Chapter 12

Collecting the neglected kingdom: Guidelines for the field mycologist with emphasis on the larger fungi

by

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Abstract

Guidelines are provided for collecting a group of organisms that has often been overlooked in earlier inventories: the kingdom Fungi and other groups that are traditionally collected by mycologists such as slime molds. After a short introduction on fungi and the feasibility of an 'all fungal taxa' inventory, the authors divide the fungi in six 'practical' groups that require specific approaches: slime molds, lichens, parasitic fungi of plants and animals, larger mushrooms, microscopic fungi. Various topics are discussed in relation to three chronological stages (before, during and after the collecting trip) and include various aspects such as equipment, photography, barcoding, documenting, storing collections, macrochemical reactions, preparation of spore deposits, humid chamber technique, ... At the end of the paper the reader will find a selection of various important web references for the field mycologist interested in various fungal groups and their taxonomic aspects.

Key words: ATBI, slime molds, ascomycetes, basidiomycetes, lichens, inventory

1. Introduction: the neglected kingdom

Mushrooms are often looked upon as some kind of odd vegetable but thanks to recent technological progress it has now been firmly established that mushrooms belong to a very diverse group of organisms we call 'fungi'. Fungi are neither plants nor animals, but represent a separate kingdom of living organisms: the 'fungal kingdom' or the 'mycota'. Because they share several biochemical and cytological features with the animal kingdom fungi are more closely related to animals than to plants (*e.g.* their cell walls contains chitin – a compound that also occurs in animals but not in plants, animals and fungi store their energy as 'glycogen', not in the form of 'amylum' as plants do, animals and fungi are both heterotrophic groups unlike plants which are autotrophic, …).

Although mycology has traditionally been taught as part of a botany course, fungi are studied by mycologists, not by botanists. Unfortunately, professional mycologists that are focusing on systematics and taxonomy of fungi are very few and their number is still declining (*e.g.* Buyck, 1999). As a result, we still know very little about the fungal diversity on this planet. Mycologists only recently realised that less than 5% of an estimated minimum of 1.5 million fungal species that inhabit this planet have been officially described (Hawksworth, 2001) and this not only concerns many microscopical or cryptic groups with simple morphologies, but also many, often even common or traditionally consumed, larger mushrooms.

The fact that we still know so little about the fungi has of course important consequences when talking about an "all taxa biodiversity inventory". The negative aspects of our general ignorance about fungi can be easily understood when being confronted with the almost complete absence of comprehensive or easily accessible identification literature for fungi in most parts of the world, as well as with the countless name changes for fungal species due to the existing confusion over classification issues (especially due to the recent repercussions of molecular evidence). One positive aspect of the recent interest in fungi, however, is that governmental and environmental decision-makers are becoming more and more aware of the important role fungi are playing for the conservation and the survival of all kinds of habitats and for that of nearly all other groups of organisms. Whereas past inventories focused principally on plant and animal biodiversity, we finally witness a growing awareness that the fungal component needs to be included as well.

2. How realistic is an 'all fungal taxa' inventory?

On top of our general ignorance concerning the amplitude of fungal diversity, mycologists are also confronted with other problems. One of the major problems for an inventory is the fact that most groups of fungi (the few exceptions include lichenized fungi for example) are not the easiest organisms to collect and to study. Indeed, many groups – particularly the larger fungi – are invisible during most of their life remaining completely hidden inside a substrate (whether this is inside living host tissue, in soil or in dead wood or even inside other fungi). When

making inventories, mycologists are usually limited to those groups that are visible above ground. For most of the larger ascomycetes and basidiomycetes this is at the moment they reproduce sexually. However, the irregularity of appearance of these sexually reproductive structures (called 'mushrooms') and the strong dependence of the latter on sufficient precipitation can make it extremely difficult to realize a good inventory (see caption below).

Above-ground visible diversity is very different from the 'actual' below-ground or host-related diversity as *e.g.* shown by a study in a Swiss forest (Straatsma *et al.*, 2001): species richness, abundance and phenology of fungal fruit bodies over 21 years in a Swiss forest plot.
Permanent plots of 1500 m² in Swiss spruce forest:

- 21 successive years give a total of 408 species of larger fungi;
- from 18 to 194 different species recovered per year;
- only 8 species (2%) fruited constantly every year;
- still 19 (5%) previously undiscovered species were found in the last year.

A second problem with inventories is usually manpower. As professional mycologists capable of identifying fungi are very few and mostly swamped by other obligations, it is in our opinion absolutely imperative to involve also the members of mycological and lichenological societies that exist in many countries. Especially for the larger fungi and for some of the other groups such as slime molds or lichenized fungi, these societies can supply the necessary expertise but may often need some guidance on particular technical or methodological requirements of a scientific approach (links to most of the important mycological societies in the world can be found at the European Mycological Association website: http://www.euromould.org).

