Chapter 13

Sampling of bryophytes

by

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Abstract

In this chapter, we provide practical guidelines for collecting and recording bryophytes. Bryophyte species exhibit a high specificity to meso- and microhabitat conditions and, although some can be observed all year-round, many are annual and/or can be identified only during a short period of the year. Completely random plot sampling (RS) or systematic sampling (SS) are therefore likely to miss important types of variation within the sampling area unless the intensity of the sampling (*i.e*. number of plots and number of visits at different seasons) is very high. Therefore, it is appropriate to use a sampling methodology, such as Floristic Habitat Sampling (FHS), that focuses on mesohabitats as the sampling unit. SS and RS offer, however, substantial advantages over FHS in terms of statistical comparisons across plots. Therefore, the combination of a systematic grid, usually of 1 to a few km², within which FHS is performed, is recommended. The size of the sampling plot is discussed depending on the goals that are followed. For recording rare species, the Area of Occupancy (AOO), defined as the area calculated by summing up all 2 x 2 km grid squares actually occupied by a taxon, is used by IUCN as a standard measure for defining species frequency. In the case of bryophytes, however, it is strongly advisable to decrease the mesh size because AOO values decline sharply as the scale of measurement reduces, as a result of the linear and frequently fragmented distribution of the species. Scientific collecting is still essential for a number of reasons, including specimen identification and herbarium collections for taxonomic studies – which is especially true for bryophytes because, although the larger species can often be named in the field, many are distinguished based on microscopic characters – and, more recently, for the constitution of DNA libraries. The collecting techniques, including information on what and how much to collect in the field, how to pack, label, dry and process specimens, are finally reviewed.

Key words: bryophyte, moss, liverwort, hornwort, floristic habitat sampling, random sampling, plot sampling, phenology, diversity

1. Introduction

Bryophyte is a generic name for plants characterized by a life-cycle of alternating haploid and diploid generations with a dominant gametophyte. They include the liverworts, mosses, and hornworts. Liverworts and hornworts comprise about extant 5,000 and 300 species, respectively. Together with mosses, which, with approximately 12,000 species, are the second most diverse phylum of land plants, bryophytes thus include a substantial proportion of the total biodiversity of land plants.

Although bryophytes are rarely the most conspicuous elements in the landscape, they play important ecological roles in terms of water balance, erosion control, or nitrogen budget, or simply by providing habitat for other organisms. Furthermore, bryophytes locally exhibit richness levels that are comparable or even higher than those of angiosperms. Lastly, and perhaps most importantly, although global biodiversity patterns tend to be congruent across taxa, especially ß diversity patterns (Schulze *et al.*, 2004; Kessler *et al.*, 2009), diversity patterns in bryophytes do not necessarily follow the patterns present in other, better-studied taxa, so that an enlarged concept of biodiversity has become increasingly necessary. As a result, there has been an increasing awareness of the necessity to include cryptogams in general, and bryophytes in particular, in conservation programs and biodiversity assessments.

In this chapter, we attempt at providing practical guidelines for collecting and recording bryophytes. From recent specialized textbooks (Goffinet & Shaw, 2009; Vanderpoorten & Goffinet, 2009), we briefly summarize the biological and ecological features of bryophytes that are relevant to their study in the field. We then review, based upon information provided in many specialized field guides, to which we refer for further information (O'Shea, 1989; Gradstein *et al.*, 2001; Wigginton, 2004), the sampling strategies and collecting techniques that are most appropriate for recording bryophyte diversity.

2. Where and when to collect bryophytes?

2.1. Where do bryophytes occur?

Bryophytes are generally seen as small plants confined to humid habitats, avoiding exposure to direct sunlight. Yet, an alert naturalist will quickly notice their presence in virtually every ecosystem. In parts of the world where short growing seasons limit plant growth, bryophytes, and especially mosses, may dominate the vegetation. Similarly, in temperate and tropical rain forests, bryophytes, and especially liverworts, compose luxuriant epiphytic communities that play important ecological functions, especially in terms of water and nutrient flow. Even in modern cities where air pollution and the man-made environment may seem unrelenting, bryophytes are able to colonize crevices in masonry.

