SAMPLING, COLLECTION, AND PREPARATION TOOLKIT FOR BIODIVERSITY SPECIMENS

GUIDELINES FOR PRE-COLLECTION, COLLECTION, AND POST-COLLECTION PROCEDURES

A PRACTICAL GUIDE FOR STANDARDIZED BIODIVERSITY SAMPLING AND SPECIMEN HANDLING

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A PRACTICAL GUIDE FOR STANDARDIZED BIODIVERSITY SAMPLING AND SPECIMEN HANDLING

This toolkit presents standardized protocols for the sampling, collection, and preparation of diverse biological specimens. It includes practical guidelines addressing pre-collection requirements, field sampling procedures, and post-collection processing, with the aim of ensuring methodological consistency, specimen preservation, and data reliability across biodiversity research and conservation initiatives.

The preparation of this toolkit would not have been possible without the support of the Bio Bridge Initiative.

CONTENT

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SEED COLLECTING MANUAL

FIELD TRIP STEPS

A. STEP 1 LOCATING THE TARGET SPECIES

The initial phase in the seed collection process involves identifying and locating populations of the target species. This requires consultation of references on species distribution, including herbarium records, botanical databases, peer-reviewed literature, and floristic surveys. Where possible, collaborating with local experts is highly recommended to improve the accuracy of site identification. In the absence of local expertise, supplementary sources such as past monitoring initiatives, ecogeographic assessments, historical botanical records, and distribution maps from regional floras should be utilized. The use of multiple, independent references is essential to verify the presence and distribution of the species.

Note: For species exhibiting synchronous fruiting and narrow seed dispersal windows, it is critical to account for phenological deviations in flowering and seed maturation periods. This is particularly relevant in Mediterranean climates, where environmental variability can influence reproductive timing. Preference should be given to species with documented desiccation tolerance, as this trait enhances seed longevity and viability during ex situ conservation and laboratory storage.

A.1.1. RECOMMENDED RESOURCES:

- RBG Kew Seed Information Database (SID)
- Lebanon Biodiversity portals
- Plants of the World Online (POWO)
- World Flora Online (WFO)

Following the selection of a collection site, a preliminary field visit is advised to assess the reproductive phenology of the species. During this visit, GPS-tagging of individual plants should be conducted to facilitate future identification during the fruiting stage. If the target species is in anthesis (flowering), a voucher specimen may be collected for taxonomic verification. Alternatively, individuals can be marked to prevent misidentification with sympatric species that may exhibit similar fruit morphologies. Optionally, a microsite map can be prepared to document spatial characteristics of the population.

B. STEP 2 SAMPLING STRATEGY

The appropriate sampling strategy depends on the specific objectives of seed collection.

If the primary goal is genetic analysis such as DNA extraction, seeds should be collected from multiple sub-populations within a defined ecological zone or geographic area. Sub-populations, understood as partially isolated breeding groups within a larger population or species, offer valuable insights into genetic variation and population structure due to limited gene flow between them.

Conversely, if the objective is Ex-situ conservation through seed banking (see fig. 1) and future germination, it is advisable to collect accessions from various locations within the same ecological region. This approach helps capture a representative range of local genetic diversity while maintaining ecological consistency, which can be critical for successful regeneration and reintroduction.

Figure 1 Seeds ex-situ conservation via seed banking (top), where seeds are dried with colored silica desiccants to reduce their humidity content (left). Source: Jouzour Loubnan Seed Bank.

B.1. SEED GERMINATION AND POPULATION SAMPLING:

Ideally, to fully capture the genetic diversity of a species, seeds should be collected from every population across its entire natural range. However, in most real-world scenarios, this is not feasible due to time, budget, and logistical limitations except in the case of very rare species with few known populations.
In practice, the number of populations sampled will depend on available resources, the biology of the species, and the specific goals of the seed collection program.

MINIMUM TARGET

A good starting point is to sample from at least five distinct populations, which can capture around 67–83% of the species' genetic diversity.

OPTIMAL TARGET FOR FULL GENETIC COVERAGE

For broader conservation efforts, aim to sample from up to 50 populations (Guerrant, 2004). This more complete coverage may need to be carried out over multiple seasons or years and should include a variety of ecological conditions (e.g., different altitudes, soil types, or habitat features like rocky slopes).

To ensure the sampling is applied to truly separate populations, look for a clear geographic break, an area between sites where no individuals of the species are present. This helps avoid overlap and ensures genetic distinctiveness between populations.
When choosing which populations to sample, consider both ecological diversity (such as variation in climate, elevation, or geology) and logistical factors, including travel time, accessibility, and proximity to base of operations.

SPECIAL NOTE FOR SHORT-LIVED OR HIGHLY DYNAMIC SPECIES

If the species tends to have rapidly changing or short-lived populations, it is advisable to collect seeds across multiple seasons to account for temporal variation in population size and structure (Begon et al., 1990).

B.2. PRE-COLLECTION PLANNING AND IN-FIELD POPULATION ASSESSMENT:

Prior to conducting fieldwork, it is essential that the collector clearly defines the objective of the seed collection. This is critical, as collecting a large number of samples may present significant challenges in terms of data management, curation, and long-term storage. Conversely, collecting too few samples may result in an inadequate representation of the species' genetic diversity. Therefore, a balance must be achieved between sample quantity and practical constraints.

Upon arrival at a collection site, a rapid survey should be carried out to assess the distribution and density of the target species. One key consideration is determining population boundaries, specifically, where one population ends and another begins. Ideally, seed collections from distinct populations should be kept separate to avoid obscuring underlying genetic differences. Pooling or merging material from genetically distinct populations can result in the loss of important genetic structure and reduce the value of the collection for conservation or research.
In practice, collectors should adopt a pragmatic approach, guided by species-specific ecological knowledge and principles of population genetics. Within a single population, seed collection may continue until a clear barrier to gene flow is encountered. Such barriers whether physical, ecological, or behavioral, may lead to genetic isolation and justify treating populations on either side as distinct units for sampling.

The nature of these barriers is influenced by the species' pollen and seed dispersal mechanisms. While long-distance dispersal events (e.g., via wind or animals) can occasionally occur, they are generally infrequent. In most cases, local dispersal predominates. For example, the majority of seeds tend to disperse less than 100 meters from the parent plant (Cain et al., 2000). Although pollen, particularly wind-dispersed or animal-mediated may travel greater distances, its genetic impact on recipient populations is often limited due to the dominance of locally produced pollen.

In line with these considerations, it is essential to verify that the population being sampled is of wild origin, and not the result of recent planting, or hybridization. Collecting from non-wild or artificially influenced populations may compromise the genetic integrity and conservation value of the seed samples.

B.3. GENERAL PRINCIPLES FOR SEED COLLECTION FOR CONSERVATION & RESTORATION:

- **Aim to collect seeds from as many individual plants as possible, without endangering the population.**
- **Random sampling is preferred to avoid bias.**
- **Cover the widest geographic and ecological range within the population.**

This ensures that the collected sample captures the full spectrum of genetic variation present in the wild population. Collecting from many individuals helps preserve evolutionary potential and adaptability.

B.3.1. STRATIFIED SAMPLING IN DIVERSE HABITATS:

- If the habitat varies significantly (e.g., different soil types, elevation zones), use a stratified sampling method.
- Keep seeds from each ecological type separate.

This method captures local adaptations by ensuring that seeds reflect the environmental conditions in which they were growing. This is especially important for future restoration efforts where local adaptation can influence success.

B.3.1. STRATIFIED SAMPLING IN DIVERSE HABITATS:

There is much guidance on the collection of plant genetic resources in the literature. Much of it is derived from work on crop species by Marshall & Brown (1975) who recommend the capture of at least one copy of 95% of the alleles that occur in the target population at frequencies greater than 5%. In order to achieve this, aim to collect:

- Outbreeding species: minimum of 30 individuals
- Inbreeding species: minimum of 59 individuals
- If breeding system is unknown: aim for at least 50 individuals

Outbreeders mix genes more, so fewer individuals are needed. Inbreeders tend to have more genetic similarity, so a larger sample is needed to capture diversity.

B.4. REINTRODUCTION AND ADAPTATION:

When collecting seeds for reintroduction (i.e., bringing a species back to a site where it used to grow), it's important that the genetic makeup of your sample matches the original population. This means your collected seeds should have alleles (gene variants) in roughly the same proportions as they existed in the wild population that was previously at that site. This genetic similarity maximizes the chance that the plants will be well-adapted to the environmental conditions at the site. To accurately reflect allele frequencies, especially in large populations, you need a larger sample size. Marshall & Brown (1983) recommend sampling at least 200 individuals to get a good representation of the gene pool. For outbreeding species (plants that typically cross-pollinate), it's important to also collect at least five seeds from each plant, since each seed could be genetically different.

Do not overlook the possibility that a population of individuals is in fact one individual joined by rhizomes or stolons, and check thoroughly for this. If in doubt, make a note in the notes field of the collecting form. In summary, try to collect at least 50 and preferably 200 plants but modify this advice based on local circumstances.

All in all, while the ideal target is to sample from at least 50, and preferably up to 200 genetically distinct individuals, this guideline should be adjusted based on species biology, population structure, and logistical constraints in the field.

B.5. FINAL SUMMARY AND RECOMMENDATIONS:

- **Aim to collect seeds from 50 to 200 genetically distinct individuals,** where feasible, to ensure adequate representation of population-level genetic diversity.
- **Adjust sampling strategies based on field conditions,** including population size, accessibility, and environmental constraints. Flexibility is essential for effective decision-making.
- **Maintain separation of individual plant samples** whenever possible, particularly in cases where full population-level sampling cannot be achieved. This allows for future analysis of intra-population variation and genetic structure.
- **Record comprehensive field data,** including habitat characteristics, ecological gradients, phenological stage, and any limitations encountered during collection. These metadata are crucial for interpreting the genetic and ecological context of the samples.
- **The primary objective is to conserve genetic diversity** for long-term research, restoration, and conservation purposes. While ideal sampling may not always be achievable, rigorous documentation, sample integrity, and transparent methodology ensure the scientific and conservation value of the collected material is retained.

C. STEP 3 SAMPLE SIZE AND SEED QUANTITY

Environmental heterogeneity across geographic regions exerts varying selective pressures on plant populations, often resulting in genetic differentiation among populations of the same taxon. To account for this, the target collection area should be divided into eco-geographic sectors based on available climatic, edaphic, and topographic data. It can be reasonably assumed that populations located farther apart or within contrasting environments are more likely to exhibit greater genetic divergence. Within each sector, populations are expected to share similar adaptive traits due to exposure to comparable environmental conditions.

The persistence of plant populations, particularly annuals, is often dependent on the presence of a viable seed bank that spans multiple years. This reliance on seed reserves diminishes in longer-lived perennial species. To mitigate the risk of negatively impacting natural regeneration, especially in rare or small populations, it is recommended to limit seed collection to no more than 20% of the mature seeds available at the time of collection (Way, 2003).

In addition, avoid consecutive annual collections from the same population unless the quantity harvested in each year is well below the 20% threshold. This helps to reduce pressure on seed banks and maintain population viability over time. Guerrant et al. (2004) advocate for lower-intensity sampling spread across multiple seasons, as this improves temporal genetic representation while minimizing ecological disturbance.

Exceptions to these guidelines may apply in cases where populations are imminently threatened by habitat destruction (e.g., urban development), in which case salvage collection is warranted regardless of the seed proportion.

D. STEP 4 SEED COLLECTION TECHNIQUES – SELECTION OF APPROPRIATE SAMPLING BAGS

Selecting an appropriate seed collection container is essential for maintaining sample integrity during fieldwork and ensuring ease of subsequent handling and processing. The choice of bag should be guided by the target species' fruit type, the environmental conditions, and the duration of the collection effort. Field teams are advised to prepare and procure suitable bags prior to deployment.

