

# A PROTOCOL FOR RAPID AND REPRESENTATIVE SAMPLING OF VASCULAR AND NON-VASCULAR EPIPHYTE DIVERSITY OF TROPICAL RAIN FORESTS

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**ABSTRACT.** A protocol for rapid and representative sampling of vascular and non-vascular epiphytes (excluding epiphylls) is presented for one hectare of tropical rain forest, including montane forest. We estimate that the inventory and morphospecies recognition (excluding species identification) can be carried out in approximately 2 weeks by a team of six persons, three specialists (one each for vascular plants, bryophytes, and lichens) and three field assistants.

**Key words:** Cloud forest, epiphytes, minimum sample size, non-vascular epiphytes, sampling, species diversity, tropical rain forest

## INTRODUCTION

The enormous diversity of epiphytes, both vascular plants (e.g., orchids, bromeliads, aroids, ferns) and non-vascular plants (mosses, liverworts, lichens), is one of the most striking characteristics of tropical wet lowland and montane forests. This feature distinguishes these forests from most temperate forests (Catling & Lefkovich 1989, Cornelissen & ter Steege 1989, Gentry & Dodson 1987, Nadkarni et al. 2001, Nieder et al. 1999). Epiphytes play a key role in ecosystem-level interactions in tropical wet forests, especially in the processes that affect the water balance and nutrient cycles of the forest (Coxson & Nadkarni 1995). They are a major source of food and habitat for birds, mammals, amphibians, and reptiles, and offer shelter to a variety of invertebrates and microorganisms (Remsen & Parker 1984, Nadkarni & Matelson 1989).

The value of epiphytes also is exemplified by their usefulness as ecological indicators of climate and forest types (Benzing 1990, Frahm & Gradstein 1990, Nadkarni & Solano 2002). Non-vascular epiphytes and “atmospheric” vascular epiphytes are indicators of microclimate and environmental quality, as their growth forms and physiology make them sensitive to changes in the environment (Benzing 1990, Bates & Farmer 1992, Nash 1996, Shaw & Goffinet 2000). Non-vascular plants lack the protective cuticle that vascular plants have, which allows the free entrance of solutions, gases, and minerals to the living cells of the plants.

As they often live high up in the canopy, epiphytes frequently have been overlooked or understudied in rain forest studies, because of difficulties of access. These limitations have been largely overcome by the development of techniques for access into the canopy (Mitchell 1982, Lowman & Nadkarni 1995, Mitchell et al. 2002). Although many vascular epiphytes may

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be spotted and identified from some distance, inventories based solely on observations from the ground will be incomplete and biased, as many small species growing in the canopy cannot be detected from the forest floor. Unless freshly logged trees are available, inventory of the canopy must be conducted with access from tree-climbing, cranes, or balloons.

Documenting the diversity of epiphytes requires uniform, repeatable sampling methods. Haphazard collecting gives a rough impression of the species richness of a forest, but it does not provide robust data for comparing biodiversity of different habitats. Historically several methods have been used (McCune 1990, Shaw & Bergstrom 1997, Nieder & Zotz 1998), but they have not been widely accepted by the canopy research community. The need for standardized sampling of tropical epiphytes was discussed at the Second International Workshop on Tropical Canopy Research of the European Science Foundation held at Ulm in 1995. The papers that came out of that meeting (Gradstein et al. 1996) were a first step toward developing a uniform method for epiphyte sampling.

In this paper, we present a standard protocol for vascular and non-vascular epiphyte sampling in tropical wet forests, including montane forests. These methods were prepared in the framework of the Global Canopy Programme (GCP), following the recommendations of the GCP workshop held in Göttingen, Germany, on 24–25 February 2002 (Secoy 2002). The protocol is designed for Rapid and Representative Analysis of Epiphyte Diversity (RRED-analysis) within a 1-ha plot of forest. It is largely based on the research experiences of the authors in tropical America (Bolivia, Colombia, Costa Rica, Ecuador, French Guiana, Guyana, and Panama).

RRED-analysis pertains to the inventory of vascular and non-vascular epiphytes of 1 ha of homogeneous forest. It is carried out by a team of six persons, three specialists (one each for vascular plants, bryophytes, and lichens) and three field assistants. The protocol for non-vascular epiphytes focuses on corticolous, bark-inhabiting epiphytes. For sampling of epiphyllous species, see Lücking and Lücking (1996).

