Bryophytes as Indicators of Forest Disturbance: a new toolset for conservation

Sarah Carvalho Stow

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Abstract

Bryophytes, though relatively understudied, are an important and diverse component of ecosystems with around 20 000 extant species. Three plant phyla make up bryophytes: liverworts (Marchantiophyta), mosses (Bryophyta) and hornworts (Anthocerophyta). The bryophyte life-cycle is unique among land plants for having a dominant gametophyte generation, a characteristic possibly retained from the first plant land-colonisers. Because of bryophytes' small size, their ecophysiology is particular and different to most other land plants, with moisture availability being a limiting factor for many species. Included in this, is the mechanism of desiccation tolerance (DT), which is almost exclusively found in bryophytes. Desiccation tolerance together with a small size means that bryophytes can occupy harsh habitats and substrates that are not available to most plants as they have the ability to efficiently utilise water in the form of water vapour. Bryophytes are therefore highly dependent on microclimate and consequently, have a high affinity to particular microhabitats. In forests, bryophyte reliance on microclimate and microhabitats make bryophytes particularly susceptible to disturbances due to a decrease in humidity and increase in insolation often associated with forest degradation. Bryophytes also have varying degrees of desiccation tolerance which means bryophytes will respond differently to forest degradation.

Tropical humid forests are one of the richest ecosystems but also, historically, one of the least protected. Currently, it is estimated that more than 50% of all tropical habitats are degraded. Madagascar is highly regarded for being a "biodiversity and endemism hotspot" but is also known for the significant human threats to its ecosystems. The level of threat makes conservation of biodiversity both necessary and urgent and so quick, cost-effective and reliable methods that measure biodiversity responses to forest degradation are vital; one such method is the use of indicators. Indicators can be taxa, groups of taxa or abiotic characteristics. This study investigates the potential of using bryophytes as indicators of forest degradation based on their morphological and life-history traits and how these traits affect their environmental preferences.

A bryophyte trait database was created for 1430 taxa, 51 morphological and reproduction traits, five environmental traits, 13 ecological and distribution traits and three conservation traits. It is the largest bryophyte trait database to date, and is also novel in that it includes Malagasy bryophytes. Portuguese bryophytes were also included to inform on Malagasy species, for which data is scarce. Studies have found that it is possible to extrapolate bryophyte data from one region to another due to the high dispersal ability of bryophytes resulting in species, genera and families common to both regions. In the specific case of Madagascar and Portugal, 34% of Malagasy genera and 64% of Malagasy families are found in Portugal.

Many traits were found to affect species' environmental preferences from large-scale traits such as life-form and plant size to cell shape and spore size. Importantly, analyses conducted on Malagasy and Portuguese species individually showed that their traits have comparable responses to environmental preferences thus confirming that results from Portuguese species can indeed be used to extrapolate to tropical ones. Two trait profiles that characterise species of dry and exposed habitats, and species of humid and sheltered habitats were identified and used to assign species an indicator value. This methodology allowed the inclusion of species with missing trait data, which was the majority of Malagasy species.

Species, genera and families were identified that indicate particular environmental conditions. Species that indicate humid and sheltered conditions are those that have open life-forms and are large. Most epiphytic species are indicators of drier and more exposed conditions. The indicator index created therefore reflects the different responses of bryophyte species. These findings were validated with sampling of bryophytes in a lowland humid forest, in southeastern Madagascar, along a gradient of degradation. Two metrics were used to quantify degradation: a categorical one of four classes of forest degradation and non-forest (cleared forest for shifting cultivation) and an index based on various disturbance variables. This showed that using a finer-scale of degradation provided greater insight into the response of bryophytes to varying degrees of degradation.

Bryophytes have potential as indicators, and the IV metric created here needs further refinements. An important finding was that certain bryophyte traits (e.g. life-form) respond predictably to environmental conditions and forest degradation. These traits could therefore be used as a quick, simple and cost-efficient measure of forest degradation.

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CONTENTS

TOOLBAR.

List of figures

List of tables

Rationale

Tropical humid forests are one of the richest ecosystems but also, historically, one of the least protected (Myers, 1981). In 1980, between 200 000 km^2 to 250 000 km^2 of tropical humid forests was estimated to have been degraded per year (Myers, 1981). Currently, it is estimated that more than 50% of all tropical habitats are degraded (Struebig et al., 2013). Madagascar is highly regarded for being a "biodiversity and endemism hotspot" (Mittermeier et al., 1998; Myers et al., 2000; Ganzhorn et al., 2001) but is also known for the significant human threats to its ecosystems (Gardner, 2011). The level of threat makes conservation of biodiversity both necessary and urgent and so quick, cost-effective and realiable methods that measure biodiversity responses to forest degradation are vital; one such method is the use of indicators. Indicators can be taxa, groups of taxa or abiotic characteristics. This study investigates the potential of using bryophytes as indicators of forest degradation. Bryophytes, commonly known as mosses, though relatively understudied and physically small, are an important and diverse component of ecosystems with around 20 000 extant species. Their small stature means they are sensitive to changes in microclimate and so could readily indicate any disturbances in a forest.

The level of threat in bryophytes varies between countries and regions, but for areas that have undertaken complete Red List assessments of the bryoflora, it has been found that a large proportion of bryophytes are at risk of extinction. Although many bryophyte species are inherently rare locally, extrinsic threats are numerous: habitat loss and degradation, pollution (air, water, soil), invasive species, fire and forest management practices. The status of knowledge on bryophytes is generally poor, with a large disparity between temperate and tropical areas, although there has been a recent small but marked increase in tropical bryology research. One of the main focuses of bryophyte conservation is improving the knowledge on bryophytes so that effective management policies and actions can be put into place. This study will focus on the bryoflora of Madagascar, which is understudied but likely highly threatened given the overall threats facing Malagasy biodiversity.

Overall research questions and aims

The overall aim of this PhD is to assess whether bryophyte species can be used as indicators of forest degradation – based on where species with different desiccation tolerance levels occur. This will be achieved by relating desiccation tolerance traits of species with their environmental preferences and creating an index from this. Thus the main research questions of this thesis are:

1. Do bryophytes have different levels of desiccation tolerance?
2. Are there known morphological and life-history traits that relate to desiccation tolerance and can these traits be related to the environmental conditions a species inhabits?
3. Can these traits be used to group species according to their trait similarity and environmental preferences?
4. How can we create an indication index based on species traits that are associated with particular environmental conditions?
5. Does this indication index vary between different microhabitats and levels of forest degradation?

Thesis Structure

Chapter one provides an introduction to bryophytes, from their morphology to their ecology and conservation. It also introduces Madagascar in terms of its biodiversity and bryoflora.

Following the background provided in chapter one, **chapter two** provides a review of desiccation tolerance (DT) in the plant world and gives further details on bryophyte morphological and life-history traits that are associated with desiccation tolerance. A detailed description of these traits and the reason they are included in this study is also provided. A summary of the application of desiccation tolerance in ecological studies and how desiccation tolerance traits may be used to select indicator species of forest degradation is provided.

Chapter three investigates the relationship between traits (morphological, reproductive and life-history characters) and the environment. The overall aim is to examine the relationship between traits and environmental preferences to determine which traits indicate desiccation tolerance. Aditionally, how to best categorise qualitative traits for subsequent statistical analyses is also determined. The traits selected for inclusion in the trait database are those that relate to desiccation tolerance, as reviewed in chapter two. The reasoning and methods for constructing the trait database are outlined and the methods for obtaining data on the traits themselves are described for traits where subjectivity is involved in their recording. An environmental index (EI) is created and an EI value assigned to each species which is then related to DT. Univariate analyses (ANOVAs) are undertaken to identify traits that are significantly related to species' environmental preferences (EI) and DT.

Building on the results in chapter three, **chapter four** links desiccation tolerance to habitat and conservation traits and assigns species indicator values (IVs). The trait database created in chapter 3 is used to identify indicator species, genera and families using multivariate analyses. An ordination and subsequent clustering analysis groups species according to shared traits, desiccation tolerance and environmental preferences. Subsequently, trait profiles that represent species of different environments, namely: dry and exposed, and humid and sheltered, are identified. These results are then used to assign all species, genera and families an indicator value (IV). These taxa are then assigned to an indicator class based on their indicator value and environmental range. The IV is further tested by seeing how it is associated with certain easy-to-measure bryophyte traits and selected habitat, distribution and conservation traits.

While chapters 3 and 4, the compilation of the trait database and deriving the environmental index (EI) and indicator value (IV) metrics, comprised the largest part of this study a fieldwork component is also necessary to apply and verify the derived metrics. **Chapter five** identifies the indicator values of different habitat degradation and microhabitats occupied by species collected during fieldwork in Madagascar. A larger-scale analysis is also undertaken testing if bryophytes in different ecoregions in Madagascar have different indicator values, using georeferenced specimen data.

Chapter six provides a synthesis of results and their application to conservation and future research to be conducted based on this study, as well as limitations of this study.

A list of acronyms and glossary can be found at the end of the thesis.

Chapter 1 General Introduction

This chapter provides an introduction to bryophytes; as they are little known, a summary of their study, evolution, morphology, physiology, ecology and conservation is given. Several recent books and articles provide detailed reviews on bryophytes, ranging from their evolution to their ecology, and so the focus in this chapter is on tropical bryophyte ecology, in particular bryology in Madagascar.

1.1 An introduction to bryophytes

1.1.1 What is a bryophyte?

Most people will have noticed bryophytes at some point in their lives as green patches growing on walls and trees and colloquially called them 'mosses'. The term bryophyte actually refers to three morphologically diverse plant phyla: Bryophyta (mosses *sensu strictu*), Marchantiophyta (liverworts, also sometimes called hepatics) and Anthocerophyta (hornworts). The three bryophyte phyla are informally grouped together as they share several characteristics: very small size (though a few species can reach up to 1 m in length); lack of lignified tissues; production of spores; a branched gametophyte; poikilohydry (unable to regulate their water content) and a life cycle with a dominant gametophyte generation. The last characteristic is unique to bryophytes. The word 'bryophyte' is a combination of the Greek words 'bryon' – moss and 'phyto' – plant and means "plants that swell with water" (Vanderpoorten & Goffinet, 2009, p. 2). This refers to how, after almost completely drying out, they appear to expand when again in contact with water. Another important character of bryophytes is that they are poikilohydric – unable to regulate their water content, in contrast to all other terrestrial plants. Their ability to lose most of their cell water content, cease metabolic activity and then resume metabolic activity when rehydrated is referred to as vegetative desiccation tolerance, a strategy that has enabled plants to adapt to life on the relative dry terrestrial environment. This mechanism, which is found almost exclusively in bryophytes and only present in angiosperms in their seeds and pollen, is what this thesis centres around and is discussed in more detail in chapters two and three.

Because of the colloquial use of the word 'moss' it is important to define how the following terms are used in this thesis: 'bryophyte' refers to all three taxonomic phyla (Bryophyta, Marchantiophyta, Anthocerophyta); 'moss' is used to refer solely to the Bryophyta phylum; 'bryophyte group' refers to a phylum (Bryophyta, Marchantiophyta or Anthocerophyta); 'plant group' refers to groupings of similar plant phyla (see Table 1.1, p. 6); and 'tracheophyte' refers to any terrestrial plant that is not a bryophyte (for further definitions see the glossary, p. 334).

Bryophytes, in comparison to other plants, have historically been understudied and misunderstood, likely due to their small stature making them easy to overlook in the field and difficult to identify. This has meant that the organism referred to as a moss has changed over time. Pliny the Elder in his Natural History uses the term 'bryon' to mean lichens, algae, berries, buds, as well as moss (Bostock & Riley, 1855). Early naturalists believed they were "(…) excrescences produced from the earth, trees etc. (…)" (Miller, 1735, p. 158) and a symptom of illness in trees (Encyclopaedia Perthensis, 1816). The founders of modern botany, Malphigi and Grew, make no mention of bryophytes in their works on plants (Malpighi, 1675; Grew, 1682). The

16th & 17th century philosopher Francis Bacon reasoned moss to be "(…) but the rudiment of a plant (…) and the mould of earth or bark" (Bacon, 1627, p. 139). By the early 18th century, however, they were recognised to be small plants and classification of species was underway (Ray, 1690, 1724; Dillenius, 1719, 1741; Miller, 1735). However, bryophytes still remained grouped together with other non-flowering plants (Dillenius, 1719; Linnaeus, 1753), and the term 'moss' could be used when referring to lichens (Watson, 1758) and vice-versa. Early classifications listed some bryophytes in the genus named 'Lichen' e.g. the liverwort *Marchantia polymorpha* was named *Lichen domesticus minor* (Dillenius, 1741, p. 527), Figure 1.1. Linnaeus (1753) included bryophytes in his seminal work *Species Plantarum* (vol. 2), guided in part by Dillenius's publications, but the German botanist Johann Hedwig was the first to undertake a thorough study of bryophytes, notably through his work on mosses, *Species Muscorum Frondosorum* (published posthumously in 1801), which included detailed coloured illustrations (Figure 1.1). Although publications on bryophytes existed before these two works, they have been designated as the baseline for bryophyte nomenclature; Linnaeus's *Species Plantarum* (1753) for liverworts, hornworts and *Sphagnum* mosses and Hedwig's *Species Muscorum Frondosorum* (1801) for all other mosses (Koch & Crum, 1956; McNeill & International Association for Plant Taxonomy, 2012). The setting of this basis for bryophyte nomenclature was needed so that bryologists could reduce the confusion in the nomenclature and subsequently create checklists of all known species.

Figure 1.1 Early illustrations of bryophytes: left – a liverwort, *Lichen domesticus minor* (now *Marchantia polymorpha* L.) from Dillenius's *Historia Muscorum* (1741), plate LXXVII; right – a moss, *Bryum dichotomum* Hedw., from Hedwig's *Species Muscorum Frondosum* (1801), plate XLII. Images from the Biodiversity Heritage Library – digitised by the Biblioteca Digital del Real Jardín Botánico de Madrid.

1.1.2 How many bryophytes are there?

As with all plant groups, the total number of species recognised varies, due to synonymy issues, identification difficulties and changes in bryological exploration (Figure 1.2) (Magill, 2010; Söderström et al., 2016). Unsurprisingly, the number of species known has increased greatly since Linnaeus (1753) and Hedwig (1801) who listed 41 liverworts, 550 mosses and 3 hornworts (Figure 1.3). The latest checklists estimate that around 20 000 bryophyte species have been described: 12 800 mosses (Crosby et al., 1999), 7200 liverworts and 215 hornworts (Söderström et al., 2016). Hornworts continue to make up a very small part of bryophytes (Figure 1.3) and it has been suggested that they may be the end of a lineage that was once more speciose (Villarreal et al., 2010). Bryophytes are the second largest group of terrestrial plants (second only to the flowering

plants) and the third largest plant group when algae are included; the Bryophyta phylum alone is the second largest of all plant phyla (Table 1.1).

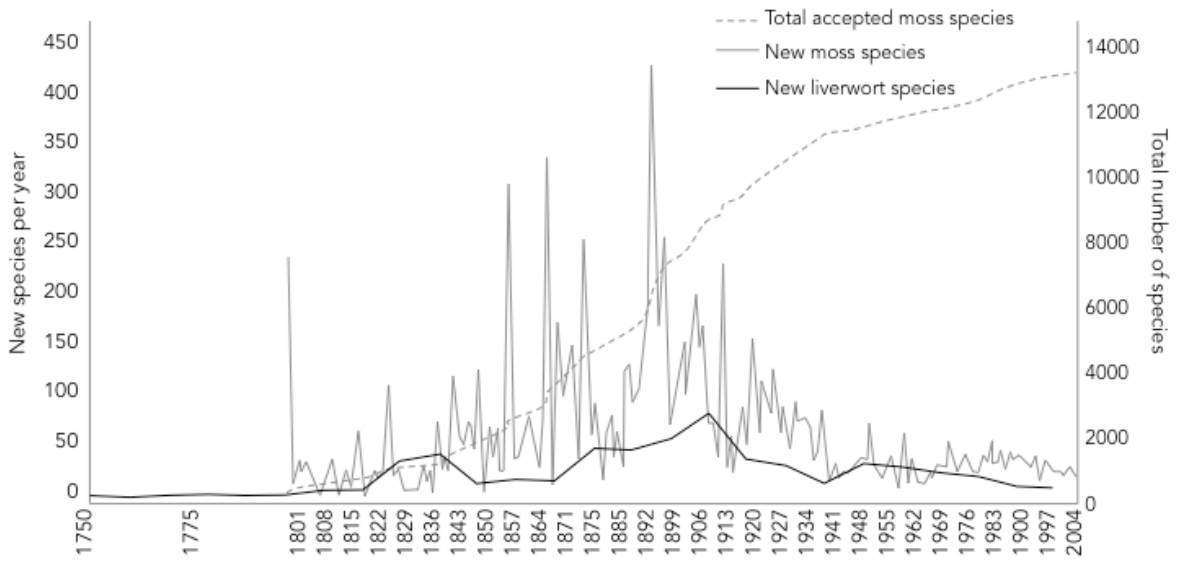


Figure 1.2 Number of new liverwort and moss species described globally over the last 250 years showing how the number of species discovered differs greatly between years. The total moss species described over time (dotted line) indicates that there has been a slowing rate of discovery since the mid-19th century. (Liverwort data redrawn from Söderström et al., 2016, fig. 2, p. 7; moss data redrawn from Magill, 2010, fig. 3, p. 170)

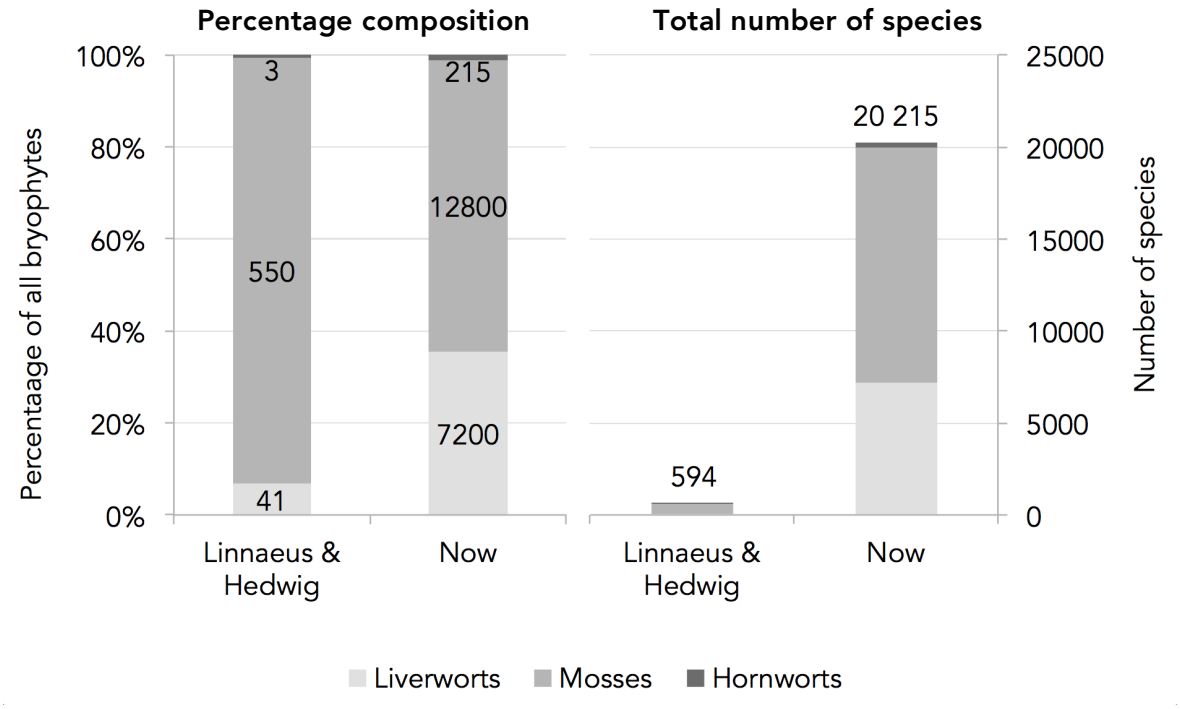


Figure 1.3 Change in amount of bryophyte species described globally over the last 250 years between baseline publications and now. Left: percentage composition by bryophyte phyla with species numbers in each phyla; right: numbers of species described per phyla with total species known at top of bars. (Data compiled from Linnaeus, 1753; Hedwig, 1801; Crosby et al., 1999; Söderström et al., 2016).

Table 1.1 Number of species in each major plant group with number of species in each plant phyla, with source for each number. The grouping of phyla into major plant groups follows those used in The Plant List (2013). The definition of algae in its broadest sense is used here following Guiry 2012; for simplification, only the larger algae divisions are specified (see Table 1.11, p. 45 for more detail on algae species numbers).

Plant group	Phylum	Number of species	Total	Source
Algae	Rhodophyta	6 131	33 260	Guiry, 2012
	Charophyta	3 470		Guiry, 2012
	Chlorophyta	4 548		Guiry, 2012
	Ochrophyta	11 571		Guiry, 2012
	All others	7 540		Guiry, 2012
Bryophytes	Marchantiophyta	7 200	20 215	Söderström et al., 2016
	Bryophyta	12 800		Crosby et al. 2000
	Antocerophyta	215		Söderström et al., 2016
Gymnosperms	Cycadophyta	92	1 104	The Plant List, 2013
	Ginkgophyta	1		The Plant List, 2013
	Pinophyta	899		The Plant List, 2013
	Gnetophyta	112		The Plant List, 2013
Ferns	Lycopodiophyta	1 285	12 285	Frey & Stech, 2009
	Pteridophyta	11 000		Smith et al., 2006
Angiosperms	Magnoliophyta	352 000	352 000	The Plant List, 2013

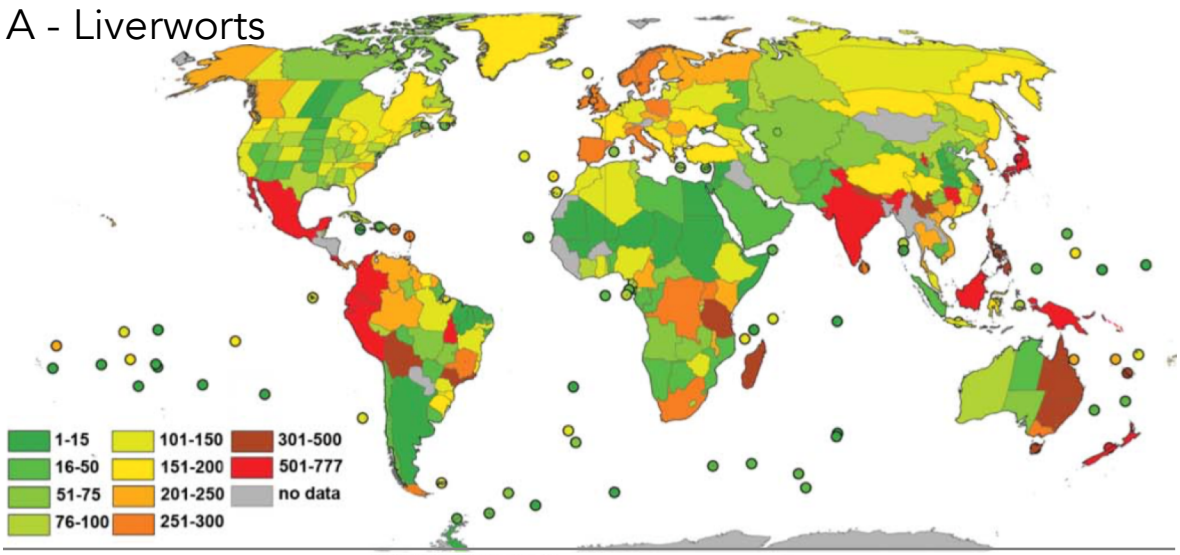
1.1.3 Where are bryophytes found? – distribution and biogeography

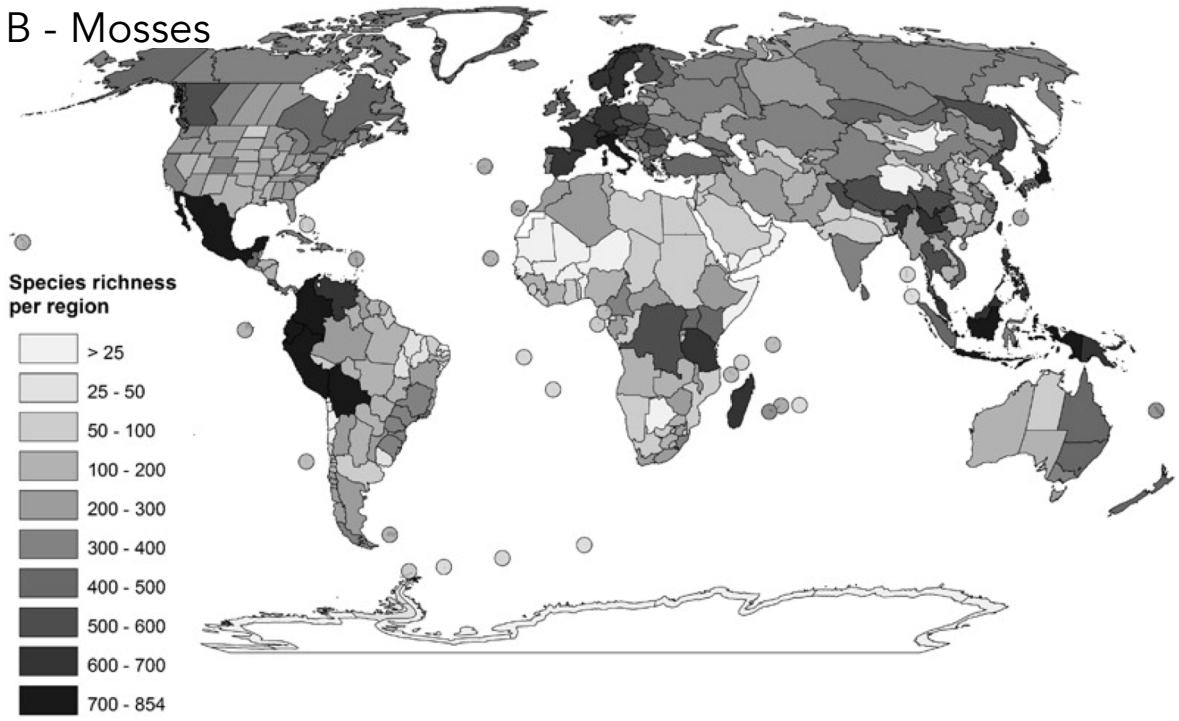
Bryophytes are one of the most successful plant groups as they are found on every continent (except hornworts, which are not known from Antarctica) (Figure 1.4 A & B) and all terrestrial habitats (Alpert, 2000a; Vanderpoorten & Goffinet, 2009; Tuba et al., 2011; Geffert et al., 2013). Some species that can tolerate low salt levels inhabit coastal habitats, although cannot be permanently submerged (Vanderpoorten & Goffinet, 2009). They have even been found to survive in permafrost (La Farge et al., 2013), with a recent experiment showing that mosses that had been buried in ice for around 4800 years were able to re-grow on the ice itself (Roads et al., 2014). Despite their small size, bryophytes can occupy large areas of a substrate making them conspicuous in many habitats; a striking example of this is the genus *Sphagnum* which alone is estimated to cover 2-3% of the terrestrial surface, notably in peatlands (Hanson & Rice, 2014).

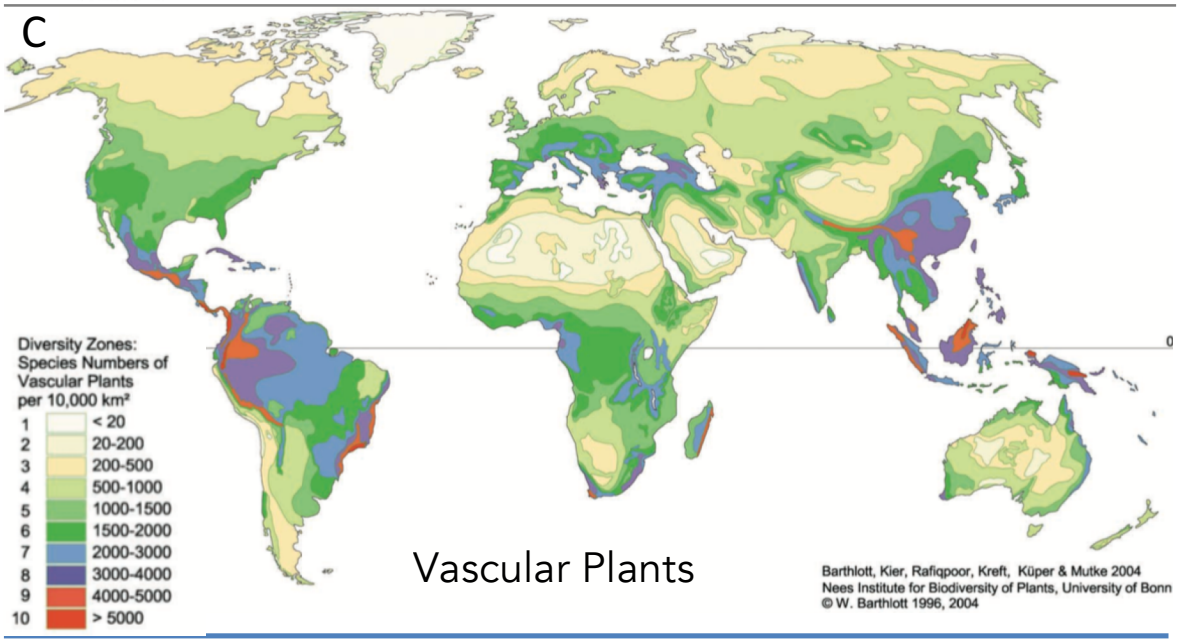
Bryophytes tend to have wide geographical ranges, that can span two or more continents (Vanderpoorten & Goffinet, 2009) and lower rates of endemism compared to vascular plants e.g. in Madagascar, known for its high endemism rates, 29% of bryophytes are endemic compared to 82% of vascular plants (Callmander, 2011; Marline et al., 2012, respectively.). Explanations for their global distribution are a combination of plate tectonics and bryophytes' dispersal capacity, as well as reproductive strategy (Vanderpoorten & Goffinet, 2009; Magill, 2010; Mateo et al., 2013).

Areas with the highest bryophyte diversity include the neotropics, 4000 species are known from here (Wagner et al., 2014), particularly in humid montane cloud forests (Pardow & Lakatos, 2013). This could be due to the high amount of bryological research undertaken in this area compared to other tropical regions, although diversity of other plant groups is known to be exceptionally high for areas in the Neotropics (Figure 1.4 C) (Myers et al., 2000; Geffert et al., 2013). Bryophyte abundance has been shown to be correlated with altitude in the tropics (Bader et al., 2013; Wagner et al., 2014) and tends to be greater where water availability is not limited. When studying African inselbergs, Frahm (2000) found that although species had low habitat specificity, the number of species was greater in wetter areas, such as rock pools, and lower in wet flushes where amount of water is lowest.

Figure 1.4 (next page) Liverwort (A) and moss (B) species richness per country showing their presence in every continent on earth. (C) shows vascular plant species richness for comparison of regional diversity levels. Units: (A) and (B) species richness per geo-political unit, (C) species richness per 10 000 km^2. ((A) taken from von Konrat et al., 2008, fig. 2 p. 97; (B) from Geffert et al., 2013, fig. 1, p. 4; and (C) from Mutke & Barthlott, 2005, fig. 3 p. 525.)


A - Liverworts
1-15
16-50
51-75
76-100
101-150
151-200
201-250
251-300
301-500
501-777
no data


B - Mosses
Species richness per region
> 25
25 - 50
50 - 100
100 - 200
200 - 300
300 - 400
400 - 500
500 - 600
600 - 700
700 - 854


C
Diversity Zones: Species Numbers of Vascular Plants per 10,000 km²
1 < 20
2 20-200
3 200-500
4 500-1000
5 1000-1500
6 1500-2000
7 2000-3000
8 3000-4000
9 4000-5000
10 > 5000
Vascular Plants
Barthlott, Kier, Rafiqpoor, Kreft, Küper & Mutke 2004
Nees Institute for Biodiversity of Plants, University of Bonn
© W. Barthlott 1996, 2004

1.1.4 What makes bryophytes unique? – the bryophyte life cycle

The Bryophyta, Marchantiophyta and Anthocerophyta are grouped together because they share the unique trait among land plants of having a dominant gametophyte generation. All land plants have alternating gametophyte (haploid) and sporophyte (diploid) generations but vascular land plants spend most of their existence in the sporophyte stage (so-called because it is the stage when spores are produced). In contrast, the gametophyte stage (so-called because it is the stage when female and male gametes are produced) dominates the life cycle of bryophytes (Figure 1.5). The stages in the life cycle are similar across the three bryophyte groups, with variation in the structure of the gametophyte and sporophyte (see section 1.1.6, p. 12 for more details). When they land upon a favourable substrate, spores (haploid - 1n) develop into protonema via mitotic division of cells. Their ability to adapt to varying habitat conditions by changing their morphology is due to the way bryophytes grow (Vanderpoorten & Goffinet, 2009). They exhibit modular growth, which simply put means that growth is via the addition of 'modules'. Each module is formed of several 'metamers' where each metamer is a group of cells that can develop into either a single branch or leaf. The metamer is created by the mitotic division of the apical cell (Crandall-Stotler et al., 2009; Goffinet et al., 2009; Vanderpoorten & Goffinet, 2009). When the adult gametophyte plant is developed (haploid - 1n) it produces gametes: egg in archegonia and diflagellated sperm in antheridia (Figure 1.5). Fertilization occurs in the presence of water, which allows the sperm to swim to the egg. The sporophyte generation is initiated with the zygote (diploid - 2n), which develops into the sporangium. Spores are produced in the sporangium, which is enclosed within a capsule, via meiotic divisions. Once mature, the spores are released via capsule dehiscence (opening) via either a "mouth" in the capsule (an operculum) or longitudinal slits in the capsule (Figure 1.5).

The organisation of sex organs on the gametophyte varies and, as with tracheophytes, bryophytes can be monoicous or dioicous. This obviously has implications for the life cycle as the distance the sperm has to travel to reach the egg affects fertilisation success. In monoicous mosses there are three main types of sex organ organisation: paroicous, the antheridia surround the archegonia; synoicous, antheridia and archegonia are mixed; and autoicous, antheridia and archegonia are held on different branches (Figure 1.6). The high levels of dioicy in liverworts and mosses (around 50-60%, see Figure 1.10) and the need for water to transport the sperm means that asexual reproduction is prevalent and many species produce vegetative reproduction structures (Vanderpoorten & Goffinet, 2009). These structures are very varied, ranging from small propagules (gemmae, see Figure 1.11 G & N) to deciduous branches.

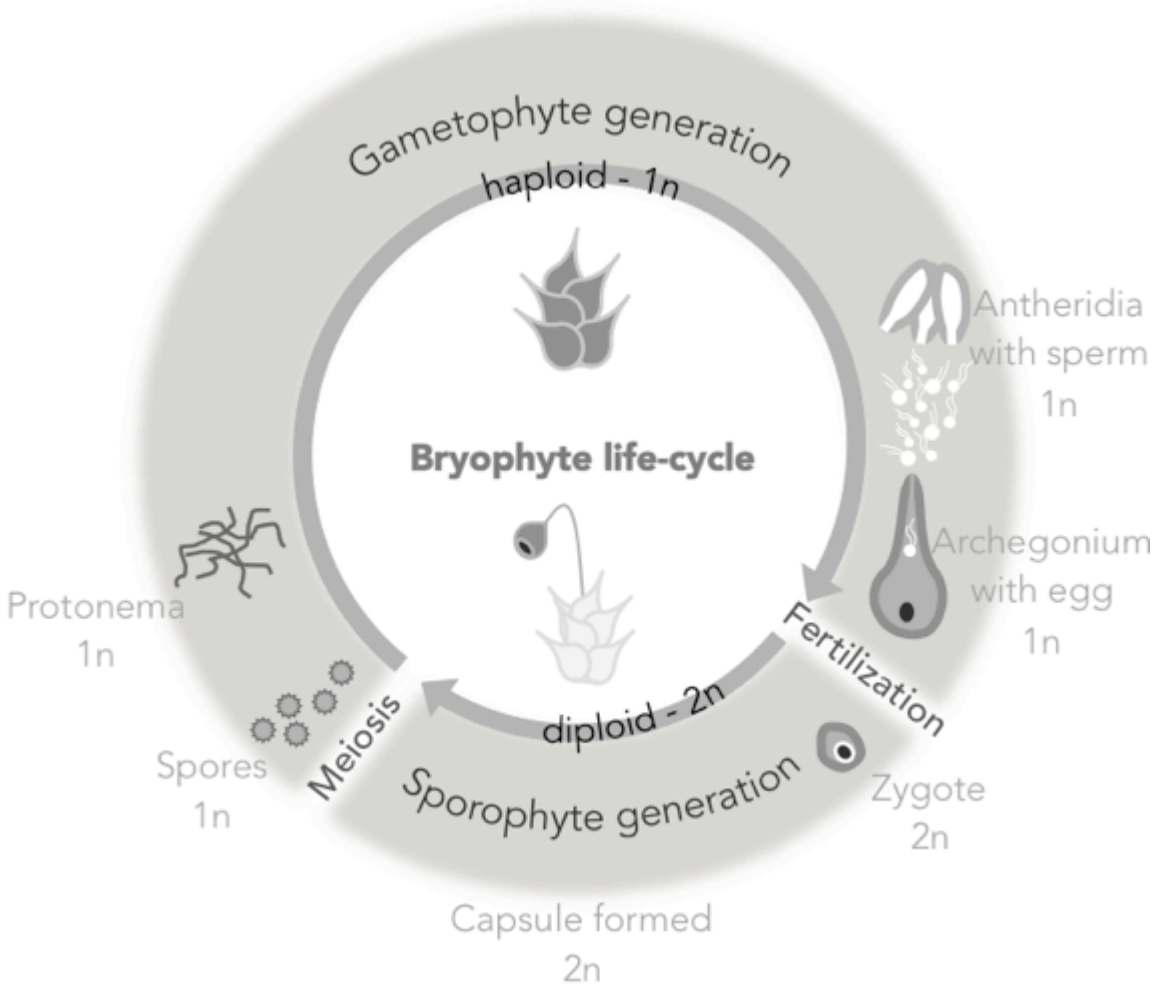


Figure 1.5 Main stages of the bryophyte life cycle. Most of a bryophyte's life cycle is spent in the gametophyte stage (haploid - 1n) that begins when spores are produced. Spores develop into protonema and subsequently into the gametophyte plant. The plant then produces gametes: egg in archegonia and/or sperm in antheridia, hence the term gametophyte. After fertilization the sporophyte generation (diploid – 2n) begins with the zygote, which develops into sporangia held within a capsule. Following meiosis in the sporangia, mature spores are released beginning the cycle again. Source: Sarah Stow

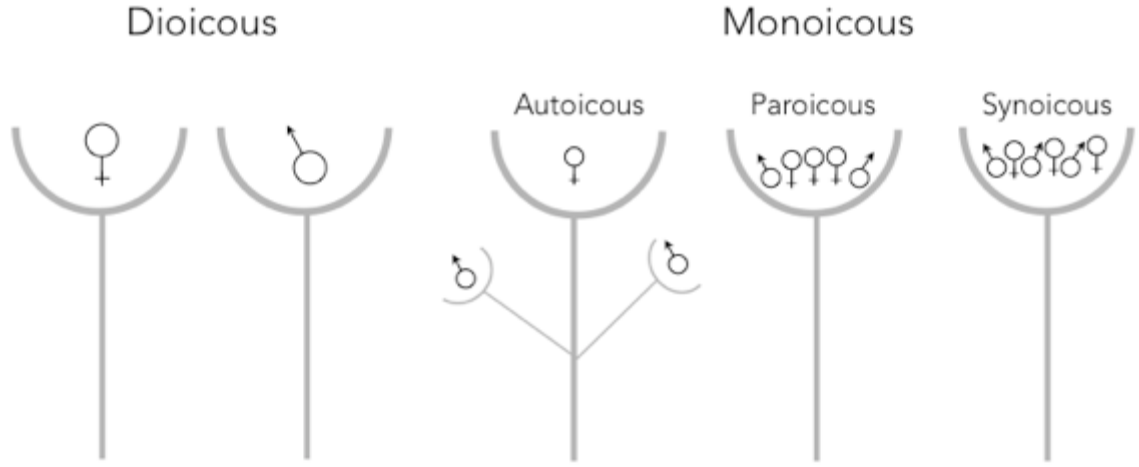


Figure 1.6 Organisation of sex organs in mosses. Dioicous species have individual female and male plants. Monoicous species have female and male organs on the same plant, in one of three configurations: autoicy, paroicy or synoicy. (Taken from Vanderpoorten & Goffinet, 2009, fig. 4.12, p. 86.)

