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HOW TO SAMPLE THE EPIPHYTIC DIVERSITY OF TROPICAL RAIN FORESTS

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CONTENTS

1. INTRODUCTION

S. Rob Gradstein

Key words: Epiphytes, tropical rain forest, epiphyte sampling.

The great diversity of epiphytic plants, both vascular and non-vascular, is one of the striking features of tropical rain forests, distinguishing these forests from temperate ones. Because they are mostly canopy dwellers, epiphytes have often been neglected in rain forest studies due to difficulties of access. These limitations have recently been overcome by the development of techniques for access into the canopy (Mitchell 1982). Using ropes, trees can be prepared for ascending in less than an hour and climbed to a height of 30 m in 5-10 minutes. The outer portions of the canopy, too fragile to climb, may be studied by sawing out branches and lowering them to the ground using ropes (ter Steege & Cornelissen 1988, Wolf 1993).

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Recent studies have shown that epiphytes may play an important role in ecosystem-level interactions in rain forests, especially in the water balance and nutrient cycles of the forest (Lowman & Nadkarni 1995). In addition, epiphytes are an important source of food and habitat for many birds, mammals, amphibians, and reptiles, and offer shelter to a great variety of invertebrates and micro-organisms. The importance of epiphytes is also exemplified by their usefulness as indicators of forest types and altitudinal formations (Frahm & Gradstein 1990, Wolf 1993).

Epiphyte diversity in tropical rain forests was a subject of discussion at the "Second International ESF-Workshop on Tropical Canopy Research" at Schloß Reisensburg (Günzburg), Germany, 27-30 July 1995, organized by Prof. Dr. Gerhard Gottsberger and his collaborators from the University of Ulm (Lücking *et al.* 1995). In the course of the discussions it was felt that there was a need for a clarifying of the methodology of epiphyte sampling in rain forests, especially the size and quality of representative samples and plots. Several specialists participating in the workshop agreed to write a small paper on the subject, each paper dealing with a different group of epiphytes and providing recommendations and guidelines for sampling.

The results are presented here in five papers. Sampling of vascular epiphytes is discussed by Peter Hietz and Jan H. D. Wolf, corticolous bryophytes by S. Rob Gradstein, corticolous lichens by Harrie J. M. Sipman, and foliicolous bryophytes and lichens by Robert & Andrea Lücking. The series is rounded off by a note on epiphyte sampling in a three-dimensional framework by Hans F. M. Vester & Eric Gardette. The manuscripts have been put together and prepared for publication by the author.

It is hoped that these papers will be a helpful introduction to the sampling of the epiphytic diversity of rain forests, and may serve as a useful contribution towards standardization of collecting methods for biodiversity assessments of these forests.

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2. VASCULAR EPIPHYTES

Peter Hietz & Jan H. D. Wolf

Key words: Vascular epiphytes, tropical rain forest, canopy, epiphyte sampling, species diversity.

INTRODUCTION

Although, in contrast to cryptogams, many vascular epiphytes may be spotted and identified from some distance, an inventory based merely on observations from the ground will almost always be incomplete and biased since many small species growing in the upper canopy will remain undetected. Tree climbing is therefore unavoidable, unless trees freshly logged for other purposes are available. Also, simply collecting as many different species as possible in an area without any systematic sampling is unsatisfactory, as no information is obtained on how biased and complete the sampling is.

The aim of this paper is to provide a brief discussion of the difficulties encountered when sampling for vascular epiphyte diversity and to suggest some methodological approaches. Inventories typically require information on 1) species composition, 2) species abundance, 3) seasonal variation.

GUIDELINES FOR SAMPLING

Species composition. Positioning small plots on tree stems and branches has proved to be a useful approach for inventorying epiphytic cryptogams. As vascular epiphytes often grow more sparsely, are irregularly distributed, and only one of a few individuals may be found on a tree, many species will escape registration even with a high number of plots on a tree.

To ensure a representative sample whole trees should be sampled, or, where the tree size does not permit this, at least the stem and a number of large branch systems. As the tree size and therefore the sample area is variable, and was often found to correlate with the number of epiphyte species it carried (Johansson 1974, Hietz & Hietz-Seifert 1995), some measure of tree size (e.g., diameter) should be recorded for all trees sampled. The species of the host tree mostly has rather little effect on the number or composition of the epiphytes on it. Host species may be of importance in the case of species offering specific or uncommon substrates like very rough bark, persistent leaf bases or thick horizontal branches accumulating large quantities of detritus. Generally, trees sampled should be representative for the forest inventoried (unless the effect of certain tree species is of key interest). Where a host species appears to show a distinct epiphyte community a number of trees of this species may be sampled as well.

The position of the trees sampled may have considerable influence on the results and should be carefully considered depending on the purpose of the study. The composition and abundance of epiphytes in a forest may change significantly over a few 100 m or less, even when the forest structure appears homogeneous. Sampling all trees (of a given minimum size, e.g., > 10 cm DBH) within a compact, more or less square-shaped plot is the best way to ensure a homogeneous forest section.

On the other hand, epiphytes often show a clustered distribution and a species may be quite common on a group of trees just outside the plot but absent within. A sample transect will be more likely to encounter species with such patchy distribution than a compact plot. Generally, the number of species found in a 100 x 4 m plot is likely to be higher than in a 20 x 20 m plot due to patchy distribution of some species and small-scale changes of the environment. Alternatively, if a larger area is to be inventoried, which may be of heterogeneous forest type, substrate and climate, a number of single trees, distributed regularly or randomly, can be sampled.

