

CHAPTER 3-1

HERBARIUM METHODS AND EXCHANGES

Janice M. Glime and David H. Wagner

TABLE OF CONTENTS

Folding Packets.....	3-1-2
Packet Machine.....	3-1-4
Followers.....	3-1-5
Herbarium Sheets.....	3-1-6
Herbarium Labels.....	3-1-6
Multiple Species.....	3-1-6
Annotations.....	3-1-8
Multiple Access.....	3-1-8
Storage.....	3-1-9
Cabinets.....	3-1-9
Packet storage.....	3-1-9
Type Specimens.....	3-1-10
Storage Containers.....	3-1-11
Palm Folders.....	3-1-11
Storage Boxes from Genus Covers.....	3-1-12
Specially Made Storage Boxes.....	3-1-13
Preservation.....	3-1-14
Cool Preservation.....	3-1-15
Minute Species and Special Structures.....	3-1-15
Herbarium Arrangement.....	3-1-15
Guide Cards.....	3-1-15
Herbarium Care.....	3-1-15
Pest Control.....	3-1-15
Agris 600.....	3-1-16
Moth Balls (Naphthalene).....	3-1-16
Microwave Oven.....	3-1-16
Bromomethane (Methyl Bromide).....	3-1-17
Freezing.....	3-1-17
Insect Traps.....	3-1-18
Drowning.....	3-1-18
Steam Sterilization.....	3-1-18
Moisture Control.....	3-1-18
Dehumidifier.....	3-1-19
Silica Gel.....	3-1-19
Herbarium Cabinet Materials.....	3-1-19
Sending Specimens for Identification.....	3-1-19
References.....	3-1-21
Current Names.....	3-1-21
Indexing.....	3-1-21
Herbarium Programs.....	3-1-22
Shipping Live Bryophytes.....	3-1-22
Sharing Images.....	3-1-23
Herbaria.....	3-1-23
Herbarium Specimen Mapping.....	3-1-23
Live Collections.....	3-1-24
Cryopreservation.....	3-1-24
When You Depart – Willing Your Herbarium.....	3-1-27
Exchange Programs.....	3-1-27
Borrowing Specimens.....	3-1-27
Type Specimens.....	3-1-27
Summary.....	3-1-28
Acknowledgments.....	3-1-28
Literature Cited.....	3-1-28

CHAPTER 3-1

HERBARIUM METHODS AND EXCHANGES



Figure 1. Benito Tan and herbarium cabinets for bryophytes at the Hattori Botanical Laboratory in Nichinan, Japan. Photo by Janice Glime.

Folding Packets

The standard for bryophyte storage is to put them in packets. These are made from a sheet of white paper, preferably acid-free, 100% cotton to reduce decomposition of brittle paper. Some herbaria use brown packets made from shelf liners or grocery bags (*e.g.* Kraft paper), and use of these is somewhat common in the field. Those are not quite as easy to read, but they do last well. Wagner uses 3" margins for the packets, but Glime finds that 1-1.5" margins work well. The size depends in part on the size of the herbarium box or drawer used to hold the packets. Having an exact size isn't critical, so after a little practice it probably won't be necessary to measure. If the housing for the packets permits larger sizes, larger packets may be desirable for some large taxa. Note that the outside (last) fold should be a little shorter than the others (Schofield 1985). This permits more space for the bryophyte and makes it clear which side is to be opened.

At CAS, curators use Strathmore ultimate white 100% pure cotton 24 lb watermarked paper, available in reams of 500 sheets, CODE 318003 (US standard size 8.5 x 11 in) (Jim Shevock, Bryonet 8 April 2015). CAS also uses Strathmore 25 percent cotton fiber paper for printing of herbarium labels and to process additional labels for specimen exchange. Both are acid free and of archival quality and print well on photocopiers. Karen Golinski (Bryonet 8 April 2015) similarly uses 100% cotton, acid free, Avon Brilliant White, wove finish, 24 basis <<http://www.neenahpaper.com/finepaper/morebrands/cottonpapers/classiccottonpapers/productdetail?color=Avon+Brilliant+White&finish=Wove>>. Mary Zimmerman (Bryonet 8 April 2015) uses Byron Weston Linen Record Ledger Paper from Talas: 100% cotton ('linen' is just the slight texture on the paper) and it has the year of manufacture watermarked into the sheets

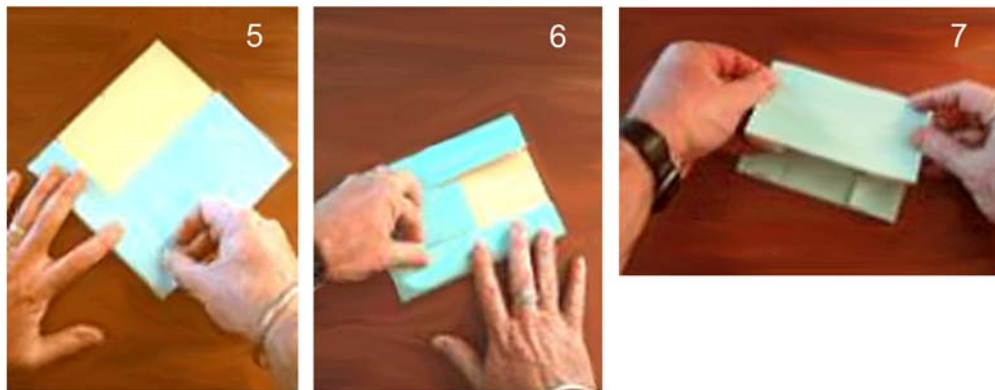
<http://apps.bnt.com/ecom/catalog/product_specific.cfm?ClientID=15&ProductID=24235>

Bryophyte specimens should be placed into the packets. An 8 1/2 x 11" (21.6 x 28 cm) sheet of paper, or size close to that such as the standard European size, should be folded in thirds like a business letter (Figure 2). After the first fold, the two open ends are folded inward. It is an important consideration that the first fold is up, then the sides are folded in before the top is folded down. This folding is less likely to lose specimens and fits more neatly

into the box or drawer than those where sides are folded last. And it is the only folding system that works well when the packet is glued to a herbarium sheet. The typical resulting packet is 4x6" (10x15 cm), a convenient size for storage in shoe boxes. These packets may be stored in boxes as packets or glued to a herbarium sheet, with the packet glued across the middle section of the back so the opening flap faces you like the flap of a pocket. See storage below.



Left to right: 1. Mark 3" (7.6 cm) in from top of 8.5x11" (21.6x25.4 cm) sheet. 2. Mark 3" in from other side at top. 3. Mark 3" from top using 3" card template. 4. Fold bottom up to mark 3" down side. [Change 3" on sides to 1.5" 3.8 cm) if you desire.]



Left to right: 5. Fold left edge to mark 3" (7.6 cm) from left. 6. Fold right edge to mark on right. 7. Fold top flap down.



Left to right: 8. Crease well. Packet is complete. 9. Packet with preprinted label data.

Figure 2. Steps for folding herbarium packets. Colors were used to make it easier to see the folds in these images. Photos by David Wagner.

Packet Machine

Miller (1988) offers an alternative way to expedite making packets. He uses a file folder to make a **packet machine**. We have modified it here to make the same type of packet as the one shown in Figure 2 and to maintain packet size close to 4x6" (10x15 cm) with maximum space on the flap for the label [3.5" (8.9 cm)] (Figure 3-Figure 13) (Schofield 1985).

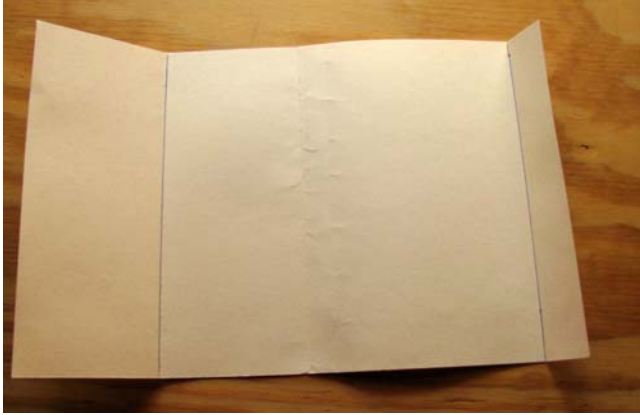


Figure 3. Cut the tabs from the folder to leave all edges straight and square. Then carefully measure 3.75" (9.5 cm) wide on one end of the opened folder, parallel to the folder fold. Score this line with a ball point pen and ruler to make it easy to fold. Photo by Janice Glime.

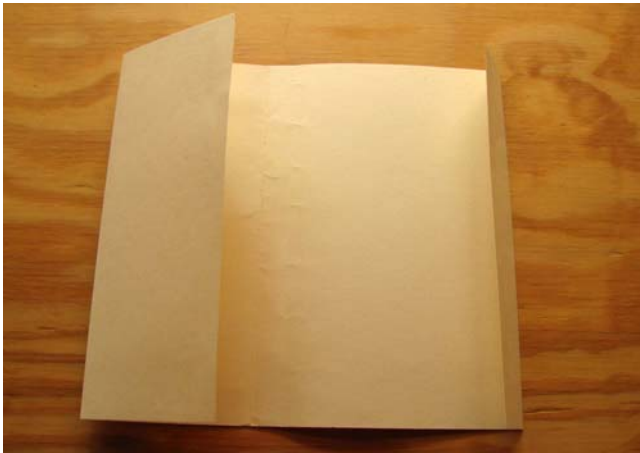


Figure 4. On the opposite end prepare a similar pocket; measure 1.25" (3.2 cm) from that end, score, and fold both ends to make pockets. Photo by Janice Glime.



Figure 5. Line up the pocket creases carefully and press them with a spoon or your fingernail. Photo by Janice Glime.

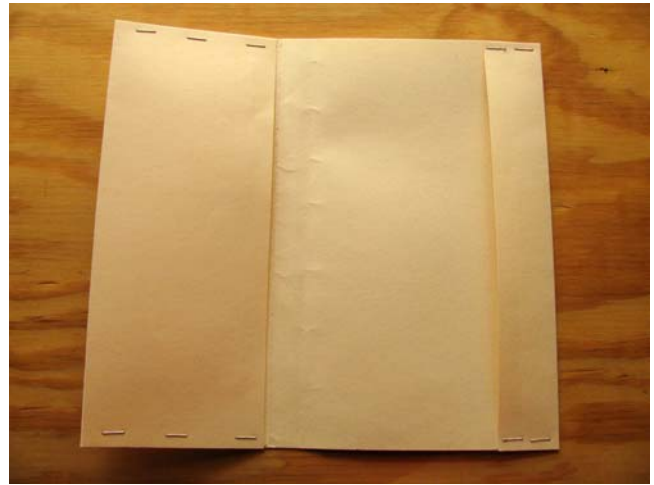


Figure 6. Staple or tape the ends so that it forms a pocket. Once stapled, this packet machine is ready to prepare packets. Photo by Janice Glime.

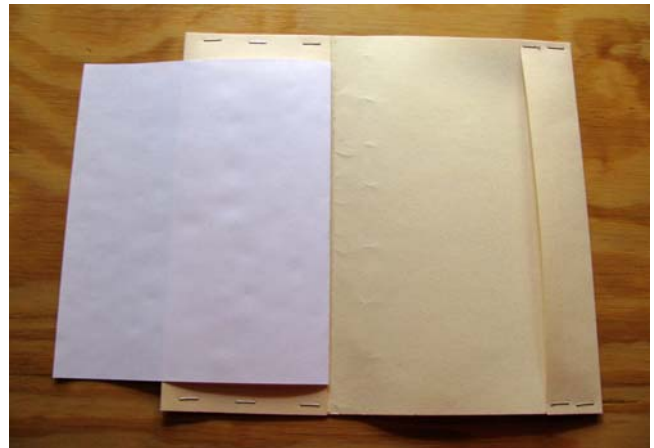


Figure 7. The machine is now complete with staples. For the first fold, 2-3 sheets can be folded together. Place one end of the 8.5" (21.6 cm) wide paper in the 3.75" (8.9 cm) pocket and fold it over the pocket. An old stainless steel spoon under the thumb or just the thumbnail helps to get a good crease on the packet. Photo by Janice Glime.



Figure 8. Separate the sheets and place one side that is perpendicular to the fold into the 1.25" (3.2 cm) pocket. Photo by Janice Glime.

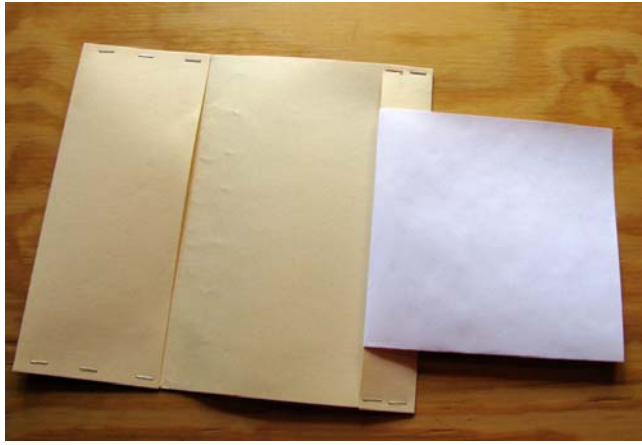


Figure 9. Fold the packet over the pocket and crease. Photo by Janice Glimme.

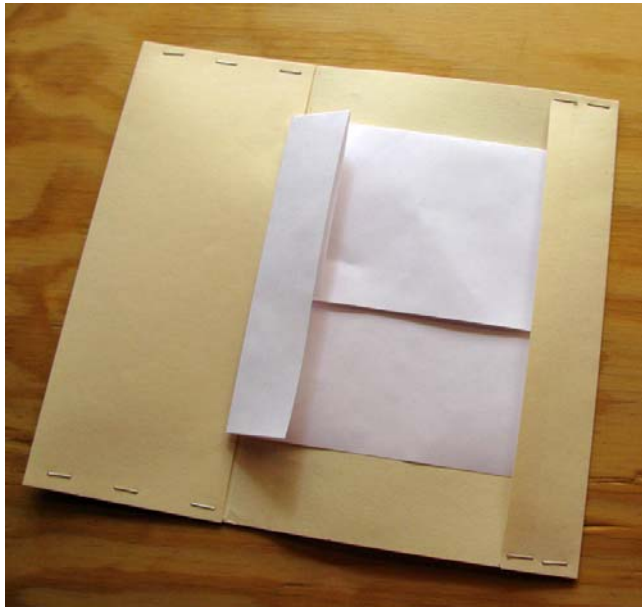


Figure 10. Repeat the operation on the other side of the packet. Photo by Janice Glimme.