## METHODS

### Sampling Design and Tree Selection

Species-accumulation curves, based on the number of epiphyte species recorded against the number of trees sampled, provide information on minimum sample size (MSS) (Gradstein 1992, Wolf 1993, Hietz & Wolf 1996, Shaw & Bergstrom 1997, Annaselvam & Parthasarathy

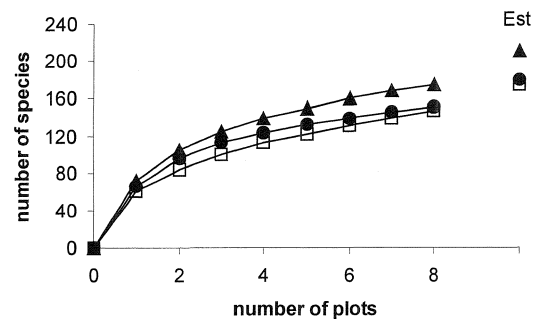


FIGURE 1. Species accumulation curves and estimated total number of species (Est) of vascular epiphytes in three 1 ha plots in a montane forest of Bolivia (after Krömer 2003), using the MMeans richness estimator (Colwell & Coddington 1995). In each hectare plot, up to eight trees were sampled, as was a 20 × 20-m plot around each sampled tree.

2001, Flores-Palacios & García-Franco 2001, Rauer & Rudolph 2001). Recent studies indicate that the MMS of vascular epiphytes is relatively small. About 80% of the total estimated number of vascular epiphyte species in 1 ha of Bolivian montane forests was tallied by sampling eight trees and a 20 × 20 m plot around each tree (Krömer 2003) (FIGURE 1). About half of the vascular epiphyte species of a 4000 km<sup>2</sup> region in Mexico was found in 0.5 ha of forest (Hietz & Hietz-Seifert 1995a); and ca. 50% of the species of the valley of Sehuencas, Bolivia, occurred in less than 0.1 ha (Ibisch 1996). Engwald (1999) recorded ca. 50% of the species of 0.1 ha of montane forest in La Carbonera, Venezuela, in 0.01 ha.

The MMS of bryophytes is significantly smaller than that of vascular plants. Sampling of 3–5 trees yielded 75–80% of total bryophyte diversity of a tropical forest stand (Gradstein 1992, 1996, Acebey et al. 2003). The MMS of lichens, however, is larger than that of bryophytes (Sipman 1996, Komposch & Hafellner 2000) and may be similar to that of vascular epiphytes.

Based on the available information on species-area relationships, we propose to sample eight mature canopy trees within a 1-ha plot of forest for RRED-analysis for vascular epiphytes and lichens, along with five trees for bryophytes. We also recommend sampling the epiphyte diversity on treelets and shrubs in a 20 × 20 m area around each selected tree (see below). The completeness of the sampling may be checked by means of species-accumulation curves and a species-richness estimator (Colwell & Coddington 1995) (FIGURES 1, 2). Herzog and his colleagues tested the accuracy of different richness estimators, including ACE, ICE, Chao1, Chao2,

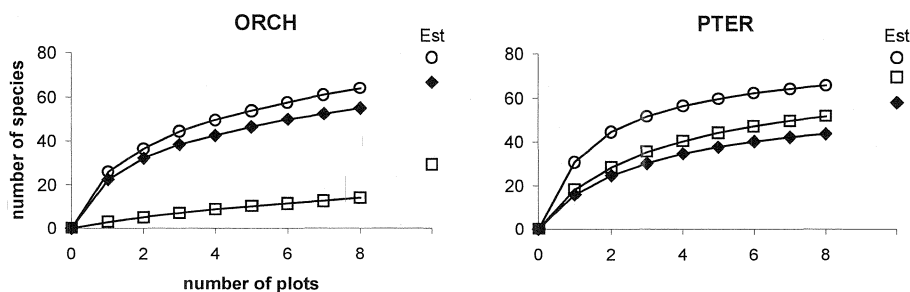


FIGURE 2. Species accumulation curves and estimated total number of species (Est) of orchids (ORCH) and ferns (PTER) on eight trees (diamond), on shrubs and treelets in eight  $20 \times 20$ -m understory plots (square), and on the trees, shrubs, and treelets taken together (circle) in a montane forest of Bolivia (after Krömer 2003).

MMMeans, and MMRuns, in a study of species richness of tropical bird communities and found that the most accurate estimation of total species richness was obtained by using the MMMeans richness estimator (for details, see Colwell 1997, Herzog et al. 2002).

