



VASCULAR EPIPHYTES

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Introduction

Taxon Definition – Vascular epiphytes are plants that germinate and live upon another plant without parasitic-roots and at least for a part of their life cycle do not take nutrients from the soil. Over 27.600 species of plants, in 73 families and 913 genera are epiphytes accounting for about 9% of all plant species. The majority of vascular epiphytes are ferns and monocots - especially orchids, bromeliads and aroids - relatively few are dicots (e.g., ericads, gesneriads, *Peperomia*), and virtually none are gymnosperms.

Why include Vascular Epiphytes in rapid biodiversity assessment? Vascular epiphytes as a study group are particularly appropriate for rapid baseline surveys because they are relatively small (allowing for high species richness on fairly small plots), physiognomically distinctive (making them easy to survey), have high species numbers (allowing for quantitative analyses) and are comparatively easy to identify.

The high diversity of vascular epiphytes is one of the most striking characteristics of tropical rain forests and humid montane forests. These organisms are of major significance for a great number of reasons: 1) they contribute substantially to ecosystem diversity, production and nutrient cycles; 2) they provide appreciable nutrient and energy sources to associated organisms such as pollinating birds, bats and mutualistic ants; 3) they act as global indicators for climate change; 4) they are of major horticultural and, hence, of economic value; and 5) they create an arena for observational and experimental studies on a wide range of biological questions including diversity patterns, systematics, plant interactions, ecophysiology and mechanisms of evolutionary change.

There is growing recognition that vascular epiphytes are increasingly threatened. The main causes for epiphyte extirpation and population reduction are overcollecting of horticulturally valuable species for commercial purposes and habitat loss due to deforestation and land use changes. Because epiphytes, especially orchids, may occupy very narrow ranges and often occur in regions of rapid development, many tropical plant species listed as “endangered” by conservationists are epiphytes.

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Due to their life high up in the forest canopy and their strong dependence on atmospheric water and minerals, vascular epiphyte species are very sensitive indicators of environmental disturbance and climate change.

Core Methods

The method of the Rapid and Representative Analysis of Epiphyte diversity (RRED-analysis), recommended here, has been specifically designed and tested for standardized, rapid assessment of the biodiversity of vascular epiphytes.

Key Publications of method:

Gradstein, S.R., Nadkarni, N.M., Krömer, T., Holz, I. & N. Nöske. 2003. A protocol for rapid and representative sampling of vascular and non-vascular epiphyte diversity of tropical rain forests. *Selbyana* 24: 105-111.

Krömer, T. & S.R. Gradstein. 2003. Species richness of vascular epiphytes in two primary forests and fallows in the Bolivian Andes. *Selbyana* 24: 190-195.

Krömer, T., Kessler, M., Gradstein, S.R. & A. Acebey. 2005. Diversity patterns of vascular epiphytes along an elevational gradient in the Andes. *Journal of Biogeography* 32: 1799-1809.

Krömer, T., Kessler, M. & S.R. Gradstein. 2007. Vertical stratification of vascular epiphytes in submontane and montane forest of the Bolivian Andes: the importance of the understory. *Plant Ecology* 189: 261-278.

Target organisms: Vascular epiphytes

Target habitats: All forest types, but especially tropical rain forests and humid montane forests

Biodiversity data provided: Species lists, richness (alpha diversity), frequency and vertical distribution

Time and personnel needed: 8 working days are needed for a complete inventory, including tree climbing and processing of specimens of eight 20 x 20 m subplots by two persons (one specialist and one field assistant)

Skills required: Regional experience on epiphyte identification and tree climbing training, preferably the single rope technique

Equipment and costs:
Tree climbing equipment: ca. 1.000 US\$

Standardized sampling protocol for rapid survey – Vascular epiphyte diversity in natural or secondary tropical forest is measured based on sampling of one hectare of homogeneous forest. Forest margins should be excluded. Eight mature canopy trees >10 cm DBH are sampled from the base to the outer portions of the crown using the single rope tree climbing technique, in order to provide a large sample of the upper canopy community from a variety of microhabitats. These methods will allow the collection of data and plant material from the entire tree without sawing branches.

Presence/absence of vascular epiphyte species is recorded in five vertical tree zones:

- 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. Zone 2 is often subdivided into a humid lower part of the trunk (zone 2a) and a dryer upper part (zone 2b);
- 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.

Epiphyte diversity on shrubs and understory trees is additionally sampled in eight 20 x 20 m subplots (zone U), one around each sampled canopy tree, using collecting poles and binoculars.