The sample size is crucial for the number of species found. Sugden & Robins (1979) sampled 98 and 100 m^2 plots, Bøgh (1992) a 175 m^2 plot, Gentry & Dodson (1987) 10 2 x 50 m plots per site, and Hietz & Hietz-Seifert (1995 and unpublished) 500-1500 m^2 plots. Sugden & Robins (1979) admit that their plot size was probably too small to include nearly all species present, and so also may be Bøgh's. Species-area curves, or the number of species plotted against the number of trees sampled, are easily obtained in the field and should be consulted to obtain the minimum sample size needed. In a variety of Mexican forests a satisfactory levelling-off of the curves was obtained in plots between 500 and 1500 m^2 (Fig. 1).

Species abundance. Various measures like cover (ter Steege & Cornelissen 1989, Wolf 1993), biomass (van Leerdam *et al.* 1990, Ingram & Nadkarni 1993, Hietz & Hietz-Seifert 1995) or the number of individuals (Sugden & Robins 1979, Gentry & Dodson 1987, Zimmerman & Olmsted 1992) have been used to quantify epiphytes. A parameter automatically collected when recording all species on a number of trees is the percentage of trees on which a species was found. This gives a useful picture of the abundance or rarity of individual species.

The number of individual epiphytes is often difficult to estimate due to the clonal growth habit of many species. In addition, it is not clear whether small seedlings should be included, since they have a high mortality rate. Cover measurements may be problematical when the plants have larger diameters than the branches on which they are growing. Tank bromeliads, for example, need only small adhesion points relative to the size of the plants. A three-dimensional approach, expressing abundance as the volume of epiphytes related to the volume of the tree crown, may be a good solution (see Vester & Garette, chapter 6).

Epiphyte volume should be correlated with epiphyte biomass and some of the more recent studies have indeed attempted to estimate epiphytic biomass (e.g., Ingram & Nadkarni 1993). The estimation of epiphytic biomass is intuitively attractive because many of the earlier mentioned problems are avoided, and is very important for studies concerning energy budgets, nutrient fluxes, etc. The approach of Hietz & Hietz-Seifert (1995), who classified epiphytic

species and growth forms in size classes for the purpose of biomass estimations, is of particular interest as the necessity of massive destructive sampling is avoided.

Seasonal variation. In tropical areas with a pronounced dry season care should be taken not to overlook epiphyte species that shed their leaves during dry periods. Biomass values may be underestimated at the beginning of the rainy season, when leaves of deciduous species are immature. Repeat sampling may be necessary during the year.

Identification. Identifying all species found, or at least being able to distinguish between different species, is essential for inventories. The help of specialists is often required and for this purpose it is important to make field-notes on the growth habit and morphology of the living plants. For orchids it is recommended that flowers be collected in alcohol.

Infertile epiphytes may be difficult or impossible to determine. Where this is the case, only fertile plants may be counted; however, when sterile material can be identified it should certainly be done. Species found only as juveniles may be present because of the influx of seeds from surrounding different vegetation, but also because they are rare and reproduce slowly. Recording fertile and infertile species may thus provide important clues as to the origin and maintenance of a location's diversity.

The best approach to the identification of sterile material is cultivation. Fortunately, many epiphytic plants can be easily removed from the bark substrate and transplanted into a growth facility. Survival rate of such transplants is usually high when the plants

