

CHAPTER 1-1

FIELD TAXONOMY AND COLLECTION METHODS

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CHAPTER 1-1

FIELD TAXONOMY AND COLLECTION METHODS



Figure 1. Zen Iwatsuki on a collecting trip in Iceland. Photo by Janice Glime.

Collection

Vanderpoorten *et al.* (2010) suggest that to find a high species richness, look for a habitat with lots of bryophyte cover (Figure 1). Species diversity has a high positive correlation with the carpet density. Such a habitat suggests an appropriate moisture level, and the large clumps of bryophytes can create microhabitats where moisture is lost more slowly, permitting smaller species to develop among them.

Stevenson (2005) reminds us that when you focus on one habitat, you begin to ask questions about how and why plants are growing there. These questions form the bases of hypotheses. And when you accept the rigors of recording your habitat observations, these hypotheses are more likely to take form. They are also much more reliable for later descriptions than your memory will be. These notes will help you to formulate better data collection sheets, and you should spend time field-testing these sheets before you begin an actual comprehensive study.

For ecological studies, there are at least two reasons to collect specimens (Vanderpoorten *et al.* 2010). First, you need to collect to verify your field identification and to look for minute species hiding among the more obvious ones. Second, you need to collect **voucher specimens** for

your collections. Collections for quantitative or systematic sampling will be discussed later in a chapter on Sampling. For vouchers, you will probably want some for your own herbarium, but you also need one for your institution or other permanent herbarium that is available to other researchers and one for the person who verifies the species for you. **If you are collecting in another country**, especially a country where the bryophyte flora is poorly known, you should also prepare an identified specimen to give to a national or other public herbarium in that country. By doing this, you help to pay back your debt of collecting there and help the field of bryology progress in that country.

Collection methods have been described many times and in multiple languages (*e.g.* Loeske 1925; Iwatsuki 1970; Kildyushevsky 1973; O'Shea 1989; Buck & Thiers 1996; Gradstein *et al.* 2003). Loeske (1925), interpreted in English by Raup (1926), stressed the importance of a systematic study, rather than a random one. Even at that early date, he opined that a region seldom offers many new or rare mosses. Instead, he espoused the value of studying a bryophyte in relation to its habitat, way of living, and relationship to the rest of the flora.

Bryophytes are the easiest of all plants to collect (Buck & Thiers 1996). They rarely need to be pressed, but rather can be placed in a **paper bag** (Buck & Thiers 1996) or **packet** and permitted to air dry (Smith Merrill 1990). Some bryologists (e.g. Ireland 1982) prefer packets made of newspaper because it allows more rapid water loss than brown paper bags. And very wet specimens may cause the bags to come unglued.

The collection depends somewhat on the substrate of the bryophyte. Buck and Thiers (1996) point out that if plants grow in loose tufts or mats or are pendent, they can be easily picked up by hand. Extra adhering soil can, and should, be removed, provided that does not cause the colony to fall apart. Small plants or those tightly adhering to their substrate will be best served if they are collected with a small portion of their substrate to keep them together and to retain the growth habit. Likewise, epiphytic species should be collected with a shallow strip of the bark. Epiphylls should be collected with their underlying leaf. Those on branches can be collected with clippers.

Some collection methods are unique, permitting collection of difficult specimens. Snider and He (1991) suggest using a **flashlight** to peer into crevices and under cliff overhangs. It should be one that can be locked into the on position rather than requiring continuous pushing of a button switch.

Obtaining the Sample

In most cases, the bryophytes can be sampled by a hand grab. However, bryophytes on bark or those that have grown for decades may require extraction with the help of a knife. As Patricia Eckel put it in Evansia (1996), using a knife can have its hazards: having plants blow away from the blade, getting cut, getting poked by the knife in the pocket, losing one's balance while balancing on a talus slope, and shutting sand in with the blade, making opening and closing more difficult.

The Sposs

After losing all her knives and seemingly suitable tools, Eckel (1996) discovered the "**sposs**" (Figure 2). This is a hybrid form of spoon boss, a tool that doesn't hurt and that catches the loosened bryophyte before the wind can carry it away. Her husband, Richard Zander, invented and named the sposs. The official sposs has its handle bent back so it can be hung over the belt and one can have a firmer grip. Eckel recommends a 30 cm cooking spoon with a 15 cm boss (bowl part) for gathering bryophytes from under cacti and avoiding snakes and other animals enjoying the cool of the same tracheophyte as the bryophyte. This tool works equally well for the bryophytes in a crevice, on a rock ledge, or in the fragile arrangement of sand in the desert. And it is not confiscated from your pack at the airport!



Figure 2. Spoon bent to make a **sposs** for collecting soil bryophytes. Image by Patricia Eckel.

Chisel

If you typically collect bryophytes on rock or bark, especially tiny ones that require bringing the substrate with them, you might want to invest in a good chisel (Schofield 1985). McCune (1994) recommends one available from Miners Inc. (catalog # AO 601). This is currently available for US \$50.80. It has a tungsten carbide cutting edge that makes it strong and durable, and it is lighter in weight than most chisels (Figure 3). On the other hand, a much cheaper putty knife will work well for soil samples and even some bark samples.



Figure 3. Carbide-tipped hand chisel for removing bits of wood or rock. Photo by Miners Inc.

Masking Tape Sampler

Some species are so tiny that the eye cannot discern them even in good light, or they may be within reach but out of view. Once a possible site for tiny bryophytes is located, extraction of the bryophyte can be accomplished with the aid of masking tape (Snider & He 1991). The tape should be at least 5 cm wide (Figure 4). The tapes differ in their ability to adhere, but none adhered well to very wet or dripping rocks. The vinyl packaging tape was least useful because it easily wrinkled, stuck to itself easily, and was unmanageable in the field. Duck tapes, bandaging tapes, and thicker vinyl repair tapes worked well in the field, but weighed more and were difficult to cut or tear; they were also difficult to work with after samples were acquired. Only painter's tape (masking tape) seemed to be adequate for the job. Even if the tape did not adhere well to moist surfaces, it did a good job of removing and holding the bryophytes.



Figure 4. Masking tape bryophyte sampler with masking tape on a strip of plexiglass. Edges of the plexiglass have been sanded to make them smooth. Photo by Janice Glime.

Snider and He (1991) prepared the tapes by cutting them in 5x17 cm strips, then folding over 2 cm at one end (Figure 5). These were attached at one end to a piece of 6 cm x 17 cm x 5 mm plexiglass. The folded end was used to

pull the tape off the plexiglass to take a sample. Once the sample was in place (Figure 6-Figure 7), they attached the sticky side to the other side of the plexiglass and wrote collection data on the non-sticky side of the tape. When the specimen is returned to the lab, it can be removed by moistening the specimen with water or a wetting agent like Pohlstoffe (See Chapter 2-2 in this volume). They used the method to discover such findings as protonemal trumpets of *Diphyscium foliosum*, protonemal flaps of *Tetraphis pellucida* (Figure 8-Figure 9), asexual propagules of various bryophytes, and several minute leafy liverworts such as *Cephaloziella* (Figure 10). These flat samples can even be photographed by a scanner without glares or need for a tripod (Figure 11-Figure 12). They can be enlarged as scanned or later in Photoshop.



Figure 5. Masking tape sampler, showing folded over ends. Photo by Janice Glime.

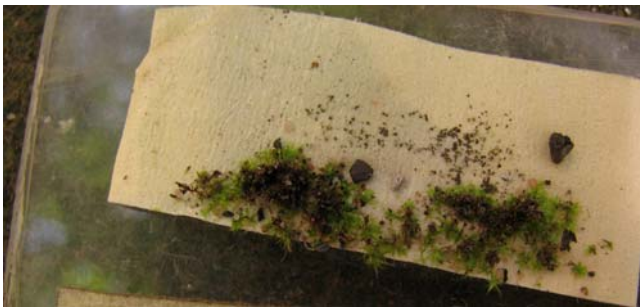


Figure 6. Masking tape sampler with sample of *Bryum* from crack in stone. Photo by Janice Glime.



Figure 7. Masking tape sampler with sample of *Bryum* sp. from crack in stone. Photo by Janice Glime using Epson V500 scanner.

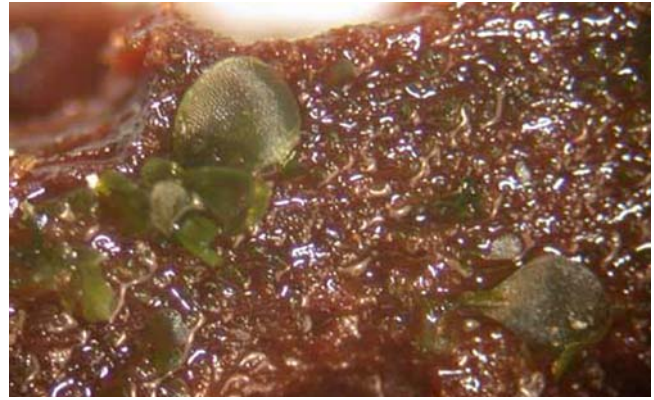


Figure 8. Protonemal flaps of *Tetraphis pellucida*. Photo from University of British Columbia website.