The diversity of bryophytes is correlated with habitat heterogeneity at two spatial scales. Mesohabitats are localized physiographic (*e.g.* streams, seeps, cliffs) or physiognomic (*e.g.* forests) features. In a forested landscape, mesohabitats are

arranged into a mosaic of dominant mesohabitats (*e.g.* forests), wherein restricted mesohabitats (*e.g.* streams, seeps, cliffs) exist (Vitt & Belland, 1997). Microhabitats (*e.g.* trees, logs, rocks, stumps) are the smallest landscape units and may be unique to one type of mesohabitat. Epiphytic communities provide a classical example of microhabitat differentiation. Epiphytes typically exhibit both a vertical and a horizontal zonation, segregating vertically from the base to the crown along gradients of humidity, pH, and nutrient content (Barkman, 1958; Sillett & Antoine, 2004). Within each ecological unit, bark microtopography further generates a mosaic of microhabitats. For example, Barkman (1958) described the mosaic of species inhabiting beech bark in The Netherlands (Fig. 1). Wound exudates induce a vertical zonation of neutrophytic species, including *Orthotrichum diaphanum, Syntrichia laevipila* and *Zygodon viridissimus*, which are normally absent from acid beech bark. The last two species grow lower, presumably due to greater moisture near the ground. In contrast, acidophilous species, such as *Lophocolea heterophylla*, develop far from the wound.

Different species thus tend to utilize different portions of the resource continuum available. The competitive exclusion principle predicts that species avoid competition by occupying different niches, creating a spatial pattern that represents habitat partitioning corresponding to habitat heterogeneity. Thus, an increasing body of literature points to the strong correlation between habitat and species diversity. Some habitats are, however, more species-rich than other and hence, request a longer investigation time. Bryophytes are poikilohydric, which means that they suspend any metabolic activity upon drying. They tend therefore to be more dominant in sheltered, humid habitats than on open ground directly exposed to irradiation and desiccation.

A good trick to find species-rich habitats is to look at the extent of species cover. There is indeed a positive correlation between carpet density and species diversity for two main reasons. First, massive cover suggests that the habitat has the appropriate humidity level for many species to establish. Second, at low to moderate densities, growth is constrained by water availability. Moderately dense stands are dehydrated less rapidly than loose stands or isolated shoots because a dense packing of shoots may reduce water loss by effectively reducing the diameter of capillary spaces among close neighbours. Bryophytes growing in dense communities are therefore able to remain physiologically active for a larger part of the growing season, resulting in greater biomass and diversity.

2.2. Can we record bryophytes all year-round?

It is often believed that bryophytes occur all year-round, and this is one of the reasons why many naturalists shift to bryology in wintertime. This is definitely true for stress-tolerant species, which invest much in gametophytic development, enabling them to survive periods of stress. As a most extreme example, large cushions of the moss *Leucobryum glaucum* on forest ground or *Sphagnum* species in peat bogs, all of which occur in stable habitats and display gametophytic adaptations to store water in dead hyaline cells, can last for centuries. Thus, bryophyte species of long-lived, stable mesohabitats such as woodlands, can in fact be recorded at any time.

