2006 Summer Workshop in Fungal Biology for High School Teachers
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Guidelines for collecting and identifying macrofungi (basidiomycetes)

I. In the field:

Gear:
- basket
- waxed paper or paper bags, collecting box
- knife
- notebook and pencil
- insect repellent
- (camera)
- (GPS device)

Before picking a mushroom, note where and how it is growing:
What is the substrate (soil, leaf litter, wood, living plants, other mushrooms, dung)?
- If the substrate is soil, the mushroom may be mycorrhizal. Note the plant species in the immediate area, especially trees and other woody plants.
- If the substrate is leaf litter, note if it is coniferous or broad-leaf in origin.
- If the substrate is wood, note if it is coniferous or broad-leaf in origin. Is the bark present, or is the wood decorticated? Can you tell if the wood has been decayed by a brown rot or white rot mechanism (white rot leaves the substrate bleached in appearance, with a stringy consistency, wherease brown rot leave the substrate brown, with a crumbly consistency; these decay modes differ in the chemical components of wood that are degraded).
- If the substrate is a living plant, note the plant species and the position of the mushroom(s).
- If the substrate is another mushroom, can you tell what kind of mushroom it is? Collect the host along with the parasite.

How are the mushrooms arranged?
- Note if they are solitary, scattered, clustered in overlapping groups, or in "troops".
- Do several mushrooms arise from a single point (in which case they are "cespitose")?
- If the mushrooms are on soild, do they form a fairy ring?

When you are ready to make the collection:
- Collect the entire fruiting body. Using your knife or a trowel, dig up the base of the mushroom; do not cut the stipe.
- Are rhizomorphs, sclerotia, or a subiculum (a mat made up of hyphae) present? If so, collect those as well.
- If possible, collect multiple fruiting bodies, including different developmental stages. But make sure that all fruiting bodies are from the same individual (to the extent that this is possible!).
- Remove excess dirt and debris before packaging to bring back to the lab.
- (This may be a good time to take a picture.)
- Note the presence and color of latex or other exudates from cut or uncut surfaces.
- Note if cut or bruised surfaces change color (this may take a few minutes).
- Note odor, if any.
- Pack mushrooms in waxed paper, paper bags, or boxes, not in plastic bags. Do not mix collections. Protect from excessive heat.

II. In the lab:

Equipment:
- identification literature
- microscopes (10x, 40x, 100x)
- immersion oil
- lens paper
- stage micrometer
- slides, coverslips
- white paper for spore prints
- agar media
- scalpels or razor blades
- forceps
- alcohol lamp
- vaseline
- silica gel
- kimwipes
- markers
- label tape
- parafilm

Identification of fungi is not a trivial task! Identification of many species requires use of specialized literature (e.g., monographs), and to confirm an identification it is often necessary to make comparisons with type specimens or other authentic material (specimens collected and identified by other workers). We will use several general guides, including *Mushrooms of Northeastern North America* by A. E. Blanchette, A. R. Blanchette and D. W. Fisher, *Mushrooms of North America* by Roger Phillips, and the *Fungi of Switzerland* series by Breitenbach and Kränzlin. Although they are not comprehensive, these references should permit identification of most of the species we encounter (to genus level, at least). There is a huge amount of jargon used to describe the macro- and microscopic features of fungi. The works cited have glossaries. For additional explanations of terms and illustrations, I recommend that you refer to *How to Identify Mushrooms to Genus I: Macroscopic Features*, by D. L. Largent, and *How to Identify Mushrooms to Genus III: Microscopic Features,* by D. L. Largent, D. Johnson, and R. Watling.