Avoid plastic or airtight containers for most seed types, particularly during overnight storage. These containers can trap moisture and lead to condensation, especially under cool nighttime conditions, which promotes fungal growth and compromises seed viability (see fig. 5).

D.1. GENERAL GUIDELINES BY FRUIT AND SPECIES TYPE:

- **Dehiscent Fruits (e.g., siliques, legume pods, capsules):**
  Collect seeds directly into paper or breathable cloth bags, or initially into a rigid container such as a bowl or bucket to remove debris before transferring them into appropriate bags.

- **Branched Seed Heads and Panicles (e.g., grasses):**
  Use secateurs or scissors to cut entire seed-bearing structures and place them head-first into collection bags.

- **Awned Grasses and Similar Species (e.g., Asteraceae):**
  Prefer heavy-duty paper bags over cloth, which better contain fine or barbed structures and minimize snagging.

- **Thorny or Rigid Species (e.g., Onopordum spp.):**
  Use durable cloth bags to withstand physical abrasion. Rigid plastic containers may be used only if the material is dry and storage duration is short to avoid heat buildup and moisture retention.

- **Fleshy or Large Fruits:**
  Collect fruits individually by hand, placing them into ventilated plastic bags left open for airflow. To prevent fermentation during extended transport, either air-dry the fruits or manually extract the seeds in the field (see figs. 2, 3 &4).

- **Tall Trees with Inaccessible Fruits:**
  Utilize canvas sheets or durable fabric laid under the canopy to capture seeds or fruits dislodged by shaking or striking branches. Refer to Schmidt (2000) for specialized tree climbing and harvesting methods.

- **Smaller Plants with Dehiscent Fruits:**
  Spread a large sheet of paper (e.g., A3 size) beneath the plant, gently dislodge seeds onto the surface, and transfer them carefully into collection bags.

Figure 2 Examples of fleshy fruits belonging to wild fruiting trees from Lebanon: Prunus ursina (orange), Juniperus oxycedrus (brown), Sorbus flabellifolia (red), Malus trilobata (pale yellow), and Styrax officinalis (green).

These fruits were placed in well-aerated bags during field collection and placed in dishes when they reached the lab, where their seeds will be extracted. Source: Jouzour Loubnan Seed Bank.

Figure 3 a close-up view at some of the fleshy fruits that need to be treated in the lab as soon as they are collected. Source: Jouzour Loubnan Seed Bank..

Figure 4 Example of fleshy fruits kept in well-aerated plastic bags during collection. Source: Jouzour Loubnan Seed Bank.

Field cleaning (e.g., removal of chaff or plant debris) may be conducted when feasible. However, unless constrained by space or time (e.g., during extended expeditions), comprehensive cleaning is best deferred to laboratory conditions to ensure precision and reduce loss.

D.2. ADDITIONAL PRECAUTIONS DURING SEED COLLECTION:

It is generally advisable to avoid collecting seeds or fruits that have already fallen to the ground, as they may be aged, damaged, or otherwise compromised in viability. Moreover, fallen seeds may not accurately represent the target plant due to secondary dispersal (e.g., by wind, gravity, or animals), leading to potential misidentification or sampling bias especially if seeds collected beneath one plant originated from a neighboring individual or a morphologically similar, non-target species.

If ground collection is unavoidable due to limited availability of canopy or attached fruits, this must be clearly recorded in the passport or field data form to inform seed bank personnel of the increased likelihood of reduced germination rates or genetic ambiguity.

Special care must also be taken when collecting from sensitive taxa such as orchids. Orchid capsules should not be handled directly, even with gloves, due to the risk of damaging the delicate structures. Instead, use a sterile razor blade or scalpel to sever the pedicel and allow the fruit to fall directly into a clean collection bag. Orchid seeds are extremely small and light (dust-like), so extra caution during handling and transport is essential to avoid loss or contamination.

An effective indicator of seed maturity is the ease with which seeds or fruits can be detached from the parent plant. Changes in fruit coloration often serve as phenological signals of maturation; for example, many ornithochorous (bird-dispersed) fruits exhibit a color transition; commonly to red that enhances visibility against green foliage.

Collection of immature seeds or fruits is generally discouraged due to their lower viability and incomplete development. However, under certain circumstances, it may be feasible or necessary to harvest slightly immature fruits, which typically retain a predominantly green coloration, and allow them to mature ex situ under controlled laboratory conditions. These fruits should be maintained in environments with moderate humidity and light exposure until full maturity is reached, at which point seeds can be extracted and subjected to drying protocols.

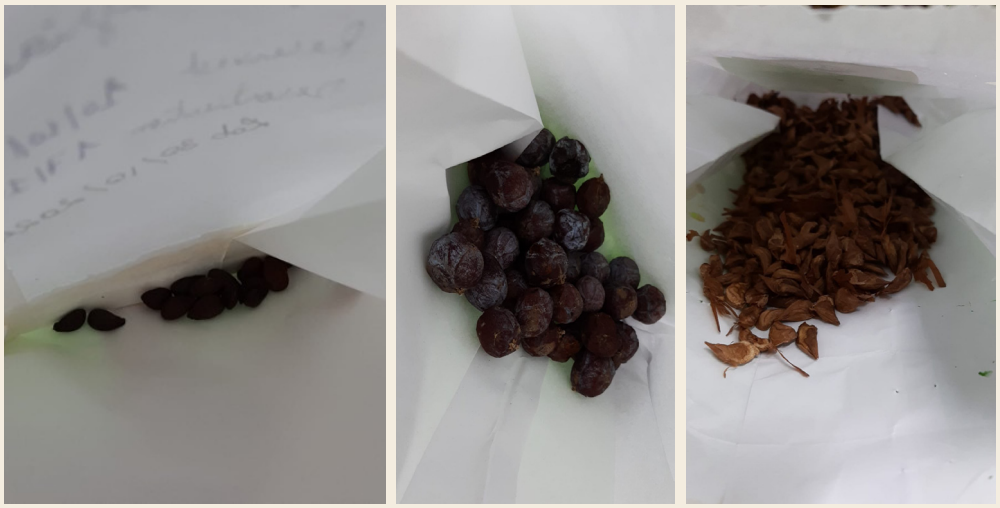

Figure 5 examples of seeds and juniper drupes (or juniper cones) collected and placed in paper bags. Source: Jouzour Loubnan Seed Bank.

D.3. PLANT IDENTIFICATION AND DOCUMENTATION:

Accurate and comprehensive documentation of seed collections is essential, as seeds lacking associated metadata have limited scientific and conservation value. Full passport data should be recorded for each collection, including detailed information on seed provenance and sampling methodology (Annex I)

Beyond basic collection data, additional information such as the estimated number of individuals at the collection site is valuable for long-term population monitoring. Data on local ethnobotanical uses and observable threats to the population should also be documented. It is critical that all recorded information remains objective and is sufficiently clear to be interpretable for several decades post-collection.

Traditionally, collectors record data on paper forms in the field; however, the increasing use of handheld digital devices or tablets enables direct electronic data capture, reducing transcription errors and data loss. Regardless of the method, it is imperative that digital data are backed up regularly in the field to prevent loss.

Precise geolocation of the collection site should be recorded using topographic maps or, preferably, Geographic Positioning Systems (GPS) to ensure accurate spatial referencing.

D.4. FIELD COLLECTION PRECAUTIONS AND BEST PRACTICES:

Some wild plant species possess toxic or irritant properties; collectors should exercise caution and use protective gloves when handling potentially hazardous species.

Seeds and fruits may adhere to clothing and footwear. Prior to departing the collection site, check and remove any such material to avoid unintentional dispersal, which could lead to gene flow between distinct populations, potentially compromising the genetic integrity of narrow endemic taxa.

When collecting rare species in publicly accessible areas, minimize disturbance and visibility to avoid attracting attention that could lead to trampling or habitat degradation.

D.5. FINAL FIELD ASSESSMENT OF SEED MOISTURE:

Before departing the site, if feasible, measure the equilibrium relative humidity (eRH) of the seed samples in-situ (refer to Probert, 2003; MSBP Technical Information Sheet No. 5: http://www.kew.org/msbp/scitech/publications/05-eRH%20moisture%20measurement.pdf). If the eRH exceeds 50%, or if ambient relative humidity is persistently above this threshold during sampling, active drying of samples using desiccants is recommended.

Because relative humidity fluctuates diurnally; typically rising overnight and declining with daytime warming; timing of measurements should be carefully considered. Collections should be protected from moisture ingress during periods of high ambient humidity, especially nocturnal intervals.

D.6. CHECKLIST OF THE IMPORTANT MATERIAL FOR FIELD VISITS:

1. **GENERAL DOCUMENTS:** bring permits and authorization, personal and vehicular documentation.

2. **CLOTHES:** suitable footwear for the terrain, water-proofs and hats. Gloves without finger ends (useful for cold locations)

3. **NAVIGATION:** bring maps, GPS, compass (if needed), altimeter (if needed), batteries.

4. **SAFETY:** include first aid kit, sunscreen, insect repellent, water in cool or hot flasks (depending on locations), spare car keys, mobile phone and its charger, power bank, two-way radio device.

5. **HABITAT AND SPECIES IDENTIFICATION:** flora and botanical field guides, list of targeted species for your trip, lenses (10x and 20x), IUCN or EUNIS habitat classification document for your terrain.

6. **SEED / HERBARIUM SPECIMEN COLLECTING EQUIPMENT:** bring a backpack, a passport data form for plants collected, binoculars, camera and batteries (with films if needed), label tags, staplers, pencils, pens, bags (different sizes and types – cloth, plastic and paper), sieves, trays, large white papers, forceps if needed, shears, scissors, work gloves, notebook, voice recorder (or handheld computer if available), pocket knife, measuring tape, silica gel (to dry seeds and samples for DNA collection), large plastic bags (to store herbarium material for a few hours), large newspapers and portable press (for herbarium).

7. **OTHER USEFUL EQUIPMENT:** flashlight, sunglasses, car (4x4 with sufficient storing capacity and spare parts).

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02

PITFALL TRAPPING PROCEDURE

PITFALL TRAPPING PROCEDURE

A TARGET ORGANISMS

HYMENOPTERA
(ants)

ARACHNIDA
(spiders, mites)

COLLEMBOLA
(springtails)

COLEOPTERA
(beetles)

CHILOPODA
(centipedes)

DIPLOPODA
(millipedes)

ORTHOPTERA
(grasshoppers, crickets)

B STANDARD EQUIPMENT FOR PITFALL TRAPPING

PER PITFALL:

- Clear plastic cup, Styrofoam plate, 3-4 skewers, wire excluder
- Trowel/spade (for digging)
- Soapy water or ethanol (for collection)
- Small aquatic/aquarium net
- Sample jar with ethanol (for preservation)

C FIELD SAMPLING PROCEDURE FOR PITFALL TRAPS

1. Excavate a hole in the ground corresponding to the diameter and depth of the plastic cup.
2. Insert the plastic cup into the hole such that the rim is level with the surrounding ground surface. Minimize disturbance to the adjacent substrate to ensure a smooth transition between the natural ground and the pitfall trap. If the hole is oversized, backfill any gaps between the cup and the soil.
3. Remove any debris from the cup and fill it to approximately half its volume with soapy water. The detergent reduces surface tension, allowing insects to be trapped without immediate preservation; therefore, specimens must be collected at least every two days. Ethanol may be used as an alternative killing and preservation agent, but traps must be monitored more frequently due to rapid evaporation.
4. To reduce the accidental capture of small vertebrates (e.g., amphibians or rodents), install a wire excluder over the pitfall opening.
5. Construct a protective cover using 3–4 skewers and a Styrofoam plate to prevent flooding during rainfall. Position the plate approximately 5 inches above the trap, inserting the skewers through the wire excluder to secure the structure.
6. Collect specimens at intervals no longer than two days. More frequent monitoring is required when using ethanol or following heavy rainfall. During collection, remove the cover and wire excluder, then extract the plastic cup from the ground.
7. Decant the contents of the cup into a fine-mesh aquatic or aquarium net. Samples from multiple pitfall traps within the same sampling unit (e.g., a transect) may be combined into a single net. If ethanol is used instead of soapy water, pour the contents directly into a labeled sample jar, using a funnel if necessary.
8. Reinstall the pitfall trap by refilling the cup with soapy water and replacing the cover and excluder in preparation for the next sampling period.
9. Rinse the contents retained in the net with water to remove residual soap, then transfer the specimens into a sample jar containing ethanol. Nitrile or rubber gloves should be worn if hands come into contact with ethanol.
10. Label each sample jar with the collection date and locality, along with any additional relevant information (e.g., duration of sampling, number of pooled pitfalls).
11. Upon completion of the trapping period, backfill all pitfall holes to restore the site and minimize habitat disturbance.

