BRYOPHYTE INVENTORY AND SAMPLING TECHNIQUES

James R. Shevock
California Academy of Sciences, Department of Botany
55 Music Concourse Dr., Golden Gate Park
San Francisco, CA 94118 U.S.A.

Last updated viii.2024

What is a bryophyte?

Bryophytes are the first land plants that include three distinct lineages: mosses, liverworts, and hornworts. They differ primarily from the flowering (vascular) plants by lacking roots, flowers, seeds, and a defined system of tissues for transporting fluids throughout the plant. Bryophytes reproduce not by seeds but by single-celled spores. Many bryophytes can also form new plants by vegetative means. Bryophytes are important in ecosystem function. They assist in protecting soil from erosion and release water stored in their cells gradually back into the environment. Bryophytes have two features that make them fairly unique; they are capable of dealing with long periods of desiccation thereby shutting down all cellular activity and then the ability to rapidly come back to life when water again becomes available. In addition the green bryophyte plant we see is a gametophyte meaning it is haploid (1N) versus the vascular plants as diploid (2N). The reproductive plant is called the sporophyte and this part of the bryophyte is 2N but it produces a capsule of haploid spores.

How different is it to collect vascular plants from bryophytes?

It is indeed easier to collect bryophytes compared to vascular plants since no plant press is required. In fact, bryophytes should not be placed in a plant press. The following questions and answers will aid you in how bryophytes should be collected, and how to make a really valuable collection that can be used by other botanists and researchers.

Frequently Asked Questions

One of the first questions botanists and plant enthusiasts ask is ***how does one collect a bryophyte?*** This answer is it's easier than making a vascular plant collection. The bryophyte sample is simply placed in a collection packet folded from a sheet of photocopy or other greater strength paper. Standard photocopy paper easily tears when moistened so if a stronger type of paper is available it would be preferred. Even newsprint will work for making collecting packets (although it is not my preferred method). While some bryologists use small brown paper bags for collecting samples, I have concluded that specimens collected and dried in packets have the following advantages:

1) specimens are much better looking since they dry relatively flat in a packet versus forming a wad at the bottom of a bag

2) specimens are handled less frequently before being dried

3) data capture at a collecting site by circling various features on a packet are more consistently recorded than writing directly on a bag

4) data remains with the specimen obtained at the time of collection

5) specimens dried in packets fit perfectly into archival herbarium packets later

6) packets are easier to dry since there is more surface area

7) draft labels can then be stapled directly onto the field packets and packets arranged by family or genus to expedite the identification step

8) specimens are easier to store and sort until identified; they will fit nicely in a flat cut from a standard photocopy box

9) specimens do not have to be transferred later from field bags to packets

Plastic bags for bryophyte collecting should generally be avoided. While bryophytes can be placed in plastic bags in the field, the specimens need to be placed as soon as possible into packets so they can begin to dry. Bryophytes kept in plastic bags will quickly mildew or become deformed looking so they will need to be transferred relatively quickly after collection. The problem with collecting in bags is specimens then have to be separated later, numbered, and data recorded. These are all activities that can create errors especially if these tasks are deferred to a later date. Relying on memory is not a good scientific method! Therefore, it's best to just start off by developing good collecting habits and techniques and use paper collecting packets. When I am collecting in the field, I place my collections (inside their pre-folded paper packets) into a lightweight plastic shoebox with lid. This keeps the specimens in good shape while on a hike and I can easily place plastic shoeboxes in my daypack. The specimens fit nicely in plastic shoe-sized boxes and they are placed in the box like a row of filing cards. Bulky specimens can be further secured in their packet with the use of a rubber band. For bryophytes collected in wet sites (like creeks, springs, etc.), I first gently place the specimen between my palms and squeeze out the excess water. Sometimes bryophytes collected on muddy soil, if a water source is available, can also be rinsed off, squeezed to remove excess water (do not wring), and then placed in the collecting packet. These collections too go directly into my field plastic shoebox ready for the trip home. Once returning from a collecting event the packets need to air dry. More about drying specimens later.