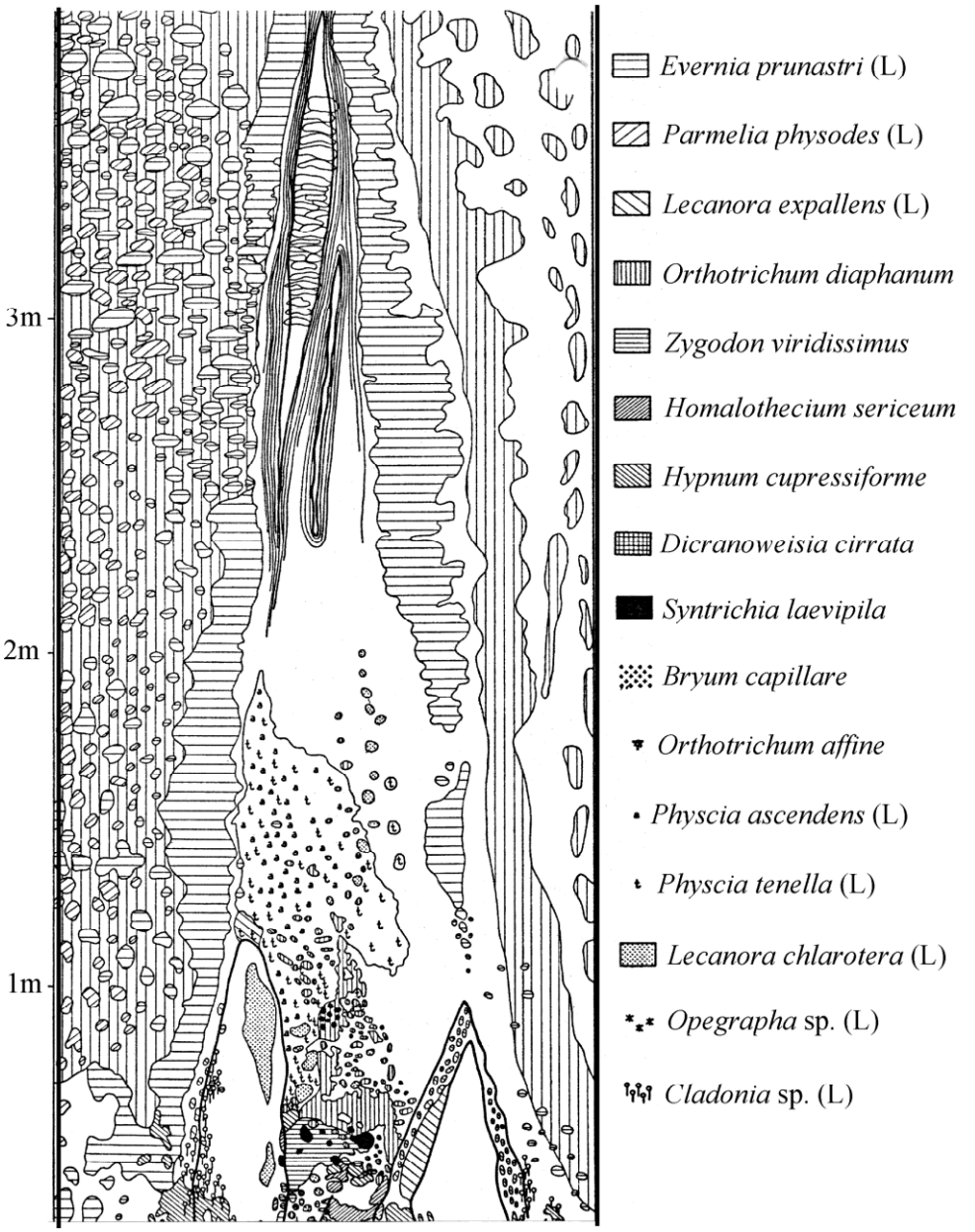

Fig. 1. Mosaic of cryptogamic vegetation comprised of lichens (L) and bryophytes along the first 4 m on an old beech trunk in The Netherlands (after Barkman, 1958).

It must be emphasized, however, that whilst perennial species can be observed regardless of the season, their identification might rely on sporophytic features that can be observed only during a short period of the year. The moss genus *Orthotrichum*, for example, includes mostly perennial epiphytic species whose identification relies on specific sporophyte features. In the northern hemisphere, the capsule reaches its full development in the spring, and taxonomically relevant characters of the peristome progressively become impossible to observe towards the summer season, during which the capsule itself eventually falls down.

In many other habitats, bryophyte species can be observed during a short period of the year only. In fact, plants have to cope with unstable habitats in time (*e.g.,* seasonal climate variations) and space (*e.g.*, habitat degradation or destruction). To face the risk of local extinction, they may either disperse in an attempt to establish new populations or remain under the form of long-lived diaspores, from which new establishment will be subsequently possible under favourable growth conditions. Parts of these diaspores may become buried into the soil, requiring light for germination, constituting a bank of diaspores. Because of the vulnerability of their gametophyte, bryophytes are, in particular, likely to rely more on stored propagules for their long-term survival than seed plants. Species of unstable habitats that recur predictably at a given site thus tend to produce a few, large spores with a low dispersal capacity but better chances of successful establishment and a longer life span in the diaspore bank. This is, for example, the case of hornworts in temperate areas, which are well adapted to regular disturbance in arable fields thanks to their diaspore bank, or of annual thalloid liverwort communities in xerotropical environments experiencing a severe drought season. On a less regular basis, habitats such as dried-out ponds are quickly recolonized thanks to the diaspore bank and their survey is often rewarded by the discovery of many specialized species.