3. What groups of organisms are composing the Fungi? … and are they all equally suitable for an ATBI?

The most recent proposal for a scientifically sound classification of the Fungi was published by Hibbett *et al.* (2007). However, in the context of an ATBI, it is more appropriate to consider practical groups that require more or less similar approaches in the field. The groups mentioned below therefore do not correspond to some actual classification of the Fungi; they represent the most common practical approaches for Fungi in mycological inventories.

3.1. Slime molds or 'myxomycetes'

Myxomycetes are no longer considered to be part of the Fungal kingdom and are now classified among the protists. Nevertheless, they correspond to a group of organisms that has traditionally been studied by mycologists. The expertise on this group remains therefore with some rare professional mycologists but is especially passed on within the various mycological societies.

The number of species is relatively low (ca 1500 worldwide). Slime molds form a natural group within Kingdom Protista and inventories mostly concern the true myxomycetes (Eumycetozoa). These myxomycetes can be considered an 'easy' group in inventories because:

- Collecting is easy once you know what to look for, but a hand lens (10x-20x) is absolutely necessary. In the absence of fruitbodies, one can collect substrates that are later kept under humid conditions to favor development (this technique is especially valuable for recovering very tiny or rare species). For more details see 'humid chamber technique' below.
- Literature resources / identification guides are very good on a world scale.
- Description requirements in the field are not or very rarely required.
- Preservation is very easy (simply air drying).
- Identification requires nevertheless a good microscope.
- There are plenty of web resources.

3.2. Lichenized fungi or 'lichens'

Lichens are dual, mutualistic, symbiotic organisms: a lichen thallus consists mainly of a fungus (generally an ascomycete, rarely a basidiomycete or a zygomycete) that harbors a species of green algae and/or cyanobacterium inside its tissue. There are also several groups of fungi that parasitize lichens or simply live inside a lichen benefiting from the moisture and protection of the host thallus! The number of species is high (ca 20,000 worldwide).

Lichens are not an easy group, especially in the southern hemisphere, although:

- Collecting is easy, at least for foliose or fruticose species (very analogous to collecting bryophytes – see chapter 13) but may be more problematic in the case of crustose species that are adhering firmly to the surface of rocks, stones and bark of living trees.
- Literature resources / identification guides are plenty for the northern hemisphere, but are limited or lacking elsewhere.
- Correct identification needs microscopic observation and often requires the use of certain chemicals and chromatography, but many species in the southern hemisphere remain to be described.
- Description requirements in the field are minimal.
- Preservation is easy (specimens are simply air dried).
- Web sources are good for the northern hemisphere.

3.3. Mushrooms and toadstools (the 'larger fungi')

The number of species is very high (some hundreds of thousands worldwide), mainly the larger ascomycetes and basidiomycetes. The larger fungi or

'macrofungi' are generally of special interest to mycological societies which usually possess both the expertise and bibliographic resources. Nevertheless, the larger fungi are difficult to study.

- Collecting is relatively easy in most cases, but the material is usually useless without a detailed description and pictures of the fresh fruitbodies, notes on spore deposits, chemical reactions, etc.
- Literature resources and identification guides are good to very good for Western Europe, Scandinavia and the Mediterranean area, but become very problematic elsewhere, even for rich western countries such as the USA where a very high proportion of the taxa remains to be described.
- Identification is difficult because of the wide array of simple to complex characters that need correct interpretation. Most often students require good microscopic skills, long standing experience, use of various chemicals and specialized literature in different languages. The complete absence of identification guides in most parts of the world is a further major obstacle.
- Preservation implies rapid drying using a desiccator.
- Web resources are limited although specialized web sites exist for many individual genera or families.

3.4. Plant parasitic fungi

The number of species is very high (several hundreds of thousands worldwide) and most species belong to specialized groups of ascomycetes (*e.g.* powdery mildews) and basidiomycetes (*e.g.* rusts and smuts) and also to several other groups of fungi (zygomycetes, chytridiomycetes) and the fungal-like oomycetes that are typically studied in the laboratory by phytopathologists (although such fungi fall mostly under 3.6 below). Depending on the group:

- Collecting is easy in most cases (similar to collecting plants – see chapter 14).
- Description requirements on fresh material are minimal.
- Literature resources can be quite good.
- Identification guides are restricted to specialized literature. Identification itself requires good botanical knowledge (you need to identify the host plant), and is impossible without the observation of microscopic features. For some of the purely microscopical groups (chytridiomycetes etc.) identification usually requires culturing on artificial media.
- Preservation is easy since specimens are dried as part of a botanical specimen.
- Web resources exist for some groups.

