeguard against re-hydration and mold growth. Delicate and fragile specimens can be dried in closed containers containing activated silica gel or other desiccant.

General Reminders:

* Collect as many individuals as possible; including a range from young (immature) to old (mature or over mature). Four to five individuals in a single collection will make one excellent specimen.

* Collect part of substrate (small portion of soil, leaf litter, wood, etc.) and note habitat, plant (tree or shrub) association. Picking a specimen while holding onto the stipe can destroy characters or leave part of the fungus behind. Use a knife or some other stout tool to dig the mushroom from the soil or cut it from a log or wood.

* In preparing descriptions of macroscopic features, describe what you see based on your experience. If in doubt, a sketch is extremely helpful. If at all possible, cut 2–3 sporocarps lengthwise in half before drying. This is essential anyway to note context color and any oxidation reactions. If sporocarps are very fleshy, this should be done for all collected in order to promote drying.

Bibliography

Largent, D.L. 1977. How to identify mushrooms to genus I: Macroscopic features. Mad River Press, Eureka. 86 pp.

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