As a result, all habitats cannot be recorded all year-round and some must be investigated during the appropriate season. During a survey of the bryophytes of arable land in Britain and Ireland for example, inventorying of the fields occurred at a time of year when the bryophytes were large enough for most of them to be identified or, in the rare cases of fields with no bryophytes, at a time of year when bryophytes would have been identifiable if present. In practice, this meant that fields were inventoried in the autumn, winter and early spring (Preston *et al.*, in press).

3. How to record bryophytes?

3.1. How to organize the sample plots?

An appropriate sampling methodology is crucial to understanding patterns of community and taxon diversity at the landscape scale. The type of sampling used for estimating diversity depends on the organism being studied, how closely that organism is associated with its substrate, and the nature of the ecological question (Krebs, 1989). In plant studies, Clements (1905) described methods for collecting plant species data using plots. Since that time, many variations of quantitative measurements using plots have been used. The bounded nature of

plots in relation to a specific sample area allows for quantitative sampling of species abundance and frequency, and later statistical analysis. This has made plot sampling a successful method for studying population and community dynamics in bryophytes and many other groups of plants.

Plots may be organized in a regular fashion, using a systematic grid, or selected at random. For instance, the combination of a systematic grid of 10 x 10 km, within which 'standard relevés' of 100 m^2 are inventoried, has been used for the standardized mapping of Swiss bryophytes (Urmi *et al.*, 1990). In each 'relevé', all bryophyte species are collected and determined, and voucher specimens are kept. This approach is most appropriate to identify the commonest species and assess their frequency and distribution, but may not allow for the recording of rare species. This is because many bryophyte species exhibit a high specificity to peculiar meso- and microhabitat conditions; a completely random plot sampling method is likely to miss important types of variation within the sampling area unless the intensity of the sampling (*i.e.* number of plots) is very high. Therefore, it is appropriate to use a sampling methodology that focuses on mesohabitats as the sampling unit. Sampling methods aimed at assessing total bryophyte diversity studies should include all of the potential habitats in an ecosystem. The method referred to as Floristic Habitat Sampling (hereafter, FHS) uses mesohabitats as the basic sampling units.

Comparisons of the efficiency of random Plot Sampling (hereafter, PS) and FHS suggested that the latter captures a greater mean species richness per stands than PS (Newmaster *et al.*, 2005). Bryophyte diversity estimates compared within the dominant forest mesohabitat were found to be much greater (*i.e.* species richness is 50% higher) when using FHS as compared to PS (Fig. 2). Although it is not made explicit, and although other data from herbarium records as well as casual observations are also included, FHS within each square of a systematic grid of one to several km is basically used in most of the European mapping programs for example in the UK (Hill *et al.,* 1991-1994), The Netherlands (van Tooren & Sparrius, 2007), Germany (Meinunger & Schröder, 2007), and Belgium (Sotiaux *et al.*, 2000; Sotiaux & Vanderpoorten, 2001, 2004).

Usually, all mesohabitats are identified from the analysis of fine-scale topographic maps. Each mesohabitat is then visited and sampled until no new species are reported. In some instance, special attention is paid to key-habitats that are identified on the basis of specific attributes, *e.g.* the known presence of rare bryophytes, special topography or soils, or, since the diversity of bryophytes most often correlates with global biodiversity patterns (Pharo *et al.*, 2000; Schulze *et al.*, 2004), the known presence of rare taxa.

The time necessary to survey an area depends of course of many factors including the number and experience of recorders, as well as the extrinsic floristic quality of the habitats. In Belgium, our experience is that the record of a grid-square of 4 x 4 km is considered complete, *i.e.* with no more than approximately 10% of missed species, takes between one (species-poor squares with low habitat heterogeneity, with approximately 50-60 species/square) and four days (species-rich squares with high habitat heterogeneity and quality with >150 species/square).