Figure 1 Steps of the Pitfall Trap construction and usage. Curtesy of the University of Guelph CDB, Canada.

REFERENCE

This protocol was extracted from the original document “Pitfall Trapping Protocol” prepared by the Centre for Biodiversity Genomics (CBG), University of Guelph,Ontario, Canada.

03

HERBARIUM COLLECTION AND PREPARATION

HERBARIUM COLLECTION AND PREPARATION

SUMMARY

The preparation of herbarium specimens begins with the accurate identification of plant species using reliable botanical references. In Lebanon, key references include the works of (Mouterde, 1984; Tohmé, G., Tohmé, H., 2014). For each specimen collected, it is essential to record its GPS coordinates, and to photograph the plant, its habitat, and surrounding vegetation. Collected specimens should include as many organs as possible buds, leaves, flowers, and fruits and must be carefully cut using pruning shears prior to pressing.

A PERMIT REQUIREMENTS FOR COLLECTING IN PROTECTED AREAS

If you plan to collect plant specimens in a nature reserve or protected area, it is essential to obtain the necessary collection permits or official approvals in advance.

A copy of the permit (printed or photographed) must accompany any specimens submitted to the herbarium. These permits typically come with strict conditions, including the requirement to notify the local ranger or relevant authorities prior to your visit.

Permit details should be clearly documented with each collection, and the permit number must be included on the specimen labels to ensure proper traceability and compliance with conservation regulations.

B FIELD SAFETY AND PERSONAL PROTECTIVE EQUIPMENT (PPE)

When conducting surveys or collections in the field, it is highly recommended to carry appropriate personal protective equipment and safety essentials, including:

SUNSCREEN

A WIDE-BRIMMED HAT

LONG-SLEEVED SHIRT AND LONG TROUSERS

STURDY, CLOSED-TOE FOOTWEAR

GLOVES
(particularly useful when handling some types of allergy-inducing parts of plants)

INSECT REPELLENT

FIRST AID KIT

SUFFICIENT WATER AND FOOD

GPS DEVICE

C HEALTH AND SAFETY CONSIDERATIONS

Some individuals may experience allergic reactions, triggered by the plant itself or by bacteria, molds, or other organisms growing on it. For this reason, handwashing after handling these plants is strongly recommended, in addition to wearing gloves while handling.

Tick prevention is important in many areas; the use of DEET-based repellents or electronic tick deterrent devices has proven effective. Be vigilant for snakes, spiders, and insects, particularly when working in dense vegetation or leaf litter.

It is advisable that field personnel receive basic first aid training, especially regarding the management of bites, stings, and allergic reactions.

D COLLECTION PERIOD

Herbarium specimens are collected at their flowering period. To do that, check in advance the flowering periods of all the species that are targeted for the herbarium to ensure that specimens are blooming at the time of collection. Flowering plants are generally collected for herbariums during springtime, when most are in bloom and different species have different flowering brackets (from a few days to a few weeks).

D.1. STANDARD EQUIPMENT FOR PLANT FIELDWORK:

- **CAMERA AND TRIPOD**
- **NOTEBOOKS, PENS AND/OR PENCILS**
- **HAND LENS**
- **POCKET KNIFE**
- **PRUNING SHEARS**
- **MIRROR** (dental or automotive telescopic inspection mirrors have proven to be very effective)
- **SMALL TROWEL FOR DIGGING UP FRUIT BODIES**
- **GPS UNIT AND/OR MAPS/ SMART PHONE**
- **RULER FOR PROVIDING SCALE IN PHOTOS**
- **TAGS FOR LABELLING COLLECTIONS.** These may be QMS numbered tags with scale, or jewellers' tags or simple sticker name tags
- **STORAGE CONTAINERS FOR SOME SPECIMENS**
- **NEWSPAPERS AND OLD UNWANTED JOURNAL PAPERS**
- **WOODEN PRESS WITH STRAPS**
- **MASKING TAPE** (if needed to secure in place some specimens)

E COLLECTION OF PLANT MATERIAL AS HERBARIUM SPECIMENS

E.1. FIELD IDENTIFICATION AND DOCUMENTATION:

BOTANICAL REFERENCES

Always consult botanical references appropriate for the region in which the collection is being conducted. These are essential for accurate plant identification. Identification is typically based on floral characteristics, followed by leaf morphology. However, all visible organs should be considered.

ORGAN-SPECIFIC GUIDELINES

Conifers: Examine leaf shape, cone structure, and overall tree form.
Fruiting Trees: Note the presence of flowers, fruits, and foliage.
Herbaceous Plants: Assess both flowers and leaves to aid identification.

IN-SITU IDENTIFICATION

Whenever possible, identify plant species in the field. Record the following data for each specimen:
- Scientific name
- Date of observation
- Name of the region or locality
- GPS coordinates of the collection site

PHOTOGRAPHY

Photographic documentation is critical for future reference and can assist in re-locating individual plants during subsequent field visits, as well as in understanding the ecological context of the species. Take multiple high-quality photographs for each plant, regardless of whether it has been identified:
- A full view of the plant (entire structure for trees or whole form for small plants)
- Close-ups of identifiable organs (flowers, leaves, buds, fruits)
- The immediate habitat and surrounding vegetation

To ensure the collection of high-quality herbarium specimens, the following best practices should be observed:

E.2. CUTTING TECHNIQUE:

Once the plant has been properly identified, carefully cut the specimen using clean and sharp pruning shears. Avoid pulling or tearing the plant, as this can cause damage to the stem and compromise the integrity of the sample. If multiple specimens of the same species are needed for replication purposes, it is acceptable to collect several individuals, provided they are from the same population and in good condition.

- Use clean, sharp pruning shears to cut specimens. Aim to collect complete individuals or branches that include:

- Specimens should be placed between ventilated plant presses or drying frames as soon as possible after collection to preserve their structural integrity and botanical value.

E.2.1. COLLECTION BY PLANT TYPE

- **Herbaceous Plants (Flowering and Non-flowering):** Collect the entire plant by cutting from the base of the stem, ensuring the specimen includes all essential organs: leaves, flowers, buds, and fruits (if available). The presence of intact flowers and leaves is crucial for proper identification. Select specimens that are healthy and free from damage or parasitism.

- **Woody Plants and Trees:** When collecting from larger shrubs or trees, take a representative branch of a reasonable length that includes flowers, leaves, buds, and fruits if possible. Ensure the cut does not harm the long-term health of the plant.

E.2.2. ETHICAL CONSIDERATIONS:

For species that are rare, endangered, or of conservation concern, refrain from collecting whole plants. In such cases, non-destructive methods of documentation (photography, GPS tagging, habitat notes) should be prioritized, and collection should only proceed with appropriate permits and ethical justification.

E.2.3. NOTE ON DNA SAMPLING:

For molecular analysis, a separate leaf sample may be collected for DNA extraction. This should be placed immediately into a sealed container with silica gel desiccant. The leaf may be in direct contact with the silica beads or separated by a thin layer of sterile cotton. This method ensures rapid dehydration of the tissue, preserving its genetic material for future laboratory analysis.

F FIELD PRESSING AND DOCUMENTATION OF PLANT SPECIMENS

During fieldwork, plant specimens intended for herbarium preservation must be carefully pressed and transported in light, portable field presses. Proper handling and documentation at this stage are essential to maintain the scientific value and physical quality of the specimens.

F.1. FIELD PRESSING PROCEDURE:

F.1.1. INITIAL PLACEMENT:

Immediately after collection (see Step 2), place each plant specimen between sheets of newspaper. These sheets serve as an initial pressing medium and help absorb moisture. The specimens, once inserted, should be placed within portable plant presses or wooden frames that allow for adequate ventilation and ease of transport.

F.1.2. LABELING AND DOCUMENTATION:

For each specimen, clearly record the following information:

- **DATE OF COLLECTION**
- **EXACT LOCATION** (region, GPS coordinates if possible)
- **FULL SCIENTIFIC NAME** (in Latin)
- **NAME OF THE COLLECTOR**
- **ADDITIONAL OBSERVATIONS** (e.g. habitat, abundance, associated species)

This data can be:

- Written directly on the newspaper sheet
- Attached on an index card placed with the specimen
- Recorded in a dedicated field notebook, provided that each specimen is labeled with a unique code or number to allow accurate cross-referencing

F.2. SPECIMEN ARRANGEMENT:

F.2.1. ORGAN POSITIONING:

Carefully arrange all visible plant organs (leaves, flowers, fruits, buds) in a natural, spread-out manner to avoid overlapping or wrinkling:

- If the specimen has a single flower, position it face up (toward the collector), especially if the flower displays distinctive patterns, colors, or structural traits.
- For specimens with multiple flowers, orient some blossoms facing up and others facing down to capture details of both the adaxial (upper) and abaxial (lower) surfaces.
- Handle flowers delicately, as petals of some species may be extremely fragile and prone to tearing or distortion.

F.2.2. INCLUSION OF BUDS AND IMMATURE STRUCTURES:

If flower buds are present, consider collecting and pressing them alongside mature blooms. This provides a more complete morphological representation of the species.

F.2.3. LEAF PRESENTATION:

Leaves are critical for plant identification and must be arranged to show clear detail:

- Spread clustered leaves to reduce overlap
- Ensure all leaves lie flat and do not curl or fold
- In specimens with numerous leaves, invert a few to expose their lower (abaxial) surfaces, revealing distinguishing characteristics such as venation, pilosity, coloration, or the presence of glands or spines

Proper arrangement and documentation at this stage ensure that the specimens retain their diagnostic features, which are essential for future identification, research, and archiving in herbarium collections.

G PRESSING, TRANSPORTING, AND LABORATORY CARE OF HERBARIUM SPECIMENS

During fieldwork, plant specimens intended for herbarium preservation must be carefully pressed and transported in light, portable field presses. Proper handling and documentation at this stage are essential to maintain the scientific value and physical quality of the specimens.

G.1. FIELD PRESSING AND TRANSPORT:

After arranging the specimens between sheets of newspaper as described in the previous step, carefully fold the newspaper sheets over the plant material. These should then be inserted between two rigid wooden frames or plant press boards. Multiple specimens (each in their own newspaper) can be stacked together within the same press.

Secure the frames using adjustable straps or belts, tightening them enough to apply uniform pressure without damaging the specimens. This pressing method ensures that the plant material is preserved in a flattened state and facilitates safe and convenient transport to the laboratory or next collection site.

G.2. POST-FIELD LABORATORY PROCESSING:

Once in the laboratory, ideally on the same day or no later than the next—conduct the following procedures:

G.2.1. SPECIMEN REVIEW AND ADJUSTMENT:

Carefully reopen the newspapers and examine each specimen. Adjust any misaligned plant parts (e.g., leaves, stems, flowers) to ensure they are lying flat and properly oriented. Then, return the specimens to the press or place them under flat weights to continue drying.