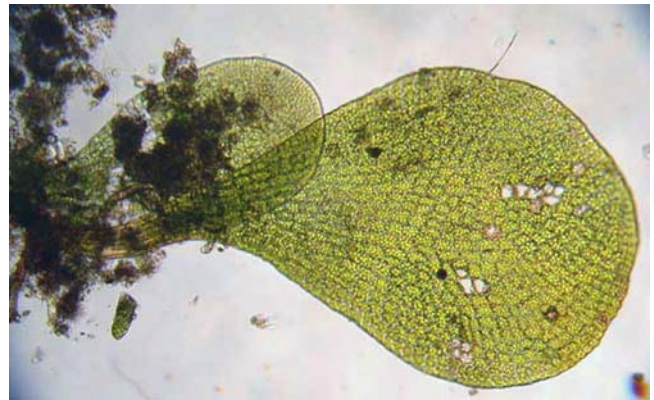


Figure 9. Microscope view of protonemal flaps of *Tetraphis pellucida*. Photo from University of British Columbia website.



Figure 10. *Cephaloziella massalongi*, a very tiny liverwort. Photo by Des Callaghan.

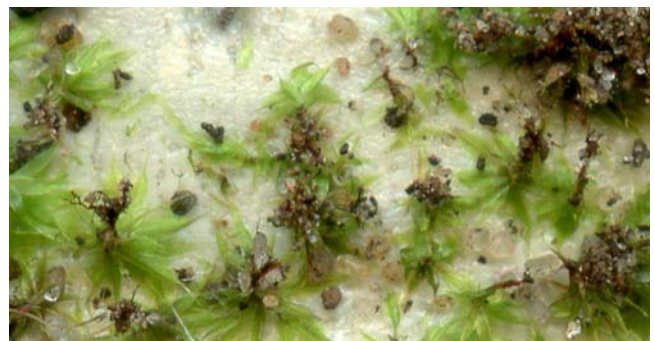


Figure 11. Masking tape sampler with sample of *Bryum* sp. from crack in stone. Photo by Janice Glime using Epson V500 scanner to make image.

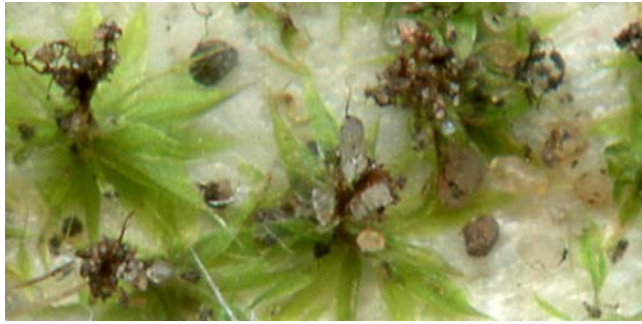


Figure 12. Enlarged view of masking tape sample of *Bryum* sp. from crack in stone. Photo by Janice Glime using Epson V500 scanner.

Seasons

Some bryophytes are seasonal or annual. Although winter is a good season for epiphytes that don't require capsules for identification, it is often not a good collecting season for other bryophytes that may be buried under snow. Flood plain species are only discernible for a period of time after the water recedes following flooding. Species of arable fields are mostly ephemerals that disappear in a relatively short period and often are present in only either spring or fall. Preston *et al.* (2010) found that autumn, winter, and early spring were suitable times to inventory fields in Great Britain. And capsules are only in a mature state with spores intact for a short time. Most of the sexual structures mature in spring or fall, or when the rainy season occurs. Nevertheless, some mature in winter. Hence, the season most suitable for collection depends on the purpose of the collection, the species, and the location.

What to Sample – the Miniscule

Many different kinds of characters are used to identify bryophytes, and reproductive structures also provide ecological life cycle strategy information. Sporophytes on bryophytes like *Orthotrichum* provide important, and sometimes essential, characters needed for identification. Additional searching can sometimes reveal local hidden capsules from a previous year or young, developing capsules from the current year. Tubers and bulbils are also important for both taxonomic and ecological purposes (Vanderpoorten *et al.* 2010). Unstable habitats such as riverbanks, arable fields, and flood plains are likely to have rhizoidal tubers buried in the soil beneath the bryophytes, so 1-3 cm of soil should be collected with the bryophytes (Whitehouse 1966; Porley 2008). Unfortunately, most countries won't permit soil to come into the country, so these must be cleaned and at least some propagules carefully preserved in a **minipacket** along with the specimen.

Minipackets are useful for a number of rare structures and species (Vanderpoorten *et al.* 2010). These can be made in advance, or as needed, so be sure you have some light-weight paper to use. If small species occur among a clump of larger species, place at least a sample of each of the smaller species in a **minipacket**. These packets can be made like the large packets (see Chapter 3 on Herbarium Methods and Exchanges in this volume). If a small species is left to dry with the larger clump, it can become glued to the larger bryophyte when it dries. It will also be brittle

when dry and easily broken if you try to remove it then. Rewetting to remove it can reduce the ability to extract DNA from the bryophyte. At least some plants of an especially small specimen like *Ephemerum* spp. should also be placed in a minipacket, and if only a few plants have reproductive structures, these, too, should be placed in a minipacket (Rothero & Blackstock 2005). Small species on soil are likely to become invisible if the soil dries and loses its cohesiveness, so extracting a few individuals into a minipacket is again useful.

Sample Size

The amount to collect is an important consideration. An ideal sample is about the size of the palm of your hand (Miller 1988; Smith Merrill 1990; Buck & Thiers 1996; Vanderpoorten *et al.* 2010), but that is not always feasible or wise. Conservation should be a foremost consideration. If you must deposit a sample in an institutional herbarium, send to someone to verify identification, and keep some for yourself, be sure to take enough for all those purposes (Buck & Thiers 1996). Only small samples of suspected rare species should be collected, and then only if absolutely necessary and more than that amount is left intact where you found it. **DO NOT collect rare species** just to add to your personal or institutional herbarium or to use for exchange. Be sure to protect the edges if you take part of a clump, at least for species that seem rare in that location or overall. You can do this by placing a rock against the exposed edge or by packing soil against it to protect against desiccation inside the clump. Even another species of bryophyte might help, but try to avoid ones that might overtake a rare species.

When I joined a field trip with the British Bryological Society (BBS), I was warned not to collect more than a thumbnail (or about a 1.5 cm diameter). On the other hand, if you are collecting for exchange or gifts to herbaria, you usually need at least half a palm size for the herbarium to accept the material. Of course if it is a small species with only small clumps, such size will not be possible, or will require several clumps. The danger of several clumps is that they could turn out to be different species or microspecies, and they should certainly all come from the same small area within a location where it is most likely that they have originated from spores or fragments of the same population.

Mixed Collections

The usefulness of mixed collections depends largely on the use of the collection. These provide us with information and should be treated somewhat differently. If the sample collection contains mixed species (Figure 13), they can be separated partially into minipackets in the field, or separation can occur later in the lab. Each packet can be given a different letter while retaining the original collection number; the species occurring together should be noted on the packets. If these are just small bits among a larger collection, they can be placed in minipackets that are kept with the original collection. It is important that packet labelling makes clear which specimen belongs with which name. This can be clarified with minipackets (David Wagner, Bryonet 20 January 2023).



Figure 13. *Hypnum jutlandicum* (pinnate) + *Hypnum lacunosum* (thick branches) + *Dicranum scoparium* (acrocarpous, bright green) in Denmark, illustrating typical species mixes one might encounter. Photo by Lars Hedenäs.

Mixed populations of closely related species can reveal both genetic differences and ecological information. Both species presumably are exposed to the same conditions, so one might assume that differences in morphology (or physiology) are the result of genetic differences. But Wyatt *et al.* (1985) remind us that the microclimate within a bryophyte clump is not uniform. Young individuals resulting from spores that germinate within the clump will experience different growing conditions than did the spores that germinated to form the original colony.

Thus the question arises as to the usefulness of mixed collections (Wyatt *et al.* 1982, 1985). Consider that whether they are all one species or distinguishable as different species, the multiple morphologies contribute important ecological information about the past history of the clump and its microhabitat conditions.

On the other hand, as common garden information, the mixed collection usually falls short. These will be discussed in more detail in the chapter on bryophyte – bryophyte interactions in the Bryophyte Interactions volume. Isoviita (1985), however, argues that in some cases they can be useful to represent common garden conditions. First of all, bryophytes can be difficult to cultivate, and morphologies of cultured bryophytes are likely to change, being unrepresentative. Secondly, the equipment to conduct common garden experiments is not always available.