1.1.5 When did bryophytes appear? – evolution & phylogeny

Plants first colonised land around 475 million years ago (Ma) and these early plants (Charophycean green algae) are no longer extant, but bryophytes, being the closest living relatives to these plants (Goffinet & Shaw, 2009; Hanson & Rice, 2014), provide insight into how the first colonising plants adapted to life in a dry environment (Shaw et al., 2011). The oldest fossil evidence for bryophytes are spores and tissues similar to those of liverworts from the Ordovician period, 470 Ma (Crandall-Stotler et al., 2009; Shaw et al., 2011). This predates the estimated origin of vascular plants during the early Devonian period by about 50 - 30 million years (Figure 1.7) showing how ancient the bryophyte lineage is and the reason why they are often termed 'primitive plants'. Bryophytes' close evolutionary relationship to the first land plants mean they play an important role in the study of plant evolution (Vanderpoorten & Goffinet, 2009).

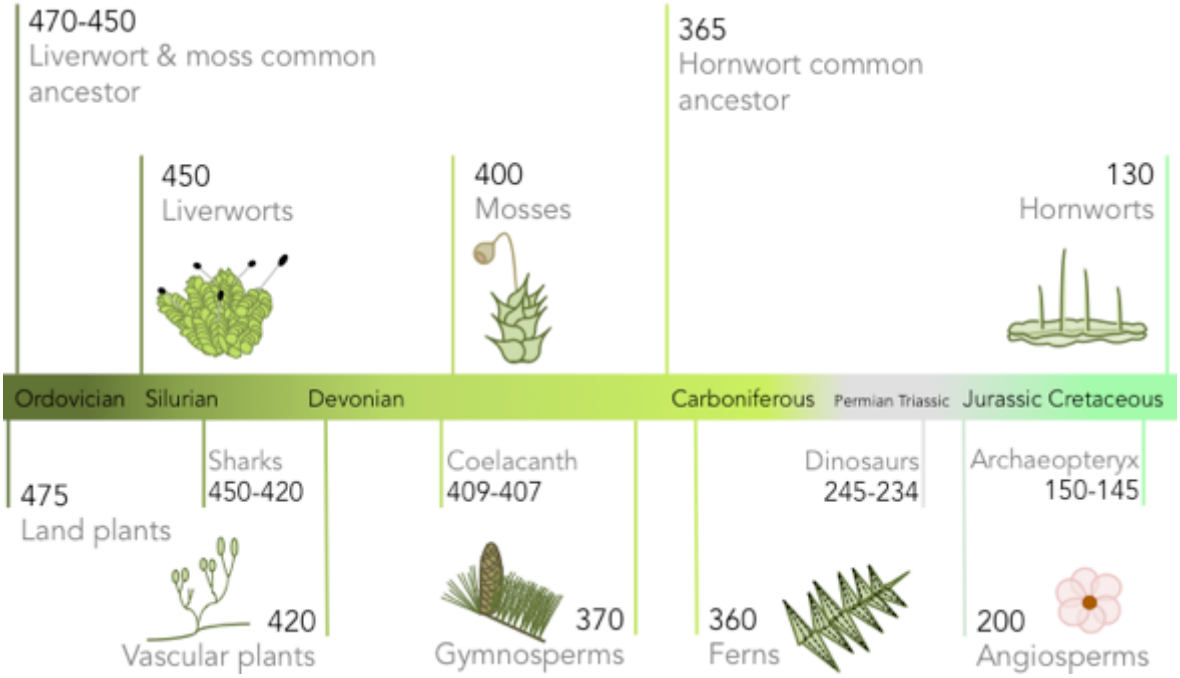


Figure 1.7 Origin of the bryophyte divisions and major terrestrial plant groups according to latest research. The origin of well-known animals is shown for reference. Numbers indicate millions of years ago. The term "ancestor" refers to earliest known fossils with affinities to a bryophyte group. (Animal data compiled from Johanson et al., 2006; Bryophyte and vascular data compiled from Crandall-Stotler et al., 2009; Villarreal et al., 2010; Brusatte et al., 2010; Shaw et al., 2011; Ligrone et al., 2012. Benton, 2014; Chatterjee, 2015; Sues, 2016) Source: Sarah Stow

Fossilisation is a rare event, even more so in bryophytes which lack lignified tissues and so the bryophyte fossil record is scarce (Goffinet, 2000; Edwards, 2000; Ligrone et al., 2012). Calibration of phylogenies and mapping ancestral character-states is therefore hampered, bringing uncertainty to the reconstruction of bryophytes' phylogeny (Mishler & Kelch, 2009; Villarreal et al., 2010). Over time various bryophyte phylogenies have been put forward (Figure 1.8) with consensus shifting between which bryophyte phylum is the sister group to tracheophytes (Shaw & Renzaglia, 2004; Vanderpoorten & Goffinet, 2009; Villarreal & Renzaglia, 2015). Advances in molecular techniques and analyses as well as the increasing number of sequenced species have led to changes in early land plant phylogenies (Figure 1.9) (Mishler & Kelch, 2000; Villarreal & Renzaglia, 2015). The first reconstructions of land plant phylogenies were based on morphological data and bryophytes were believed to form a monophyletic group (Figure 1.9 A) (Goffinet, 2000; Vanderpoorten & Goffinet, 2009). Subsequent analyses which included ribosomal DNA as well as

morphological data revealed that they are in fact paraphyletic (Shaw et al., 2011). Once phylogenetic analyses began to include more data types and species, a wider array of phylogenies were proposed (Figure 1.8 and Figure 1.9). Knowing which phylogeny is the correct one has not been straightforward as the type of data used, the number of taxa used and the analyses applied will yield different topologies (Goffinet, 2000; Mishler & Kelch, 2009; Vanderpoorten & Goffinet, 2009; Villarreal & Renzaglia, 2015). The current consensus is that liverworts are the basal land plant group and hornworts are a sister lineage to the tracheophytes (Figure 1.9 C) (Villarreal & Renzaglia, 2015). This is based on studies that have used a large number of plant species as well as different data (e.g. morphological; chloroplast, mitochondrial and nuclear DNA; genomic structural data; amino acid sequence DNA). The phylogenies within each bryophyte phylum are also not fully resolved and classifications are continuously being revised (Crandall-Stotler et al., 2009; Goffinet et al., 2009; Renzaglia et al., 2009).

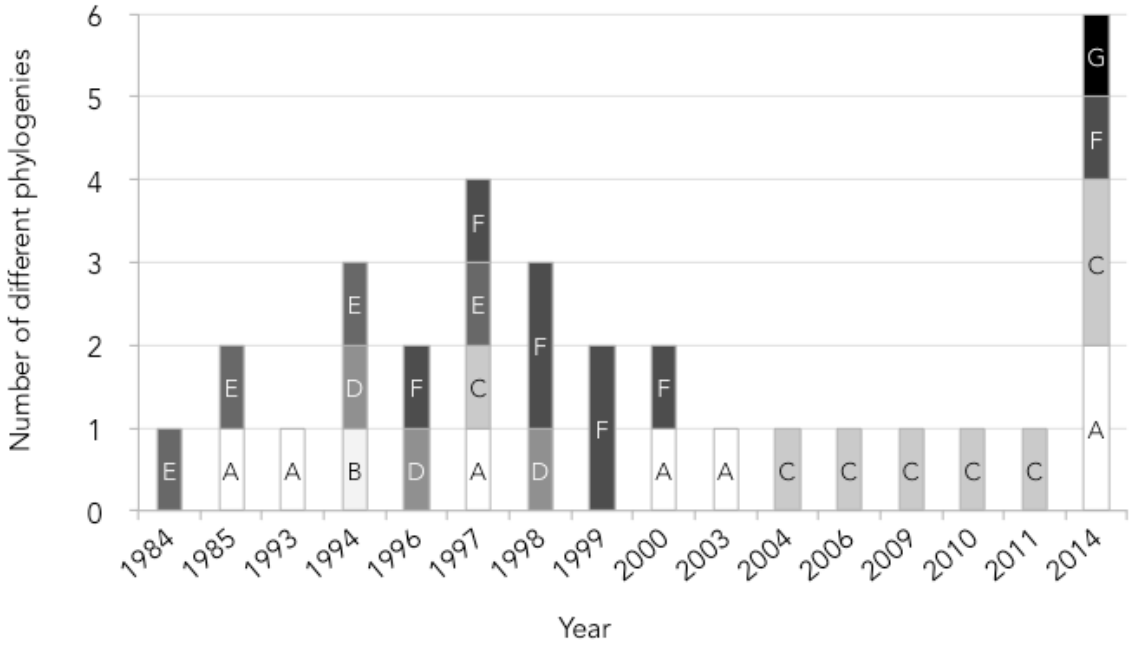


Figure 1.8 Number of phylogenies and phylogeny topology (A, B, C, D, E, F, G) in research papers over the last 30 years. For phylogenetic cladograms see corresponding letter in Figure 1.9.

1.1.6 Morphology

In each of the three bryophyte phyla the gametophyte and sporophyte generations have distinct morphologies. Morphological traits affect species' survival (Violle et al., 2007) and so adaptation to the wide variety of microhabitats inhabited by bryophytes has lead to their great morphological diversity. As there exist good overviews of bryophyte morphology in Vanderpoorten and Goffinet (2009) and in Goffinet and Shaw (2009) and detailed descriptions, including their development and phylogeny, in several publications (e.g. mosses - Shaw et al., 2011; hornworts - Villarreal et al., 2010; Desirò et al., 2013; Villarreal & Renzaglia, 2015), I focus on the defining characteristics of each phylum and what separates these three phyla morphologically. Vitt et al. (2014) provide a succint summary of the main differences between orders in each of the three phyla, but see chapters in Goffinet and Shaw (2009) for a more detailed description. A summary of the main differences between the three phyla can be found in Figure 1.10, p. 16). Further details on some morphological characters relevant to this study are provided in Chapter 2.

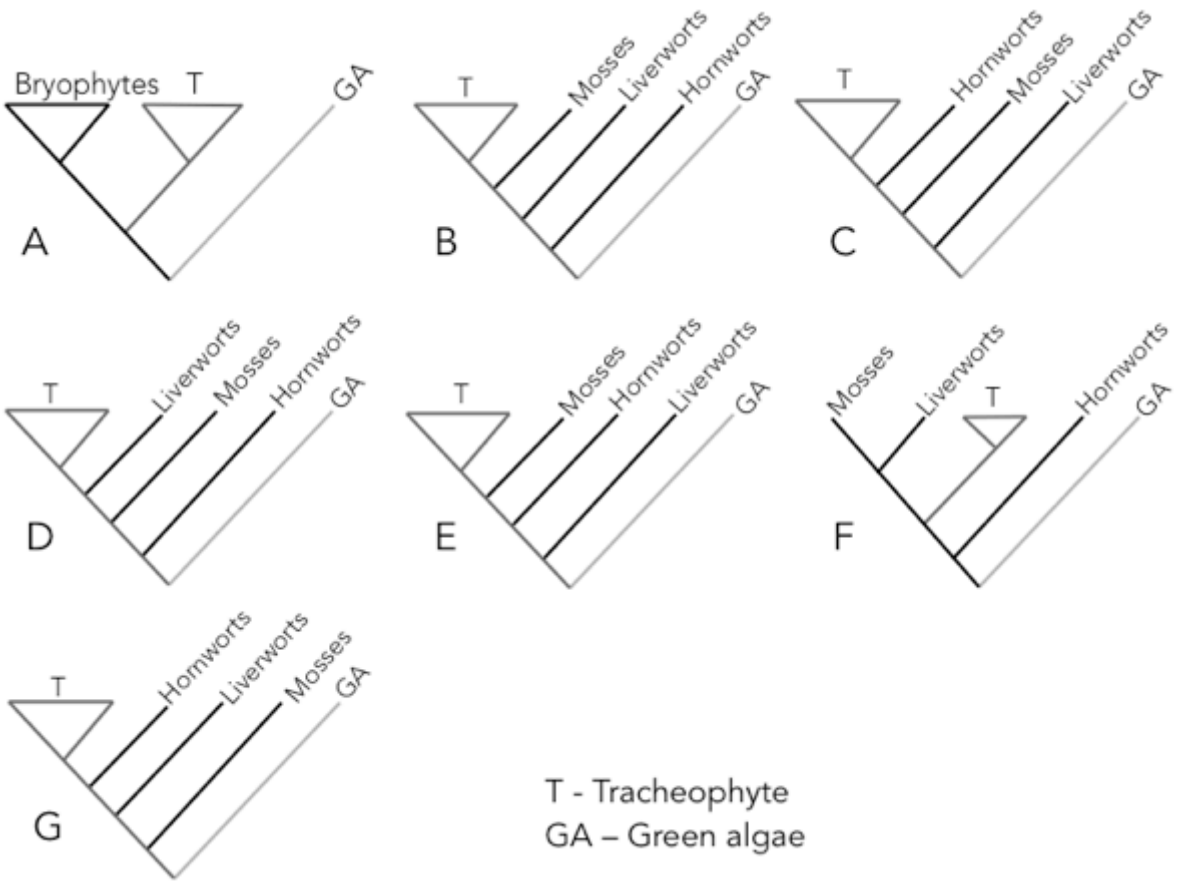


Figure 1.9 The various proposed topologies of early land plant phylogenies. (A) bryophytes are monophyletic and the sister group to tracheophytes. (B) paraphyletic bryophyte assemblage where mosses are the sister lineage to tracheophytes and hornworts are the earliest divergent land plants (basal). (C) paraphyletic bryophyte assemblage with hornworts as the sister lineage to tracheophytes and liverworts basal; this is the currently accepted phylogenetic relationship. (D) paraphyletic bryophyte assemblage with liverworts as the sister lineage to tracheophytes and hornworts basal. (E) paraphyletic bryophyte assemblage with mosses as the sister lineage to tracheophytes and liverworts basal. (F) liverworts and mosses are a monophyletic sister clade to tracheophytes and hornworts are basal. (G) paraphyletic bryophyte assemblage with hornworts as the sister lineage to tracheophytes and mosses basal. For information on data types used to construct the phylogenies and research papers see Table 1.12, p. 47, Appendix 1. (Adapted from: Goffinet, 2000, fig. 4.1, p. 136; Villarreal & Renzaglia, 2015, fig. 1, p. 158.)

1.1.6.1 Marchantiophyta – liverworts

Liverworts are broadly divided into two types based on morphology: the leafy liverworts and the thalloid liverworts. The latter are further sub-divided into simple and complex thalloids. As the names suggest, leafy liverworts have stems with leaves (Figure 1.11 A & B) and thalloid liverworts are composed of thalli – loosely differentiated fleshy lobes, which can be arranged in rosettes or be spreading (Figure 1.11 C). Simple thalloids usually have a midrib and two unistratose lateral wings but no specialised tissues (Figure 1.11 H). In contrast, complex thalloids have storage cells, air pores and air chambers (Figure 1.11 G) (Crandall-Stotler et al., 2009; Vanderpoorten & Goffinet, 2009).

Leafy liverworts have various morphological characters unique to them such as lobules and underleaves (see Figure 2.12, p. 76) – these structures have implications in bryophyte ecology and desiccation tolerance and are discussed further in section 1.2.1.3, p. 23 and section 2.2.3, p. 65,

respectively. Another unique character to all liverworts is the presence of oil-bodies (membrane-bound organelles that contain terpenoid oils and aromatic compounds) in 90% of species (Crandall-Stotler et al., 2009; Vanderpoorten & Goffinet, 2009). These are not known from other extant plant groups.

Sporophytes consist of a capsule on the end of an elongated seta (stalk) with a foot (Figure 1.11 E&F) or in a few species the capsule remains embedded in the thallus. In the genus *Riccia* capsules do not emerge from the thallus and the sporophyte lacks a foot and seta (Figure 1.11 D). The shape of the capsule varies considerably from spherical to ovoid to cylindrical and also star-shaped (Figure 1.11 E & F). Liverworts have several structures that protect the sporangium as it develops: calyptra, shoot calyptra, pterygynium, involucre, perianth, pseudoperianth (Figure 1.11 C); no other land plant has such a variety of structures protecting the sporangium (Vanderpoorten & Goffinet, 2009). The presence of these varies across the liverwort genera and can be used as a diagnostic feature. Following capsule dehiscence (release of the spores via opening of the capsule), the seta, which is composed of thin-walled cells, dehydrates and collapses (Figure 1.11 F) (Crandall-Stotler et al., 2009).

1.1.6.2 Bryophyta – mosses

This bryophyte group is perhaps the one that most resembles tracheophytes when in the field. They are composed of leaves (referred to as laminae in bryology) arranged spirally around a central stem (except in *Fissidens* where leaves are distichous - in two opposite rows) and can be branched or not. Like liverworts, their morphology can be divided into two types: pleurocarpous (Figure 1.11 I & J) and acrocarpous (Figure 1.11 K & L). Pleurocarpous mosses are branched, sporophytes develop on the stem/branch and they tend to grow horizontally along the substrate whereas acrocarpous mosses are unbranched, sporophytes develop at the end of the stem and they tend to grow upright.

Moss leaves are different from liverwort leaves in that they usually have a costa (nerve or midrib), and unlike in liverworts and hornworts, cell size in a leaf varies – this is discussed further in Chapter 2. A morphological character unique to mosses is the presence in some genera of lamellae from the base to the apex of their leaves. These lamellae are rows of photosynthetic cells that project outwards from the costa and are a character used in the identification of some species (e.g. *Pogonatum* species).

The sporophyte possesses a foot, seta and capsule, but unlike in liverworts, the seta remains in place following spore release due to the presence of conducting cells, hydroids (for water) and leptoids (for photosynthates), providing structure to the seta (Goffinet et al., 2009; Vanderpoorten & Goffinet, 2009). In most mosses, spores are released via the operculum (opening with lid-like structure) at the end of the capsule (Figure 1.11 M) (except in four genera: *Andraeae* and *Acrochisma* (4 longitudinal slits); *Takakia* (spiral slit); *Andreobryum* (various longitudinal slits)). Many species also have a peristome: a ring of teeth surrounding the operculum thought to regulate the release of spores. Mature moss capsules of some species retain their calyptra (Figure 1.11 K & M) which protects the capsule while it is developing, and may also control its development (Vanderpoorten & Goffinet, 2009). Calyptra morphology in mosses is an important diagnostic character in some genera (e.g. *Orthotrichum*). A unique feature in moss capsules is the presence of a peristome (a ring of teeth surrounding the capsule

operculum) that controls the release of spores, though it is not present in all moss species (Goffinet et al., 2009). It is also an important diagnostic character.

Interestingly, mosses do not form fungal symbioses, although 80% of other land plants, including liverworts and hornworts, have arbuscular mycorrhizae symbionts (Field et al., 2015). Mosses have multicellular rhizoids (used for anchoring and absorption) whereas in the other two bryophyte groups they are unicellular, which could explain the absence of fungal symbionts in mosses (Field et al., 2015).

1.1.6.3 Anthocerophyta – hornworts

The most distinguishing feature of hornworts in the field is their dark-green coloured thallus (Figure 1.11 O & P), which is why they were initially classified with liverworts (Renzaglia et al., 2009). Within the chloroplasts of most hornworts are pyrenoids, protein structures which contain high concentrations of the photosynthetic enzyme RuBisCO, unlike in other land plant chloroplasts where RuBisCO is found on starch grains (Renzaglia et al., 2009). This fundamental difference in the chloroplasts is shared with algae and it is not fully understood what physiological purpose it serves (Villarreal & Renzaglia, 2015).

When fertile they can easily be distinguished as, unlike liverworts and mosses, the sporophyte does not have a round capsule or stalk but is instead composed of "an elongated cylindrical spore-bearing region" (Renzaglia et al., 2009, p. 157) with a foot at its base (Figure 1.11 Q). This is formed by the longitudinal division of the zygote, contrasting with the transverse division in liverworts and mosses. Again in contrast with the other two bryophyte phyla, spores do not all mature at the same time but instead mature progressively from the apex to the base (Figure 1.11 Q) (Villarreal et al., 2010) meaning that spore dispersal takes place over a longer time (Renzaglia et al., 2009; Vanderpoorten & Goffinet, 2009).

Another distinguishing feature of hornworts is the presence of cyanobacteria endosymbionts. All hornworts have *Nostoc* spp. (cyanobacteria) colonies within their thalli that fix nitrogen and so provide the hornwort with this essential nutrient (Vanderpoorten & Goffinet, 2009). The dark-green appearance of hornworts is due to the presence of these *Nostoc* colonies and the 'canals' formed in the thalli into the colonies to access the nitrogen (Renzaglia et al., 2009). Symbiotic mycorrhizae are also present in hornworts and recent research has yielded interesting discoveries such as the fact that hornworts have the highest diversity of fungal symbionts in any land plant (Desirò et al., 2013; Villarreal & Renzaglia, 2015). This suggests that early land plants had a much wider association with fungi than present-day tracheophytes in order to maximise their chances of successful adaptation to land (Field et al., 2015).

As summarised above, the three bryophyte phyla clearly possess very different morphologies but their small size, lack of lignified tissues, life cycle and poikilohydry means that they share most of the same physiological mechanisms to adapt to their environment.

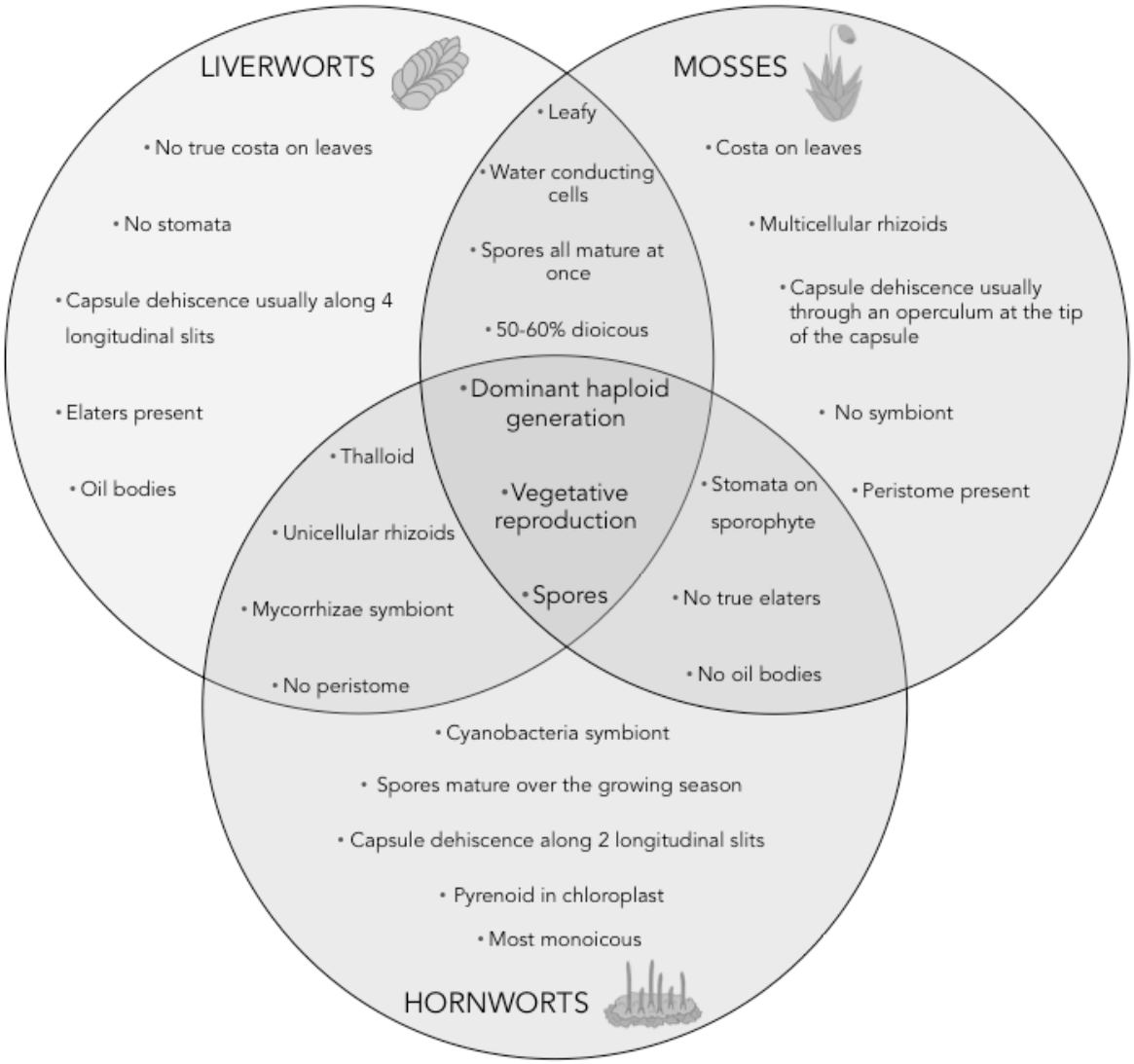


Figure 1.10 Similarities and differences in some key characteristics between the three bryophyte phyla. (Compiled from: Vanderpoorten and Goffinet 2009; Crandall-Stotler, Stotler and Long 2009; Goffinet, Buck and Shaw 2009; Renzaglia, Villarreal and Duff 2009; Villarreal *et al.* 2010; Ligrone, Duckett and Renzaglia 2012; Field *et al.* 2015; Villarreal and Renzaglia 2015). Source: Sarah Stow.

Figure 1.11 *(next three pages)* The various bryophyte morphologies: A-H different liverwort morphologies; I-N different moss morphologies; O-Q hornwort morphology. (A) leafy liverwort *Frullania* sp. (B) leafy liverwort *Bazzania* sp. (C) complex thalloid liverwort, *Riccia* sp., arranged in rosettes and fertile thalloid liverwort (*Sphaerocarpos* sp.) with bottle-shaped pseudoperianths which enclose the capsule (white circle). (D) complex thalloid liverwort *Riccia atromarginata* var. *jovet-astiae* with mature sporophytes embedded in thalli visible. (E) Maturing sporophytes of the complex thalloid *Reboulia hemispherica* developing from midrib at end of the thalli. (F) Capsules of a simple thalloid, *Fossombronia* sp.: a mature capsule (right) and dehisced capsule (left) showing the brown spores. Note how the seta of the dehisced sporophyte is wilting. (G) Splash-cup on the end of a *Marchantia polymorpha* thallus with discoid gemmae inside, note also the air pores appearing as small white dots, it is a complex thalloid. (H) A simple thalloid liverwort, *Metzgeria* sp. All photos by Sarah Stow.

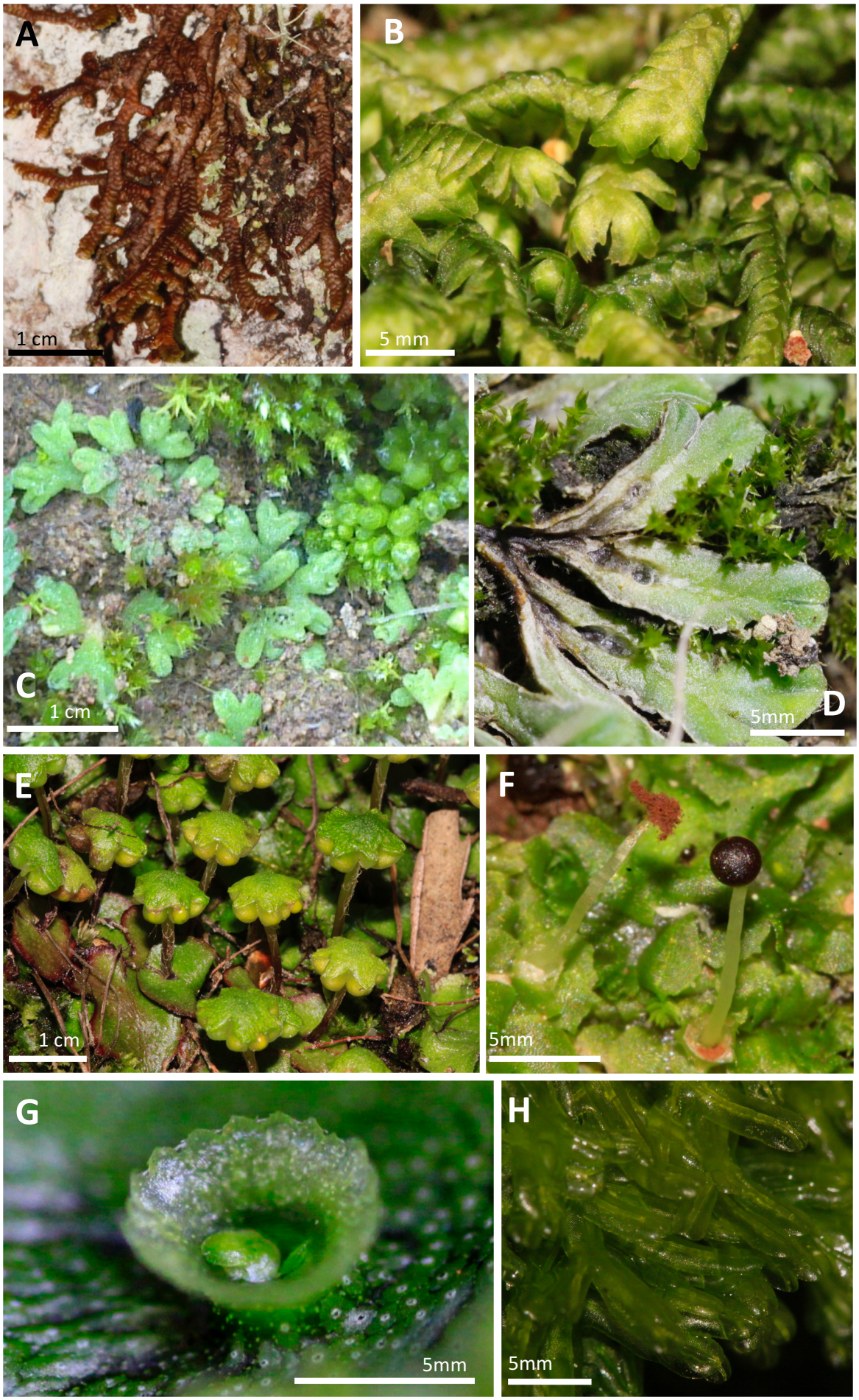

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Figure 1.11 *(cont.)* I-N Different moss morphologies. (I) pleurocarpous moss *Thuidium tamariscinum*. (J) pleurocarpous moss with sporophytes (*Hypnum* sp.). (K) acrocarpous epiphytic moss with capsules (*Ulota* sp.). (L) acrocarpous terrestrial moss *Polytrichastrum formosum*. (M) sporophyte of a moss showing calyptra (top left), seta and mature capsule with operculum (*Macromitrium* sp.). (N) gemmae on the end of a stalk (*Aulocomnium* sp.). All photos by Sarah Stow.

Figure 1.11 ***(cont.)*** **O-Q Hornwort morphology (O) hornworts on an earth embankment. (P) developing sporophytes. (Q) sporophytes showing direction of spore maturation: the tips are brownish-yellow with mature spores being released, further down the sporophyte is yellow-orange and at the base it is still green; inset shows mature yellow spores on the dehisced sporophyte. All photos by Sarah Stow.**

1.1.7 Life at a smaller scale – physiological ecology

Although bryophytes' physiology underpins their ecology, I shall only briefly outline some main points, not only because it is a vast topic and there are several recent reviews on different aspects of their physiology (e.g. overall physiology in Cornelissen et al., 2007; Glime, 2007; Proctor, 2009; Vanderpoorten & Goffinet, 2009; mineral nutrition in Bates, 2009; desiccation tolerance in Proctor et al., 2007; Oliver, 2009) but also because chapters 2 & 3 deal with aspects of their physiology specific to this thesis and so more details are given there. Most bryophyte ecology studies have traditionally come from temperate areas but since the 1970s there has been an increase in tropical bryophyte research (Mervin & Nadkarni, 2001) particulary within the last 20 years.

The origin of bryophytes between the first land colonisers and tracheophytes means that they possess biochemical and cellular biology features from these two groups (Hanson & Rice, 2014). However, their small size means that the physics of gravity, surface area, surface tension and boundary layer apply differently in bryophytes than in tracheophytes (Hinshiri & Proctor, 1971; Proctor, 2000a, 2009). Poikilohydry and desiccation tolerance govern the response of bryophytes to environmental conditions and hence their ecology (Proctor, 1990, 2009).

1.1.7.1 Water, Light & Temperature

One of the most important characteristics affecting bryophytes' physiology is that they are poikilohydric – unable to regulate their water content – they are therefore dependent on their immediate ambient environment, a trait retained from the first terrestrial plant colonisers (Bates, 1998). Poikilohydry allows bryophytes to lose nearly all their cellular water and vegetative desiccation tolerance, an adaptive strategy to life on dry land, allows them to survive in a state of suspended animation (Proctor, 2009). Whereas other major land plant groups have lost their poikilohydry, bryophytes have maintained this in part because this is the optimal strategy for their size (Tuba et al., 1998; Proctor et al., 2007; Proctor, 2009). Their lack of thick cuticle and thin leaves/thalli (with the exception of some species) allow them to take in water throughout their whole surface (Proctor, 2009; Vanderpoorten & Goffinet, 2009) and water conduction takes place in capillary spaces on the plant – they are ectohydric (Proctor, 2009). Bryophytes can lose and gain water quickly, in contrast to most tracheophytes, which has implications for respiration and photosynthesis (Proctor & Tuba, 2002). Water is not only necessary for metabolic processes but also for fertilisation so that the sperm can reach the archegonia; the small size of bryophytes minimizes this distance (Shaw & Renzaglia, 2004; Goffinet & Shaw, 2009).

Bryophytes exhibit a range of tolerance to light from those that live in permanent shade to those that live in full sun. As photosynthetic rate is limited by light availability, two parameters govern light relations in plants: the light compensation point - the minimum light level required for positive net photosynthesis (photosynthesis and respiration rates are equal), and the light saturation point - the light level at which photosynthetic rate does not increase (no more photons can be accepted by the photosynthetic apparatus) (Vanderpoorten & Goffinet, 2009). At light levels above the saturation point, damage can occur due to oxidation, requiring plants to use photo-protection mechanisms (Oliver, 2009; Vanderpoorten & Goffinet, 2009).

Many bryophyte species have broad optimum temperature ranges between 15 and 25^{o}C, but some species can survive extreme temperatures (cold and hot) and some have a very narrow

optimum temperature range (Vanderpoorten & Goffinet, 2009). The optimal temperature is determined by the net photosynthetic rate and bryophytes tend to achieve net photosynthesis at lower temperatures than tracheophytes (Vanderpoorten & Goffinet, 2009). Damage that occurs at high temperatures is similar to that of high light levels: disintegration of membranes and bleaching of the photosynthetic apparatus through the loss of pigments (Vanderpoorten & Goffinet, 2009). As well as damage, higher temperatures increase photorespiration which is energetically inefficient due to loss of carbon (Glime, 2007; Proctor, 2010). Freezing tolerance, an important feature in boreal species, is less relevant in this thesis (which focuses on temperate and tropical bryophytes) although it should be noted that even tropical bryophytes have been shown to be able to survive below 0^{o}C temperatures (Vanderpoorten & Goffinet, 2009). The retention of this ability in bryophytes that do not experience freezing temperature indicates the evolutionary importance of freezing tolerance in the first plant land-colonisers.

There is a trade-off between having sufficient water for metabolic processes and capturing enough light for photosynthesis. More details on water and light relations in bryophytes are given in Chapter 2.

1.1.7.2 Nutrients

Bryophytes differ significantly from vascular plants in how they acquire nutrients (Bates, 2009). Bryophytes can take in mineral nutrients from the atmosphere, particularly epiphytic species (atmospheric dust, salt particles, ammonia and nitric acid (Barkman, 1969)), and the substrate they grow on (Vanderpoorten & Goffinet, 2009). Desiccation greatly affects nutrition as nutrients are lost when cellular water is lost (Bates & Baaken, 1998) and upon rehydration leaking of solutes occurs; consequently, most bryophyte growth occurs when moisture availability is high (Vanderpoorten & Goffinet, 2009). Additionally, water availability determines nutrient uptake ability and rates (Bates, 2009). A consequence of poikilohydry is that bryophytes accumulate mineral nutrients and chemicals, which can become toxic (Bates, 2009; Vanderpoorten & Goffinet, 2009); this trait has led to bryophytes being successfully used as indicators in biomonitoring studies (Bates, 2009). For a detailed review of bryophyte nutrient requirements, capture, transport, and ecology see Bates (2009).

1.1.7.3 Life-strategy

Life strategy is a concept that brings together different aspects of bryophyte morphology and life-history: life-span, reproductive effort, reproduction type, age of first reproduction, spore size, longevity and growth-form (Table 1.2); species are categorised together based on shared values of these characteristics (During, 1979; Bates, 2009). Life-strategy is a useful concept as it helps explain and determine bryophyte distribution and aspects of their ecology, although the delimitation in life-strategy is not as strict as the categories defined and variation exists (Bates, 2009).

Table 1.2 The six main life-strategies of bryophytes. Taken from Bates 2009, table 8.2, p. 327.

Life span	Spore number and size		Reproductive effort
	Many small	Few large	
<1 year	*Fugitive*	*Annual shuttle*	High
A few years	*Colonist*	*Medium shuttle*	Medium
Many years	*Perennial stayers*	*Dominant*	Low

1.2 Bryophyte ecology & conservation

1.2.1.1 Substrate

Because of their ability to absorb water through their leaves and stems, rather than restricted to the roots as in tracheophytes, bryophytes can occupy substrates unavailable to other plant groups (Proctor, 2009); they can occupy a wide range of substrates, see Table 1.3. Bryophytes are often among the first colonisers of bare soil and rock providing a subsequent habitat for other plant groups and animals. Though many bryophyte species are specific to their substrate type (only occupying one type), others can occupy a range of substrates (Barkman, 1969; Bates, 2009). The life-strategy of a bryophyte and three main substrate factors affect whether a bryophyte inhabits a particular substrate: longevity, chemical properties, and water-holding capacity (Bates, 2009). Bryophytes that have short-life spans are able to colonise ephemeral substrates whereas species with long life-spans require stable substrates (Bates, 2009).

Table 1.3 Substrates occupied by bryophytes, terminology used and relative number of species occupying those substrates. Compiled from Smith (1982) and Bates (2009).