Another question frequently asked is ***how much plant material should I collect?*** The answer here is fairly simple too. The amount that would fit on the palm of your hand is ample for a bryophyte collection. If you want to provide more (and in doing so you would not adversely affect that population), then a more robust packet or additional packets for additional duplicates of that collection would be desirable. Under most conditions, a single packet can accommodate 2-3 duplicates depending on the size and amount of the bryophyte collected. Collecting samples where a duplicate is available is desirable for the following reasons:

1) Many agencies, as a requirement of granting a collecting permit, will request either a duplicate sample for their herbarium or a synoptic set (one good labeled specimen to accommodate each species documented) to be deposited in a designated herbarium. I prefer to provide a synoptic set (one well labeled collection per species) if agreed upon set since most agencies do not have a lot of storage space for specimens and it also reduces my workload.

2) Having ample material of a collection offers a duplicate sample to be placed in a major herbarium so your specimens are available to a wider research community and can provide material for molecular study.

3) Offers you an option to provide a "gift for determination" specimen to another bryologist.

4) Extra duplicates can be used for exchange purposes to build up your reference collection and,

5) Used as a tax donation to a non-profit scientific or academic institution that maintains a herbarium.

What other information do you need? Each plant collection needs to have a few items recorded to make it valuable for a scientist. The key items for any plant collection includes: <u>who</u> collected it, <u>when</u> was it collected, <u>where</u> was it collected (as detailed a description as possible so a botanist decades from now could return to the site or it could be readily plotted on a topographic map), <u>what</u> was it growing on (a rock, a log, on soil, submerged in a creek etc.) and any other important feature as an indication of the basic habitat type (i.e. mixed tropical rain forest etc.). So this information needs to be gathered at the time of collection and recorded with the specimen. This information eventually will be placed on the herbarium label for that collection. If you do not have a field notebook or a collecting numbering system, I suggest you just number your bryophyte packets in chronological order starting with "number 1, 2, 3 etc." and place all of this information into a pocket-sized field notebook while in the field. The important thing is that each collection is a unique number, and therefore, no number is used more than once. Avoid embedding the date in the collection number or start with a new No. 1 each year.

On the front flap of the collecting packet I use, I have a template that lists a suite of attribute fields. All one has to do (once the packet is folded in such a way as to form a "pouch" so the specimen does not fall out) is to circle all of the appropriate attribute fields for that specific collection and place the specimen in the packet. That's all there is to it. I have attached a copy of the template on the last page that can then be photocopied onto paper and folded into packets. Simply copy, cut off the template and align it (centered) to the bottom of a standard 8.5 x 11 inch piece of paper. Then re-photocopy. Simply fold the sheets of paper into thirds (as if you were to mail a letter). First place the template (printed side face down) then by lifting up the template, crease the paper on the horizontal black line and then place the other end of the paper into that crease). This should have the piece of paper folded into 3 equal sized portions. The top flap now has the template. Lift up, and then fold in about an inch on each side (along the vertical lines). This will now form the collecting pouch. Now return the template flap over the packet. The use of the template form on the front flap of the collecting packet ensures that each collection obtained has the same level of ecological data recorded directly **at the time of collection**. Also, once the specimen is dried, identified and ready for processing into the herbarium, the front flap of the field collecting packet with the circled ecological data can be removed with a pair of scissors and then placed inside the labeled archival quality herbarium packet thereby adding further to the scientific value of the collected specimen. This data form (the template) can also be photocopied by placing 4 templates on a sheet of paper then copy. These sheets of templates can then be cut with a paper cutter so the ecological data form can be replicated so every duplicate specimen for that collection has the same ecological data provided with it.

The next most commonly asked question is ***how are bryophytes dried?*** This too is simple. They are air dried just like you would for a vascular plant collection except that bryophytes should not be placed in a plant press (except for liverworts fused to the surface of leaves in the tropics). As bryophytes dry many species have diagnostic features (such as how leaves curl or twist). These are valuable clues for identification later. If placed in a plant press and flattened, the bryophytes "loose" many of these drying features. The key to drying bryophytes is to have air flow across and between the packets (a small fan works wonders), but they will dry quite nicely in any place with air circulation. Just about any method used for drying vascular plants will work for drying bryophytes as long as the specimens can air dry in a few days. Note: never place bryophyte specimens in a microwave or bake them in the oven. You want to ensure they dry only with air so as not to destroy DNA within their cells. If the packets remain wet for longer periods than a few days they will probably begin to mildew or discolor. Wet packets can also be separated and laid out to aid in drying. The paper packets absorb moisture from the bryophyte so placing the packet with the ecological data flap face down will expose the wettest part of the packet first (that part of the specimen that was affixed to the substrate). As the paper dries then flip over the packet and repeat this procedure. Another drying technique for specimens is to have each packet stand up forming a triangle (like a tent). Air will flow between the specimens further aiding in the drying

process. Once dried, bryophytes are basically indestructible. Key is to get them dry as soon as possible.