The method is based on the observation, obtained by means of species-accumulation curves and diversity estimates in natural and secondary lowland and montane forests in different tropical regions and climate zones, that sampling of eight mature canopy trees and a 20 x 20 m subplot that follows the terrain around each tree yields a representative inventory (ca. 80% of the total estimated number) of vascular epiphyte species within one hectare of forest (Fig. 1).

RRED-analysis was developed and tested by the authors and their associates in Bolivian and Mexican rain forests and humid montane forests, in the framework of doctoral and postdoctoral research of the first author and in consultation with the Global Canopy Programme [www.globalcanopy.org] and the International Canopy Network [<http://internationalcanopynetwork.org>].

A standard method for rapid sampling of vascular epiphyte diversity has been lacking and the one presented has been newly and specifically developed for the purpose. This is necessary, because haphazard collecting only gives a rough impression of the species richness of a forest, but it does not provide robust data for comparing biodiversity of different habitats. Vascular epiphyte inventories based solely on observations from the ground are also incomplete and biased, as many small species growing in the canopy cannot be detected from the forest floor. Therefore, inventory of the canopy must be conducted with access from canopy climbing. Furthermore, vascular epiphytes on shrubs and small trees must be sampled/recorded additionally, because the epiphyte flora in the forest understory is usually different from that on the large canopy trees.

RRED-analysis focuses on species richness and frequency, but does allow for assessment of species abundance and biomass (for that see Wolf *et al.*, 2009). Completeness of the sampling is influenced by the observer's knowledge of vascular epiphytes. Lack of knowledge of these plants may result in overlooking of species that are difficult to recognize for small size, lack of flowers or fruits, or other reasons. Therefore, the team carrying out RRED-analysis should contain at least one vascular epiphyte specialist.

RRED-analysis is the most recent and comprehensive standard method available, including suggestions of the following papers on epiphyte sampling published before:

Gradstein, S.R., Hietz, P., Lücking, R., Lücking, A., Sipman, H.J.M., Vester, H.F.M., Wolf, J.H.D. & E. Gardette. 1996. How to sample epiphytic diversity of tropical rain forests. *Ecotropica* 2: 59-72.

Nieder, J. & G. Zotz. 1998. Methods of analyzing the structure and dynamics of vascular epiphyte communities. *Ecotropica* 4: 33-39.

Shaw, J.D. & D.M. Bergstrom. 1997. A rapid assessment technique of vascular epiphyte diversity at forest and regional levels. *Selbyana* 18: 195-199.

Research Design

Sampling design:

Basic Set-up: Select eight mature canopy trees with a high epiphyte load, each surrounded by a 20 x 20 m subplot, within a 1.0 ha plot of homogeneous forest.

Placement of the sampling design: Trees in close vicinity of each other tend to have similar vascular epiphyte flora resulting from clumped distribution of many epiphyte species. Therefore, canopy trees standing well apart (separated by at least 25 m) and with crowns not overlapping should be selected. Trees at forest margins should be avoided because of edge effects. However, natural edges, as those along rivers, should be used, because the epiphyte diversity can peak or different species are found in riparian trees. In habitats that are not very rich in epiphyte species, the number of sampled trees and surrounding 20 x 20 m subplots can be reduced in accordance with the leveling-off of the species-accumulation curve. Tree-climbing might be dispensable in some dry forests, young secondary forests, scrub or mangrove, e.g., in many locations where species richness and canopies are low and when good binoculars are available.

Time and effort: About eight working days are needed by an experienced working group for a complete inventory, including tree climbing and processing of specimens of eight 20 x 20 m subplots.

Each canopy tree and 20 x 20 m subplot is sampled once. RRED-analysis is carried out during the daytime. RRED-analysis should not be attempted during heavy rain for safety reasons. However, if possible one or several visits are recommended in different seasons to detect or collect fertile plant material necessary for plant identification (e.g., bromeliads, orchids).

It is recommended that at least two people carry out this protocol. The vascular epiphyte specialist does the collecting of species data by tree climbing, the field assistant takes care of securing the climbing operation at ground level and the recording of the species data.

Field Methods

How to implement the protocol in the field

Basics Steps:

1. Selection of homogenous 1-ha forest plot.
2. Selection of eight canopy trees to be climbed by Single Rope Technique (SRT).
3. Carrying out of the inventory, usually by sampling of one tree and its surrounding 20 x 20 m subplot per day.

Sampling:

Species diversity of vascular epiphytes is scored by recording presence-absence of all species in each of the five vertical tree zones and in the understory subplots. Small trees and shrubs in the 20 x 20 m plot should be inspected during the climbing of the mature tree. To avoid damaging the vascular epiphyte populations within the sampled trees, all species encountered in the 1.0 ha plot (but not in every single tree or subplot) should be collected only once in triplicate.