G.2.2. MOISTURE MANAGEMENT:

Every 24 hours, remove the specimens from the press and inspect the newspapers for signs of moisture or damping. Even slight dampness is sufficient reason to replace the newspaper with fresh, dry sheets. This step is essential to prevent mold and decay and to facilitate efficient drying.

G.2.3. MONITORING FOR DAMAGE OR INFESTATION:

Examine each specimen for signs of parasitism (e.g., mites, insects) or fungal growth. Any specimen showing advanced decay or infestation that threatens the integrity of the rest of the collection should be removed and, if possible, recollected at a later time.

G.2.4. DRYING PERIOD:

Continue changing newspapers daily, or as needed, until the specimens are fully dried. Fully dried specimens will no longer transfer moisture to the newspaper. At this point, the specimen is ready to be mounted or stored in final herbarium sheets.

G.2.5. FINAL PREPARATION FOR STORAGE:

Once dry, transfer each specimen into clean herbarium paper and include a data label that clearly presents the following information (see fig.1 & 5):

- Scientific name (Latin binomial)
- Name of the collector
- Date of collection
- Geographic location (including GPS coordinates)
- Description of the specimen and habitat
- Additional notes, if relevant

H SPECIAL HANDLING INSTRUCTIONS: FERN SPECIMENS

Ferns require particular care due to their delicate structure and rapid wilting:

H.1. FIELD COLLECTION AND TRANSPORT:

- Collect ferns from humid, shaded areas, avoiding exposure to direct sunlight.
- Select healthy specimens with intact fronds and, if possible, visible sporangia on the undersides. Fructification periods should be considered when planning fieldwork.
- Use shears to cut specimens cleanly—do not pull or tear.
- When appropriate, include rhizomes (see fig. 5).
- Store collected ferns in plastic sandwich bags to retain moisture. Keep them out of heat and direct sunlight. If available, use a portable cooler or refrigerated storage to preserve freshness during transport.

H.2. DOCUMENTATION:

- Record detailed information: scientific name, date, GPS coordinates, collector's name, site description, and microhabitat.
- Take high-quality photographs of various parts of the fern: fronds, fiddleheads (young, curled fronds), mature and juvenile forms, and any visible reproductive structures (sporophytes).

H.3. LABORATORY PROCESSING:

- Upon arrival in the laboratory, press the ferns between newspapers following the same procedures outlined above (see figs 2, 3 & 4)
- Do not fold fronds to expose both sides within a single specimen. Instead, collect multiple individuals from the same species, arranging some with the upper surface visible and others with the lower surface visible. This ensures diagnostic details (e.g., venation, texture, spore arrangement) are preserved without distorting the specimen.

Figure 1 example of a flowering plant in herbarium, along with corresponding labels. Courtesy of Royal Botanic Gardens of Kew.

Figure 2 Adiantum capillus-veneris fern placed in newspaper where it slowly dries. Note how its leaflets are not folded. source: Jouzour Loubnan Seed Bank

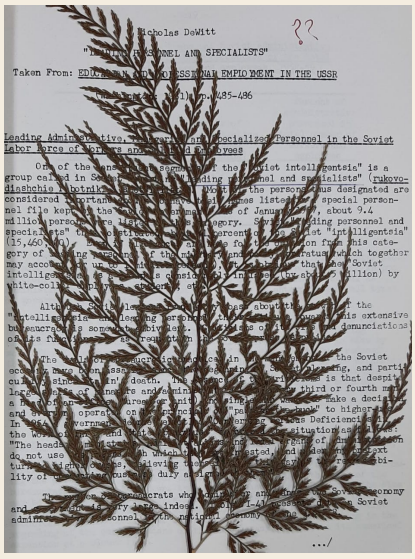

Figure 3 another example of an unidentified fern sample drying. Note that leaflets are as much as possible separated and flat. source: Jouzour Loubnan Seed Bank.

Figure 4 Example of a fern sample drying between newspapers, with leaflets still a bit entangled and folded in places. Source: Jouzour Loubnan Seed Bank.

Figure 5 Example of specimens on clear herbarium paper ready for storage; including several individuals of the same species as well as their rhizome. source: Jouzour Loubnan Seed Bank.

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04

FUNGARIUM COLLECTION AND PREPARATION

FUNGARIUM COLLECTION AND PREPARATION

A PERMIT REQUIREMENTS FOR COLLECTING IN PROTECTED AREAS

If you plan to collect fungal specimens in a nature reserve or protected area, it is essential to obtain the necessary collection permits or official approvals in advance.

A copy of the permit (printed or photographed) must accompany any specimens submitted to the fungarium. These permits typically come with strict conditions, including the requirement to notify the local ranger or relevant authorities prior to your visit.

Permit details should be clearly documented with each collection, and the permit number must be included on the specimen labels to ensure proper traceability and compliance with conservation regulations.

B FIELD SAFETY AND PERSONAL PROTECTIVE EQUIPMENT (PPE)

When conducting fungal surveys or collections in the field, it is highly recommended to carry appropriate personal protective equipment and safety essentials, including:

SUNSCREEN

A WIDE-BRIMMED HAT

LONG-SLEEVED SHIRT AND LONG TROUSERS

STURDY, CLOSED-TOE FOOTWEAR

GLOVES
(particularly useful when handling some types of allergy-inducing parts of plants)

INSECT REPELLENT

FIRST AID KIT

SUFFICIENT WATER AND FOOD

C HEALTH AND SAFETY CONSIDERATIONS

While the risk of poisoning from handling fungi is minimal, tasting is strongly discouraged, especially for beginners. Serious illness and fatalities have occurred in Lebanon due to the ingestion of toxic fungi.

Some individuals may experience skin or respiratory allergic reactions, triggered by the fungus itself or by bacteria, molds, or other organisms growing on the fruiting body. For this reason, handwashing after handling fungi is strongly recommended.

Tick prevention is important in many areas; the use of DEET-based repellents or electronic tick deterrent devices has proven effective. Be vigilant for snakes, spiders, and insects, particularly when working in dense vegetation or leaf litter.
It is advisable that field personnel receive basic first aid training, especially regarding the management of bites, stings, and allergic reactions.

D COLLECTION PERIOD

Most fungal species produce fruiting bodies (sporocarps) that persist for a few hours to several days, although some hard bracket fungi can remain for many years. Fruiting may occur at any time of the year, depending on the species, provided that moisture levels are adequate and temperatures are favorable. Typically, a peak in fruiting activity is preceded by a gradual increase in fungal productivity over several days or weeks. These flushes of activity are often triggered by rainfall events.

E STANDARD EQUIPMENT FOR FUNGAL FIELDWORK

CAMERA AND TRIPOD

NOTEBOOKS, PENS AND/OR PENCILS

HAND LENS

POCKET KNIFE

MIRROR
(dental or automotive telescopic inspection mirrors have proven to be very effective)

SMALL TROWEL FOR DIGGING UP FRUIT BODIES

TRUFFLE RAKE

PAINT BRUSH TO DUST OFF SOIL

SCISSORS TO CUT GRASS AND NUMBER TAGS (if not cut previously)

GPS UNIT AND/OR MAPS/ SMART PHONE

RULER FOR PROVIDING SCALE IN PHOTOS

TAGS FOR LABELLING COLLECTIONS. These may be QMS numbered tags with scale, or jewellers' tags

STORAGE CONTAINERS

ESKY WITH ICE BRICKS TO STORE SPECIMENS
(not directly on the ice brick).

F COLLECTION OF FUNGAL MATERIAL AS FUNGARIUM SPECIMENS

F.1. SELECTING THE FRUITING BODIES:

- **Select fruit bodies in good condition,** avoiding specimens that are over-mature, decayed, deliquescent, infested with maggots, dried out, withered, or extensively damaged by herbivory (see Figure 1).
- **Aim to collect fruit bodies at various developmental stages**, from immature (button stage) to fully mature specimens with open caps. The majority of the collection should consist of specimens that are sufficiently developed to be fertile. If a microscope is available, the presence of spores can be used to confirm fertility.
- **Ensure that each specimen includes all morphological features,** such as the cap, hymenium (spore-bearing tissue), stipe, ring, and volva when present. Where feasible, include a small portion of the mycelium.
- **Ideally, collect fruit bodies from a single substrate or defined location,** such as the same log, the trunk of a single tree, or the same patch of lawn around one tree. Limiting the collection area to approximately one square meter reduces the risk of assembling a mixed-species collection.
- **Avoid mixing specimens from different collection sites** to maintain the integrity and scientific value of each sample.
- **When possible, collect duplicate specimens.** Many herbaria maintain duplicate collections for exchange or distribution to specialists working on specific genera or taxonomic groups.

F.2. HOW MANY FRUITING BODIES TO COLLECT?

- New genetic techniques have increased the need for well-annotated and correctly identified specimens (the mycologist's role) to be stored in herbaria and collected in sufficient quantities to allow limited destructive sampling. These specimens need to be processed and stored so that the DNA is preserved for future study.

- The number of fruit bodies required will depend on the size of the species: smaller fungi require larger sample sizes to capture morphological variation and allow for proper study.

- As a general guideline, aim to collect at least 20 fruit bodies of small species (e.g., Marasmius, Mycena), 5–10 fruit bodies of medium-sized species (e.g., Russula), and a single specimen of large fungi may suffice. For exceptionally large species (e.g., Phlebopus marginatus), a representative cross-section may be adequate.

- Photograph all specimens to document morphological variation, including features such as color changes, chemical reactions, and texture. Each photograph should be clearly labeled or otherwise associated with the corresponding specimen to ensure accurate referencing.

Figure 1 examples of an adequate collection of fruiting bodies. Source ©Christine Bailly / Presse.inserm.fr 2020

F.3. COLLECTION METHOD:

Use a knife or trowel to carefully excavate the base of the stipe, ensuring it remains intact along with some attached mycelium and substrate. Take care not to leave behind important underground structures—some fungi, such as Laccocephalum species, develop large subterranean sclerotia, which are essential for identification but may be missed if not fully excavated.

Small gilled fungi (agarics), including genera like Marasmius and Mycena, often feature a distinctive basal disc that should be preserved during collection.

In the case of small Ascomycetes, the substrate should also be collected to observe the point and nature of attachment. This is especially important for species such as bird's nest fungi, which possess an "emplacement" or foot—an important diagnostic feature (see Figure 2).

For fungi growing in soil, use a small trowel or pocketknife to excavate the specimen completely. Avoid pulling fungi from the ground by hand, as this often results in a broken stipe and loss of key diagnostic features at the base. If full excavation is not possible, collect a portion of the substrate with the fungus still attached.

Fungi growing on wood can be collected either by removing the entire piece of wood (if manageable in size) or by carefully cutting or chiseling away a thin section of substrate containing the specimen. A high-quality pocketknife is usually sufficient for this task.