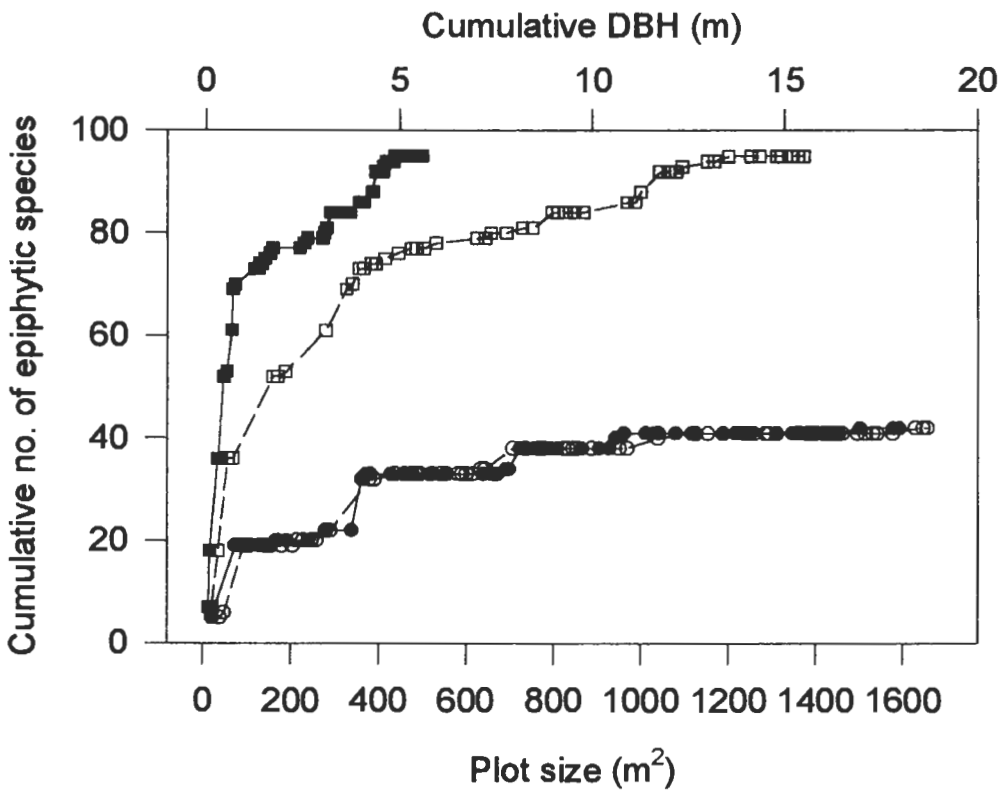

FIG. 1. Species-area curves (closed symbols) and the cumulative number of species plotted against the cumulative diameter of all trees sampled (open symbols) of a humid montane cloud forest (squares) and a humid lowland forest (circles) in Mexico (Hietz & Hietz-Seifert, unpublished). The two forests shown represent the extreme cases of a steep and a shallow rise of the species-area curves for vascular epiphytes found in a number of Mexican forests.

are protected against desiccation. Problems may arise when plants are transplanted from high to low elevation (1000 m or more elevational difference). Plants transferred from lowland to mountain areas have a better chance of survival.

Flowering often occurs within a few months of transplantation, but may take a year for a species with a well-defined flowering season, and several years in the case of juveniles or damaged individuals. When fieldwork does not extend over a period of several months, the collaboration of a local field-station may be sought for the maintenance of the living collection over a longer period of time.

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3. CORTICOLOUS BRYOPHYTES

S. Rob Gradstein

Key words: Epiphytic bryophytes, tropical rain forest, canopy, epiphyte sampling, species diversity.

INTRODUCTION

Bryophytes (mosses, hepatics) often have rather narrow ecological ranges and may occur in very specific habitats. Owing to the sensitivity to water loss of these rootless plants and their often relatively slow growth, these organisms may be good indicators of environmental conditions and microclimate. Bryophytes do not have a protective cuticle like flowering plants, and this allows the free entrance of solutions and gases to most of the living cells of the plant. They can also absorb minerals through the leaves and accumulate large amounts of heavy metals. These characteristics make bryophytes quite sensitive to changes in the environment and are reasons for their usefulness as bio-indicators of habitat quality.

Most of the bryophytes of the rain forest are corticolous epiphytes, inhabiting the bark of trees, lianas, shrubs, saplings, etc. As argued by Wolf (1995), the study of epiphytic bryophytes of tropical forests has several advantages compared with that of vascular epiphytes:

1. Species density of epiphytic bryophytes is usually very high and minimum areas thus relatively small. Complete sampling of 4-5 trees may yield most species of the local flora.
2. Sample plots can be small (a few square decimeters), due to the small size of the plants.
3. Sterile plants can usually be identified to species.
4. Geographical ranges of species are usually very large, allowing for comparisons of species diversity between distant areas.

In spite of these research advantages, our understanding of the species richness of the bryophyte flora of tropical forests is still very poor. To some extent, this ignorance is due to incomplete taxonomic know-

ledge and difficulties with identification. Another important reason is the fact that most studies have been done at ground level in the forest understory, neglecting the canopy. Recent studies indicate that the rain forest canopy harbors a much richer flora than the forest understory. In a lowland rain forest of Guyana, Cornelissen & Gradstein (1990) found that about 50% of the corticolous bryophyte species were restricted to the tree crowns, while 14% were exclusive to the understory. It is evident, therefore, that an assessment of bryophyte diversity should include sampling of the canopy flora. This can be achieved either by tree climbing (see below) or by sampling of felled trees. In the latter case, it is important that trees have been freshly cut or have been lying on the ground for no more than a few days. Older felled trees are less suitable for complete inventories since many small species may be missed due to the rapid desiccation and decay of the canopy branches of the fallen tree.

How many species are to be expected? One of the most complete bryophyte inventories in lowland rain forest is the study by Montfoort & Ek (1990) in French Guyana. Using ropes for access into the canopy, 28 mature standing trees belonging to 22 species were sampled, from the bases of the trunks up to the highest canopy twigs. In total, 154 species of bryophytes were identified (66 mosses, 88 hepatics). Species density was very high and 4-5 trees yielded about 75% of the total number of bryophyte species gathered. By comparison, lichens were much more sparsely distributed (Fig. 1).

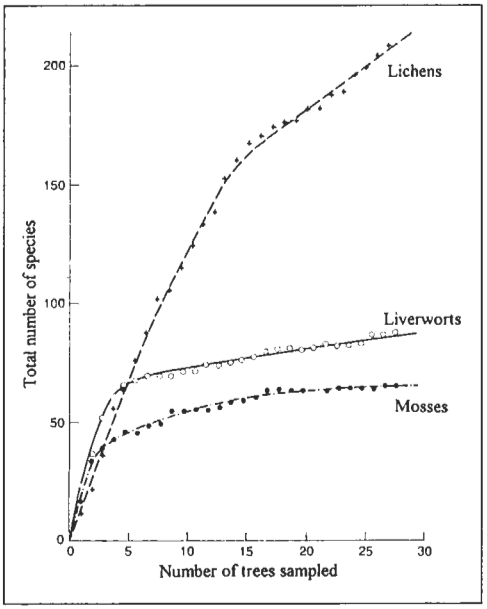

FIG. 1. Species-area curve for epiphytic bryophytes (mosses, liverworts) and corticolous lichens in a lowland rain forest of French Guiana (after Montfoort & Ek, in Gradstein 1992).