To understand when mixed collections might be useful, we can consider the arguments of Wagner and Wagner (1983). "Cohabitation of two or more species without successful interbreeding demonstrates biological discreteness and confirms that the character differences are most likely genetically fixed." They used the technique in their study of the fern genus *Botrychium*. This is a fascinating genus with underground prothalli that depend on mycorrhizal fungi. The sporophyte of some species spends little time above ground. This genus can occur intermixed in ways that have little effect on the environment of each other, thus possibly providing information on niche separation. But their most convincing argument is that most of the species in the genus are endangered, so that this is a means of gaining ecological information with minimal disturbance that could create further endangerment in a species that is difficult to culture.

In Papua, New Guinea, handfuls of *Frullania* often produced two or more species of *Frullania* (Glime *et al.* 1990). Multiple collections of these indicated associations that were rather frequent. Other mixed pairs of species in the same genus (**congeneric**) include *Syntrichia laevigata* and *S. papillosa* (Figure 14) (Robert Klips, pers. comm. 10 August 2012) and *Grimmia anodon* and *G. plagiopodia* growing intermixed on sandstone outcrops in western Montana (Roxanne Hastings, pers. comm. 10 August 2012). Intermixed species will be discussed in detail in the Bryophyte Interactions volume in the chapter on bryophyte – bryophyte interactions.



Figure 14. *Syntrichia laevigata* and *S. papillosa* growing intermixed in Columbus, Ohio, USA. Photo by Robert Klips.

In short, for ecological work intermixed collections can be useful and should not be totally avoided. Rather, for verification purposes, use minipackets to store a small sample of each species, but leave most of the mix intact for whatever use might later be needed, including DNA analysis. All identified species should be listed on the packet.

Epiphytes and Epiphylls

In some habitats, especially the tropics, the greatest diversity and abundance occur in the canopy. These require special collecting (Perry 1978) and preservation techniques. Furthermore, only outer bark should be collected with the bryophyte, keeping enough of the bark on the tree to protect the wood against disease. Nevertheless, at least the outer layer of bark should be collected to maintain the slender species that would otherwise be lost (Buck & Thiers 1996).

Epiphytic bryophytes often have directional, vertical, and bark type preferences, and these need to be noted on the herbarium label. Hence, when collecting these, note the host species, the type of bark (rough, smooth, flaking, fissured), height on the tree, and side/aspect of the tree (N-S-E-W). It is also important to note if the substrate was vertical, on a branch or lean, and whether it was on the top, side or bottom of leaning or horizontal structures.

Some bark bryophytes will come off easily, but for some you will need to make a slice of the underlying bark with a sharp knife or chisel in order to keep the growth form of the bryophyte intact.

Canopy

Bryophytes in the canopy present the greatest challenges. These are typically out of reach, so short of bringing a trained monkey, one needs to develop special

techniques. Several researchers have been successful using a single rope to aid tree climbing (Perry 1978; Ter Steege & Cornelissen 1988; Gradstein *et al.* 1996, 2003) (to be covered in chapter on Sampling in this volume). Smaller branches can be sawed off and lowered by ropes.

But not all of us are so agile. Developing archery skills can help, allowing you to shoot epiphytes from the tree, but not all bryophytes will cooperate, and your arrow may lodge in the canopy without returning the prize. Ropes with a weight or hook on one end can sometimes help; with a little skill you might be able to toss it over a branch to pull the branch down. But this method is limited to lower branches because there is too much congestion to be successful in reaching an adequate number of epiphytes in upper branches. Some studies (for insects and other animals) have used a helium-filled dirigible (Hallé 1990) to reach the canopy, but that has another set of dangers.

You (but not the forest) may get lucky and have the advantage of a hurricane or other wind storm to bring branches down from the canopy, but Gradstein *et al.* (2003) point out that fallen branches are inadequate to sample the canopy diversity. One needs to be careful that these are recent falls and represent canopy colonizations rather than post-fall additions. This should be recognizable by the newness of the break on the branch. This method of collecting has the disadvantage that you don't know the height from which the branch has fallen, and sampling is likely to be biased by size class, position in the canopy, and species of tree. Even the age of the tree can be a factor, especially in heavy wind storms.

Epiphytes with their bark substrate may be subject to squashing, especially if you collect in packets, so you might want to pack paper wads around them to protect against such flattening.

Epiphylls should be collected on their substrate leaves to keep the colonies intact, to help in identification of the substrate leaf, and to recognize patterns of colonization. If the leaf is too large, it can be cut so that your collection includes the base, the middle, and the tip (tip morphology is often important in determining the species that collect there). These should be kept in a plant press or other means of keeping the leaf flat for later examination. These are sites for tiny liverworts, especially those in the **Lejeuneaceae**, and should be explored in the lab with the dissecting microscope. Since there are likely to be fungi and Cyanobacteria as cohabitants, the collected leaves need to be dried quickly. Newspapers are useful absorbent materials, but they or other absorbents must be changed daily, especially in humid climates, to discourage overgrowth by the Cyanobacteria and fungi. Especially wet leaves should be blotted dry before the leaf is put in the plant press.

Aquatic Samples

Aquatic bryophytes tend to be quite "dirty." When the bryophytes dry, this mix of silt, bacteria, fungi, and algae becomes glued to the plants, making it difficult to see cells. Hence, aquatic bryophytes need to be washed in the water of their habitat to remove as much of the adhering material as possible. Once the adhering material is removed as best as practical, the bryophyte should be squeezed or pressed, but not wrung, to remove excess water. Then it should be shaken lightly to loosen up the branches and leaves so they

don't all stick together. It may be helpful to remove a few branches and dry them in a minipacket where they can be spread out singly. Otherwise, you may find leaves hopelessly glued together by the adhering algae and bacteria.

For some of the more delicate species, like *Fontinalis flaccida*, the plants can be floated on a 3"x5" (~7x13 cm) card and branches arranged so that some are clear. This may be especially useful for herbaria that glue specimens to sheets, but the cards can also be put in packets and the specimens are easily removed from the cards.

Conservation Issues

Collecting anywhere that is not slated for another destruction is always a conservation issue. Not only is sampling a potential means of destroying the entire colony by disturbing rhizoids and increasing moisture loss, the trampling involved can also be destructive. Wagner () follows the 1-in-20 rule. That is, don't remove more than 1 plant in 20 or more than 5% of a plant to conserve its life. This same rule has been adopted independently in Australia and at the New York Botanical Garden, suggesting that 1-in-20 is a good rule.

Removal depends partly on the moss species and its growth habit. There are a few "rules" you can follow:

1. If you have permanent plots, sample pieces outside, but adjacent, to the plot when sampling is essential, such as reproductive phenology studies.
2. Don't disturb the cushions and other growth forms that are needed to maintain water relations of the bryophyte.
3. Collect pleurocarpous branches from the edge of the colony.
4. Generally don't remove specimens from the center of a colony, especially of acrocarpous species, because it changes moisture-holding ability.
5. If there is only a small population, avoid removing any, except perhaps a small branch if it is absolutely essential, placing it in a mini packet so it doesn't get lost.
6. Be aware of potentially rare species and take only pictures.
7. Be careful what you tread on and how often. Sometimes a plastic sled can be used for standing or squatting to minimize damage.
8. The objective is to collect in a way that the bryophytes can fill in the vacated space before other species invade.
9. For teaching, I kept a teaching collection (plus photography) that students could use for study without having to collect their own.
10. Some herbaria won't accept specimens smaller than the palm of your hand, but I found that the British get upset if you collect a sample larger than your thumbnail on their forays.
11. Always have permission before collecting.

Collecting Permits

The temptation to pick up a bit of moss anywhere you find it is compelling, especially if it looks new and interesting. And, unfortunately, most land owners don't care about the bryophytes. But in many places, especially parks at any level, a collecting permit is required. At the very least, you need permission of the land-owner. It would be futile to try to list places where one might obtain such a permit, but it is very important. Not only is it embarrassing to be caught "stealing" a specimen, but there may be fines and even sanctions. As a representative of your institution, you can bring bad publicity to that institution and even to your country if you are in another country from your own.

A search of Google for **collecting permit** will get you lots of addresses and websites, but a narrower search for the country, state, or municipality may get just what you need. If you are unable to find anything for that country or state, you can usually get pointed to the right place by contacting a local bryologist. If there is no bryologist, try the Department of Agriculture website to see if it provides any leads – or contact them directly with an explanation of what you want to collect, how much, the purpose of the collection, and a query about who to contact for permission to collect and export. A useful website telling you contact information for various countries and various agencies in the USA is called The Skeptical Moth <<http://skepticalmoth.southernfriedscience.com/techniques/collecting-permits/>>.

One of the most embarrassing things you could do is to take a class collecting somewhere when you don't have permission. And even if you have standing permission, it is often a good idea to notify the owner you are coming so you don't inadvertently enter upon an event where it would be dangerous or awkward.

Don't be surprised if there is a fee for a collecting permit. And that may differ, depending on who you are and where you are from! For example, West Virginia, USA, provides the permits free of charge to academics, students, and researchers from West Virginia, USA, but charges \$25 for the same group out of state. Permits for commercial use are much higher and apply to everyone.