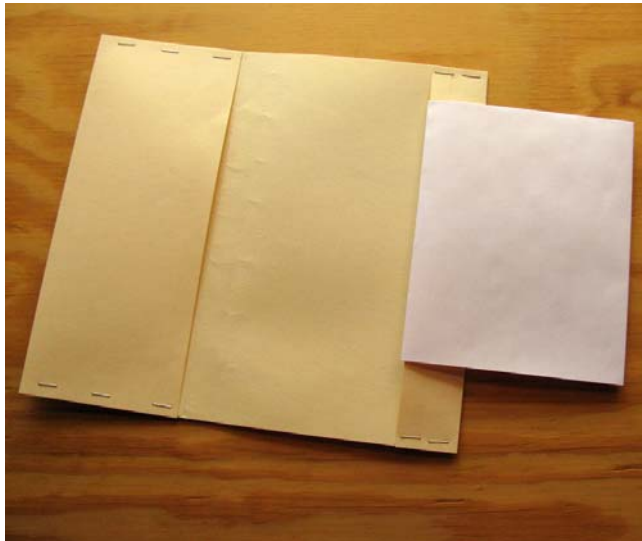


Figure 11. After you fold this side of the packet, you have an envelope and only the top flap needs to be folded down. Photo by Janice Glimme.

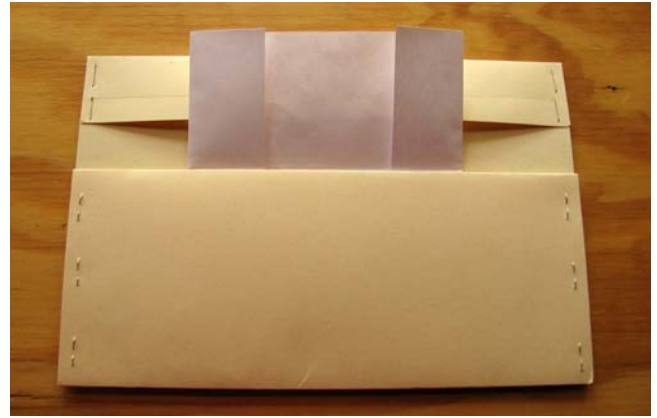


Figure 12. Place the bottom folded edge of the packet into the 3.75" (9.5 cm) pocket of the folder and fold the exposed part of the sheet over the pocket just above the pocket top edge so that when folded the dimensions are 3.75x6" (9.5x15 cm) with the last flap being 3.5" (8.9 cm). You won't be able to fold along the edge of the folder pocket this time, but must fold just above it. Photo by Janice Glimme.

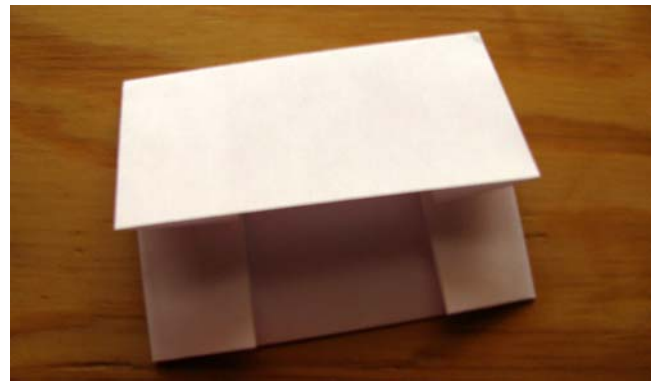


Figure 13. Now it is ready to use. The label should be placed on the top flap. Photo by Janice Glimme.

Followers

David Wagner (pers. comm. 2009) has found a way to keep folded packets neatly stacked, in order, under constant but light pressure. This also works for sorting, since specimens can be added anywhere in the row with ease and it will expand readily to fit. The trick is to use a cylinder (can of beans in this case) in a tray that is propped up to provide an incline for the can to roll against the packets (Figure 14).



Figure 14. Packets held in place with food can in inclined box. Photo by David Wagner.

Card files (4x6" card size \approx 10x15 cm) have a movable back on the drawers that can be adjusted to hold the packets upright. Shoe boxes can be packed with wadded paper in back to keep packets upright.

Herbarium Sheets

Jerry Jenkins (Bryonet 31 January 3013) reports getting good herbarium paper from Herbarium Supply <http://www.herbariumsupply.com/nu_listCategoriesAndProducts.asp?idCategory=31> in Bozeman, Montana.

Herbarium Labels

Rob Gradstein (pers. comm. 26 July 2012) states that "labels should be a little smaller than herbarium packets and glued on the outer surface (top, not bottom!) of the packet." But we agree with Schuster (1966) that the label should be printed directly on the front flap of the packet. This saves time, and glued-on labels have a tendency to come loose from packets after time in storage. This can result in loss of data, or worse, incorrect information when the label is matched to the wrong specimen. (Glime inherited a herbarium where loose and lost labels were a serious problem.) If the specimen needs to be put in a new packet, the label can always be cut from the original packet and glued to it or stored inside if a new label is printed on the packet. In either case, the label should be on the opening face of the packet.

Label data should include **name of the species** (if known), the **author** of the scientific name, **altitude**, **habitat**, **substrate**, **date** of collection (with month written out), and **location** (country, state, county, distance to nearest town), **GPS** coordinates, name of **collector**, **collection number**, **determiner** (name of person identifying or verifying identification). Persons adding identifications or verifications to specimens often precede their names with an **exclamation mark (!)** to indicate determined by. Additional information may include name of associated species, color, height of plant, abundance or other information not evident from the pressed specimen. For liverworts, it should include descriptions of the oil bodies because these will disappear upon drying.

The family name is less commonly used for bryophyte labels because the family concept is less stable than in flowering plants and there are fewer families and genera. Specimens are usually stored alphabetically by genus (see Herbarium Arrangement below).

The label will usually also include the name of the **herbarium** and the **accession number** for that herbarium. The herbarium name aids in getting loans back to the rightful owner.

A sample herbarium label is shown in Figure 15. Note that the **date is written out in full** to avoid confusion among different country annotations, and a detailed collection location is included.

CRYPTOGAMIC HERBARIUM OF MICHIGAN TECHNOLOGICAL UNIVERSITY	
FAMILY: Fontinalaceae	
SPECIES: <i>Fontinalis duriaei</i> Schimp.	
DATE: 1 May 1969	
LOCATION: USA, New Hampshire, Grafton Co., 1 km. north of Plymouth in woods on left of Texas Hill Rd.	
45°8'N, 71°40'W, R21, T15, sec 6	ELEV. 300 m
HABITAT: on granite rock in mountain stream in <i>Tsuga canadensis</i> woods	
NOTES: few dark capsules with ends abraded away; plants dark green with little algal growth	
COLLECTED BY: Janice Glime	COLLECTION NUMBER: 281
DETERMINED BY: Janice Glime	! Winona Welch
ACCESSION NUMBER: 12896	

Figure 15. Sample herbarium label from Michigan Technological University. Designed by Janice Glime.

Multiple Species

Bryophytes often grow intermixed (Figure 16). Here need of ecologists and taxonomists/systematists differ. For ecological studies, the associations contribute important information. I (Glime) am reminded of a letter I received from Sin Hattori, along with his careful notes on the species in a set of collections of *Frullania*. Most of the collections contained multiple species. He encouraged me to "do something" with the information of the mixes – so I did (Li *et al.* 1989; Glime *et al.* 1990).

On the other hand, when Niels Klazenga (Bryonet 15 July 2013) collected bryophytes in Borneo in 1997, he grabbed what he could – as told to by his PhD supervisor, hence including many mixed collections. Curation officers at the museum spent ten years cleaning up the mess.



Figure 16. At least 3 species are tucked in between lobes of *Conocephalum conicum*. When mixes like these are in collections it is best to make a minipacket if the mixed-in species are important. In any case, they should be noted on the label. Photo by Janice Glime.

Jean Faubert (Bryonet 15 July 2013) disagrees. Rather, he admonishes not to try to make single species collections. Grab the lot, put it in the bag and DO identify everything you see in it when back in the lab. Sure enough, that is when the goodies show up. He declares that most of his lifetime big finds were made that way. Philip E. Hyatt (Bryonet 15 July 2013) agrees and adds that since most info is slowly going on line, someone who is desperate to find a specimen won't have too much problem running it down in the future like we might have had to do with mixed collections in the past. In 1990, if you were not in a herbarium you probably didn't know the specimen existed. Life changes.

In habitats like the Sahara Desert or epiphylls, species are often not separable. Tamás Pócs (Bryonet 14 December 2015) makes a packet with one good specimen of the mix, then makes as many copies of the label as the number of species present. Packets with just labels (no specimens) or just labels are filed by each species, with the appropriate species for that location underlined.

As Philip Hyatt suggested, the herbarium in Trondheim is databasing every species (present in the same packet) as a separate record. They have a way to track which of the species is the 'main' species in the packet, *i.e.* in what cabinet the specimen is stored. This is more practical for the data users (and less practical for the herbarium curators), and curation of synonyms is easier this way, too.

The practices and reasons are varied, as demonstrated by a Bryonet discussion in mid July 2013. There are certainly pros and cons for both approaches. Separating the species is likely to lose the growth habit. Parts may be broken and underground structures lost. One loses the information gained by determining which species form associations and how reliable those associations are. Baranabas Malombe (Bryonet 15 July 2013) also considers it important to collect and retain all the species in the collections to demonstrate the diversity of the site.

If it is desirable to have archival specimens of more than one species, then removal to a separate packet is necessary. If only one species is of interest, it is safest to make minipackets to represent the accompanying species and to include their names in the notes on the packet label, or at least indicate that it is mixed with other species. Keep in mind that beginners may use this collection to learn species. Rod Seppelt (Bryonet 16 July 2013) agrees. "While it may be desirable to have monospecific collections, in practice it is rarely possible." For example, Seppelt states "I have been looking at *Fissidens* (collected by the late Ilma Stone); the label clearly indicates that the packet also contains fruiting material of two, sometimes three, additional *Fissidens*. It would be impossible to separate these into separate collections and still have a meaningful herbarium voucher." As Seppelt points out, "if all threads/plants of a particularly species are removed from a mixed collection, what can be important information about associated taxa is lost."

While it is desirable to separate species into separate packets, exemplars of intermixed species can be housed in minipackets within the herbarium packet. When accompanying species are removed to separate packets, the collection numbers should be retained, but individualized by adding a letter at the end of the collection number. The

parent packet should retain the letter **a**, alerting the researcher that there are other packets. If the other taxa have been identified, they should be listed in the notes along with their collection number and letters.

David Wagner suggests a way to have all the specimens catalogued in the herbarium: Make duplicate labels and file the duplicates for the subordinate species in their appropriate places, but indicate the specimen label where the actual specimens are located. This does cause problems when the systematics are updated, but can be helpful in locating the smaller associates.

Alas, in large herbaria, as noted by Ambrose Baker (Bryonet 16 July 2013), 1 specimen = 1 species at 1 location at 1 collection date. This is also true for higher plants, but it is easier to do for them. As stated by Niels Klazenga (Bryonet 15 July 2013), "mixed collections are not okay." But Jon Shaw (Bryonet 15 July 2013) disagrees.

My (Glime) own solution to the mixed collection is to make minipackets in which a bit of each minor species is placed. A sample of the dominant species can also be placed in a minipacket to assure the right specimen/species is examined. If the community is important, only samples of each species are removed, but if the specimen is important for taxonomic purposes, I might attempt to remove all the minor associates. Blanka Shaw, herbarium curator at Duke, likewise treats the plants that are mixed together and a separation is not an option by making small fragment packets with a few plants of each named species separated from the rest. "If you spend the extra time to name more than one taxon in the packet, definitely do make a fragment packet. There is nothing more frustrating than having a specimen with a rare liverwort in it, that is present in few stems only, and there's no way to find it out among all the other dried species that look identical in the dissecting scope." Blanka Shaw further distinguishes between plants associated in the same microhabitat in the field and those associated in the collection/packer.

Blanka Shaw (Bryonet 15 July 2013) does issue a warning about only listing the species on the same packet as associated species. These species might not be searchable in some databases. The bryophyte portal <<http://bryophyteportal.org/portal/index.php>> currently doesn't enable one to search the associatedTaxa field. However, the field is available there for this purpose, and you can get at the data by downloading the result of your search. In the Duke database, there are about 5,000 specimens with the associatedTaxa field filled in (out of ca. 160,000 records). But this information is rarely used – she has never considered it when preparing species lists. When a species name is updated, the name(s) in the associatedTaxa field does not get updated automatically (=you have to search for every synonym). So, it is not very practical for the data users. Hence, David Wagner's method of making a separate label to be filed as if it were a herbarium packet would put it into the database and enter it in both the search and nomenclatural updates.

Dorothy J. Allard (Bryonet 15 July 2013) suggests the following from the perspective of a bryophyte collector and curator:

- If you have enough material, split all of it into separate packets and establish one collection for each species. Then in an "associated taxa" field, indicate which other

species are present. Separating material can sometimes be difficult and destructive.

- If it is easy and non-destructive you can separate the material into individual packets. Information on the associated taxa is still useful to express on the label.
- If you don't have enough material, label the specimen with a single species and include information about the other species in the packet in an associated species field. If possible include one smaller packet inside for each of the associated species with its own label.

It is not unusual for one of the minor species to be the one of interest. Dorothy J. Allard (Bryonet 15 July 2013) reports that sometimes she collects a specimen because of a small and interesting liverwort, for example, embedded within a clump of *Brachythecium*. In this case she labels the specimen with the name of the liverwort and indicates that it is within a matrix of the *Brachythecium* in a habitat field, but she also lists the *Brachythecium* in an associated taxa field. In essence she treats the *Brachythecium* as the liverwort's substrate.

Ken Kellman (Bryonet 15 December 2015) separates a collection out when the identification is made. Each will become a separate collection with a suffix like a,b,c etc. On the front of the label, you can then put "growing with *Tortula muralis*, *Didymodon fallax*...) and this information is included on all the separated packets. But he only does this if there is some special reason to separate the collection: 1) the separated plant needs to be documented for a study; 2) the separated plant is rare or disjunctive etc. It would take so much time to separate out all collections that you have to prioritize. In any event, he tries to put all species found in a collection on the label so future workers can read what he was seeing.

Genevieve Lewis-Gentry (Bryonet 14 December 2015) and coworkers use three different options for a mixed collection:

1. Put a separate barcode for each different biological organism on the single packet. Use this to track name changes/annotations for each.
2. Use a single barcode that corresponds to the packet. Add many biological organisms to this one identifier.
3. Only use a single barcode, pretend it is a simple specimen, and note all the other species in a remarks field. (This is exclusively what was done with older systems, but this is never done now.)

Their database and workflow centers around barcodes. Everything has to have at least 1 to go into their computer. Their system is flexible so that depending on the situation they can choose to either put many barcodes on a single packet/sheet/slide/etc. or to put a single barcode with the packet etc. and tell the computer that this preparation has multiple organisms they would like to track separately.

As Claudio Delgadillo-Moya (Bryonet 15 July 2013) summed it up, "What and how you collect mosses and other small plants depends on where you live, the purpose of your research, or what you want the herbarium for."

Annotations

Sometimes labels are filled with information and little room remains for further annotation. A common practice is to glue one end of a slip of paper to the edge of the packet

label for name changes, verifications, or other notes. However, this slip of paper can easily come loose, so several options are used. One is to glue the packet to a larger card and attaching the paper, fully glued, to that. This seems to defeat some of the advantages of the packets and can create storage problems, unless the packets are in palm folders, but packets could get tangled with each other, causing glue to come loose. Another alternative is to place the annotations in a waxed envelope and to place that inside the packet. (Putting it in without protection could result in smudging or mold.) The disadvantage is that one must open the packet to know that something has been added. If the addition is extensive, one could place a note on the outside label instructing one to see inside.