Trees in close vicinity of each other tend to have a similar epiphyte flora resulting from the clumped distribution of many epiphyte species (Hietz & Hietz-Seifert 1995b, Sipman 1996, Engwald 1999, Nieder et al. 2000). Thus trees standing well apart (separated by at least 25 m) and with crowns not overlapping should be selected for species richness estimates. Trees at forest margins should be avoided because of potential microclimatic edge effects. To maximize the information on species richness, preferably the oldest or largest trees (with the largest trunks) should be selected. These trees are usually richest in epiphyte species because of their large and highly diversified crowns; they also have been available for establishment by epiphytes during the longest period of time (Hietz & Hietz-Seifert 1995a, Shaw & Bergstrom 1997, Zotz et al. 1999, Krömer 2003).

Many studies have shown that bark and canopy structure can have a strong influence on species composition of epiphytes. Trees with rough bark have epiphyte species that those with smooth bark lack (Cornelissen & ter Steege 1989, ter Steege & Cornelissen 1989). Trees with oblique canopy branches tend to collect less detritus than thick horizontal branches, which in turn may affect epiphyte community composition and abundance (Ingram & Nadkarni 1993). For these reasons, we sampled tree species that differed in these respects. Tree sampling can be achieved by visual selection and by sampling of tree species belonging to different genera or families. We recommend that not more than half of the selected trees belong to the same species or genus (Krömer 2003).

### Sampling of Trees

Representative sampling of the epiphyte diversity of tropical rain forests requires sampling of whole trees, from the base to the outer canopy. Trees may be ascended using the single rope technique (SRT) (Perry 1978). Ground-based inventory (GBI), using binoculars and sampling of fallen branches, is inadequate to assess the diversity of the epiphyte communities (Gradstein 1992, Flores-Palacios & García-Franco 2001). Using SRT, Krömer (2003) recorded more species of vascular epiphytes—including three times as many orchids—in one  $20 \times 20$  m plot of mountain forest than did Sugden and Robins (1979) in fourteen  $10 \times 10$  m plots using GBI. In a Mexican oak forest, 20% more species were found using STR than by using GBI (Flores Palacios & Garcia-Franco 2001). Sampling of the forest canopy by SRT is particularly important for assessment of orchid (FIGURE 2) and non-vascular epiphyte diversity. About 50% of the bryophyte species of the rain forest may be restricted to the canopy (Gradstein 1992, Gradstein et al. 2001b), and 60% of orchid species in Bolivian montane forest can be exclusive to the tree crowns (Krömer 2003). In a Venezuelan lowland rain forest, 87% of corticolous lichens occurred exclusively above 2 m height on the trees (Johansson zones 2–5, see below; Komposch & Hafellner 2000).

To analyze species richness, we subdivided trees into the following five vertical zones according to Johansson (1974) (FIGURE 3):

**Zone 1.** Basal part of trunk (0–2 m high);

**Zone 2.** Trunk up to the first ramification and excluding isolated branches originating on the trunk zone. Following Longman and Jeník (1987) and others (e.g., ter Steege & Cornelissen 1989, Ek et al. 1997, Engwald 1999), zone 2 is subdivided into a humid lower part of the trunk (zone 2a) and a dryer upper part (zone 2b);

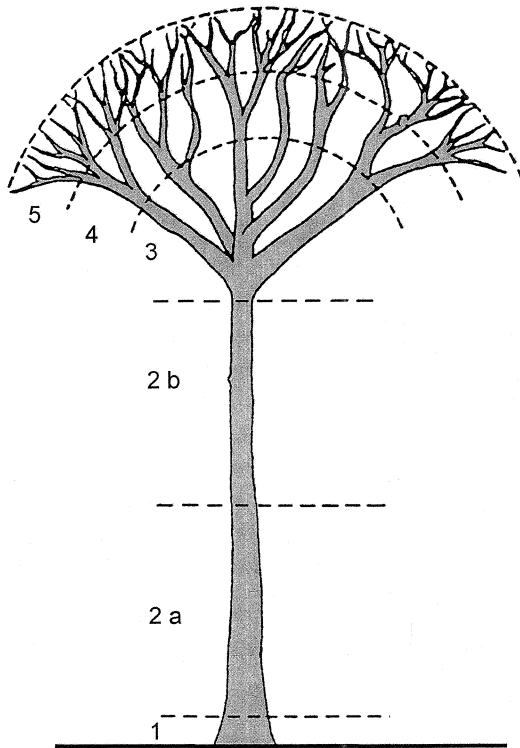


FIGURE 3. Subdivision of the tree into vertical zones after Johansson (1974) and ter Steege and Cornelissen (1989).