Keep your permit with you in the field. You might want to keep it in a Ziploc bag so it remains legible. When we were in Yellowstone, off trail and out of sight, a ranger approached us and we had to show our permit. Our parked car had attracted his attention.

Bryological Collector Arrested

Collecting without permission is taken seriously, at least in New Zealand. One eager collector in New Zealand was arrested for collecting without a permit. The arrested collector became temporarily famous through journals such as *Commercial Horticulture* (January 1993), with the article titled "US botanist fined for taking native mosses" (Alan Whittemore, Bryonet 29 September 1999). The botanist was collecting material to screen it for natural products, not for herbarium records. In addition to his infamy, he was fined. The mosses had been collected in national parks in quantities for which personnel would not have issued a permit.

In some countries you will be asked to leave your collections behind with a local herbarium or museum and may never see them again (Willem Meijer, Bryonet 28 September 1999). Meijer suggests working with young students from that country who are eager to learn. They may be more willing to send a portion of your specimens from a herbarium just to get them identified.

Record-keeping

When in the field, do fieldwork. Minimal time should be spent doing other record-keeping chores. BUT, do keep complete records. A common way for bryologists to do this is to prepare packets or small paper candy bags in advance (Figure 15). This is done by numbering them consecutively and keeping a small record book (Figure 16). If you keep a life list of numbers, you also have a record of how much collecting you have done. There are numbering machines that use stamp pad ink. These allow you to set the starting number and each time you press it onto a bag or packet, the number advances.



Figure 15. Candy bag collection bags, pre-numbered. Photo by Janice Glime.

When you arrive at a collecting site, record in the notebook the **starting collection number**, **date**, **location**, general features of the **habitat**, **altitude**, and **GPS coordinates** (Figure 17). It is also important to record characters that might change as the specimen dries, including **color**, **growth form**, and **fertility** (Rob Gradstein, pers. comm. 28 July 2012). Be aware that different nationalities abbreviate dates differently, so 3/5/12 means 5 March 2012 in the USA, but means 3 May 2012 in Germany. It is safest to **write out the month**. A good way to be sure your information is not lost due to rain or other mishap is to photograph the beginning page if you have a camera. This also serves to mark the beginning of pictures taken at the site.



Figure 16. Record book showing dates of collection included. Photo by Janice Glime.

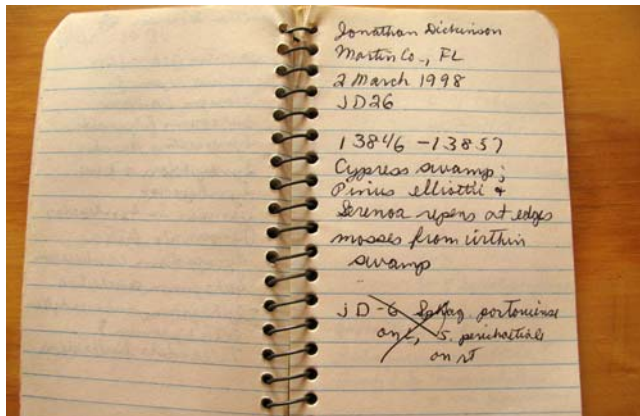


Figure 17. Field notebook record of a collection site, including general habitat description and record of collection numbers. GPS was not available. Photo by Janice Glime.

A partial alternative to notebooks or writing on bags is a field packet labelled with habitat characteristics to circle (Figure 18-Figure 19). I was introduced to this in Japan by Zen Iwatsuki. I found I could write just about as fast as I could locate the right word to circle, but I suspect that after one uses the method for awhile it would be faster. It does provide the advantage that the collector is more likely to include more detail about habitat information.

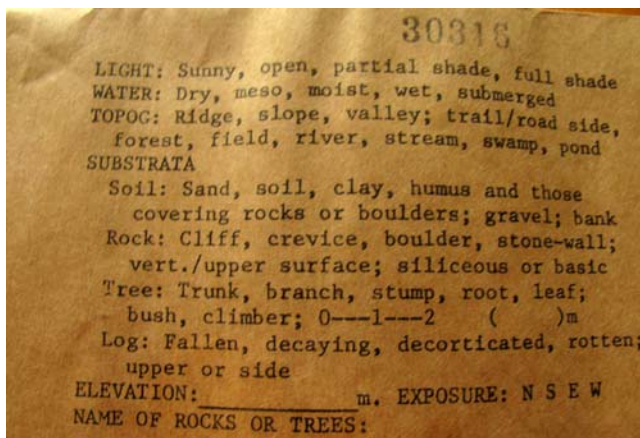


Figure 18. Collecting packet from Zen Iwatsuki, demonstrating a habitat circling system used by some bryologists. Photo by Janice Glime.

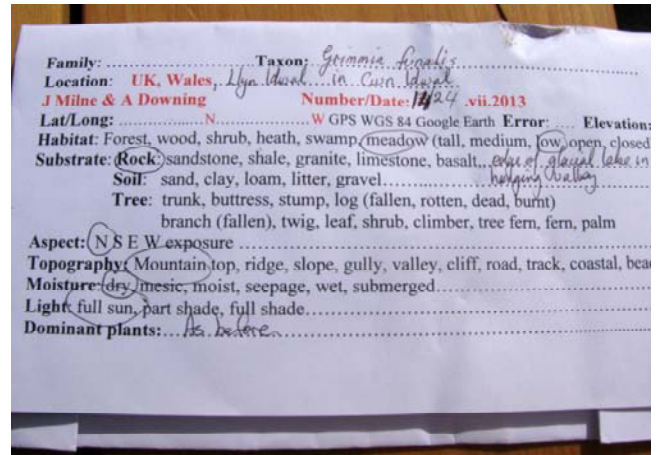


Figure 19. Field packet used by Allison Downing and Pina Milne. Photo by Janice Glime.

When collecting the bryophyte, squeeze out excess water and put the bryophyte in the bag or packet. (See chapter on Herbarium Methods and Exchanges to learn one way of folding a packet.) Be sure the numbered packets or bags are kept in order before use. I do this by having an apron with pockets (see Figure 32-Figure 36 below). The pocket is long enough and wide enough for the bags I use to fit easily with an end sticking out for easy grabbing. Always remove the bag or packet from the top of the pile, then record the **elevation, substrate, exposure, indication of moisture, and specific habitat and microhabitat** information that is not included with your general habitat information (Buck & Thiers 1996; Figure 20). It is helpful to put your **best guess name** on the packet, with a question mark if there is any doubt. That can make it easier to find the specimen later when you want a specific one, and it also makes identification easier because you have used the clues provided by growth habit and microhabitat. Try to avoid putting more than one collection or species in the same bag or packet unless they are tightly intermixed or the mix is needed for ecological study.

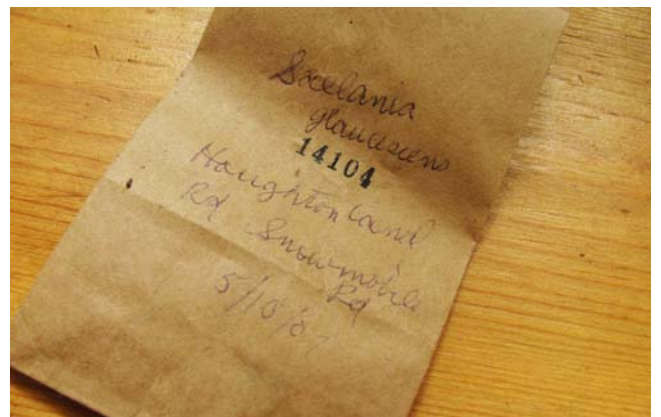


Figure 20. Bag with sample and tentative name. It is missing substrate information; hopefully that is in the field notebook. Photo by Janice Glime.

When you are ready to leave the site, finish your notebook page by recording the last collection number. Add any further observations that might help. It is also helpful to take another picture of the page to mark the end of that collection site among your pictures. If you take

bryophyte pictures along the way, you might want to photograph the packet or bag with your identification guess to help you recognize your pictures. If you are on an extended collecting trip, it might be awhile before you are able to process them, and bryophytes in pictures are not easy to recognize. And don't forget to include some pictures of the habitat for your collections.

An alternative option for the age of technology is to use a mobile phone app such as EpiCollect (Franks 2013). This app was originally designed for recording epidemiological data, but can be used conveniently for plant field records (Aanensen *et al.* 2009). You can design your own database for a specific project, as Franks has done. Data recorded on your phone can be synched into a Google Cloud that is available through the internet anywhere. The phone GPS system can assign the coordinates, date, and elevation, and you can even link a picture, taken by the same phone, to the data entry. For closer images, a hand lens over the phone's lens can magnify your image considerably. The only drawbacks are carrying extra batteries, risk of getting the phone wet, and having to spend a bit more time entering data while in the field. Thus far, the app cannot duplicate location and habitat from one record to the next, but it is only a matter of time before someone designs a repeat button for that purpose. Franks has created a bryological app that permits you to click on a specific point on Google Maps or Google Earth to see all the data fields for that point and any linked photographs. This application is part of the QBry project at <<http://epicollectserver.appspot.com/project.html?name=QBry>>.