A drier type of lowland rain forest inventoried by Cornelissen & ter Steege (1989) in Guyana, using the same sampling technique, yielded about half the number of species (26 mosses, 53 hepatics). The lower figure is probably explained by the lower humidity of the forest and by the fact that foliicolous bryophytes were not taken into account; moreover, fewer host trees (11), belonging to only 2 different species, were inventoried.

It is generally assumed that montane rain forests are much richer in epiphytic bryophyte species than lowland forests. A recent whole-tree inventory along an elevational gradient between 1500 and 3500 m in montane forests of Colombia (Wolf 1993), however, shows that this may be a myth. About 100 species on average (minimally 55, maximally 140) were obtained on sets of 4 trees sampled at different elevations. Minimum area analysis indicated that the sample size (4 trees) was representative. A random inventory of several hectares of montane cloud forest (1500 m) in Costa Rica, involving sampling of tree crowns as well as understory, yielded an average of 88 species per ha (minimally 75, maximally 117), hence little more than in Guyanan lowland forest (Gradstein *et al.* in prep.).

GUIDELINES FOR SAMPLING

To allow comparison of data on bryophyte diversity, it is very important that sampling methods are uniform. It should be emphasized that for the purpose of diversity assessment, complete inventories of small areas are much more useful than incomplete inventories of large areas.

Within-forest diversity. A first impression of corticolous bryophyte diversity in the forest understory may be obtained by random inventorying of bark substrates (trunks, lianas, shrubs, saplings) near ground level, in square plots of minimally 25 x 25 m and maximally 100 x 100 m (Frahm 1994), or in

elongated plots of, e.g., 10 x 100 m. Although previous studies usually employed square plots, elongated plots may be advantageous as redundance in vegetation structure is lower and distances between trees larger (see Sipman, chapter 4, and Lücking & Lücking, chapter 5). It is recommended that the minimum area of the diversity be checked by means of a species-area curve. Plots of 1 ha and larger may have more species than 25 x 25 m plots but may be ecologically more heterogeneous and therefore less useful.

To obtain a representative sample of the entire corticulous bryophyte diversity of the forest, whole trees should be inventoried. As for vascular epiphytes and lichens, it is important to select trees that are not too close to each other and that differ in roughness of bark, possession of buttresses, etc. (see Hietz & Wolf, chapter 2, and Sipman, chapter 4). Full sampling of 4-5 mature standing (or freshly felled) trees selected on the basis of the above criteria may be sufficient to obtain a representative sample (Fig. 1).

Trees may be climbed using the standard rope techniques described by Perry and others (see ter Steege & Cornelissen 1988). Trees may be subdivided into 5-6 height zones following Johansson (1974) and Longman & Jeník (1987). Within each height zone, one or several small plots of a few to maximally 20 square decimeters are sampled. The total number of plots should be sufficient to allow for statistical analysis of species composition and abundance.

A rectangular plot shape may be used to fit the small branches. Sampling of the outer canopy branches, which are too fragile to climb, may be achieved by sawing off a canopy branch, lowering it carefully by means of ropes and studying it on the ground (ter Steege & Cornelissen 1988).

Species diversity may conveniently be calculated by means of a presence-absence analysis of the plot data. Measurement of percentage cover of species, undertaken by some recent authors, is awkward and usually unnecessary for the purpose of determining diversity. When a sufficiently large number of plots is sampled, presence-absence of species should be adequate.

It is strongly recommended that each species is collected in a separate bag, in order to speed up the identification process.

Diversity along elevational gradients. For studies of diversity along elevational gradients, it is recommended that sites at 200 m elevational intervals be inventoried. This approach has been employed in almost all recent studies (e.g., van Reenen & Gradstein 1983, Wolf 1993, Frahm 1994). Sampling may done by inventorying forest plots of minimally 25 x 25 m as described above, or by analysis of 4-5 whole trees.

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4. CORTICOLOUS LICHENS

Harrie J. M. Sipman

Key words: Epiphytic lichens, tropical rain forest, epiphyte sampling, species diversity.

INTRODUCTION

The majority of the corticolous lichens of tropical rain forests are crustose lichens, forming a thin layer over the bark or even occurring inside the outermost bark cells. While the bare bark is usually grey, these crustose lichens form often greenish, whitish or yellowish spots, measuring about 1-20 cm in diameter. Crustose lichens may often cover the entire surface of the tree and normally cannot be collected unless the supporting piece of park is cut off. More conspicuous and better known are the foliose and fruticose lichens which are more loosely attached to the substrate, may become several decimeters long, and can easily be collected without substrate. Foliose and fruticose lichens are usually referred to as *macrolichens*, while the less conspicuous crustose lichens are called *microlichens*.

How many species are to be expected? Very little information is available on the number of lichen species in tropical forests. Montfoort & Ek (1990) found 209 species on 28 trees (belonging to different species) in a lowland rain forest in French Guiana, including 12-55 species per tree. They indicated that the actual number of species existing in the forest was probably higher. Cornelissen & ter Steege (1989) reported 34 macrolichen species from 11 Eperua trees in a lowland rain forest in Guyana (macrolichens constitute a minority of the lichen flora of lowland rain forests) and Wolf (1993) found 140 macrolichen taxa in montane rain forests of Colombia.

How complete can/should an inventory be? Lichen diversity much depends on the number of available "microhabitats" (= larger or smaller sites with identical growth conditions). These may cover considerable parts of tree trunks or branches, or be restricted to a few square cm. Microhabitats may differ considerably in humidity and light exposure, e.g., dry, overhanging parts of tree trunks, wet tree bases, etc. They may be very localized and unpredictable, e.g., a small patch of callus on a canopy branch may have a lichen vegetation very different from the surrounding normal bark. Any microhabitat is likely to have at least some specialized lichen species.