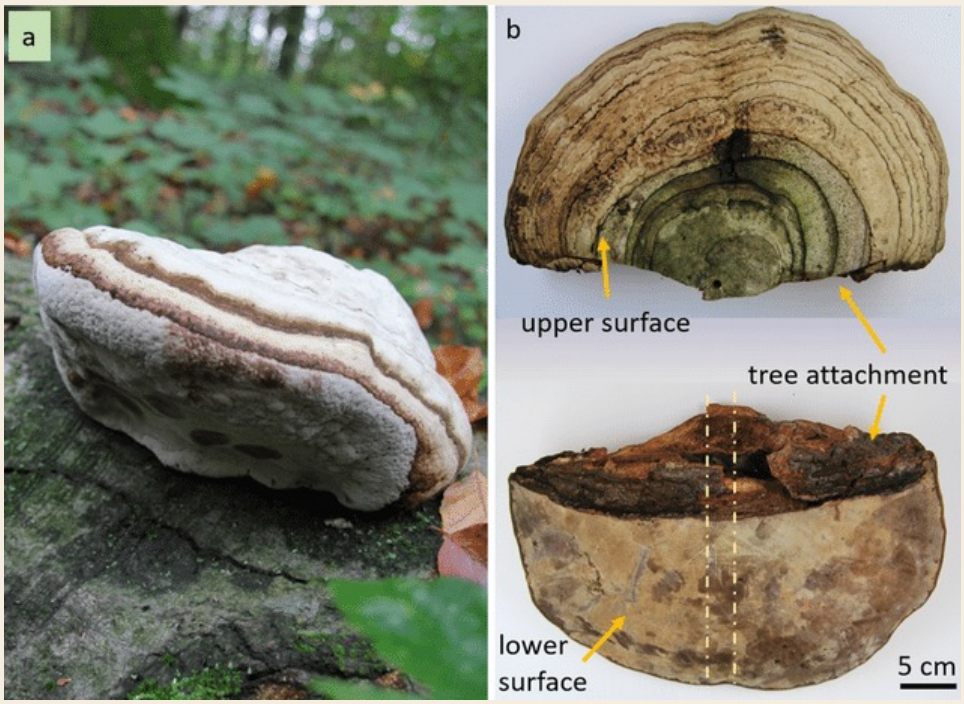

Figure 2 Examples of Fomes fomentarius mushroom collections with their attachment points. a) F. fomentarius growing on fallen log. b) different surfces of the fungi. Some may have small quantity of their substrates included. Source: Müller, Klemm & Fleck 2021. https://doi.org/10.1007/s00339-020-04270-2

G TRANSPORTING AND STORING FUNGARIUM SPECIMENS

F.1. SELECTING THE FRUITING BODIES:

- Do not store fresh fungi in plastic bags. Instead, specimens may be wrapped in waxed paper or aluminum foil for transport from the field to the processing location.
- Clean, compartmentalized toolboxes or fishing tackle boxes are highly useful for collecting and safely transporting small or fragile specimens (see Figure 3).
- Alternatively, takeaway food containers, ice cream tubs, or sample jars can be used as transport vessels.
- To prevent cross-contamination of spores, it is essential that fruit bodies from different collections do not come into contact with each other. When using compartmentalized boxes, take care to avoid turning them over.
- To avoid specimen mix-ups, label each collection immediately upon collection. At a minimum, labels should include the date, location, collection number, and collector's initials, and must always remain with the specimen.
- In warm conditions or when collections will be stored for over an hour before processing, using an insulated bag or cooler (Esky) can help keep specimens cool and slow deterioration.
- Note that some fungi deteriorate rapidly regardless of storage conditions, and others may harbor insect larvae that are initially inconspicuous but can develop into maggot infestations by the time you return to base.
- Do not press fungi. Specimens are dried whole, as outlined in 'Drying Specimens'.

Figure 3 Organizing fungi collected in a tackle box. Source: The Guardian, 2024.
https://www.theguardian.com/environment/2024/jan/05/mushrooms-amazon-ecuador-rare-fungi-aoe

H FIELD NOTES (CHECK APPENDIX 1)

Accurate and detailed field notes are just as essential as the fungal specimens themselves. Undocumented or poorly documented collections hold limited scientific value. Specimens may be used in future research by other mycologists and thus require comprehensive contextual information. While photographs are helpful, they are often insufficient on their own and may degrade or be lost over time. A written record is indispensable.

H.1. DOCUMENTATION OF FUNGAL SPECIMENS:

PROVISIONAL IDENTIFICATION
(temporary name of the fungus)

COLLECTION NUMBER (ID)

DATE OF COLLECTION

PRECISE LOCALITY AND GEOGRAPHIC COORDINATES
(include the name of the old municipality, if applicable)

HABITAT DESCRIPTION, INCLUDING:
- Vegetation type and plant species present
- Accompanying fungi
- Nearest tree species

SUBSTRATE (e.g. soil, leaf litter, dead or living wood). If on wood, specify whether it is bark-covered or exposed.

ODOR, COLOR, and any observable **COLOR CHANGES** over time

NAMES OF ALL ACCOMPANYING COLLECTORS

When collecting mushrooms, carefully note the type of substrate they are growing on and the surrounding vegetation, as many fungi are substrate-specific or form associations with particular tree species.

H.2. IMPORTANT NOTE ON GEOLOCATION DATA:

Include GPS coordinates in the form of latitude and longitude, or other geocoding formats such as easting/northing or a detailed map reference. This enables accurate georeferencing of your collection site.

Important:

Always submit field notes or specimen labels along with the corresponding specimen. Failing to do so makes it difficult—and sometimes impossible—to correctly associate data with samples, which may result in the specimen being discarded.

1 PHOTOGRAPHY

- Photography is a very useful method for recording the appearance of fleshy fungi, especially colour, texture and other features that are lost on drying. Benefits include showing the specimens in their natural habitat in natural daylight.
- Take an initial photo with the specimen in situ, without disturbing the surroundings. Include a number tag and a scale. It is best to place the tag so that it can be cropped out of the image. If using jeweler's tags, write the number on them with a felt pen.
- Take another photo with distractions removed but try to maintain a natural look to the location.
- Do not shift the specimen to a different substrate, e.g. from soil onto a log, even though this may give a better photo, it will convey misleading information.
- The photographs should show all aspects of the fruit body: cap surface, margin, fertile surface, stipe, and any other unusual, distinctive or interesting features.
- If you are collecting several fruit bodies, you can show all views by creating a "group" shot. Do this by laying some on their side, some upside down and leaving some in situ. This will allow the cap, gills and stipe to be visible in a single photo. You can take close shots of ornamentations on all parts of the fruiting body, as well as the texture of the cap and the stipe (see Figure 4).
- You can also cut the mushroom in half in length to take pictures of the flesh. Cutting the mushroom should be quick, careful and neat (see Figure 5).
- A picture displaying the gill attachment can be obtained from a longitudinally sectioned cap.
- Natural backgrounds may not provide sufficient contrast to display the details of the specimen.
- A dull grey card often works well as a background for lab shots. These shots can be taken later, at home or in the lab, utilizing a "light box".

Figure 4 Shots of fungi specimens in-situ on the field as well as using a light box in the lab. Sources: fundis.org & Prance et al., 2017.

Figure 5 Example of cut mushrooms with their flesh changing color as it comes into contact with air Gyroporus cyanescens. Source: © Pauline Sutter, 2025. https://actus-limousin.fr/tourisme-et-patrimoine/2025/10/24/premiere-fois-pauline-decouvre-enfin-le-monde-merveilleux-des-champignons-dans-la-foret-de-sedieres/ .

After downloading your photos, give the files meaningful names that will allow the images to be readily matched with the specimens.

J WRITING A DESCRIPTION

Many characters of the fresh fruit body are lost when specimens are dried. A detailed written description is a useful way of recording such information while you are still processing the specimens. The description should provide information on the size, shape, color, texture, and surface features of all parts. Use language you are comfortable with. As you become more experienced, the technical terms will become more familiar. It is better to use accurate plain English than to get confused using an incorrect technical term. See Annex 1 for a sample record sheet (see Figures 10 and 11).

- Shape and size e.g. shape, diameter & height of pileus, length and diameter of stipe, and the radius measurement (distance it projects from the substrate) of a bracket polypore should be recorded. Take a range of measurements using the smallest and largest mature specimens.
- Examination with hand lens will enable you to describe the indumentum (surface covering).
- A longitudinal section through the fruit body will allow you to describe the gill attachment and the colour and texture of the cap and stipe flesh, and any bruising reactions that may occur.
- Describe colour changes occurring as a result of handling, cutting, or drying out – both externally and internally and don't forget to describe the smell.
- Make note of the substrate, habitat and associated plant species.

Many collectors describe colours by referring to standard reference charts. If you are using a chart, please name the chart, name the colour, and add the colour / numeric code to your notes. Many collectors today use the Online Auction Colour chart available from the Fungimap bookstore

http://fungimap.org.au/index.php/bookshop.

J.1.

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.

J.2.

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).

J.3.

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.

J.4.

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.

J.5.

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.

K POST-FIELD AND LABORATORY CARE FOR FUNGARIUM SPECIMENS

K.1. DRYING FUNGAL SPECIMENS:

Dry specimens as soon as possible after completing photographs and descriptive notes. While it is generally best practice to avoid collecting more specimens than can be processed within the same day, delays may occasionally be unavoidable.

If immediate drying is not possible, store specimens temporarily in a refrigerator for up to 48 hours. Ensure the storage containers are breathable and do not trap moisture, which can cause the specimens to "sweat" and degrade. This method is only effective if the fungi were in good condition at the time of collection.

However, refrigeration is not suitable for all fungi, particularly for fleshy species such as boletes or auto-digesting agarics, which deteriorate rapidly. With experience, you will learn to distinguish between species that can tolerate short-term storage and those that must be dried immediately.

Important:

Always submit field notes or specimen labels along with the corresponding specimen. Failing to do so makes it difficult—and sometimes impossible—to correctly associate data with samples, which may result in the specimen being discarded.

- Fungi should be dried using gentle, controlled heat to preserve their structural integrity and, where relevant, DNA quality. Food dehydrators and clothes drying cabinets are among the most effective and reliable options. Commercial fruit and vegetable dehydrators are particularly suitable, as they provide consistent airflow and temperature control (see Figure 9).
- Alternatively, specimens can be placed on a rack in front of a heater with a fan, over ducted heating vents, or near a heat source such as an electric or tent heater, hot plate, light bulb, kerosene stove, or lantern. The heat should be directed upward, creating a chimney effect that allows moist air to escape. In dry, sunny weather, small specimens can sometimes be air-dried by placing them in a paper bag pegged to a clothesline, though this method is less reliable and not suitable for all species.
- Regardless of the method, it is critical that specimens are dried slowly and never cooked. The drying temperature should not exceed 65°C, and for specimens intended for DNA extraction, a lower temperature range of 42–55°C is recommended. The drying process is complete only when the fungi are crisp and brittle. Avoid placing specimens in enclosed, oven-like spaces, which can cause them to bake and become unusable.
- **Important:** Never press mushroom specimens between newspaper sheets or in a plant press, as this will destroy essential morphological features.
- Once fully dried, fungal specimens must be kept in a dry environment to prevent rehydration, which can lead to mold development and render the material scientifically unusable. To maintain

- dryness, it is advisable to transfer freshly dried specimens directly from the dryer into sealed plastic bags (see example below, image courtesy of G. Mueller, Field Museum) that are appropriately sized to accommodate the specimens without compression.
- In high-humidity environments, the addition of a small quantity of desiccant (e.g., silica gel) to the storage container can provide additional protection against moisture absorption and fungal deterioration. Particularly delicate or fragile specimens may also be dried and stored within airtight containers containing activated silica gel or other suitable desiccants to ensure both structural preservation and long-term viability.
- Larger fruit bodies (with stipes >5 mm in diameter) should be sectioned into halves or quarters to accelerate and ensure even drying.
- Remove excess soil from the base of the stipe using a soft brush, taking care not to damage diagnostic features.
- Keep each collection separate at all times and ensure the identification label or reference number remains attached to its corresponding specimen throughout the drying and storage process.
- Do not press fungal specimens as you would vascular plants, as this will distort or destroy key morphological features.
- If using a food dehydrator, always turn off the unit before removing the lid, as the air current may displace lightweight or fragile specimens.
- Ensure that drying stations are kept out of reach of small children and pets to prevent contamination, loss, or injury.
- The duration of drying will vary depending on the species, as well as the size, shape, thickness, and type of fruit body, along with ambient humidity and the efficiency of the drying apparatus. Drying may take a few hours, overnight, or several days.
- Properly dried specimens will be crisp and brittle; the stipe should feel firm and stiff. If the material remains pliable, it is not fully dried.
- To confirm dryness, weigh the specimen, return it to the dryer for 1–2 additional hours, then weigh it again. When the weight remains consistent between measurements, the specimen can be considered fully dried. Note: Transfer specimens quickly between the dryer and the scale to avoid moisture reabsorption from ambient air.