Data Sheets

If consistent habitat information is needed, especially if more than one person is collecting the information, field data sheets can be useful (Shevock 2021; , . For ecological studies, it is best to create a preliminary list of species, allowing plenty of space to add to it as needed. This can be done by a reconnaissance trip and lab identifications prior to a more detailed study, or by a quick reconnaissance on the day of the data collection. In the latter case, the team should combine their lists and discuss possible identification conflicts and annotations for unknown species. At the end of the day, the added species should be coordinated and their temporary names unified to avoid confusion later. Data sheets will be discussed in more detail in the chapter on Sampling in this volume.

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: _____	

Figure 21. Field data sheet from Shevock 2021; circle descriptors that fit.

Permanent Ink

I (Glime) learned as a graduate student to use a Rapidograph pen with India ink to write labels. This permanence was especially important because I was placing labels into 1 dram vials that housed preserved insects I had removed from stream bryophytes. Since ball point pen ink was readily soluble in the alcohol preservative, and external labels frequently came off the vials, the Rapidograph solved both the permanence problem and the need to write very small on a label small enough to fit in the vial and still be legible.

Zander (2004) pointed out the problems in using Rapidograph pens. The ink easily clogs in the small diameter point, filling them is not easy, and they are expensive. He suggests using a modified ball point pen. In particular, the Beifa "Tank" pen is available in dollar stores and is cheap (Figure 22).



Figure 22. Permanent ink Beifa ball point pen. Photo by Richard Zander.

Although the ink is supposed to be "permanent," Zander replaces it with India ink (Figure 23). To do this, he removes the point stem with its disks using a pair of pliers, then replaces the ink with India ink such as the Rapidograph ink (it comes in a handy squeeze bottle). The stem is then replaced in the pen. Zander has used this modification for a long time without experiencing a point jam.



Figure 23. Permanent ink ball point pen, with original ink replaced with permanent India ink. Photo by Richard Zander.

If your bags and notebooks get wet, not much will work for record-keeping. I keep a felt pen (Sharpie) with me because it has a little more success on wet paper. Pencils just dig up the paper, although they are somewhat more successful on damp paper than most pens. I haven't tried India ink on wet surfaces. Richard Zander (pers. comm. 12 August 2012) recommends a crayon or wax pencil as backup. Zander also suggested waterproof paper such as that from Forestry Suppliers (Rite in the Rain® Field-Flex Notebooks). If you can keep a notebook in a dry place and be able to write in it without getting wet, you can put your notes on a sheet of paper there and put that page into the packet or bag. For this purpose, it helps to have a plastic bag that is large enough for you to write within it. The notebook can be wrapped inside it.

GPS Coordinates

Technology has even improved fieldwork in bryology. A simple hand-held GPS unit permits one to record exact locations, with degree of accuracy depending on the quality of the meter, and of course, its price. And many of the new digital cameras will automatically record GPS coordinates with you pictures. Now even cell phones come with GPS software. Once this information is recorded with the specimen, it is possible to relocate the population much more easily than was possible in most cases before this technology. Furthermore, Jan-Peter Frahm (Bryonet 31 May 2012) reports that he has had a program created that permits him to record a list of species in *.txt format. By clicking on the name, one can record the name with its coordinates, date and hour of collection, and altitude. The records can be transferred to a PC in Excel or a Google file, then imported to the database FLORKART (in German meaning plant map) to produce a map output or to display on Google Earth. This can be used with Android smart phones or with Windows Mobile Smart phones that have a built-in GPS. Unfortunately its website is no longer available.

Voucher Specimens

As noted by Shevock *et al.* (2014), understanding of biodiversity is critical to determining distribution, abundance, rarity, and conservation priorities. To document these, one must place voucher specimens in stable herbaria for later verification and new species concepts. As already mentioned, **every** study, whether it is taxonomic, ecological, physiological, or biochemical, should provide voucher specimens so that later researchers can verify or compare the identifications. This does not imply that you have misidentified the species. Rather, it adds to our comparisons by providing material for species to be checked for possibility of a segregate when they are later split. This will undoubtedly become more common as we increase our DNA knowledge base. And of course if someone studies the same location later, but finds species differences, the voucher specimens will permit checking to be sure the two studies haven't determined different names for the same species. This collection can also be studied by the next researcher before embarking on the field study to learn to recognize the species and prepare the mind for spotting them. Storage of these specimens should take into account that they might be later used for DNA testing,

baseline records of pollutants, or other purposes that require careful treatment.

For voucher specimens to be useful, any publication on the study should clearly state where the specimens are located and how they can be identified as belonging to that study. This is typically done by specifying the **collection numbers** (your field numbers) or **accession numbers** (numbers assigned by the herbarium) in the publication. It also helps to label them as voucher specimens and identification of the study name. This can help to protect them from being discarded or moved without notifying the bryological community. With the digitizing of herbarium records, it should eventually be easier to track such collections.

Field Preservation

Most specimens are easily kept in paper packets or in paper bags until such time as herbarium packets are made, but some require special attention. It is important that the specimens are dried relatively quickly. Schuster (1966) warns that keeping them moist, especially in a confined, warm place, will encourage growth of fungi, and the bryophytes may continue to grow, becoming **etiolated**. **Never** store them in plastic bags as that encourages mold.

It is useful to maintain the growth form, but this will be disturbed when leaves are removed. To facilitate examination, it is helpful to separate a few specimens from the edge of the clump or from a neighboring clump so that these can be used for close observation and leaf removal.

Vanderpoorten *et al.* (2010) advise that material collected for DNA extraction should be cleaned and immediately air-dried, then kept dry. Subsequent moistening can lead to degradation of the DNA so that it cannot be used for molecular analysis.

Liverworts will lose their oil bodies upon drying, so if at all possible they should be kept hydrated until they have been examined. Make drawings or take pictures of the oil bodies, or at least make a detailed description, because these cannot be preserved. In some cases, they will become reestablished after a number of hours of rehydration.

Chris Cargill (Bryonet 12 August 2021) reports using A4 sheets of used photocopy paper to enclose the bryophytes while they dry. This prevents curling in soil specimens as they dry and also reduces or eliminates the effects of light during drying, making the specimens more useful for later study. Wire baskets can be used for faster drying. She further recommends keeping loose soil from the specimens in small Ziploc bags, stored with the specimen. The separate packaging prevents abrasions. Specimens with soil can be wrapped in tissue paper inside the packet to prevent further loss of soil.

Liverworts and other Flat Plants

Although some liverworts, especially *Riccia* species of flood plains, can revive after long periods of desiccation, many thallose liverworts can dry out, break, or become irrevocably distorted when they dry. These are best identified while still fresh and moist, but if this is impossible, add water to rehydrate them. Herbarium specimens should not be preserved in any preservative

because it makes them unusable for DNA or other molecular analysis. If one is concerned about maintaining the natural habit, a small portion of the sample could be preserved (Ohta 1991) or stored in the preservative phenylacetic acid-alcohol (Rob Gradstein, pers. comm. 26 July 2012), with the bulk of the specimen being kept dry and having a cross reference to indicate where the preserved specimen is located.

Liverworts typically need light pressing. This can be done between sheets of a newspaper, or in a phone book, but do not apply pressure, *i.e.*, do not put them in a tight plant press. Buck and Thiers (1996) suggest removing excess soil and debris and placing them between papers or in a folded packet, then placing them in a plant press with light pressure and no heat for 24 hours.

Oil bodies typically disappear upon drying. Flash freezing and other methods that work in the lab might not be available for prolonged fieldwork. Jeff Duckett (Bryonet 5 October 2021) recommends keeping a bottle of 25% glutaraldehyde or formaldehyde on hand and diluting to 1% for use. ETOH should not be used if preservation of oil bodies is desirable.

Tiny Bryophytes

Tiny bryophytes can also be a problem. Richard Zander (pers. comm. 27 July 2012) was kind enough to contribute to dealing with this problem. He suggests that one can use a squirt bottle of water to wash away powdery soil from small plants. In some cases, especially on wet clay, one might be able to put these on a card (3x5" is a good size) and have the clay substrate glue itself to the card. This won't work with dry sand. Using an empty squirt bottle or other type of hand air pump to blow away powdery soil might expose enough of the plant clump that it can be separated from the soil and placed in a minipacket or small envelope so it doesn't get lost. In fact, Zander (pers. comm. 29 July 2012) triple-packets them. He puts the sample (dust and bryophytes) into a large inner packet, then puts each bryophyte species into a small packet inside that.