**Zone 3.** Basal part of the large branches, up to the second ramifications (about a third of total branch length);

**Zone 4.** Second third of branch length; and

**Zone 5.** Outer third of branch length.

These zones, used frequently in epiphyte research, are a useful approach for analysis of vertical diversification of epiphyte communities (e.g., Cornelissen & ter Steege 1989, ter Steege & Cornelissen 1989, Wolf 1993, Ibisch 1996, Ek et al. 1997, Nieder & Zotz 1998, Engwald 1999, Freiberg 1999, Krömer 2003). The scheme is based on tree structure and conspicuous differences in epiphyte community composition, although each Johansson zone may not coincide with distinguishable epiphyte communities (Nieder & Zotz 1998). The three principal communities of vascular epiphytes of the rain forest occur in zones 1–2, zone 3, and zones 4–5. These communities differ in species richness (low in 1–2, high in 3–5), biomass (low in 1–2 and 4–5, high in 3), and frequency of succulence (low in 1–3, high in 4–5) (Kelly 1985, ter Steege & Cornelissen 1989).

Species diversity of vascular epiphytes is

scored by presence-absence of species in each Johansson zone and in the understory plots. Outer canopy branches too fragile to be climbed can be cut, carefully lowered to the ground with ropes, and sampled on the ground (ter Steege & Cornelissen 1988, 1989).

Species diversity of bryophytes and lichens, because of their small size, are scored by analyzing five small plots within each Johansson zone. Plots in zones 1–3 are 30 cm × 20 cm (6 dm<sup>2</sup> total) randomly positioned at each cardinal direction (N, W, S, E). Plots in zones 4–5 are 60 cm long (total surface depending on branch diameter) positioned on the upper surface (three plots) and the lower surface (two plots) of the branch (Holz et al. 2001). Plots in zones 4–5 usually are studied from cut-off or naturally fallen branches on the ground. Each species of bryophyte and lichen is collected in a separately labelled paper bag, and studies can be conducted in the field or in the laboratory.

#### Sampling Shrubs and Treelets

The epiphyte flora on shrubs and treelets growing in the shaded understory of the forest usually differs from that of large canopy trees (Shaw & Bergstrom 1997, Gradstein et al. 2001b, Krömer 2003). About 20% of the vascular epiphyte species recorded in 1 ha of montane forest (including many species of *Peperomia* and hemiepiphytic aroids and ferns but few species of orchids and bromeliads) occurred exclusively on shrubs and treelets (Krömer 2003; FIGURE 2). Therefore, understory shrubs and treelets (< 10 m in height) within a 20 × 20 m area around each sample tree also are sampled. This area corresponds to the plot size commonly used in floristic inventories in tropical montane forests (e.g., Van Reenen & Gradstein 1983, Van der Hammen & Ruiz 1984, Kessler & Bach 1999). Vascular epiphytes on shrubs and treelets may be inventoried using collecting poles and binoculars. This approach also may be used for analysis of trees in secondary forests that are too fragile to be climbed safely (Krömer 2003).

#### Ecological Parameters

A single tree represents many different microclimates and substrates for epiphytes in “a physical mosaic” (Benzing 1995). To document the habitat of the epiphytes, researchers measure the following characteristics of the host tree: 1) tree height (using a clinometer and measuring tape); 2) tree diameter at breast height (dbh or 1.3 m above the ground) or height above buttresses, if present; and 3) general architectural form of the host tree (Hallé 1995). For non-vascular epi-

phytes, other important characteristics include 4) plot height above ground; 5) inclination, cardinal direction, and diameter of branch; 6) bark texture (smooth, rough, scaling); and 7) thickness of arboreal soil. Measurements of other physical parameters, such as light, moisture content of bark, and pH of bark, are beyond the scope of RRED-analysis.

#### Assessing Species Richness and Abundance

Species richness of epiphytes is determined by means of enumerating presence-absence. Abundance is determined by the number of trees or plots in which a species occurs. Estimation of percent cover of species is time-consuming and therefore omitted in RRED-analysis. Epiphyte volume and biomass, critical for studies of ecosystem processes (e.g., water and nutrient cycling) but laborious to measure, are not discussed here (see Van Leerdam et al. 1990, Ingram & Nadkarni 1993, Wolf 1993, Hietz & Hietz-Seifert 1995a).

#### Identification

Taxonomic specialists often are needed to identify plants to species, especially in species-rich groups and for those poorly known taxonomically. Notetaking in the field on growth habit, morphology, and flower colors of living plants is essential to support efficient identification of vascular epiphytes. For orchids, flowers should be collected in 70% alcohol. Collections should include mature sterile plants, since these can be divided into morphospecies. Some of the sterile material can be cultivated, which may result in positive identification. Many epiphytic plants can be easily removed from the substrate and transplanted. Survival rate of such transplants is usually high when the plants are well-protected against desiccation. Flowering of cultivated orchids usually occurs within a few months after transplantation but can take up to a year for larger plants, such as bromeliads. When fieldwork does not extend over a period of several months, staff at a local field station may be enlisted to maintain the living collection over a longer period of time (Hietz & Wolf 1996).