3.5. Animal parasites above microscopic level (Hypocreales such as *e.g. Cordyceps*, Laboulbeniomycetes, …)

The number of species is high (especially if considering Laboulbeniomycetes) and the experts or mycologists capable of identifying these groups are very limited. These groups are therefore too specialized to be covered in most ATBI's.

- Collecting is similar to collecting the animal host (insects and their larvae normally, spiders, …).
- Description requirements *in situ* are minimal for such tiny organisms as Laboulbeniomycetes but their observation and preparation requires a stereomicroscope (25x-50x). For some of the larger Hypocreales complete documentation of fresh material is needed.
- Literature resources / identification guides are restricted to specialized literature or non-existent.
- Identification requires a microscope and a good knowledge of the host animals (you need to identify the host).
- Preservation is easy (either in liquid or dried) and corresponds to those used for the animals.
- Web sites exist for *e.g. Cordyceps* and other hypocrealean fungi but other groups are less fortunate.

3.6. Microscopic fungi (molds, aquatic, coprophilous, nematophagous, yeasts, endophytic, …)

The number of species is very high (many hundreds of thousands worldwide) and because interest in these groups is usually restricted to commercially or industrially important species, their inclusion in an ATBI is exceptional. Some rare artificial assemblages requiring particular substrates, such as coprophilous or nematophagous fungi have attracted the interest of rare amateurs.

- Collecting itself is easy in most cases as one collects in fact only the substrates, but then isolation and identification require laboratory conditions and plating or pure culture techniques for obtaining individual species.
- *In situ* description is not needed.
- Literature resources / identification guides are problematic (specialized literature).
- Identification is very difficult, requires experience in microscopy, and mostly laboratory conditions for culturing and testing specimens, in case of coprophilous fungi humid chamber technique is also a valid alternative.
- Preservation of dried specimens after culture is easy, maintaining living cultures is expensive and difficult.

As these guidelines are only intended for the non-experienced mycologist, the purely microscopical fungal groups are not further discussed here.

4. Preparing for the field

Good preparation begins with reading about the places and types of vegetation you will be visiting. Know what to expect in the field!

- Find out about harmful or dangerous animals or toxic plants (collecting in the USA without knowing what poison ivy looks like (http://www.poison-ivy.org), may stop your participation in an ATBI right on the first day).
- Respect customs / traditions in the collecting area.
- Consult host / habitat lists in the area (know what fungi to expect or to look for!). This is especially important when composing the team of participants to your inventory. It is of no use to include many experts on beautiful larger basidiomycete genera such as *Russula*, *Amanita*, *Cortinarius*, various boletes, … when there are no ectomycorrhizal trees in the area.
- Study as many maps as possible from the area (including phytogeographical and geological maps).
- Detect the possibly best sites for your purpose and know how to get there (driving, walking).
- Hire a local guide and/or local specialist (especially in the tropics).
- Find out about places to stay and what they can offer (*e.g.* a separate space to work on your collections in the evening).

4.1. What to take with you in the field?

4.1.1. Transporting your specimens

Old newsprint suffices to wrap pieces of wood carrying lichens or resupinate fungi. Well-wrapped, you can stuff all of them together in a plastic or other bag you carry with you in the field, which will keep them sufficiently humid when in the field (don't keep them that way for more than one day since molds will develop very quickly!). It is advisable to take photographs before wrapping up your specimens.

A plant press and newsprint is mostly used for collecting and carrying plant parasitic fungi with you. Compartmented plastic boxes are useful for small species (eventually add moss for humidity). Larger fleshy fungi are best wrapped in aluminum foil or wax paper bags (in the tropics an icebox can be used to keep such collections on ice when traveling by car). When using an open basket to stack collected mushrooms, put the mushrooms upside down so that falling spores do not contaminate other species underneath.

Group	Collecting recipients
Myxomycetes	Boxes of various sizes, eventually with cork or similar materials in the bottom and pins to attach fragile, small or immature samples
Lichens	Paper or paper bags
Plant parasites	Old newspapers / large plastic bags
Animal parasites	Insect containers / liquid / chloroform. Note that bigger quantities of these (flammable) liquids (>90% ethanol) cannot be transported by plane. They will have to be purchased locally or even ordered locally (in some countries denaturated ethanol cannot be bought without a prescription)
Mushrooms and toadstools	Aluminum foil, wax paper bags, plastic containers of various sizes – wide basket or laundry net
Molds / Aquatic / coprophilic / endophytes	Bags for substrate collecting

Table 1. Overview of different collecting recipients.