At the University of Colorado Museum, William Weber reports that annotations are placed on the back of the packet (with packets stored in boxes or palm packets, not on herbarium sheets).

NEVER DISCARD THE ORIGINAL LABEL. Handwritten and even typed labels must be interpreted, and sometimes that interpretation is in error. Keeping the original label permits researchers to check for possible alternative interpretations. And there is always the possibility of transcription error.

Multiple Access

Guido van Reenen (Bryonet 15 December 2015) has refined his relational database to overcome the problems mentioned above. In his database structure the specimen information is basically stored in two tables, an 'Observation' table and a 'Collection' table. In the 'Observation' table the information of the specimen is stored (taxon name, determiner, date, substrate, phenology, if there is a microscope slide and/or a photo, etc.). If the specimen is also collected (that is not necessarily the case) a link is established to the 'Collection' table. In database jargon: the 'Observation' table has a many-to-one relation to the 'Collection' table. In other words a collection can contain one or more specimen.

In the case of multiple specimen in a collection, van Reenen defines one specimen as the 'main' specimen. Mostly this is the most abundant specimen in the collection. Under this specimen the collection is stored in the herbarium. The herbarium label also lists the names of the other specimens in the collection. He also uses the method of placing cards in the herbarium to reference to the physical collection, but that takes too much time and requires a lot of discipline, especially after a name change. And it is not necessary anymore because all the information is now in the database. He does most of the queries in the 'Observations' table, because all other tables in the database are linked to this table and it gives him all the information I need, including herbarium information.

Van Reenen also maintains a 'Taxa' table with information on the name, if it is a synonym, if it contains synonyms, place in the taxonomic hierarchy, etc., a 'Location' table, a 'Persons' table and a 'Relevé' table, to name the most important. The last one gives some headaches as well, because when collecting in a 400 m² relevé, often one species was collected more than once. And a percentage cover should only be attached to every unique species in the relevé.

The printing of the herbarium labels is done from the 'Collections' table (Figure 17). The collection is stored in the herbarium under *Fissidens ornatus*.

BRYOPHYTES OF COLOMBIA	
Collected by G.B.A. van Reenen & C. Schulte Fischedick	
No. 04366	15 August 1981
<i>Fissidens ornatus</i> Herzog	
+ <i>Fissidens flexinervis</i> Mitt.	
+ <i>Fissidens intermedius</i> Müll. Hal.	
Identified by R.A. Pursell, 1988.	

Figure 17. Sample herbarium label for a mixed collection from Colombia. Courtesy of Guido van Reenen.

Storage

Cabinets

Herbarium cabinets are the standard method for storage of preserved plant material. For most tracheophyte specimens, pest control is essential and it is important that the cabinets be sealed or nearly so to keep specimens dry and to discourage pests. Bryophytes, on the other hand, are usually not bothered by pests, so in less humid climates, less expensive storage cabinets are acceptable.

But cabinets require lots of space, so many larger herbaria with larger budgets have converted to compactors (Figure 18) that are used for both bryophytes and other plants. Although these can be a nuisance at times, they are great space savers and also make it somewhat easier to control humidity and pests because access is reduced.

Packet Storage

Nearly everyone stores bryophytes in packets, but some herbaria glue the packet to a standard size herbarium sheet. This has the advantage that the herbarium can use the same storage method for the bryophytes as they use for tracheophytes. But the packets take much more room this way, and a herbarium sheet is difficult or impossible to put under the microscope for closer inspection. It also makes your working space more crowded. My biggest concern is that the large format forces me to remove the specimen to observe it under the microscope, and when comparing several specimens, it is easy to mix them up, returning specimens to the wrong packet. Single packets can be placed under the microscope without removing the bryophyte from the packet.

Dale Kruse conducted a survey of bryonnet members in 2008 and got a mixed response. Susana Rams Sánchez has worked with specimens at MA, MUB, BM, E, S, MO and others. She finds the method at MO (Missouri Botanical Garden) to work the best, *i.e.*, packets. Others using packets included Noris Salazar Allen (Herbarium, University of Panama), Chris Cargill (Canberra), Stephen Rae (MUSCI Natural Resource Assessment). Rudolf Schuster (1966) considered packets in shoe boxes, trays, or drawers to be "much better" than pasting the packets to herbarium sheets. He also recommended that if the packet must be affixed to a herbarium sheet, it should be stapled

rather than glued so that it can be removed without destroying the packet.



Figure 18. Herbarium compactor at Missouri Botanical Garden showing cabinet with open door. Labels on the ends denote the contents of that section. Photo by Paul J. Morris through Flickr Creative Commons.

Cargill (Canberra) reports that one can prevent specimens from falling to the bottom of the packets by storing the specimens in polypropylene archival bags. In some cases they are also wrapped in Kimwipes® before placing them in the bag.

Kerry Barringer (Brooklyn Botanic Garden) reported that they were changing their method from packets on sheets to packets in cardboard boxes (51 x 16.5 x 6.3 cm). The boxes are open and two will fit lengthwise on a standard herbarium cabinet shelf. They made new packets and photocopied disintegrating old ones to store inside the packet.

Those who disliked the placement of packets onto herbarium sheets cited concerns such as glue yellowing the packet, glue coming loose, packets getting caught and being torn off, glue catching dirt, difficulty in removing packets from the sheet (resulting in loss of specimens), greater cost for sending loans, more storage space required. To this list, one must consider where the packet is to be placed on the sheet. If it is placed in the lower right corner, where a label would normally go, then the stack becomes very lopsided. If packets are arranged at random on different sheets, then it makes sorting through the sheets to find a particular specimen a more difficult job. Placement of more than one packet on a sheet brings its own problems – renaming some, but not all, specimens; shipping for loans or

verification of identification, and still has the problem of locating the labels when sorting through to find something.

David Long (Royal Botanic Garden, Edinburgh), a proponent of herbarium sheets, cited advantages of gluing packets to herbarium sheets: being able to use standard herbarium cabinets, species covers, and genus covers; specimens do not get lost as easily as those in loose packets; it is easier to flick through sheets to find individual specimens (if packets are in a standard position and only one per sheet); hunting for specimens requires less handling and thus less chance for damage; specimens are kept horizontal so that soil does not collect at the bottom of the packet and damage specimens; specimens are better protected when sent on loan; there is greater ease to arrange packets geographically by sheets (this could also be accomplished in a palm folder); types can have the traditional red folder and be easier to spot; useful literature can be placed in the folder with them (Bryonet July 2008).

Bill Buck (New York Botanical Garden) further supports the use of packets glued onto herbarium sheets. The greater protection of the specimen seems to be a primary concern for supporters of this method, including problems with settling in vertical packets and provision for extra padding without tight packing. The herbarium sheet also will accommodate large packets for such taxa as *Spiridens* (Figure 19) and *Polytrichum*; when just packets are used, large specimens must either be cut into sections or stored elsewhere. And packets, due to their small size, are more easily lost, especially when sent out on loan. Catherine La Farge England (Bryonet 18 July 2008) reports the same reasoning for the University of Alberta Herbarium, an approach established by Dale Vitt.

Type Specimens

Colored folders are traditionally used for tracheophytes to indicate special collections. Red is standard for type specimens, whereas blue or other color may be used to indicate a particular geographic area. The same system can be used if bryophytes are stored on herbarium sheets and provides one of the arguments in favor of this method. A red felt pen run across the top of a packet will serve the same purpose (Figure 20-Figure 21), or a red herbarium folder can be cut to fit around the packet (Figure 22).



Figure 19. *Spiridens flagellosus*, a large epiphytic moss. Photo by John Game through Flickr Creative Commons.



Figure 20. Type specimen packet (red top) among other packets. The red top is made by a red felt pen. Photo by Janice Glime.



Figure 21. Close view of type specimen packet among other packets. The red top is made by a red felt pen. Photo by Janice Glime.

When a palm folder is used, a felt pen can be used to make a colored dot on the folder to indicate the presence of a type specimen (Figure 25).

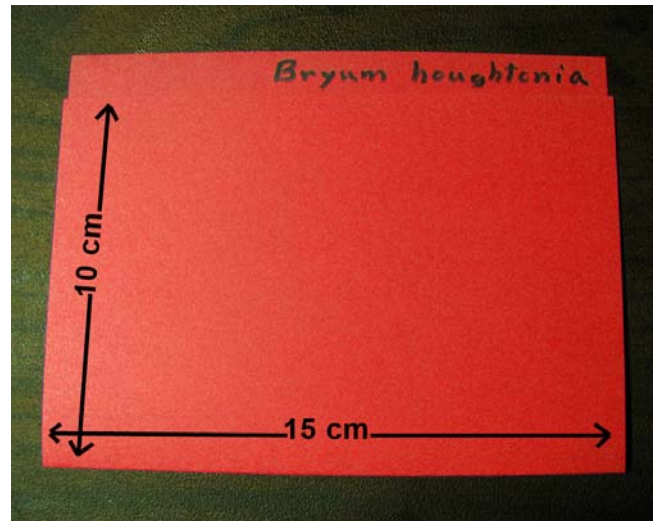


Figure 22. Sample type specimen folder for bryophyte packet. Photo by Janice Glime.

When slides are made of the type specimen, it is important to retain the slide with its specimen (Singh Deo & Majumdar 2020). These can be mounted in a permanent medium, or allowed to dry on the slide. For the latter, in particular, the slide should be placed in a small packet to prevent loss of the specimen. These latter ones can be rewet to position the specimen as needed.

It might be tempting to keep a "**kleptotype**" (stolen specimen or part of specimen of type) when working with type specimens, but this should never be done. With other herbarium specimens, it should be done only with permission of the owner/curator (Katagiri & Majumdar (2020). In the International Code of Nomenclature for Algae, Fungi and Plants, Rec. 7A.1 (Turland *et al.* 2018), it is strongly recommended that the material on which the name of a taxon is based, especially the holotype, be scrupulously conserved. Nevertheless, the Code does not explicitly prohibit the taking of fragments of a type specimen. Katagiri and Majumdar have proposed the following language be added to the Code:

"8A.n. The unauthorized division of a type specimen should be prevented to avoid a loss of the type specimen. Such action most likely complicates the work of future generations and the resulting reduced collections may give a misleading concept of the species. When found, such "kleptotype" specimens should not be destroyed but preserved as duplicates. Formal notification of the existence and location of these specimens should also be published."

Storage Containers

For those using 4x6 (10x15 cm) packets, a 4x6 card file cabinet can be used to hold the packets. It has a pull-out drawer that can be removed and a movable back that can hold the packets up even when the drawer is not full.

Brian Eversham uses plastic boxes that can hold a double row if the packets are folded small enough (Figure 23). I use shoe boxes because they are free at the local shoe stores and keep the packets covered, avoiding excess dust. It is easy to attach a label to the end of the box to indicate the part of the alphabet contained therein. I try to leave enough room for half as many more packets to be added, *i.e.*, 2/3 full.



Figure 23. Herbarium drawer with packets. Photo by Brian Eversham.

Packets on herbarium sheets can be stored in a standard herbarium cabinet, and that seems to be the main asset for those who prefer them. The boxes or drawers, however, can also be stored in a herbarium cabinet and require much less space than a packet plus herbarium sheet.

Bryophytes are seldom eaten by pests in a herbarium, unlike tracheophytes, so most bryologists store them without mothballs or other deterrents.

Palm Folders

Palm folders were originally constructed to handle large or thick tracheophyte specimens like palms, hence the name. Palm folders can hold 10-20 packets, or even more, depending on the size and thickness of the packets. Those using packets placed in palm folders (Bryonet July 2012) included Jaakko Hyvönen (Plant Biology, Helsinki), Dan Norris (Berkeley University Herbarium), Xiaolan He-Nygren (Helsinki), and Jim Shevock (California Academy of Sciences). This method permits the packets to lie flat, overcoming the crushing problem and the problem of having specimens collect at the bottom of the packet in a pile of soil.

Dan Norris (Bryonet July 2012) cites the flexibility offered by palm folders for having different sizes of packets to accommodate large specimens. The folders are 30.5 mm x 56 mm and have additional flaps on each side, top, and bottom (Figure 24). The large size of the folder, like the large herbarium sheet, can accommodate large specimens like *Spiridens* (Figure 19) or *Dawsonia*.

Palm folders can be stacked so that 6-7 will fit on one standard herbarium shelf (Figure 25). The folders will allow specimens up to 27 mm thick. This permits a collection of various sizes to remain together. Jim Shevock points out that a further advantage is that the 27 mm thickness permits labelling the end of the folder (Figure 25), making it easier to find the right folder.

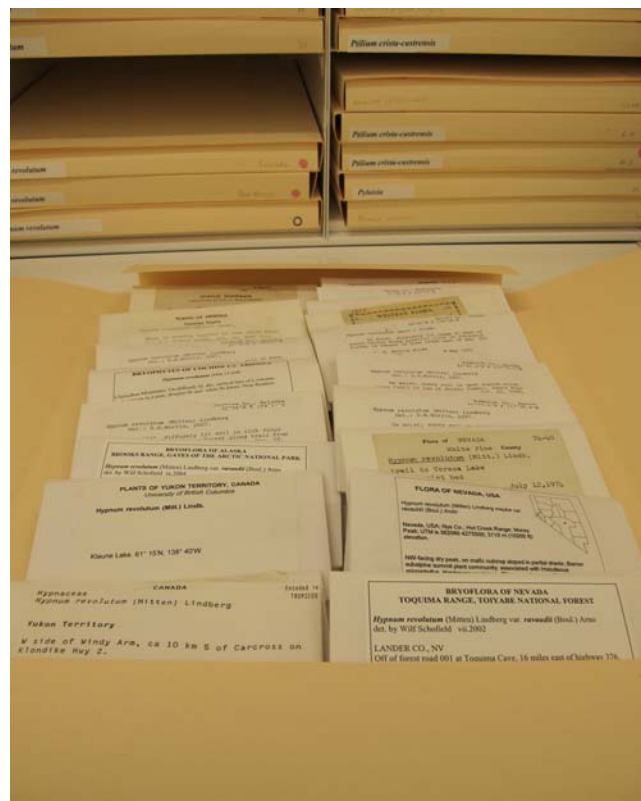


Figure 24. Herbarium palm folders showing arrangement of packets with a variety of labels, some as part of the packet, others glued on. Note the map on one of the labels indicating its location in the state of Nevada. Photo by Jim Shevock.

Susana Rams Sánchez warns against making species sheets with more than one packet glued to them. These will soon be a problem as identifications change with revisions. And when specimens are sent for loan, all the packets must be shipped, making them unavailable at the home herbarium and increasing shipping costs.



Figure 25. Herbarium cabinet with palm folders. Photo by Jim Shevock.

Storage Boxes from Genus Covers

If you are familiar with large herbaria, you are familiar with the heavy poster board or Manila folder quality of genus covers used for storing tracheophytes. Davison (2002) suggests using these for making storage boxes for bryophyte packets. These are similar to the palm folders, but the ones Davison has designed are the width of a "standard" packet and are not covered. The following instructions (Figure 26-Figure 28) are only slightly modified from his:

The finished box occupies the full length of a standard herbarium cabinet. Two boxes fit side by side on the shelf. The boxes can hold 40-130 upright specimens, depending on the size of the specimens. Be sure to measure the shelf size of your cabinet before making the boxes because the cabinet sizes can vary somewhat.