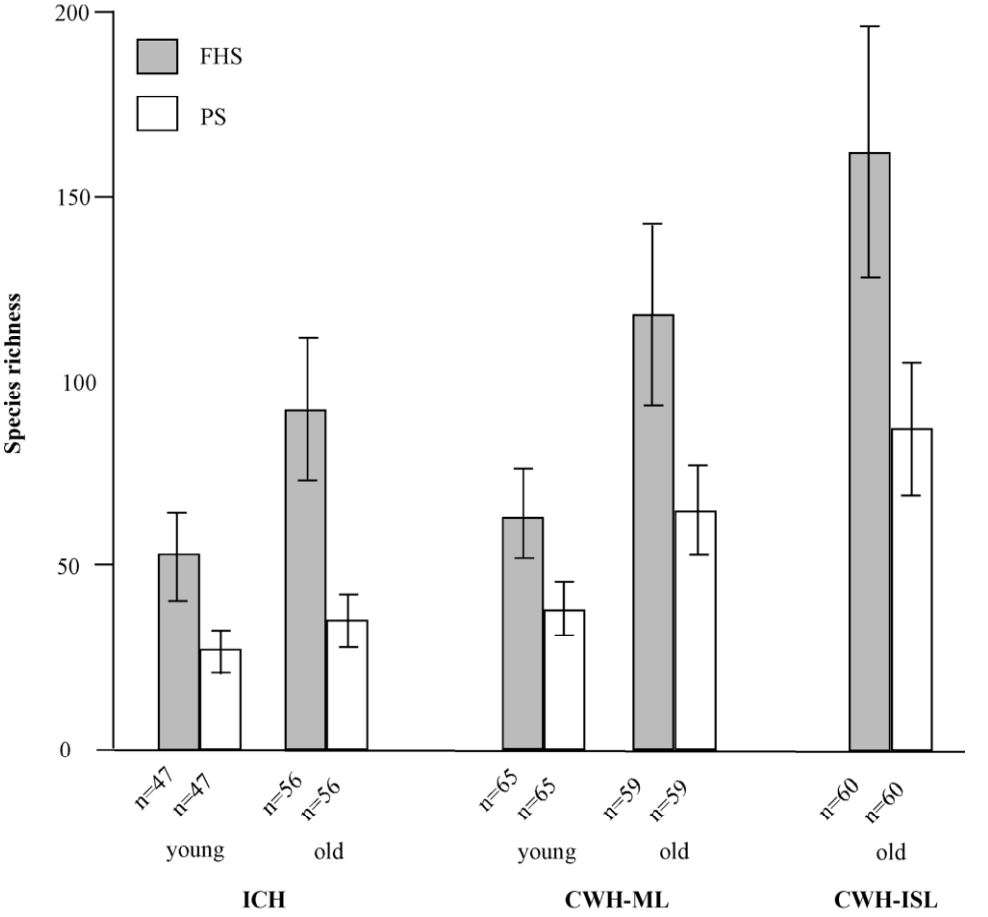

Fig. 2. Alpha diversity of stands assessed using floristic habitat sampling (FHS including all mesohabitats) and plot sampling (PS). Cedar hemlock forests are divided into inland (ICH), coastal mainland (CWH-ML), coastal oceanic (CWH-ISL), and by age classes (class 4, young = 80 years and class 9, old > 250 years). Error bars represent two standard errors on either side of the mean (reproduced from Newmaster *et al.,* 2005 with permission from Blackwell).

3.2. What size should sample plots have?

The size of the sampling plot depends on the goals that are followed. For biodiversity inventories, large plots should be favored since species richness typically increases with sample area (Fig. 3). In a comparative study of bryophyte forest diversity in Canadian forests, Newmaster *et al.* (2005) found that the 20 m-diameter plot used in the PS method sampled 314 m^2 of forest mesohabitat resulting in a mean species richness of 35 (± 5) species. Expanding sampling area to 1000 m^2 increased mean species richness by only 18 species.