Paying attention to the available microhabitats is the most suitable and effective approach to complete lichen inventories. Some lichen species can easily be recognized in the field by their colour or other conspicuous morphological features, but others can be recognized only by microscopic and/or chemical analysis. Knowledge of potential microhabitats of species can speed up the inventory.

In view of the difficulties of access, complete inventories of standing trees will be almost impossible. A representative inventory, treating the regular microhabitats on the commoner trees and using a limited number of sample plots, may therefore be advisable.

GUIDELINES FOR SAMPLING

Selection of trees. One method of facilitating tree selection is to define a study plot and restrict the selection to trees within this plot. The most suitable geometry for a plot seems to be an elongated transect, as it is more likely to include a good representation of the natural heterogeneity of the forest, e.g., gaps, streamlets. An area of 100 x 10 m was used in French Guiana (Montfoort & Ek 1990).

It is not known how many trees should be examined for a reasonably complete list of epiphytic lichens. Sipman (in prep.) observed in Guyana that two adjacent *Licania densiflora* trees shared only 50% of their total foliicolous lichen flora. If this figure is applicable to corticolous lichens, it would be worth investigating more than one tree per species. Corticolous lichens seem to be much more sparsely distributed than corticolous bryophytes. A minimum area curve for corticolous lichens based on an inventory of 28 trees in a lowland rain forest of French Guiana did not reach saturation, whereas for bryophytes near-saturation was reached with 4-5 trees (see also Gradstein, chapter 3: Fig. 1).

Trees in close proximity tend to have a similar lichen flora. Therefore, it is recommended that trees standing well apart be selected.

Tree age seems to be an important factor. The canopy flora of young trees, growing in the shaded and humid lower levels of the forest, is very different

from that of large, emergent trees. Old trunks tend to have a specialized lichen flora.

Bark structure is another important habitat factor for epiphytic lichens. Therefore tree species with different types of bark should be selected. Bark may be smooth, and with a very thin cork layer, or rough and with a thick, more or less spongy cork layer. Trees with flaking bark tend to be poor in epiphytic lichens. In view of the intricate relationship between bark structure and epiphyte flora, it is advisable to investigate as many different tree species as possible, even when their bark structure seems to be identical.

Since many lichen species are rather unspecific, it is usually unnecessary to investigate all tree species. Additional lichen species may be found by searching for special microhabitats. They may be found on trees with an unusual type of bark, on slanting or irregularly formed trees with overhanging sides, and on dead trunks. Isolated trees near houses, fields, parking lots, etc. also have a different lichen flora.

Tree analysis. Instead of inspecting the whole tree, plots of maximally 0.5-1 m may be investigated in each of the main elevational levels of the tree (Johansson 1974, Longman & Jeník 1987): tree base, lower trunk, upper trunk, inner canopy, middle canopy, outer canopy. Cornelissen & ter Steege (1989) used plots of 0.4-35 dm^2, based on a minimal area analysis. When the outer canopy is difficult to reach, branches should be cut and sampled on the ground (ter Steege & Cornelissen 1988). These branches should be lowered gently to prevent damage and loss of epiphyte species. Canopy inventory cannot be done on the ground by analysis of old, fallen branches. Most fallen branches are from the lower part of the canopy. They are often overgrown with mould and carry a modified lichen flora characteristic of such conditions. Most canopy lichens die quickly in undergrowth conditions, certainly within a few weeks. The same is probably true for bryophytes (Gradstein, chapter 3).

For each species absence/presence should be noted; detailed abundance estimates may be omitted. Since the taxonomy of tropical lichens is very incomplete and different species may look very similar, extensive sampling for laboratory investigations will be necessary.

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5. FOLIICOLOUS BRYOPHYTES AND LICHENS

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INTRODUCTION

Leaves overgrown by foliicolous bryophytes and lichens are highly attractive to the casual collector and therefore often gathered by non-specialists. Such collections are usually not very representative and data important for the taxonomist or ecologist are often missing. The following guidelines should give both the casual collector and the taxonomist information as to how to make representative collections of foliicolous bryophytes and lichens, and how to gather supplementary data that may serve to evaluate the local diversity of foliicolous cryptogams. It appears that a rather complete inventory of a forest stand can be made by collecting a maximum of 250 leaves from different phorophytes and in different microhabitats (= microsites, cf. Lücking 1995a).

PATTERNS OF DIVERSITY DISTRIBUTION

The aim of an inventory is a species list which might be accompanied by abundance data. To answer questions about the number of leaves and where to collect them in order to obtain as complete as possible data on species diversity, one must understand the pattern of foliicolous cryptogam diversity within the forest stand. The following observations are based primarily on studies by the authors in Costa Rica (R. Lücking 1994, 1995a, 1995b; A. Lücking 1995).