Although genera and morphospecies of bryophytes and lichens can frequently be recognized in the field with a handlens, microscopic analysis usually is required for species identification. Identification manuals are available for tropical bryophytes (e.g., Gradstein et al. 2001a) and macrolichens (H. Sipman unpubl. data: [www.bgbm.fuberlin.de/BGBM/staff/wiss/sipman/keys](http://www.bgbm.fuberlin.de/BGBM/staff/wiss/sipman/keys)), but are lacking for

tropical crustose lichens. For RRED analysis, therefore, it may be necessary to exclude the microlichens.

#### Time Frame

RRED analysis of vascular and non-vascular epiphytes, including preliminary identification of morphospecies but excluding full species identification, can be completed in 14 days by six persons, three specialists (one each for vascular plants, bryophytes, and lichens), and three field assistants. Tree analysis consumes 8 days (1–2 days per tree), and processing of collections and identification of morphospecies takes 6 days. The proposed RRED-analysis may become the standard protocol for rapid and representative sampling of vascular and non-vascular epiphytes.

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## Editorial

### The Ethics Of Peer Review

Peer review—that valuable tool for scientific authors and journal editors—is being abused in the field of plant taxonomy. Botanical manuscripts benefit from peer review for style and clarity, including organization, presentation, and compliance with the International Code of Botanic Nomenclature. Plant taxonomy, however, is a competitive field, where the first to publish gets to name a new species or rename a known one. For this reason, peer review of taxonomic manuscripts can do a disservice to editors and authors. The following case studies show how the system can fail.

A young author completed a DNA study of a plant group using 28 taxa and one gene region for her thesis, which revealed a polyphyletic genus requiring a new combination to make it monophyletic. The author submitted a manuscript on the study to a prominent journal, as she was a member of the professional society publishing that journal. It was rejected by the editor as reporting on too small a study for that journal. She next submitted the manuscript to a journal whose editor sent it for peer review to an established taxonomist working with that plant group at a prestigious institution (reviewer A). He recommended rejecting it as a preliminary study, noting that his working group was working on that genus in a large complex study. Despite a recommendation by reviewer B to accept with corrections, the journal rejected it. Three months later, an article by colleagues of reviewer A appeared in the prestigious institution's in-house journal, publishing the new combination proposed by the young author. The combination was based on a study of two gene regions using eight taxa, hardly a large complex study. Circumstantial evidence points to a reviewer who recommended rejection to delay publication of a young author's new combination, thus allowing his working group to publish first.

In another case, a plant taxonomist submitted a manuscript describing a new genus to a journal, whose editor sent it for review by a taxonomist working in the same family. The reviewer happened to sit on the funding panel of the group backing the author's research. As a panelist, he had progress reports of the author's research, reports considered confidential by the

funder. The reviewer recommended rejection, reasoning that the author's findings did not support the conclusions. He also sent the editor a copy of a confidential phylogeny from an initial progress report to support his recommendation. Not only did the reviewer release confidential material, he selected a preliminary unresolved report on eight taxa when he had a final report of 67 taxa that supported the author's conclusions. The reviewer's motivation was unclear, until it became known that he was a disgruntled ex-employee of the institution where the author worked. There was a happy ending, when the manuscript was submitted to another journal and published.

In yet a third case, an author, after completing fieldwork in Cameroon, using the French-sponsored Radeau des Cimes dirigible and treetops raft, submitted a manuscript to a national journal for biology educators. The manuscript reviewed the educational aspects of the international expedition and offered educators "backyard" activities and applications for use in high school curricula. Although the manuscript was accepted with revisions and published, one anonymous reviewer condemned it, writing: "If this article appears in any publication I read, I will cancel my subscription." Yet two other reviewers recommended publication. The editor ignored the spiteful comments from this opinionated reviewer.

Even though reviewer comments can improve manuscripts, and editors rely on them to maintain the standards of their journals, the competitive nature of taxonomy lends itself to peer-review abuse. For this reason, editors are well-advised to choose reviewers who do not work in the same plant area as the author, and reviewers are well-disposed to cast off anonymity and allow their names to be published with the articles they review, if requested.

Currently peer review favors established authors over neophytes. Yet rather than discouraging young taxonomists and biologists, we need to be recruiting them to meet the challenges at hand and those ahead. *Selbyana* allows reviewers to remain anonymous but encourages signed reviews.

—Wesley E. Higgins