For those working in tropical environments with high humidity, bryophytes can be dried exceedingly well and quickly if dried in an air-conditioned room. Basically, air conditioning sucks out the moisture out of a bryophyte collection, so if you have access to an air conditioned room that would be the best option for drying specimens. Specimens are dry once the collecting paper packet is dry to the touch.

The next question is ***how do I know what to collect?*** This is a bit more difficult to answer. If the goal is to do a biodiversity inventory, then the collector(s) need a systematic approach to sample all of the available habitats within the project area. If on the other hand one only has time to make limited collections, then I recommend you to seek-out microhabitats that are unique or uncommon on the landscape. Many bryophytes are habitat specific. With a bit of practice one will recognize the common species for a particular area being inventoried then one can concentrate on the unique and less common species that may be present at that location. Most non-bryologists generally collect bryophyte specimens as a random collecting event. They do not generally plan to go collect bryophytes, but rather, have packets in their daypack in the event something catches their eye that they would like to document.

The next question concerns reproductive structures (the spore-bearing plant). ***Are sporophytes required?*** The answer here is absolutely not! Nearly every bryophyte can be identified (at least to genus) without the sporophyte attached. Not all sporophytes are erect on a stalk (called a seta). For some bryophytes, the sporophytes can be hidden among the leaves but these structures can be easily seen with a hand-lens. So while sporophytes are nice to have, do not worry if your bryophyte collection lacks them. For some closely related species, sporophytes may be required to reach species identification. But don't let a lack of sporophytes deter your collecting inventory efforts. For some bryophytes, sporophytes remain unknown.

So what should happen to your bryophyte specimens? As soon as possible after returning from a collecting event a draft label for each collected specimen should be developed. You should get in the habit of doing this at the end of each trip otherwise a backlog is created and the task may later become daunting. Besides keeping up with your collecting activities, you are likely to have a clearer memory of the collecting sites etc. in the event you overlooked some particular item during data collection. Once the specimens are dried, then a draft herbarium label is simply stapled directly to the front flap of the field collecting packet. Next review the specimen under the dissecting microscope and place it in a genus or family if you do not know what it is. For really puzzling collections, you may want to contact a bryological expert and send a small sample of the collection with a draft label off to be examined and named. So as you can see, some specimens can be named rather quickly while others will have to wait until they can be studied in far greater detail at a later date. The key is to have high quality draft labels attached to each collection so you do not create a backlog of specimens without labels. It is very easy and quick to go from draft to final labels once specimens are named.

There are many ways to make a herbarium label. Some botanists have a spreadsheet and labels are developed from those fields in a merge and print routine. Others just have a word document setup to make a label and it is simply a cut and paste routine to make the next label. Other botanists have some type of database program for generating herbarium labels. Regardless of how one makes the label, it should not be larger than 3.5 long by 5 inches wide in size when it is printed. Labels larger than this will not fit comfortably on archival museum specimen packets used by most herbaria.

Once a collection is named, then a final label is produced (use 25 percent cotton fiber). Extra labels can also be printed for that specimen number to accommodate all of the extra duplicates available. You may want to maintain a synoptic set for your own reference. In this case it is best to place the specimens in a 100 percent cotton fiber acid free packet. Archival packets are folded from standard sized 8.5 x 11 inch paper. Labels can either be printed directly onto the packet or a label can be glued onto the front flap of the archival packet. Any duplicates you wish to distribute can be sent in the original field collecting packet, or if one is splitting a collection into duplicates, additional packets are simply folded from standard photocopy paper

(even previously used photocopy paper can be recycled for this purpose). Just place a final label inside the packet (text side face down) with the specimen on top. This will keep the data side of the label clean. A copy of the ecological data (the front flap of the collecting packet) should also be enclosed with the duplicate sample. There is no need to place duplicates that you plan to provide to another herbarium in archival quality paper since each herbarium has its own procedures and its own archival packets for adding specimens into their collection. So any remaining duplicates you have of that number can be used for specimen exchange programs or specimen donation to a non-profit scientific institution for tax purposes.