Substrate	Terminology	Species occupying substrate
Rock	Epilith or saxicolous	Many
Alkaline	Calcicole	Many
Acidic	Calcifuge	Many
Metal-rich	Metallophyte	Few
Bark	Epiphyte or corticolous	Many
Leaf surface	Epiphyll	Many (mostly tropical)
Soil	Epilith or terricolous	Many
Salt-marshes & coastal dunes	Halophyte	Few
Dead vegetation		
Non-ligneous	Litter species	Some
Logs and stumps	Epixylic	Many
Dead animals	Coprophile	Few
Dung	Coprophile	Few

Epiphytes and saxicoles are the best-studied groups (Barkman, 1969; Smith, 1982; Bates, 2009). Substrate specificity has been linked to chemical properties of the substrate and environmental variables as well as to the ecophysiology of the bryophyte itself (Bates, 2009). Bryophytes can be classified as substrate obligates (specialist, occupying only one substrate type) or facultatives (occupying two or more substrate types) (Smith, 1982).

1.2.1.2 Habitat

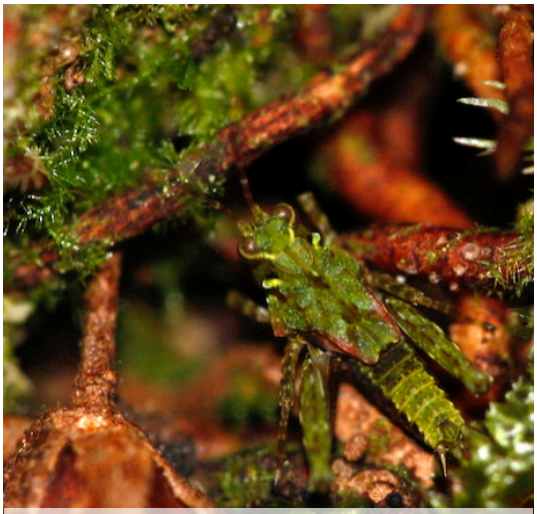
Whereas vascular plant distribution is mostly dictated by edaphic and macro-climatic variables (Barkman, 1969), epiphytic bryophyte distribution is determined by microclimatic variables, predominantly moisture availability (Barkman, 1969; Proctor, 2009). Certain bryophytes, particularly rare ones, are associated with specific microhabitats (Vanderpoorten & Engels, 2003). This affinity to microhabitat can be illustrated by *Riccia cavernosa*, typically a species of dried ponds in grasslands, but that can be found in pavement cracks with weeds including the very common mosses *Bryum argenteum*, *B. dichotomum*, and *Funaria hygrometrica* (Vanderpoorten &

Engels, 2003) showing that microhabitat, rather than habitat, is the determining factor in this species' distribution. However, some bryophyte species may occupy different niches in different regions (Mateo et al., 2013). In Alberta, Canada, rare mosses are mostly composed of acrocarps, stress tolerators and rare species prefer rock and soil microhabitats as well as cliff and alpine mesohabitats (Vitt & Belland, 1997).

1.2.1.3 Interactions with other species

Bryophytes interact with a range of other organisms, from protozoa to vascular plants to large mammals (see Glime (2017a) for a thorough and fascinating review of the interactions of bryophytes with various animal taxa). Competition with other plants is generally low (Bates, 1998; Proctor, 2000a; Vitt et al., 2014) due to the fact that bryophytes occupy microhabitats that most vascular plants cannot – those with low water availability or high exposure (Proctor, 2000a; Alpert, 2000a). This is particularly true of hornworts which tend to be habitat pioneers (Vanderpoorten & Goffinet, 2009). However, simply due to the larger size of vascular plants, some bryophytes may be out-competed due to the creation of shade (Rydin, 2009). In a study comparing a bryophyte and a tracheophyte from the same habitat, their phenology was found to be complementary: the bryophyte was most productive at the coldest time of year, and the tracheophyte at the hottest time of year (Vanderpoorten & Goffinet, 2009). This was explained by the fact that the tracheophyte herbaceous cover is lower in winter allowing more light to reach the ground-dwelling bryophyte. In their turn, bryophytes can out-compete vascular plants by preventing the germination and establishment of seedlings either by creating a physical barrier or modifying the soil's environmental conditions (Rydin, 2009; Vanderpoorten & Goffinet, 2009). A prime example of this is the accumulation of *Sphagnum* leading to the creation of bogs which have both a low pH (chemical barrier) and a thick organic layer that prevents vascular plant roots from reaching the mineral soil layer (physical barrier) (Rydin, 2009).

Bryophytes interact extensively with invertebrates and protozoans, with the term "bryofauna" used to describe animals that associate with bryophytes, whether occasionally or throughout their whole life-cycle (Gerson, 1982). Some leafy liverworts have helmet-shaped lobules which effectively act as water storage "sacs" but which can also provide a habitat for invertebrates (Hess et al., 2005; Crandall-Stotler et al., 2009). Recently, a new species of mite was found in the water sacs of an Australian *Frullania* species, using the liverwort for shelter and feeding (Colloff & Cairns, 2011). Further to this, zoophagy has been documented in *Colura* and *Pleurozia* species which have water sacs with a lid that can open and close thus trapping protozoans (Hess et al., 2005). Whether the liverworts are actively attracting the animals or their trapping is incidental is debated, but the decomposition and excreta of the animals is thought to provide a source of nutrients (Hess et al., 2005; Crandall-Stotler et al., 2009). Bryophytes can also indirectly interact with animals by providing them with the ability to camouflage e.g. amphibians and invertebrates (Figure 1.12). In Papua New Guinea weevils have been found to encourage the growth of bryophytes (among other cryptogams) on their backs in order to have a permanent camouflage plant layer; the bryophytes either make the weevils inconspicuous to predators or add an unpleasant taste to the weevil (Gressitt et al., 1965).



Ground-dwelling grasshopper,
Anjozorobe, Madagascar.
S. Stow

Tree frog (*Platypelis* sp.),
Tsitongambarika Forest, Madagascar.
S. Stow

Figure 1.12 Animals camouflaging on bryophytes in Madagascar: a grasshopper and a frog.

Whereas many plant species have evolved strategies to employ animals as vectors of pollen, seeds and fruits, bryophytes are more reliant on water and wind for dispersal of their spores and vegetative propagules. However, bryophyte species have been found to benefit from animal dispersal, particularly coprophiles, e.g. slugs transporting vegetative branches of the epixylic moss *Dicranum flagellare* Hedw. and flies dispersing sticky spores of coprophilous mosses (Bates, 2009).

1.2.2 Ecosystem services

The underpinning ecosystems and their biodiversity provide to humans, in terms of economic development and sustainability, has been formally recognised and assessed in the Millenium Ecosystem Assessment (Alcamo et al., 2003; Millennium Ecosystem Assessment, 2005). Despite their small stature, bryophytes play an important role in various ecosystem services and are fundamental components of biodiversity. Table 1.4 summarises some of the services they provide and a few of these are expanded upon below.

Table 1.4 Summary of some ecosystem services provided by bryophytes showing that despite their diminuitive size they are important contributors to ecosystems. Service types based on those in the Millenium Ecosystem Assessment (2005).

Service type	Service provided	Source
Provisioning Services		
Food	Mushroom cultivation; shipping of food; hydroponic gardening; air-layering of fruit trees; pesticides & herbicides	(Asakawa, 2007; Vanderpoorten & Goffinet, 2009)
Fresh water	Water filtration	(Glime, 2017b)
Fuel	Household heating; electricity production	Glime, 2017b
Fiber	Bedding; packing material; absorbing (bandages & nappies); building material	(Harris, 2008) (Toms & Devoto, 1734)
Biochemicals, natural medicines, pharmaceuticals	Biological control; desiccation tolerance induced in human cells; antimicrobial; antibiotic; anticancer	(Sabovljević et al., 2001; Alpert, 2005)
Genetic resources	Genetic research; bioengineering	(Glime, 2017b)
Regulating Services		
Climate regulation	Long-term storage of carbon – 33% of global terrestrial carbon	Vanderpoorten & Goffinet, 2009
Water regulation	Precipitation interception; water storage; increasing local water table	Vanderpoorten & Goffinet, 2009
Erosion regulation	Protect soil from wind and water erosion; protecting soil from extreme air temperatures and drought	Vanderpoorten & Goffinet, 2009
Water purification and waste treatment	Removing heavy metal contamination; filtration to remove microbes, pesticides and odors; oil spill cleanups	(Glime, 2017b) Hallingback & Hodgetts
Disease regulation	Medicinal properties of chemical compounds	Hallingbäck & Hodgetts, 2000
Pest regulation	Biological control; pesticides & herbicides	Glime, 2017b
Cultural Services		
Spiritual and religious values	"Moss men" procession in Spain; Bhuddist temple moss gardens	(Martínez-Abaigar & Núñez-Olivera, 2001) Hallingback & Hodgetts
Recreation and ecotourism	Aquariums; tourists visiting "moss men" procession	(Martínez-Abaigar & Núñez-Olivera, 2001); Glime, 2017b
Aesthetic values	Horticulture; moss walls; moss tables, moss bath mats	(Hallingbäck & Hodgetts, 2000)
Inspirational	Literature; graffiti	(Budke, 2015) Hallingback & Hodgetts
Educational	Model organisms for physiological and biochemical experiments	(Hallingbäck & Hodgetts, 2000)

Service type	Service provided	Source
Cultural heritage	Japanese moss gardens; protecting architecture from weathering	(Hallingbäck & Hodgetts, 2000)
Supporting services		
Soil formation	As habitat pioneers they create suitable conditions for the establishement of other organisms	Vanderpoorten & Goffinet, 2009
Nutrient cycling	Mineral nutrient storage and source	Vanderpoorten & Goffinet, 2009
Primary production	Major biomass component	Vanderpoorten & Goffinet, 2009

1.2.2.1 Provisioning services

Biochemicals, natural medicines, pharmaceuticals

Despite the wide use of vascular plants in medicine throughout human history, there has been very little traditional use of bryophytes – only 235 species are recorded to be used in ethnobotany (Harris, 2008). However, with modern techniques it has been found that bryophyte chemical compounds can have a range of applications (Table 1.5), particulary antimicrobial; antibiotic (Basile et al., 1998; Olofin, 2013) and anticancer (Sabovljevic et al., 2016), with over 400 chemical compounds known (Asakawa, 2007).

Table 1.5 Bryophytes and their medicinal properties. (Taken from Asakawa, 2007, Table 1, p. 558.)

Species	Medicinal application
Mosses	
Bryum argenteum	Antidotal, antipyretic, antirhinitic activity; for bacteriosis
Cratoneuron filicinum	For malum cordis (heart disease)
Ditrichum pallidum	For convulsions, particularly in infants
Fissidens japonicum	Diuretic activity; for growth of hair, burns, and choloplania (jaundice, icterus)
Funaria hygrometrica	For hemostatis, pulmonary tuberculosis, vomitus cruentus (hematemesis), bruises, and athlete's foot dermatophytosis dermatomycosis, dermomycosis)
Haplocladium catillatum	Antidotal and antipyretic activity; for adenopharyngitis, pharyngitis, uropathy, mastitis, erysipelas (rose), pneumonia, urocystitis, and tympanitis
Leptodictyum riparium	Antipyretic; for choloplania and uropathy
Mnium cuspidatum	For hematostasis and nosebleed
Oreas martiana	For anodyne (pain), hemostasis, external wounds, epilepsy, menorrhagia, and neurasthenia (nervosism, nervous exhaustion)
Philonotis fontana	Antipyretic and antidotal activity; for adenopharyngitis
Plagiopus oederi	As a sedative; for epilepsy, apoplexy, and cardiopathy
Polytrichum species	Diuretic activity; for growth of hair
Polytrichum commune	Antipyretic and antidotal; for hemostasis, cuts, bleeding from gingivae, hematemesis, and pulmonary tuberculosis

Species	Medicinal application
Rhodobryum giganteum	Antipyretic, diuretic, and antihypertensive; for sedation, neurasthenia, psychosis, cuts, cardiopathy, and expansion of heart blood vessels
Rhodobryum roseum	As a sedative; for neurasthenia and cardiopathy
Taxiphyllum taxirameum	Antiphlogistic; for hemostasis and external wounds
Weissia viridula	Antipyretic and antidotal; for rhinitis
Liverworts	
Conocephalum conicum	Antimicrobial, antifungal, antipyretic, antidotal activity; used to cure cuts, burns, scalds, fractures, swollen tissue, poisonous snake bites, and gallstones
Frullania tamarisci	Antiseptic activity
Marchantia polymorpha	Antipyretic, antihepatic, antidotal, diuretic activity; used to cure cuts, fractures, poisonous snake bites, burns, scalds, and open wounds
Reboulia hemisphaerica	For blotches, hemostasis, external wounds, and bruises

Science

In science bryophytes have been used in important plant physiology and genetics experiments such as the identification of sex chromosomes in plants (Anderson, 1963) and a moss, *Physcomitrella patens* (Hedw.) Bruch & Schimp., has become a model organism in the study of genetics (Cuming, 2009). Analyses of radiocarbon dates cores from bryophyte deposits (mainly peatland) provides historical data on the earth's climate (Glime, 2017b). The proportion of different species along a core's profile can indicate if that time period was wet and cold or warm and dry (Vanderpoorten & Goffinet, 2009). Aditionally, analyses of populations' genetic diversity and structure can allow researchers infer how bryophytes were affected by glaciations (Vanderpoorten & Goffinet, 2009).

Because bryophytes absorb water and nutrients directly through their leaves and are not able to regulate water uptake and gas exchange, they can accumulate large amounts of chemical compounds present in their surrounding environment (Vanderpoorten & Goffinet, 2009). As such, since the 1960s bryophytes have been used as successful indicators of air pollution (Winner & Bewley, 1978), heavy metal pollution (e.g. Burton & Peterson, 1979; Figueira et al., 2002) and water pollution (Heino et al., 2005), overwhelmingly in temperate regions of the world (Frahm, 2003). Researchers can either record the presence and abundance of species growing naturally within an area (and potentially monitor them over time) or place specific bryophyte species at particular locations to monitor the levels of pollutants by subsequently measuring the pollutant concentrations in the bryophyte's tissues (e.g. (e.g. Meyer et al., 2012). The former method either relies on creating indices based on species community composition and species abundance (e.g. (e.g. Aguiar et al., 2010; Delgado & Ederra, 2013) or pollutant concentrations can also be measured from collected samples (e.g. Aceto et al., 2003).

More recently, bryophytes have been put forward as indicators of biodiversity (Salazar Allen et al., 1996) and habitat change (Drehwald, 2005) with a few studies showing that they can be useful indicators of diversity levels in other organisms (e.g. Frego, 2007). The rationale behind the usefulness of bryophytes to indicate habitat change lies in their rapid responses to changes in

insolation and relative humidity (Frahm & Gradstein, 1991; Sporn et al., 2009). Desiccation-intolerant shade epiphytes are particularly susceptible to increases in air circulation and solar radiation which result from anthropogenic habitat degradation (Acebey et al., 2003). A handful of studies have shown that bryophytes have great potential as indicators of habitat change (Drehwald, 2005; Frego, 2007) yet this important application remains under-studied, particularly in tropical Africa.

1.2.2.2 Regulating services

Bryophytes can play an important role in altering the habitat they occupy such as in forests with a heavy epiphyte layer or in peatlands. In forests, a large amount of nutrients are stored in the bryophyte layer (Bates & Baaken, 1998) and in some tropical montane forests they can make up as much as 12% of the above ground biomass and 90% of the epiphyte biomass (Hallingbäck & Hodgetts, 2000). This significant amount of biomass means bryophytes affect the cycle of nutrients, carbon and water (Vanderpoorten & Goffinet, 2009).

Water regulation

Most tropical forest bryophytes are epiphytes (Wagner et al., 2014) and their interception of precipitation (from 22% to 63% of total precipitation (Frahm, 1990)) means they act as important water reservoirs, more so in tropical than temperate forests, providing a water source for other forest species when it is dry (Pócs, 1982). The amount of water stored varies but has been calculated to reach 15 000 kg/ha in tropical forests (Vanderpoorten & Goffinet, 2009). Another ecosystem where bryophytes play a central role in the water budget is peatland – a *Sphagnum*-dominated ecosystem. Due to their cell structure, *Sphagnum* species can hold large amounts of water leading to a rise in water tables locally (Vitt & Wieder, 2009) and are a vital component .

Climate regulation

The most significant way in which bryophytes contribute to climate regulation is through the long-term storage of carbon – one third of the world's carbon is stored in bryophyte "ground layers" (Smith et al., 2015) such as peatlands. Despite their diminuitive size, *Sphagnum* stores more carbon than any other plant genus (Vanderpoorten & Goffinet, 2009). The extraction and burning of peat and the conversion of peatland to other land-uses therefore has a significant effect on the amount of carbon released into the atmosphere. It has been estimated that it would take 692 years for the carbon lost through peatland removal to be recaptured (compared to 93 years for the same area of tropical forest) (Danielsen et al., 2009).

Erosion regulation

Bryophytes can protect soil from wind and water erosion; extreme air temperatures and drought (Vanderpoorten & Goffinet, 2009). They are a major component of "cryptogamic crusts" which, as the name indicates, are a layer composed of bryophytes, lichens, green algae, cyanobacteria and fungi found commonly in grasslands and arid and semi-arid habitats (Eldridge et al., 2000; Vanderpoorten & Goffinet, 2009). By binding soil particles and creating a more heterogenous soil topology water surface runoff is reduced through an increase in the soil's permability and water capacity (Eldridge et al., 2000; Vanderpoorten & Goffinet, 2009). Cryptogamic crusts can mitigate the effects of over-grazing by providing a source population of bryophytes that can colonise bare soil patches and subsequently create conditions for vascular plants to establish (Eldridge et al.,

2000). These crusts also provide a physical barrier that protects against wind erosion of surface particles and the dispersal of soil when raindrops hit (Eldridge et al., 2000).

1.2.2.3 Cultural services

Bryophytes have long been used by humans for a number of varied purposes including, most commonly (and still to this day), in horticulture for transporting and propagating plants (Edwards et al., 1757; Glime, 2008); as a substitute for mortar in walls (Toms & Devoto, 1734); during the preparation of quicksilver (mercury – an essential part of alchemy, the starting point of modern chemistry and physics) (Hill, 1773); as stuffing for mattresses (Encyclopaedia Perthensis, 1816) and recently even used in graffiti art (Budke, 2015) (see Table 1.4 for further cultural uses). Despite their uses, bryophytes have not always been appreciated: in Miller's gardening dictionary (1735) he states that "(…) they are plants of no use or beauty [in gardening] (…)" and Edwards et al. (1757) oscillate between recommending them "Moss is vastly preferable to straw [when packing plants]" to instructing how to get rid of them: "(…) rub off all the moss and other foulness from the trunk (…)".

1.2.2.4 Supporting services

Soil formation

Pioneer bryophytes are among the first organisms to establish on bare soils (e.g. volcanic deposits) creating conditions for vascular plants to establish themselves (Vanderpoorten & Goffinet, 2009).

Nutrient cycling

Because bryophytes absorb large quantities of water, they also play an important role in ecosystem nutrient cycling and accumulation (Frahm, 1990). Mineral nutrients stored in forest ground and epiphyte layers (of which bryophytes are often the majority (Nadkarni, 1984) provide not only nutrient storage but also a readily available source of nutrients. Although a larger amount of nutrients is stored in standing trees (see lower montane forest values in Table 1.6, p. 30), these have much slower decomposition rates than bryophytes (Vanderpoorten & Goffinet, 2009).

Primary production

Bryophytes can contribute significantly to ecosystems' biomass, up to 12% of total above-ground biomass in tropical montane forest (Hallingbäck & Hodgetts, 2000). They are the major component of epiphytic biomass in tropical forests and are therefore vital to nutrient and water cycles in these ecosystems (Gehrig-Downie et al., 2011; Pardow et al., 2012). Unsuprisingly, bryophytes have a much lower rate of productivity than vascular plants: mosses have a CO_2 uptake of 3 mg dm^{-2} $hour^{-1}$ compared to 40 to 80 mg dm^{-2} $hour^{-1}$ in vascular plants (Glime, 2017c). However, they still provide around 7% of terrestrial net primary production and about 50% of terrestrial biological nitrogen fixation (Glime, 2017c).

1.2.3 Threat

1.2.3.1 Why are bryophytes threatened?

Although the extraction of bryophytes from the wild is a significant factor in the decline of some species (notably *Sphagnum* species in peatlands), the greatest threats are habitat destruction and change; soil, water and air pollution; and forestry practices (afforestation, exotic species and

introduction of invasive species) (Hallingbäck & Hodgetts, 2000; Vanderpoorten & Hallingbäck, 2009; Sérgio et al., 2013). Bryophytes of wetlands and peatlands decrease or even disappear when the water table drops as a result of wildfires and a drier climate due to climate change (Smith et al., 2015). Their sensitivity to environmental conditions, a useful trait for bioindication, makes them particularly susceptible pollution, particularly sulphur dioxide and heavy metals (Hallingbäck & Hodgetts, 2000).

Table 1.6 Mineral nutrient inputs and accumulation in different forest types. (Oakwood and spruce forest data taken from: Bates, 2009, table 8.1, p. 317; montane forest data taken from: Nadkarni, 1984)

	Mineral nutrient - kg ha^{-1} $year^{-1}$					
Temperate oakwood - ground	Ca	Mg	K	Na	N	P
Throughfall & litterfall input	31.0	18.1	29.2	106.9	-	-
Bryophyte accumulation	4.1	3.90	14.3	1.6	-	-
Bryophyte accumulation as percentage of throughfall & litterfall	13%	22%	49%	1.5%	-	-
Black spruce forest - ground	Ca	Mg	K	Na	N	P
Throughfall & litterfall input	29.0	5.0	4.0	-	24.0	0.6
Bryophyte accumulation	14.0	12.0	16.0	-	92.0	5.0
Bryophyte accumulation as percentage of throughfall & litterfall	48%	240%	400%	-	383%	833%
Lower montane forest - epiphyte	Ca	Mg	K	Na	N	P
Total aboveground capital	432.0	159.0	259.2	-	432.0	25.9
Total foliar capital	46.5	24.8	41.1	-	78.7	4.2
Bryophyte capital	5.0	1.7	9.5	2.9	43.3	1.2
Bryophyte capital as percentage of total aboveground	1.0%	0.9%	3.2%	-	8.5%	4.1%
Bryophyte capital as percentage of foliar	10.6%	6.7%	23.1%	-	55.0%	29.5%

As well as extrinsic factors, bryophyte life-history, ecology and evolution may determine their level of threat by making the species naturally rare. Bryophyte rarity can be defined within Rabinowitz's (1981) widely used "forms of rarity" classification (Table 1.7): bryophytes tend be habitat specialists with narrow ranges (Birks et al., 1998). Their small size means that most bryophytes are dependent on particular microhabitats and therefore have narrow habitat specificity. As such, most species are not locally abundant and therefore rare (Birks et al., 1998). Species with disjunct distributions and endemic species are also rarer (Vitt & Belland, 1997). However, other factors besides geographical range, population size and habitat specificity affect bryophyte rarity and must be taken into account.

Table 1.7 Species rarity according to Rabinowitz (1981) and applied to bryophytes by Gabriel et al. (2011, Figure 1, p. 161). Three variables can be used to decide if a species is rare: geographical range, habitat specificity and local abundance. These leads to seven forms of rarity and only one combination which makes species common.

Geographical range		Wide		Narrow	
Habitat specificity		Generalist	Specialist	Generalist	Specialist
Local abundance	Large	Common	Narrow ecological tolerance	Restricted (rare by range)	Restricted with narrow ecological range
	Small	Scarce (rare by adundance)	Scarce with narrow ecological tolerance	Restricted and scarce	Restricted & scarce with narrow ecological tolerance

Bryophyte life-strategy has an impact on rarity e.g. species that produce small spores may be more widespread than ones with large spores (During, 1979; Söderström et al., 2007); dioicious species tend to be rarer than monoicous ones (Longton, 1992; Laaka-Lindberg et al., 2000); and species with short life-spans are also rarer (Vellak et al., 2007). In fact, due to the dispersal of bryophytes by spores, studies have shown that rare bryophytes tend to have wide geographic distributions (Gabriel et al., 2011). There is also taxonomic bias as rarity is more prevalent in certain taxonomic lineages e.g. the Bryales (Vitt & Belland, 1997). Aditionally, historical climate change has an impact on rarity as many rare species are found in glacial refugia (Sérgio et al., 2013). Rare mosses tend to be found in rare habitats (Cleavitt, 2005; Vellak et al., 2007) and the number of mesohabitats can also be a determining factor in the presence of rare species (Vitt & Belland, 1997). It has been noted that the ability to predict extinction probability would be useful tool for the preventative management of habitats (Davies et al., 2000). Additionally, Rabinowitz's (1981) system may not always apply well to bryophytes as studies have shown that there is usually a lack of data on bryophyte abundance (Gabriel et al., 2011) and that bryophytes do not have the same rarity patterns as vascular plants (Söderström & Séneca, 2008). To overcome the issue of lack of abundance data, Söderström & Séneca (2008) created a "Rarity Index" which identifies how important an area is for restricted species. Range restricted species were identified using a diversity index based on the proportion of areas occupied by a species in a region. Subsequently, the Rarity Index is calculated using the relative proportion of restricted species in an area compared to the overall number of restricted species.

Although rarity is an important aspect to consider when assessing a species' level of threat, it is equally important to take into account common species (Gaston & Fuller, 2008). Despite their "common" status, historical and current significant declines of common species have been recorded (Gaston & Fuller, 2007), perhaps most famously the extinction of the Passenger pigeon in North America, with implication in the abundance of associated species (Gaston & Fuller, 2008). The significant decrease in number of individuals, or even extinction, of common species shows that just because a species is common does not mean it is safe from threat (Gaston & Fuller, 2008). Common species are fundamental components of the ecosystems they are part of simply due to their abundance and any changes in this can affect the functioning of the ecosystem

(Gaston & Fuller, 2008). Due to the dispersal capacity of bryophytes (because of the use of spores), there are many examples of species that are common in one region of the world but rare in another (e.g. Hallingbäck, 2002). Monitoring common species in one region could therefore ensure their global preservation. This is especially useful if the species is rare in an understudied region of the world, but common in a region where data availability and expertise is high.

1.2.3.2 How threatened are bryophytes?

To assess the extinction risk of species the Red List system was created by the International Union for the Conservation of Nature (IUCN); a system which classifies species into one of nine threat categories (Figure 1.21, p. 49, Appendix A1.2) based on a set of five criteria (A to E) focussed on abundance and distribution (IUCN, 2012). Species are assessed against all categories and criteria and to be assigned a threat category a species must meet the requirements of at least one of the criteria – see Figure 1.22, p. 50, Appendix A1.2 for the full criteria (Hallingbäck & Hodgetts, 2000). A conservative approach is used meaning a species is assigned to the most threatened category it fulfils e.g. if a species fulfils criteria A for Vulnerable but also fulfils criteria B for Endangered it is categorised as Endangered (Hallingbäck & Hodgetts, 2000; IUCN, 2012), IUCN 2012).

Because bryophytes tend to be naturally rare and there is a lack of data, the IUCN Red List criteria have been adapted for the assessment of bryophytes – see Figure 1.23, p. 51, Appendix A1.2 (Hallingbäck & Hodgetts, 2000; Ah-Peng, Wilding, et al., 2012; Sérgio et al., 2013). The criteria which are harder to apply are A, C and particularly E (Population Viability Analysis) due to the lack of data on populations, generation time and mature individuals (Hallingbäck et al., 1998). Six additional categories can be applied to bryophytes (see Figure 1.21, p. 49, Appendix A1.2): Regionally Extinct (RE) – when a species is extinct in the area of assessment but not globally (Hallingbäck & Hodgetts, 2000); Least concern – attention (LC-att) when a species is not threatened but is an important species in the bryoflora due to being a local endemic (national or regional) or phytogeographically unique (Sérgio et al., 2013); Data Deficient – new (DD-n) species that have been discovered in the ten years prior to the assessment and so there is insufficient data for the region (Sérgio et al., 2013); Data Deficient – taxonomy (DD-t) when the species taxonomy is not well known (Ah-Peng, Bardat, et al., 2012); Data Deficient – distribution (DD-d) when there is a lack of distribution data (Ah-Peng, Bardat, et al., 2012); Data deficient – vanished (DD-va) when, based on a recent revision of the species, it is likely the species does not exist in the flora (this does not mean it is extinct, but that it likely was erroneously recorded for the area) (Sérgio et al., 2013).

Red Listing provides a method of setting conservation actions for bryophytes, especially considering other approaches (namely using umbrella, keystone, and flagship species) are harder to apply. Although only 102 bryophyte species (about 0.5% of species) (Figure 1.13) are currently listed on the IUCN World Red List (IUCN, 2016; IUCN SSC Bryophyte Specialist Group, 2016), several regional and national Red Lists have been published advancing our knowledge on the conservation status of bryophytes and the threats they face. The lists show that the number of threatened species varies greatly between countries or regions e.g. 3.8% of liverworts and hornworts are threatened in New Zealand compared to 9.5% in Reunion and 38% in Portugal (Figure 1.14) (Fife et al., 2010; Ah-Peng, Bardat, et al., 2012; Sérgio et al., 2013, respectively). This variation arises for several reasons including: difference in data availability for assessments (including variation in research effort); different level and pattern of threats; presence of habitats that are more threatened; inherent susceptibility to extinction of certain species or species groups

(Hallingbäck & Hodgetts, 2000). Level of threat is not homogenous across species as certain groups of species more threatened than others e.g. 50% of Portuguese endangered species (EN) are found in *Sphagnum* communities (Sérgio et al., 2013).

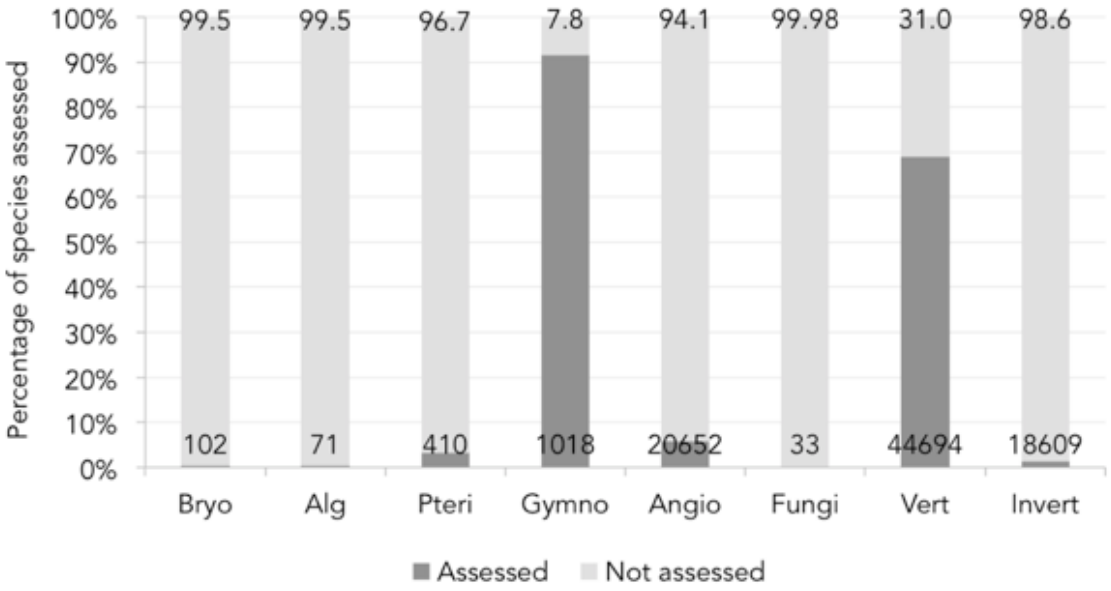


Figure 1.13 Percentage of assessed vs. not assessed species on the latest World Red List for each major plant and animal group. Number of species assessed shown at base of bars and percentage remaining to be assessed at top of bars. Bryo - Bryophytes; Alg - Algae; Pteri - Pteridophytes; Gymno - Gymnosperms; Angio - Angiosperms; Fungi - Fungi; Vert - Vertebrates; Invert - Invertebrates. For phyla included in the plant groups, see Table 1.1. (Data compiled from: The Plant List, 2013; IUCN, 2016)

Though there has been progress in assessing the conservation status of bryophytes, they are still far behind other taxonomic groups in global assessments, and along with algae and fungi, they have less than 1% of their species assessed (see Figure 1.13). Overall, 6.3% of all land plant species have been assessed (including bryophytes), up from 3.2% in 2007 (Brummitt et al., 2008). However, all plant groups have less than 5% of their species assessed. An exception is the gymnosperm group which has 92.2% of species assessed. Almost 70% of vertebrate animals have been assessed, with some groups such as mammals with all species assessed, but only 1.4% of invertebrates are on the Red List (IUCN, 2016). Although clearly many groups remain poorly assessed, the Red List is an invaluable tool for monitoring changes in extinction risk and the Red List Index (RLI) has been developed to track changes and trends in species' threat (Butchart et al., 2004). The underlying idea is that a set of species is repeatedly assessed at set intervals using the IUCN Red List Criteria (IUCN, 2012) and an index is then calculated based on the threat category the species are in; by comparing the index between assessments, changes in extinction risk can be tracked (Butchart et al., 2004, 2007). The lower the index value, the more threatened the group of species. Though the index works well for groups that have a high assessment completion rate, e.g. birds, mammals and amphibians (IUCN, 2016), it is harder to apply to groups with large species numbers and that have few assessed species, as is the case of plants (Brummitt et al., 2008). To address this issue, the Sample Red List Index (SRLI) was developed (Baillie et al., 2008) which calculates the RLI for a sample of species from an animal or plant group and uses that sample to monitor trends for the group overall (Baillie et al., 2008; Brummitt et al., 2015).

For plants, SRLI assessments have been completed for random samples of 1500 species from each of the following groups: pteridophytes, monocots, legumes and all 1028 species of gymnosperms; assessments are currently underway for bryophytes (Brummitt et al., 2015). In total 5528 species have been assessed and have shown that about 22% of plants species are threatened with an SRLI value of 0.86. As it stands, plants are more threatened than birds (SRLI value 0.91) but less so than amphibians (SRLI value 0.76), although the level of threat varies between plant groups and geographical regions (Brummitt et al., 2015). The index value calculated from the world bryophyte Red List is low, 0.49, but species that are known to be threatened or with narrow ranges were targeted so a low index value is expected. Values calculated from national Red Lists that have assessed all species show great variation (Figure 1.14). Given that many bryophytes are dependent on particular microhabitats and therefore have narrow habitat specificity, it will be important to assess the level of threat they face. Plants from tropical regions are under greater threat than those of temperate regions (Brummitt et al., 2015) and so the study of tropical bryophytes is necessary not only for the sake of bryophyte conservation itself, but also to inform on the state of biodiversity as a whole in the habitats they occupy.

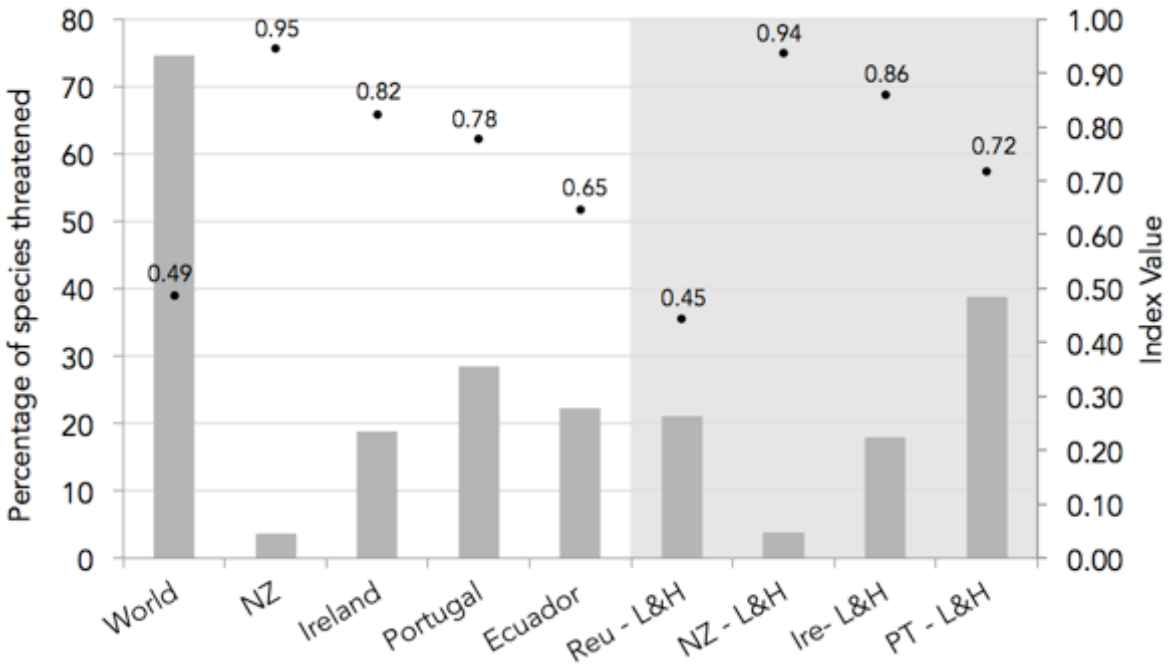


Figure 1.14 Percentage of threatened bryophyte species (bars) and Red List Index value (the closer to 1 the less threatened) of different recent Red Lists. The Ecuador Red List includes only endemic bryophytes. Values on grey background are for liverworts and hornworts (L&H) only, as the Reunion checklist includes only these phyla. REU- Reunion, NZ- New Zealand, Ire- Ireland, PT- Portugal. (Data compiled from: Fife et al., 2010; Gradstein & León-Vargas, 2011; Ah-Peng, Bardat, et al., 2012; Lockhart et al., 2012; Sérgio et al., 2013; IUCN, 2016)

1.2.4 Conservation

Bryophytes have historically been overlooked in conservation actions due to a lack of knowledge and awareness of bryophytes (Hallingbäck & Hodgetts, 2000). Although bryophytes are widespread globally and locally abundant in certain ecosystems, the attention given to them in the conservation and ecological literature has been minimal (Table 1.8). However, there has been a marked upward trend in the prevalence of bryophyte conservation studies throughout the last

20 years (Figure 1.15) as well as an increase in conservation studies in the bryophyte literature (Table 1.8).

Table 1.8 Number of bryophyte-focussed articles in the conservation literature per decade from 1970 to 2017 and number of conservation-focussed articles in the bryophyte literature per decade from 1970 to 2017 - excluding book reviews and corrections. Data from a Web of Science search: conservation literature data from thirty-three top-ranked conservation and ecology journals[§] (excluding animal-specific journals) using the ranking of (Bradshaw & Brook, 2016; Bradshaw, 2017); and bryophyte literature data from bryophyte-specific journals*. Excludes book reviews and corrections.