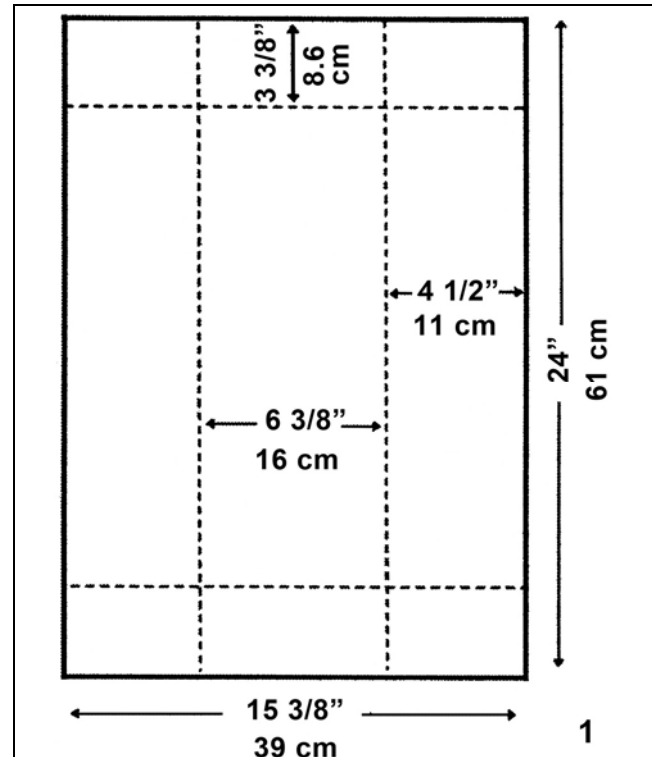


Figure 26. To make boxes, use scissors, razor knife, or paper cutter to remove 4.2 cm from long side of a 43x61 cm genus cover. Save trim as template for trimming additional genus covers. Score with hard metal edge such as door key and fold/unfold along indicated lines. Scoring controls exact line of fold. Rub smooth, hard object down folded edge to make creases sharp. To save measuring scoring lines, create template strips from cardboard or genus covers to guide scoring tool.

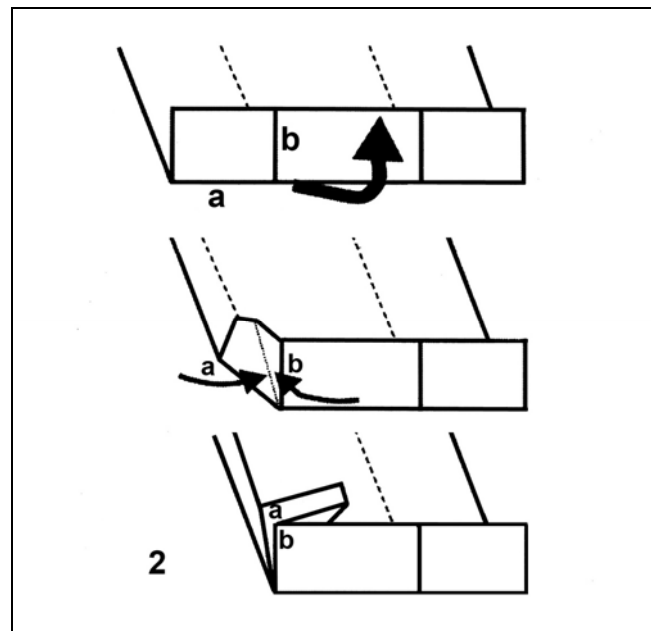


Figure 27. To form corners of box, push slightly inward at arrows and align edge **a** with edge **b**. Hold edges **a** and **b** firmly together and crease from inside. The corner crease will find itself as you align, meet, and hold edge **a** to **b**. Press firmly while creasing. Fold/unfold all four corners.

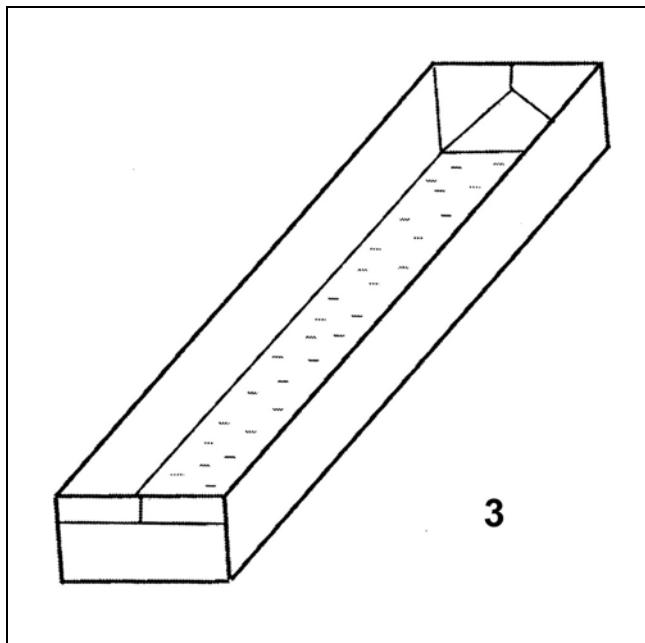


Figure 28. Once all folds have been made, shape the box and adhere each end with tape, glue, or staples. Davison uses clear 2" (5 cm) wide commercial-grade box packing tape and cover the entire outer face of each end. The tape provides a tear-resistant surface for taping and removing labels that identify the box contents. If handled carefully, as specimens should be, the boxes are adequately sturdy. Placing cardboard inside the upright ends strengthens the boxes but is not required.

Size 3 ($4 \frac{3}{4} \times 3 \frac{3}{8} \times \frac{7}{8}$): ~pricing range \$0.72 to \$0.88

Size 4 ($7 \frac{5}{16} \times 4 \frac{15}{16} \times \frac{7}{8}$): ~pricing range \$0.93 to \$1.19

Size 5 ($9 \frac{5}{8} \times 6 \frac{7}{8} \times \frac{7}{8}$): ~pricing range \$1.59 to \$1.91

Purchase is direct from:

Jay Cordeiro

Northeast Natural History & Supply Co.

Distributor: HH Elements, Inc.

24 North Grove Street

Middleboro, MA 02346 USA

<unionid@comcast.net>



Figure 29. Wooden tray. Photo by Jay Cordeiro, Northeast Natural History Supply Co.

Specially Made Storage Boxes

Jay Cordeiro of the Northeast Natural History & Supply Co. supplies herbarium drawers and trays to your specifications (Figure 29-Figure 34). Specimen drawers and unit trays are designed for curation, storage, rehousing, and display. They can be used for shells, minerals, skeletal material, feathers, eggs, skins, anthropological objects, fossils, glass vials, and memorabilia, as well as bryophytes. Archival trays are custom manufactured to any dimension; they are rigid, unbuffered, and acid-free with neutral pH. Trays are constructed of white corrugated cardboard, come free-assembled (not flat and self-folding), are overwrapped, and nested for maximum storage efficiency. Archival drawers are available in standard sizes to fit typical Cornell, California Academy, and National Museum of Natural History style storage cabinets. Trays can be lined with plastazote or ethafoam, unbleached cotton, or polyester batting for use with delicate specimens. Lids are optionally available for better protection from ambient environmental damage and for use in layered storage. The trays are sturdy and affordable.

The company does not have an online catalog because their product is custom designed. The trays and drawers can be purchased in sets of 100 or more. Sample sizes and prices include:

Size 1 ($2 \frac{3}{8} \times 1 \frac{5}{8} \times \frac{7}{8}$): ~pricing range \$0.50 to \$0.63

Size 2 ($2 \frac{3}{8} \times 3 \frac{5}{16} \times \frac{7}{8}$): ~pricing range \$0.62 to \$0.76



Figure 30. Wooden tray. Photo by Jay Cordeiro, Northeast Natural History Supply Co.



Figure 31. Wood pull of wooden tray. Photo by Jay Cordeiro, Northeast Natural History Supply Co.

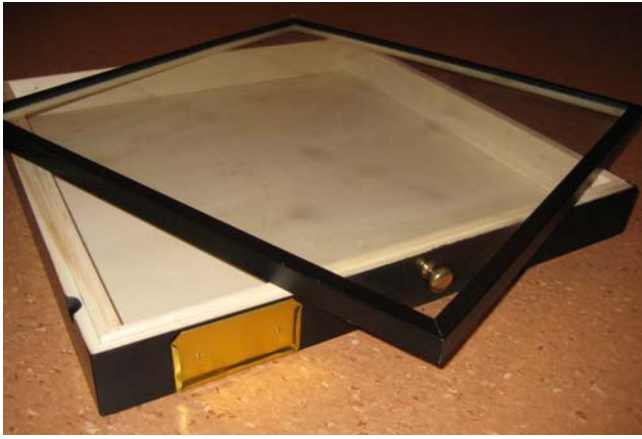


Figure 32. Box opener of wooden tray. Photo by Jay Cordeiro, Northeast Natural History Supply Co.



Figure 33. Herbarium drawer with boxes. Photo by Jay Cordeiro, Northeast Natural History Supply Co.



Figure 34. Trays. Photo by Jay Cordeiro, Northeast Natural History Supply Co.

Preservation

Most specimens will keep well in packets if they are not packed together too tightly. If a specimen has fragile parts sticking out, it helps to pack crumpled tissue paper around the specimen. An alternative is to cut out space for the specimen in a piece of styrofoam or corrugated cardboard of appropriate thickness and dimensions. However, if the specimen falls out of the styrofoam, it may suffer even greater damage than with no packing, so it might be necessary to staple or tape a minipacket in the cut out space. Small jewelry boxes can sometimes be useful.

Glime once stored *Splachnum ampullaceum* in a plastic film can.

Preservatives should be avoided so the material can be used later for DNA or chemical analysis. If preservation is necessary for maintaining the morphology of a liverwort, maintain some of the specimens in preservative and others dried, and in some cases (flat thallose species), like *Conocephalum* (Figure 35), pressed. Rob Gradstein (pers. comm. 26 July 2012) suggests using FAA (feryl-acetic-alcohol). This preservative served Barbara Crandall-Stotler for her morphological work and Rudy Schuster for making the drawings used in his liverwort volumes.



Figure 35. *Conocephalum conicum*, a mostly flat thallose liverwort that preserves better if it is pressed. Photo by Robert Klips.

Gradstein (pers. comm. 26 July 2012) also suggests that dry, shrunken herbarium material of thallose liverworts can be rehydrated and stained with methylene blue (see Rico 2011), a method that works well for him in studying *Riccardia* (Figure 42). Rico developed this method of rehydrating the moss in a solution of sodium hypochlorite (commercial bleach) diluted to 20% in distilled water. This restores the form of the liverwort and the structure of the cells. The cells are cleared, making observation easier.

Species like *Riccia fluitans* (Figure 36) can be teased apart and floated onto a 3x5 (7.6x12.7 cm) card. The algae on these aquatic plants will serve as a glue to make them adhere to the card. Once affixed, they will retain their shape and remain flat.

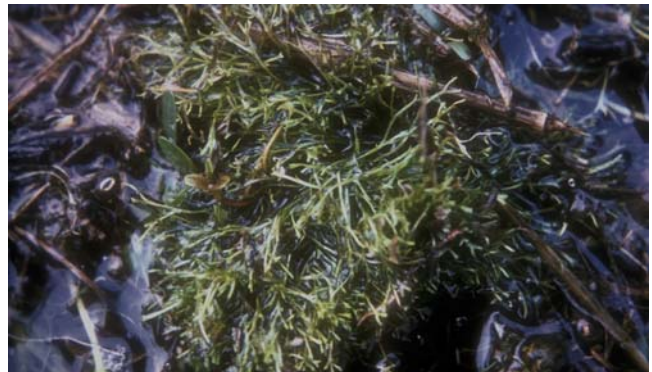


Figure 36. Floating form of *Riccia fluitans*, a species that can be floated on a card before putting it in a packet. Algae help to glue it to the card. Photo by Janice Glime.

Since leafy liverworts will lose their oil bodies upon drying, one should preserve them by a photograph that shows details of the oil bodies. In addition, describe the oil bodies in detail.

Cool Preservation

Bryophytes prosper in cool temperatures, so it is not surprising that cooling them during drying can improve the quality of the specimen over air-drying. Victor Ardiles H. (Bryonet 25 September 2013) reports that both gametophytes and sporophytes look better when fresh samples, still in their paper bags, are placed in a freezer for 15 days at 7°C, 37% Relative humidity. The method discourages fungal growth and retains colors, leaf details, structure of thallose and leafy liverworts, bottle liverworts, and hornworts. The low temperature slows the dehydration of the tissues, a more natural approach.

Minute Species and Special Structures

The really tiny species can present special problems because they are easily lost among the soil in the packet. Several methods can help to make these locatable in the future. One popular method is to remove some of the specimens from the soil and place them in a minipacket (Rothero & Blackstock 2005) or small envelope without the soil. Another possibility, suggested by Richard Zander (pers. comm. 1 August 2012) is to glue the plants (without soil) to a white card with a polyvinyl-alcohol-based glue. That glue is water soluble, so the specimens can be soaked loose.

In some cases, only a few plants may have capsules, antheridia, archegonia, or propagules. To help avoid loss of these important structures, make a small packet or use a small envelope to store these within the species packet (Rothero & Blackstock 2005). Microscope slides can also be put in a small packet and stored within the species packet. They may survive better in a waxed paper envelope because the slide can be sticky and the paper may stick to the slide. The waxy surface can reduce this but won't necessarily eliminate it.

Herbarium Arrangement

There are two choices in widespread use in the arrangement of bryophyte herbaria – systematically or alphabetically. They each have their advantages and disadvantages, so one needs to choose based on resources and needs. The majority of those who commented on this to Dale Kruse in his survey preferred a strictly alphabetical system.

The systematic arrangement provides groupings that make it easier for someone making a systematic study. All members of a family would be grouped together. This method is further divided into choices – systematic or alphabetical arrangement of genera. Richard Zander (Bryonet 13 November 2008) considers this family grouping with alphabetical arrangement of genera to be "a nice compromise." Rod Seppelt (Australian Antarctic Division) practices a further compromise to group genera into the family, but to arrange the families alphabetically. This solves the problem of trying to linearize the non-linear systematics of families. One could also arrange the species systematically, but that does not seem to be a common practice.

The disadvantage of systematic arrangements is that our knowledge of bryophyte systematics is constantly changing. The publication of Shaw and Goffinet (2000) moved a lot of genera to other families and split some families. Because of the instability of our understanding of the systematics, the cabinets would require an updated list of the locations of each genus and family. Flora North America is making further changes. As we gather more molecular information we keep moving things. Hence, this arrangement can be expensive because it would require constant monitoring and rearrangement whenever a taxon has been moved or redefined.

The alphabetical arrangement is more practical. In some cases, the packets are arranged in families with an alphabetical arrangement of families. In other herbaria, the genera are arranged alphabetically with no family groupings. The latter arrangement is the most stable arrangement.

Jim Shevock reports that the University of California herbarium files their bryophytes alphabetically by genus (Figure 25). My own experience is that most bryological herbaria use that method because it is easier and less expensive to maintain. Missouri Botanical Garden uses family groupings. At the California Academy of Sciences the genera are filed by family, but the genera and species are filed alphabetically within the family, and the families are arranged alphabetically.