Figure 9 Drying large (top right) and small (bottom left) fungi, using a food dryer machine. Source: Prance et al., 2017.

K.2. MAKING A SPORE PRINT:

As the name indicates, spore prints are pieces of paper covered with mushroom spore deposits. A spore print is useful for establishing the colour of the spores and can be used as a source of spores for microscopic examination. Prints can be obtained from gilled, pored, toothed and, with perseverance, coralloid fungi. It is not necessary to get a spore print from puffballs and earth stars as these fungi produce copious masses of spores.

1. Place the fruit body on a piece of white paper with the fertile surface facing down. Use plain white paper. You may need to separate the cap from the stipe or you may be able to stand the mushroom up in a glass and use paper with a hole cut for the stipe (see Figures 6 and 7). 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). 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. Spore prints can also be made directly onto glass microscope slides, although for storage purposes, those on paper are preferred (see Figures 7 and 8).

2. Note that polypores have their spores in tubes, so ensure the tubes are vertical so the spores drop. If the tubes are not perpendicular to the paper or slide, the spores may get stuck in the tubes.

3. Place a small amount of wet tissue paper on the cap and cover the specimen with a moisture-resistant container (e.g., a drinking glass or jar or plastic sandwich bag; even wrapping in wax paper can be sufficient). Leave for a few hours or overnight (see Figure 6).

4. Circle the outline of the specimen to help identify light-colored spores on the paper.

5. Fold the paper in half with the spore print facing inward. Label it with the collector's initials, the collection number, and the date, then place it on the drying rack with the specimen.

6. Avoid using acetate for spore prints. Although it can help show spore color, it is not suitable for herbarium storage. Instead, note the spore color and discard the acetate.

7. For microscopic study, transfer some spores from the original spore print (the "master" slide) to a temporary slide for easier examination, which can be discarded afterward.

8. To preserve the master slide, secure a cover slip over the spores with tape and place the slide in a protective slide mailer. Make sure both the slide and mailer are clearly labeled with the specimen information.

Figure 6 Using a fruiting body with wet tissue paper, and waterproof cover. Source: Santa Cruz Museum. https://santacruzmuseum.org/how-to-make-a-spore-print/

Figure 7 different spore prints: on white paper (left) and on a glass slide (right). Source: Prance et al., 2017

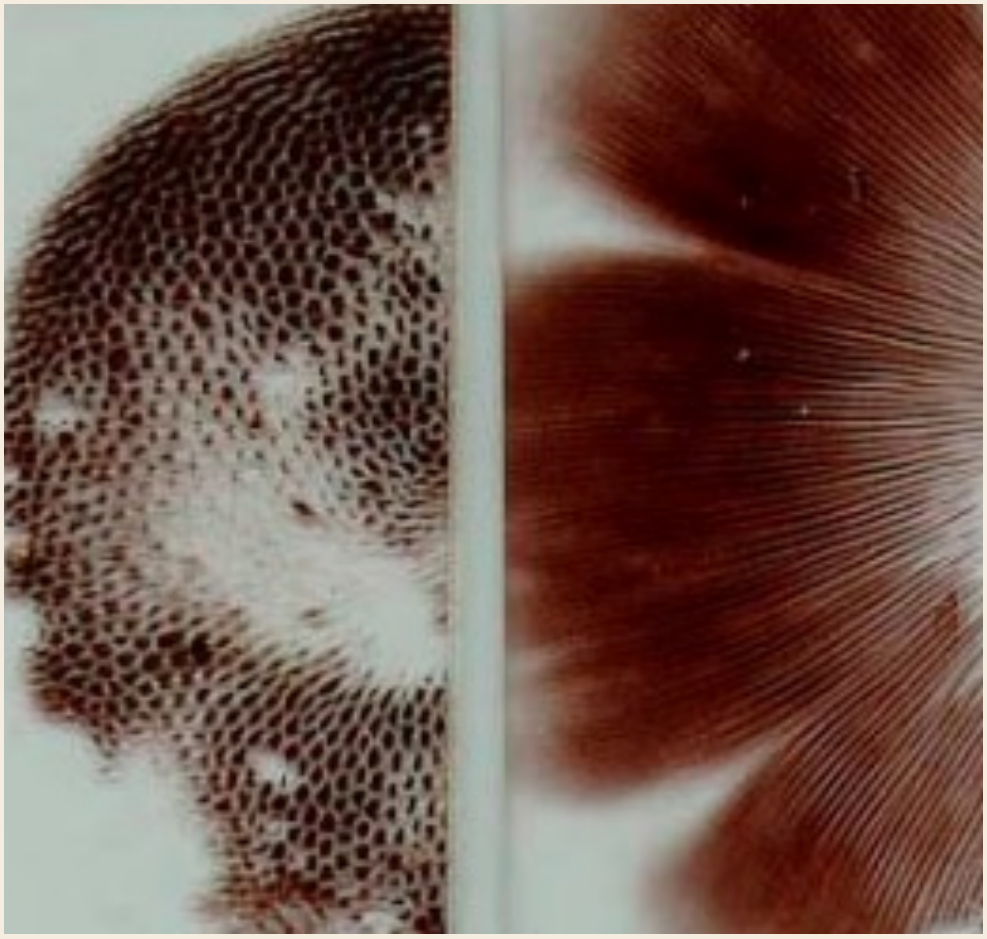

Figure 8 Examples of two different spore prints. Source: Hailing R. E., 1996.

ANNEX 1 ILLUSTRATED CHART OF FUNGI ANATOMY

Figure 10 Description of the different organs of the fruiting body of fungi. Source: Prance et al., 2017.

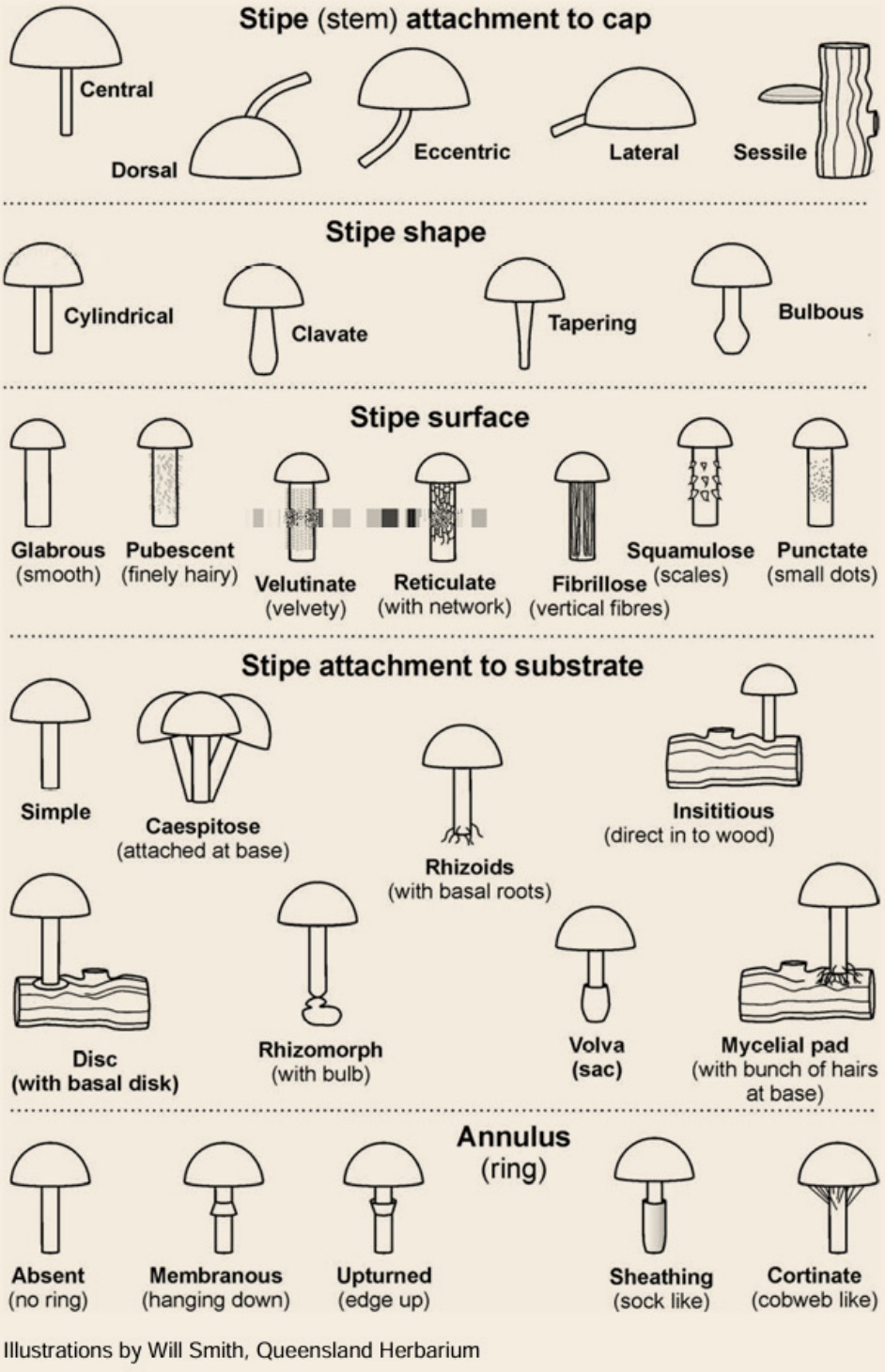

Figure 11 Description of the different organs of the fruiting body of fungi. Source: Prance et al., 2017.

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05

SOIL SAMPLING PROCEDURE

SOIL SAMPLING PROCEDURE

A STANDARD EQUIPMENT FOR SOIL SAMPLING

DISPOSABLE RUBBER GLOVES

CLEAR CLEAN PLASTIC BAGS FOR MIXING SOIL SAMPLES

PAPER GLASSINE BAGS

SAMPLE ID STICKERS / PENCIL

AIRTIGHT PLASTIC RESEALABLE BAGS

SILICA GEL

GARDENING SPADE OR 5 CM CORER OR KNIFE (sharp and strong, at least 5 cm blade) **AND TABLESPOON**

TABLESPOON

RULER TO MEASURE 5 CM DEPTH

95% PURE ETHANOL / FLAME

PAPER TISSUES

COOLER BOX

FREEZER (PREFERRED) OR REFRIGERATOR FOR SAMPLE STORAGE

B PRE-FIELD WORK

Ensure that all proper specimen permissions are obtained (i.e. from local authorities, property owners, etc.).
Consider possibilities of wildlife disturbance and/or human vandalism

C PRE-FIELD WORK

Adapted from ISO 10381-1:2002 – Soil Quality: Sampling

This document provides standardized procedures for the design and execution of soil sampling programs for environmental assessment, soil quality monitoring, land use planning, contamination assessment, and legal documentation.