	Decade				
	1971-1980	1981-1990	1991-2000	2001-2010	2011-2017
Conservation & Ecology journals[§]					
Total number of publications	55 967	66 364	94 993	121 083	100 230
Number of bryophyte publications	20	25	67	122	91
Bryophyte publications as percentage of all publications	0.04%	0.04%	0.07%	0.10%	0.09%
Average number of bryophyte publications per year	2.0	2.5	6.7	12.2	13.0
Bryophyte journals*					
Total number of bryophyte publications	235	456	659	1139	750
Number of conservation articles	0	0	11	59	70
Conservation publications as percentage of all publications	0%	0%	1.7%	5.2%	9.3%
Average number of conservation publications per year	0	0	1.1	5.9	10.0
Total bryophyte conservation articles	**20**	**25**	**78**	**173**	**154**

[§] Conservation and ecology journals included in the search (in alphabetical order): Annual Review of Ecology Evolution and Systematics; Aquatic Biodiversity Conservation and Ecosystem Services; Aquatic Conservation Marine and Freshwater Ecosystems; Basic and Applied Ecology; Biodiversity and Conservation; Biological Conservation; Biological Invasions; Conservation Biology; Conservation Genetics; Conservation Letters; Current Biology; Ecological Monographs; Ecology Letters; Environmental Conservation; Frontiers in Ecology and the Environment; Functional Ecology; Global Change Biology; Global Ecology and Biogeography; Journal For Nature Conservation; Journal of Applied Ecology; Journal of Ecology; Methods in Ecology and Evolution; Molecular Ecology; Nature; Nature Climate Change; Oryx; Philosophical Transactions of the Royal Society B Biological Sciences; Plos Biology; Proceedings of the National Academy of Sciences of the United States of America; Restoration Ecology; Science; Trends in Ecology Evolution; Tropical Conservation Science.

* Bryology journals included in the search (in alphabetical order): The Bryologist; Bryology and Lichenology in Belgium; Bryophyte Diversity & Evolution; Cryptogamie Bryologie; Cryptogamie Bryologie Lichenologie; Genomes and Evolution of Charophytes Bryophytes Lycophytes and Ferns; Herzogia; Journal of Bryology; Journal of the Hattori Botanical Laboratory; Molecular Systematics of Bryophytes; Lindbergia; Nova Hedwigia; Nova Hedwigia Beiheft 114; Transactions of the British Bryological Society.

Some bryophyte-specific conservation measures have been devised and put into action, but knowledge-gaps means that bryophytes in certain regions, particularly in Tropical regions (Figure 1.15) remain in need of urgent action (Hallingbäck & Hodgetts, 2000). These knowledge-gaps arise from a lack of resident bryologists, specimens (both historical and recent), literature and floras

(Hallingbäck & Hodgetts, 2000; Ah-Peng, Wilding, et al., 2012). Hallingbäck & Hodgetts (2000, p. viii) put forward the following seven actions to ensure the conservation of bryophytes:

1. increasing inventories in the tropics to determine bryophyte richness in different regions and habitat types and to determine which species are locally common, rare, or threatened;
2. establishing protected areas or national systems of protected areas where endangered bryophytes occur;
3. incorporating bryophyte conservation in development and industrial activities;
4. comparing bryophyte floras of undisturbed and disturbed habitats to determine the impact of disturbance, and to identify those species unable to survive in disturbed areas. Without reliable information on the habitat requirements of species, including information on the quality of the habitats, it is impossible to determine appropriate conservation actions;
5. studying the taxonomy and distribution of individual species to determine how species can be identified, to determine their ranges, and to help identify those that are narrowly endemic (i.e., occur only within a small region);
6. training local people to become specialists. Because of the speed at which natural environments are disappearing worldwide, this initiative is extremely urgent and should be implemented immediately; and
7. creating user-friendly regional identification guides.

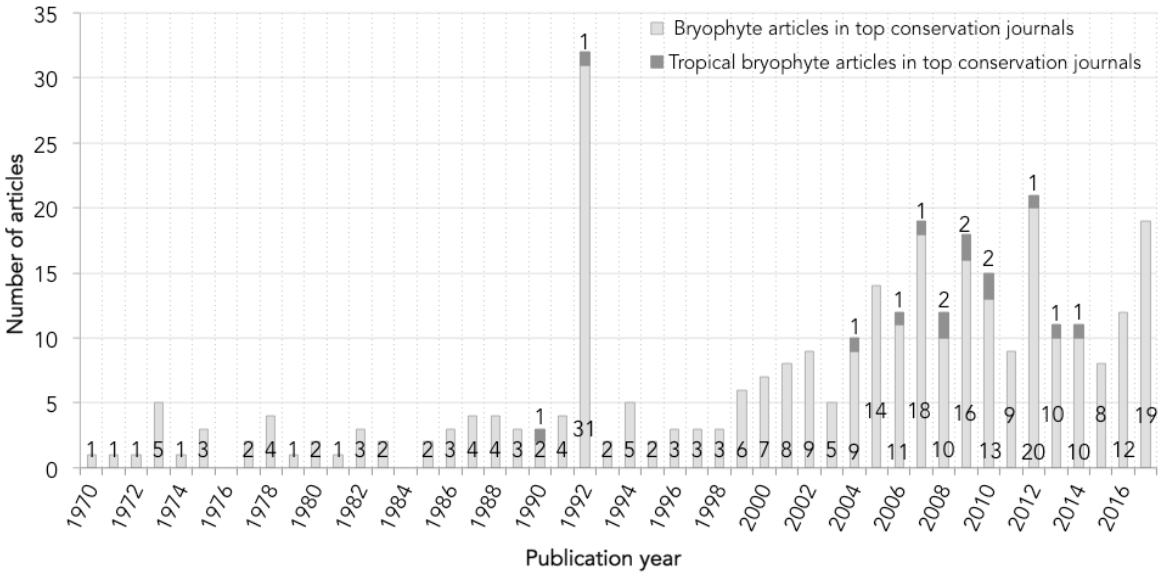


Figure 1.15 Number of bryophyte-focussed articles in top conservation and ecology journals between 1970 and 2017 with the number of tropical studies also shown. The very high number of publications in 1992 relative to other years is due to the publication of the conference proceedings from the Symposium on Endangered Bryophytes in Europe (September 24-28, 1990) in Biological Conservation (1992, 59:2-3); 25 articles alone were from this issue. Data obtained from a Web of Science search of thirty-three top-ranked conservation and ecology journals (excluding animal-specific journals) using the ranking of (Bradshaw & Brook, 2016; Bradshaw, 2017); excludes book reviews and corrections. See Table 1.8, p. 35 for list of journals.

The approach of using common species as indicators (Gaston & Fuller, 2008) could be perhaps easily applied to bryophytes due to the lack of data for bryophytes e.g. 16% DD in Portugal; 33% of Reunion liverworts and hornworts; 4% Ireland; 18% of mosses in New Zealand. The application

of using common species in bryology could provide a more complete indication of bryophyte status by complementing existing Red List assessments (Gaston & Fuller, 2007).

1.3 The study of bryophytes

The seven actions to conserve bryophytes put forward by Hallingbäck & Hodgetts (2000) mainly concern improving the knowledge of bryophytes through species inventories (particularly in the tropics), taxonomic studies, improving distribution data, and training local taxonomists. There has been an increase in tropical bryophyte research in the last 30 years, which mirrors an overall increase in tropical studies in the conservation literature (Figure 1.16). However, yet again this research is geographically biased with most taking place in the Neotropics (Mervin & Nadkarni, 2001). Compiling a database with complete trait data for sufficient Malagasy bryophytes to ensure a robust analysis would be beyond the time-frame of this PhD. Therefore, trait data from a relatively well-known bryoflora, Portugal, is used in conjunction with Malagasy species to ensure there are enough species for statistical analyses. A brief summary of bryology in Portugal is presented to provide context for subsequent methodologies and analyses.

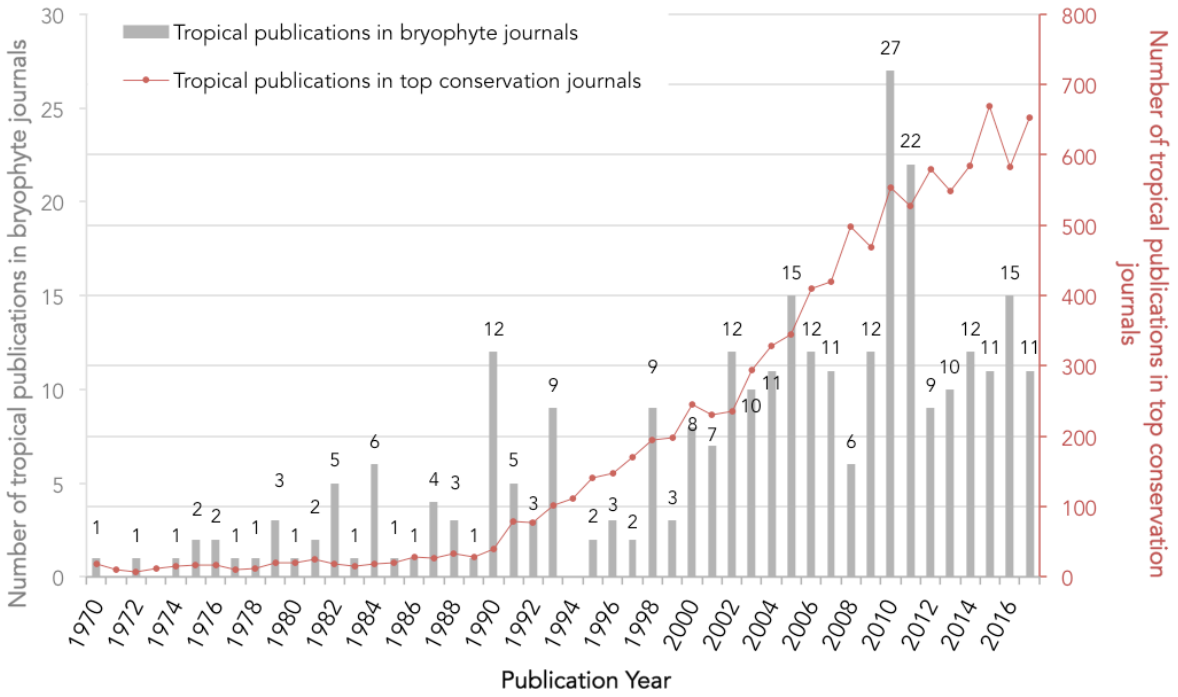


Figure 1.16 Tropical-focussed publications in the conservation literature and bryophyte literature from 1970 to 2017 showing an increase since the 1990s in tropical studies. Data from a Web of Science search: conservation literature data from thirty-three top-ranked conservation and ecology journals (excluding animal-specific journals) using the ranking of (Bradshaw & Brook, 2016; Bradshaw, 2017); and bryophyte literature data from bryophyte-specific journals. Excludes book reviews and corrections. See Table 1.8, p. 35 for list of journals.

1.3.1 Bryology in Madagascar

Madagascar's unique flora has attracted many botanists throughout the centuries (Figure 1.17) (Dorr, 1997) but few bryologists. Consequently, most botanical research has traditionally focused on vascular plants with little mention of cryptogams in publications on the Malagasy flora; only 2.3% of plant science publications in Madagascar since 1970 have focussed on bryophytes (Table

1.9). The latest synthesis of species diversity and richness in Madagascar does not include bryophytes; although other cryptogams are listed: algae, ferns and diatoms (Goodman & Benstead, 2003; see Table 1.13, p. 52 in Appendix A1.3).

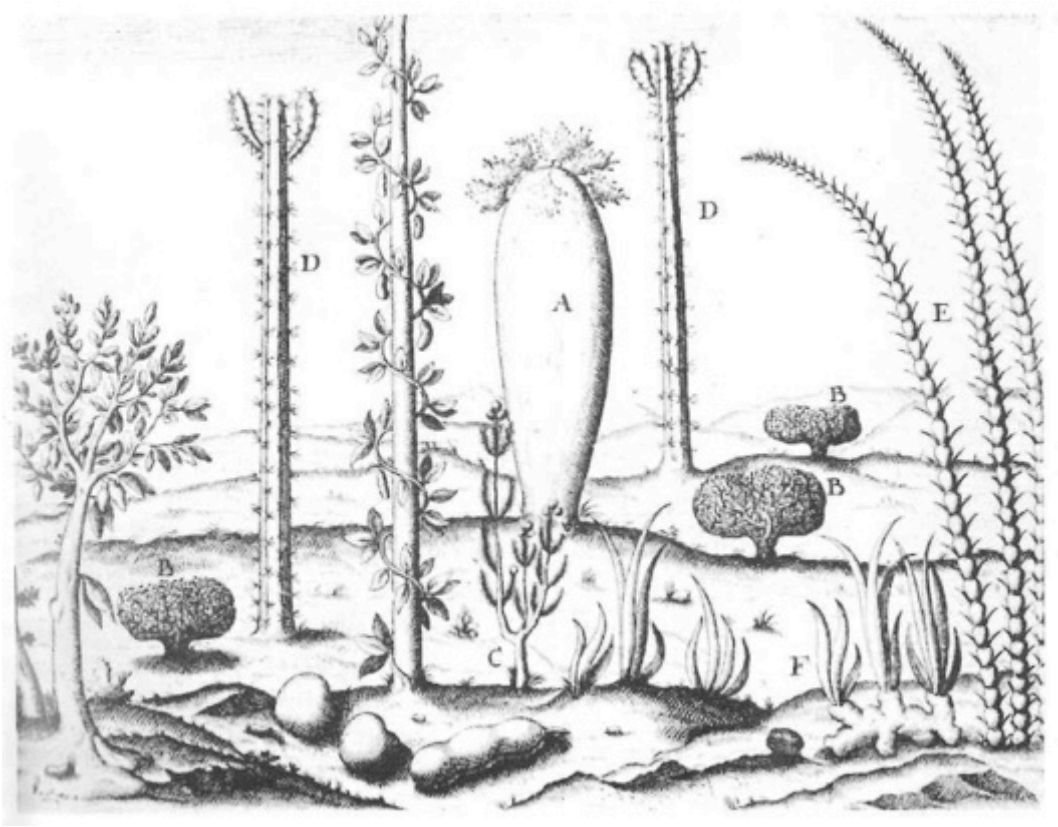


Figure 1.17 17th Century engraving of the Madagascar Spiny Forest. Taken from Koechlin 1974.

Table 1.9 Number of cryptogam-focussed articles in Madagascar plant science publications between 1970 and 2017. Data from a Web of Science search of all Madagascar publications in the plant sciences subject area (n=1494).

Group	Number of articles	Percentage of all plant science articles
Bryophytes	34	2.3%
Ferns	69	4.6%
Algae	19	1.3%
Fungi	21	1.4%
Lichen	21	1.4%
Vascular	1330	89.0%

The first significant bryophyte collections were made by A. Pervillé in 1837 on the small island of Nosy Be (off the northwest coast), followed by L.-H. Boivin in 1849, M. Borgen in 1874, J. M. Hildebrandt in 1876 and M. Marie in 1878, the latter on the island of Ste. Marie (on the eastern coast). Émile Bescherelle published a flora on the bryophytes of the nearby island of Réunion and "other African Islands of the Indian Ocean" [translated from original French] (Bescherelle, 1880, 1881) using the collections of Pervillé and Borgen, along with a smaller collection by Bernier from 1835 (Bescherelle, 1880). One of the first efforts towards an overall understanding of the Malagasy flora was Richard Baron's publication "The Flora of Madagascar" (1889) although

bryophytes are mentioned only in a footnote stating they "(…) are as yet very imperfectly known. Of Mosses about 250 have been described (…)" (Baron, 1889, p. 251).

The first comprehensive bryological flora on Madagascar was published in 1897 by the French bryologist Ferdinand Renauld (Renauld, 1897, 1909). He based himself on Bescherelle's work but included additional large collections from the following collectors: R.P. Camboué (1890-1894), G. Chénagon (1890), Perrot (1890-1894), G. Arbogast (1891), L. Besson (1891-1892) and F. Sikora (1891). Together with another French bryologist, Jules Cardot, Renauld published a flora specific to Madagascar (Renauld & Cardot, 1915) within the monumental publication "Histoire Physique, Naturelle et Politique de Madagascar [Physical, Natural and Political History of Madagacar]", a 39 volume work published between 1875 and 1915 (Grandidier, 1885). This flora listed 550 species of mosses (31 families and 130 genera) in 1915, of which over half were endemic, and provided a description of their habitats in Madagascar. They state that the Malagasy Bryoflora, together with that of the neighbouring Indian Ocean Islands, constitutes its own element due to the presence of endemic genera and species. Because the collections used were made mostly by non-botanists (usually soldiers or missionaries), few details were collected on their habitat making it difficult to gain a true understanding of their ecology (Chevalier, 1922).

The french botanist Henri Perrier de la Bâthie travelled throughout most of Madagascar over 25 years and included bryophytes in his collections (Chevalier, 1922). Based on his collections and field observations, he published the first comprehensive description of the Malagasy vegetation (Perrier de La Bâthie, 1921), although there is little specific mention of bryophytes. When classifying the flora into two types – "Wind Flora" in the East and Centre and "Sub-wind Flora", in the West – Perrier de la Bâthie states that the former has abundant bryophytes and the latter very few. These two "wind" zones correspond roughly to the major humid and dry climatic zones of east and west Madagascar. The nomenclature refers to the eastern trade winds, which mediate seasonal rains. Although many references are made to the abundance of epiphytes in certain forest types, only vascular species are discussed. An exception is when he states that humid forests at higher altitudes are covered in bryophytes and lichens; a moss carpet is mentioned in the 'Lichen forests' of high altitude; and in *Erica* bushland, mosses and lichens are the dominant epiphytes (Perrier de La Bâthie, 1921). However, in his later publication on the biogeography of Malagasy plants (1936), Perrier de la Bâthie provides a summary of cryptogams including bryophytes and lists literature on Malagasy bryophytes. He states that there have been few studies on cryptogams as a whole, although bryophytes have received more attention than other cryptogamic groups. Already at that time he remarked that the level of endemism in Malagasy bryophytes was high, despite not yet being well known.

Although Perrier de la Bâthie did not focus on bryophytes, between 1920 and 1932 H. Thériot published a series of "contributions" to the Malagasy bryoflora, based on specimens sent to him by collectors, particularly Perrier de la Bâthie. His identification of specimens led to the total species of mosses known rising to approximately 650 (Thériot, 1932). The amount of bryological collection and research in the latter half of the 20th century decreased significantly compared to the first half (Figure 1.18). In 1948 Jovet-Ast published biogeographical studies on both Malagasy mosses and the long-neglected liverworts, listing 250 species of the latter (Léandri, 1952; Abbayes et al., 1959). There was no significant bryophyte research undertaken in Madagascar until the 1970s.

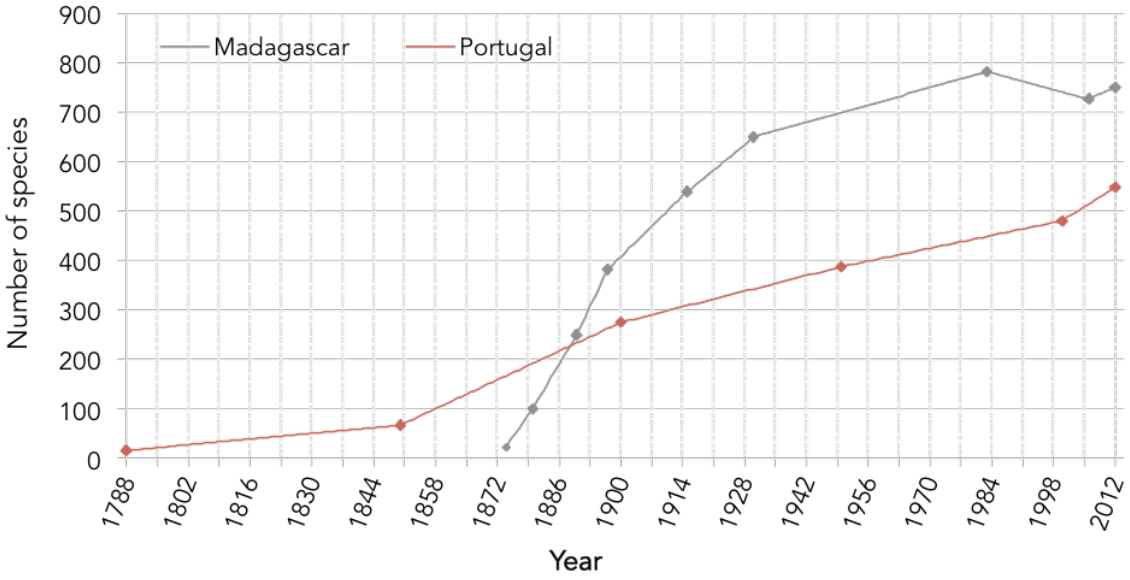


Figure 1.18 Total number of moss species described over time in Madagascar and Portugal. A slowing rate of discovery since the early 20th century can be seen for Madagascar. The decrease in species number in Madagascar from 1983 to 2006 is due to a revision of names yielding several synonyms (Crosby et al., 1999). (Madagascar data compiled from: Hampe, 1874; Bescherelle, 1880; Baron, 1889; Renauld, 1897; Renauld & Cardot, 1915; Thériot, 1930; O'Shea, 2006; Marline et al., 2012; Portugal data compiled from: Vandelli, 1788; Sérgio et al., 2013).

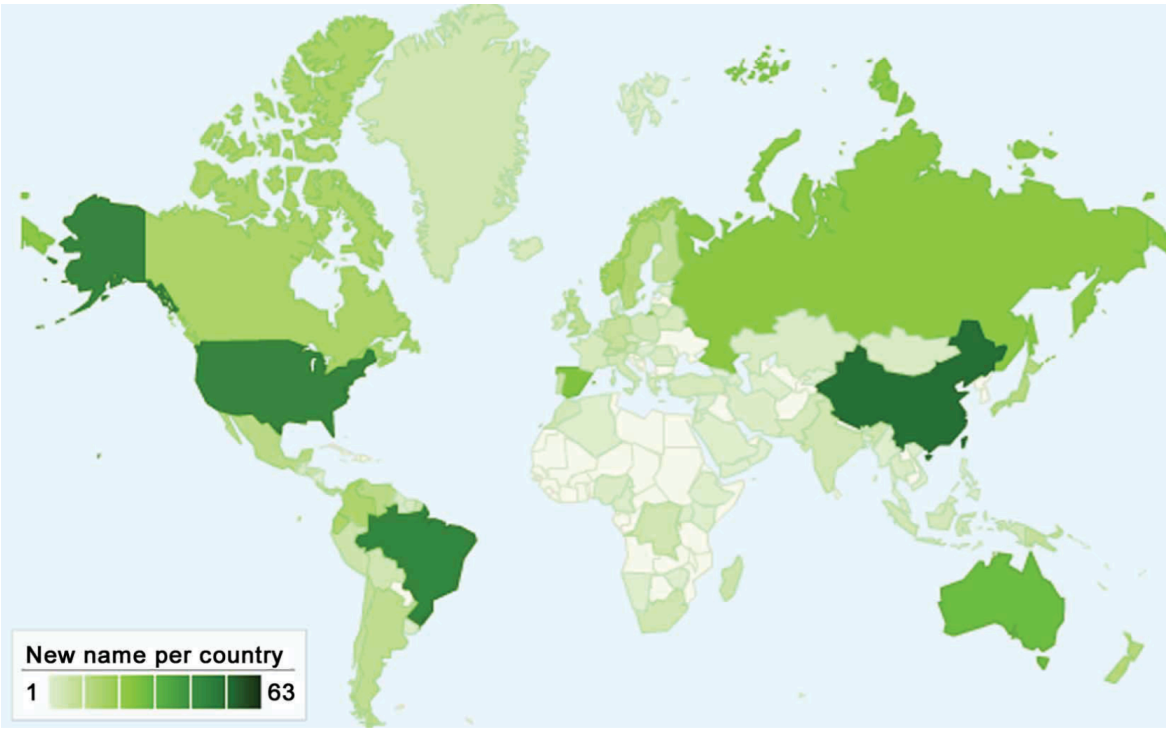


Figure 1.19 New moss (Bryophyta) names published between 1989-2009 per country. (Taken from: Magill, 2010, figure 4, p. 171)

Missouri Botanical Garden has been undertaking botanical research, including bryophytes, in Madagascar since the 1970s and has undertaken collecting expeditions at several locations. Between 1989 and 2009 Madagascar had one of the highest number of new published moss names in Africa, although still low compared to other tropical regions (Figure 1.19) (Magill, 2010). Separate to this work by MBG, bryophyte research currently being conducted includes a much-

needed checklist of the Malagasy bryoflora has been published and with it the hope of "stimulating and facilitating" work in this area (Marline et al., 2012).

This latest checklist lists 1144 *taxa* – 751 mosses, 390 liverworts and 3 hornworts – of which 28.7% are endemic, a much lower endemism rate compared to other plant groups in Madagascar as most groups have levels of 80% and above (Table 1.10). However, this rate of endemism is high relative to other bryofloras e.g. 1.7% of all bryophytes in the Azores (Gabriel et al., 2011); 9% of liverworts in Europe; 10% in Reunion (Ah-Peng, 2007). Madagascar has the highest bryophyte species richness of all Indian Ocean islands (Figure 1.20), and also of other oceanic islands, although it also has the largest area – however, the question of whether Madagascar should be regarded as a continental landmass or oceanic island remains (Wit, 2003). It is likely this endemism rate will decline as further studies are conducted on the Malagasy bryoflora – for example, in 1915 the endemism rate was over 50% (Renauld & Cardot, 1915).

In their study of Malagasy inselbergs, Fischer & Theisen (2000) recorded several genera of bryophytes from various habitats of the central highland. Species of *Leucobryum*, *Polytrichum* and *Frullania* were found in the lichen forests (above 2000 m) and in wet flushes species of *Philonotis* and *Campylopus*. However, when recording cryptogamic vegetation on rocks and boulders, many lichen species were found but few bryophytes. At tropical latitudes it is uncommon to find many bryophyte species on exposed lowland rocks due to domination by lichens and cyanobacteria (Frahm, 2000).

It is interesting to note that no books describing the Malagasy vegetation types mention the large expanse of coastal *Sphagnum* beds with *Nepenthes* species found along the southeast coast (personal observation). Perrier de la Bâthie (1921) describes a vegetation formation he calls xerophytic "lawn" with rocks and boulders where there is a dense carpet of mosses and lichens (one species of each is listed without naming each – most likely *Sphagnum* and *Cladinia* from personal observation), but these are at altitude in the central plateau. No description exists of these *Sphagnum* beds likely due not only to bryophytes being an understudied group but also to that area of the southeast being understudied as the only access to this region is through a dirt track that is periodically flooded during the wet season. There is also the possibility that this coastal area used to have much greater forest cover (Fischer & Theisen, 2000; Goodman & Benstead, 2003) and so these large areas of *Sphagnum* may not have existed when this region was at its climax vegetation.

As well as a taxonomical bias, there has been a geographical bias in botanical collections with most taking place in mid to high altitude humid forest and along main roads. This study therefore focuses on lowland humid forest and further details on Madagascar's biodiversity and its threats are provided in Chapter 5.

Table 1.10 Species richness and endemism among plant groups (families and phyla) and lichens in Madagascar, ordered from highest to lowest percentage of endemics, showing that bryophytes have the lowest endemism. Cryptogam groups are highlighted. Taken and adapted from Goodman & Benstead, 2003, Table 1, p. 74 except for: bryophyte data which is taken from Marline et al., 2012; lichen data compiled from (Aptroot, 2016).

Group	Endemism species number	Endemism %	Species richness
Myristicaeae	10	100%	10
Balsaminaceae	149	100%	149
Pandanaceae *(Pandanus)*	99	100%	99
Poaceae (grasses), Bambuseae (bamboos)	34	100%	34
Melastomataceae	318	99%	321
Rubiaceae	637	98%	*c.* 650
Arecaceae (palms)	167	98%	170
Sapotaceae	81	96%	84
Annonaceae	83	93%	89
Anacardiaceae	38	93%	41
Gentianaceae	62	93%	67
Bombaceae *(Adansonia)*	6	85–100%[1]	7
Euphorbiaceae		mostly endemic	*c.* 700
Leguminosae	459	80%	573
Moraceae *(Ficus)*	15	60%	25
Scrophulariaceae	40	51%	79
Pteridophyta (ferns & allies)	**265**	**45%**	**586**
Aquatic plants	128	38%	338
Bacillariophyceae (diatoms)		**some endemic**	**134**
Bryophytes (mosses, liverworts & hornworts)	**328**	**29%**	**1144**
Marine algae		**not stated**	**c. 200**
Lichen		**unknown but >2%**	**500**

1 One species may be naturalised

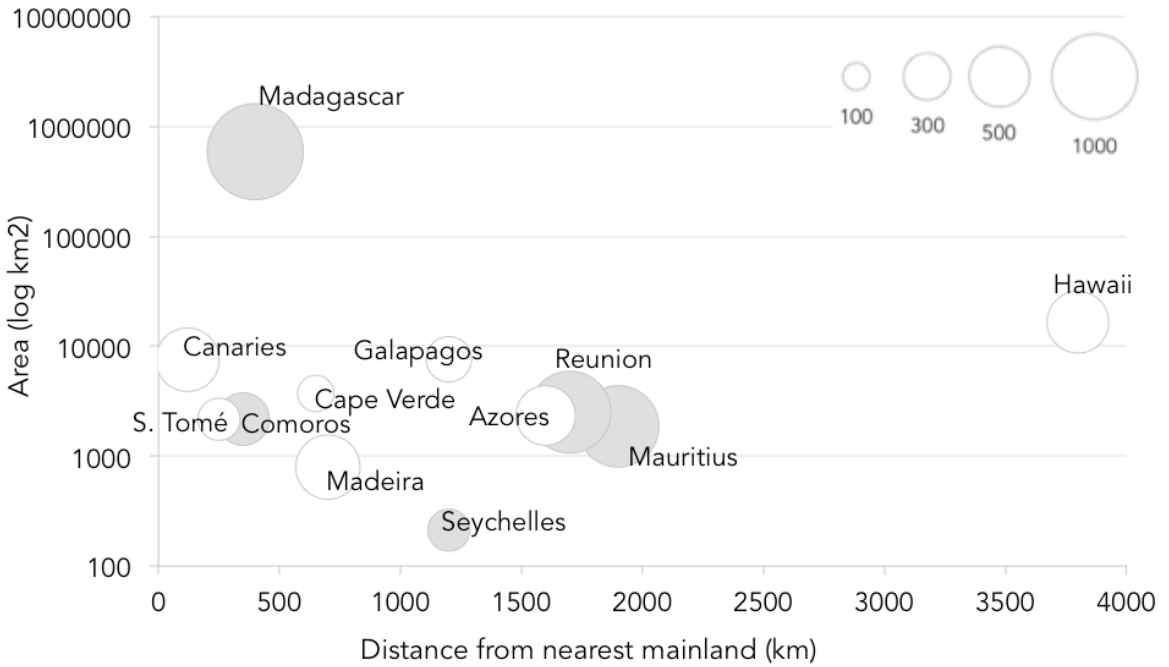


Figure 1.20 Number of bryophyte species (circle size), area (log km^2) and distance to nearest mainland (km) of oceanic islands and archipelagos. Circle size represents species number. Indian Ocean Islands are indicated in grey. Seychelles represents only the 5 main islands of the Inner Seychelles; S. Tomé includes São Tomé and Principe.

1.3.2 Bryology in Portugal

Mainland Portugal, despite its relatively small size of 89 060 km^2, has a diverse range of habitats. It is located between 37° 42°N and 6.5° 9.5°W and most of continental Portugal lies in the Mediterranean region, with a part of the North located in the Euro-Siberian region. Its location on the south-western tip of Europe and position between the European continent and the Atlantic means it has a high level of biodiversity. Its bryoflora comprises 65% of all Iberian species and 40% of all European species (Sérgio et al., 2013). As part of the Iberian glacial refuge it is home to several endemic Iberian and rare European species (Sérgio et al., 2013). Portugal, like Madagascar, is part of a biodiversity hot spot (Myers et al., 2000) and there is a recent flora (Guerra & Cros, 2006) and Red Data Book (Sérgio et al., 2013) providing accurate and sufficient information to complete the trait data for these species (see Chapter 3 for further details).

The first publication of Portuguese bryophytes was by D. Vandelli in 1788 but the oldest targeted bryological collections in Portugal date from the beginning of the 19th century by the botanist Felix Brotero who published the first Portuguese flora in 1804 (Sérgio et al., 2000, 2013). Since that time until the beginning of the 20th century collections were few and tended to be located in the same localities, referred to as "classical localities". Towards the end of the 19th century and beginning of the 20th new areas were explored and the first checklists of liverworts and mosses were published in 1886 and 1889, respectively, by J. Henriques and together numbered 315 bryophytes (Sérgio et al., 2000). From 1980 the knowledge on the Portuguese bryoflora has increased in depth and breadth with fieldwork being carried out in previously unstudied areas. In

the 21st century there have been several bryological works (mainly PhD theses) that have added to this knowledge, particularly in aquatic, saxicolous and epiphytic habitats (Sérgio et al., 2013). For a complete history of bryology in Portugal see Sérgio et al., 2013.

Currently there are around 35 000 bryophyte specimen records held at Lisbon University Herbarium (LISU), all of which are on an electronic database (BROTERO), and are accurately georeferenced. Many of these specimens have been reviewed for various studies and during the preparation of red lists and floras e.g. the Iberian Bryoflora and Portuguese Red Data Book (Guerra & Cros, 2006; Sérgio et al, 2013). This provides us with a wealth of reliable spatial and taxonomic data that can be used to answer ecological and conservation questions.

1.4 Summary

Bryophytes, though relatively understudied, are an important and diverse component of ecosystems with around 20 000 extant species. Three plant phyla make up bryophytes: liverworts (Marchantiophyta), mosses (Bryophyta) and hornworts (Anthocerophyta). The bryophyte life-cycle is unique among land plants for having a dominant gametophyte generation, a characteristic possibly retained from the first plant land-colonisers. Because of bryophytes' small size, their ecophysiology is particular and different to most other land plants, with moisture availability being a limiting factor for many species. Included in this, is the mechanism of desiccation tolerance (DT), which is almost exclusively found in bryophytes. Desiccation tolerance together with a small size means that bryophytes can occupy harsh habitats and substrates that are not available to most plants as they have the ability to efficiently utilise water in the form of water vapour. Bryophytes are therefore highly dependent on microclimate and consequently, have a high affinity to particular microhabitats. A wide range of ecosystem services is provided by bryophytes ranging from biochemicals and genetic resources to climate regulation, nutrient cycling and primary production, among many others.

The level of threat in bryophytes varies between countries and regions, but for areas that have undertaken complete Red List assessments of the bryoflora, it has been found that a large proportion of bryophytes are at risk of extinction. Although many bryophyte species are inherently rare locally, extrinsic threats are numerous: habitat loss and degradation, pollution (air, water, soil), invasive species, fire and forest management practices. The status of knowledge on bryophytes is generally poor, with a large disparity between temperate and tropical areas, although there has been a recent small but marked increase in tropical bryology research. One of the main focuses of bryophyte conservation is improving the knowledge on bryophytes so that effective management policies and actions can be put into place. This study will focus on the bryoflora of Madagascar, which is understudied but likely highly threatened given the overall threats facing Malagasy biodiversity.

Appendix 1 Background to Bryophytes

A1.1. Background data

Table 1.11 Number of algae species in all divisions and classes. Taken from Guiry 2012, table 1, p. 1058

Phylum and classes encompassed	Vernacular name	Class total	Phylum total
Cyanobacteria	Blue-green algae		3300
Cyanophyceae	Blue-green algae	3300	
Rhodophyta	Red algae		6131
Bangiophyceae	Bangiophytes	138	
Cyanidophyceae	Cyanidophytes	4	
Pophyridiophyceae	Porphyridiophytes	11	
Stylenomatophyceae	Stylonematophytes	25	
Rhodellophyceae	Rhodellophytes	5	
Florideophyceae	Florideophytes	5948	
Glaucophyta	Glaucophytes		14
Charophyta	Charophytes		3470
Charophyceae	Charophytes	690	
Coleochaetophyceae	Coleochaetophytes	18	
Klebsormidophyceae	Klebsormidophytes	39	
Mesostigmatophyceae	Mesostigmatophytes	14	
Zygnematophyceae	Zygnemophytes	2709	
Chlorophyta	Chlorophytes		4548
Bryopsidophyceae	Bryopsidophytes	520	
Chlorodendrophyceae	Chlorodendrophytes	43	
Chlorophyceae	Chlorophytes	2292	
Dasycladophyceae	Dasycladophytes	50	
Mamiellophyceae	Mamiellophytes	16	
Nephroselmidophyceae	Nephroselmidophytes	26	
Pedinophyceae	Pedinophytes	22	
Pleurastrosphyceae	Pleurastrophytes	3	
Prasinophyceae	Prasinophytes	97	
Siphonocladiophyceae	Siphonocladiophytes	402	
Trebouxiophyceae	Trebouxiophytes	546	
Ulvophyceae	Ulvophytes	531	
Cryptophyta	Cryptophytesa		148
Cryptophyceae	Cryptophytes	148	
Haptophyta	Haptophytesa		510
Coccolithophyceae	Coccolithophorids	371	
Pavlovophyceae	Pavlovophytes	15	
Incertae sedis		124	
Cercozoa			12
Chlorarachniophyceae	Chlorarachniophytes	12	
Ochrophyta	Ochrophytesa		11571
Aureanophyceae	Aureanophytes	1	

Phylum and classes encompassed	Vernacular name	Class total	Phylum total
Bacillariophyceae	Diatoms	8397	
Bolidophyceae	Bolidophytes	14	
Chrysomoerophyceae	Chrysomerophytes	4	
Chrysophyceae	Chrysophytes	431	
Dictyochophyceae	Dictyochophytes	51	
Eustigmnatophyceae	Eustigmatophtes	35	
Pelagophyceae	Pelagophytes	12	
Phaeophyceae	Brown algae	1792	
Phaeothamniophyceae	Phaeothamniophytes	33	
Picophagophyceae	Picophagophytes	4	
Pinguiophyceae	Pinguiophytes	6	
Placidiophyceae	Placidophytes	2	
Raphidophyceae	Raphidophytes	35	
Schizocladiophyceae	Schizocladiophytes	1	
Synchromophyceae	Synchromophytes	1	
Synurophyceae	Synurophytes	252	
Xanthophyceae	Xanthophytes	500	
Choanozoa	Choanoflagellates		79
Choanoflagellatea	Choanoflagellates	79	
Euglenozoa	Euglenoid flagellates		1189
Bodonophyceae	Bodonozoans	32	
Euglenophyceae	Euglenozoansa	1157	
Loukozoa	Loukozoans		3
Jakobea	Jakobids	3	
Metamonada	Metamonads		5
Trepomonadea	Trepomonads	5	
Myzozoa	Myzozoans		2277
Dinophyceae	Dinoflagellates	2270	
Perkinsea	Perkinsids	7	
Percolozoa	Percolozoans		3
Heterolobosea	Heterolobosids	3	
Total			**33260**

Table 1.12 Studies that have published early land plant phylogenies, in chronological order, with type of data used and colour coded according to which phylogeny topology the study supports: light blue- A; purple- B; brown- C; red- D; green- E; orange- F; dark blue- G (see Figure 1.8, p. 12 for topologies).