I like the advice of Jaakko Hyvönen (Bryonet July 2008) regarding phylogenetic vs alphabetical: we are...still too far away from the classification that would enable arrangement accordingly. Alphabets have been pretty stable for quite some time and this makes it easy for ALL people (most of whom are NOT bryologists) to locate specimens in collections. On the long run one would be able to save a LOT of precious volunteer, student *etc.* herbarium time by adopting this simple system. At the same time, need for rearrangement is minimized.

Guide Cards

A practical way to help the user is to provide guide cards. William Weber (University of Colorado Museum) uses blue cards for Colorado material and yellow for other areas. A salmon guide card indicates the genus, yellow the species. Alternatively, one could color code the top of the packets with a felt pen. Note that red is reserved for type specimens.

When a herbarium is rearranged or names change, guide cards can be placed where the alternative name would occur, directing the user to the location of that group. This can be useful if staff lack the time to rearrange the collection. A guide card can be placed where the new name should be, directing users to the name on the packets.

Herbarium Care

Soil

More information is retained if the substrate is collected with the specimen. However, this brings its own problems. It can be bulky, bring plant pests, and prevent you from taking or sending specimens across borders. But Brent Mishler (Bryonet 2 March 2021) warns us that if it is removed, rhizoids, tubers, and other underground structures can be damaged or removed. Furthermore, the soil helps to keep the clump intact. Hence, for taxonomic and

ecological purposes, it is best not to remove soil in contact with rhizoids or stems. Mishler recommends using window screening to gently rub away excess soil.

Wet soil is likely to cling to the clump, but dry soil may have dried to fine parts like glue. Sometimes a gentle washing by dipping the bases of the clumps in water can help to remove water with minimal damage to the underground parts, but caution is needed. Heavy soil clumps can pull on the rhizoids and break them loose.

Chris Cargill (Bryonet, 2 March 2021) faces similar problems at the Australian National Herbarium (CANB), especially with specimens like *Riccia* (Figure 37) and hornworts that typically have soil attached. The soil detaches over time as researchers access the collections, often just by disturbing the packet without removing anything. To improve maintenance of the collection integrity, they wrap terricolous collections carefully in archival tissue paper, sometimes on a piece of card for rigidity, and then place the whole wrapped specimen into an archival quality polypropylene bag. This reduces the movement of the specimens and inside the packet and therefore any rubbing from loose soil or small pebbles. They place any loose soil in a separate small archival plastic ziplock bag and place it with the specimen.



Figure 37. *Riccia* showing rhizoids clinging to soil. Photo by Bernd Haynold, through Creative Commons.

Ken Kellman (Bryonet, 2 March 2021) agrees with the importance of keeping soil. If there is a lot of powdery soil, wrap the sample in a smaller packet inside your herbarium packet. Fragment packets work well. That tends to minimize the inevitable settling of the soil.

Richard Zander (Bryonet, 2 March 2021) finds that, within limits, the more soil the better:

1. Rhizoidal gemmae, fallen calyptrae, and other plant parts may be found in the debris
2. Other organisms are present in the packet, and I think maybe in the future the best place to search for new taxa or significant species will be among soil in packets already in the herbaria.

Brent Mishler (Bryonet, 2 March 2021) raised an additional concern. The specimens may be used later for extracting DNA [or heavy metals in soil vs bryophytes], so washing can give false results. Initial wetting can cause a

lot of damage and loss of cellular contents. This is particularly true if they dry out again before being able to repair the damage. Hence, if specimens must be cleaned, Mishler recommends doing it dry.

Brent Mishler, Bryonet 2 March 2021

Pest Control

Pests can be a problem in a herbarium, and methods to eliminate or minimize them can be detrimental to future studies that rely on untreated material for historical pollution studies or DNA testing. In November of 2010 there was a discussion on bryonet-L regarding means of eliminating pests without compromising future studies.

Historically, most bryophyte herbaria have not treated for pests with the same care as that used for vascular plants. For example, beetles can be real pests among tracheophytes and some algae, but are usually not rampant among bryophytes. The popular belief that nothing eats them let of a somewhat false confidence in storing the with no pesticide treatments. However, if you have ever tried to import them into a country, you know that the border quarantine agents are concerned about pests in the soil, and this alone should suggest that the bryophytes may introduce pests into the herbarium. Scattered publications, and especially more recent ones, as cited in the interactions volume on this website, demonstrate that our assumption that nothing eats bryophytes was incorrect.

Agral 600

As mentioned in the Laboratory Techniques subchapter on Slide Preparation and Stains, Tom Thekathiyil (Bryonet 12 May 2010) submerses the bryophytes in **Agral 600** (horticultural wetting agent). It kills the animal life that often accompanies the bryophytes but does not seem to affect the plants.

Moth Balls (Naphthalene)

For tracheophytes, the standard treatment has been to put **moth balls** in the cabinets. These have contained such compounds as **naphthalene** (highly flammable and carcinogenic), **1,4-dichlorobenzene**, or **camphor**. These all have strong odors that are very offensive to some people, especially when they work for many hours in that environment.

Rod Seppelt (Bryonet 26 November 2010) reports using fumigation with *Pyrethrum* in a spray. The plant that produces the Pyrethrin[™], however, is known to cause human health problems among long-term growers of the plant.

Microwave Oven

A more recent method for killing bryophyte inhabitants has been to put them in the microwave oven, but such treatment renders the bryophytes unusable for future DNA studies due to the ability of the gamma rays to alter the DNA. Lars Hedenäs (Bryonet 30 November 2010) reports that the Swedish Museum of Natural History would never send material to another herbarium if there is the danger that the material on loan would be subjected to microwaves. The risk of destroying DNA would "seriously reduce its value for future research."

Wagner finds that the microwave is not effective, largely because of the uneven distribution of microwaves inside the oven. The oven has the further problem of being

too small unless you purchase a commercial grade oven. Wagner had a friend who trapped a fly inside his otherwise empty microwave, turned it on for 60 seconds, and when he opened it the fly flew out. It had survived by cowering in a safe corner. Some herbarium material absorbed microwaves and overheated. Wagner has even had charred herbarium specimens, and blackened paper under them, that resulted from too long a treatment.

Bromomethane (Methyl Bromide)

Juan Larraín (Bryonet 13 April 2016) raised concerns that Chile requires that all specimens with soil, including bryophytes, must be treated with bromomethane before entering the country. While this may be a good means to prevent the introduction of agricultural pests, Rod Seppelt (Bryonet 13 April 2016) warns that it should not be used on specimens to be used for molecular work. Rather, he recommends freezing.

Freezing

It appears that the safest and most common method in current use is freezing. And this is standard practice in many herbaria (Figure 38). In this method, one recommendation is to freeze the packets for 24-48 hours; the process should be repeated annually to maintain the pest-free environment (Denis Oliver, Bryonet 26 November 2010). Rod Seppelt (Bryonet 26 November 2010) recommended three days at -18°C for material collected in the region or -18°C for seven days if it has come from a different biogeographic region or outside the country. He later modified this (Bryonet 6 February 2012), based on a response from the herbarium in Auckland, New Zealand. They found that a more effective treatment was to use cycles of room temperature to -15°C over a few days. It is kinder to the bryophytes and more effective against insects. At Christchurch (CHR), freezing is for 7 days at -20°C (Allan Fife, Bryonet 15 August 2002).

At the University of Alberta Herbarium (ALTA) specimens are frozen at -20°C (Catherine La Farge England, Bryonet 15 August 2002). The specimens are stacked as single sheets or only a few sheets overnight; larger stacks are stored at that temperature for four days to be sure the center gets cold enough. The specimens are sealed in poly freezer bags in the freezer and kept in them until they reach room temperature afterwards, for up to a day for larger stacks. A similar procedure is followed at the New York Botanical Garden (NYBG) and Missouri Botanical Garden (Marshall Crosby, Bryonet 15 August 2002), where freezing is for 3-4 days (Barbara Thiers, Bryonet 15 August 2002).

At the Helsinki Herbarium (H), all loans are frozen for at least a week before putting them in herbarium cabinets (Johannes Enroth, Bryonet 15 August 2002). The same procedure is followed at the British Museum (Brian O'Shea, Bryonet 15 August 2002). At the Chicago Field Museum (Matt von Konrat, Bryonet 15 August 2002), new specimens and loans are frozen unless they are to be used for DNA analysis or study of oil bodies in liverworts. The concern is more for the protection of other plants and fungi in the herbarium since bryophytes are seldom eaten by herbarium pests. Fungi are particularly vulnerable.



Figure 38. New specimens are being placed into the freezer at Beaty Biodiversity Museum in Vancouver, BC, Canada. Photo by Derek Tan, copyright Beaty Biodiversity Museum

Lloyd Stark (Bryonet 15 August 2002) warns that freezing as described above may be too effective, killing the bryophytes as well as the pests. In regeneration tests on specimens from UC, MO, and Cal Acad, mosses were not able to regenerate, but members of Pottiaceae that had not been frozen were able to regenerate several years later. At the University of Nevada Herbarium (UNLV), the dry climate makes freezing unnecessary. David Wagner agrees that low humidity is almost as effective as low temperature for controlling typical herbarium pests.

Rod Seppelt (Bryonet 16 August 2002) reports the additional precaution of freezing specimens that have been taken out of the herbarium cabinets for more than a few hours. If the specimens are kept in the herbarium facility, overnight freezing is usually adequate. If they reside anywhere else while outside the cabinets, they are frozen for several days.

At the Provincial Museum of Alberta (PMAE), the procedure is even more extreme. They do a quick freeze to -70°C for small accessions (fewer than 50 specimens). For larger collections they fumigate. Roxanne Hastings (Bryonet 16 August 2002) reports that creatures are killed within 24 hours at the very low temperature and have no chance to acclimate to it.

Herbarium personnel have done some experimenting, although it may not appear in the literature. John Braggins reported to Rod Seppelt (Bryonet 26 November 2010) that **multiple freezing events** were more effective than a single event. He found at AK that silverfish could be killed with a number of cycles, from room temperature down to -6°C or -10°C and back to room temperature. That procedure was more effective than just one cycle to -10°C . Freezing overnight is most likely useless. After all, these organisms survive such cycles in nature in many parts of the world. Rod Seppelt (Bryonet 6 February 2012) also reported that he had greater success with several low temperature (-1°C)/warm temperature cycles for several days. The multiple freezing event treatment seems to be gaining popularity, and many of the herbaria cited above may already be using it.

Domestic freezers vary in their temperatures, but generally only go down to about -15°C , and depending on their arrangement may have zones that are warmer or slightly colder.

Jeff Duckett (Bryonet 26 November 2010) points out that one advantage to freezing the bryophytes is that it does not **always** kill the bryophytes, despite killing their inhabitants. These are plants that can spend the winter, often for three months, under snow, or in many cases exposed with no snow above below freezing temperatures. In the polar regions they survive in areas that may be snow-free for some time at very low temperatures. Yet these species survive. Such is probably not the case for tropical bryophytes.

Adequate freezing facilities are not available in many herbarium locations. David Wagner (Bryonet 16 August 2002) suggests that baking or poisoning, coupled with closely contained quarantining may be necessary instead, particularly in the tropics. An alternative in temperate climates is an air-conditioning system that chills the air before heating it, thus dehumidifying it. Keeping vulnerable specimens, especially fungi, away from the bryophytes solves a lot of the problems, especially if low humidity can be maintained.

The downside to all this pest control is that the specimens are no longer suitable for longevity tests on spores or plant tissues and might not be usable for DNA testing. The **specimen label should indicate treatments** such as these to protect against faulty conclusions by people using the specimens for physiological purposes or DNA analysis.

Eva Krab (Bryonet 3 February 2012) found that a number of approaches did not work. After a number of failed attempts, she took the approach of flushing the moss cores [*Sphagnum fuscum* (Figure 39) and *Hylocomium splendens* (Figure 40)] in a gas-closed chamber with 100% CO₂ for 12 hours, then leaving the cores at room temperature for 24 hrs (so that eggs would hatch) before freezing them at -20°C. But even after 3 rounds of all those treatments – and still no success – the springtails were still active! (It worked a lot better in the *Hylocomium* cores than in the *Sphagnum* cores.) The mosses actually survived these treatments surprisingly well. These were subarctic springtails, so maybe temperate springtails might be more sensitive to the freezing part of the cycle.



Figure 39. *Sphagnum fuscum*, a hummock moss that survives cryopreservation with a pretreatment in 100% CO₂ to eliminate pests. Photo by Michael Lüth.

I like the suggestion from Javier Martínez-Abaigar (Bryonet 3 February 2012). He suggested using a Berlese

funnel (Figure 41) to chase the springtails out of the moss, then returning them to their natural habitat.



Figure 40. *Hylocomium splendens*, a moss that survives cryopreservation with a pretreatment in 100% CO₂ to eliminate pests, but invertebrates do not survive as well as those on *Sphagnum*. Photo by Janice Glime.

Insect Traps

Some passive means include insect traps, apparently somewhat standard procedure in large herbaria, but these are ineffective against eggs that may be dormant for long periods, causing new outbreaks when new material is introduced. What traps adults may not work for larvae that sit and chew on bryophytes and packets for weeks or months.

Drowning

Eleanor Edge (Bryonet 2 February 2012) found that washing the collections with a surfactant before drying them increases the effectiveness in killing them. She reports that springtails usually have a very hydrophobic cuticle and thus tend to float. Forced immersion in water will reduce their populations. If bryophytes are the only concern, some of the predatory mites will eat the springtails but not the bryophytes.

Steam Sterilization

Soil can be sterilized with steam. While this will most likely kill the pests, it will likewise kill the bryophytes. Rod Seppelt (Bryonet 16 December 2009) reported that Alison Downing found that some bryophyte spores, such as the thick-walled spores of *Riccia* species, survive standard autoclaving of soil.

UV radiation can be used to sterilize the air and even for a short distance (a few cm) into water (Javier Martínez-Abaigar, Bryonet 16 December 2009). However, soil shields it, so it is not an effective tool for sterilizing soil adhering to bryophytes, and most likely will not kill invertebrates hiding among the bryophytes.

Moisture Control

Moisture is another challenge in some herbaria, especially in the tropics. Fungi may appear as tiny hairs projecting upward or as a mass of hairs forming a mat. In worse cases they may form spores that spread easily to

other specimens and that are not healthy to breathe. Roxy Hastings (Bryonet 26 November 2010) found that fungi could be a problem at relative humidities above 40%.

Dehumidifier

Use of a dehumidifier may be sufficient in some cases to prevent the growth of fungi and bacteria, but it adds to the operating expenses and may be insufficient in large herbaria in very humid climates.