Keeping a sand-dwelling or clay-dwelling colony intact is a special challenge. Zander (pers. comm. 29 July 2012) tells me he used Elmer's glue once. He says the polyvinyl alcohol available now is soluble in water, so bryophytes can be glued to paper, then removed with water for examination later. I haven't tried it.

Aquatic Species

If wet aquatic species are stored with other bryophytes, they will keep the others from drying. For species like *Sphagnum* and other wet bryophytes, remove as much water as possible by squeezing them (Vanderpoorten *et al.* 2010). If possible, fluff them out again before putting them in their packets or bags. Make their containers triple thick so the water is less likely to cause the container to tear or come apart. If the bryophyte is really wet, put it in a plastic bag, but be sure to take it out as soon as you reach a place where you can dry your collections.

Fornwall (1977) compared three storage methods for the aquatic moss *Fontinalis duriaei*. He found that storage dry at room temperature, and dry packed in coolers with

cold packs both caused the mosses to lose color and appear to be quite unhealthy after being rehydrated and cultured in fresh stream water at 10°C for seven days. However, those mosses that were stored in bags of stream water with cool packs for three weeks (and opened every night to allow gas exchange) exhibited levels of photosynthesis and respiration after storage that did not differ from the measurements prior to storage.

Drying Specimens

Getting specimens dried before they have an opportunity to mold or curl can be a challenge on extended field trips in faraway places. Generally, they can be dried by opening the bags and spreading them around your room or laboratory (Figure 24-Figure 25). If you are travelling by car, bryophytes in their collection bags or packets can be placed in a net or burlap bag and affixed to the top of the car to air dry. It is best not to leave them there when you are not in attendance because it could rain. They also should not be baked in the hot sun.



Figure 24. Jim Shevock in "drying room" with packets on left and specimen bags opened for drying on right. Photo by Blanka Aguero.



Figure 25. Drying bryophytes during Nordic Bryological Society foray. Photo by Michael Lüth.

Croat (1979) addressed this problem in a big way by modifying a truck into a processing lab and modifying a refrigerator by adding a portable propane gas oven to use for field drying. Fortunately, such elaborate equipment is usually not necessary for bryophytes, but in humid warm climates of the tropics, drying can still at times be a challenge.

Frahm and Gradstein (1986) constructed a dryer that is light weight and inexpensive for use in such humid climates (Figure 26). The drying source is a pair of kerosene stoves. The legs of the dryer are made of aluminum, making them light weight. They are about 1 m high and extend above the platform where they support a cotton curtain to hold in the dry heat. The shelf is made of wire screening and packets or open bags can be distributed across it. Of course, these must be protected against wind or your prized collection will escape to freedom! Frahm and Gradstein warn against use of polyester or nylon for the curtains or screen because they are more flammable. Be sure to do a little experimenting so you know just how high to place your shelf and how often the apparatus should be checked or your specimens could turn to charcoal – or worse.

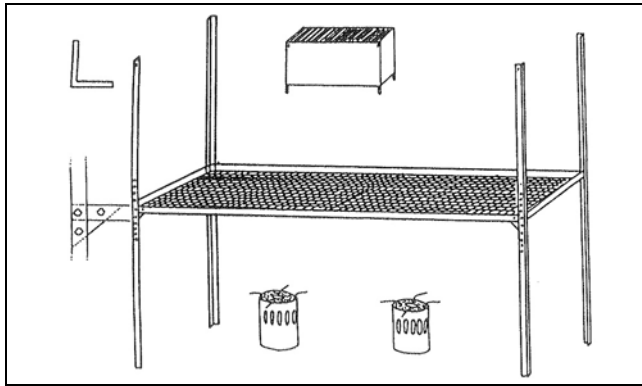


Figure 26. Field drying rack for bryophytes. Note the two kerosene stoves beneath and the inset of the curtained part of the platform above. Image from Frahm & Gradstein 1986, *Bryological Times* 38: 5.

David Wagner (2014), a constant innovator of bryological methods, has devised a simple, rapid, and inexpensive method for drying bryophyte specimens. He uses the spring type of clothespins to attach specimens first to a rod or rope, than to attach additional ones to the specimen above (Figure 27). This method has the advantage of permitting air to reach both sides of the specimens. He devised this method for field packets, but it should work as well for paper bags, provided they are not so wet that they tear under the added weight and pressure of the clothes pins. Bulldog clips (Figure 28) are more compact for travelling and may even be easier to find for purchase. A fan can be used to speed up drying even more. The paper in the packets is kraft paper.

Once the specimens are **thoroughly** dry, they should be packed in sealed plastic bags (unless the air is dry) to avoid having these hygroscopic plants once again take up water. Please note that if they are not completely dry, they are likely to mold inside the plastic bags.



Figure 27. Drying packets clipped together with wooden clothes pins. Photo by David Wagner.



Figure 28. Drying packets clamped together with bulldog clips. Photo by David Wagner.

Once dry, specimens can be kept dry by sealing them in bags containing silica gel ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) (Greene 1986). Greene reports that the method worked excellently in the southern Chilean rainforest. If the silica gel has absorbed moisture prior to use, it can be dried along with the bryophytes on the drying rack.

If floating species like *Riccia fluitans* (Figure 29) are to be kept, a good way to collect them is to float them on an index card that will fit inside a packet. The algae and other detritus will glue them to the card. Once dry, they can be put in a packet like other bryophytes.

Field Stains

Occasionally you may want to see something more clearly in the field. For ecological studies, being able to identify every individual can sometimes be tedious but necessary. In some cases, field stains can help in this endeavor, such as seeing fimbriate stem leaves on *Sphagnum*. Jan Janssens (Bryonet 4 October 2012) suggested using crystal violet or gentian violet solution. It works well when filled into a rinsed and dried felt-tip pen. He suggests pulling off the *Sphagnum* capitulum and squeezing the *Sphagnum* somewhat dry before applying

stain at the top of the broken stem. This technique also works well in the lab. If no stain is available, you can hold plants up to diffuse skylight to get a somewhat better view.



Figure 29. *Riccia fluitans* that is "floating" on paper. Photo by Kristian Peters, with permission.

Adam Hölzer (Bryonet 4 October 2012) likewise uses **crystal violet** (Merck Art. 1408), enabling him to see pores under the microscope. Dissolve some powder in 50 ml distilled water and add alcohol to preserve. Add new alcohol from time to time as the alcohol evaporates. Use alcohol for cleanup.

Field Gear – Collecting Equipment

If you have the motto "Be prepared" you might want to keep your collecting pack ready to go, or at least keep a checklist. Loeske (1925), interpreted by Raup (1926), suggested that essential equipment consisted of a good lens, paper envelopes, and notebook. I would suggest a bit more to increase efficiency. Here is what I would recommend.

Field Gear Checklist (essentials are in bold)
hand lens on lanyard or string (Figure 40- Figure 43)
indelible pen
pencil
knife with protected point (Figure 36)
prenumbered packets or bags (Figure 15)
bag for collections
Ziploc plastic baggies (Figure 34)
field notebook (Figure 16)
masking tape sampler (Figure 4-Figure 7)
back pack
collecting pockets (Figure 31-Figure 36)
squirt bottle for moistening specimens
cloth measuring tape (Figure 30)
GPS
altimeter
metric ruler
water
sun glasses
hat
pocket raincoat
bug repellent
food (added the day of the trip)
field guide



Figure 30. Cloth measuring tape with metric units on one side and English units on the other. This can be helpful for measuring height on tree, diameter of tree, size of population, and various other distances. Photo by Janice Glime.

Attire

Although attire is mostly dictated by preference and the climate of the collecting location, one might consider a few accessories. Sun protection is important for those working in the open sun, and Eckel (1996) suggests carrying a small, collapsible umbrella to gain some relief from an intense sun. I prefer a wide-brimmed cloth hat that I can fold into a pocket or pack when it isn't needed. Sun glasses that flip up during hand lens use are important for protecting one's eyes.

Collecting Apron

Keeping bags, pens, hand lenses, camera, knife, record book, and other items close at hand but out of the way during a field trip can be a challenge. Back packs can hold a lot, but they are not handy, and sometimes there is no place to set one down. In others, they may throw you off balance, causing a fall and even endangering your life. To solve the problem, I created a set of pockets that I tie around my waist (Figure 31-Figure 34). These can be designed to meet your own needs with pockets to hold your equipment while holding it secure against loss. I recommend a heavy cloth like denim, or even double cloth. Stitching should be in double lines, and ends and corners should be reinforced with criss-cross stitches or other means of reinforcement. Mine are designed to tie, and my last set has the pockets sewn onto the ties. I like my former design better, where the pockets, or at least the front ones, are threaded onto the ties like curtains on a rod. When this is done, don't use loops, as they are easily torn if the pocket gets caught when you are hiking through brush. You could also use a belt, but with some clothing it can be uncomfortable. In either case, try the pockets on before stitching them down to be sure the pockets locate themselves where you want them. The two flank pocket panels provide easiest access when they meet near the middle of the front.