Authors	Year	Data type	Bryophytes	Sister to tracheophytes	Sister to all land plants	Topology	Cited in
Mishler & Churchill	1984	Morphological	Paraphyletic	Mosses	Liverworts	E	Goffinet, 2000
Bremer	1985	Morphological	Paraphyletic	Mosses	Liverworts	E	Goffinet, 2000
Hori et al.	1985	Ribosomal	Monophyletic	Bryophytes	Tracheophytes	A	Goffinet, 2000
Garbary et al.	1993	Sperm ultrastructure	Monophyletic	Bryophytes	Tracheophytes	A	Goffinet, 2000
Mishler et al.	1994	18S	Paraphyletic	Liverworts	Hornworts	D	Goffinet, 2000
Mishler et al.	1994	Morphological & 18S	Paraphyletic	Mosses	Hornworts	B	Goffinet, 2000
Mishler et al.	1994	Morphological, 26S & 18S	Paraphyletic	Mosses	Liverworts	E	Goffinet, 2000
Hedderson et al.	1996	18S	Paraphyletic	Mosses & Liverworts	Hornworts	F	Goffinet, 2000
Malek et al.	1996	cox3	Paraphyletic	Liverworts	Hornworts	D	Goffinet, 2000
Crowe et al.	1997	pbsA	Paraphyletic	Mosses & Liverworts	Hornworts	F	Goffinet, 2000
Kenrick & Crane	1997	Morphological	Paraphyletic	Mosses	Liverworts	E	Goffinet, 2000
Lewis et al.	1997	rbcL	Paraphyletic	Hornworts	Liverworts	C	Goffinet, 2000
Maden et al.	1997	Sperm ultrastructure	Monophyletic	Bryophytes	Tracheophytes	A	Goffinet, 2000
Garbary & Renzaglia	1998	Morphological	Paraphyletic	Mosses & Liverworts	Hornworts	F	Goffinet, 2000
Garbary & Renzaglia	1998	Sporophyte	Paraphyletic	Liverworts	Hornworts	D	Goffinet, 2000
Hedderson et al.	1998	18S	Paraphyletic	Mosses & Liverworts	Hornworts	F	Goffinet, 2000
Qiu et al.	1998	Mitochondrial	Paraphyletic	Hornworts	Liverworts	C	Villarreal & Renzaglia, 2015
Duff & Nickrent	1999	19S	Paraphyletic	Mosses & Liverworts	Hornworts	F	Goffinet, 2000

Authors	Year	Data type	Bryophytes	Sister to tracheophytes	Sister to all land plants	Topology	Cited in
Nishiyama & Kato	1999	18S, rbcL, psaA, psaB, psbD, rpoC2	Paraphyletic	Mosses & Liverworts	Hornworts	F	Goffinet, 2000
Renzaglia et al.	2000	Morphological & ontogeny	Paraphyletic	Mosses & Liverworts	Hornworts	F	Goffinet, 2000
Renzaglia et al.	2000	Sperm ultrastructure	Monophyletic	Bryophytes	Tracheophytes	A	Goffinet, 2000
Nishiyama et al.	2003	Chloroplast DNA	Monophyletic	Bryophytes	Tracheophytes	A	Vanderpoorten & Goffinet, 2009
Kelch et al.	2004	Chloroplast genome	Paraphyletic	Hornworts	Liverworts	C	Vanderpoorten & Goffinet, 2009
Qiu et al.	2006	Nucleotide	Paraphyletic	Hornworts	Liverworts	C	Vanderpoorten & Goffinet, 2009
Mishler & Kelch	2009	rpoA, tRNA	Paraphyletic	Hornworts	Liverworts	C	Mishler & Kelch, 2009
Karol et al.	2010	Plastid genes	Paraphyletic	Hornworts	Liverworts	C	Villarreal & Renzaglia, 2015
Chang & Graham	2011	Plastid genes	Paraphyletic	Hornworts	Liverworts	C	Villarreal & Renzaglia, 2015
Cox et al.	2014	Review of Qui et al., 2006 & Karol et al., 2010	Monophyletic	Bryophytes	Tracheophytes	A	Villarreal & Renzaglia, 2015
Liu et al.	2014	Amino acid	Paraphyletic	Hornworts	Liverworts	C	Villarreal & Renzaglia, 2015
Liu et al.	2014	Mitochondrial	Paraphyletic	Hornworts	Mosses	G	Villarreal & Renzaglia, 2015
Wickett et al.	2014	Nuclear genes - analysis 1	Paraphyletic	Mosses	Hornworts	B	Villarreal & Renzaglia, 2015
Wickett et al.	2014	Nuclear genes - analysis 2	Monophyletic	Bryophytes	Tracheophytes	A	Villarreal & Renzaglia, 2015
Wickett et al.	2014	Nuclear genes - analysis 3	Paraphyletic	Hornworts	Liverworts	C	Villarreal & Renzaglia, 2015

A1.2. Red list categories and criteria

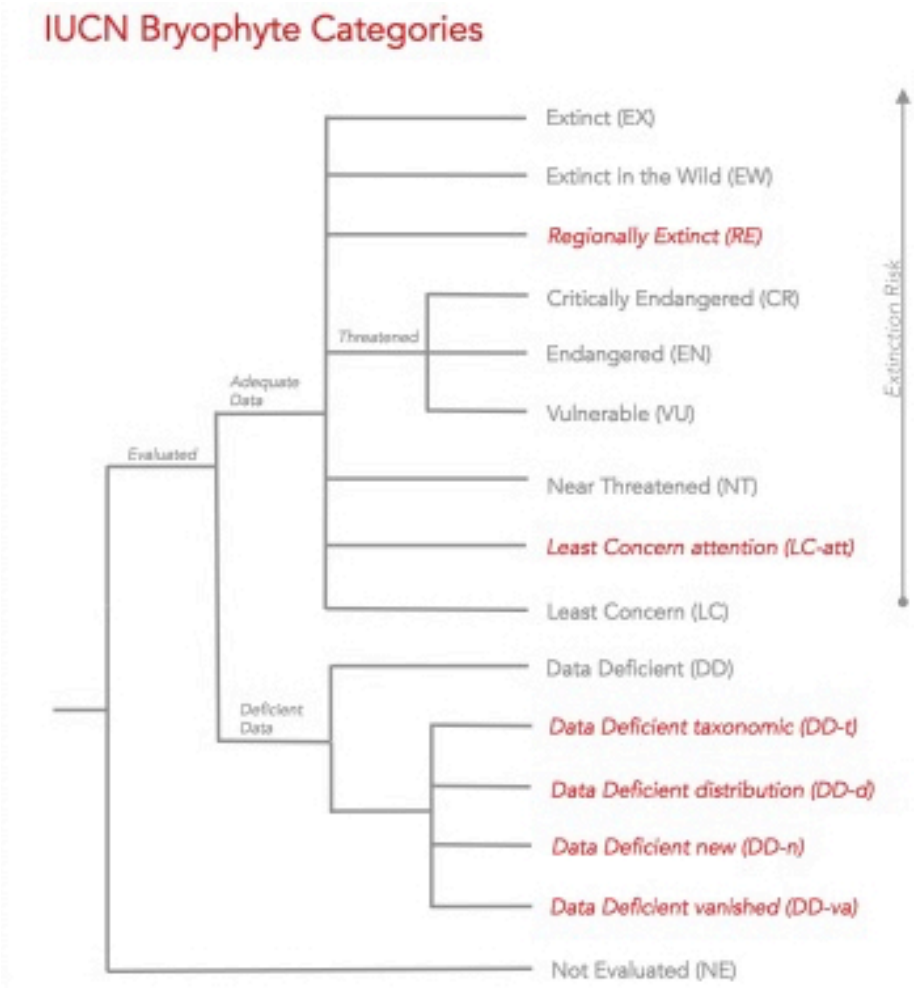


Figure 1.21 Outline of the IUCN Red List Categories and the additional categories (in red) that are used when assessing bryophytes. Adapted from IUCN, 2012, Figure 1, p. 5.

A. Population size reduction. Population reduction (measured over the longer of 10 years or 3 generations) based on any of A1 to A4

	Critically Endangered	Endangered	Vulnerable
A1	≥ 90%	≥ 70%	≥ 50%
A2, A3 & A4	≥ 80%	≥ 50%	≥ 30%

A1 Population reduction observed, estimated, inferred, or suspected in the past where the causes of the reduction are clearly reversible AND understood AND have ceased.

A2 Population reduction observed, estimated, inferred, or suspected in the past where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.

A3 Population reduction projected, inferred or suspected to be met in the future (up to a maximum of 100 years) *[(a) cannot be used for A3]*.

A4 An observed, estimated, inferred, projected or suspected population reduction where the time period must include both the past and the future (up to a max. of 100 years in future), and where the causes of reduction may not have ceased OR may not be understood OR may not be reversible.

based on any of the following:

- **(a)** direct observation *[except A3]*
- **(b)** an index of abundance appropriate to the taxon
- **(c)** a decline in area of occupancy (AOO), extent of occurrence (EOO) and/or habitat quality
- **(d)** actual or potential levels of exploitation
- **(e)** effects of introduced taxa, hybridization, pathogens, pollutants, competitors or parasites.

B. Geographic range in the form of either B1 (extent of occurrence) AND/OR B2 (area of occupancy)

	Critically Endangered	Endangered	Vulnerable
B1. Extent of occurrence (EOO)	< 100 km²	< 5,000 km²	< 20,000 km²
B2. Area of occupancy (AOO)	< 10 km²	< 500 km²	< 2,000 km²
AND at least 2 of the following 3 conditions:			
(a) Severely fragmented **OR** Number of locations	= 1	≤ 5	≤ 10
(b) Continuing decline observed, estimated, inferred or projected in any of: **(i)** extent of occurrence; **(ii)** area of occupancy; **(iii)** area, extent and/or quality of habitat; **(iv)** number of locations or subpopulations; **(v)** number of mature individuals			
(c) Extreme fluctuations in any of: **(i)** extent of occurrence; **(ii)** area of occupancy; **(iii)** number of locations or subpopulations; **(iv)** number of mature individuals			

C. Small population size and decline

	Critically Endangered	Endangered	Vulnerable
Number of mature individuals	< 250	< 2,500	< 10,000
AND at least one of C1 or C2			
C1. An observed, estimated or projected continuing decline of at least (up to a max. of 100 years in future):	25% in 3 years or 1 generation (whichever is longer)	20% in 5 years or 2 generations (whichever is longer)	10% in 10 years or 3 generations (whichever is longer)
C2. An observed, estimated, projected or inferred continuing decline AND at least 1 of the following 3 conditions:			
(a) **(i)** Number of mature individuals in each subpopulation	≤ 50	≤ 250	≤ 1,000
(ii) % of mature individuals in one subpopulation =	90–100%	95–100%	100%
(b) Extreme fluctuations in the number of mature individuals			

D. Very small or restricted population

	Critically Endangered	Endangered	Vulnerable
D. Number of mature individuals	< 50	< 250	**D1.** < 1,000
D2. *Only applies to the VU category* Restricted area of occupancy or number of locations with a plausible future threat that could drive the taxon to CR or EX in a very short time.	-	-	**D2.** typically: AOO < 20 km² or number of locations ≤ 5

E. Quantitative Analysis

	Critically Endangered	Endangered	Vulnerable
Indicating the probability of extinction in the wild to be:	≥ 50% in 10 years or 3 generations, whichever is longer (100 years max.)	≥ 20% in 20 years or 5 generations, whichever is longer (100 years max.)	≥ 10% in 100 years

Figure 1.22 Summary of the five criteria (A-E) used to evaluate if a taxon belongs in an IUCN Red List threatened category (Critically Endangered, Endangered or Vulnerable). Taken from IUCN, 2012, p. 28-29.

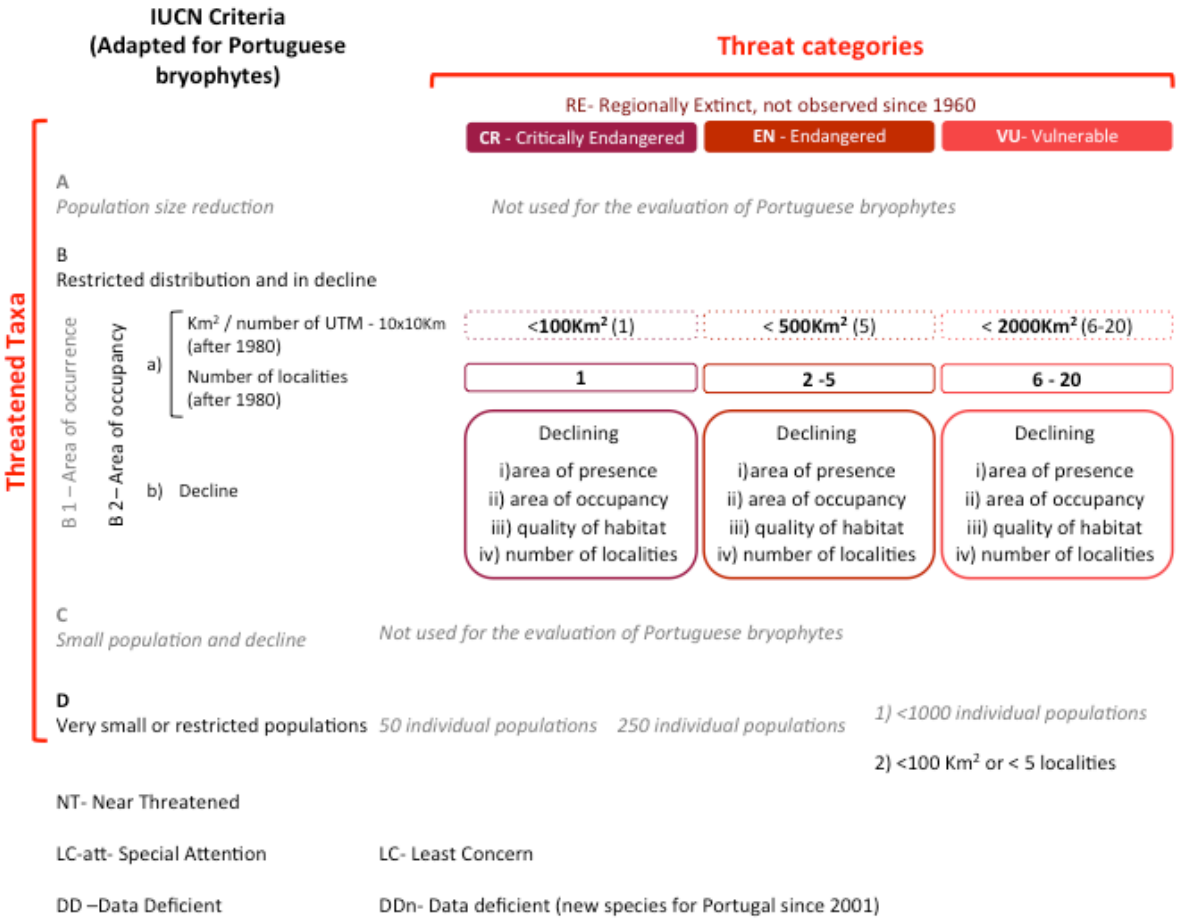


Figure 1.23 The adapted IUCN criteria used to evaluate if a bryophyte belongs in an IUCN Red List threatened category (Critically Endangered, Endangered or Vulnerable). Adapted for the assessment of Portuguese bryophytes by Sérgio et al., 2013 (Figure 40, p. 70, translated by Sarah Stow) based on Hällingback & Hodgetts, 2000.

A1.3. Madagascar species richness and endemism

Table 1.13 Species richness and endemism in certain phyla, classes and families of Malagasy flora and fauna. Taken and adapted from Goodman & Benstead, 2003, Table 1, p. 74 except for bryophyte data which is taken from Marline et al., 2012 and lichen data taken from Aptroot, 2016.

Group	Richness	Endemism
Non-marine plants	**2984**	**2463 (83%)**
Bryophytes (liverworts, mosses, hornworts)	1144	328 (29%)
Aquatic plants	338	128 (38%)
Bacillariophyceae (diatoms)	134	some endemic
Pteridophyta (ferns & allies)	586	265 (45%)
Annonaceae	89	83 (93%)
Myristicaeae	10	10 (100%)
Moraceae (*Ficus*)	25	15 (60%)
Bombaceae (*Adansonia*)	7	6 (85–100%)
Sapotaceae	84	81 (96%)
Leguminosae	573	459 (80%)
Melastomataceae	321	318 (99%)
Euphorbiaceae	*c.* 700	mostly endemic
Anacardiaceae	41	38 (93%)
Balsaminaceae	149	149 (100%)
Gentianaceae	67	62 (93%)
Scrophulariaceae	79	40 (51%)
Rubiaceae	*c.* 650	637 (98%)
Arecaceae (palms)	170	167 (98%)
Pandanaceae (*Pandanus*)	99	99 (100%)
Poaceae (grasses), Bambuseae (bamboos)	34	34 (100%)
Lichens	**500**	**>11 but not yet assessed**
Non-marine invertebrates	**5808**	**4976 (86%)**
Land vertebrates	**879**	**739 (84%)**
Amphibia (frogs)	199	197 (99%)
Reptilia (reptiles)	340	314 (92%)
Aves (birds)	209	109 (52%)
Mammalia (non-volant mammals)	101	101 (100%)
Mammalia (bats)	30	18 (60%)
Marine	**>5100**	**generally very low**
Fishes (including elasmobranchs)	*c.* 1110	very low
Marine algae	*c.* 200	not stated
Porifera (sponges)	>300	none
Cnidaria (corals & anemones)	>400	very low
Octocorallians (soft corals sea fans etc.)	222	62 regional endemics
Hexacorallians (hard corals)	208	some regional endemism
Mollusca & Crustacea (molluscs & crustaceans)	c. 2300	some regional endemism
Echinoderma (echinoderms)	*c.* 400	>80 regional endemics
Chelonidae (sea turtles)	5	none
Mammallia (whales, dolphins, seals, dugongs)	28	none

Chapter 2 Desiccation Tolerance & Bryophyte Traits

Abstract

Desiccation tolerance is a wide and fertile field of study with several books (Black & Pritchard, 2002; Jenks & Wood, 2007; Lüttge et al., 2011) and reviews available (e.g. Oliver & Bewley, 1996; Alpert, 2005, 2006; Oliver et al., 2005; Wood, 2007; Proctor et al., 2007; Vitt et al., 2014) which focus on particular plant groups as well as different aspects of desiccation tolerance. It is present in a range of organisms but in the plant world is almost exclusively found in bryophytes. Species with this mechanism are able to survive long periods of drought but recover full metabolic activity once water is available. Bryophyte DT is conferred at a molecular level, but certain morphological traits can indicate how desiccation tolerant a species is based on how they affect its ecophysiology. Different bryophytes have varying levels of DT, including within the same habitat, and so their distribution could be used to indicate changes to forest integrity and forest bryophytes more susceptible to extinction could be identified based on their traits and DT level. Although certain traits are related to the environment a species is found in and can indicate the likely DT level of a species, this relationship is not clear-cut and needs further study, particularly among tropical bryophytes.

2.1 "Drying without dying" – desiccation tolerance

Animal and plant life on earth began in the sea and despite their migration and adaptation to land all terrestrial organisms still require water to survive (Alpert & Tuba, 2000). Water availability is therefore a determining factor in where and how organisms live (Alpert & Tuba, 2000). To cope with living in an environment which is mostly dry (global average is 77% relative humidity (NASA & SSE, 2009)) terrestrial plants have developed three main adaptations to prevent damage as a consequence of drought: 1) drought escape – increases their growth rate and productivity; 2) drought avoidance – expands the range of conditions they can survive in and 3) desiccation tolerance – expands the range of habitats they can survive in (Proctor, 2000a; Alpert & Tuba, 2000; Vanderpoorten & Goffinet, 2009). The latter is the strategy largely used by bryophytes.

Drought escape plants restrict their growth and reproduction to periods of the year when water is available. Spores, seeds and vegetative propagules produced during periods of water availability remain in the ground and resist the lack of water whilst the adult plant dies (Vanderpoorten & Goffinet, 2009). Some annual bryophyte species living in dry climates employ this strategy (Table 2.1). Drought avoidance (or drought resistance) plants are able to maintain a higher internal water balance than the external environment through the internalisation of water transport (water conducting vessels) and a waterproof surface with stomata. There are some species of bryophytes that have water conducting cells (hydroids) and others with large dead cells (hyalocysts) that can hold water, but their ability to retain a high internal water balance is poor (Vanderpoorten & Goffinet, 2009). Both these mechanisms are uncommon in bryophytes, particularly drought avoidance, though some bryophytes exhibit characteristics of these strategies (Table 2.1) such as a spore bank or completing their life-cycle when water is available (see 2.2.3.3.2 below, p. 79). Plants that rely on either of these strategies are referred to as desiccation sensitive (Alpert, 2006).

Table 2.1 Bryophyte genera, with family in parentheses, that exhibit characteristics of drought-escape or drought-avoidance strategies. (Vanderpoorten & Goffinet, 2009; Vitt et al., 2014)

Strategy	Moss	Liverwort
Drought escape	*Physcomitrella* (Funariaceae), *Acaulon*, *Aloina*, *Crossidium, Pterygoneuron* (Pottiaceae), *Pleuridium* (Ditrichaceae)	*Riccia* (Ricciaceae)
Drought avoidance	*Campylopus*, *Leucobryum*, *Octobelapharum*, *Paraleucobryum* (Dicranaceae), *Sphagnum* (Sphagnaceae), *Leucophanes* (Calymperaceae)	*Anthelia* (Antheliaceae), *Conocephalum* (Conocephalaceae), *Scapania* (Scapaniaceae)

Desiccation tolerance (DT) is the "ability to reach equilibrium with air that is moderately to extremely dry and then regain normal function after rehydration" (Alpert, 2005, p. 686); "drying up without dying", in its simplest terms (Proctor et al., 2007, p. 596). In order to do this, cellular structure, certain proteins and genetic components must remain undamaged during dehydration as well as hydration so that respiration and photosynthesis are restored soon after rehydration (Proctor & Tuba, 2002). This mechanism was first noticed in rotifers at the beginning of the 18th century but was only confirmed and accepted by the scientific community in the mid-19th century following further experiments (Alpert, 2000a). DT has since been documented in a wide range of small organisms such as bacteria, protozoa, nematodes, tardigrades, crustaceans, fungi (including yeasts and lichens) and plants (Treonis, 2005; Alpert, 2006). It is prevalent in bryophytes and lichens (currently classified as fungi) with most species in these two groups exhibiting some level of DT (Table 2.2) (Tuba et al., 1998; Alpert, 2000a). It can be considered a very successful adaptation strategy due to the presence of bryophytes in almost all habitats on earth, including microhabitats that tracheophytes cannot inhabit (Proctor, 2012). Although DT in bryophytes has been the subject of research since the early 20th century (Proctor & Smirnoff, 2000; Wood, 2007), the ability of bryophytes to inhabit dry habitats and survive drought has long been noticed; Francis Bacon made several observations of instances where there was insufficient moisture for plants to germinate but 'moss' would grow (Bacon, 1627, p. 139).

2.1.1 How many species are desiccation tolerant?

Although DT is present in all plant phyla, except gymnosperms (Table 2.2), less than 0.1% of angiosperms have vegetative parts that are tolerant (vegetative DT) (Oliver, 1996; Alpert, 2000a; Proctor et al., 2007; Gaff & Oliver, 2013). It should be noted that although most adult tracheophytes do not exhibit DT, pollen (Illing et al., 2005) and 90% of angiosperm seeds (Kranner et al., 2008) are DT. Very few fern species are known to have vegetative DT (Table 2.2), but a study on tropical fern gametophytes suggests that the gametophyte stage may be DT even if the sporophyte stage is not (Watkins et al., 2007). However, as the dominant life-phase in tracheophytes is the sporophyte stage, tracheophytes are considered DT if it is present in this stage and therefore in their vegetative tissues.

Table 2.2 Number of desiccation tolerant species per major taxonomic plant and fungi group, with respective references for figures.

Group	Number of species	Percentage of group	Reference
Algae	176	0.53	Gaff & Oliver 2013
Cyanobacteria	59	1.79	Gaff & Oliver 2013
Bryophytes	210 - Most	1 - 95	Wood 2007 - Alpert 2000
Gymnosperms	0	0	Oliver et al., 2000
Pteridophytes	64 - 1200	0.5 - 9.8	Watkins et al., 2007 - (Porembski, 2011)
Fungi		Some	Alpert 2006
Lichen		Most	Kranner et al., 2008
Angiosperms	135 - 300	0.038 - 0.085	Gaff & Oliver 2013 - Porembski, 2011

The number of DT species varies between authors, see Table 2.2, with some citing only species that have had their vegetative DT levels experimentally assessed although studies experimentally assessing DT have focussed on a small number of species (Wood, 2007; Holzinger & Karsten, 2013). Wood (2007) provides a useful synthesis of bryophyte species that have been experimentally assessed and found that fully-DT species (defined in this case as those that can survive desiccation in extremely dry air for at least 6 hours, 0-30% RH) are found in 6 of 13 bryophyte classes. Moss orders and classes that have as yet not been found to have any DT species are: Archidiales, Bryoxiphiales, Buxbaumiales, Funariales, Ptychomniales, Scouleriales (Bryopsida), Andreaeobryopsida, Oedipodiopsida, Sphagnopsida and Takakiopsida; within the liverworts there are no DT species in the Blasiales, Spaerocarpales (Marchantiopsida) or Haplomitriopsida (Wood, 2007). It is likely, however, that most bryophytes have some level of DT as suggested by studies looking at aspects of DT, but not directly assessing DT level (e.g. increased DT induced in *Funaria hygrometrica* (Funariales) following exposure to ABA (abscisic acid) in Werner et al., 1991) and by the fact that most bryophytes occupy periodically dry microhabitats (Stark & Brinda, 2015a). Hornworts are not known to be DT and only one epiphytic species from New Zealand, *Dendroceros granulosus*, has experimentally shown to be DT (Wood, 2007). Altogether this yields only 1% of bryophyte species that have experimentally confirmed to be DT (Wood, 2007; Oliver, 2009) showing that there is a large gap to fill in terms of quantitatively assessing and measuring DT in bryophytes.

The presence of DT may be linked to size as most DT tracheophytes are perennial herbs (Alpert, 2000a). Also, DT tracheophyte species that are quickest to rehydrate following desiccation (1.5 hours) are the species of the small herb genus *Craterostigma* (Alpert, 2000a). One of the reasons could be because low lying plants are exposed to higher temperatures (irradiation from the ground) meaning that the usual drought avoidance mechanism used in other vascular plants, e.g. closing stomata, is not suitable as it would not allow them to transpire (Proctor et al., 2007).

2.1.1.1 Evolutionary need for desiccation tolerance

The ability to lose cellular water and survive clearly provides protection in a dry environment, albeit at the cost of limited growth and reproduction. The fact that many plant species are DT at

some stage of their life cycle indicates that most land plants have retained the genetic potential for DT (Proctor, 2009). The loss of vegetative DT in adult tracheophytes is thought to stem from their migration into habitats less exposed to drought (Alpert, 2006) and the development of a water conducting system together with a waterproof cuticle and stomata allowing them to control their water uptake and loss more efficiently (Oliver et al., 2005) and continue metabolising in times of water-stress (Bartels et al., 2011). Bryophytes, on the other hand, retained the ancient trait of vegetative DT, the ability to dry out and suspend metabolic activity when water is scarce (Proctor et al., 2007; Proctor, 2009). However, even within bryophytes species have different levels of DT, ranging from those that are highly DT (e.g. *Tortula* spp.; Proctor et al., 2007) to those that have a very low DT (e.g. *Physcomitrella patens*; Vitt et al., 2014). Generally, bryophytes of drier and more exposed (xeric) habitats are more DT than those from more humid and sheltered habitats (mesic). This range of DT is illustrated throughout section 2.2 and discussed in section 2.2.5, p. 80.

2.1.1.2 Geographical distribution

In angiosperms, DT species have an uneven global distribution and are mostly located in the tropics: sub-Saharan Africa, Madagascar, western Australia and south America (Porembski & Barthlott, 2000a). Within these regions, they are mostly found on rock outcrops (Porembski & Barthlott, 2000a). On the other hand, DT bryophytes have a wide geographic distribution (Alpert, 2000a).

2.1.2 How tolerant is tolerant?

Tracheophytes normally have relative water contents (RWC) of between 85%-100% and begin to die once RWC reaches 30%; DT tracheophytes have RWCs of between 5-13% (Gaff & Oliver, 2013). Bryophytes can recover from RWC as low as 5% (Lakatos, 2011), similarly to lichens (Kranner et al., 2008). It is estimated that metabolism ceases when there is 0.1g water per 1g of tissue and so DT bryophytes can be quantitatively defined as those that survive "drying to 10% water content or less" (Alpert, 2005, p. 686, 2006). Damage caused by desiccation is varied (see table Table 2.4, p. 64 for more details) but centres around oxidative damage due to production of oxygen reactive species and disintegration of cellular structures.

Ecologically, DT mechanisms can be classified as constitutively DT (CDT) - they can survive rapid drying with minimal damage - or inducibly DT (IDT) – they require slow drying in order to minimise damage and can be considered to go through a hardening process (Stark et al., 2014; Stark & Brinda, 2015a). Put simply, CDT species recover fast following desiccation as the mechanisms are already in place, whereas IDT species recover slowly. Bryophytes have been considered to be mostly CDT with a few species considered to be IDT as they cannot escape damage following rapid drying (Proctor et al., 2007; Oliver, 2009). Recently, however, this has been questioned due to the fact that IDT bryophytes that have been hardened in the field appear to be CDT whereas in fact they are IDT species whose DT mechanisms were induced in the field; this raises the suggestion that specimens should be acclimatised in the laboratory before measuring DT and that IDT bryophytes may be more common (Stark et al., 2014).

DT tracheophytes differ from lichens and bryophytes in that most can survive desiccation only if the water loss is gradual - they are IDT - while lichens and several highly DT bryophytes are able to survive rapid rates of water loss (Oliver et al., 2000; Stark & Brinda, 2015a). However, even in

highly DT bryophytes (those that can survive desiccation in "extremely dry air (0-30% RH)" (Wood, 2007, p. 165), rapid drying can cause more damage than slow because of the shorter time available to activate protection mechanisms (Oliver & Bewley, 1996; Alpert, 2006; Proctor et al., 2007; Oliver, 2009; Stark & Brinda, 2015a). Further, while DT bryophytes are able to resume metabolic activity within minutes of rewetting (Proctor & Tuba, 2002), recovery in DT tracheophytes takes much longer, from several hours to days (Alpert, 2000a). For further details on the differences between tracheophytes and bryophytes, Proctor and Tuba (2002) provide a comparison of water relations and Oliver and Bewley (1996) provide a review on metabolic processes.

Some bryophyte species show survival in extreme conditions under laboratory experiments (Table 2.3) but it is unlikely such extreme survival occurs in the field (Alpert, 2000a) and so is of limited use for investigating the real level of bryophyte DT in the field. Although present in many habitats, bryophytes do not occur in areas without regular precipitation (Proctor et al., 2007) and so extreme conditions of drought simulated in the laboratory are not a true depiction of the environmental extremes experienced by bryophytes. There have been a few studies investigating DT directly in the field (e.g. Proctor, 2004; Stark et al., 2005; Léon-Vargas et al., 2006) and the longest period without precipitation was 191 days (Stark et al., 2005). As a result of these studies, a more accurate method for assessing bryophyte DT in the field is to conduct experiments with alternating short wet and dry periods of days to minutes (Proctor et al., 2007).

Table 2.3 Survival of mosses in extreme environmental or temporal conditions. (Taken and adapted from Alpert, 2000a, Table 1, p. 8, with new data added).

Species	Environmental condition	Exposure time	Test for survival	Source
Syntrichia ruralis	-198°C	24 hours	RNA and protein synthesis	
Grimmia & *Barbula* (leaves)	Less than 0.05 °C above absolute zero	2 hours		Alpert, 2000
Riccia macrocarpa	Air dry (herbarium)	23 years	New cells at apices	
Grimmia laevigata	Air dry (herbarium)	7-10 years	New shoots or protonema	7)(Keever, 1957)
Racomitrium lanuginosum	32% RH	1 year	Recovery of photosynthetic function	Tuba et al., 1998
Andraea rothii	32% RH	1 year	Recovery of photosynthetic function	Tuba et al., 1998
Syntrichia caninervis	2-4% RH	6 years	Metabolic activity & new growth	Oliver et al., 2005

2.2 What makes bryophytes desiccation tolerant?

The underlying biochemical and physiological mechanisms of DT are complex as evidenced by the many studies on the topic. Bryophytes are one of the best studied plant groups in terms of DT (Kranner et al., 2008; Holzinger & Karsten, 2013) with a number of studies addressing specific aspects of bryophyte DT (e.g. molecular pathways: Werner et al., 1991; water relations: Santarius, 1994; Proctor et al., 1998; photosynthetic recovery: Proctor & Smirnoff, 2000; Proctor, 2003; León-Vargas et al., 2006; cytology: Pressel et al., 2009; Pressel & Duckett, 2010; morphology: Proctor, 2004; Song et al., 2015) as well as several recent reviews (Proctor, 2000b, 2009; Proctor & Tuba, 2002; Oliver et al., 2005; Glime, 2007; Proctor et al., 2007; Oliver, 2009). This section outlines the main aspects of bryophyte physiology that relate to DT. The physiological processes involved in DT have been well documented, and detailing them all is beyond the scope of this thesis. Therefore the main physiological processes are outlined – water intake, gas exchange and photosynthesis – and how they are affected by desiccation. A brief summary of the biochemical molecular mechanisms involved in desiccation is presented as this is the level at which DT is conferred (Oliver, 2009); it will also provide an understanding of the challenges bryophytes face when drying out. The relationship between DT and morphological traits is reviewed, as these are the most pertinent to this study, with a more detailed description of how morphological traits affect DT (section 2.2.3), as well as life-history and ecological characters. Finally, the methods used to quantitatively measure DT in bryophytes and how they are used to define the different DT levels exhibited by bryophytes are summarised.

2.2.1 Physiological ecology

Most studies on bryophyte physiological ecology have focussed on bryophytes of temperate forests and regions but there has recently been an increasing number of studies on tropical or temperate rainforest bryophytes (e.g. Proctor, 2004; León-Vargas et al., 2006; Pardow & Lakatos, 2013; Song et al., 2015). As mentioned above, responses to desiccation are complex and vary according to drying speed, length of time exposed to desiccation and environmental conditions (Proctor et al., 2007; Oliver, 2009; Stark et al., 2014). Level of DT varies between species but can also vary between populations of the same species and even across generations (Oliver, 2009) Oliver et al. 1993 in (Alpert, 2000a). Respiration seems to be less affected by desiccation than photosynthesis and so measuring photosynthetic performance could be a better indicator of a species DT level (Holzinger & Karsten, 2013). Essentially, the goal of metabolic processes is the accumulation of carbon (net carbon gain) through photosynthesis; the water relations and gas exchange outlined below come together with light capture and microclimatic variables to determine the photosynthetic and respiration rate of bryophytes.

2.2.1.1 Water interception, conduction and storage

As water is a requirement for photosynthesis, the ability of bryophytes to intercept and store water is central to their physiology. Their interaction with water is defined by the fact that they are poikilohydric – unable to regulate their water content – a trait retained from the first terrestrial plant colonisers (Bates, 1998). Whereas other major land plant groups have lost their poikilohydry, bryophytes have maintained this in part because this is an optimal strategy for their size (Tuba et al., 1998; Proctor et al., 2007; Proctor, 2009) although it exposes them to regular desiccation (Proctor, 2009; Lakatos, 2011). There is also a trade-off between surviving desiccation by suspending metabolism, and growth and reproduction. Their lack of a comprehensive vascular

system is explained by the fact that, for small organisms such as insects, lichens and bryophytes, surface tension is a greater force than gravity so equilibration with surrounding air is the optimal strategy (compared to the water pumping of other plants) (Proctor et al., 2007). Bryophyte poikilohydry means that their physiology is directly controlled by the ambient air humidity (Bates, 1998; Proctor, 2009) and enables them to utilise water vapour (fog) as well as liquid water (dew or rain) (Barkman, 1969; Lakatos, 2011; Song et al., 2015). Bryophyte water loss rates are therefore dependent on the ambient relative humidity (Oliver, 2009) as well as the boundary layer surrounding the plant which affects the gaseous diffusion of water (Proctor & Tuba, 2002).

Despite sometimes possessing conductive tissues (e.g. in the genus *Polytrichum* (Proctor et al., 1998), water conduction takes place mostly in the external capillary spaces of the plant – bryophytes are ectohydric (Proctor, 2009; Lakatos, 2011). These external interconnecting capillary spaces are found in various places: between leaves, rhizoids, tomentum, paraphyllia, papillae and between shoots (Proctor, 2009). Their lack of cuticle and thin leaves/thalli (with the exception of some species such as in the Polytrichaceae) allow them to take in water throughout their whole surface (Proctor, 2009; Vanderpoorten & Goffinet, 2009). Within the bryophyte tissues, water conduction is through diffusion between cells and within cell walls, similarly to small tracheophytes (Proctor, 2009).

Bryophyte water content is composed of apoplast (within cell walls and between cells), symplast (within cells e.g. hyalocysts in *Sphagnum* species) and the external capillary water (Dilks & Proctor, 1979; Proctor et al., 1998; Proctor & Tuba, 2002). Unlike drought-tolerant plants (e.g. succulents) that store symplast water, most water in bryophytes is stored in the external capillary spaces (Proctor & Tuba, 2002) and this external capillary water is equally important in physiology (Proctor, 2009). External capillary water acts as a buffer allowing bryophytes to remain at full turgor (and therefore at maximum photosynthetic rate) for a period of time after atmospheric humidity has decreased (Proctor & Tuba, 2002); consequently the time spent “wet” and “dry” is less than in tracheophytes (Proctor, 2009). Once external capillary water is lost, cell water potential decreases and metabolic processes will cease (Proctor & Tuba, 2002). Bryophytes that have low water storage capacity will cease photosynthesis rapidly, in relation to species that are able to store greater amounts of water in their capillary spaces (Proctor, 1990; Song et al., 2015). The water relations of bryophyte cells are similar to those in other plants (Figure 2.1) (Proctor & Tuba, 2002; Proctor, 2009) but bryophytes can have extremely high water contents – up to 2000% of their dry weight – and osmotic potential at full turgor is usually between -1.0 to -2.0 MPa, though they can reach -9 to -10 MPa (Barkman, 1969; Proctor et al., 1998; Proctor, 2009). Bryophytes experience turgor loss at between 60% – 80% of RWC (Proctor et al., 1998). A species’ level of desiccation is quantified in the literature as the minimum external water potential (MPa) or relative humidity (%) a bryophyte can survive (Oliver, 2009).