4.1.2. Barcoding and tissue sampling for later molecular work

Although it is not absolutely imperative to sample tissues of fresh material for barcoding purposes (ribosomal genes can usually be obtained without too much problems from recently dried material), we recommend storing fresh tissue samples in an appropriate buffer as quickly as possible for later molecular research as an added value for your specimens.

If you have sufficient time and/or manpower, start taking tissue samples directly in the field, in this case you will need:

- Sterile Eppendorf tubes filled with 250 or 500 µl of 2x CTAB buffer for taking tissue samples of fleshy fungi *in situ* (the recipe and protocol for preparing CTAB can be found at *e.g.*: http://www.umich.edu/~mycology/protocols_assets/DNAminipreps.doc).
- Cleaning alcohol, paper tissue, tweezers.
- A permanent marker or pencil (fine tip) to annotate the tubes.
- Labels to go with the specimens.

Another recently developed method that seems excellent for barcoding uses so-called FTA cards (more information available on: http://www.whatman.com/FTANucleicAcidCollectionStorageandPurification.aspx)

4.1.3. Photographic equipment

Documenting collections often starts in the field and for many groups of fungi it is the only moment that you will see the specimens in a really fresh or clean condition, so think of taking the necessary photographic equipment with you. Needed are:

- A solid, small tripod.
- A (digital) camera, *e.g.* with macrolens (50-60 mm) or a wide-angle (18 mm) for larger species; a reflector or a piece of aluminum foil or white paper to provide light from underneath.
- Spare batteries and memory cards.

4.1.4. Geographic referencing

Take your maps, GPS and spare batteries or a compass in areas with insufficient coverage.

If you have little or no sense of orientation, start by taking the GPS coordinates of your car or camp before going out on a collecting trip! It is usually impossible or too time consuming to take coordinates for every collected specimen. Taking coordinates that correspond to homogeneous habitats or niches with a certain radius (10 to 30 m) is therefore often more advisable.

4.2. What else to take?

When collecting, you will need to take notes or write down various information concerning the specimens, their location and habitat. Therefore a **small notebook and pencil** are indispensable (and safer than a dictaphone) for taking notes on host or substrate, references to pictures, geographic coordinates, for writing labels, etc.

A **firm knife** or other digging tool should preferably be used to collect mushrooms from soil (always take care you have the very base of the mushroom!); in other cases a **small folding handsaw** or **pruning knife** (secateur) is needed to collect specimens such as pyrenomycetes or crust-like basidiomycetes growing on twigs or bark of living or dead trees.

Tweezers, **nets**, **pooter**, etc. for those collections parasitized by insects, spiders, etc.

Lichens growing on rocks or stones may require a **hammer** and **cold chisel** for collecting (in this case the use of **protective glasses** and **gloves** is recommended as well!). Also for lichens a **small spray-bottle with water** can be useful (damp thalli are more likely to remain intact during collection).

It is also very important to carry a small magnifying glass or hand lens to observe your specimens when collecting in the field. Taking a **hand lens (10x-20x magnification)** is absolutely recommended, especially for smaller species, myxomycetes, pyrenomycetes, etc.

A **walking stick** is useful not only for walking, but is also very handy with regard to uncover snakes or wasp nests, discarding spider webs, turning over litter, etc. especially in tropical and subtropical areas (any stick will do).

Don't forget to bring a suitable **repellent** against leaches, ticks, mosquitoes, chiggers, etc. especially since mycologists don't move a lot!

5. In the field

5.1. Collecting specimens

Always collect good quality material. Look around for a good specimen rather than collecting the first one you see. In some groups, it will be important to gather all different life or maturity stages you encounter, while in others fully mature material will suffice. Collecting young, mature and old specimens can indeed be very important because identification keys may be based on features only visible in very young or very old specimens.

- **Lichenized fungi** and **slime molds** are straightforward to collect. You can see practically the entire organism and it is usually easy to collect at least part of it. Slime molds can be extremely small and without a hand lens (minimum 10x magnification) you will not be able to do very much.
- Collecting **lichenized fungi** is very much as collecting bryophytes (see chapter 13). Place specimens in breathable bags or folded packets made of brown, white, or wax paper or even newsprint. Put only one species from one substrate in each bag or packet (eventually more than one specimen for common species). Collect entire, intact and preferably fertile thalli, part of a specimen is sufficient for uncommon to rare species.
- Collecting **plant or animal pathogens** is very similar to collecting the host plants (with the difference that you collect now only the parts that look attacked or ill) or the host animals. Collect parts with different aspects or stages of the disease if possible. Use newsprint and a plant press for plant pathogens. Screen collected hosts with a hand lens (if appropriate). Host animals are best stored in small vials. The latter need to be labeled clearly.
- For **fleshy fungi**, mycologists usually only collect the above-ground (or below-ground truffles), sexual fruiting bodies or 'mushrooms' of the fungus. The first thing you have to realize when collecting the softer, fleshy mushrooms, is that they are mainly composed of water (up to 90%) and that they will start to rot the moment you collect them. In a tropical climate this happens within hours or less, in more temperate or cool areas it may take days. Therefore, avoid long distances to and from the field and try to keep your specimens as cool as possible. Use different types of containers, boxes or bags to ensure a good storage and transport of specimens of variable shapes and sizes. Do not collect more than what you and your drier can handle after you return from the field! It is a waste to spend time collecting too much material. Be selective in the field and go for representative and well-documented specimens, instead of throwing away in the evening what you collected in the morning.