I carry 3x5" cards with me for a variety of uses (Figure 33). They can be helpful for scooping floating bryophytes from the water. They can be used to mount these wet specimens by floating them on the card and letting them

dry there. This permits the specimen to spread out and glue itself to the card instead of the plants gluing themselves together. This may also be useful for some small liverworts that may otherwise get lost in the bag, although minipackets are usually a better way to handle these. Cards can also provide a smooth surface for epiphylls and thallose liverworts and they help create rigidity for packets with thin soil layers.



Figure 31. Janice Glime wearing collecting pockets in geothermal field, Karapiti, NZ. Photo by Zen Iwatsuki.



Figure 32. Set of three pocket panels. Note the bulky middle pocket that hangs over one's rump to hold collected specimens and field guides. The whistle on the tie is for calling missing students or calling for help when you are lost from your companion. Photo by Janice Glime.



Figure 33. Pockets for numbered bags, cards, field notebook. Note the small pockets above the bag pockets. These are suitable for batteries, hand lenses, and other small items. See detailed image of these in Figure 35. Photo by Janice Glime.



Figure 34. Pockets for pens, knives, flash, phone, or camera, or, in this case, plastic bags in case very wet species are collected. Note that the back pocket on the left is gathered at the top to make the inside larger to keep the contents from falling out. Photo by Janice Glime.

It is useful to have small sheets of paper to make mini-packets for small species or small objects such as capsules that might get lost in the collecting bag or among other bryophytes. Pages from the field notebook can serve this purpose, provided that their removal does not cause the remaining pages to come apart.

Small pockets help to keep tools in easy reach and avoid tangling (Figure 35). Long, narrow pockets can hold knives, pens, or pencils (Figure 36).



Figure 35. Small pockets with potential uses shown by the lenses sitting on them. Note the double stitching at the bottom of the pocket. Photo by Janice Glime.

My back pocket is large and is not flat, being larger across the bottom edge (Figure 34). It can hold packets with mosses in them and a field guide. It's a good idea to keep the field guide in a Ziploc bag to protect it from dirt and water.

Some carpenters' aprons may serve your purposes and are made to carry heavy tools, so they are durable. You may have to add your own back pocket and some small pockets if you need them. It depends on your needs – and how ambitious you are.



Figure 36. Long, narrow pockets house pens and knives. Photo by Janice Glime.

Collection Bags

Bryophyte collections can be damp or even soggy. If you are staying in a hotel or have much travelling to do, these must be placed where they won't mold and can begin to dry. In Japan, I was introduced to hand-made collecting bags for holding the paper bags (Figure 37). If you don't go too many places, you can use a separate bag for each collection site. It is usually possible to tie these to your belt or to the collection apron (Figure 38). Bright colors help you to locate a bag you have left on the ground.



Figure 37. Bryophyte collection bag. Photo by Janice Glime.



Figure 38. Zen Iwatsuki warming his hands over a geothermal vent in Iceland. Note the collecting bag hanging from his belt. Photo by Janice Glime.

Eckel (1996) extols the benefits of a Naugahyde (vinyl-coated fabric) flat bag. In the morning it is filled with empty packets that are replaced during the day with filled packets. It can double as a pillow for sitting, a cushion for sliding down a slope, a shield against cacti, and protection for crossing a barbed wire fence.

Hand Lenses (Loupes)

Hand lenses are essential for seeing the details needed for identifying bryophytes. And they also reveal the beauty of the bryophyte world. Lenses come in a variety of magnifications and sizes (Figure 39). The most commonly used is a 10X loupe, but you might even be able to use one up to 30X. The small ones are the most convenient because they weigh less and are often easier to focus, especially if you wear glasses. A reading magnifying glass offers some help but is not nearly as helpful as a 10X hand lens, and it is heavy and bulky.

When using a hand lens, hold it close to your eye and bring the bryophyte toward you until it is in focus (Figure 40). One advantage of the lens is that it permits you to focus on something close to your eye. The exact positioning will depend on the correction in your glasses.



Figure 39. Small 20X and larger 10X hand lens with nylon string. Photo by Janice Glime.



Figure 40. Janice Glime demonstrating the use of a hand lens while wearing eye glasses. Photo by Jill Nissila.

It is a good idea to carry several hand lenses with you. In damp, cold, or rainy weather, the lens can fog up and it may take an hour before it is usable again. And there is always the chance you will lose one. By all means attach your hand lens to something. A lanyard is good, but a heavy string will work well and is flexible and light weight. If your lens is hanging around your neck, you can tuck it inside your coat when it is raining or cold, and it will always be handy without being lost easily.

A few bryophytes have been shown to have fluorescent propagula, and such propagula are often difficult to see in the field. For the taxonomist, the solution is to collect and identify later, but for the ecologist, field identification is important. More importantly, the same species needs to be distinguished from similar species during field studies, even if verification must come later. Zimmermann (2011) introduced us to a 10X hand lens that provides the UV light needed to see this fluorescence in the field Figure 41- Figure 42) (Zimmermann 2012). The lens now is available at 10X, 15X, and 20X with color temperatures of 4500, 600 (neutral), and 8000 K. Norbert Stapper (Bryonet 16 July 2013) recommends the neutral, with cool white not showing the typical yellow color of a the lichen *Flavoparmelia*. Nick Hodgetts (Bryonet 1 December 2011) adds his endorsement to this lens. The lens is a bit costly at 195 Euros plus postage.



Figure 41. Lichen candelaris UV hand lens. Photo by Erich Zimmermann.



Figure 42. Lichen candelaris UV hand lens showing inside. Photo by Erich Zimmermann.

Technical details:

- Cold white light through two laterally shifted LEDs (prevents shadows). LED with low power consumption and high lifespan
- Operation time: 8,000 x 5 sec flashes until low battery indicator lights up, additional 300 flashes to battery empty
- 3V Lithium-batteries (3 pcs. CR 2023 Renata): High energy density and extended shelf life
- Active power source results in constant luminous intensity over the whole battery life cycle and extended temperature range
- Lens system x10, $\text{\AA}20\text{mm}$, (triplet, aplanate, achromate, closely glued)
- Submerged key, anodized Alloy-box, water spray proof IP67, your name is laser labelled on special order.
- Weight 76 g
- Swiss made, 1 year warranty
- Included in delivery: 10X magnifying glass, 2 pcs Lanyard, 3 replacement batteries, instruction manual with technical details

Hand lenses have been a popular topic on Bryonet, and members have their own preferences that may help you in your consideration. Werner Pflaum (Bryonet 30 November 2011) recommends the Lichen candelaris despite its high price. He considers the light source to be excellent. Norbert Stapper (Bryonet 30 November 2011) warns that the lens is not waterproof because it lacks an O-ring to seal the electronics and battery compartment. The lens system is a sealed triplet, which eliminates fogging, an important consideration for rainy days or cold weather.

David Wagner (Bryonet 16 July 2013) recommends the 20X hand lens by Iwamoto, claiming it is worth the \$100 or so it costs because of exceptional clarity and wide field of the lens. In USA it can be ordered from Minerox <<http://www.minerox.com>>.

Less expensive lenses lack the light source and generally have only two lenses, not three. David DuMond (Bryonet 28 November 2011) recommends a hand lens with LED source from Miners <https://minerox.com/index.cfm?fuseaction=category.display&category_ID=2>. This 20X lens has a 21 mm diameter and triplet glass (Figure 43). It is only US \$24.95, complete with leather carrying case.



Figure 43. Handlens with LED. Photo by Miners.

The Weinschenk hand lens has excellent optics with sealed triplet lenses, available in 10X and 20X, but no light source. Norbert Stapper (Bryonet 15 July 2013) combines

the 20X Weinschenk hand lens with the Lichen candelaris 10X to obtain 28X magnification. The Weinschenk lens is available through Industrieoptik Fischer, Wetzlar, Germany <<http://www.iof-wetzlar.de>> or from <http://www.kruess.de/shop/Lupen/Weinschenk-Lupe:::21_44.html>. Rune Halvorsen (Bryonet 15 July 2013) considers it "an absolute must for bryologists!" Martin Godfrey (Bryonet 29 November 2011) recommends hand lenses from Quicktest <www.quicktest.co.uk>. This company supplies lenses for the jewelry trade and carries hand lenses that range in cost from £1.50 to £95.00 for a 10X lens. Marshall Crosby (Bryonet 2 February 2012) recommends BioQuip for hand lenses at a range of prices <http://www.bioquip.com/specials/product_special.asp>.

McCune (1994) recommended an illuminated lens by Bausch and Lomb, available through Forestry Suppliers for US \$43.75, but it no longer seems to be available from them. However, they now have one that is 10X instead of the original 7X lens, also by Bausch and Lomb, but for only US \$28.25. It requires two AA batteries and is the size of a fountain pen (if you remember what that is!). McCune found it very useful in the field for examining bryophytes and lichens in a permanent plot when you must get so close you block the daylight.