1. Species-area curves based on leaves. A comparison of species-area curves for foliicolous lichens from a low- and a high-diversity area in Costa Rica, constructed by random arrangement of leaves (Fig. 1),

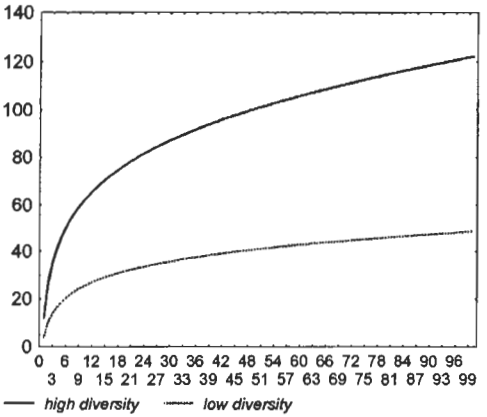

FIG. 1. Comparison of species-area curves of foliicolous lichens based on random arrangement of leaves in a low-diversity area (Costa Rica, Rincón, Centro Boscosa, 50 m alt., exposed *Citrus* stand: 48 spp.) and a high-diversity area (Costa Rica, Braulio Carillo National Park, Botarrama trail, 480 m alt., primary forest: 177 spp.).

shows that in a low-diversity area (48 species), 50% of the species are found on 9 leaves, whereas 62 leaves are necessary to obtain 90% of the total diversity. In a high-diversity area (177 species), 50% of the species occur on 31 leaves, and 90% on 360 leaves. The number of leaves to be collected to obtain a representative sample thus increases with total diversity in a non-linear relationship. Apparently, collecting in high-diversity areas is much more time-consuming. In the present case, to obtain 90% of the diversity six times more leaves would have to be collected in the high-diversity area than in the low-diversity area, even though total diversity in the high-diversity area is only 3.7 times higher.

2. Species-area curves based on phorophytes. Species-area curves constructed by random arrangement of the sampled phorophytes have been found to be steeper in lichens than in bryophytes, indicating that in bryophytes saturation of species diversity is reached earlier (Montfoort & Ek 1990, R. Lücking 1994, A. Lücking 1995). A similar difference in minimum area has been found between corticolous bryophytes and lichens (see Gradstein, chapter 3). In foliicolous lichens, 50% of the total diversity (177 species) was reached by sampling 15 phorophytes (of 321), and 90% by 110 phorophytes. In bryophytes, 50% (79 species) was reached by sampling 4 phorophytes (of 68), and 90% by 33. This indicates that for obtaining a representative sample of follicolous bryophytes fewer leaves need to be collected than for lichens.

3. Species-area curves based on fixed arrangement of phorophytes. When constructing a species-area curve, the sampled phorophytes are not arranged randomly but by decreasing order of species richness (beginning with the species-richest phorophyte). In this case, 50% of the diversity of foliicolous lichens was obtained from 2 (instead of 15) phorophytes and 90% from 13 (instead of 110). In a study of 321 phorophytes in a forest stand (Lücking 1994), 28 phorophytes (or less than 10% of the total number sampled) yielded 100% of the foliicolous lichen diversity in the area, whereas the remaining 293 phorophytes carried redundant information as regards species richness. The problem is that it is virtually impossible to detect in the field the phorophytes that carry the maximum diversity. Strategies towards effective selection of phorophytes are discussed below.

4. Distribution of diversity on single branches. On single branches, leaves with the highest diversity do not carry more than 40-70% of the total diversity of the branch (Table 1). The possible reasons for this are (a) succession, i.e. species change along the branch, or (b) stochastic influences on species composition, due to the fact that one leaf is usually too small to carty the complete branch diversity. In truly foliicolous bryophytes and lichens there is usually no real succession along branches since early colonizers are not

replaced but persist on mature leaves. There is, however, often a decrease in individuals of early colonizers with leaf age and some early colonizers may disappear on old leaves (e.g., Winkler 1967, Pócs 1978). On the other hand, species changes occur when facultatively foliicolous taxa start colonization. This occurs also on very old leaves. It should be pointed out that the contribution of facultatively follicolous species to total foliicolous diversity is much greater in bryophytes than in lichens, since many corticolous bryophytes, especially members of the family Lejeuneaceae (Hepaticae), are able to grow on living leaves under certain conditions (Montfoort & Ek 1990, Gradstein 1994, A. Lücking 1995).

Another type of succession can be found on branches of light-gap phorophytes, where species composition on old leaves is somewhat different from that on young leaves, due to the fact that old leaves are usually more shaded. This means that for the purpose of collecting as many species as possible it is usually unnecessary to sample very young leaves; very old leaves, however, may contribute substantially to total diversity. Furthermore, it appears that a higher number of species is obtained by collecting a few leaves per branch from a higher number of branches, instead of collecting many leaves from a single branch (R. Lücking 1994).

5. Recognition of high-diversity leaves in the field. To detect high-diversity leaves in the field is difficult since high diversity is not necessarily correlated with high area cover. In our experience leaves exhibiting a highly diverse foliicolous community, with clear borders between individual plants, usually have the highest diversity. Leaves that are completely covered with a more or less homogeneous foliicolous community, however, are usually less diverse in terms of foliicolous species richness.

6. Microclimatic preferences in the shrub layer. In foliicolous cryptogams, species composition mostly depends on microclimatic parameters. Three microhabitats with distinctive species composition can be distinguished in the shrub layer: the shaded understory, the margins of light gaps and the centers of light gaps, the latter corresponding to the forest margin (R. Lücking 1994, 1995a, 1995b; A. Lücking 1995). Diversity is usually highest along the margins of natural light gaps and decreases towards the shaded understory, the centers of light gaps, and the canopy.

7. Differences between the shrub layer and the canopy. Several recent investigations have shown that there is a gradual change in species composition from the shrub layer to the canopy (Montfoort & Ek 1990, R. Lücking 1994, 1995a, 1995b; A. Lücking in prep., Sipman in prep.). Besides the shrub layer, with its gradient from the shaded understory towards light gaps, distinctive foliicolous communities can usually be found in the lower canopy (trees partly shaded by other trees) and in the upper canopy (fully exposed crown periphery).