Beware to collect the whole ‘mushroom’, particularly if the stipe base is hidden in the substrate.

Include both young and mature specimens in a sample.

Particularly fragile or tiny mushrooms should be transported in closed, rigid containers that maintain maximum humidity (adding moss is a very efficient way to achieve this!).

Very large specimens are difficult to transport, dry and preserve as such. Usually they are sliced into smaller pieces, making sure every section retains a representative part of every structure (cap, stipe, gills or tubes, etc.).

We recommend the use of aluminum foil or wax paper to wrap your specimens whenever possible. It will keep them fresh for a longer time, reduces the risk of contamination and makes it easy to add a label. However, if you decide to stack gilled mushrooms, boletes, etc. in an open basket without packing them individually, put your specimens always with the stipe pointing upward so that no clouds of falling spores can contaminate the specimens that are just underneath. Always protect the mushroom basket from direct sunlight and don’t leave it in the car when you stop for a break as your collections will quickly deteriorate in an overheated vehicle.

5.1.1. Which are the best sites for collecting fungi?

Of course the answer to this question depends on what group you are interested in. Moreover, a site can have an excellent reputation for the fungi you are looking for, but if the season is wrong or if the rains fail, it may leave you empty-handed.

> BEWARE! Collecting can be a sensitive item. In most countries, collection / picking of larger fungi is usually restricted or forbidden because of excessive and destructive collecting of several commercial species in the past. Because of this commercial value and the fact that they are considered to be some sort of wild ‘fruits’, larger fungi are considered the property of the landowner in many countries. Therefore, be sure to have the necessary permissions to collect in the places you visit, and avoid offending or provoking other visitors and tourists by collecting in crowded or public places for example. This also applies when cutting lichens from tree bark or other substrates: keep in mind that other people can take offense when someone cuts into a tree.

Ascomycetes with apothecia – so-called discomycetes or ‘cup-fungi’ – should be collected following the same principles as other macrofungi, and careful notes on colour, size, presence of hairs and other external features should be taken. The nature of the substrate, and if at all possible the host (if present) should be described or identified. Species vary from very tiny (less than 100 µm in diameter) to huge complex structures as those of morels (*Morchella* spp.). Many fruit directly on soil, and can be ectomycorrhizal as many basidiomycetes, others produce ascomata on living or dead bryophytes, on fallen leaves from trees, on dead herbaceous stems, on dead wood on dry land or in stagnant or running water and in a range of other habitats. Some are adapted to withstand

desiccation and can be collected high up in trees (just as a range of corticioid basidiomycetes). Such tiny species are usually collected together with the substrate they are growing on. Ascomycetes with perithecia constitute another very large and diverse group. Some have single perithecia seated directly on or immersed in the substrate, be it rotten wood, still corticated twigs, herbaceous stems, dung, etc. while other species have so-called stromatic structures that may house up to several thousands of individual perithecia. Such stromata can be very large (up to more than 20 cm), and develop either well above the substrate on some kind of 'stipe' or they can be hidden under bark or develop as a layer on top of the substrate.

For myxomycetes, good places you may want to explore include highly decomposed or rotten wood, litter layers, undergrowth of dense bushes, heaps of decomposing *Urtica*, *Salvia* spp., various ombelliferous plants, … and especially after heavy rains also vertical surfaces such as moss-covered rocks or walls and tree trunks.

5.1.2. Collecting substrates for later observation in humid chamber

For some groups, in particular slime molds and coprophilous fungi, the technique of the humid chamber is frequently used. It consists in the collection of substrate (soil, decomposing litter, pieces of rotten or fresh bark or wood for myxomycetes, animal excrements for coprophilous fungi, soil or other substrates for microscopic fungi) that are simply dried to be rehumidified later and examined regularly under the dissecting microscope for freshly fruiting structures within the humid atmosphere of a Petri dish or other plastic containers or in culture media.