Furthermore, species richness steadily increases even after 5000 m^2 has been sampled, increasing mean species richness in the dominant forest mesohabitat to just over 80 (± 6) species (Fig. 3). Using FHS, the mean species richness within the dominant forest mesohabitat was 106 (± 9) species. In fact, intensifying PS or simply sampling large areas using randomly placed plots will not necessarily include the natural variety in microhabitats. This is because PS within a mesohabitat will exclude important microhabitats and their respective bryophyte communities even after sampling unconventionally large sample areas. These results clearly suggest that the size of the sampling units depends on the sampling strategy itself, and that, in any case, the size of each sampling unit should be determined by means of species-area curves. In tropical rain forest, Gradstein *et al.* (2003) found that full sampling of 4-5 mature trees may yield 75-80% of the tree-inhabiting bryophytes in a forest stand (excluding epiphylls).

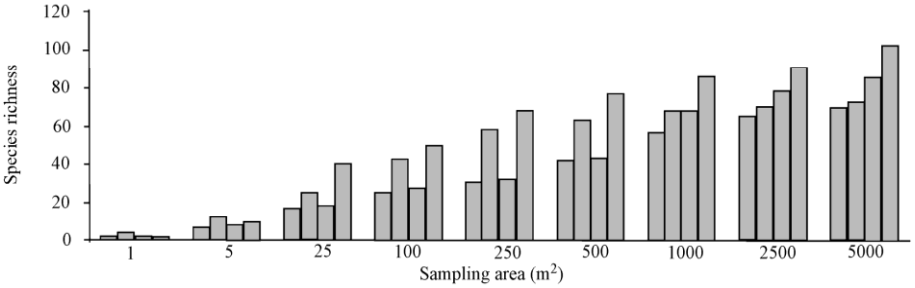

Fig. 3. Mesohabitat alpha diversity (species richness) within increasing sample size areas for 287 temperate rainforest stands (SP = seep, CF = cliff, FS = forest, ST = stream) (reproduced from Newmaster *et al.,* 2005 with permission from Blackwell).

For the record of rare species, the Area of Occupancy (AOO), which is defined as the area, calculated by summing up all grid squares with the mesh size of 2 x 2 km that are actually occupied by a taxon, excluding cases of vagrancy, is used by IUCN as a standard measure for defining species frequency. In the case of bryophytes, however, it is strongly advisable to decrease the mesh size because AOO values decline sharply as the scale of measurement reduces, as a result of the linear and frequently fragmented distribution of the species (Callaghan, 2008).

3.3. What to measure in each plot?

Depending on the time available and the goals followed, presence-absence or increasingly complex abundance indices can be used to document the frequency of each species in each sampling unit. The 'relevé' sampling method involves the attribution, to each species within the plot, of a coefficient of abundance-dominance, sometimes associated with a coefficient of sociability (see chapter on vascular plant recording), which serve to describe the cover of each species on the ground and its distribution mode, from lose, isolated plants to densely packed cushions.

In some tropical areas characterized by a very lush and species-rich bryophyte vegetation, however, this method may not be applicable and alternative

strategies must be used. One such strategy is to sub-divide each sampling unit into smaller sub-plots of a few dm², select some at random, perform complete species lists in each, and assess the frequency of each species across the sub-plots in each sampling unit. Alternatively, the same procedure of sub-division of the main sampling unit can follow a systematic scheme. This is, for instance, the method applied by the Hungarian Bryophyte Monitoring Program (Papp *et al.*, 2005) for the record of epiphytes. Within each sampling unit, each standing tree (living or dead) with a diameter of at least 19 cm at breast height is included in the sampling of epiphytic bryophyte vegetation. The sampling of epiphytic bryophytes is carried out at three levels: 10 cm (1. level), 70 cm (2. level), 140 cm (3. level) upwards from the base of the tree. A 10 cm wide cylinder is examined at each level (from the marked level 5-5 cm upward and downward), where the occurrences of the species are recorded (presence/absence data).