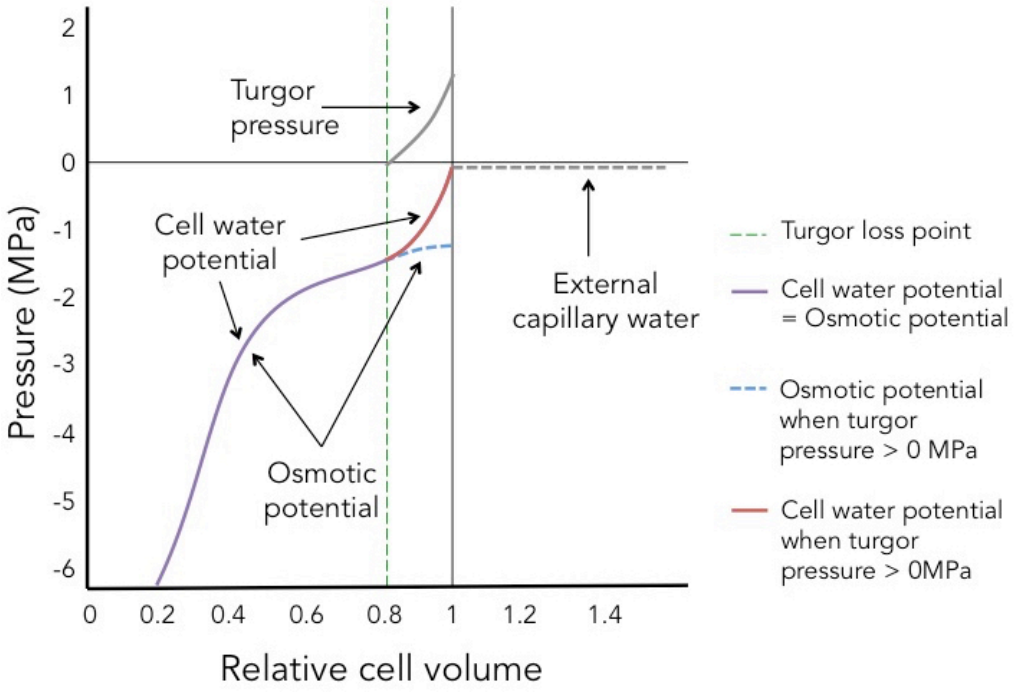


Figure 2.1 Cell water relations depicted by the Höfler diagram showing the relationship between external water potential, cell water potential, cell osmotic potential and turgor pressure. The water potential of a cell is the sum of its osmotic potential and turgor pressure. When in full turgor the cell's osmotic potential is balanced by the turgor pressure of the cell wall and it is in equilibrium with the external water potential. The cell's water potential is therefore 0 MPa. The water held in the plant's capillary spaces is shown by the grey dotted line, and occurs when cell water potential is zero as no more water can pass into the bryophyte's cells. As the amount of external water decreases, turgor pressure decreases and causes the initial decrease in cell water potential, as well as a reduction in cell volume. Osmotic potential becomes negative, and when the plant's turgor loss point (green dashed line) is reached, the cell water potential and osmotic potential are equal (purple). Based on data measured from the leafy liverwort *Porella platyphylla*. Taken and adapted from Proctor 2009, fig. 6.1, p. 240.

Water potential of bryophyte cells is correlated with the cell wall thickness to lumen ratio (Proctor, 2009), in other words species with small, thick-walled cells have less negative osmotic potentials. Other morphological traits play a role in water relations and are discussed in section 2.2.3, p. 65. There does not seem to be a pattern between osmotic potential of a species and the humidity of the habitat they occupy but relative water content when in full turgor (as percentage of dry mass) tends to be greater in species of humid habitats (Proctor, 2009). Poikilohydry and ectohydry allow bryophytes to lose and gain water quickly, in contrast to most tracheophytes, which has implications for respiration and photosynthesis, as well as for DT (Proctor & Tuba, 2002; Proctor, 2009). Although water is vital for metabolic processes, having the leaf surface completely covered by water would prevent gas exchange (Proctor, 2009; Vitt et al., 2014), another fundamental component of photosynthesis and respiration.

2.2.1.2 Gas exchange

On entering plant cells from the atmosphere, CO_2 needed for photosynthesis faces two resistance mechanisms: "liquid-phase diffusive resistance" in cell walls and cytoplasm (due to water leaving cells in the opposite direction) and "carboxylation resistance" in the chloroplasts (Proctor, 2009). There is therefore a trade-off between water uptake and gas exchange, as shown by the changes in photosynthetic rate as water content changes (Figure 2.2), with water content limiting CO_2 absorption (Zotz et al., 2000). However, if CO_2 concentration increases, high water content is no longer a limiting factor (Glime, 2007). To reduce liquid-phase diffusive resistance, bryophytes tend

to have relatively high leaf-area index values (total leaf area/plant occupied area) and can increase this through morphological structures such as lamellae, papillae or wax which keep surface areas free of water (Proctor & Tuba, 2002; Proctor, 2009; Vanderpoorten & Goffinet, 2009).

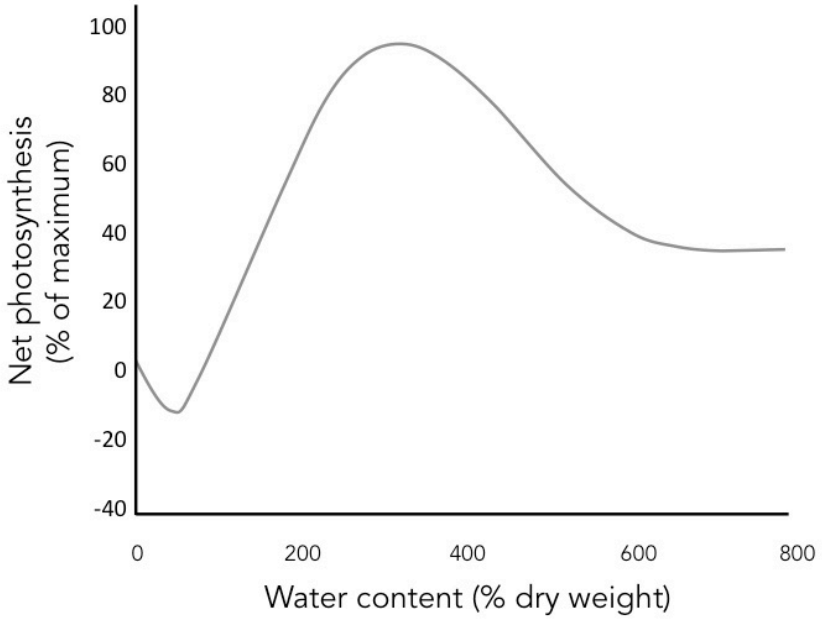


Figure 2.2 Changes in net photosynthesis at different water contents (% dry weight) in the moss *Grimmia pulvinata*. An optimum photosynthetic level is reached between 200-400% water content illustrating the trade-off between water and gas exchange. (Taken from Zotz et al., 2000, figure 5, p. 63.)

2.2.1.3 Light

Although water is a determining factor in DT, light also contributes to bryophyte response (Seel et al., 1992a; Marschall & Proctor, 2004). Bryophytes have to adapt to both extremes of light availability: being able to photosynthesize at low light levels and prevent damage at high light levels (due to oxidation (Oliver & Bewley, 1996). Light as used in this thesis refers to the amount of insolation (incident solar radiation) that is available for photosynthesis and is measured as the amount of photons: Photosynthetic Photon Flux Density (PPFD, µmol m^{-2} s^{-1}). In order for light to enter cells (in the form of photons), surface area must be available. The light levels required for bryophytes to achieve net positive photosynthesis, the light compensation point, are comparatively lower than those in tracheophytes (often 20% of full sunlight available (Marschall & Proctor, 2004)). Similarly, the light saturation point (the point at which no more photons can be accepted by the photosynthetic apparatus) is lower, it usually occurs at about 600 µmol m^{-2} s^{-1} (Vanderpoorten & Goffinet, 2009) but can reach 1000 µmol m^{-2} s^{-1} (Proctor, 2004); full sunlight is around 1800 µmol m^{-2} s^{-1}. Bryophytes of wet habitats tend to have higher light saturation levels than those of dry habitats (Vanderpoorten & Goffinet, 2009). When in high light conditions, there is an increase in heat particularly close to the ground or substrates due to irradiation; this is therefore a problem for bryophytes with their small stature (Proctor & Tuba, 2002). Oxidation can also reduce the amount of chlorophyll pigments, photobleaching, causing damage to the photosynthetic apparatus (Seel et al., 1992a). Bryophytes can prevent heat damage at the molecular level (e.g. dissipating energy as heat by non-photochemical quenching (NPQ), see 2.2.2 below) and also at the morphological level by curling their leaves when drying (Porembski & Barthlott, 2000b; Alpert, 2006). However, the level of light even the most DT bryophytes can

survive is limited as noted by Frahm (2000) when observing the absence of bryophytes on exposed lowland rocks.

CO_2 diffusion is limited by light as well as by the amount of external surface covered in water water (Proctor, 2009). Species of exposed habitats tend to tolerate higher insolation thresholds than forest species as well as having larger leaf areas and higher chlorophyll a and b contents (Proctor, 2009). Within forests, species with higher NPQ are found in areas where light intensity is greater (Proctor, 2004). It has been shown that the recovery of photosynthetic ability varies depending on whether species are from dry, exposed habitats or humid, sheltered ones (Proctor & Smirnoff, 2000; Proctor, 2009), providing a potential use as indicators of habitat change. NPQ values are higher in bryophytes from exposed, dry habitats due to the higher insolation levels requiring them to use heat dissipation to protect cells from oxidative damage (Proctor, 2009). Several studies have found that respiration rate changes less with different water contents when compared to photosynthetic rate (Proctor, 2009).

2.2.1.4 Micro-climate variables

Water loss is the governing factor for bryophyte growth, reproduction and survival and is affected by relative humidity (RH), insolation and wind (Seel et al., 1992a; Oliver, 2009). The small size of bryophytes means they live within the boundary layer, either of the bryophyte colony or of their substrate, and therefore the physics of wind currents apply differently to bryophytes than in tracheophytes (Proctor, 2009). Evaporation increases with wind speed and the rate of water loss is slower when the air flow is laminar than when air flow is turbulent (Figure 2.3) due to the decreases in the thickness of the boundary layer (Proctor, 1990). This has implications at the macro- and micro-habitat scale as bryophytes of dense canopy forests will be exposed to lower wind speeds than those in forests with canopy gaps (such as in disturbed forests) (Proctor, 2012). Within a habitat, bryophytes on the lower trunk will also be exposed to lower wind speeds than those in the upper trunk and canopy. In terms of water availability, it is the frequency of humidity rather than the amount that is most important for bryophytes in forests (León-Vargas et al., 2006); this clearly has implications at the microhabitat level due to the variation in microclimate variables mentioned.

At high temperatures, the photorespiration rate, which uses carbon, is greater than the photosynthetic rate reducing productivity and making it energetically inefficient to remain metabolically active at high temperatures (Glime, 2007). Bryophytes of dry and exposed habitats will desiccate faster and so become metabolically inactive quicker than those that occupy humid and sheltered habitats (Proctor & Tuba, 2002; Proctor, 2004; Song et al., 2015).

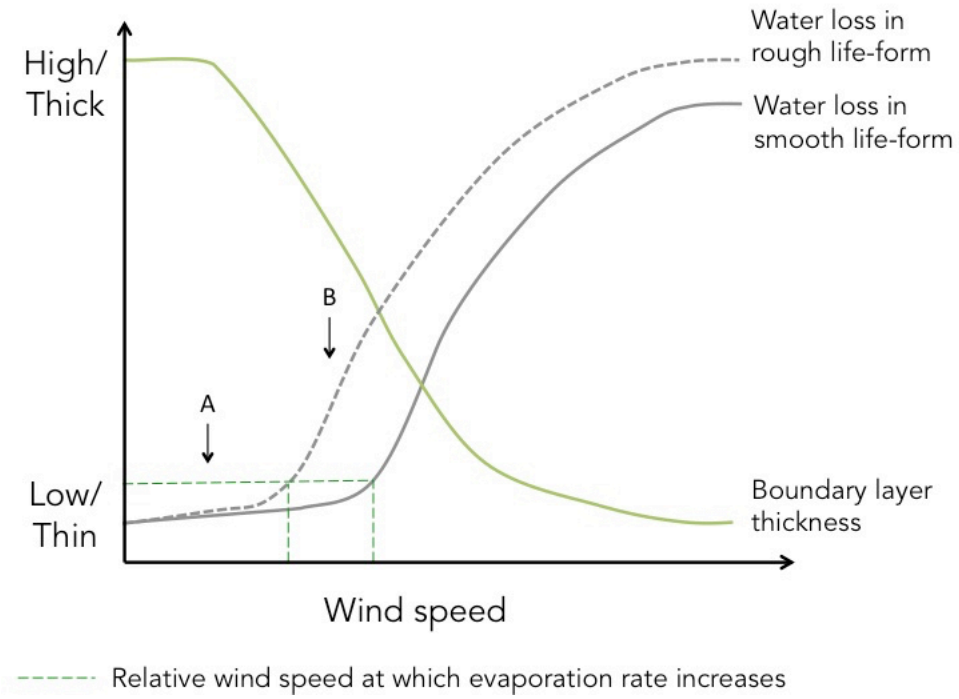


Figure 2.3 Schematic diagram showing relative bryophyte water loss and boundary layer thickness in relation to wind speed. A- At low wind speeds the colony acts as a leaf and evaporation is low; air-flow is laminar. B- As wind speed increases so does evaporation rate, the bryophyte surface generates turbulence and evaporating area increases due to decreasing boundary layer; air-flow is turbulent. Water loss increases at lower wind speeds in bryophyte colonies with rougher surfaces. Drawn with information from Proctor 2009.

2.2.2 Biochemical molecular mechanisms

Alpert (2006) and Oliver (2009) provide detailed reviews of the damage caused to organisms and bryophytes, respectively, by drying and the mechanisms employed to prevent or repair the damage. Most of the knowledge on molecular mechanisms comes from studies on the highly DT moss *Syntrichia ruralis* (Oliver et al., 1998; Proctor & Tuba, 2002) and mosses are much better understood than liverworts (Pressel et al., 2009; Vitt et al., 2014). Some damage occurs as a result of photosynthesis ceasing and others as a direct consequence of water loss (Table 2.4). Bryophytes protect their tissues, and hence their metabolic processes, during desiccation but also employ repair mechanisms following hydration (Oliver, 1996, 2009; Maxwell & Johnson, 2000; Alpert, 2006) though these seem less numerous and critical than those in tracheophytes (Oliver et al., 1998; Proctor & Tuba, 2002; Illing et al., 2005). As the focus of this study is not at the molecular level, a summary table of main effects and molecular processes is provided (Table 2.4). The speed, exposure time and amount of desiccation in bryophytes is important in determining the level of damage they sustain (Oliver, 2009). The main components involved in DT are: sugars, protective proteins and antioxidants (Alpert, 2006; Oliver, 2009) although exactly how these mechanisms confer DT is not yet fully known (Oliver, 2009). The extent to which these processes are present and the speed at which they are 'switched on' determine a bryophyte's response to desiccation and hence its DT (Oliver, 2009). For example, in the highly DT *Syntrichia ruraliformis*, there is a higher level of anti-oxidant enzymes than in the less DT *Dicranella palustris* (Seel et al., 1992b).

Some DT plants lose their chlorophyll and thylakoids (photosynthetic apparatus) when drying out, others do not; they are termed poikilochlorophyllous and homoiochlorophyllous, respectively (Tuba et al., 1998; Oliver et al., 2000; Porembski & Barthlott, 2000a). Each strategy has different advantages: retaining chlorophyll reduces the amount of photo-oxidative stress but homoiochlorophyllous species can survive rapid drying and recover photosynthetic activity faster (Tuba et al., 1998; Oliver et al., 2000; Porembski & Barthlott, 2000a). Bryophytes tend to be homoiochlorophyllous, and the photosynthetic apparatus is maintained through some of the protective mechanisms listed in Table 2.4 (Proctor & Tuba, 2002). The maintenance of the photosynthetic apparatus allows bryophytes to survive rapid cycles of drying and rehydration; cycle lengths that tracheophytes are less likely to be exposed to as they cannot rehydrate from water vapour or dew alone (Tuba et al., 1998; Proctor & Tuba, 2002).

Table 2.4 Main biochemical molecular mechanisms involved in DT of plants and their presence in bryophytes according to latest research. Compiled from Proctor & Tuba 2002 and Oliver 2009.

Stage	Mechanism	Molecular protection or repair process	Damage/process	Present in bryophytes
Dehydrating	Constitutive Cellular Protection	Slow drying induced by production of abscisic acid (ABA)	Fast drying	Yes – some species
		Supressing enzyme activity following ceasing of photosynthesis	Reactive oxygen species (ROS) generated	Yes – amount varies
		Emitting energy from light as heat (non-photochemical quenching, NPQ)	Reactive oxygen species (ROS) generated	Yes – liverworts & mosses
		Sugars - sucrose content maintained	Hydrogen molecular bonds broken	Yes
		Sugars - biological glass formation	Disintegration of membranes and aggregation of macromolecules	Not verified
		LEA proteins (Late Embryogenesis Adundant - proteins that protect other proteins)	Enzymes denature	Yes
			Membrane disintegrates	Yes
			Disordered cellular collapse	Yes
		Ordered cell collapse due to microtubular cytoskeleton	Disordered cellular collapse	Yes
Rehydrating	Induced Recovery and Repair Mechanism	Control water re-entry into cells	Fast rehydration	No – angiosperm seeds
		Rapid repair of cellular leakage	Solute leakage from protoplasm due to membrane disintegration	Yes
		Rapid recovery of cell structure	Disordered cellular collapse	Yes

Stage Mechanism	Molecular protection or repair process	Damage/process	Present in bryophytes
	Rapid recovery of protein synthesis due to presence of already transcribed protein mRNA (transcribed during dehydration)	Protein synthesis metabolism slow to recover	Yes
	Alteration of gene expression	Protein synthesis metabolism ceased	Yes
	LEA protein gene expression increased		Yes
	Rapid recovery of photosynthesis function	Photosynthesis slow to recover	Yes

2.2.3 Bryophyte desiccation tolerant traits

Many definitions of "trait" have developed over time but a good definition is: "Any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level, without reference to the environment or any other level of organ." (Violle et al., 2007, p. 884). Traits are representative of how species biochemistry functions; for example, photosynthesis is affected by water content and CO_2 intake and certain traits such as specific leaf area (Albert et al., 2010) can maximise, or minimise, the amount of these.

Some studies have looked at how morphology relates to DT and environment, either observationally or experimentally (Clee, 1937; e.g. Bischler & Jovet-Ast, 1981; Proctor, 1982, 2004; Song et al., 2015). As briefly mentioned in the previous section, although DT is conferred by biochemical mechanisms, certain morphological traits can indicate how desiccation tolerant a species is based on how they affect its ecophysiology i.e. water uptake and storage and surface area available for gas exchange and light capture (Vanderpoorten & Goffinet, 2009; Vitt et al., 2014). Technically, this is a type of drought-avoidance or drought-escape as they are using morphology to avoid or reduce the effects of desiccation, whereas true DT is conferred at the biochemical level. However, morphological traits are representative of how species biochemistry functions e.g. photosynthesis is affected by water content and CO_2 intake and certain traits such as specific leaf area (Albert et al., 2010) can maximise, or minimise, the amount of these. Therefore species with traits that allow them to avoid/reduce desiccation effects (e.g. smaller leaf size to reduce transpiration) will inhabit drier and more exposed habitats than species that do not, and therefore their ecological DT is greater (Alpert, 2000b). Additionally, there is no strict delimitation between DT and drought-avoidance in bryophytes (Vitt et al., 2014). Other factors beside species traits affect the presence of species in particular habitats (e.g. environmental factors, survival ability, competition and stochastic events), but DT of species also has an impact on the likelihood of establishment (Bates, 2009; Rydin, 2009).

Morphological traits affect DT by essentially either prolonging metabolic activity when the surrounding environment gets drier or reduce potential for damage due to desiccation or high light levels. The traits discussed below are not an exhaustive list, but focus on those that are present in many species, are observable at the light microscope level and have sufficient known variability between species. They are discussed in the context of how they interact with water, light capture and DT level, but it should be noted that certain traits may also play a role in other

aspects of bryophyte ecology (e.g. protection against herbivory). The traits are divided into gametophyte, sporophyte and life-history traits as they are different types of traits: gametophyte traits are present throughout a bryophyte's life-cycle and so are those most responsive to environmental conditions; sporophyte traits are only present for a short period, if at all, and inform reproduction success; and life-history traits inform species phenology (Violle et al., 2007). For information on a wider range of morphological traits see the reviews in Glime (2007) which provide a thorough overview and illustrative photographs.

2.2.3.1 Gametophyte traits

Most of the traits used in this study are gametophytic traits as this is the dominant life phase of bryophytes, hence these traits are more exposed to the environment than sporophytic ones (Hedenäs et al., 2014) and so may be more representative of a bryophyte's adaptation to environmental conditions. It is also easier to find data on these traits than on sporophyte ones due to the lack of sporophyte observation in some species and because some species rarely or never produce sporophytes. The longer exposure to the environment also means that there is wider plasticity in gametophytic traits (Hedenäs et al., 2014) allowing for differences in species to be found, which may not be there when looking at sporophyte traits. This is in slight contrast to phylogenetic studies where sporophyte and gametophyte characteristics are used due to the morphological variation displayed in both generations (Shaw et al., 2011). It is also likely that bryophyte gametophytes exhibit a wider range of DT than their sporophytes, as demonstrated in ferns (Watkins et al., 2007), and by the fact that liverwort setae dehydrate as soon as the spores are released. The traits below refer to all three bryophyte phyla as many traits behave the same way across the phyla, but where there are traits specific to a phylum these are indicated.

2.2.3.1.1 Plant colour

Although there have been no studies looking specifically at how bryophyte colour varies with environmental conditions, it is known that certain plant colour can be associated with particular environmental conditions due to changes in the ratios of photosynthetic pigments. In DT tracheophytes of rocky outcrops, it has been observed that they turn a greyer colour when dried out (Porembski & Barthlott, 2000a). When exposed to high light levels, bryophytes that are less DT suffer a greater reduction in chloroplast pigments, known as photobleaching (Seel et al., 1992a). Highly DT species vary little in pigment quantities or ratios (chlorophyll a:b) when desiccated or not (Seel et al., 1992a) and chlorophyll content is higher in less DT bryophytes and those of sheltered habitats (Seel et al., 1992a; Marschall & Proctor, 2004) giving these plants a "greener" appearance. Yellow, orange, red or purple pigmentation could indicate species of more exposed environments as carotenoid pigments provide photo-protection in mosses (Heber et al., 2001). *Sphagnum* species of open habitats have been found to have higher concentrations of these pigments (Rice et al., 2008). Liverworts of drier habitats tend to have darker colours, with pigments protecting chlorophyll from high light levels (Proctor, 2010). Plant shine, i.e. reflectiveness of light, has been suggested to provide photo-protection and some species of dry and exposed habitats exhibit this trait (Glime, 2015a).

2.2.3.1.2 Life-form

Life-form is one of the morphological traits that has been most studied in relation to DT as it is easily observable (Rice et al., 2001; e.g. Proctor, 2004; Song et al., 2015). A bryophyte's habit or form is influenced by the environmental conditions it is found in, meaning that this trait can indicate the humidity and insolation of a bryophyte's habitat and plays an important role in a

species' DT (Proctor, 1990; Kürschner et al., 1999). To describe a bryophyte's form several characters exist ranging from those at the small-scale level, e.g. position of leaves on the branch, to the largest organization level i.e. how individual plants are arranged in the colony. These two levels can be referred to as the growth-form and the life-form of a species, respectively, and it is important to make the distinction between them. As defined by Bates (1998, p. 224) growth-form refers to the "(…) positions of [a plant's] growing points, its mode of branching, leaf orientation, etc." whereas life-form "(…) combines the features of growth-form with the assembly of shoots into colonies and modification of the resultant form by local environmental conditions." Several authors also state that life-form is more determined by the ecology of a species (Bates, 1998). The first published study on plant growth-forms was in 1806 by Alexander von Humbolt who produced a broad classification of fifteen types. Since then there have been several life-form classifications produced including ones specific to bryophytes. Mägdefrau (1982) devised 9 categories for bryophytes' life-forms and this system, or an adaptation of it, is most commonly used. Both life-form (Figure 2.4) and growth-form are used as traits in this study, but the characters that make up growth-form are individually recorded and are discussed further below (see 2.2.3.1.4, p. 68).

Generalizations on the relationship between life-form and DT can be made, although there are other factors that affect the relationship between environment and life-form and there is no strict system for categorising life-form according to ecophysiology (Bates, 1998; Song et al., 2015). Generally, DT decreases from: cushions, tufts and mats to dendroid, fan, pendant and weft (Proctor, 2004). Species that have more tightly packed forms (e.g. cushions and smooth mats) can slow down the loss of water from the plant due to maintaining the boundary layer (Oliver et al., 2005) whereas 'rougher' and more open life forms create more turbulence in the air-flow around a bryophyte and so evaporation is greater (Rice et al., 2001; Proctor, 2004, 2009). Although open life-forms have higher evaporation rates than other life-forms (Proctor, 2004), the trade-off is that the area for gas exchange and light capture is greater. As open life-forms tend to be found in more sheltered areas where light levels are low they need to maximise surface area for light capture, and as wind speeds are lower their exposure to evaporation will be lower also, so the trade-off is worthwhile (Proctor, 2004; Song et al., 2015). Open forms have less external capillary spaces and so water storage is minimal (Song et al., 2015), but again, as they are found in sheltered habitats, water storage is not a priority as water is more available than in exposed habitats due to higher humidities. Essentially, life-form is a trade-off between water interception and storage and light capture (Proctor, 1990).

Globally, certain types of life-forms predominate at particular elevations and are related to the varying humidity and insolation levels (Kürschner et al., 1999). Lowland forests, where humidity is lower, tend to be dominated by mat-forming bryophytes but species of the Calymperaceae and Rhizogoniaceae families, which form cushions and tufts, can be found along forest edges and in more open areas of forests (disturbed) (Kürschner et al., 1999). In forests at a higher elevation, the higher humidity gives rise to species with weft, fan, dendroid and pendant life-forms and at the highest elevation turf and cushion life-forms appear due to the decrease in humidity (Kürschner et al., 1999). Open forms (dendroid, fan, pendant and weft) are found in the more sheltered areas of forests (Proctor, 2004).

2.2.3.1.3 Plant size

The size of a plant will affect how it interacts with the environment and should be taken into account when conducting comparative physiological or ecological studies (Proctor, 2000a; Zotz et

al., 2000). Not much research has used bryophyte size as a variable when investigating species traits and environment although one study found that larger plants tend to be found at higher elevations, within the same species (Benassi et al., 2011). Walker & Preston (2006) use plant height in their study of vascular plant extinction risk and found that most species that had become extinct in their study region were short. Larger bryophytes may have a competitive advantage, not only because they physically occupy a larger area, but also as they are able to intercept a larger amount of moisture and if a branched form they reduce evaporation rates from sheltered branches (Vitt et al., 2014).

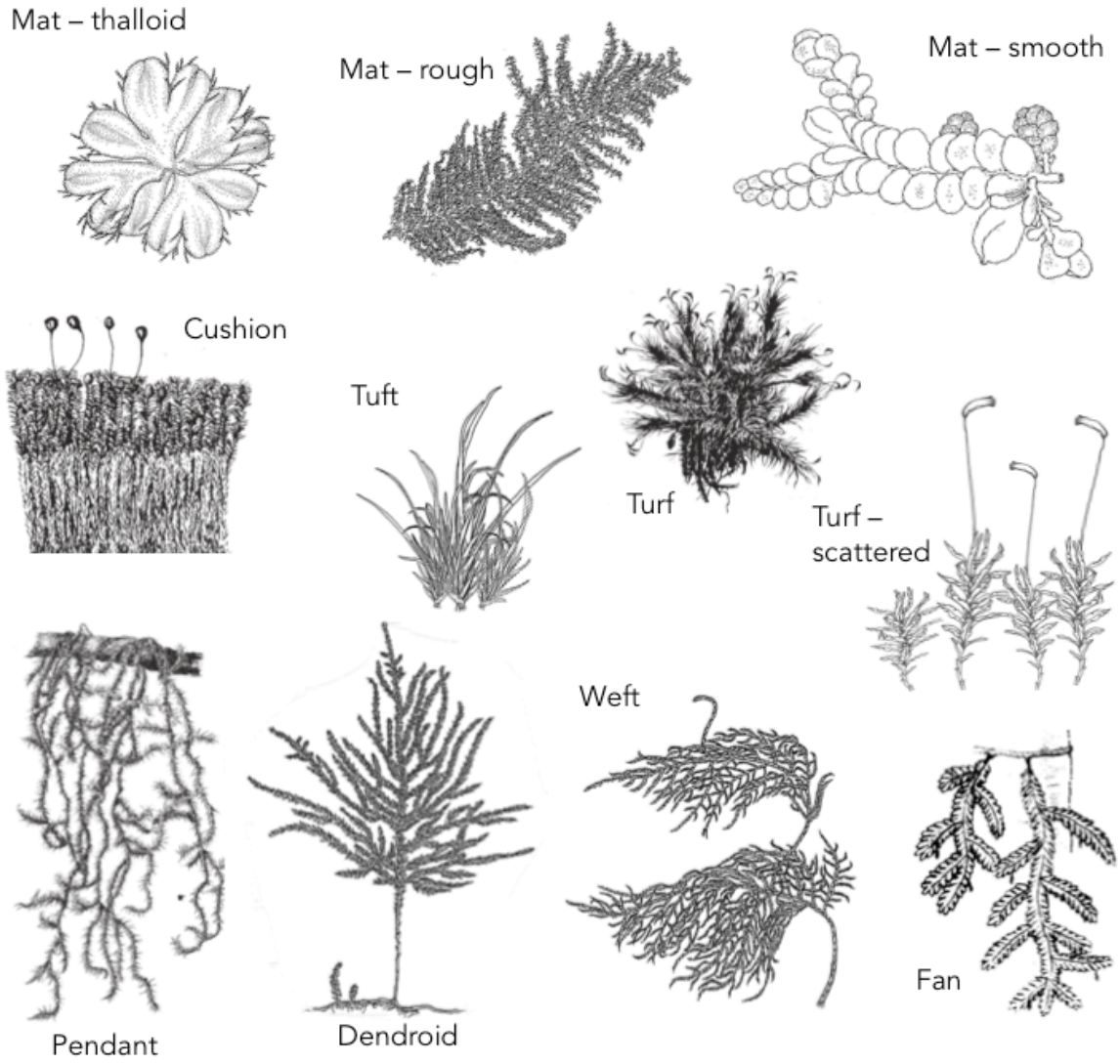


Figure 2.4 Bryophyte life-forms. Sources for illustrations: Cushion: Frahm, 2003, p. 30; Dendroid: *Thamnobryum alopecurum*, Casas et al., 2006, p. 314; Fan: *Neckeropsis undulata*, Mägdefrau, 1982, fig. 2, p. 50; Mat - rough: *Ctenidium molluscum*, Casas et al., 2006, p. 287; Mat – smooth: *Lejeunea lamacerina*, Paton, 1999, p. 492; Mat - thalloid: *Riccia crozalsii*, Casas et al., 2009, p. 50; Pendant: Meteoriaceae, Frahm, 2003, p. 30; Tuft: *Astomum levieri*, Casas et al., 2006, p. 157; Turf: *Dicranum sp.*, Frahm, 2003, p. 30; Turf – scattered: *Atrichum undulatum*, Casas et al., 2006, p. 72; Weft: *Hylocomium splendens*, Casas et al., 2006, p. 299.

2.2.3.1.4 Leaf characters

Bryophyte leaves are the part of the plant that interact most with the environment therefore it may be expected that leaf traits would closely indicate environmental conditions. Their shape and

structure affect water flow and accumulation. Water conduction between and along leaves is as important, if not more important, for water relations of a bryophyte as cell to cell conduction (Proctor, 2009). The leaf traits below were considered to be the most relevant to DT, either because they are known in the literature to relate to DT or because it is thought they might. Unlike life-form, many leaf traits have had less research in how they relate to DT or environmental parameters. The term lamina (plural laminae) used in bryology refers to "the flat blade of a leaf not including the nerve" (Casas et al., 2006, p. 324).

Leaf orientation and overlap

The orientation of leaves in relation to the stem (Figure 2.5) affects the amount of water a plant can hold or how quickly it uptakes water following rehydration as well as playing a role in water conduction by capillary action (Proctor, 2009). Species of arid habitats tend to have appressed leaves when dry and then spreading leaves when hydrated (Vitt et al., 2014). This means a greater surface area becomes exposed when water is available, increasing light capture and therefore photosynthesis (Glime, 2015a). Leaf orientations closer to the stem (appressed, imbricate or erect) may also hold more water than other orientations (Glime, 2015a). Orientation also affects water loss rates due to the amount of stem that is exposed: water loss is reduced in species with appressed or imbricate leaves (Vitt et al., 2014).

In liverworts, overlapping leaves are either succubous (upper leaves overlap lower leaves) or incubous (lower leaves overlap upper leaves), Figure 2.5. It was first thought that this trait was related to speed and direction of ectohydric water transport: water transport is faster in succubous plants and direction of transport in these is from from base to apex, resulting in succubous forms being more prevalent in habitats with water available substrate surface (e.g. soil dwelling species) and incubous forms more prevalent in habitats where water comes from above (e.g. epiphytic species) (Clee, 1937). However, leaf overlap is now considered more likely to be related with water loss rates (due to exposure of the stem, similarly to leaf orientation) as well as providing capillary space (Proctor, 2009; Vitt et al., 2014). Species with succubous leaves have higher water loss rates as not only is more stem exposed, but leaves are not as appressed as in incubous species (Vitt et al., 2014), and are therefore found in more moist and sheltered habitats and have a lower DT level (Schuster, 1966).

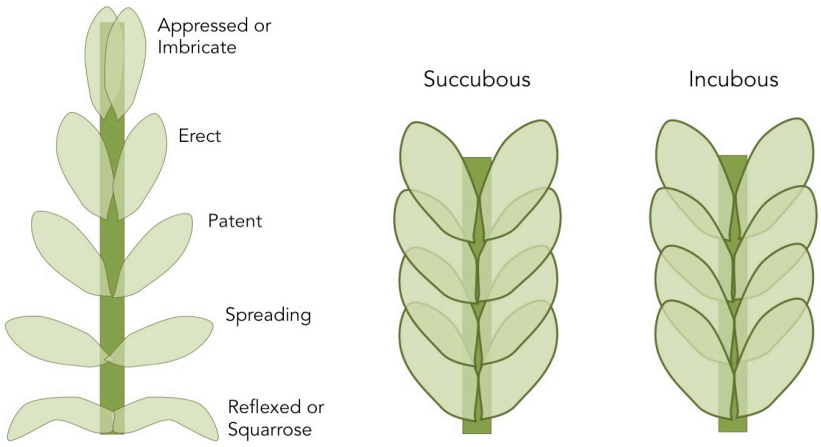


Figure 2.5 Schematic representation of the main leaf orientation and overlapping types. Source: Sarah Stow.

Leaf insertion

This trait applies to liverworts (as mosses overwhelmingly only have one insertion type); leaves that are transversely inserted are able to trap more water than those that are longitudinally inserted (Figure 2.6). The association of this trait with environment and DT is not clear-cut as there exist both highly DT liverworts and species of exclusively moist habitats that have transverse leaves (Vitt et al., 2014). Sheathing bases (in mosses) provide capillary space for water (Proctor et al., 1998).

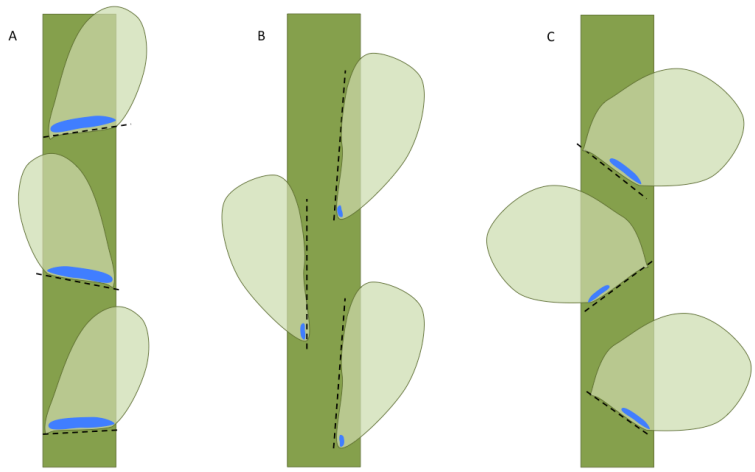


Figure 2.6 Schematic representation of main leaf insertion types in liverworts with relative amount of water trapped (blue). A – Transverse; B – Longitudinal; C – Oblique. Source: Sarah Stow.

Leaf transverse profile shape

Leaf concavity aids water conduction and allows water to be retained on the bryophyte leaf surface (Frahm, 2000; Proctor, 2009). This provides water for metabolic processes while allowing gas exchange to take place on the convex outer leaf surface (Proctor, 2009). Pleats on leaves may also help with water conduction and retention and are often found on species of harsh environments (Vitt et al., 2014). Keeled leaves conduct water rapidly (Glime, 2015a) due to the presence of capillary space created by the keel. A plicate lamina helps with desiccation by reducing the area exposed and creating capillary spaces between the folds (Glime, 2015a).

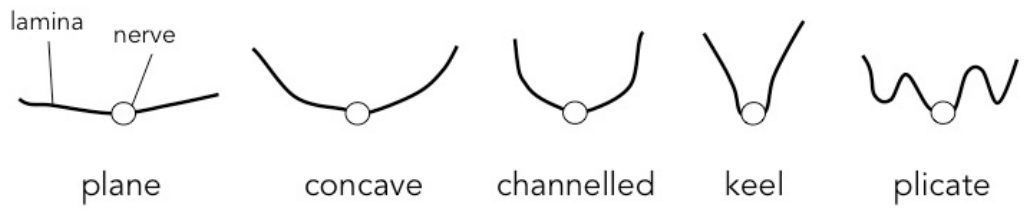


Figure 2.7 Schematic representation of leaf transverse profiles. Source: Sarah Stow.

Leaf longitudinal profile shape

It is known in tracheophytes that curling leaves when dry protects the plant by exposing a smaller leaf area to the drier atmosphere and higher insolation (Porembski & Barthlott, 2000a; Alpert, 2006; Proctor, 2010). It has also been shown to occur in bryophytes as a protection against high heat levels due to high insolation levels (Proctor & Tuba, 2002) as several bryophytes of dry habitats have curved or twisted leaves (Proctor et al., 1998). Leaves that are falcate or secund are

associated with water retention (Vitt et al., 2014). The curling of leaves when drying also retains water that is on the leaf surface, hence providing water for maintenance of metabolic functions (Glime, 2015a).

Leaf lamina thickness

Although bryophytes are characterised by a single-cell thick lamina, some species are bistratose or pluristratose (more than 2 cell layers thick) and these species are found in drier habitats (Vitt et al., 2014). One suggestion is that a thicker lamina allows a species to tolerate drier environments by reducing evaporative water loss due to the reduced surface to volume ratio and also provides protection to photosynthetic cells (Vitt et al., 2014; Glime, 2015a). This is particularly the case in species of the Dicranaceae family (e.g. *Leucobryum* and *Octoblepharum* species) that have large hyaline cells (hyalocysts) surrounding chlorophyll filled cells (leucocysts).

Leaf apex

Leaf apices are the part of the leaf most exposed to environment, particularly light, as lower parts of the leaf are usually covered by other leaves (Glime, 2015a). Hair points can affect the micro-climate surrounding a moss, either by interacting with the air flow by increasing their boundary layer (trapping air) or creating an albedo effect due to the white colour of these hairs (Proctor, 2009). This has implication for evaporative loss (reducing it by increasing the boundary layer) and photo-oxidative damage (reducing it via the albedo effect). Hair-points also provide condensation points for water vapour or collection points for dew allowing the plant to use small amounts of moisture; this has been widely demonstrated to be a trait of bryophytes from very dry habitats, both from field observations and environment manipulation experiments (Glime, 2015a).

Leaf surface

This refers to waxy deposits, cilia, papillae, hairs, lamellae and scales. Papillae (projections of leaf cell walls) are perhaps one of the best studied leaf traits and have various interactions with water and light: they can create capillary spaces for water transport and speed up leaf hydration (Proctor et al., 1998; Crandall-Stotler et al., 2009; Vitt et al., 2014); they can provide a location for gas exchange when their apices remain free of water (Proctor, 2009); they can increase the rate of water loss allowing species to reduce stress on their metabolism while drying (Pressel et al., 2010); and they reflect UV light providing protection at high light intensities (Glime, 2015a). Papillae are usually found in species that occupy dry habitats (Proctor et al., 1998) but can also be found in species of wet habitats (Glime, 2015a) suggesting they not only help with desiccation, but also with excess water due to the capillary spaces they create or provide protection when these species become exposed (Glime, 2015a). Scales (in liverworts) may also create capillary spaces through which to draw water and are present in xeromorphic taxa (*Riccia*, *Targionia*, *Plagiochasma*) that curl up when desiccated and are protected by the scales (Crandall-Stotler et al., 2009).