5.2. Photographing specimens in the field

For some groups of mushrooms, in particular very fragile species (*e.g. Leucocoprinus*, *Coprinus*, *Psathyrella*) or species with evanescent parts or structures (presence of powdery or arachnoid veils, glutinous surfaces, local exudation of droplets), *in situ* pictures are the only guarantee for a good picture of the fresh specimens.

Although you may want to show the mushrooms exactly as you found them and thus leave them untouched for the picture, there are very few situations in which this will result in an informative, scientific picture. It will usually be necessary to 'cheat' and move some of the specimens closer to one another to have them all in focus (sharp), and to turn others so that details of the gills and stipe become clearly visible in the photograph. A cross section of one fruit-body may often be useful to highlight diagnostic features of context and stipe.

A mycologist should be particularly attentive to the following aspects:

- Use a small but stable tripod in the field. It will allow for longer exposure times and thus result in considerably more depth of field and less blurry pictures.
- Avoid direct sunlight on your subject as it results in too much contrast.

- Avoid using a flash in the field to obtain correct colours and better contrast.
- Use a reflector instead to brighten up the dark parts of the fungus (underneath the cap mostly).
- Photograph from close-by and frame the fungus to fill the image as much as possible. Tiny mushrooms in a large landscape convey little information. Using a macro lens is therefore a good solution.
- To appreciate the importance of these aspects, you can check out the photographs explaining 'how to do it' and 'what to avoid' on http://www.mtsn.tn.it/russulales-news/tc_photographs.asp

6. Back from the field

Depending on the collected species, you will have to work on the collected specimens before processing them for later identification and preservation.

Some recommendations for working on fungal collections:

- Start by assigning a unique number to each of your specimens. These numbers can be continuous throughout your herbarium. Label all related documents (pictures, tissue samples, spore deposits, descriptive notes, etc.) from this specimen with the same number.
- Decide on priorities in function of fragility and ephemeral character of specimens and set up for spore deposits.
- Team-work! It is more efficient to have a single person taking all the pictures, another person doing all tissue sampling, …
- Mycology does require a certain comfort!
- Good (white or natural) light is required for good description and appreciation of colours. Therefore, taking a good lamp with you for evening work is absolutely recommended (best fitted with a day light bulb).
- A lot of space is needed to sort collections and taking pictures, spore deposits and tissue samples… Be sure you have enough space available!
- Work protected from rain and wind (Fig. 1).

Fig. 1. Remember that a fungal inventory is for the greater part taking notes, spore prints, sampling tissues; therefore, an adequate, sufficiently large, dry and wind-free space for doing these various activities is hardly a luxury. Camping offers not really the ideal solution for a fungal inventory. (Photo by T. Laessoe).

6.1. Barcoding (using CTAB method)

If you want to add scientific value to your specimens by optimizing future sequencing possibilities, take small parts (see Table 2) of the specimens and put them in an Eppendorf tube with 0.5 ml or 0.25 ml CTAB buffer (or preferably CTAB 2x for fleshy mushrooms as they contain up to 90% of water).

Group	Tissue to be collected
Myxomycetes	Entire sporocysts
Lichens	Reproductive structures
Plant parasites	Various types of spores
Animal parasites	Small parts of spore producing surfaces
Mushrooms and toadstools	Small parts of spore producing surfaces or context
Molds / Aquatic/ coprophilic / endophytes	n.a.

Table 2. Tissue to be collected per group.

6.1.1. Sampling protocol example for larger fungi

- Sample the tissues **as soon as possible** after collecting the fungus (you can even do it in the field if there is time for it).
- Use **clean tweezers** (with tips not necessarily sterilized, but at least well cleaned with soft paper tissue (eventually drenched in alcohol 70% or higher).
- Choose parts of the gills that look **perfectly clean**, that are not parasitized by molds and not attacked by animals or other microorganisms (insect larvae, collembolla, mites, etc.). If gills seem not very clean, you can also cut the mushroom lengthwise and take tissue sample from the firm parts of the flesh inside cap or stipe.
- Take about the quantity of gill or flesh **tissue that corresponds to half of the amount of CTAB** liquid in the tube, not more.
- Close the Eppendorf tubes **very tightly** when finished.
- Write the **collection number on the side** of the tube, and **also on top** of the lid, using a fine permanent marker.
- Repeat for **a second tube** or eventually up to 3-4 tubes for very rare species.

6.2. Documenting collections

6.2.1. Morphology

As you can see from Table 3, most groups of fungi do not need to be described in detail immediately after collecting.

Group	Need for immediate documentation	Preservation method
Myxomycetes	No	Air dried immediately
Lichens	No	Air dried immediately
Plant parasites	No	Air dried as for botanical specimens
Animal parasites	No	Micr. prep. / liquid (alcohol, formol)
Mushrooms and toadstools	Yes	Dried after description
Molds / Aquatic / coprophilic / endophytes	No	Needs lab work for isolation, later dried after culturing/ kept as micr. prep. / or living culture

Table 3. Preservation methods.