8. Phorophyte preferences. Phorophytes usually differ in species diversity, but only partly in species composition. Most of the diversity is usually found on phorophytes with "normal" rain forest leaves ("Normalblatt" according to Vareschi 1980) and with leaves or leaflets of the palm type (R. Lücking 1994, A. Lücking 1995). A single phorophyte species can cover up to 73 % of the overall foliicolous lichen diversity

TABLE 1. Comparison of branch and leaf diversity of 13 plants of *Ocotea atirrensis* (us = shaded understory, lg = light gap). Note that the percentage of overall diversity on the species-richest leaf is lower in light-gap plants (except in no. 9, which occurred in a humid gap) than in understory plants.

Phorophyte:	1	2	3	4	5	6	7	8	9	10	11	12	13
Microsite:	us	us	us	us	us	us	us	lg	lg	lg	lg	lg	lg
Leaf number:	8	8	6	9	10	7	5	25	7	16	15	9	13
Species number on whole branch:	52	31	29	40	43	36	39	65	35	59	57	27	64
Species number on richest leaf:	28	18	22	22	30	25	28	38	27	27	26	13	27
Percentage [%]	54	58	76	55	70	69	72	58	77	46	46	48	42

(R. Lücking 1994). Distinctive foliicolous lichen communities can be found on thick, leathery leaves of the aroid type (*Dieffenbachia, Monstera, Philodendron, Anthurium*) and on fern fronds (e. g., tree ferns), especially those with pinnae of the "Regenwaldblatt" type (Vareschi 1980). In bryophytes, which often show more pronounced phorophyte preferences than lichens, distinctive communities are often present on hairy leaves, on fern fronds (e. g., tree ferns) and on filmy ferns (Hymenophyllaceae; see Pócs 1978, A. Lücking 1995).

9. Spatial differences between microhabitats in the shrub layer. In foliicolous lichens, it was found that in the shaded understory nearby phorophytes belonging to different species may have a more distinctive species composition than different phorophyte species growing closely together in light gaps. The species of phorophyte thus seems to be a more important factor in the understory than in light gaps. On the other hand, understory microhabitats are usually more similar to each other than light gap microhabitats are to each other (R. Lücking 1994).

10. The problem of rare species. When sampling for a complete list of species, rare species are the most troublesome to collect. They may account for up to 25% of the total diversity (R. Lücking 1994). In our experience, the number of rare species detected usually increases with area, and leaves with rare species are often homogeneously distributed within a stand. Some species that are rare in one forest stand may be common elsewhere. In the case of the foliicolous lichens of the high-diversity area in Costa Rica, the 34 rarest species - present on only one phorophyte each - were distributed among 23 different phorophytes, in 9 different microhabitats (of 16 in total; R. Lücking 1994). The most effective strategy to detect as many rare species as possible seems to be to collect at sample sites as far apart as possible.

GUIDELINES FOR SAMPLING

The following guidelines are based on the above considerations and concern questions such as 1) where to collect, 2) which phorophytes and microhabitats to consider, 3) how many leaves to sample.

1. Evaluation of the collection site. In view of possible harmful effects of sampling on the local foliicolous flora, it is important to determine whether the collection site is a low- or a high-diversity area (or is intermediate) before starting to collect. Low-diversity areas are usually recognized by the aggregate distribution of epiphylls, that means that epiphylls are restricted to particular microhabitats, e.g., along creeks, on certain phorophyte species, etc. In high-diversity areas, epiphylls may be present almost everywhere and easily detected by the collector. In such rich areas, collecting of 250 leaves should scarcely harm the local foliicolous cryptogam flora. In low-diversity areas, where the number of leaves carrying a well-developed foliicolous flora is much less, damage may be reduced by sampling at greater distances between microhabitats and by reducing the number of microhabitats.

2. Size of the collecting site. Collections can be made within a square plot or along a transect. Since transects have lower redundance in vegetation structure and in possible gradients within the stand, and higher maximum distances, they are preferred. Transects might follow a trail within the stand or, if possible, a constructed straight line. They should have a length of 500-1000 m and should be in a homogeneous forest stand.

3. Selection of phorophyte species. Collections should be taken from different leaf types, preferably at least five different types from more than one host species each. Thus, leaves of the palm type should be collected from different palm species. Possible leaf types include (1) "normal" rain forest leaf (dicotyledon), (2) palm leaf, (3) aroid leaf, (4) hairy leaf, (5) fern frond with linear leaflets (*Blechnum* type), (6) fern frond with highly divided leaves, (7) filmy fern leaf (Hymenophyllaceae), (8) small sclerophyllous leaf (especially in montane forests), (9) large leaf (especially in the canopy, e. g., *Cecropia* type).

4. Selection of microhabitats. Concerning the shrub layer, it is recommended that a total of about 17 microhabitats be selected: 3 in the shaded understory, 7 in the margin of light gaps, and 7 in the center of light gaps. These microhabitats should be as distant from each other as possible. As to the canopy, 10 microhabitats - 5 in the lower canopy and 5 in the upper canopy - should be selected where collecting of leaves is possible (by climbing, by sampling freshly fallen trees, or by sampling fallen leaves which can be assigned to their place of origin in the canopy).

5. Selection of phorophytes per microhabitat. In the shaded understory, at least two phorophytes (be-

longing to different species, if possible) with high diversity should be selected for each leaf type. At the margins and the center of light gaps, it is sufficient to sample one phorophyte, with high diversity, for each leaf type. For both the lower and the upper canopy, one phorophyte per leaf type, each at the greatest possible distance from the others, should be sampled.