How should bryophyte specimens be sent to herbaria or to other bryologists as gift for determination? Mailing packets (with either a draft or final label) can be done at any time once the specimens are completely dry. A few specimens can be placed on a sheet of standard photocopy paper and wrapped in a similar fashion as one would wrap a small package using tape to secure the ends. Another way is to simply place a rubber band around a few packets so the contents of the packets do not fall out during shipment. These samples can then be placed either in a padded envelope or a cardboard box for shipment in the mail. For specimens you wish to mail overseas, a Customs Form will be required. The customs form section titled detailed description of contents should state **"dried plant herbarium specimens for scientific study---No commercial value"**. If you seek a determination or confirmation of one or more of your bryophyte collections, contact the bryologist before sending any specimens to ensure they are willing to take the time to examine them. Clearly state if the specimens to be sent are gifts for determination. Most bryologists will not accept specimens for identification without a draft label attached or provided with the specimen and most are going to want to keep the sample(s) for their herbarium. The expectation is you will send final labels once determinations have been provided to you. Not doing this in a timely manner will likely result in the bryologist who provided the initial determinations not to accept future requests from you to examine your collections. Note also that some countries have strict policies about shipping plant materials through the Post so it is best to ask the person you wish to send specimens if any special documentation is required on the package. In sending duplicate specimens through the Post remove as much loose soil, litter and debris as possible before shipping, especially internationally.

Special things to do for liverwort collections. Distinction between mosses and liverworts can at times be difficult to distinguish in the field especially if a collection appears to lack a costa based on a hand-lens examination or the collector is relatively new to the study of bryophytes. The liverworts have many critical diagnostic features that are either lost in the drying process or are harder to determine later. Once the specimens return home for drying, the collections can be quickly examined under the dissecting scope to validate the liverwort samples collected. Each liverwort collection should then be examined under the compound microscope to capture key diagnostic features that will then be used later during the identification process. Taking the time to gather these data while the specimen is still fresh will expedite the eventual identification of the specimen later. Oil bodies

in many liverwort genera are critical to successful identification; however, oil bodies can disappear quickly during the drying process or be altered substantially. For your liverwort collections, examine cells across the leaf and record presence or absence of oil bodies, their form, number, color, and their distribution in the cell. Place these data into the packet so it is retained and continue drying the specimens. Underleaves (shape and size) are also easier to examine on fresh material and these data too can be added to the examination data set along with the oil body information.

You can keep liverworts in the refrigerator (not the freezer) for several days until the examination under the microscope can occur. One strategy is to simply remove a small piece from a liverwort collection (wrapped in moist paper towel) and place it in a sandwich-sized plastic bag and with a Sharpie or other permanent marker record the collection number from the packet on the plastic bag. The remainder of that collection can begin the drying process with the other samples collected. Mosses on the other hand have no oil bodies or internal cellular features that are lost in the drying process. However, some species have gemmae and other types of vegetative propagules. These too are best seen on fresh materials and these data can also be added to the collection documentation and label information.

Coll. No. _____ Taxon _____

LIGHT: sunny, open, filtered, partial shade, full shade
WATER: dry, mesic, moist, seep, wet, saturated, submerged to ___ m
TOPOG: ridge, slope, swale, valley, trail, roadside
HABITAT: dense/open/cut forest, woodland, savannah, grassland, heath, chaparral, tropical, sub-tropical, temperate, subalpine, alpine, desert, riparian, spring/seep, meadow, bog/fen, swamp, pond, lake, vernal pool, river/stream/creek bank, intermittent streamlet, rivulet, rheophytic
SUBSTRATE: igneous (granite, volcanic), metamorphic (slate, shale, marble, limestone), sedimentary (sandstone), other _____
Soil: sand, loam, gravel, clay, rocky, litter, humus, peat
Rock: outcrop, cliff, crevice, top/wall of boulder, rock-slab, underhang
Tree: base, buttress, trunk, stump, snag, log, fallen/dead/rotten trunk, branch, exposed roots, bark, leaf, shrub, climber, liana, ___ m above ground
Type: conifer, podocarp, hardwood, tree fern, palm, bamboo, _____
Dominant plants: _____