Data to Record:

Species presence in each tree zone and in the understory plot is recorded on the data sheet (see attached). For any specimens collected, label each with collection number and provisional scientific name. Minimally, presence/absence of individual species must be recorded for each of the five tree zones and the understory plot.

To document the habitat of the epiphytes, characteristics of the host tree such as tree height, trunk diameter (diameter at breast height or DBH), bark structure (rough, smooth, flaking) and crown architecture (main branches horizontal or oblique, etc.) should preferably be recorded (see attached data sheet).

Voucher specimens are dried in newspaper, in a plant press. Collection number is written on the paper containing the voucher specimen and, preferably, on small field labels attached to the specimen. Succulent materials are sliced to enhance drying.

The following additional voucher data are recorded in the field note book under the collection number: Scientific name, Name of the person responsible for the scientific identification, Location, Habitat, Field characters of the species (e.g., growth form, color of flower, fruit, etc.), Collector(s) name(s), Collection number, Date of collection.

Data Management

Identifying Specimens – Vouchers should preferably be collected in triplicates, one for the local herbarium, one for the collector’s herbarium and one for mailing to a specialist for final identification (if needed). Furthermore, cultivation of vouchers may be necessary for species identification when flowers are lacking, especially sterile material of orchids.

With basic botanical knowledge, specimens with flowers may usually be fairly easily identified to family or genus, occasionally even to species, by using published keys or by comparison with dried reference material. This will allow you to seek experts on specific epiphyte groups for help with species identification.

For identification of species, the following experts may be contacted for help: T.B. Croat, Missouri Botanical Garden (aroids); E. Gouda, Utrecht University and W. Till, University of Vienna (bromeliads); R.L. Dressler, University of Costa Rica, Lankester Botanical Gardens (orchids); G. Mathieu, Ghent University (*Peperomia*); and J.T. Mickel, New York Botanical Garden and A.R. Smith, Jepson Herbarium Berkeley (ferns). Furthermore, the Bromeliad and Orchid Research Centers of the Marie Selby Botanical Gardens may provide help with identification of orchids and bromeliads.

Data treatment and interpretation – Single RRED-analysis provides species lists, richness (alpha diversity) and frequency for one hectare of forest. Multiple RRED-analyses allow for biodiversity comparisons across habitats and regions. The data can be analyzed with conventional techniques for diversity comparisons and richness estimation, as those based on frequency records (e.g., Chao 2).

Species frequencies, e.g., in the various height zones, may be statistically analyzed in various ways. A simple and effective approach would be by using contingency tables (see Krömer *et al.*, 2007, Vergara-Torres *et al.*, 2010).

Results of RRED-analysis can be interpreted at different levels: at the level of individual trees or height zones, canopy versus understory, single hectares of forest, etc.

Context-Dependent Sampling Considerations

RRED-analysis is impeded by heavy rain or storms, therefore is best carried out during dry spells in the rainy season. Sampling during the dry season may underestimate epiphyte richness because some epiphyte species lost their leaves (e.g., ferns) or lack reproductive structures; however, the cultivation of sterile plants to obtain herbaria vouchers or several visits to the sampling locality might complete the inventory.

The forest canopy can be a hazardous environment to work in, but need not be when appropriate safety precautions are taken. For a detailed safety protocol the website of Tree Climbers International [<http://treeclimbing.com/index.php/climbing/rules>] should be consulted. Potential hazards are the following: Hazardous trees and branches-- Branch or tree falls pose a danger to RRED-analysis. Inspection should be made of the area around the tree prior to access of the canopy for any hazardous neighboring trees or branches that might impede safe climbing. Also, RRED-analysis should not be carried out on instable fast-growing pioneer trees, and under conditions of high winds or storms.

Hazards from other organisms – In many forest hazardous species of plants and animals may occur. Plant hazards include thorns, stings, and poison (e.g., *Rhus radicans*). Personnel should be trained to identify these hazardous species (e.g., avoiding trees with vines, honeycombs or dry branches with bee activity). Hazards from animals include feral attacks, snake bites, and insect stings. Bees can be especially dangerous in the canopy. Although most snakes are harmless, they should be treated with caution and avoided. Where possible appropriate anti-venom should be available on site and arrangements should be made for quick evacuation of a casualty.

Heat exhaustion and stroke – Working in the canopy for extended periods of time under hot and humid conditions may cause risk of heat exhaustion or heat stroke. Precautions to be taken include wearing of hats and carrying of adequate water supplies.

In RRED-analysis there is always a second person at ground level for safety (see above!).