C.1. OBJECTIVES OF SAMPLING AND CORRESPONDING DEPTHS:

OBJECTIVE	RECOMMENDED SAMPLING DEPTHS	JUSTIFICATION
GENERAL SOIL QUALITY ASSESSMENT	TOPSOIL: 0–30 cm SUBSOIL: 30–100 cm	To analyze root zone nutrients, pH, and organic matter; deeper layers for leaching trends
SOIL MAPPING AND CLASSIFICATION	STRATIFIED BY HORIZON (e.g., A, B, C layers)	Characterization requires natural layer distinctions preserved
REGULATORY OR LEGAL BASELINE STUDIES	SITE-SPECIFIC DEPTHS, OFTEN DOWN TO 1.5 m OR MORE	May be determined by environmental law or type of suspected contamination
RISK/HAZARD ASSESSMENT (E.G., POLLUTION)	SURFACE (0–30 cm) AND SUB-SURFACE (30–150 cm+); INCLUDE HOTSPOTS	To understand vertical migration of contaminants and exposure potential

C.2. TYPES OF SAMPLES AND USE ACCORDING TO OBJECTIVE:

SAMPLE TYPE	DESCRIPTION	BEST SUITED OBJECTIVES
GENERAL SOIL QUALITY ASSESSMENT	A SAMPLE FROM A SINGLE POINT	Site-specific contamination analysis; regulatory or legal compliance
SOIL MAPPING AND CLASSIFICATION	MIXTURE OF SEVERAL SAMPLES FROM ONE ZONE OR AREA	Average conditions for general soil quality assessment fertility studies
REGULATORY OR LEGAL BASELINE STUDIES	SAMPLE COLLECTED TO MAINTAIN IN-SITU STRUCTURE	Physical property testing: porosity, permeability, bulk density
RISK/HAZARD ASSESSMENT (e.g., pollution)	SOIL COLLECTED WITHOUT MAINTAINING STRUCTURE	Chemical and biological analysis; nutrients, metals, microbial content

D COLLECTION OF SOIL SAMPLES

D.1 PRE-SAMPLING PREPARATIONS:

- Define sampling objectives, strategy, and pattern (grid, random, stratified).
- Coordinate with laboratory analysts to ensure compatibility of sampling and analysis methods.
- Select appropriate tools (auger, corer, shovel) based on soil type and required depth.

D.2 SAMPLING SITE PREPARATION:

- Remove surface debris (organic material, stones).
- Identify and mark sampling points using GPS.
- Install barriers if sampling in hazardous or restricted areas.

D.3 SAMPLE COLLECTION:

- Excavate or drill to the required depth.
- For undisturbed samples, use appropriate cylindrical corers to maintain structure.
- For composite samples, collect 5–10 subsamples and mix thoroughly in a clean container.
- Record depth, coordinates, and any unusual features (e.g., color, smell, moisture).

D.4 SAMPLE HANDLING AND PRESERVATION:

- Use clean, inert containers (polyethylene or glass jars).
- Label each sample with ID, location, depth, date, and time.
- Store in coolers if volatile or microbial parameters are analyzed.
- Complete field forms and chain-of-custody documents.

E SAFETY AND LEGAL COMPLIANCE

- Wear appropriate PPE (gloves, boots, masks if needed).
- Notify landowners or authorities before accessing the site.
- Avoid or manage environmental risks like exposure to contaminated dust or gases.
- Properly dispose of surplus soil and decon-taminate tools between samples.

F DOCUMENTATION AND REPORTING

Every field sampling should be accompanied by a Sampling Report including:

- Site identification and purpose of sampling
- Coordinates and depth of each sample
- Methods used (equipment, pattern, preservation)
- Observations (e.g., soil structure, color, odour)
- Transport and storage conditions
- Any deviations from the protocol

LIFEPLAN PROJECT

Adapted from Lifeplan soil sampling protocol: dx.doi.org/10.17504/protocols.io.5jyl8pw9rg2w/v2

G SAMPLING / COLLECTION PERIOD

At each site, soil samples are taken four times a year. The four sampling events should be distributed evenly over the entire plant growing season.
This protocol, we describe the method used within the Lifeplan project for collecting soil samples on a global scale in a wide variety of environmental conditions and habitats. The aim is to use this data to identify species of fungi and create species lists for the different locations across the globe. This protocol contains a detailed description on the materials needed to take soil samples, the steps for preparing the samples for further analysis (e.g. DNA sequencing), as well as necessary information to help in species identification.

- Prepare the sampling materials by wearing rubber gloves and labeling a clear plastic bag with the sample ID and sampling date using a permanent marker.

- Decontaminate the spade, spoon, or knife by applying ethanol and wiping thoroughly with paper tissue; alternatively, sterilize the tool using a flame.

- Remove the surface layer of living cryptogams (including mosses, lichens, and algae) and any loose debris from the sampling area (e.g., dry or uncompacted leaves, branches, twigs, or loose needles). Retain the litter layer that remains attached to the soil surface. In litter-rich habitats, such as tropical rainforests, collect samples only where the litter layer does not exceed 2 cm above the soil. In peatland habitats, begin sampling at the point where decomposed organic material is less than 1 cm in size.

Figure 1 curtesy of Hardwick et al., 2024, LIFEPLAN project.

Figure 2 curtesy of Hardwick et al., 2024, LIFEPLAN project.

/// SOIL SAMPLING PROCEDURE

- Using a knife or spade, cut a hole with a diameter of 5 cm. Excavate the soil to a depth of 5 cm using a tablespoon or spade and transfer the sample into a clear plastic bag. If available, a 5 cm diameter soil corer may be used to collect a core to a depth of 5 cm.

- Collect two additional replicate samples at distances of 1–2 m from the initial sampling point, removing any green plant material during collection. Combine all three replicate samples into the same plastic bag.

Figure 3 curtesy of Hardwick et al., 2024, LIFEPLAN project.

- Using gloved hands, remove large roots, leaves, and branches present in the sample. Intact leaves should not be fragmented; however, partially decomposed litter and soil aggregates should be gently separated.

- Homogenize the soil within the bag by sealing it and manually kneading the exterior to break down larger soil or litter clumps.

- While continuing to wear gloves, remove and discard coarse materials greater than 1 cm in size, including stones, litter, wood fragments, and plant roots.

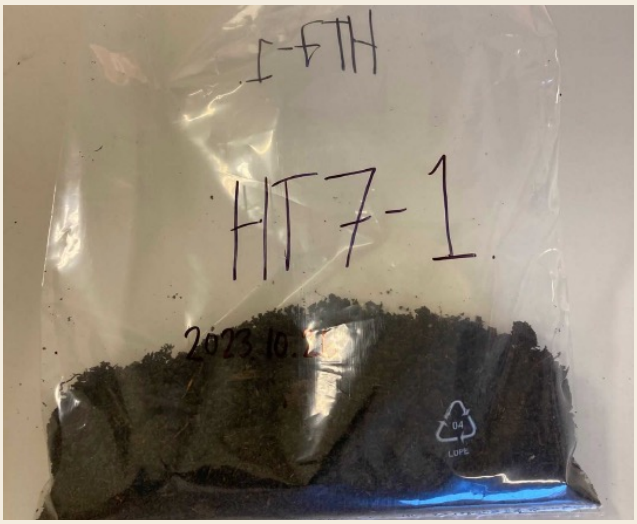


Figure 4 curtesy of Hardwick et al., 2024, LIFEPLAN project.

- For soil samples with high organic matter content (e.g., peat), the removal and sorting process may be limited to a maximum of 90 seconds.

- Measure three tablespoons of homogenized soil and transfer them into a paper bag, ensuring the sample is free of particles, aggregates, or fragments larger than 1 cm.

Figure 4 curtesy of Hardwick et al., 2024, LIFEPLAN project.

- Decontaminate the spoon between samples using an appropriate disinfectant (e.g., flame sterilization, bleach, or Virkon), followed by wiping with a sterile paper tissue (e.g., Kimtech).
- Clearly label each bag with the sample identification code and all other required metadata.

Figure 6 curtesy of Hardwick et al., 2024, LIFEPLAN project.

- Repeat steps 3–13 for each sampling location within the site, ensuring that new disposable gloves are worn for each new sample (but not for individual replicates).
- Place each soil sample into a separate resealable plastic bag and seal it securely.
- Store the samples in a cooler during transport until arrival at the laboratory.
- Store the samples in a cooler during transport until arrival at the laboratory.
- Upon returning to the laboratory, open each resealable bag and add approximately 50–100 g of dry silica gel. Expel most of the air by flattening the bag before resealing and allow the samples to dry for approximately 24 hours.

Figure 7 curtesy of Hardwick et al., 2024, LIFEPLAN project.

- When the silica gel changes color, reopen the bag and replace the saturated silica with dry silica gel. This process should be repeated every few days until the silica gel remains dry. The total drying duration will depend on the moisture content of the soil and the ambient environmental humidity.
- Once the silica gel remains dry, transfer the sample to a −20 °C freezer. Retain a small number of silica beads in the bag to maintain a dry environment until the samples undergo freeze-drying.
- Freeze dry the soil samples.

REFERENCES

This protocol was extracted from the original document prepared by the LIFEPLAN project by Hardwick et al., 2024, as well as the ISO 10381-1:2002 – Soil Quality: Sampling document.

- Part 1: Guidance on the design of sampling programmes ISO 10381-1:2002 – Soil Quality: Sampling https://www.iso.org/standard/32423.html

- Hardwick B, Kerdraon D, Rogers HMK, Raharinjanahary D, Rajoelison ET, Mononen T, et al. (2024) LIFEPLAN: A worldwide biodiversity sampling design. PLoS ONE 19(12): e0313353. https://doi.org/10.1371/journal.pone.0313353

06

DNA SAMPLE COLLECTION – FECES, URINE, BLOOD AND TISSUE

DNA SAMPLE COLLECTION - FECES, URINE, BLOOD AND TISSUE

A INTRODUCTION

The application of genetics in wildlife biology has grown rapidly in recent years. To maximize the value of genetic data, however, proper handling and storage of biological samples are essential. This guide outlines best practices for collecting a variety of sample types for DNA analysis. Across all methods, common principles emerge such as the importance of sterile techniques, accurate labeling, and effective preservation.

DNA can be extracted from a wide range of materials, including suboptimal samples like scat or hair, provided they are stored correctly. Improper storage, on the other hand, can compromise DNA integrity. One major threat comes from naturally occurring enzymes in animal cells and bacteria that degrade DNA. These enzymes typically require moisture to function, so drying or freezing samples are key to inhibiting their activity.

In addition, environmental factors such as repeated freeze-thaw cycles, ultraviolet (UV) exposure, and high temperatures can cause physical damage to DNA, including fragmentation or degradation. Fortunately, with appropriate storage techniques, both chemical and physical damage can be minimized. The following sections detail how to store different types of biological samples to preserve DNA quality and ensure robust genetic analysis.

The collection and preservation of scat samples often depend on the main objective of collection and the specific requirements of the laboratory performing the DNA extraction. Each lab typically provides a detailed collection and preservation protocol tailored to the extraction techniques they use. In this document, we have compiled the most used field techniques for collection and preservation. These practices serve as a standardized reference but should be adjusted according to the specifications provided by the recipient laboratory whenever possible.

B COLLECTING DATA/LABELING SAMPLES

Sample collection is useless unless the samples are well labeled and documented.
The following data should be recorded for all samples on a field form and/or on the vial itself:

1. **UNIQUE SAMPLE ID**
2. **COLLECTION LOCATION**
3. **COLLECTION DATE**
4. **SAMPLE NUMBER**
5. **TYPES OF SAMPLES TAKEN** *(e.g., ear plug, scat, muscle, hair, blood etc.)* **WITH THEIR RESPECTIVE NUMBERS**
6. **COLLECTORS INITIALS**
7. **ANY COMMENTS ON CONDITION OF SAMPLES,** *etc.*
8. **SEX** *(if known)*

C TREATING SCATS WITH DESICCANT AND /OR LYSIS BUFFER {1}

C.1. COLLECTION OF SCATS OF CARNIVOROUS ANIMALS:

Scat can be a useful source of DNA, but it is critical to dry the scat out to prevent degradation (and growing mold etc.). When scat is found, collect the scat (fresher tends to work better) and place it in a new unwaxed brown paper bag, (or plastic vial/ specimen cup with desiccant). Which option you use may depend of the size of the scat. Only place one scat in each bag/ container to ensure that samples are from only one individual. After scat is collected, take it to a warm, dry place and allow it to dry for 1-4 days (either in paper bags or vials with desiccant).