Observe the macroscopic features of the fruiting body:
The following incomplete list is intended to introduce some of the major characters used to identify fungi and their terminology. See Largent for illustrations.
Fruiting body habit:
- pileate-stipitate (with pileus [cap] and stipe [stalk])
- pileate-sessile (with pileus, but no stipe)
- resupinate (flattened, crustlike; ="corticioid" if the hymenophore is smooth)
- coralloid (branched, like a candelabra; ="ramarioid")
- clavarioid (clublike, unbranched)
- gasteroid (with spores produced internally; e.g., puffballs, stinkhorns, bird's nest fungi)

Hymenophore (the spore-producing surfaces) configuration:
- gills (=lamellae; "agaricoid")
- teeth/spines ("hydnoid")
- pores/tubes ("poroid")

- smooth
- wrinkled ("cantharelloid"/"merulioid")

Attachment of the stipe to the pileus (pileate-stipitate forms only)
- central/excentric/lateral

Attachment of the stipe to the substrate
- presence/absence of basal tomentum, radicating (rootlike) stipe

Presence/absence of veils (pileate-stipitate forms only)
- presence/absence of partial veil, connecting stipe and pileus margin, often leaving an annulus (ring) on the stipe
- presence/absence of universal vail, enclosing entire fruiting body, often leaving a volva at the base of the stipe, and remnants on the pileus surface (may appear as "warts")

Pileus
- shape
- margin
- texture

Attachment of lamellae to the stipe
- free/attached
- if attached, decurrent, notched, adnate

Spacing of lamellae
- distant, subdistant, close, crowded

Other macroscopic characters:
- consistency/texture
- presence/absence of latex (color)
- odor
- spore print color (see below)

<u>Observe the microscopic features of the fruiting body:</u>

Again, this is an incomplete list that introduces some of the important sources of characters for identification. Use compound microscope. Mount material in water or KOH, and Melzer's reagent. The latter can reveal a bluing reaction, called an amyloid response. See Largent, Johnson, and Watling for illustrations

Spores:
- size
- shape
- ornamentation
- number per basidium
- reactions in Melzer's or other stains

Cystidia (sterile cells on the surface of the fruiting body):
- location (cheilocystidia=on gill edge; pleurocystidia=on gill face; pileocystidia=on pileus surface; caulocystidia=on stipe)
- shape
- staining, presence of refractive or oily-looking contents (gloeocystidia)

Pileipellis (cells covering the surface of the pileus):
- shape
- orientation

Hyphal construction (presence of specialized hyphae in the context of the fruiting body):
- generative hyphae
- gloeoplerous hyphae
- binding hyphae

- skeletal hyphae

Hymenophoral trama (context of the hymenophore):
- orientation of hyphae in the trama

Clamp connections (visible at the septae of the hyphae)
- presence/absence/frequency

Basidia (cells that produce spores)
- shape
- number of sterigmata (stalks on which spores are produced)
- presence/absence of a basal clamp connection

Make a spore print:
- Place a pileus with the hymenophore facing down over a sheet of white paper. You may cover this with a beaker to limit drying and prevent spores from being dispersed.
- Leave overnight to collect spores en masse.

Make a culture by the spore-drop method:
- Place a small dab of vaseline on the inside of the lid of a petri plate of agar media.
- Attach a small portion of the hymenophore to the vaseline.
- Turn the lid about 30 degrees every 10-20 minutes until you have gone all the way around the plate. If the fruiting body is fertile, it should produce enough spores to provide a good inoculum.
- Remove the fragment of hymenophore, seal the plate with parafilm.
- Observe daily with dissecting microscope for germination; subculture new mycelia.

Make a culture by the tissue-explant method:
- Tear (do not cut) the fruiting body open.
- Dip forceps in ethanol and flame, then allow to cool briefly.
- Pluck out a small amount of hyphae from the interior of the fruiting body and place directly on agar media.
- Check daily for hyphae growing out from the inoculum.
- When mycelium spreads onto plate, subculture to a new plate.

Dry a portion of the fruiting body using silica gel for DNA isolation:
- Wrap a small (1cm) piece of the fruiting body in a kimwipe, label with tape and place into container with excess of silica gel.
- Should dry completely overnight.

Dry the rest of the collection for deposition in the herbarium:
- Place the fruiting bodies in the food dryer with a paper label and leave overnight. Larger fruiting bodies make take longer to dry, and may need to be cut into sections.