A protocol for rapid and representative sampling of epiphytic bryophytes growing on bark of trees in tropical rain forest was designed by Gradstein *et al.* (2003). Within a core area of one hectare, 5 mature rain forest trees (standing well apart and differing in bark structure) are sampled from the base to the outer canopy using the single rope technique (ter Steege & Cornelissen, 1988) or some other method for sampling of the forest canopy. Species are collected in 4 small plots within each of 6 height zones, the so-called “Johannson zones” (1: tree base, 2a: lower trunk, 2b: upper trunk, 3: lower crown, 4: middle crown, 5: outer crown). Plots in zones 1-3 are 20 x 30 cm and positioned in each cardinal direction, those on thin branches in zones 5-6 are ca. 60 x 10 cm long and positioned on the upper and lower surfaces of the branch. For safety reason, plots in zones 4 and 5 are sampled on the ground from cut-off branches.

A protocol for sampling of epiphyllous bryophytes in tropical rain forest was designed by Lücking & Lücking (1996).

4. Collecting techniques

Scientific collecting is essential for a number of reasons, including specimen identification, herbarium collections for taxonomic studies, and, more recently, constitution of banks of DNA. This is especially true for bryophytes because, although the larger species can often be named in the field with a 10-20x hand-lens, many are distinguished based on microscopic characters. Reference collections of specimens are thus invaluable in the study of bryology, but in order to obtain useful specimens for research, the correct techniques for collecting and processing should be employed. It must also be emphasized that, although bryophyte species rarely legally protected, it is necessary to obtain permits to collect bryophytes and an export licence if the material is to be taken out of the country. Herbarium staff can often advise on what is needed, but obtaining necessary papers and permissions can be a lengthy process, so should be investigated well in advance.

4.1. Packeting

Bryophytes are among the easiest plants to collect (Buck & Thiers, 1996). Since they lack roots, they can often be readily collected by hand, although some species closely attached to their substrate will have to be scratched using a knife. Specimens should be selected to include all the parts of the plant needed for identification. Sporophytes are often useful, if not necessary, for identification, and should be searched for. Several mosses from unstable habitats, *e.g.* riverbanks, arable fields, have rhizoidal tubers buried in the soil. As these are often diagnostic, these bryophytes should be collected with 1-3 cm of the substrate (Whitehouse, 1966; Porley, 2008).

Individual species within a collection should be packed-up separately, so far as this is possible. It is in fact generally easier when the material is still fresh than later, when several collections jumbled together in a single packet have to be separated. The specimens are normally put into envelopes. A standard envelope can be folded from an A4 paper to be (10-)12 x 14 cm in size (Fig. 4). Particularly small specimens should be wrapped separately in mini-packets before being put into normal size packets. If sporophytes or fertile structures are rare, these should also be placed in mini-packets, but attached to a piece of the gametophyte to avoid any subsequent confusion. If specimens are very wet, as is often the case with *Sphagnum*, they should be gently pressed to remove most of the water, and packed into a double or treble thickness packets. As for ground-dwelling species, it is often more appropriate to keep them in stiff boxes for transportation and storage to avoid ending up with a mixture of soil particles and plant fragments.

For collecting of epiphyllous bryophytes in tropical rain forest, whole leaves on which the epiphylls are growing are collected in new papers in a plant press, lightly pressed and dried. The epiphyllous species are subsequently sorted, and leaves cut up, in the laboratory using a dissecting microscope. For collecting of thalloid liverworts and hornworts it may also be recommendable to dry the specimens in a plant press instead of in collecting bags, in order to keep them flat and avoid them from becoming rolled inwards. Pressing of the specimens should be lightly only, to avoid damage to the plants.

4.2. How much to collect?

Collecting of specimens for scientific purposes is usually highly selective and seldom constitutes a real threat to the survival of species. The extinction of species by a targeted over-collecting has been, however, already documented. It is difficult to provide exact guidelines since everything depends on species size, local and overall abundance, etc. As a general rule, collecting enough to fill a 12 x 8 cm packet should be plenty for a robust species. On the other hand, too small specimens are of no value if there is insufficient material to allow identification and, perhaps, DNA extraction. In addition, the really important plant in a collection may not be what the collector actually saw in the field, but some minute plant sparsely mixed with it, and only discovered later in the laboratory.