The method is already established and has therefore been widely applied. The method has been tested in different tropical forest types, including lowland and montane forests as well as primary and secondary forests, and its robustness for analysis of species richness and frequency in one hectare plots has been shown. We envisage further development of the method towards representative sampling of abundance and biomass of vascular epiphytes, which is not yet facilitated by RRED-analysis.

Conservation Implications and Limitations

RRED-analysis can be applied to the study of the responses of vascular epiphytes to forest disturbance and environmental change, by comparing species richness in natural vegetation and sites with anthropogenic influence. Numerous studies have shown a drastic decrease of epiphyte species richness in secondary vegetation (see Köster *et al.*, 2009) This decrease is especially notable among orchids and certain groups of ferns (filmy ferns, grammitid ferns), which are extremely sensitive to human disturbance.

Richness of epiphytes in terms of species number per given area can thus be a good indicator of environmental quality, and a useful measure for determining the conservation status of an area (see Krömer *et al.*, 2014). It goes without saying that the use of epiphyte richness as a bioindicator requires a rigidly uniform protocol for sampling of epiphyte richness data. RRED analysis was exactly designed for this purpose and has shown its usefulness.

This method can target rare and uncommon species of conservation importance. The authors have discovered several new species to science and many new species records on the regional level by using RRED-analysis.

Long-term monitoring of changes in epiphytic species richness and community structure over time, in permanent sites or plots, is highly desirable but is beyond the scope of RRED-analysis. However, these kinds of studies can provide useful information about the resistance and resilience of epiphytes to the loss, alteration and deterioration of their natural habitat as well as the effects of climate change.

Acknowledgments

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Helpful References

There are several websites of general interest for epiphyte researchers:

The Global Canopy Programme [<http://www.globalcanopy.org>]

The International Canopy Network [<http://internationalcanopynetwork.org/>]

The Big Canopy Database Project [<http://canopy.evergreen.edu/BCD/>]

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Equipment

The following list includes the basic tree-climbing equipment (and its cost in July 2015), which can be ordered online at <http://www.newtribe.com/store>, <http://www.sherrilltree.com/tree-climbing-gear>. However, there are other providers, where similar equipment from other brands can be obtained (e.g., <https://www.forestry-suppliers.com/>).

Product Name	Quantity	Price per unit (US \$)	Sum (US \$)
New Tribe TWIST Adjustable Saddle	1	149	149
CMI Large Left & Right Ultrascenders	1	140	140
1" Nylon Tubular Webbing (to connect Ascenders)	10 ft	10	10
Petzl Rig Self-Braking Descender	1	185	185
Petzl Carabiner	5	20	100
Petzl Elios Adult Tree Climbing Helmet	1	65	65
Grippy Rappel Gloves	1	20	20
New Tribe Nifty Throwing Kit	1	42	42
New Tribe Shot Pouch 12 oz	1	15	15
New England 11 mm Static Kernmantle Rope	150 ft	132	132
NewTribe Medium Ropebag	1	49	49
Big Shot line launcher two 4' poles Kit Specs	1	120	120
		Total (US\$)	1.027

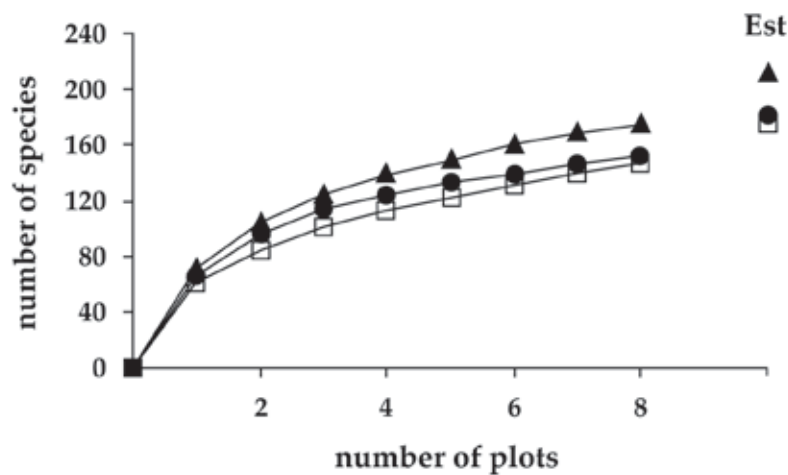


Figure 1
Species accumulation curves and estimated total number of species (Est) of vascular epiphytes in three 1 ha plots in a montane forest of Bolivia, using the Michaelis-Menten richness estimator. In each hectare plot, up to eight trees were sampled, as was a 20 x 20-m plot around each sampled tree (taken from Gradstein *et al.*, 2003).

Data Sheet

Collection Number	(Provisional) Scientific Name	Tree Zone						Additional Notes
		U	1	2	3	4	5	