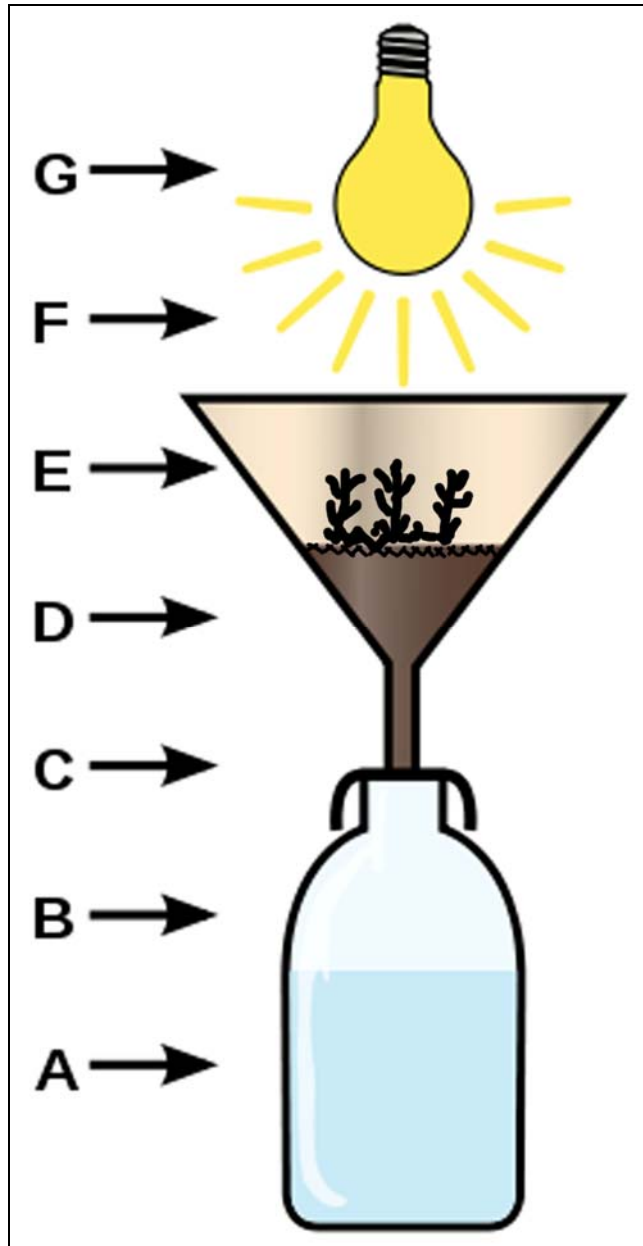


Figure 41. Berlese funnel showing its parts. **A** = liquid for trapping insects (not needed if you want to keep them alive). **B** = air space above liquid in jar or bottle. **C** = lid with hole for funnel end. **D** = open funnel area with screen or wider mesh on top. **E** = area of funnel where sample is placed. **F** = space above funnel to avoid scorching bryophyte sample. **G** = light/heat source that causes invertebrate inhabitants to go downward to escape. Drawing from Creative Commons.

Silica Gel

Modest problems can be controlled with silica gel packs, available from herbarium suppliers (Roxy Hastings, Bryonet 26 November 2010). They are available from "Herbarium Supplies" to maintain various humidity levels in the range of 25-40%. These packs can be "recharged" by putting them in an oven to dry and usually provide a color indicator of their state of moisture.

Herbarium Cabinet Materials

If you choose to make your own cabinet for herbarium specimens, be aware that particle board can contain formaldehyde in the glue, presenting a long-term health risk (Rod Seppelt, Bryonet 26 November 2010). Herbarium cabinets are usually made of metal with a somewhat spongy material around the door to seal it. A good cabinet will not allow pests to gain entry unless they travel with the herbarium specimen.

Sending Specimens for Identification

Understanding accepted courtesy and rules for sending bryophyte specimens can make it easier for one to get much needed help. These guidelines should keep you out of trouble and avoid misunderstandings:

1. Assign each of your collections a unique collection number. Many bryologists pre-number collection bags and keep a life list of numbers to avoid ambiguity.
2. When mailing a specimen for identification, keep part of the sample yourself and be sure it has the same collection number on both yours and the identifier's packets. This will permit correspondence with the least ambiguity.
3. Be prepared to donate the collection to the person doing the identification (Loeske 1925; Raup 1926; Zander 1993). This is a courtesy for the time that person spends helping you. Be aware that some recipients will assume that the specimen is now theirs.
4. Make it clear who will be considered author(s) of any scientific publications resulting from the identification. If possible, offer to make the determiner a co-author. If there is a reason you can't do this, explain why you must be the only author. Establishing this at the onset can avoid awkward misunderstandings.
5. Include details of name of collector, collection number, date, substrate, habitat, and location on the packets, including latitude, longitude, and more precise coordinates, including GPS if possible.
6. Include on the packet label any notes that might be important. Information included in an accompanying letter will usually not be added to the label by others.
7. Check and follow the import/export laws regarding herbarium specimens in both yours and the receiving

countries. Usually it is sufficient to label a package as "herbarium specimens, no commercial value," but some countries have very rigorous import standards to protect against introducing soil organisms and disease, and more recently, against collections of rare or endangered species. For example, specimens entering Australia and New Zealand require paperwork in advance and treatment protocols (Rod Seppelt, Bryonet 12 July 2012). The sender or recipient may have to pay inspection and/or fumigation costs, the alternative being destruction of the specimens. And in some countries you could get the recipient in trouble because the necessary paperwork is lacking. A Google search for **plant import regulations** and the name of the country can be a good start.

8. Remove as much soil as possible.
9. Be sure the specimen is dry and in paper, not plastic, to avoid mold.
10. Get permission from the recipient before sending the specimen. Otherwise, you might never see your specimen or any identification again.
11. Provide a clear address and email address for providing you with the names of bryophytes identified.
12. If you have a target deadline, be sure you discuss that with the person identifying before you send the specimens.
13. Try not to send more than three specimens at a time so the task will not seem so daunting to the recipient (Zander 1993).
14. Don't include more than one species in a packet if you can avoid it. If not, tease out the individual species and put some branches of them in small packets within the larger one, giving each the same collection number but a unique letter to distinguish it (and keep duplicates of the individuals). It is important to maintain the growth form to help in identification.
15. If you are borrowing samples for DNA analysis or other destructive purpose, be sure the person/institution loaning them understands that, and be sure that at least some material is left for verification by anyone later. Actual plants from which DNA analysis is made should be maintained in minipackets (triangular packets) and kept both in the original voucher specimen packet, entered in the herbarium database, and linked to the voucher specimen in Genbank (Brent Mishler, Bryonet 20 January 2023).
16. Include in your packet a carefully prepared slide with a semi-permanent or permanent mount of the specimen of interest, including stem leaves, branch leaves from the middle of the branch, a short branch from which the middle leaves have been removed, and if available, a peristome (Holzinger 1900). It is also very helpful to provide a permanent mount slide of leaf cross sections. These inclusions will save

considerable time for the identifier and make it more likely that you will get your identifications in a timely manner. These should be protected in a small envelope within the packet.

17. Karen Golinski (Bryonet 12 July 2012) suggests providing a spreadsheet with the collection numbers and collection information with space for adding the name. This makes it easy for the identifier to provide you the names and makes it easier for that person (or you) to add the information to a herbarium database.
18. Make an attempt to identify your specimens before you send them to experts. Not only will you learn more this way, but it makes the task less daunting for those helping you. And some bryologists will take the time to tell you where you went wrong in those that are identified incorrectly. David Wagner (Bryonet 12 July 2012) states "First, for anybody sending specimens to an expert you have not had communication with before, send only one or two specimens with your best guess as to identification. This is far more likely to get a prompt response. As soon as somebody sends me a box with a dozen or more specimens, it gets put on a shelf as a 'when I get time to do it' task. This shelf has accumulated specimens for many years with most boxes advancing only very slowly to the top of priority tasks. Send me one specimen and I'll look at it and respond within 24 hours. Offering to pay helps to advance priority ranking, of course." Wagner also points out that your identifications will give the expert some idea of your level of expertise.
19. Make use of the internet to see if your identification matches the images there. But of course be aware that some ID's shown there may be wrong.
20. If you took pictures of the bryophytes and their habitats, send the best of these to the person doing identification (Figure 42-Figure 44). Field growth habit can help in the identification, and if the pictures are good, they are an additional way of saying thank you to the one helping you. Photographs of microscopic characters are even better.



Figure 42. *Riccardia cf. elata*, posted to Bryonet by Zhang Li for help in identification. With only this view, Bryonetters suggested the fern *Hymenophyllum*. A view showing its habitat and growth habit might have helped. Cellular structure and other microscopic views are needed. Photo by Zhang Li.

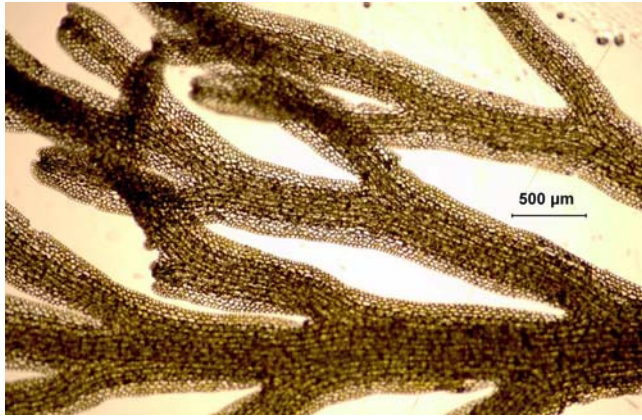


Figure 43. *Riccardia cf. elata*, posted later to Bryonet by Zhang Li for help in identification. With the addition of this view, Bryonetters could be more certain the species was one of *Riccardia*. Photo by Zhang Li.

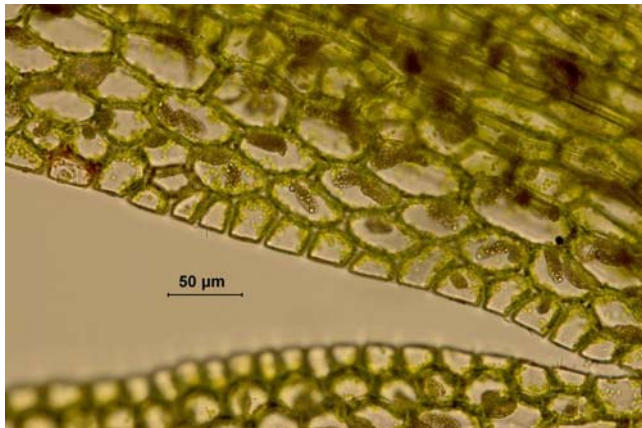


Figure 44. *Riccardia cf. elata* microscopic view posted to Bryonet by Zhang Li for further help in identification. This view enabled Bryonetters to be certain the species was not a fern, but rather one of the liverwort *Riccardia*. Note the oil bodies. The suggestions were narrowed to *Riccardia elata* or *R. prehensilis*. Photo by Zhang Li.

References

Current Names

The choice of references depends on your geographic location, so it would most likely be of little help for us to make suggestions. Old references can be useful if you check the names in TROPICOS <<http://www.tropicos.org/>> for nomenclatural changes. If you can't locate them there, Google might help, or The Plant List <<http://www.theplantlist.org/tpl/record/tro-35156923>>. Be careful of your spelling – these lists don't find similar spellings. They don't care about capitalization. For liverworts and hornworts, the best source currently is Söderström *et al.* (2016).

Bryologists will always disagree among themselves about generic placement using the Linnean naming system. This generates healthy discussion about relationships, but creates problems for a herbarium and the ability to relocate a specimen. It is best for a herbarium to choose a published classification system and be consistent in its use. If a different system is chosen, then the entire collection should be updated. This might be at the generic or family level, not necessarily at the level of the entire herbarium. But it

should not be store partly by phylogeny and partly by alphabet at the same hierarchical level.

Easy sources for names, authors, synonyms, and currently accepted legitimate names are Söderström *et al.* (2016), TROPICOS <<http://www.tropicos.org/>>, a service of the Missouri Botanical Garden, and The Plant List <<http://www.theplantlist.org/>>, a collaboration between the Royal Botanic Gardens, Kew, and the Missouri Botanical Garden. I (Glime) don't like the higher level classification used by the Missouri Botanical Garden (it puts the bryophytes in the class Equisetopsida to use classification levels considered commensurate with those of animals). At least I don't have to look at them in the Kew list!

Indexing

Wagner recommends indexing your taxonomic reference books. He found the books more inviting when they were easier to use, and found that the accuracy of his work definitely increased as a result. With bryophytes he indexes the major genera. Figure 45 shows an indexed copy of Paton's "The Liverwort Flora of the British Isles." Figure 46 shows the thumb tabs in greater detail and Figure 47 shows the method for cutting them. An index card serves as template for the area to be cut out. A cutting mat is placed ON TOP of the page to be indexed. Wagner uses a #11 scalpel blade to cut through forty pages (twenty sheets of paper). The important part is to plan which pages will be indexed. It is easy to want to do too many and run out of space on the outer margin of the book.



Figure 45. Index tab indentations in The Liverwort Flora of the British Isles. Photo by David Wagner from Bryological Times #136 (2012).



Figure 46. Close view of index tab indentations in The Liverwort Flora of the British Isles. Photo by David Wagner from Bryological Times #136 (2012).



Figure 47. Tools for cutting indentations in page edges. Photo by David Wagner from Bryological Times #136 (2012).

The second type of index Wagner uses is an invention that began with an address book. The index is printed on only one side of the paper and stapled on the right side. This might seem counterintuitive because most booklets have the binding on the left side when it is face up. However, because our writing is from left to right, it means the words to be indexed will appear lined up on the left side of a sheet. (This might need to be reversed for some Asian countries.) By staggering the sheets and trimming on the left, any item is quickly found. There are two examples here. The first (Figure 48) shows an index to the five most common references Wagner uses for liverworts, directing one to the pages for species of liverworts found in Oregon. The species are designated by six letter codes. This index is kept with the appropriate books on the workbench or book shelf.



Figure 48. Excel file printout of six-letter liverwort codes, showing reference where it can be found and page. Photo by David Wagner from Bryological Times #136 (2012).

The second example of this kind of index is an older one, made in 1998 when Wagner was doing extensive cryptogam inventories in southwestern Oregon. It has all the mosses and liverworts known from the entire state, almost 700 names (Figure 49). It is useful to check spelling or authority of a name when typing memos, labels, or annotating. Again, the names are designated by six letter codes manufactured for rapid data entry both in the field

and when databasing. By using small type all 678 names fit onto 14 pages.



Figure 49. List of all bryophytes found by David Wagner in Oregon, used to check spelling and supply authors. Photo by David Wagner from Bryological Times #136 (2012).

Herbarium Label Programs

There are several programs on the internet, *e.g.* <http://www.flmnh.ufl.edu/herbarium/pl/>, to help make it easier for you to produce labels and make a herbarium database. If you can use Access, UC Davis has a free Herbarium Management System to download <http://herbarium.ucdavis.edu/database.html> that allows you to print up labels from Access data (Stephen Lodder, Bryonet 12 July 2012).

Specify 6 is another free herbarium program <http://specifysoftware.org/>, funded by the U.S. National Science Foundation. This program can handle specimen data for computerizing collections, tracking specimen and tissue management transactions, and moving species data to the internet. It runs on Windows, Mac OS X, and Linux operating systems.

One consideration for computer programs is that their database is compatible with other programs in widespread use. Current efforts to create a national database of US holdings are underway, supported by government funding. Part of this effort includes converting the many individual databases into a single one that is accessible online. This will permit researchers to find the location of needed specimens and to determine the contact person(s) for loans.