Jesús Muñoz (Bryonet 15 July 2013) uses both the 14X and 20X Bausch & Lomb Hastings Triplet hand lenses, available from Forestry Suppliers <http://www.forestry-suppliers.com/product_pages/View_Catalog_Page.asp?mi=52491&title=Bausch+%26+Lomb%AE+Hastings+Triplet+Pocket+Magnifiers>.

Sean Edwards (Bryonet 1 August 2013) has found the 10X Ruper triplet lenses from Summerfield Books <www.summerfieldbooks.com> to be excellent for all his uses. These are aplanatic Japanese lenses at a reasonable cost. This company also stocks lanyards. They are also stocking an ultraviolet LED triplet hand lens. Although this is designed for detection of mineral fluorescence, they may be helpful for detecting fluorescent structures such as *Pohlia* bulbils on bryophytes. This is also a triplet lens that corrects for both aplanatic aberrations to improve the field of view (21 mm) and achromatic distortion for true color viewing at the reasonable price of only £21.

Des Callaghan (Bryonet 1 August 2013) advises that one should be sure the lenses are cemented together (usually sold as cemented doublet or cemented triplet). Otherwise, one must seed assurance that the housing is waterproof. And some of the cheaper models have lenses held by a threaded ring that can easily unscrew, causing you to lose the lenses. When lenses are not sealed, they easily steam up inside, especially in cold or wet weather.

Field Microscopes

When you are examining small plots for total bryophyte cover, and you must name every species and determine how much cover it provides, a field microscope can be useful. But when looking with such closeness, it becomes more difficult to avoid missing some parts and overlapping others.

Rod Seppelt (Bryonet 9 February 2012) reports on a field microscope that Gert Steen Mogensen introduced to him many years ago. This microscope was mounted on a miniature train track, maintaining a consistent distance of the lens from the ground and facilitating a consistent

movement. When the train track is on the ground, one can move the microscope along the track. A camera could even be attached to an eyepiece, especially if a trinocular microscope is used. This system provides stability and helps to solve the problem of vibration. It should be adaptable for stacking. I haven't tried it, but the ability to photograph and enlarge the picture later might even permit one to do the cover estimates accurately later in the lab.

You are less likely to need a compound microscope for the field, but you might want to check some things for verification in the evening after a day in the field. Tamás Pócs (Bryonet 10 February 2012) reports great satisfaction with the BioLux NV (Figure 44), made by BRESSER, Meade Instruments Europe Bmbh & Co. KG, Gutenbergstrasse 2, DE-456414 Rhede/Westf. Germany at a price of about 100 Euro. Its magnification ranges 20-128 X. It is lit by LEDs (with transmission and overhead light) and also has a digital camera ocular. It has a movable stage and several built in filters. More information is available at <<http://www.astroshop.eu/monocular-microscopes/bresser-microscope-biolux-nv/p,14667>>.



Figure 44. BioLux NV field microscope, made by BRESSER. Photo modified from Bresser website.

Norbert Stapper (Bryonet 11 February 2012) suggests two good sites for light-weight field microscopes: <http://www.blam-hp.eu/swift_br.pdf> and <<http://dominique.voisin.pagesperso-orange.fr/technique/pyrennee/index.htm>>.

Return at the End of the Day

Rob Gradstein (pers. comm. 28 July 2012) recommends that all information gathered on the specimens should be written in the field notebook. This depends in part on whether legible information with indelible ink is on the collecting packet or bag. Certainly general habitat notes should be in the notebook since that was most likely not repeated on each collection bag. Each specimen should receive a unique collection number, preferably already done in advance. (If the collection contains mixed species

and is separated later, each packet can be given a different letter while retaining the original collection number.) If there wasn't time to clean the specimens in the field, they **should be cleaned as well as possible and excessive substrate removed**. The specimens need to be spread to dry. I have many memories of collecting bags spread around hotel rooms at bryological meetings. It might be a good idea to alert the hotel staff so your specimens don't get tossed or mixed up. Be sure these collections remain in a paper container that won't permit them to escape if someone opens a window or door. If there is such a danger, place the collection packets/bags in a broad weave bag(s) and hang these where they get good air circulation. I still recall bags of mosses blowing around when we opened our hotel room balcony doors to cool the room (no air conditioner) and a storm came up. First the packets were blown around and some were dumped. Then some got soaked when the rain came in. We were scrambling in the middle of the night to move and protect the collections.

Thallose liverworts and epiphylls should be pressed lightly between sheets of absorbent paper (newspaper works well) and the paper changed daily. Place a sample of any liverwort capsules in a small envelope or minipacket with the rest of the sample to help keep some of the capsules unopened (but keep some intact as well). These capsules tend to release when they dry. It is also important to keep part of the liverwort sample alive/moist for later study of **oil bodies** (Rob Gradstein, pers. comm. 28 July 2012). You can accomplish this by keeping them in plastic at a cool (but not freezing) temperature. Examine them as soon as possible with a microscope and carefully describe the oil bodies. If possible, photograph them through a microscope; if not, draw them. Oil bodies will begin to disintegrate within a few days, or hours in a dry climate, and their morphology can change, even if they don't disappear.

Getting your Specimens Home – Customs and Inspection

Transporting your specimens requires a little attention. Some become brittle when they dry, so they should be packed to protect them. The bags or packets help to protect them. They should be tight enough that they will not move around in their shipping or transporting box, but loose enough that they don't crush each other (Buck & Thiers 1996).

Getting your specimens back into your own country can sometimes be problematic. Be sure you know both the import and export requirements for your home country and the country you are sending specimens from. As a courtesy, you should always provide a set of specimens, preferably identified, to the country where you collected them. Ask permission from a national herbarium or other prominent herbarium to give them the specimens. If you are sending them later, follow the protocol for "Sending Specimens for Identification" in Chapter 3 of this volume.

Hedenäs (1993) raised questions about various requirements of some countries. It is important that you understand these. Some countries require deposit of a duplicate set of specimens before you leave the country. This is impractical in most cases, as it is unlikely that you will be able to identify positively all of the specimens in a

country where the flora is poorly known. Nevertheless, if that is the law, it is important that you comply. You can send a list of species and collection numbers later. Instead, if leaving a set is not required, it might be better to send a duplicate set after they have been examined in the lab and identifications verified.

One aspect that can cause import/export problems is rare or protected species (Willem Meijer, Bryonet 28 September 1999). You might need proof that each specimen is not an endangered species. Customs agents are not familiar with mosses and may not even recognize that it is a moss, much less a liverwort.

Frahm (2000) reports on difficulties with specimens mailed to him from other herbaria, requiring him to go to the customs office at the German port of entry. It was no longer sufficient to label the package as "dried specimens" or "dried plants for scientific study." Rather, it is necessary to include a CITES certificate. This requires a declaration of the species enclosed. But bryophytes are not yet on the CITES list. Frahm suggests that the bryophytes be assigned a monetary value below the customs limit. He further suggests that it might help to make the statement that the enclosed bryophytes are not on the CITES list. Therefore Frahm concluded that a customs declaration indicating "Dried herbarium specimens – bryophytes: no CITES required, value \$10" could solve most problems.

When sending collections, divide them into small sets. Large sets (many specimens) may discourage inspectors, causing delays in getting the specimens to you.

Bill Buck (Bryonet 19 July 2012) assures us that there are no restrictions about bringing bryophytes into the United States, nor are any permits required. But importation of soil is problematic. Even a small amount attached to your specimens can result in having your specimens confiscated and destroyed. The process for bringing soil is complex and may include an onsite inspection. Buck showed the customs agents the freezers where new material is placed, and then the herbarium, with its multiple levels of security, where the material will ultimately be stored. But, as Wim Meijer warned, any bryophytes that get on the CITES list will most likely change the whole process. Jim Shevock (Bryonet 19 July 2012) fully agrees that it is best to mail the specimens back, at least to the USA. If you bring specimens into the USA and do not have the needed paperwork, you risk having the entire collection destroyed. In any case, specimens are likely to cause delays at customs at the port of entry and could cause you to miss a connecting flight. It is best if you mail them to yourself in care of the herbarium.

Summary

Collect in individual paper bags or envelopes. Ecological collections should include voucher specimens. Recorded data should include location, date with month written out, GPS coordinates or latitude and longitude, elevation, habitat, substrate, and collection number. Sample size depends on abundance and expected use, with palm-size collections being best for common species. Permission of the owner or a collection permit is important.

A collecting apron can make field equipment orderly and handy without being in your way. Cloth bags for collection bags facilitate drying.

A hand lens is usually essential in the field to permit tentative identification. Care should be taken to obtain one with sealed lenses. A light source (LED or UV) may be useful, depending on expected use. Field dissecting microscopes on a track can also be useful for finding small species and to facilitate consistent and thorough sampling.

If specimens must pass through customs, it is best to mail them to your herbarium. Be aware of customs guidelines for all countries in your travels before collecting.

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