Lamellae (Figure 2.8) increase the photosynthetic area available (Vitt et al., 2014) and also provide a surface area free of water between the lamellae for gas exchange to take place (Proctor, 2009). The air spaces created also reduce water loss (Glime, 2015a) although based on experiments Marschall and Proctor (2004) conclude that they are more important for gas exchange. They are usually found in species of dry and exposed habitats such as the Polytrichaceae family (Glime, 2015a).

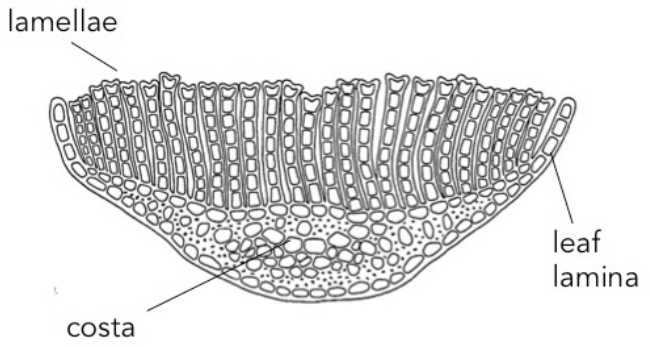


Figure 2.8 Transverse profile of *Polytrichum commune* showing lamellae. Copied and adapted from Casas et al. 2006, fig. 8.14, p. 75.

Wax is found on some thalloid liverworts and Polytrichaceae mosses and functions as a barrier to water enabling gas exchange to take place in interlamellar spaces (Proctor, 1979, 2009; Proctor & Tuba, 2002). Another hypothesis for the role of repelling water, is that when water is available but of insufficient duration or quantity for carbon accumulation, the plant is protecting itself from initiating metabolic processes that would not be energetically efficient (Proctor, 2010). Surface wax was not used as a trait as its presence is restricted to very few species, or is invisible under the light microscope (Heinrichs et al., 2000) and is therefore not listed as a character in most floras.

Leaf decurrence

Leaf decurrence, the extension of the lower leaf margins onto the stem, is related to water conduction and retention as it creates a capillary space (Figure 2.9). Species with longly decurrent bases uptake water more quickly (Glime, 2015a).

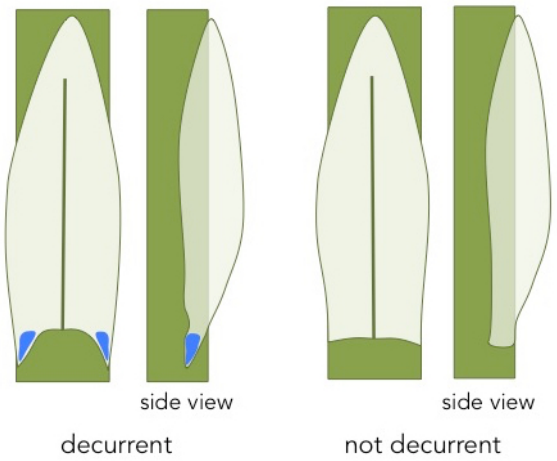


Figure 2.9 Schematic representation of water retention in decurrent and not decurrent leaf bases. Source: Sarah Stow.

Leaf margin

Four leaf margin traits are considered in this study: denticulation, cell shape, curvature and thickness. Similarly to leaf apices, margins are more exposed than interior parts of the leaf (Glime, 2015a). Cilia (in liverworts) or teeth on leaf margins (Figure 2.10) create capillary spaces increasing water uptake and its ectohydric transportation (Crandall-Stotler et al., 2009; Vitt et al., 2014). One study found that species with teeth began to photosynthesize earlier in the season

than those without teeth (Royer & Wilf 2006 in Glime 2015b). This suggests that teeth maximises carbon gain and so may be a trait associated with species that inhabit environments with low light levels. However, this study was conducted on tracheophytes and so may not be applicable to bryophytes (Glime, 2015a).

Tixier & Guého (1997) suggest that hyaline marginal cells in liverworts may facilitate the uptake of water and may also provide storage (Glime, 2015a). Marginal cell shape also plays an indirect role in physical photo-protection by helping leaves to curve when drying out (Glime, 2015a). Margin curvature may aid in the conduction of water, by channelling water from the leaf apex to its base, in the case of bryophytes from dry environments (Vitt et al., 2014). It may also provide photo-protection in species with revolute or involute margins (Figure 2.10) by providing physical shelter to marginal cells (Glime, 2015a). Many species have a margin that is bi- or pluri-stratose and this trait provides support for the leaf, but also reduces water loss and plays a role in water conduction (Glime, 2015a). Glime (2015b) suggests that water travels more quickly in leaves with borders (elongate cells) as there are less walls to cross along the water's path, but states that there are no experimental data to confirm this.

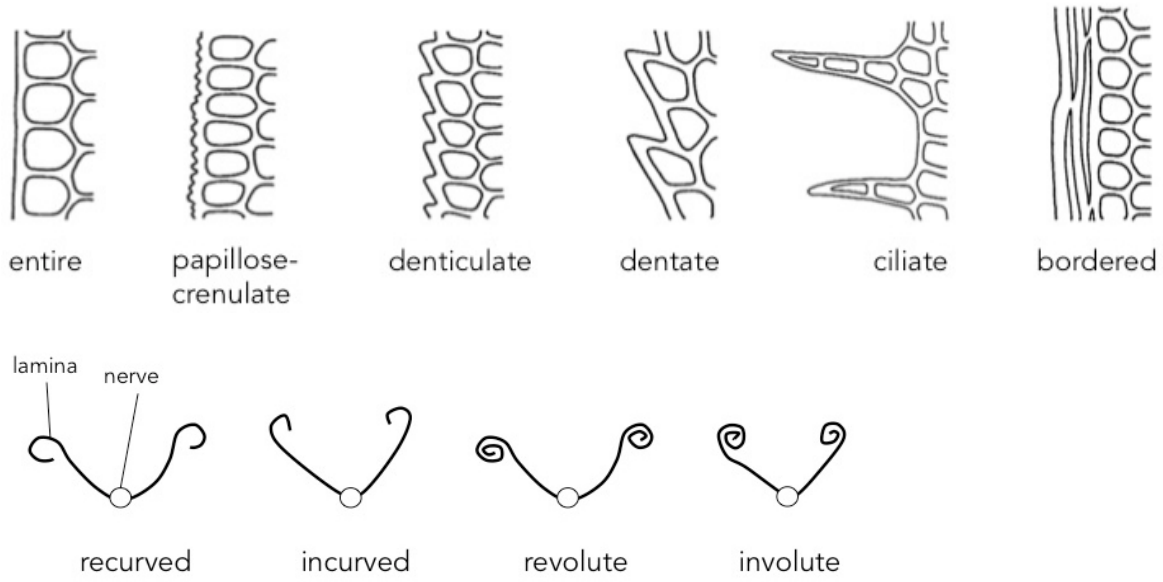


Figure 2.10 Leaf margin traits – denticulation (longitudinal section) and curvature (transverse section). Denticulation drawing taken from (Casas et al., 2006, 2009); curvature source: Sarah Stow.

Lamina cell shape

A smaller cell size allows bryophytes to utilize small amounts of water (vapour and dew) and also means most of the plant's water is held outside the cells allowing them to lose water quickly and avoid cell damage (Tuba et al., 1998). Elongate cells, as mentioned when discussing margins, provide rapid water transport when compared to shorter or wider cells. However, elongate cells are usually present in species of wet habitats and it is not known exactly what role they play (Glime, 2015a). Large cells may serve the function of water storage to prolong metabolic function when conditions are dry (Vitt et al., 2014). However, large hyaline cells can be found in species of both dry and humid habitats (Proctor et al., 1998; Vitt et al., 2014), notably in *Sphagnum* species of wet habitats (marshes and bogs). Large and hyaline cells are usually found in leaf bases; their thin walls facilitate water uptake and in some species may increase the surface area available for

light and water capture by physically pushing the leaf away from the stem due to swollen cells (Glime, 2015a).

Alar cell differentiation

Similarly to enlarged and hyaline basal cells, enlarged alar cells uptake water quickly (Glime, 2015a). Another possible purpose of differentiated alar cells, is the formation of air bubbles on their leaf surfaces to provide an area for gas exchange in species that are often saturated with water (Glime, 2015a).

Cell wall

As briefly mentioned above, species with small, thick-walled cells have less negative osmotic potentials (Proctor, 2009) and are associated with drier and more exposed habitats; these species are highly DT and the thick cell wall allows water storage, in mosses and liverworts (Vitt et al., 2014).

Bulk cell elastic modulus (ε) is a measure of how elastic cells are and, though not widely studied in bryophytes, it is suggested that a low ε (high cell elasticity) is found in bryophytes with poor water storage capabilities (Proctor, 2009; Song et al., 2015) and consequently in bryophytes of humid habitats with moderate levels of DT. The ability of cells to shrink while drying out prevents plasmolysis (Moore et al., 1982). This trait was recorded from the literature although data for very few bryophytes is available (Beckett, 1997; Song et al., 2015).

Some species have pores in their cell walls and this could be to allow photosynthates to pass from photosynthesing cells to storage cells in the leaf base (Vitt et al., 2014). However, exactly how porose cell walls affect water relations in a bryophyte is not known (Glime, 2015a).

Costa

The costa (mosses), or midrib (liverworts), provides structural support to leaves and shows great variability in terms of length and width, and is absent from some species. Species with absent or very reduced costas are found in wet habitats; this has been found both through observation of field specimens and also in manipulation experiments (where the same species is grown in wet and dry conditions) indicating the role of the costa in transporting water (Glime, 2015a). Broad costas (those that occupy a third or more of the width of the lamina) transport more water and so are likely associated with species of drier habitats.

Oil bodies

Oil bodies, present in liverworts, are membrane-bound organelles that, as the name suggests, contain terpenoid oils and aromatic compounds (Crandall-Stotler & Crandall-Stotler, 2000) and are thought to be important in DT (Pressel et al., 2009; Glime, 2015a). Tixier & Guého (1997) note that in areas of forest with high light intensities liverworts present have oil bodies. However, their exact role in DT is difficult to identify, as oil bodies disappear when a liverwort is desiccated and the rate of disappearance varies between species (Pressel et al., 2009).

2.2.3.1.5 Specialised structures

Hyalocysts (large hyaline cells) and hyaline cells act as reservoir for water in bryophytes allowing them to maintain their metabolic functions for longer when the environment becomes drier

(Frahm, 2000; Proctor, 2009). The most well developed hyalocysts (and most studied) are in the *Sphagnum* genus, and though these are famously species of wet habitats (marshes and bogs), they require water storage structures so that they can survive the periodic desiccation of their habitat (for further details on *Sphagnum* hyalocysts see Glime, 2015a).

Hydroids, which are specialised cells that conduct water, are present in species of the Polytrichaceae and Mniaceae and allow bryophytes to remain at full turgor when the atmospheric humidity has decreased (Proctor & Tuba, 2002). The presence of conducting tissues affects the mechanism of water uptake in a plant but very few bryophyte species have these and so this trait was not included. Also, despite the presence of these structures, the plant still does not have significant control of its water regulation (Proctor, 2009).

The *Fissidens* genus are characterised by a conduplicate part on their leaves: a second smaller lamina that creates a pocket (Figure 2.11) providing a space for water retention and may also provide protection to cells by creating a double cell layer (Glime, 2015a). *Fissidens* species are mostly associated with humid and sheltered habitats and so the conduplicate part may serve to maintain metabolic function once humidity decreases.

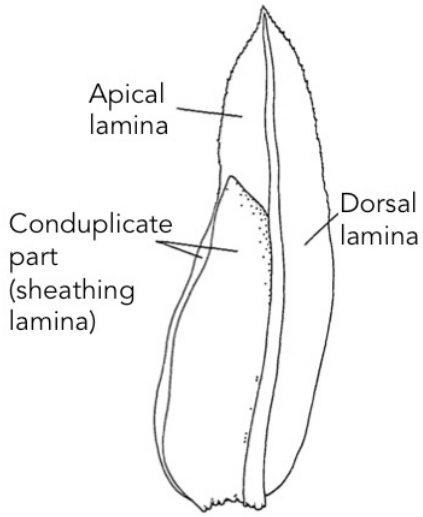


Figure 2.11 *Fissidens dubius* leaf showing how the conduplicate part (referred to also as a sheathing lamina) creates a pocket. Copied and adapted from Casas et al. 2006, fig. 23.9, p. 123.

Some species of the tropical Calymperaceae family possess intra-marginal, elongate and hyaline cells known as teniolae. They may function in facilitating water transport (Glime, 2015a) from the leaf base to the apex.

Liverworts of the Porellales order possess lobules that are helmet-shaped (Figure 2.12) which function to retain water (Glime, 2015a) although the importance of this role has been questioned as experiments have shown that water is quickly lost from these structures when humidity decreases and that they may be more important for nutrient capture than water storage (Vitt et al., 2014). Underleaves (also only in liverworts) (Figure 2.12) play a role in water retention by providing capillary spaces, although they may not be effective for long-term storage (Clausen, 1952 in Vitt et al., 2014). Species of drier habitats have larger lobules and underleaves than those of humid and sheltered habitats (Glime, 2015a).

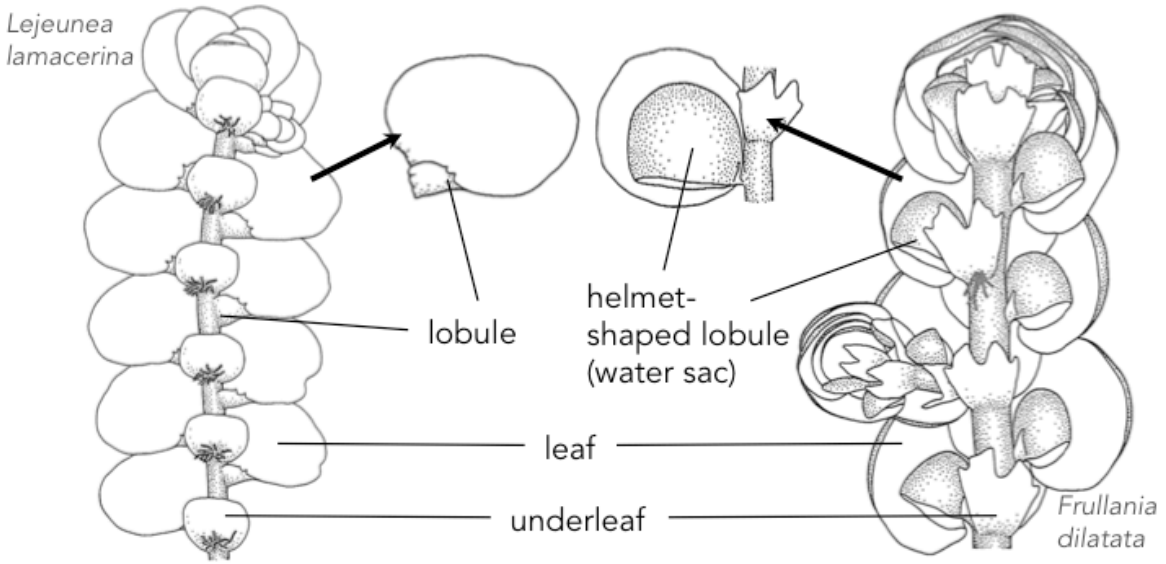


Figure 2.12 Structure of leafy liverworts with lobules and underleaves. Copied and adapted from Casas et al., 2009.

2.2.3.1.6 Vegetative reproduction propagules

Vegetative propagules allow species to reproduce when environmental conditions are not favourable for sexual reproduction (e.g. lack of water for sperm to reach egg) (Proctor et al., 2007) and occurs both in dioicous and monoicous bryophyte species (Vanderpoorten & Goffinet, 2009). They tend to be DT so that they can survive both dispersal and the time waiting until germination (Glime, 2014). There are many different types of propagules, and the number varies depending on the author (Glime, 2014). For simplicity, five main categories are used here (following the classification in Hill et al., 2007) which group several propagules types (Table 2.5 and Figure 2.13). For a description of all different vegetative propagules types, Glime (2014) provides a good review and includes other aspects of vegetative propagules. Fragments of mosses and liverworts can give rise to new plants (Crandall-Stotler et al., 2009; Glime, 2014) and in hornworts younger parts of a thallus that have become detached can also grow into new plants (Vanderpoorten & Goffinet, 2009). In liverworts, caducous leaves, bulbils and discoid gemmae are produced mostly by epiphytic species (Crandall-Stotler et al., 2009). As the different propagules differ in size and shape, the amount of water needed for dispersal will vary suggesting that certain propagules types may be more common in different environments (Goffinet et al., 2009). Additionally, the shape of the propagules can change based on the environmental conditions (Glime, 2014). A disadvantage of vegetative propagation is that as new individuals are clones, they have limited potential to adapt to new environmental conditions and so may reduce plant fitness (Laaka-Lindberg et al., 2000). This trait has been used in the study of extinction probabilities and in UK vascular plant species decline has been shown to correlate with absence of vegetative reproduction (Godefroid et al., 2014).

Table 2.5 The main categories of vegetative propagules present in bryophytes, the propagules types included in each category (where there is more than one) and the bryophyte group they occur in. Modified refers to leaves or branches that are different in shape or size from other leaves or branches on the plant. Data compiled from (Data compiled from: Vanderpoorten & Engels, 2003; Hill et al., 2007; Crandall-Stotler et al., 2009; Glime, 2014).

Vegetative propagules category	Types of vegetative propagules included	Bryophyte group
Gemmae	Leaf tips, leaf axils (multicellular, discoid, lenticular, spherical)	Liverworts, Mosses, Hornworts
Leaves	Caducous, fragments, modified and unmodified	Liverworts, Mosses
Bulbils		Liverworts, Mosses
Branches	Caducous, modified and unmodified	Liverworts, Mosses
Tubers		Liverworts, Mosses, Hornworts

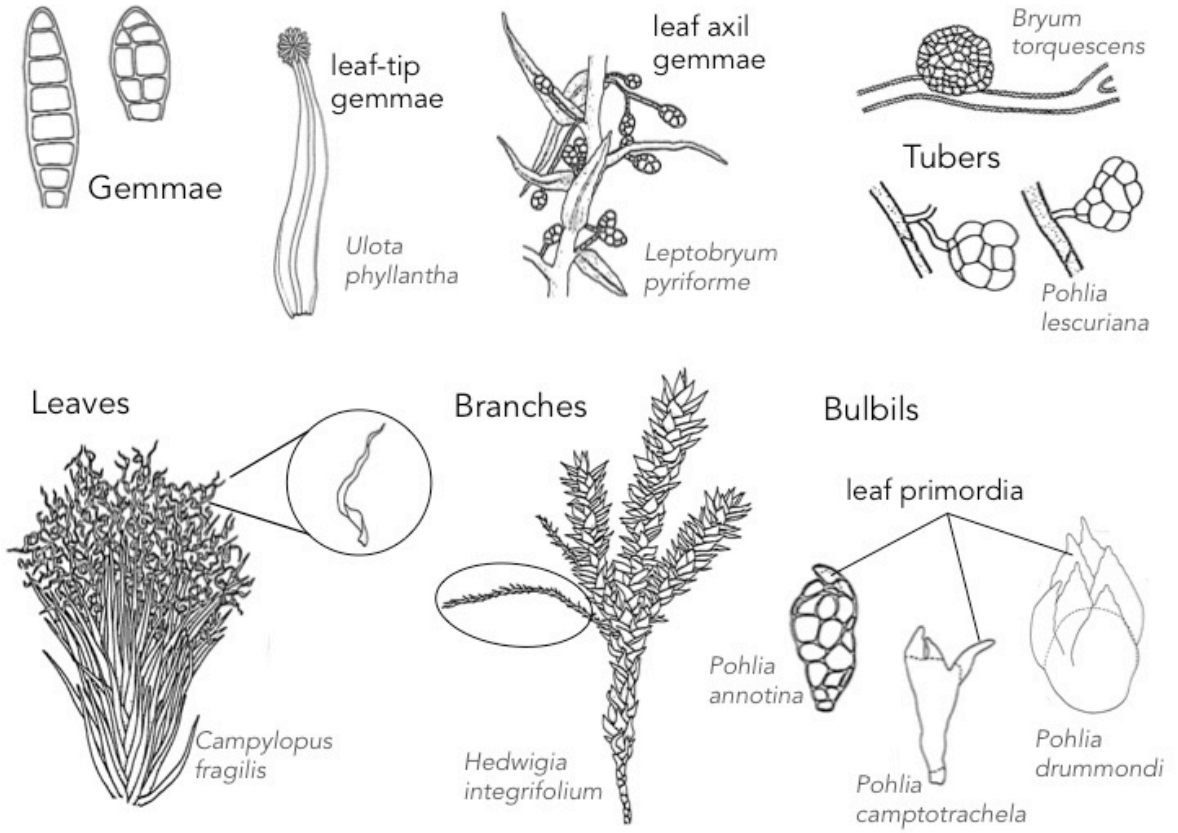


Figure 2.13 Different vegetative propagules in mosses. The leaves and branches shown here are modified. Though some bulbils may look like gemmae, they can be differentiated by the presence of developing leaves (leaf primordia) at their apices. NB- not to scale. Copied and adapted from Casas et al. 2006.

2.2.3.2 Sporophyte traits

Despite stating above (2.2.3.1, p. 66) that gametophyte traits are more likely to indicate DT than sporophyte traits, some sporophyte traits were included as little is known about DT in sporophytes (Stark & Brinda, 2015a) and so this study is an opportunity to provide some insight. A recent experimental study showed that sporophytes that had been exposed to rapid drying were

smaller and hence had fewer spores than those that were slowly dried (Stark & Brinda, 2015a) suggesting that spore number, seta length and capsule size could be used to determine environmental conditions a species is exposed to. As well as relating to DT, certain traits have been found to be related to species threat status (Sérgio et al., 2013).

2.2.3.2.1 Stomata

In bryophytes, stomata are only present on the sporophyte of mosses (usually on the capsule base) and hornworts; exactly what role they play is unknown though several ideas exist (Goffinet et al., 2009; Renzaglia et al., 2009). Stomata are lacking in three moss genera (*Takakia*, *Andraeae* and *Andreaeobryum*) suggesting that in mosses stomata may play a different role to those in tracheophytes (Goffinet et al., 2009). One role is in the control of water uptake into the capsule: while the capsule is developing, water is required for meiosis but when the capsule is mature the capsule needs to desiccate to allow spore release (Goffinet et al., 2009; Glime, 2015b). Stomata may also allow nutrients to be drawn up from the gametophyte to the capsule by creating a transpiration stream (Glime, 2015b). How they relate to environmental conditions or habitat is not clear with some studies showing no relation to stomata presence and habitat (Glime, 2015b). If stomata are required to allow the capsule to desiccate, then it would be expected that species of drier habitats have less stomata.

2.2.3.2.2 Seta and capsule

Some publications have noted that seta length is related to DT (e.g. Stark & Brinda, 2015b) with species with very short setas found in drier and more exposed habitats, but there are few data on this (Vitt et al., 2014). Capsule shape may also be related to environmental conditions as globose and spherical capsules seem to be prevalent among mosses of dry environments (Vitt et al., 2014). The capsule peristome prevents water entry into the capsule due to the waxy surface of the peristome teeth (Glime, 2015b).

2.2.3.2.3 Spores

Spore production, size and number are closely associated with the life strategy of a bryophyte (see 2.2.3.3.2 below). Spore colour has been shown to be related to how long they are able to survive once released (Renzaglia et al., 2009). In hornworts, yellow and brown spores survive longer than greener spores as the latter have thinner walls and less oils (Renzaglia et al., 2009) but there have been no studies measuring spore DT in hornworts (Vitt et al., 2014). Species with larger spores tend to be those living in dry habitats.

2.2.3.3 Life-history traits

Many studies have looked at life-history traits as they can be central in determining species survival (Söderström & During, 2005).

2.2.3.3.1 Reproduction system - monoicy or dioicy

Several studies have looked at how the reproduction system relates to other life-history traits such as spore size, seta length and plant size (Longton, 1992; Crawford et al., 2009; Manyanga et al., 2011). Monoicous species produce spores more frequently that diocious ones (Rydin, 2009). Some studies have looked at how the reproduction system relates to threat or rarity (Longton, 1992; Laaka-Lindberg et al., 2000) and from these it seems that monoicous species tend to be rarer. This trait was included as it is one that indicates threat, rather than due to DT. However, analyses will be carried out to see if this trait does also relate to DT. This is also a trait that is

relatively well documented for in the literature and so data availability should be high. Interestingly, male plants of dioicous species have been found to be less DT than female plants (Stark et al., 2005) and that the sex ratio is skewed in favour of female plants at lower elevations (Benassi et al., 2011).

2.2.3.3.2 Life strategy

Kürschner et al. (1999) found only three types of life-strategies among epiphytic bryophytes: colonists, perennial shuttle species and perennial stayers. Species that are colonists are often habitat pioneers and can therefore indicate forest disturbance (Kürschner et al., 1999). Short-lived species (annuals) avoid drought by completing their life-cycle quickly when moisture is available and surviving the drought period through a large spore bank (Frahm, 2000; Vitt et al., 2014). An example of where life strategy enables species to inhabit dry habitats can be found in the genus *Riccia*. In west Africa, members of the genus often grow on exposed rocks in rock pools and rely on an annual life strategy whereby they survive desiccation from one year to the next due to the presence of a large spore bank (Frahm, 2000). In these annual species, spore germination and sexual reproduction leading to spore production take place in the rainy season, the thallus then decomposes in the dry season and their spores persist during the dry season in the soil. This strategy has enabled them to colonise large areas, including in Madagascar.

2.2.4 Quantitatively measuring desiccation tolerance

Measuring DT in bryophytes centres around measuring physiological parameters when they are either desiccating (survival) or rehydrating (recovery). There are several experimental methods used for quantitatively assessing DT in bryophytes (see Table 2.6) with the most widely used ones being: water relation parameters (e.g. water potential, water content, water loss) (Pardow & Lakatos, 2013), gas exchange (Proctor et al., 2007) and photosynthetic parameters (e.g. photosynthetic efficiency, non-photochemical quenching) (Wood, 2007). For details on water relations, gas exchange and fluorescence methodologies see Appendix A2.1, p. 88. Initial studies looked at features that were visible by light microscopy and most widely used was plasmolysis as an indicator of cell recovery (Proctor, 2001; Wood, 2007) but this is now thought to overestimate DT (Proctor, 2009). Respiration has also been used as a measure of recovery from desiccation, but as it varies little is not considered the most suitable parameter to use (Hinshiri & Proctor, 1971). Aside from measurements of physiology, some studies measure cellular chemistry to determine DT, most commonly the plant hormone ABA (Proctor & Tuba, 2002) and chlorophyll pigments as they play a role in the protective molecular mechanisms (Table 2.4, p. 64) and abscisic acid (ABA) has been shown to be central in conferring DT to some bryophytes (Pressel et al., 2009).

The general protocol for measuring DT involves collecting specimens from the field, exposing species to different desiccation regimes, times, temperatures and humidity levels (Proctor, 2001; Wood, 2007; Bader et al., 2013; Stark et al., 2014) and measuring a combination of the parameters above. Because some species are able to become “hardened” to desiccation during slow drying or partial drying, acclimatisation prior to carrying out desiccation experiments is recommended so that measurements are carried out on dehardened species; otherwise we may be comparing values between species in hardened and dehardened states which will lead to misleading conclusions (Wood, 2007; Stark et al., 2014). However, how long to acclimatise species for is not standardised as species require different acclimatisation times (Stark et al., 2014) and very few studies have addressed this. The stresses that a species was exposed to before

specimens are collected, the "field effects", also play a role in the value obtained from physiological experiments and may not reflect the species' DT response in the field (Proctor, 2000a; Stark et al., 2014).

Due to the many methods of measuring DT in bryophytes, Wood (2007) outlined a standard protocol (the Austin protocol, see Appendix A2.2, p. 90) for measuring DT and advocates its use to allow comparability between species. However, none of the 145 studies published on DT and bryophytes since 2007 have used this protocol. The only mention is in Stark et al. (2014) to suggest an improvement to it (changing the acclimatisation period).

Table 2.6 Parameters used to quantitatively measure DT with examples of studies that have used them and the taxa they studied. This does not indicate that each study looked at just that one parameter, as most tend to use more than one.

Parameter	Taxa	Study
Water content at full turgor and external capillary water storage	Tropical	(Pardow & Lakatos, 2013)
Electrolyte leakage - plasmolysis	Tropical bryophytes	(Bader et al., 2013)
	Temperate liverworts	Clausen, 1962
Maximum duration of desiccation tolerated	Tropical mosses & liverworts	(Bader et al., 2013)
Chlorophyll fluorescence	*Crossidium crassinerve*	(Stark et al., 2014)
IRGA – infra-red gas analysis	5 temperate bryophytes	(Dilks & Proctor, 1976, 1979)
Respiration rate	*Anomodon viticulosus* *Porella platyphylla*	(Hinshiri & Proctor, 1971; Dilks & Proctor, 1979)
Cytoskeleton structure	6 liverworts (5 temperate and 1 subtropical)	(Pressel et al., 2009)
ABA	2 liverworts, 3 mosses	(Proctor & Tuba, 2002)
Protein synthesis	*Tortula ruralis*	(Oliver, 1996)
Leaf damage	*Crossidium crassinerve*	(Stark et al., 2014)
Leaf regeneration	*Syntrichia caninervis*	(Stark et al., 2005)
Protein synthesis	*Tortula ruralis*	Oliver, 1991 in Oliver & Bewley, 1996
Anti-oxidant enzymes	*Tortula ruraliformis* *Dicranella palustris*	(Seel et al., 1992b)
Photosynthetic pigments	39 temperate mosses and 16 temperate liverworts	(Marschall & Proctor, 2004)

2.2.5 Variation in desiccation tolerance and defining thresholds

Despite common protective and repairing molecular mechanisms, the behaviour of these is not equal among bryophytes (Oliver et al., 1998; Proctor & Tuba, 2002; Stark et al., 2014) and together with their varying morphologies, bryophytes exhibit different levels of DT. Bryophytes of drier and exposed habitats are considered to have constitutive DT (CDT) – they can survive rapid drying with minimal damage - whereas species of more sheltered habitats have inducible DT (IDT)

– they require slow drying in order to minimise damage and can be considered to go through a hardening process (Stark et al., 2014). Formerly, CDT bryophytes were called "fully DT" and IDT bryophytes "modified DT" (Oliver et al., 1998; Stark & Brinda, 2015a). While researchers are inherently prone to classifying natural phenomena into discrete categories, the current consensus is that DT in bryophytes is likely to be a continuum between CDT and IDT, and that a species can display different levels of DT (Pressel et al., 2006; Stark & Brinda, 2015a). This has recently been named the 'continuum hypothesis of ecological DT in bryophytes' (Stark & Brinda, 2015a).

In addition, the previous environmental conditions a bryophyte has been exposed to and the rate of drying prior to measuring DT will have an effect on the molecular and physiological response (Oliver et al., 1998; Oliver, 2009; Proctor, 2009; Stark et al., 2014). However, bryophytes from dry habitats are less affected by these two factors (Proctor & Tuba, 2002). Rate of drying varies among bryophytes with forest bryophytes drying out more slowly than bryophytes from exposed habitats (Proctor & Tuba, 2002; Proctor, 2004; Song et al., 2015) suggesting that they are less DT as they require more time to enable their protective molecular mechanisms.

Recovery of physiological function also differs, even within the same habitat, Figure 2.14 (Proctor & Tuba, 2002). Forest bryophytes tend to show less extreme DT (Figure 2.14) as they live in an environment where desiccation intensity is lower and of shorter duration than in other habitats (Proctor, 2004; Vanderpoorten & Goffinet, 2009; Song et al., 2015). Slow drying allows hardening to desiccation (Stark et al., 2014) meaning species will be more DT than at times when drying is fast. Species that are highly DT usually recover full photosynthetic function within 15-20 minutes (Proctor, 2001). Interestingly, the more DT species will survive desiccation at very low water potentials, but do not survive when kept at full turgor compared to forest species (Proctor, 2001). This could be due to a reduction in exposed surface area available for gas exchange or the growth of pathogens e.g. fungi (Proctor, 2001).

As well as differences in recovery response, the time a species can maintain metabolic function (i.e. its survival time) following desiccation varies, and is affected by intensity of desiccation (Proctor, 2001). Species of drier and more exposed habitats maintain their metabolic functions for longer (Figure 2.15). In addition, they are also able to endure very negative water potentials (lower RH) whereas bryophytes from sheltered habitats quickly decline at the same potentials (Figure 2.15) (Proctor, 2001). These responses, again, vary between species of the same habitat type.

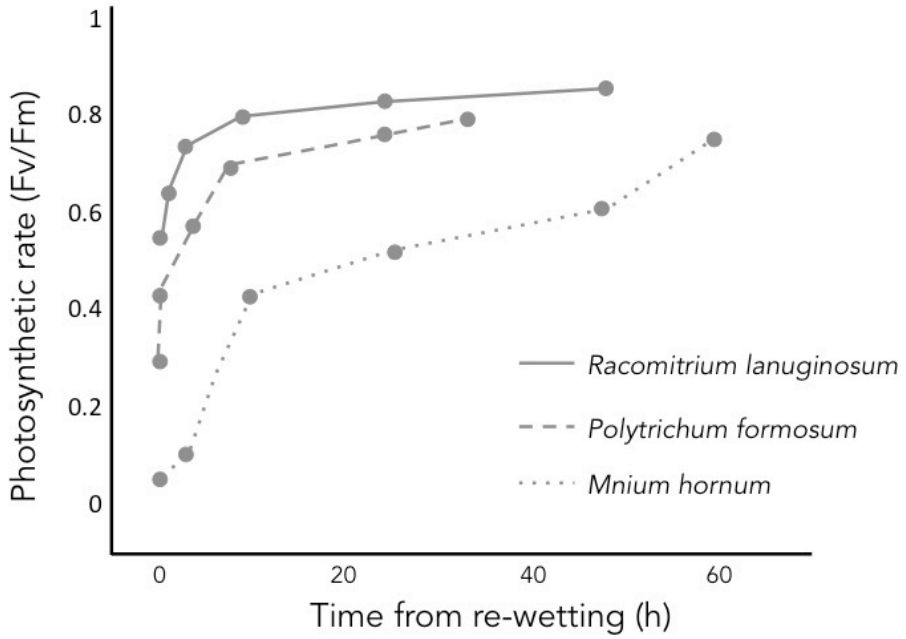


Figure 2.14 Photosynthetic recovery rates of three temperate bryophytes following rehydration showing how recovery rates vary, even within species from the same habitat type. *Polytrichum formosum* and *Mnium hornum* are forest floor bryophytes whereas *Racomitrium lanuginosum* is a saxicolous bryophyte from open habitats. (Redrawn from: Proctor & Tuba, 2002, p. 343, figure 5.)

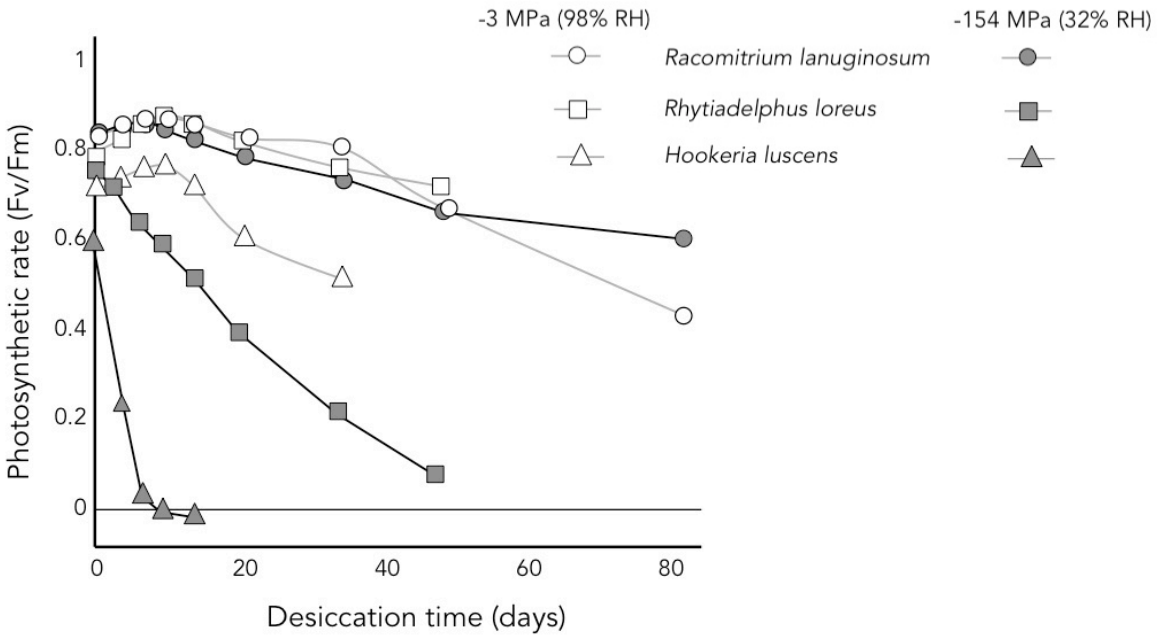


Figure 2.15 Survival based on photosynthetic rate (Fv/Fm) of three temperate bryophytes following desiccation at different water potentials showing how persistence of metabolic function varies among species. *Hookeria luscens*, *Plagiotheium undulatum* and *Rhytiadelphus loreus* were collected from a forest; *Anomodon viticulosus*, *Racomitrium lanuginosum* and *Tortula (Syntrichia) muralis* were collected from rocks on open habitats. (Redrawn from Proctor, 2001, figure 5, p. 150)

2.2.5.1 Defining DT levels

Although the range of DT in species is not sharply defined and is a gradient (Wood, 2007; Stark & Brinda, 2015a), the physiological measurements outlined in section 2.2.4 have been used by some authors to quantitatively define different DT levels (Table 2.7). When desiccated, DT bryophytes can survive cell osmotic potentials of -100 to -400 MPa (Lakatos, 2011), with extremely DT species surviving to -540 MPa (Oliver, 2009). Species that do not survive beyond -40 MPa are considered desiccation sensitive in terms of bryophytes (Oliver et al., 2005; Proctor et al., 2007; Oliver, 2009). The maximum efficiency of the photosynthetic apparatus (F_v/F_m) of a healthy, unstressed bryophyte exhibits values of between 0.76-0.83 (Proctor, 2003); values below this therefore indicate that plants have been subject to stress and are photo-inhibited. However, Pardow & Lakatos (2013) did not record values above 0.75 in their study, suggesting that tropical bryophytes have different threshold levels due to the microclimatic conditions: high temperature, low light intensity and high humidity limit photosynthetic efficiency (Frahm, 1990). Also, lowland forests have a higher temperature than those at higher altitude, which limits net productivity (higher temperatures lead to higher respiration rates).

Table 2.7 Dessication tolerance levels delimited in chronological publication order. Within a study, categories are listed from most DT to least DT.