Shipping Live Bryophytes

Bryophytes don't like to be wet and hot at the same time, and this can be exacerbated by also being dark. Such conditions are ideal for fungi to grow, and once a fungus attacks the bryophytes, they most likely won't recover. Hence, shipping live bryophytes can be a major challenge. To reduce these ideal fungal conditions, whenever possible pack some of those frozen picnic cooler gels with your bryophytes to keep them cool and use an insulated container, or insulate one with something like crumpled paper or styrofoam peanuts. If the bryophyte is drought tolerant, send it dry. If you are shipping aquatic

bryophytes, seal them with clean water in plastic bags and keep them cool. Use a rapid shipping method to ensure the best results. Don't provide nutrients as they will encourage growth of algae, fungi, and bacteria on the surface. I have had some success packing aquatic mosses with wet paper towels or newspaper, but heat will quickly spoil all your efforts.

Your first concern may be to keep the bryophytes alive, but getting them across the border might be even more challenging. Even within the same country, it might be necessary to have a **nursery license** to ship plants across state borders. For example, in North Carolina, the Department of Agriculture and Consumer Services requires a nursery license for shipping within the US (Annie Martin, Bryonet 8 December 2010). All live plants and bryophytes need to be inspected for nematodes, insects, or diseases in advance. A certificate documenting certification must be included in any shipment of live plants (bryophytes). For international shipments, at least from the USA, a local inspector must examine each and every shipment that leaves the country. Shipping overseas is a laborious process and shipping is costly.

Sharing Images

Many herbaria have web pages where they provide images of bryophytes. If you choose to set up your own web page, a few guidelines will make it more useful.

Make it clear what you consider fair use. If you prefer restricted use or permission, provide contact information for those seeking permission and make clear what information you will need to give that permission (*e.g.*, intended use, size and resolution, whether it will be modified, attribution, web address). When I (Glime) request images for this book, I state that the image is for an online book on Bryophyte Ecology <www.bryoecol.mtu.edu>, sponsored with no financial support by the International Association of Bryologists and the Department of Biological Sciences of Michigan Technological University. I clearly state that I will give credit for the image and ask if there is additional attribution they would like included besides the name of the photographer.

Sending large images by email can really slow down the system at both ends, so you might want to share images with specific individuals through a free downloadable program called DropBox <www.dropbox.com>. There are also a number of websites where you can post images that are available to everyone, or by becoming a "friend" for that group, much like FaceBook. If you give full permission for use, provide the attribution information you would like the user to include.

BE SURE OF IDENTIFICATION! It is okay to post species where your identification is doubtful, but be clear that it is doubtful, or ask for help when you post the picture.

Don't post pictures taken by anyone else without getting their permission and all the information discussed above.

Some posters restrict the resolution and size of the images they post to avoid having them used commercially for profit as posters, calendars, or advertisements. Many posters give permission for educational use, but not for other purposes. If you have no plans of publishing your

pictures, or using them for profit, why not give permission for all but commercial use? This book is built on the willingness of people to share. And the less time one must spend hunting for a contact person to gain permission, the more time can be spent on creating and sharing the final product.

Herbaria

There are numerous herbaria around the world, and many of them are able to loan specimens to other herbaria. When requesting specimens, it is important to state the use you will make of them and anticipated return date. If you need them for DNA or chemical analysis, or any other destructive sampling, be sure the loaning herbarium understands that. NEVER use type specimens for destructive sampling. And likewise, avoid using voucher specimens unless the destruction is necessary to verify identification or compare then and now. Try not to use the entire specimen.

Index

Herbariorum <<http://www.nybg.org/bsci/ih/ih.html>> provides a list of the registered herbaria of the world. The index lists 1610 herbaria in 117 countries. The site permits searching by institution, city, state, acronym, staff member, correspondent, and research specialty.

Herbarium Specimen Mapping

Some herbaria include a dot map on the herbarium label (Figure 50). Phytogeographers need to understand plant distributions, and floras typically include the distributions of the species. The size of the map depends on the level of detail needed for that herbarium or project. For instance, specimens collected for the BBS vice county records will have a dot in the county of collection.



Figure 50. Dot map for Michigan, USA, indicating location of a specimen in one county. From Voss 1996.

Computers have brought us mapping programs that greatly facilitate these tasks. Brent D. Mishler (Bryonet 13 July 2008) has alerted us about the free program BerkeleyMapper <<http://berkeleymapper.berkeley.edu/>>. This program uses Google maps and places GIS-based points on the maps.

The best way to look at a map is to run the query first at <http://ucjeps.berkeley.edu/bryolab/UC_bryophytes.html>. For example:

1. Search for Scientific name *Mnium* (or any other)
2. Submit query
3. Select on the return page the link: "Map the results using BerkeleyMapper (192 records with coordinates [those with a light green checkbox])"

Live Collections

Maintenance of live collections requires a solid background in the ecological and physiological needs of the species to be cultured. These details will be covered elsewhere in this volume. In the present chapter, we wish to caution you that cultured species may not look like the same species in the field. For genes to be expressed, the right nutrients must be present for development. Hence, caution should be used in using cultured bryophytes for taxonomic identifications. Nevertheless, live cultures are one way to maintain rare species on the verge of extinction.

An alternative to living, growing cultures, is cryopreservation. Michael Christianson (Bryonet 10 June 1999) reported that he had taken over the culture collection established by Malcolm Sargent and that he had begun using cryopreservation of the species, including successful cryopreservation of liverworts.

Cryopreservation

Before we have scratched the surface of the complexity of evolution and biogeographic pathways, many plants and animals are disappearing from the planet forever. We have struggled with our fossil record to make sense of the small samplings we have through time and we do not want to compound our struggle for understanding by losing the species we have today. Nature does not preserve species as fossils on a regular basis, so to ensure these disappearing taxa remain available for study, we as scientists must help out.

We have known for a very long time that most bryophytes have the ability to survive being frozen (Gubin *et al.* 2003), so our knowledge about cryopreservation for this group of animals already has a sound scientific basis. Some of the early studies on cryopreservation for scientific purposes have included bryophytes (Sugawara *et al.* 1980). But several bryologists led the way toward building a collection of cryopreserved endangered and rare bryophyte species (Burch & Wilkinson 2002; Burch 2003; Burch & Ramsay 2003).

Developing such a collection requires considerable testing to be assured that most of the cultures will survive and begin growth again. However, this method for conservation has advantages over the traditional live culture methods. It requires much less maintenance time once the species has been cryopreserved, and it is less

likely to get contaminated while frozen. Furthermore, cultured bryophytes tend to lose vigor over time and both their physical and physiological characters may change in the unnatural conditions of culture, making them look like a different species (Christianson 1998).

As in standard culture, it is desirable to obtain a pure culture free of algal and fungal contaminants. Burch and Ramsay (2003) and Christianson (1998) suggest eliminating algae by growing protonemata **in** (not on) a medium where they will grow toward the light. The photosynthetic ends of these protonemata will emerge from the medium free of algae.

Dehydration prior to freezing will minimize the formation of ice crystals that damage cells. Desiccation-tolerant species are able to survive the prolonged dehydration that makes this successful, but desiccation-intolerant species may not (Burch 2003). Survival of these intolerant species is more likely to be successful if the growth medium is supplemented with abscisic acid (ABA) and sucrose (see volume 1 for a discussion of desiccation tolerance in bryophytes) (Christianson 1998; Burch 2003; Burch & Ramsay 2003). Exact levels needed will require experimentation, with needs differing by species.

Preparation of the bryophytes can be important to their survival, and as you might expect, the ones from wet habitats lack desiccation tolerance, making them more difficult to preserve through cryopreservation (Burch 2003). Christianson (1998) found that only 3-4 days in a medium supplemented with 10^{-5} M ABA and 100 mM proline prepared the mosses *Ceratodon purpureus* (Figure 51), *Funaria hygrometrica* (Figure 52), *Physcomitrella patens* (Figure 53), and two species of *Sphagnum* (Figure 39) to survive at least one year in cryopreservation at -80°C .



Figure 51. *Ceratodon purpureus*, a widespread moss that survives when treated with ABA and proline prior to cryopreservation. Photo by Michael Lüth.



Figure 52. *Funaria hygrometrica*, a disturbed habitat soil moss that survives when treated with ABA and proline prior to cryopreservation. Photo by Janice Glime.



Figure 53. *Physcomitrella patens*, a disturbed habitat soil moss that survives when treated with ABA and proline prior to cryopreservation. Photo by Janice Glime.

In a study of a desiccation-tolerant, a non-desiccation tolerant, and an intermediate-tolerant bryophyte, Burch verified this expectation (Figure 54Figure 57). Burch tested a protocol in which the moss protonemata were cultured on sucrose-free 1/2 strength MS medium (Murashige & Skoog 1962), pH 5.8, solidified with 3.5 g L^{-1} Gelrite®. These were cultured in 5 cm Petri plates sealed with Micropore® tape and maintained at $20 \pm 2^\circ\text{C}$ with 16:8 hour light:dark cycle. Light was provided by Growlux® and cool white fluorescent tubes ($22\text{--}29 \mu\text{mol m}^{-2} \text{ s}^{-1}$). After sufficient cultured material developed, the protonemata were air dried for 18 days with half the cultures encapsulated and half not. The **encapsulation** process started with a double thickness sterile filter paper cut into $0.5 \times 1.5 \text{ cm}$ strips placed into sucrose-free 1/2 MS, 3% sodium alginate (from Sigma) encapsulation medium. This medium was solidified using 100 mM calcium chloride solution. The two pieces of filter paper were separated so that one side was coated in alginate. 2-4 mm diameter circles of protonemata were embedded in the alginate, re-immersed in 3% sodium alginate, and set again using 100 mM calcium chloride solution (Wilkinson *et al.* 1998). Each strip of filter paper had only one sample protonemata, and each strip was placed separately in a 5 cm Petri plate. An equal number of samples was cultured the same way, but without the encapsulation procedure. When these were transferred onto fresh control media, and little

difference was visible between the cultures. After 18 days of dehydration in empty Petri plates sealed with Micropore® tape, they were again tested for viability. The three species exhibited 100% survival of the desiccation-tolerant species, 40% for the intermediate species, and 0% survival for the desiccation-intolerant species. After 18 days, one strip was placed in each cryovial and immersed directly into liquid nitrogen, cooling rapidly to -196°C . After 20 hours of cryopreservation, the protonemata were warmed rapidly by immersing the vials in a 40°C water bath for two minutes. The thawed samples were transferred once again to 12 MS medium and returned to the original cultural conditions. This procedure indicated that encapsulation did little to affect the survival of cryopreservation in these species. Hence, Burch concluded that for desiccation-tolerant species, pretreatment may be unnecessary.



Figure 54. *Bryum rubens*, a desiccation-tolerant bryophyte that survives dehydration and cryopreservation. Photo by Michael Lüth, with permission.



Figure 55. *Ditrichum cornubicum*, a bryophyte with intermediate desiccation tolerance that has partial survival following dehydration and freezing. Photo by Des Callaghan, with permission.



Figure 56. *Cyclodictyon laetevirens*, a bryophyte that lacks desiccation tolerance and that has no survival following dehydration and freezing. Photo by Sean Edwards.

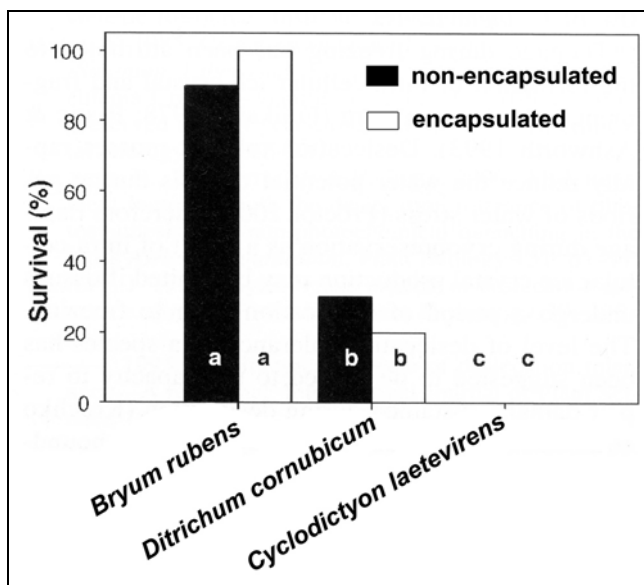


Figure 57. Comparison the effects of encapsulation in alginate on survival in a desiccation-tolerant (*Bryum rubens*), an intermediate-tolerant (*Ditrichum cornubicum*), and an intolerant (*Cyclodictyon laetevirens*) bryophyte species. Bars with the same letter are not significantly different from each other ($\alpha = 0.05$). Redrawn from Burch 2003.

Pence (1998) developed a protocol similar to that of Burch (2003), testing three liverworts and one moss. The thallose aquatic *Riccia fluitans* (Figure 36) was sensitive to desiccation and required either abscisic acid (ABA) pretreatment or encapsulation in alginate beads with 0.75 M sucrose to achieve 100% survival of drying. ABA had little effect on the leafy liverwort *Plagiochila* sp. (Figure 58); it survived with simply drying, encapsulation, and liquid N exposure. The thallose liverwort *Marchantia polymorpha* (Figure 59) required both ABA and encapsulation. Hence, ABA was needed as pretreatment for both thallose species to avoid total mortality upon drying. Rowntree and Ramsay (2009) reported that pretreatment methods, including ABA and encapsulation, were successful for 22 species of bryophytes having a broad range of moisture and other ecological requirements. Some species had 100% survival, and overall regeneration rates were more than 68% for all species tested.



Figure 58. *Plagiochila asplenoides*, member of a genus for which ABA had little effect on survival of cryopreservation. Photo by Dick Haaksma.

Duckett *et al.* (2004) suggest ways of streamlining the cryogenic process. Spores, gemmae, and vegetative fragments can be surface sterilized and grown in Petri plates on media with inorganic salts. Phytogel or Gelrite are preferable to most traditional agars because these are often toxic due to impurities. And some bryophytes benefit from dilution of nutrients. Spore availability can be extended by storing ripe capsules at 4°C. Temperatures >25°C can cause excess respiration and reduce the health of the propagule/culture; light intensity should be much lower than that in nature to prolong the culture viability.



Figure 59. *Marchantia polymorpha*, a thallose liverwort species that requires both ABA and encapsulation before cryopreservation. Photo by Jan-Peter Frahm.

Bryophytes such as *Ditrichum plumbicola* that produce specialized propagules may be easier to preserve through desiccation and cryopreservation (Rowntree *et al.* 2007). Some species cultured in preparation for cryopreservation will produce protonemal gemmae hitherto unknown in nature (*Ditrichum cornubicum*, *Saelania glaucescens*, *Seligeria camiolica*, and *Zygodon gracilis*) (Duckett *et al.* 2004). Protonemal gemmae suspensions are an ideal way to re-introduce these species to the natural environment.

Ditrichum plumbicola protonemata exhibited unexpectedly low survival of cryopreservation (Rowntree *et al.* 2007). Rowntree *et al.* (2007) found that pretreatment of *Ditrichum plumbicola* protonemata with ABA and sucrose caused protonemal growth to be arrested and propagules induced. Most protonemal cells died, but those that survived were char by thick, deeply pigmented walls, numerous small vacuoles, and lipid droplets in their cytoplasm. The protonemal propagules were highly

desiccation- and cryopreservation-tolerant, behaving like the desiccation tolerant rhizoids in the natural environment where they are induced by extreme conditions.