C.1.1. PRIOR TO THE FIELD OBTAIN:

a) Clean (new) brown paper bags
b) Extra latex gloves
c) 50 ml polypropylene screw-cap vials or specimen cups filled 1/3 with silica desiccant

C.1.2. SAMPLE TREATMENT (FIELD):

a) Put on new gloves
b) Place the scat in the paper bag or vial
c) Label with Sample ID, Date, Location, etc.

C.1.3. SAMPLE TREATMENT (FIELD CAMP SEVERAL HOURS LATER):

a) If the scat is in a vial, check the silica desiccant. If the color of the desiccant has turned from the original color, change the silica desiccant under sterile conditions. Desiccant that has turned color is water saturated and is no longer working to preserve the sample. This is very common for scat samples which often have a lot of moisture. Check the desiccant in the vials often, (every few hours initially) to replace exhausted desiccant until the color no longer changes. Make sure scat is surrounded by desiccant to effectively dry it. It is ok if the desiccant beads stick to the scat.

b) If the scat is in a brown paper bag, ensure scat is in a warm, dry room to dry out for several days.
Optional: the bag with the scat can be placed in a Tupperware container with silica desiccant to help it dry (check the desiccant often, and replace it until it stops turning color, if you are using a silica desiccant with color-indicators).
Optional: after the scat is dry (good indicator is that it's not very stinky), it can be transferred to a 50mL vial/specimen cup filled ~1/3 with desiccant if desired.

c) Store samples at room temperature, in a dry environment out of direct sunlight until shipped to the lab.

C.2. COLLECTION OF SCATS OF LARGE HERBIVOROUS ANIMALS (LARGE ANIMAL):

Scats from herbivores (frequently referred to as pellets) can also be a useful source of DNA. In addition to collecting pellets, if the pellets are fresh/moist, these pellets can also be swabbed to gather DNA from the mucous. If swabbing samples, use 3 separate Dacron (or similar) swabs to sample different pellets from the same pile, then store all swabs dry in a coin envelope or plastic packaging that came with the swab. Ideally, the swabs should be lightly stained but not covered with fecal material. Collect up to 6 individual pellets (if swabbing pellets, these should be ones that weren't swabbed) and place them in paper envelopes. Pellets and swabs (if collected) should be sent to the lab. {1}

C.2.1. PRIOR TO THE FIELD OBTAIN:

a) Pre-labeled envelopes
b) Dacron Swabs or similar
c) Extra latex gloves

C.2.2. SAMPLE TREATMENT (FIELD):

a) Put on new gloves
b) Place up to 6 individual pellets not swabbed into paper envelopes.
c) Optional: Swab 3 different pellets with 3 swabs and place in coin envelope/swab packaging

C.3. COLLECTION OF SCATS OF SMALL HERBIVOROUS ANIMALS (SMALL ANIMAL):

For smaller animals (such as rodents or bat), it is recommended that the pellets are placed in 1.5ml or 2.0ml vials containing either silica desiccant or lysis buffer. If the samples will be shipped, we recommend using desiccant or screw-cap vials if using lysis buffer to avoid leakage.

C.3.1. PRIOR TO THE FIELD OBTAIN:

a) Pre-labeled vials (pre-filled with desiccant beads or lysis buffer)
b) Extra latex gloves

C.2.2. SAMPLE TREATMENT (FIELD):

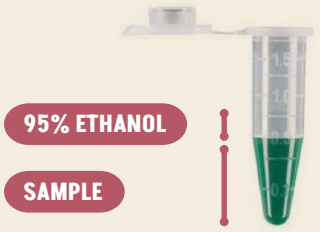
a) Put on new gloves
b) Place up to 10 pellets in a pre-filled tube

C TREATING SCATS WITH PRESERVATION AND SUBSEQUENT STORAGE AT –20 °C.{2}

Prey DNA found in fecal and stomach content samples is typically highly degraded. Therefore, the primary objective during sample collection and storage is to prevent any additional degradation. This is achieved by rapidly inhibiting microbial and enzymatic activity. The two most effective preservation methods are freezing and/or storing samples in ethanol.

D.1. SAMPLE COLLECTION:

- Collect samples as soon as possible after defecation.
- Ideally, samples should be chilled or frozen at –20 °C shortly after collection. If available, a portable refrigerator or freezer should be used in the field.
- If freezing is not feasible, adding 95% ethanol to the sample immediately is recommended.
- The optimal approach combines ethanol preservation with subsequent storage at –20 °C.



D.2. SMALL TO MEDIUM-SIZED SAMPLES:

- Do not fill more than half of the 1.5 mL tube with sample material (see illustration).
- Add approximately 50–100 µL of 95% ethanol to aid in preservation.
- Ensure the final volume in the tube does not exceed half the tube's capacity.
- Tubes must be clearly labeled with a unique sample ID and a corresponding digital inventory must be submitted.

D.3. LARGE SAMPLES:

- Collect the entire fecal sample in a sterile container; new resealable (e.g., Ziplock) bags are acceptable.
- Seal and thoroughly homogenize the sample by kneading through the bag to ensure even consistency. If needed, a small amount of 95% ethanol may be added to soften the material and assist in mixing, while also serving as a preservative.
- After homogenization, transfer approximately 300 µL of the sample into a clean 1.5 mL LoBind tube. Add 50–100 µL of 95% ethanol if not already added.
- Ensure that the tube is no more than half full.
- Tubes should be clearly labeled, and a digital list of sample IDs must accompany the submission.

E SAMPLING OF URINE

Urine can be a source of DNA. This sample is usually collected in the snow during winter surveys. In the field, obtain yellow snow in a vial or plastic container. Try to maximize urine and minimize extra snow collected. {1}

E.1. PRIOR TO THE FIELD OBTAIN:

a) 50 ml screw-cap vials/ specimen cups or similar
b) Extra latex gloves
Urine preservation buffer (recommended by the Lab)

E.2. SAMPLE TREATMENT (FIELD):

a) Put on new gloves
b) Scoop yellow snow into vial
c) Add dose of urine preservation buffer; can be done as soon as come back from the field
d) Store urine with preservation buffer in refrigerator. It's best to store the urine with preservation buffer in the refrigerator until shipping; ship samples in a cooler with blue ice.

F SAMPLING OF TISSUE/MEAT/EAR PUNCHES

Tissue can be stored in several ways. The two preferred methods are {1}:

1) **IN A SILICA DESICCANT**
2) **FROZEN**

We avoid storing tissues in ethanol as the concentration must be 95% ethanol for effective preservation and ethanol can present challenges for the field and shipping.

The most important thing for effective tissue preservation when using desiccant is to have the size of the vial allow for the tissue to float freely in the desiccant. Use a color-changing desiccant so that the desiccant can be swapped out or more added for wet tissues. Continue to monitor and change the desiccant until the color doesn't change.

Obtain a piece of muscle tissue (avoid sampling organs) and place in vial with desiccant. Ear punches from study animals can be placed directly into vials with desiccant. When sampling from a carcass, target a pencil-eraser/dime-sized piece (~1 – 2cm3) to cut. If the carcass is old/ decomposing, try and find a piece of tissue that doesn't look rotten. If dealing with a carcass such as from roadkill, a sample of tongue is often a convenient tissue to obtain. DNA from old carcasses can sometimes yield DNA, so always take a sample.

/// DNA SAMPLE COLLECTION – FECES, URINE, BLOOD AND TISSUE

F.1. STORAGE IN SILICA DESICCANT:

This is the preferred method as samples are easily stored at room temperature

F.1.1. PRIOR TO THE FIELD OBTAIN:

a) Clean (new) 2ml-50ml polypropylene, screw-cap vials (the size depends on the size of the tissue)
b) Silica desiccant with color indicator
c) Forceps/tweezers; razor blade or other cutting tool
d) Ethanol (95%) for cleaning tweezers/ forceps and blades
e) Wipes (or other tissue paper to wipe tweezers and prevent contamination)
f) Permanent Ink Markers

F.1.2. PRIOR TO CAPTURE:

a) Add desiccant to vials; fill to about 1/3
b) Clean the tweezers/forceps and blade with the ethanol and wipe with Wipes
c) Sample Collection
d) Slice the tissue and place in sample vial with desiccant
e) Label sample vial

F1.3. SAMPLE TREATMENT (LATER):

a) It is important that the tissue is fully immersed in the desiccant; ideally the tissue should be free to move as the tube is turned (avoid cramming tissue in the vial and topping off with desiccant as the tissue will almost certainly rot).
b) Check the silica desiccant. If the color of the desiccant has turned from the original color, change the silica desiccant or add more if the size of the vial allows under sterile conditions. Desiccant that has turned color is water saturated and is no longer working to preserve the sample. Check the desiccant in the vials often, (every few hours initially) to replace exhausted desiccant until the color no longer changes.
c) Store samples in the desiccant at room temperature. Keep the sample out of direct sunlight. Tissue samples stored in desiccant can be shipped at room temperature to the lab

F.2. STORAGE IN FROZEN CONDITIONS:

F2.1. PRIOR TO THE FIELD OBTAIN:

a) Clean (new) 2ml-50ml polypropylene, screw-cap vials or for larger tissues use a Whirlpack bag
b) Forceps/tweezers; razor blade or another cutting tool
d) Ethanol (95%) for cleaning tweezers/ forceps and blades
e) Wipes
f) Permanent Ink Markers (e.g., Sharpie Brand)

F2.2. PRIOR TO CAPTURE:

a) Clean the tweezers/forceps and blade with ethanol and wipe with Wet Wipes

F.2.3. SAMPLE COLLECTION:

a) Slice the tissue and place in sample vial/ Whirlpack
b) Label sample vial/Whirlpack
c) Place sample in freezer

Ship samples in a cooler with ice packs /dry ice. DNA can degrade if it goes through freeze thaw cycles, therefore, avoid thawing of tissue once it has been frozen.

G SAMPLING OF BLOOD

For mammal genetic studies, it is critical that whole blood be collected since mammalian red blood cells themselves do not contain DNA. Fish and birds have DNA in their erythrocytes, though collection of whole blood is preferred {1}.

For submitting blood samples to the lab, blood stored in blood storage vials (e.g. purple-top EDTA tube) should be shipped on dry ice.
A more convenient method is to transfer some whole blood (by dropper or syringe) to a Whatman FTA Micro Card. Ideally, enough blood should be applied to the filter paper such that a little bit of red color comes through the back. The cards should be completely air dried (at room temperature) and then placed in bags with desiccant and stored in a dark location at room temperature until they are sent to the lab.

The above 2 methods are preferred although there are also protocols for storing blood with lysis buffer.

* REFERENCES

- {1} Protocols for Collecting and Storing DNA Samples National Genomics Center for Wildlife and Fish Conservation USFS Rocky Mountain Research Station 800 East Beckwith, Missoula Montana 59801 Created: March 28, 1998 Revised April 26, 2018

- {2} Protocol Adapted from guidelines established by the Canadian Centre for DNA Barcoding (CCDB)

* DEFINITION

- Whatman FTA Micro Card is a specialized paper-based sample collection card used for the collection, transport, and long-term storage of nucleic acids (DNA and RNA) at room temperature.
- Whirl-Pak® (often written as Whirlpack) is a brand of sterile, sealable sample bags widely used in scientific, medical, environmental, food, and forensic fields for sample collection, transport, and storage.
- Dacron swabs are medical swabs made with a synthetic polyester fiber called Dacron
- LoBind tube (short for Low Binding tube) is a specially designed microcentrifuge tube—often made by Eppendorf or similar brands—that minimizes the binding of biomolecules like DNA, RNA, and proteins to the plastic surface. This is especially important for low-concentration samples or highly sensitive molecular analyses.