In particular the larger fleshy mushrooms require elaborate description before being dried because the conservation method (implying rapid drying using a desiccator) will completely change their general aspect!

- Do not collect too much specimens at a time since description afterwards is very time-consuming.
- Process specimens as soon as possible because they loose their features rapidly after collecting.

In view of later identification, it is essential to record those features that will disappear once the specimen is dried, in particular:

- Dimensions of all parts (cap diameter, stipe length and width, gill spacing, gill height, etc.).
- Colour and colour changes (we recommend to use a colour code for precise notation, but these printed colour book sare becoming increasingly difficult to find).
- Taste (it is safe to taste a very small part of the mushroom, including toxic species, on the condition to spit out all the parts! The mastication should take at least 60 sec).
- Check the smell.

The use of description forms is recommended as it avoids omitting features. It also offers a standard and usually much faster way of documenting your specimens when a pre-established list of possibilities (using correct terminology) is given for every character.

The easiest way to document your collections is by taking additional digital pictures of all informative details (*i.e.* sections of fruitbodies, colour changes, young and older specimens, interesting details of veils, droplets, excretions, etc.) using good lighting, long exposure, circular flash (if you must). In this way, a full image record of your specimens is made by taking macro-photographs of all possible aspects of the fruitbodies (habitus, surfaces, sections, scales, pores, insertion of tubes or lamellae, chemical reactions, etc.). This is best done by placing the specimens or sections together with a reference for (i) dimensions, (ii) the collection number and (iii) colour using *e.g.* a Pantone colour strip (see De Kesel, 2004).

Ascomycetes should be collected along the same principles as other macrofungi, and careful notes on colour, size, presence of hairs and other external features should be taken. If at all possible, groups such as Pyrenomycetes should be cultivated from spores or tissue when collected (or later after gentle air drying) and whilst more carbonized groups (because of stroma tissue) require less work on the fresh material, you should always try to provide details on a section through the stroma to annotate the colour and texture of the interior of the stromatic tissue. In an ideal world, the microscopical features should be studied in water mounts whilst spores are still living (so-called vital taxonomy, see Baral, 1992), but this is hardly possible when collecting under primitive field conditions.

6.2.2. Chemical reactions on various parts of the larger fungi

Chemical reagents are often applied on fresh material of larger fungi for identification purposes. Since a number of chemical tests are only used in selected genera, the choice of chemicals used and the part(s) of the fruitbody where they will be applied on, will depend from the fungal group at hand. A complete list and illustrated examples of their application can be found at http://www.champignons-passion.be/main.htm

The following reagents are often used on fresh material to help identify various groups of larger fungi:

- Ammonium (pure)
- Anilin (Schaeffer's reaction)
- Nitric acid (Schaeffer's reaction)
- Ammonium hypochlorite solution ('Eau de Javel')
- Formol 38% (pure, laboratory quality, not commercial)
- Phenol (3% solution in distilled water)
- Potassium (10-20% solution in distilled water)
- Gaïac (10% solution in 80° alcohol)
- Ammonium (10% solution in distilled water)
- Iron sulfate (crystal)
- Dr. Henry's TL4
- Sulfuric acid twice diluted (50%)
- Vanillin (pure)

6.2.3. Preparing spore deposits

Another important aspect for later identification, and again mainly restricted to the larger fungi, is the precise colour of the spore deposit. A spore deposit (or 'spore print') should thus be obtained whenever possible. An illustrated explanation on how to do this can be found at http://www.mushroomexpert.com/spore_print.html or in De Kesel (2004).

We recommend the use of transparencies (transparent plastic film) for making spore deposits. Cut to smaller pieces they constitute a very light support for the spore print and allow a more accurate determination of the colour (you can superimpose it on existing colour codes) and easier preparation of microscopic slides for spore observation (by cutting a part of the plastic film for direct observation with the appropriate reagent).

- Use plastic film for exact colour notation (better than on white paper).
- Use closed recipients (no air currents).

- Allow for sufficient but not excessive humidity (adding some moss in the container is perfect)..
- Wait for 6-12 hours and note the colour of the fresh spore print immediately. For fragile, tiny specimens this often implies sacrifying an entire specimen.
- For resupinate fungi on wood, it is recommended to rehydrate the specimens prior to making a spore print, and to keep the fragments used for spore printing slightly away from the plastic film by placing them on light matches.

6.3. Conditioning specimens for conservation

6.3.1. Drying specimens (herbarium)

Myxomycetes, Lichens, plant pathogens are mostly air dried.