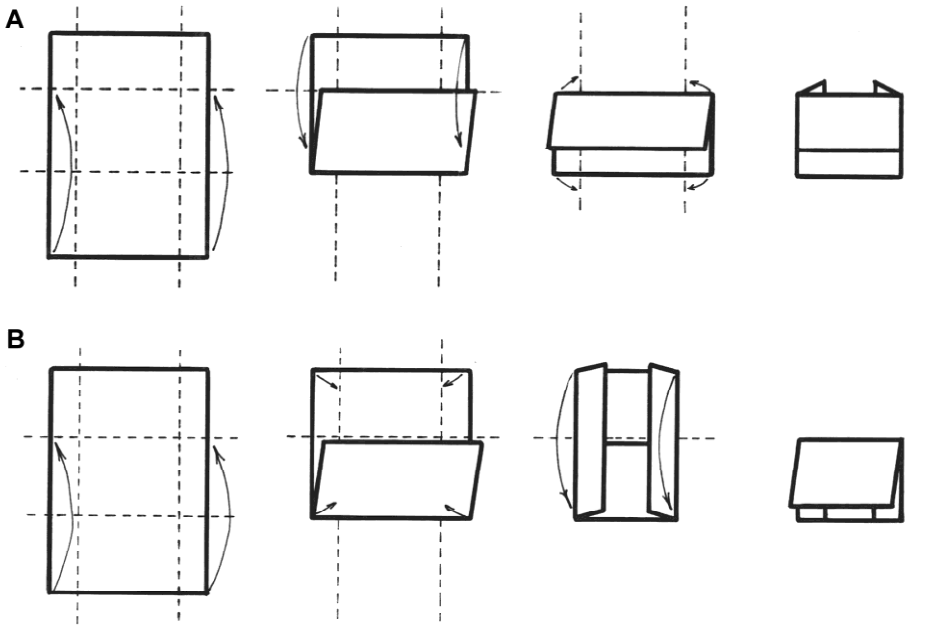

Fig. 4. Folding procedure for packing-up bryophytes.

4.3. Data and labelling

The information record is similar to that of other plants, and includes habitat information (for instance, if a species occurs on tree or rock, the tree species or rock type should be recorded), nature of the surrounding vegetation, elevation, and locality details, including GPS coordinates. For rare species, information on population size is often useful but might be difficult to assess in the case of bryophytes. Indeed, many bryophyte species are highly clonal, and several gametophytes can develop from a single protonema following the germination of a single spore.

Thus, what is the entity that best corresponds to discrete individuals like animals? For practical reasons, a purely pragmatic definition can often be used. For species that depend on discrete substrate entities (such as tree trunks or droppings), each substrate entity can be considered to contain one or two individuals. For bryophyte species growing on ground or rocks, one individual may be assumed to occupy a surface of 1 m^2. However, in some rare cases of some very small mosses (*e.g.* the genera *Seligeria* and *Tetrodontium*), one individual might be associated with a surface of 0.1 m^2.

4.4. Drying and processing

The collected specimens should be dried as soon as possible to avoid fungal damage. In most cases, the packets can be left to air-dry. In wet areas during extended expeditions, however, drying might become a major issue and

preoccupation, and the use of a plant dryer can sometimes become necessary (Frahm & Gradstein, 1986). As liverwort capsules tend open when drying, releasing their spores, it is recommended that some specimens with capsules be placed in a small paper envelope before drying together with the rest of the sample, to ensure that at least some unopened capsules are preserved.

These is no need to give a descriptive account of the plant, as one does systematically for fungi and sometimes for higher plants, since most bryophyte species recover their primary appearance upon remoistening. A special care must, however, be taken with liverworts. Indeed, the identification of many species relies on the size, shape, number, colour, and distribution of oil bodies, which are unique organelles among land plants. Because of the volatility of the oils they contain, oil bodies progressively disappear upon drying in the laboratory. In some taxa, the process takes only a few hours, so that fresh material must be studied, whereas in other, oil-bodies last for some years and can still be studied on herbarium specimens. In any case, it is advisable to take a micro-photograph of the cells to keep a record of the oil body morphology.

For preservation of DNA, fresh material should be cleaned and quickly air-dried, and subsequently kept dry. Any moistening of the material must be avoided as this might lead to degradation of the DNA, making the material unsuitable for molecular analysis.

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