Not all mosses need to be cultured as protonemata to preserve well. Schulte and Reski (2004) used fresh plants to preserve 140,000 mutants by cryopreservation (Figure 60-Figure 61). They used a combination of several of the pre-treatment techniques described above, but with some additions. They used a complete Knop medium (Egener *et al.* 2002), amended with 920 mg L⁻¹ ammonium tartrate, 87 g L⁻¹ mannitol (Grimsley & Withers 1983), 10 µM ABA dissolved in DMSO (dimethyl sulfoxide), and 100 mM proline (Christianson 1998). The liquid medium was filter sterilized; the solid medium was supplemented with 1.2% (w/v) agar. Macro- and microelements, FeSO₄ x 7H₂O, glucose, and mannitol were autoclaved. The other supplements were filter-sterilized with a 0.22 µm millipore filter and added to the medium after it was autoclaved. The medium pH was adjusted to 5.8 before autoclaving and before filter sterilizing.



Figure 60. Cryopreservation equipment in Ralf Reski's IMSC lab <<http://www.moss-stock-center.org/>>. Photo by Ralf Reski.

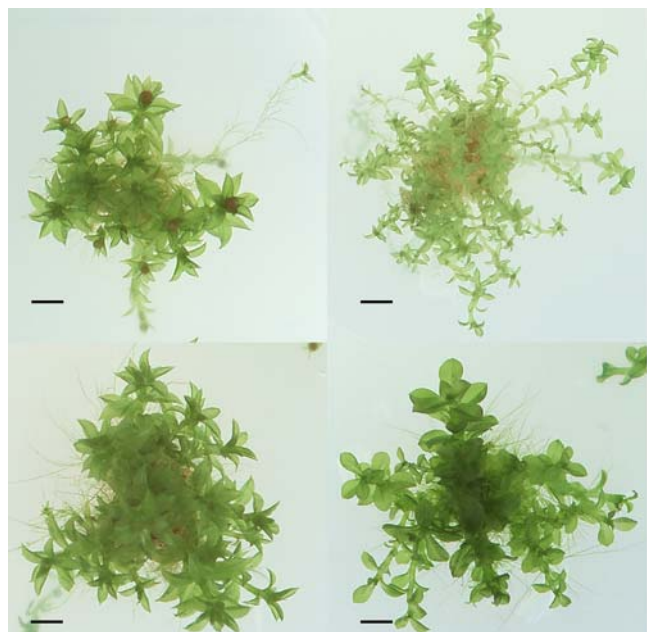


Figure 61. Four ecotypes of *Physcomitrella patens* in culture in Ralf Reski's IMSC lab <<http://www.moss-stock-center.org/>>. Photo by Ralf Reski.

When You Depart – Willing Your Herbarium

Your personal herbarium is valuable, but non-botanists might not recognize its value (Miller 1988). Therefore, it is wise to be sure you have either included it in your will or your heirs understand its value and where it should go. Since herbaria will not always be willing to accept collections, it is wise to make arrangements with the receiving herbarium so you know they will accept your specimens. It is the responsibility of the receiving herbarium to let the bryological community know that they have received your herbarium. If your herbarium is a personal herbarium and is duplicated elsewhere, consider giving it to an exchange program or to a struggling bryologist where the herbarium is inadequate. And be sure provisions are made for return of any specimens you might have on loan.

Exchange Programs

Several of the bryological societies sponsor bryophyte exchange programs. For example, the ABLS (American Bryological and Lichenological Society) program has separate liverwort and moss exchanges. To join the program, one needs to send several species with five duplicates to the current appropriate director of exchange. For each specimen you send, you can select a species from the next exchange list. Hence, if you send six species with five specimens of each, you are eligible to receive 30 specimens from among the forthcoming lists. Specimens contributed must be of adequate size, typically palm size, but this depends on the abundance and size of the species. Sending rare species for exchange should be avoided. The packets must have complete label information, as discussed earlier in this chapter.

Borrowing Specimens

Funk (2007), US National Herbarium, has provided a good introduction into the many uses of a herbarium <<http://www.virtualherbarium.org/vh/100UsesASPT.html>>. Top among these uses for ecologists is to compare your specimens with those of others to verify your identification.

If you are not near with a large herbarium, it may be necessary to borrow specimens to verify your identifications. There is an etiquette for borrowing and asking in the right way is more likely to get you the specimens you need. Visit the New York Botanical Garden website for instructions on how to borrow specimens <<http://sciweb.nybg.org/science2/herb/tips.asp.html>>.

Type Specimens

You should only borrow type specimens when non-types will not do. This would include revisions of a genus or species when you must verify the original description. Type specimens must be handled with utmost care and returned to the loaning herbarium quickly. This method of verification may change somewhat as our use of molecular identification becomes more common and a larger database is available.

The first problem in borrowing a type specimen is to locate it. Generally there are multiple paratypes placed in multiple herbaria, but there is only one holotype. The location of the holotype can be determined by checking the Index

Herbariorum <<http://sciweb.nybg.org/science2/hcol/bryotypes/index.asp.html>>. Index Herbariorum provides the physical location of a herbarium, its web address, holdings (number and type of specimens), history, staff, areas of expertise of associated staff, and contact information. Only permanent collections with active management and accessibility to scientists are included.

When using Index Herbariorum, you can locate herbarium personnel by entering the person's name on the **Text Search** page. For example, when I entered "Deguchi," it provided me Person: Hironori Deguchi; Herbarium Acronym: HIRO; Institution: Hiroshima University; Location: Japan, Hiroshima; Research Pursuits: Taxonomy; morphology; and ecology of bryophytes.

To locate a herbarium where a type specimen is housed, one can use the Virtual Herbarium for Bryophytes and visit the **Type Specimen Catalog**.

Always return type specimens as quickly as possible by a method that insures they won't get lost.

Summary

Most bryophytes are stored in packets folded in thirds of a standard sheet of 100% rag paper. It is easy to make your own packet folding machine. Labels can be designed on a word processor or produced by a herbarium label program. Labels need to include **name of species**, **author** of the scientific name, **altitude**, **habitat**, **substrate**, **date** of collection, **location** (country, state, county, distance to nearest town), **GPS** coordinates, name of **collector**, **collection number**, and **determiner** (name of person identifying or verifying identification). Once they are placed into the herbarium collection, an accession number should be added. Packets with multiple species should indicate so; methods of storing and labelling depend on the purpose of the collection. Storage cabinets need to protect from pests but usually do not require moth balls. Keeping specimens dry is most important.

Herbaria have preferences for specimen storage, including boxes, drawers, folders, and herbarium sheets. Cool preservation works best, but is expensive. Minute species and special structures may require liquid preservation or minipackets. Arrangement in the herbarium may be alphabetical (for ease of filing) or phylogenetic (useful for systematic studies). Type specimens are usually indicated by red folders, but other marks of red can be used.

Killing inhabitants and soil pathogens is necessary for new collections, whether fresh from the field or obtained from another herbarium. This can be accomplished by Agral 600, moth balls, microwave, freezing, steam, insect traps, moisture control, or drowning.

A herbarium should be equipped with both dissecting and compound microscopes and equipment named in Chapter 2-1. Its workspace should include

good taxonomic references, and it helps to add indexing tabs. A computer station is useful for entering data, using online keys, updating nomenclature, making dot maps, and finding images, as well as making herbarium labels.

When shipping specimens to other countries, be sure you know and comply with pertaining laws. Most prohibit soil. Be sure the recipient knows they are coming, and whenever possible, ship to a herbarium where the recipient can receive them. There are many acts of courtesy that can help when you ask others to identify your specimens. Posting pictures online to ask for identification help should include as much information as possible, show habit, plant, leaf, and cross sections, and be kept small so as not to clog inboxes or be slow in loading. Be sure you have permission to post pictures that are not yours.

Living culture can maintain rare species and permit testing without decimating the extant populations. Cryopreservation can also maintain the genome for later study and cultivation.

Exchange programs are available through some of the societies, e.g. the American Bryological Society, where members of the program can exchange specimens with others in the group to build the diversity in a herbarium.

Herbaria can borrow specimens from each other, but loans to individuals might be refused. Type specimens are more likely to be carefully protected, so you might have to travel to the host herbarium.

Acknowledgments

Dale Kruse kindly sent me a printout of all the emails he received on Bryonet and offline regarding herbarium practices.

Literature Cited

- Burch, J. 2003. Some mosses survive cryopreservation without prior pretreatment. *Bryologist* 106: 270-277.
- Burch, J. and Ramsay, M. 2003. The *ex situ* conservation of bryophytes. *Bryol. Times* 108: 7-8.
- Burch, J. and Wilkinson, T. 2002. Cryopreservation of protonemata of *Ditrichum cornubicum* (Paton) (*sic*) comparing the effectiveness of four cryoprotectant pretreatments. *Cryo-letters* 23(3): 197-208.
- Christianson, M. L. 1998. A simple protocol for cryopreservation of mosses. *Bryologist* 101: 32-35.
- Davison, P. G. Date: 2002. Making storage boxes for bryophyte packets. *Evansia* 19: 125-127.
- Duckett, J. G., Burch, J., Fletcher, P. W., Matcham, H. W., Read, D. J., Russell, A. J., and Pressel, S. 2004. *In vitro* cultivation of bryophytes: A review of practicalities, problems, progress and promise. *J. Bryol.* 26: 3-20.
- Egener, T., Granado, J., Guitton, M. C., Hohe, A., Holtorf, H., Lucht, J. M., Rensing, S., Schlink, K., Schulte, J., Schween, G., Zimmermann, S., Rak, B., and Reski, R. 2002. High frequency of phenotypic deviations in *Physcomitrella patens* plants transformed with a gene-disruption library. *BMC Plant Biology* 2: 6.

- Funk, V. 2007. 100 Uses for a Herbarium. US National Herbarium. Accessed 28 February 2013 at <<http://www.virtualherbarium.org/vh/100UsesASPT.html>>.
- Glime, J. M., Hudy, P. S., and Hattori, S. 1990. Diversity and altitudinal niche width characteristics for 35 taxa of the Papua New Guinea *Frullania* flora with consideration of sibling pairs. *Trop. Bryol.* 2: 103-117.
- Grimsley, N. H. and Withers, L. A. 1983. Cryopreservation of cultures of the moss *Physcomitrella patens*. *Cryo-Letters* 4: 251-258.
- Gubin, S. V., Maksimovich, S. V., Davydov, S. P., Gilichinskii, D. A., Shatilovich, A. V., Spirina, E. V., and Iashina, S. G. 2003. O vozmozhnosti uchastiia pozdnepleistotsenovoi bioty v formirovani bioraznoobraziia sovremennoi kriolitozony. [The possible contribution of late Pleistocene biota to biodiversity in present permafrost zone.]. *Zhurn. Obshchei Biol.* 64(2): 160-165.
- Holzinger, J. M. 1900. Suggestion for glycerine jelly mounts. *Bryologist* 3: 42.
- Kitagiri, T. and Majumdar, S. 2020. Recommendation for prohibiting unauthorized division of a type specimen. *Taxon* 69: 1381.
- Li, X., Glime, J. M., and Hattori, S. 1989. Ecological gradient analysis of the genus *Frullania* on Mt. Albert Edward, Papua New Guinea. *J. Hattori Bot. Lab.* 66: 359-369.
- Loeske, L. 1925. Wie man Moose sucht und sammelt. Sonder-Abdruck aus den Verhandlungen des Botanischen Vereins der Provinz Brandenburg. Vol. 67: reprint pages 1-7.
- Malcolm, B. and Malcolm, N. 2006. Mosses and Other Bryophytes: An Illustrated Glossary, 2nd ed. Micro-Optics Press, New Zealand, 336 pp.
- Miller, H. A. 1988. In: Glime, J. M. *Methods in Bryology*, Hattori Botanical Laboratory, Nichinan, pp. 369-374.
- Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. *Physiol. Plant.* 15: 473-497.
- Pence, V. C. 1998. Cryopreservation of bryophytes: The effects of abscisic acid and encapsulation dehydration. *Bryologist* 101: 278-281.
- Raup, L. C. 1926. Hunting for and collecting mosses. *Bryologist* 29: 73-74.
- Rico G., R. R. 2011. Methods for cell clearing and rehydration in thalloid liverworts. *Trop. Bryol.* 33: 12-18.
- Rothero, G. and Blackstock, T. 2005. Recording and conservation. Submitting new vice-county records. *Field Bryol.* 85: 41-43.
- Rowntree, J. K. and Ramsay, M. M. 2009. How bryophytes came out of the cold: Successful cryopreservation of threatened species. *Biodiv. Conserv.* 18: 1413-1420.
- Rowntree, J., Duckett, J. G., Mortimer, C. L., Ramsay, M. M., and Pressel, S. 2007. Formation of specialized propagules resistant to desiccation and cryopreservation in the threatened moss *Ditrichum plumbicola* (Ditrichales, Bryopsida). *Ann. Bot.* 100: 483-496.
- Schofield, W. B. 1985. *Introduction to Bryology*. Macmillan Publishing Company, New York, 431 pp.
- Schulte, J. and Reski, R. 2004. High throughput cryopreservation of 140,000 *Physcomitrella patens* mutants. *Plant Biol.* 6: 119-127.
- Schuster, R. M. 1966. The Hepaticae and Anthocerotae of North America, East of the Hundredth Meridian. Vol. I. Columbia University Press, New York, 802 pp.
- Shaw, A. J. and Goffinet, B. (eds.). 2000. *Bryophyte Biology*. Cambridge University Press, Cambridge, UK, 476 pp.
- Singh Deo, S. and Majumdar, S. 2020. Recommendation for preserving dissected parts of holotype specimens of names of bryophytes. *Taxon* 69: 625.
- Sugawara, Y., Matsushima, H., and Takeuchi, M. 1980. Cryopreservation of plant protoplasts and their differentiation after thawing. *Cryobiology* 17: 623.
- Turland, N. J., Wiersema, J. H., Barrie, F. R., Greuter, W., Hawksworth, D. L., Herendeen, P. S., Knapp, S., Kusber, W.-H., Li, D.-Z., Marhold, K., May, T. W., McNeill, J., Monro, A. M., Prado, J., Price, M. J., and Smith, G. F. 2018. International Code of Nomenclature for Algae, Fungi, and Plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress, Shenzhen, China, July 2017. *Regnum Vegetabile* Volume 159. Koeltz Botanical Books, Glashütten, 254 pp.
- Voss, E. G. 1996. Michigan Flora: Part III: Dicots Concluded. The Regents of the University of Michigan, Ann Arbor, MI.
- Wagner, D. 2012. Tools, tips, and techniques: Practical indexing. *Bryol. Times* 136: 6-7.
- Wilkinson, T., Wetten, A., and Fay, M. 1998. Cryopreservation of *Cosmos atosanguineus* shoot tips by a modified encapsulation/dehydration method. *Cryo-Letters* 19: 293-302.
- Zander, R. H. 1993. Genera of the Pottiaceae: Mosses of Harsh Environments. *Bull. Buffalo Soc. Nat. Sci* 32: 378 pp.