- Use small cardboard or plastic boxes to keep myxomycetes, and permanent packets folded from acid-free paper with 25% or higher rag content for lichens, or herbarium sheets from acid-free paper for plant pathogens.
- After drying, we recommend you to freeze lichens for five days at -20°C (-5°F). This will kill most arthropods without damaging the lichens.

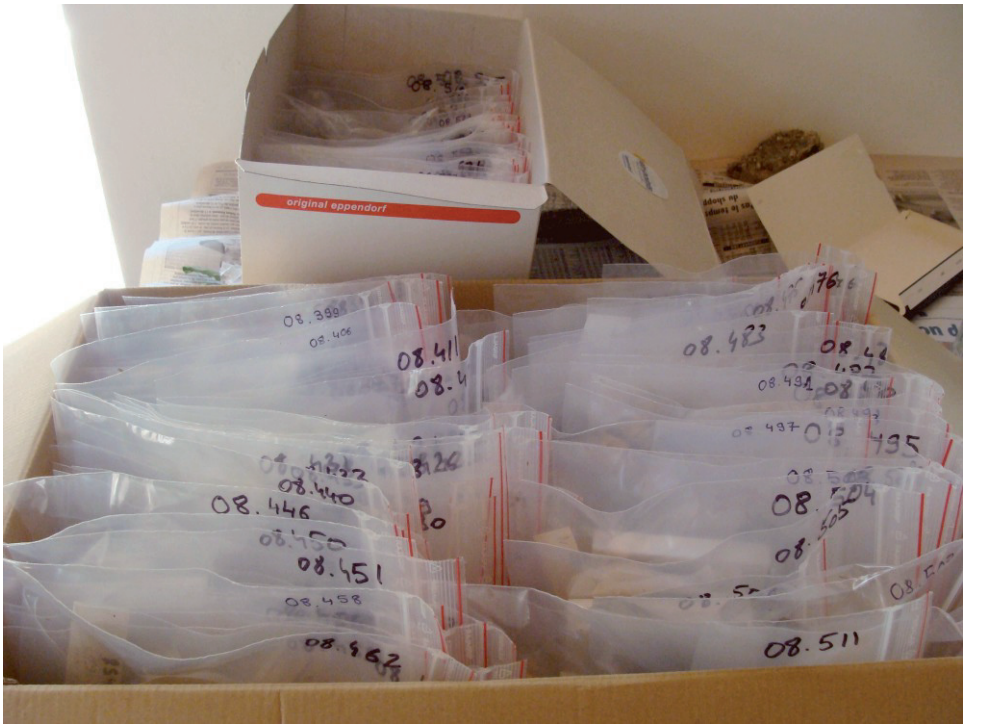

Fig. 2. Ziplocks (plastic bags that can be hermetically closed) are probably the best solution to keep and store your fungi once well dried. Just remember to keep them out of the sun to avoid eventual condensation. (Photo by B.Buyck).

Larger fungi should be quickly dried using a dessicator at 40-50°C. There exist several commercial models that work on electricity. In more modest working conditions a field dryer (De Kesel, 2001) or a cardboard box purchased locally, both using kerosene lamps or with a simple light bulb at the base, can help you out as well.

- Cut or slice large or very hard specimens.
- Use ziplock plastic bags (they come in various sizes) for storing your dried specimens in order to avoid rehydration when working in humid conditions (Fig. 2).

6.3.2. Store in liquid (alcohol, Wasson liquid)

- Very tiny or fragile specimens.
- Jelly fungi (although these can also be dried).
- Insects and other arthropods.

6.3.3. Permanent microscopic preparations for microscopic fungi

- From cultures mostly / parasites / symbionts (not further detailed here).

7. Some important identification or other web-resources for a fungal ATBI

Directory of mycological resources on the net:

- http://mycology.cornell.edu/

Fungal pages or photographs on the net:

- http://www.grzyby.pl/fglobal-directory.htm

Digital archive for books, journals, thesauri, indexes and other publication important to systematic mycology:

- http://194.203.77.76/LibriFungorum/Resources.asp
- http://www.cybertruffle.org.uk/cyberliber/

Various information on mycologists and fungal taxa:

- http://www.cybertruffle.org.uk/eng/index.htm

Index to published fungal names

- http://www.speciesfungorum.org/Names/Names.asp
- http://www.cybertruffle.org.uk/cybernome/eng/index.htm
- http://www.mycobank.org/MycoTaxo.aspx

Search for articles by either author or fungal genus

- http://www.speciesfungorum.org/BSM/bsm.asp

Synoptic multi-access key for identification of fungi and many links to other resources

- www.mycokey.com

Lichen related topics

- http://www.lichens.ie/links

Mycological societies in the world

- http://www.euromould.org

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