6. Collection of leaves per phorophyte. In the shrub layer, two leaves should be collected from each phorophyte, one with high diversity in the middle of the branch and one (possibly with colonization of facultatively foliicolous species) at the end of the branch. In the canopy, 5 leaves with as much diversity as possible should be sampled from each phorophyte. In divided leaves, such as palms or ferns, or in large leaves, it is recommended that 3 leaflets per leaf or separate parts of the leaf be collected, each distant from the other. The total number of leaves collected in this procedure is 250.

7. Preparation of the material. Leaves covered with epiphylls should be carefully prepared in a way similar to the drying of higher plants. That means leaves should be pressed between newspaper and, if possible, dried in a modest way (bulb dryer, etc.). If leaves are collected during rain or are still wet, their surface should be dried before pressing, e. g. by exposing them for some minutes to open air.

8. Measuring abundance. Various measures of abundance can be used, including biomass, area cover, number of individuals, or number of leaves or phorophytes on which a species is present. Investigations on foliicolous lichens have shown that the use of different measures does not necessarily lead to different results with respect to the arrangement of the species according to their frequency. However, the results may differ as to relarive dominance of common species, which are particularly pronounced in measures of biomass or area cover, less so when number of individuals, number of leaves or number of phorophytes on which a species is present are measured. In most cases, the number of phorophytes or leaves on which a species is present is sufficient as a measure of abundance, but then one must consider that rare species are more pronounced. Measures of area cover or biomass are much more time-consuming and can usually be applied only to a limited number of collections, reducing the completeness of the inventory.

In bryophytes, area cover is difficult to establish since species often grow intermingled and the size of individual species may vary considerably. The easiest way to measure abundance is to count or estimate the number of individuals (e. g., Pócs 1978) and to record their sizes in relation to the average size of the species. The developmental state of individuals should also be recorded if possible (sterile, fertile, etc.). The data can be combined into a "representation index" (A. Lücking 1995).

9. Comparison of diversity data. When comparing diversity data from different sites, it is important that sample sizes are similar. Diversity of cryptogams on a single leaf seems to be a good indicator for the overall diversity of a forest stand. Since leaves are of different sizes, it seems appropriate to agree on a standardized size for diversity evaluation, similar to the 1 ha plots used for the determination of tree diversity. Most leaves have an area of between 20 and 180 cm^2 (mesophyll leaf size according to Vareschi 1980); 100 cm^2 can therefore be used as average area for diversity calculations. This area might be calculated exactly based on the leaf type, or a standard shape might be used. Since most leaves are elongate, it is proposed to use an ellipsoid grid of 16 (or 20) cm length and 8 (or 6.4) cm width, covering an area of 100.5 cm^2. This grid should be placed on the leaf in such a manner as to cover the leaf asymmetrically, preferably with one edge of the grid touching the margin of the leaf.

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6. EPIPHYTE SAMPLING IN A THREE-DIMENSIONAL FRAMEWORK

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Key words: Epiphytes, tropical rain forest, 3-dimensional mapping.

The tree is a dynamic substrate, spreading itself in three dimensions (within the fourth dimension of time). Epiphytes occur within this 3-dimensional framework on the trunk, branches and leaves of the tree, on substrates with or without soil formation. The occurrence of epiphytes at a site on the tree is determined to various degrees by environmental factors and by the ability of the epiphyte to germinate, grow, reproduce and survive in that particular site. This site dependence may be weak or strong.

Epiphyte ecologists are interested in knowing which site factors determine the occurrence of the epiphytes within the tree. Also, they need adequate measures to compare the spatial distribution of epiphyte assemblages on the trees. In order to arrive at a proper understanding, it is important to register the 3-dimensional position of the epiphytes within the tree. How can this be done?

One of the problems faced is scale; another, related problem is the question as to what is a community. Concerning scale, it is evident that epiphytes can be of very different sizes, from large, tree-like hemi-epiphytes to microscopically small lichens. They may co-occur on the same branch but the direct environment of a moss patch is different from that of a full-grown hemi-epiphyte. Therefore, these scales must be treated separately, conserving however their hierarchical structure. The presence of a hemi-epiphyte on a tree can be compared only with that of other epiphytes of the same size. In order to study the spatial distribution of these "macroepiphytes", several trees should normally be surveyed since single trees may harbor only one individual. On the other hand, one tree may be sufficient for a study of the "microepiphytes", since several similar patches of small moss or lichen species may be found on a single tree.

An epiphyte patch is an indivisible group of plants of a defined scale. It has no definite relation to trunk or branch diameter; when the same epiphyte assemblage is found on several neighboring trees, then the patch stretches over all these trees. Within the patch there may be smaller patches, which are indivisible on a smaller scale.

As the parts of the tree inhabited by epiphytes are generally spatially limited in relation to the diversity of species present in the environment, similar epiphyte patches on a branch scale can be expected to show little overlap in species composition. Each epiphyte patch may be treated as a community; this can result in the recognition of many different communities. By grouping similar patches, however, fewer communities, more diverse in composition, are created.

One of the methods used to obtain greater insight into the composition, structure and environmental relations of the epiphyte community (defined in whatever way), is 3-dimensional (3D) mapping. By this method, large individuals are mapped individually and patches of small individuals or colonies are mapped by indicating form, extension and position of the patch. The maps provide detailed spatial information that may be of importance in quantitative assessments of the biodiversity and ecology of epiphytes (Gardette, in prep.).