Study	Parameter	Threshold	DT level
(Oliver & Bewley, 1996)	Survival at rate of water loss	Extremely rapid	Fully DT
		Slow	Modified DT
Wood 2007	Lowest RH survival – using Fv/Fm as survival indication	≤30% (< -162MPa)	Category A
		70-80% (-30 to -48 MPa)	Category B
		70-80%, and at 0-30% if hardened	Category B(A)
		>80% but can survive at 0-30% if hardened	Category (A)
		>80% but can survive at 70-80% if hardened	Category (B)
Wood 2007	Lowest RH survival– using Fv/Fm as survival indication	≤23%	Fully DT
		≤67%	Modified DT
(Pardow & Lakatos, 2013)	Maximum efficiency of the photosynthetic apparatus (Fv/Fm) or number of cells alive	Upper quartile (75-100%)	4
			3
			2
		Lower quartile (0-25%)	1

Pardow and Lakatos (2013) produced the first (and only) DT index (DTI) using published physiological studies (and their own data) of 65 species from different habitats and regions worldwide. They used maximum photosynthetic rate achieved or number of cells alive after desiccation at 30-50% RH (-94 to -162 MPa) at 20°C, and assigned species to four categories based on the percentage quartile a species' value is in i.e. the most DT species are in category 4 (upper

quartile) and the least DT are in category 1 (lower quartile). Though they noted the problems of comparing data from different datasets, the index still showed variation between and within species of different habitats suggesting that this could be a useful approach to monitoring effects of habitat change, and even climate change. A search for publications using this index, or a similar approach, yielded no results showing this approach remains understudied, potentially due to the lack of physiological measurements in many species, especially tropical ones. A way to circumvent this problem would be to attempt to relate easy-to-measure morphological traits to DT and then create an index based on this.

2.3 Ecology, conservation and desiccation tolerance

Most DT research to date has focussed on the mechanism itself with many DT studies using extremely DT species (such as *Tortula ruralis*) or focussed on temperate species and rarely tropical ones (Proctor & Smirnoff, 2000; Wood, 2007). Additionally, few liverworts have been studied and so DT is much better known in mosses (Vitt et al., 2014). Studies range from those looking at the biochemistry of desiccation, through to the genetics, an emerging field, and the ecophysiology of DT, with a few studies from the perspective of ecology or conservation. Stark et al. (2014) related physiological measurements of DT to their ecological implication in the field by highlighting the potential for hardening and dehardening of species to DT based on the length of time they are exposed to desiccation and hydration. Pardow and Lakatos (2013) undertook one of the few studies relating DT with threat in tropical bryophytes suggesting that the less DT understorey species are likely to become threatened through habitat and climate change.

Studies have found that tracheophyte extinction risk can be related to their environmental preferences, with species inhabiting extreme and specific environmental parameters (e.g. extreme dry or wet habitats) being most at risk (Walker & Preston, 2006). But whereas vascular plant distribution is mostly dictated by edaphic and macro-climatic variables (Barkman, 1969), bryophyte distribution and species richness is determined by microclimatic variables, predominantly moisture availability (Frahm, 2000; Vanderpoorten & Goffinet, 2009). Poikilohydry has implications for the habitats that bryophytes can occupy as water in the form of vapour is available to them but not to most tracheophytes (Barkman, 1969). Therefore, for bryophytes, more damaging than long exposure times to low humidity, is exposure to fluctuating humidity where partial metabolic activity (as opposed to total inactivity during long exposure) can be more damaging through carbon leakage or pathogen activity (Proctor, 2001; Bader et al., 2013). Being DT, bryophytes are able to occupy environments where most other plants cannot survive. This provides bryophytes with a competitive advantage although it limits the time available for growth. For example, bryophytes dominate exposed rock landscapes as the impenetrable rock surface means water is not available for most tracheophytes (Proctor & Tuba, 2002), whereas bryophytes can utilise vapour and morning dew.

In a study of bryophytes on inslebergs from four African countries, those found on this exposed habitat were highly desiccation tolerant, such as *Riccia* with xeromorphous thalli and Bryaceae species (Frahm, 2000). Abundances of DT bryophytes on granitic boulders at a semi-arid site in California were strongly negatively correlated with insolation – this could be linked to temperature, carbon balance and damage by light (Alpert, 2000a). DT level reflects the conditions the species is usually exposed to (Bader et al., 2013) but species that exhibit ability to resist

extremely dry environmental conditions are not always necessarily found in the driest habitats – either other factors also determine distribution of species or length of drought is not the best indicator of DT (Alpert, 2000a). Similarly, bryophytes of moist, sheltered areas have lower DT although this is not always the case as shown in some studies. In California it was found that out of six species from moist areas, five that were restricted to sheltered and moist sites had a relatively high DT (Cleavitt 2002b *in* (Proctor et al., 2007). The trait of "tolerance of drought period" is due to a combination of morphological and life-history traits that have been positively selected for in bryophytes (Alpert, 2000a).

Tropical montane forest mosses in Africa and Venezuela have been shown to have tolerance to long drought periods (Proctor, 2002; León-Vargas et al., 2006; Bader et al., 2013). This could be explained by the fact that to ensure long-term survival, mosses need to be prepared for potential longer periods of drought than is normal in a tropical humid forest. Epiphytic bryophytes (branches and canopy) are more tolerant of rapid and frequent drying than forest floor and mesic grassland species (Proctor et al., 2007) and there is a range of microclimates on the epiphytic substrate (Pardow & Lakatos, 2013). Because of this, community composition is more similar within a height bracket over hundreds of kilometres than within a tree (Pardow & Lakatos, 2013).

Frahm (2000) found a difference in the amount of "structural adaptation" of traits between bryophytes inhabiting forest and savannah inselbergs. Interestingly, there was little adaptation of bryophyte traits to the dry environment of the savannah and more in those of humid forest inselbergs, e.g. leaf papillae were only found in 2 out of 30 species recorded in Cote d'Ivoire but most species in the rainforest of Zimbabwe had water-storage structures.

Tolerance to desiccation of bryophytes in lowland forest is relatively unknown therefore making prediction of their response to changing climatic conditions difficult (Pardow & Lakatos, 2013). There are very few studies on DT of tropical bryophytes but it is an important study to undertake due to changing climate conditions – especially in lowland forests (Pardow & Lakatos, 2013). Most studies that have looked at tropical bryophyte DT and traits have focussed on life-form. Studies measuring recovery following desiccation of particular life-forms (Proctor, 2004; Song et al., 2015) show that life-forms have different DT although Bader et al (2013) found that life form does not seem to dictate DT in tropical montane species.

As well as climatic conditions, substrate and altitude affect bryophyte distribution and DT. Water retention of a substrate will impact the DT of a species with epiphytic species usually being less DT than those occupying rock surfaces, as an example (Bates, 2009). However, there are few studies looking into the relation between substrate and DT. In forests worldwide, epiphytic bryophytes make up a large part of the bryophyte biomass, and even the overall biomass in some forest types (Bates, 2009). Although moist and shaded forests support a higher number and biomass of epiphytic bryophytes, in areas of very low light availability this richness decreases (Bates, 2009), due to the insufficient light level to achieve net photosynthesis. Within a tree, species occupying branches and the canopy are more DT as they are exposed to higher light levels and wind (Bates, 2009; Alvarenga et al., 2010). A few studies have looked at DT and altitude (Benassi et al., 2011; e.g. Bader et al., 2013) with contradicting results. Benassi et al (2011) found that less DT male plants were found at greater proportions at higher altitude, whereas Bader et al (2013) found no pattern between DT tolerant tropical bryophytes and altitude.

Although bryophytes are widely used as indicators of environmental pollution, only recently has their potential as biodiversity indicators begun to be exploited, albeit by few and geographically restricted studies (Diekmann, 2003; Drehwald, 2005; Frego, 2007). In tropical rainforests, community composition of epiphytic bryophytes changes rapidly in response to changes in insolation and relative humidity (Frahm & Gradstein, 1991; Sporn et al., 2009). Epiphytes with low DT are particularly susceptible to increases in air circulation and solar radiation in the lower vegetative layers which result from anthropogenic habitat degradation (Pardow & Lakatos, 2013). Bryophytes thus have great potential as indicators of forest integrity yet this important application remains under-studied.

2.4 Conclusions

DT is present in many terrestrial organisms, but predominantly in those that are very small or microscopic and is an adaptation to life in a relatively dry terrestrial environment. In the plant world, almost all species with vegetative DT are bryophytes; most angiosperms have DT pollen and seeds. Bryophytes' survival, as with all plants, is determined by how effectively they can photosynthesize and maintain metabolic processes in certain microclimatic conditions. There is therefore interplay between water uptake and storage, gas exchange, insolation and relative humidity. In bryophytes limited water availability and higher temperatures increase photorespiration, which is energetically inefficient, (Glime, 2007; Proctor, 2010) therefore shutting the metabolism down when there is insufficient water provides bryophytes an advantage over tracheophytes during drought.

Within bryophytes it seems that humid adapted species will desiccate slower than arid adapted species (Proctor & Tuba, 2002; Proctor, 2004; Song et al., 2015). Conceptually this is quite non-intuitive when thinking about plants. With tracheophytes, typically a species that is adapted to a humid environment will dry out much faster when exposed to an arid environment than a xerophytic species. In effect, most tracheophytes preserve their internal water during times of moisture stress but will continue photosynthesising and respiring during this time – even if it is energetically inefficient to do so (Proctor, 2010).

The poikilohydry of bryophytes reflects their distinct advantage in dry environments over tracheophytes (Alpert, 2005; Vitt et al., 2014) as it enables them to lose water quickly and shut down their metabolic activities and wait out periods of drought - desiccation tolerance - and only metabolise when conditions are optimal. The more DT a species is, the quicker it loses its water and shuts down metabolic activity, rather than remaining metabolically active when water availability is low or light levels are high which leads to a net loss in productivity (Proctor, 2010). The degree of DT varies among bryophytes; species' DT ranges from "fully desiccation tolerant" to those that are "desiccation sensitive" (Proctor & Smirnoff, 2000; Wood, 2007; Oliver, 2009; Vanderpoorten & Goffinet, 2009).

From studies quantitatively measuring DT in bryophytes, it can be concluded that bryophytes that occupy dry and exposed environmental conditions are more desiccation tolerant than those of more sheltered and humid habitats. However, there is also variation within habitats. Within a forest, bryophytes of varying DT will be found depending on the insolation and humidity of the microhabitat, with epiphytes being more DT than ground-dwelling species. Their distribution

could therefore be used to indicate changes to forest integrity and forest bryophytes more susceptible to extinction could be identified based on their traits and DT level. It is important to bear in mind that conclusions made as to how traits respond to DT or the environment are based on a relatively few number of species, and generally temperate ones. Although certain traits are related to the environment a species is found in and can indicate the likely DT level of a species, this relationship is not clear-cut and needs further study, particularly among tropical bryophytes.

The next chapter will assess if the presence of the traits described in this chapter can be related to the environmental preferences of bryophytes, and if their environmental preference can be used as an indication of their DT.

Appendix 2 DT physiology

A2.1. Physiological measurement methodologies

Relative water content (RWC) indicates the amount of water a plant can uptake and can be used to measure the plant's water capacity when dry relative to its capacity at full turgor (maximum water capacity) (Proctor et al., 1998). It is widely used in physiological experiments of DT (e.g. Proctor, 2004). Relative water content (RWC) is expressed as percentage of dry weight (Proctor et al., 1998):

$$RWC = \frac{Wt}{Wd} * 100$$

where Wt is weight at full turgor and Wd is dry weight.

Specimens are hydrated, excess water was blotted with filter paper and weighed. Specimens are then placed in an oven at 105°C for 30 minutes and then weighed. Another method involves exposing samples to air following hydration and weighing specimens at 1 minute intervals (Rands & Davis, 1997)

Chlorophyll fluorescence

A widely used and reliable method to measure a plant's photosynthetic activity is chlorophyll fluorescence (Maxwell & Johnson, 2000; Wood, 2007; Proctor, 2009) (Proctor 2007; Bader et al., 2013; Pardow & Lakatos 2013). One of the reasons it is so popular is because it is easily measured, both in terms of equipment, time and interpretation of results. Interpretation can be complex (Maxwell & Johnson, 2000) if the readings taken are misunderstood but due to the existence of several clear methodologies and reviews on the subject (Wood, 2007; Proctor et al., 2007) this is easily avoided.

When light enters a leaf its energy is transferred three different processes: heat dissipation, photosynthesis and 1 to 2% of it is re-emitted as red fluorescence (Maxwell & Johnson, 2000; Proctor, 2009). As these three processes share the energy they are in competition with each other and it is this that allows us to use the fluorescence to measure photosynthetic activity. The parameter estimated is the efficiency of, or the damage to, photosystem II (PSII) (Maxwell & Johnson, 2000; Wood, 2007; Vanderpoorten & Goffinet, 2009).

When a plant is moved from dark to light there is a "spike" in chlorophyll fluorescence over a time period of about one second (Figure 2.16) (Maxwell & Johnson, 2000). This is caused by a reduction of electron acceptors (downstream of PSII) and the reaction centre is "closed" – this results in a decrease in photochemistry efficiency and an increase in fluorescence yield (Maxwell & Johnson, 2000). After a few seconds the fluorescence yield decreases – called fluorescence quenching (Figure 2.16). This is due to two mechanisms: photochemical quenching (PQ) where electrons are carried away at a faster rate from PSII (mainly due to enzymes in the carbon metabolism that have been light activated and opening of stomata (Maxwell & Johnson, 2000)); and non-photochemical quenching (NPQ) where light energy is converted to heat to avoid light damage (oxidative stress) (Vanderpoorten & Goffinet, 2009). All experiments need a dark-adapted, non-

stressed reference point (F_0 and F_m) (Figure 2.16, steps 2 & 4). In the field this is difficult unless the value is taken before dawn. However, the pre-dawn F_m can be influenced by the previous condition of the plant e.g. if exposed to stress (Maxwell & Johnson, 2000).

By using a modulated fluorometer (where the light source is turned off and on) the fluorescence is measured in background light conditions and full light conditions; the stages in this process and parameters measured are shown in Figure 2.16.

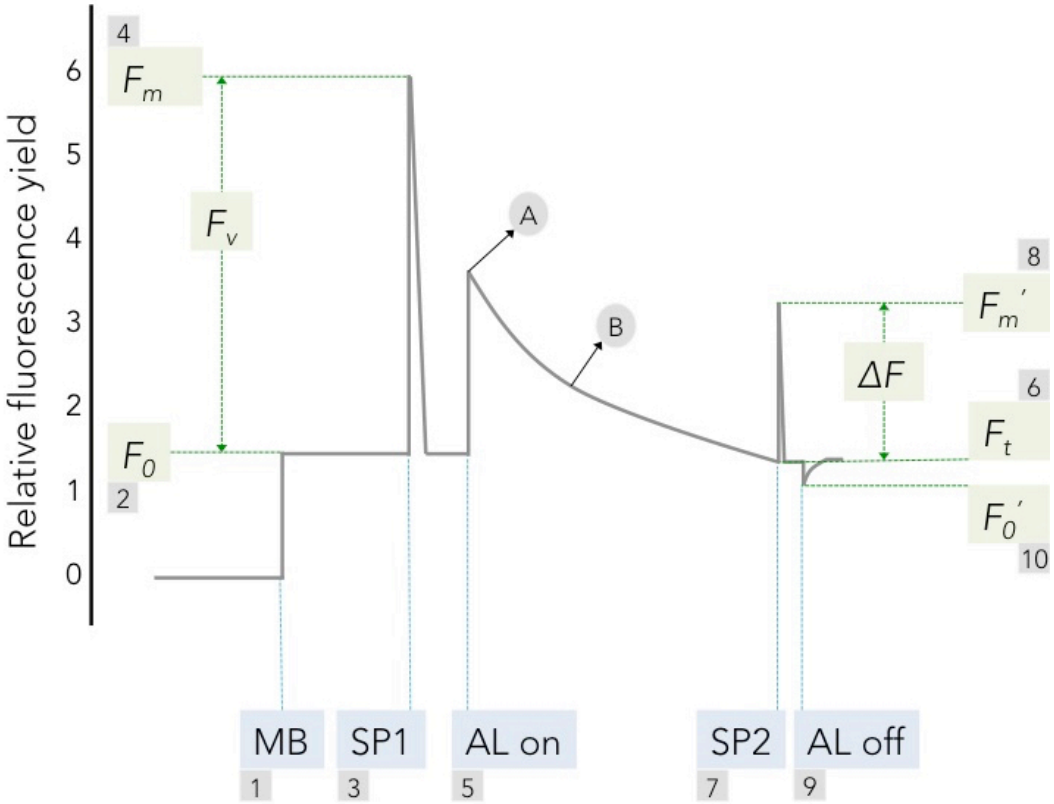


Figure 2.16 Schematic diagram showing relative changes in chlorophyll fluorescence yield as light is applied to a plant, as well as the steps of the measurement process and parameters measured. 1- MB – measuring light switched on – low intensity. 2- F_0 – minimal fluorescence level measured. 3- SP1 – short saturating light flashes are applied. This progressively closes the PSII and reflects the fluorescence in the absence of photochemical quenching – maximum fluorescence. 4- F_m – the maximum fluorescence yield measured in the dark-adapted state. 5- AL on – actinic light switched on, a photosynthesis inducing light. A peak in fluorescence occurs (A) and then decreases to a steady level due to fluorescence quenching (B). 6- F_t – steady-state fluorescence yield, measured immediately before a second saturating light is applied (SP2). 7- SP2 – short saturating light flashes are applied again. 8- F_m' – the maximum fluorescence yield in the light; measured the presence of photosynthetic light. 9- AL off – actinic light switched off. 10- F_0' – zero fluorescence level in the light; measured by applying a far-red light (650nm). Taken and adapted from Maxwell & Johnson 2000, fig. 1, p. 661 and Proctor 2009, fig. 6.5, p. 251.

Four values indicating efficiency or damage to PSII can then be calculated from the measured parameters:

1. The intrinsic or maximum efficiency of PSII (quantum efficiency if all centres are open) is the ratio of variable fluorescence to maximum fluorescence: $F_v/F_m = (F_m - F_0)/F_m'$.
2. The efficiency of PSII photochemistry measures the proportion of light absorbed by chlorophyll in PSII following photochemical quenching i.e. the amount of light used in the photochemistry of PSII: $\Phi_{\mathrm{PSII}} = (F_m' - F_t/ F_m')$.

3. Photochemical quenching indicates oxidation state or the proportion of PSII centres that are open and is calculated using: $qP = (F_m' - F_t)/(F_m' - F_0')$.
4. Non-photochemical quenching of PSII as a measure of heat dissipation is calculated as: $NPQ = (F_m - F_m')/F_m'$.
5. Electron flow calculated by multiplying Φ_{PSII} by the PPFD.

As the values calculated are quotients, values can then be compared between plants as sample size used does not affect results (Maxwell & Johnson, 2000; Proctor, 2009) and measurement of chlorophyll fluorescence is suggested as the best method when assessing many samples (Wood, 2007). Fluorescence can be measured in the field with portable fluorometers (Bader et al., 2013). Chlorophyll-fluorescence values tend to be plotted against relative humidity (RH) or amount of photosynthetic light available (PPFD µmol m^{-2} s^{-1}). One experimental method is to desiccate species at different relative humidity (RH) levels for a set amount of time and measure their photosynthetic performance at different time intervals following hydration (Proctor, 2001).

A2.2. The Austin Protocol

As described in Wood (2007, p. 173-174):

> Plant material will be freshly collected (or obtained from culture collections) and maintained as fully hydrated material under controlled conditions (14°C, 50 µE m^{-2} s^{-1}). Alternatively, for difficult to obtain plants, dried material will be maintained at 58C, and rehydrated for 24 h (14°C, 50 µE m^{-2} s^{-1}). To ensure proper drying, it will be critical to use small quantities (i.e., approximately 200 mg FW) of isolated shoots that have been blotted completely dry. Hydrated plant material will be equilibrated at two relative humidity ''set points,'' 67–75% RH or 20–30% RH using either saturated salts or diluted sulphuric acid to control humidity for both 24 h and seven days with five replicates per treatment. In my research program, we have used 67% RH (saturated solution of ammonium nitrate at 208 °C) or 23% (saturated solution of potassium acetate at 20 °C) (Zeng et al., 2002).
>
> The recovery of photosynthesis, as determined by of Fv/Fm, will be measured after 2 recovery times—1 h and 24 h. Short-term recovery (0–60 min) will be measured by rehydrating dried plant material placed within the leaf clip (i.e., spraying with de-ionized water ensuring that plants are saturated). Longer-term recovery (as long as 24 h) will be determined on rehydrated plant material maintained at 14°C, 50 µE m^{-2} s^{-1} within a growth chamber, and transferred to leaf clips for dark adaption (10 min). The parameters of Fo, Fm, and Fv/Fm will be measured on both fresh (i.e., rehydrated but not desiccated) and rehydrated pant material (i.e., rehydrated, desiccated and rehydrated). Photosynthetic recovery (i.e., an increase in the measured Fv/Fm value from near 0 to more than 0.700) will be taken as an indication of vegetative desiccation-tolerance. Bryophyte species that recover from equilibration at 67% RH are not ''desiccation sensitive'' and will be considered to be ''modified desiccation-tolerant.'' Those species that recover from equilibration at 23% RH will be considered to be ''fully desiccation-tolerant.''

Chapter 3 Desiccation tolerance traits and species' environmental preferences

Abstract

Trait databases are widely used in ecology to understand relationships between species and their habitat and environment. Increasingly, studies are using traits to inform conservation management decisions. As such, many plant trait databases exist, from local to global datasets, and there is a concerted effort to collate trait data and make it readily available online to promote research in this area.

However, these databases overlook bryophytes and only two bryophyte databases currently exist, both from temperate zones. In this chapter, the largest bryophyte trait database to date was created for 1430 taxa, 51 morphological and reproduction traits, five environmental traits, thirteen ecological and distribution traits and three conservation traits. It is also novel in that it includes Malagasy bryophytes. Portuguese bryophytes were also included to inform on Malagasy species, for which data is scarce. Studies have found that it is possible to extrapolate bryophyte data from one region to another due to the high dispersal ability of bryophytes resulting in species, genera and families common to both regions. In the specific case of Madagascar and Portugal, 34% of Malagasy genera and 64% of Malagasy families are found in Portugal.

In this study, desiccation tolerance traits (morphological and life-history) were selected in order to investigate how they may affect species's environmental preferences and if they therefore play a role in DT. Many traits were found to affect species' environmental preferences from large-scale traits such as life-form and plant size to cell shape and spore size. Sporophyte traits had a smaller effect on overall environmental preferences and so are less informative for desiccation tolerance than gametophyte traits. Importantly, analyses conducted on Malagasy and Portuguese species individually showed that their traits have comparable responses to environmental preferences thus confirming that results from Portuguese species can indeed be used to extrapolate to tropical ones.

Mosses had many more traits that were significantly associated with environmental preferences than liverworts. This is likely due to a combination of sample size (due to data availability) and that many traits were not appropriate for liverworts. It was decided to therefore continue further analyses on mosses alone, but that future studies should not overlook liverworts. The univariate tests provided some level of insight into how traits relate to the environment, but due to the presence of traits with a lot of states and the potential interaction of traits with each other, it was concluded that a multivariate approach is also needed.

3.1 Introduction

3.1.1 Why use traits?

Species traits can inform a number of topics and issues from physiological questions to conservation practice (Kattge, Ogle, et al., 2011). Species vary in their natural abundances which is not only explained by environmental factors but also potentially by the traits of the species

themselves; for example, vascular plant species requiring specific vectors for pollination will be less abundant than wind-pollinated ones (Godefroid et al., 2014) and species' dispersal distance is often dictated by seed mass (Vazačová & Münzbergová, 2014). In ecology, traits can be used to understand relationships between species and their habitat allowing predictions to be made on ecosystem changes (Albert et al., 2010). Trait databases exist for the British flora, for both tracheophytes and bryophytes (Hill et al., 2004, 2007) and have formed the basis of several studies on plant interactions with the environment (e.g. Walker & Preston, 2006). From a conservation perspective, knowing which traits make species more susceptible to threats (e.g. habitat fragmentation, climate change) and extinction allows practitioners to put in place effective protection measures. However, a particular trait will not always indicate that a species has low or high abundance as shown by Godefroid et al. (2014) who compared British and Belgian vascular flora and found that the response of species rarity to different traits was different in the two regions. Trait data can also be used as environmental and biodiversity indicators (Kattge, Ogle, et al., 2011)

3.1.2 What trait research has been done?

Many plant trait databases exist, form regional to global scales (Table 3.1), and in light of the increase in trait research, efforts to compile these data into standardized databases are underway (Kattge, Ogle, et al., 2011). Although some databases include tropical regions, most trait data is from temperate regions. On the TRY database (a compilation of 93 smaller plant trait databases), there is data on 175 traits from up to 1627 species from Tropical Africa, however, 87% of species have data on 10 traits or less. Two databases specific to tropical flora exist, although one is focussed on tree species only (*Mariwenn*, Ollivier et al., 2007) and the other has very few traits (*RAINBIO*, Dauby et al., 2016). However, they indicate the increase in trait research in tropical areas and provide an important starting point.

Most plant trait research has focussed on vascular plants (Díaz et al., 2016) although the number of studies on bryophyte traits has been growing. Of the latter, many focus on the role of traits in bryophyte physiology or the relationship between different traits (e.g. Crawford et al., 2009) with a few looking at trait-environment relationships (e.g. Rice et al., 2008; Kangas et al., 2014). Categorisation of life-history, life-forms and ecomorphology measures has been attempted in the study of bryology to allow comparison between species of different geographic regions (Kürschner et al., 1999). Functional traits commonly used in the study of vascular plant ecology (e.g. leaf nitrogen content) have been shown to not be transferrable to bryophytes (Rice et al., 2008), which is not surprising given the very different morphology and ecophysiology of these two plant groups.

Of the major plant trait databases, only the PLANTSdata database (Green, 2009) includes bryophytes (Table 3.1). Only taxonomic data is available for these 2365 bryophyte species and additional conservation data for 85 of these bryophytes. Currently, two trait databases exist specifically for bryophytes, BRYOATT (Hill et al., 2007) and BRYOTRAIT-AZO (Henriques et al., 2017), and Dierßen's (2001) publication lists ecological and distribution data; these all focus on European bryophytes. Trait data on bryophytes is therefore scarce and is non-existant for tropical bryophytes. Alpert (2000b, p. 9) stated that: "One of the most promising avenues for future research will be further comparisons of the physiology and ecology of (…) congeneric species that differ in ability to tolerate desiccation." There is some data on drought tolerance, but only on

vascular plant species: the TRY database lists the drought tolerance of 3324 vascular species. The trait database created in this study includes 1430 taxa, 51 morphological and reproduction traits, five environmental traits, thirteen ecological and distribution traits and three conservation traits.

3.1.2.1 Geographic focus

As mentioned above and in the previous chapters, most focus on bryophyte traits has been in temperate regions with little research into tropical bryophytes. This study will therefore look at bryophytes in Madagascar, which is one of the least studied tropical bryofloras (see Chapter 1) as well as potentially being highly threatened. Compiling a database with complete trait data for sufficient Malagasy bryophytes to ensure a robust analysis would be beyond the time-frame of this PhD. To record traits for species without recent flora descriptions (as the case with most of the Malagasy species) requires the consultation of herbarium specimens, original species publications and taxonomic revisions. Therefore, trait data from a relatively well-known bryoflora, Portugal, are used in conjunction with Malagasy species to ensure there are enough species for statistical analyses. Portugal, like Madagascar, is part of a biodiversity hot spot (Myers et al., 2000) and there is a recent flora (Guerra & Cros, 2006) and Red Data Book (Sérgio et al., 2013) providing accurate and sufficient information to complete the trait data for these species. It is possible to extrapolate bryophyte data from one region to another due to the high dispersal ability of bryophytes resulting in species, genera and families common to both regions (Vanderpoorten & Goffinet, 2009) and due to the fact that the ecology and community dynamics of a species found in two regions is comparable (Rydin, 2009). In the specific case of Madagascar and Portugal, 34% of Malagasy genera and 64% of Malagasy families are found in Portugal. Although there are no studies directly comparing DT traits in bryophytes from different regions, a study of DT filmy ferns (Hymenophyllaceae family) showed that the responses to DT are similar in species that occupy similar habitats regardless if they are from different geographical regions (Proctor, 2012). It can be assumed that bryophytes will behave similarly, as filmy ferns are very similar physiologically and ecologically to bryophytes (they are poikilohydric also). Additionally,

Table 3.1 Examples of large-scale plant species trait databases with the number of traits and taxa present in each; taxonomic groups represented; type of traits; and geographical coverage.

Database name	Available digitally	Number of traits	Number of taxa	Taxonomic group	Type of traits	Geographical coverage	Reference
BROT	Yes	14	952	Vascular plants	Morphological, life-history, geographical	Mediterranean Basin	(Paula et al., 2009)
LEDA	Yes	26	~3000	Vascular plants	Morphological, life-history	Northwest Europe	(Kleyer et al., 2008)
BiolFlor	Yes	66	3659	Vascular plants	Morphological, phylogenetic	Germany	(Kühn et al., 2004)
ECOFLORA	Yes	130	3842	Vascular plants	Ecological, morphological	British Isles	(Fitter & Peat, 1994)
BIOPOP	Yes	51	4700	Vascular plants	Ecological, life-history	Central Europe	(Poschlod et al., 2003)
PLANTSdata	Yes	~50	38 000	Vascular plants and bryophytes	Morphological, life-history, geographical, conservation	North America	(Green, 2009)
Databases that include tropical species							
Mariwenn	Yes	32	>60	Tree species	Ecological, morphological, physiological, phylogenetic	French Guiana	(Ollivier et al., 2007)
Wood Density	Yes	4	8412	Woody plants	Wood density, geographical	Global	(Chave et al., 2009)
InsideWood	Yes	57	>10 000	Hardwoods	Anatomy, geographical, photographic	Global	(Wheeler, 2011)
RAINBIO	Yes	5	26 694	Vascular plants	Habit, taxonomic, geographical	Continental Tropical Africa	(Dauby et al., 2016)
SID	Yes	10	33 346	Vascular plants	Seed biological characteristics	Global	(Royal Botanic Gardens Kew, 2016)
TRY	Yes	52	~69 000	Vascular plants	Morphological, life-history, ecology	Global	(Kattge, Díaz, et al., 2011)
Bryophyte trait databases							
Dierßen	No	11	~1600	Bryophytes	Geographical, ecological	Europe	(Dierßen, 2001)
BRYOATT	Yes	28	1057	Bryophytes	Morphological, life-history, geographical, conservation	British Isles	(Hill et al., 2007)
BRYOTRAIT-AZO	Yes	41	488	Bryophytes	Morphological, taxonomic, geographical	Azores	(Henriques et al., 2017)

Portugal's location at the southwestern tip of Europe means its bryoflora has some subtropical affinities (Sérgio et al., 2013) and so reducing the disparity between European and Malagasy species in terms of trait responses to the environment allowing data from Portuguese bryophytes to be applied to Malagasy bryophytes.

3.1.3 Conservation

Some studies have related species DT to either distribution or conservation, with varying conclusions. One study looking at the DT of tropical bryophytes to explain their distribution along an elevation range found that montane species which are not found in lowland forest do not have different levels of DT, and that therefore it is not DT that determines their distribution (Bader et al., 2013). However, it is known that the DT exhibited by bryophytes in experimental conditions may not reflect their DT in the field (Stark & Brinda, 2015a). In contrast, Pardow & Lakatos (2013) found that understorey bryophytes have lower DT and so are likely to be more threatened in the face of habitat change, as well as climate change.

As briefly introduced in Chapter 1 (section 1.2.3.2, p. 32), the Sampled Red List Index (SRLI) project is currently assessing a worldwide selection of 1500 bryophyte species (Brummitt et al., 2015). Results from this thesis will feed into it, and subsequently traits identified here will be correlated with the threat level assigned to those 1500 species. This will provide a large-scale global analysis of whether there are traits that can be used to indicate extinction risk and investigate the potential of using morphological traits as an indication of extinction risk for species that have no conservation assessments.

3.1.4 Environmental indicator values

Plants occupy niches defined by abiotic and biotic factors, and so plants have long been used as bioindicators for various purposes including determining changes in the local environment (Diekmann, 2003). Scientists have defined these niches in several ways, most commonly by quantifying abiotic variables such as light, humidity, pH, and temperature, among others. Ellenberg in 1950 developed defined "indicator values" based on vascular plant species' environmental preferences (Diekmann, 2003) and these values have been widely used in plant ecology. The first such system for European bryophytes was developed by Düll in 1969 and revised in 1990 (Ellenberg, 1992). Ellenberg then further refined this system for bryophytes with indicator values for light, temperature, moisture, pH, continentality, and also the morphological trait life-form (Ellenberg, 1992). This system was expanded upon by Dierßen (2001) and provides values for 12 indicators, see Table 3.2.

Indicator values can be used to: determine the environment at a particular site; assess habitat quality; compile species lists for particular localities (based on occurrence probability and potential distribution); and environmental risk assessments (Schaffers & Sýkora, 2000). Ellenberg values, assigned to vascular plants, are used to inform species' habitat preferences and therefore to define species' niches. Similarly to studies using indicator values to inform species distribution, this study will use Dierssen's (2001) moisture and light indicator values to relate morphological traits with the species' environmental preferences.

Table 3.2 Indicator values assigned to European bryophytes by Dierßen (2001).

Type of indicator	Indicator
Geographic	Vegetation zone
	Continentality
Conservation	Threat category
	Pollution
	Human impact
Environmental	pH
	Nutrient availability
	Humidity
	Heat balance
	Light
	Substrate
Life history	Life strategy

3.2 Aim

The aim of this chapter is to investigate whether bryophyte traits that can be relatively easily observed and measured are significantly related to different environmental conditions, so allowing for DT to be estimated more easily – this is particularly useful for poorly studied species.

1. Identify and collate bryophyte traits that could potentially indicate DT and create appropriate trait states for analyses.
2. Create an environmental index (EI) based on the humidity and light preferences of each species.
3. Test if the EI relates to bryophyte physiological DT measurements and so can be used as a surrogate for DT.
4. Examine the relationship between different traits and environmental preferences.
5. Determine whether temperate and tropical species' traits have similar effect on species' environmental preferences.

3.3 Methods

This section provides details on how the trait database was produced including how species were selected, which data sources were used to obtain the traits and a summary of how certain traits, mentioned in Chapter 2, were quantified and/or categorised – Table 3.6, p. 106, provides a summary of all the traits recorded (section 3.3.1.3, p. 99). Information for whether a trait indicates desiccation tolerance is taken mainly from the bryophyte literature but also from vascular plant studies. Although vascular plants do not exhibit desiccation tolerance (with a few exceptions) and therefore traits used in these studies are not related to desiccation tolerance, they do relate to drought tolerance and so may relate to desiccation tolerance in bryophytes.

3.3.1 Building the database

A database of desiccation-tolerance traits and ecological data for species from two study areas (Portugal and Madagascar) was compiled from various sources. The structure of the database follows guidelines outlined in (Kattge, Ogle, et al., 2011) to ensure a level of standardisation, thus facilitating analysis, and make the data accessible by other researchers, either on their own or

integrated into other trait matrices. Most standardisation suggested to date has been for vascular plants but where possible these standards have been adopted or adapted.

Although environmental, habitat, geographic, and conservation data for a species are not strictly species "traits" because they are ancillary to the species, for the purposes of a trait database they can be treated as such as they are all measurements of a characteristic (Kattge, Ogle, et al., 2011); i.e. a value of 1 is a measurement of the variable "humidity" in the same way "0.5 cm" is a measurement of the trait "plant size". In this chapter only the environmental variables are discussed; ecological, distribution and conservation traits are discussed in Chapter 4.

The criteria and process for quantifying and categorising traits is outlined in sections 3.3.1.3 to 3.3.1.5 below. For traits where subjectivity was unavoidable, problems were encountered (e.g. vague information on a trait) or where only part of the trait was used in analyses the process is detailed in these sections - for all other traits, their definition in this study, states and categories are listed below in Table 3.6, p. 106. It is important to include an explanation of this process as due to the variation and qualitative nature of most of the traits, this process is not self-evident. This is also important because the manner in which traits are quantified and/or categorised will obviously have an impact on analysis, results and interpretation.

3.3.1.1 Species selection

The list of species to be included in the trait database changed over the course of inputting data. Initially all Madagascar bryophyte species were to be included in the trait matrix, however due to the high number of bryophyte taxa that exist in Madagascar (1144, (Marline et al., 2012)), comparatively lower research effort there, time constraints and the varying amount of data available for different species meant that epiphyte and forest species in Madagascar were prioritised. All Portuguese species were included as it is a much more thoroughly studied flora. All species that belong to a genus known to occur in forests or is epiphytic, either in Madagascar or another region, were included. Although an epiphytic species may not necessarily be a forest species, including all epiphytes maximizes the species pool from which indicators will be chosen. Also, it is possible that a species that may not be found in intact forests could be found in disturbed forests. Focussing on this group of species was deemed appropriate as fieldwork was to be carried out in forest habitat (see Chapter 5). Further, some epiphytic families are taxonomically well known such as the families Calymperaceae and Orthotrichaceae, meaning that more trait data are available for them and their identification is relatively easy. These families are also widely distributed in the humid and littoral forests of Madagascar and in Portugal.

SRLI species

The Sampled Red List Index (SRLI) bryophyte list was cross-checked with the most recent species checklists for Madagascar (Wigginton, 2004; O'Shea, 2006; Marline et al., 2012) and Portugal (Sérgio & Carvalho, 2003; Sérgio et al., 2013). This yielded a list of 125 species: 79 species for Madagascar, 45 for Portugal and one species common to both (see Table 3.22, p. 163). *Bryum argenteum* Hedw., common to both countries, is one of the most globally widespread bryophyte species. These species were all included in the database, even if they are not forest or epiphytic species in order to contribute to the bryophyte Sampled Red List assessments. Before selecting the species, the SRLI names were checked against the accepted nomenclature and corrected where necessary.

Taxonomy

Taxonomy for families and genera follows Renzaglia *et al.* (2009) for hornworts, Crandall-Stotler *et al.* (2009) for liverworts and Goffinet *et al.* (2009) for mosses. For each taxon, the phylum, order, class, genus and species was recorded. Nomenclature of all species included in the database was checked – all recent synonyms were recorded so that literature and herbarium searches were conducted using all recent synonyms of a species. Following this check, twenty species were excluded from the database due to taxonomic doubt.

3.3.1.2 Data sources

Varying amounts of data available for species meant that multiple sources were consulted; the variety of sources and their prioritisation is shown in Figure 3.1. Sources included specimen record data from herbaria (including online herbaria databases), literature and field data (particularly in the case of the Malagasy species where herbaria and literature data are scarce for many species). The latter data yield information on the species' distribution and ecology as well as trait data. For sources used see A3.1, p. 149 and A3.2, p. 150, in Appendix 3. Literature specific to each study area was prioritized over sources from other geographical distributions. The reason for this is that some traits within the same species may vary between regions due to climatic differences (as described in floras (e.g. Smith, 2004; Guerra & Cros, 2006)).

Literature data

Morphological and life-history traits were taken from floras, species publications and taxonomic treatments, prioritising the most recently published of each. These sources were also used for environmental, ecological and distribution data where available. In addition, for Portuguese species, a European phytosociological classification of bryophytes (Dierßen, 2001) was used for environmental preferences, life-strategy and distribution. As the bryoflora of Portugal is well studied (Sérgio et al., 2013), it was possible to find information for most of the traits of all Portuguese species. For Malagasy species, trait data was lacking for many species due to the overall lack of study of the Malagasy bryoflora, and particularly in relation to their ecology and distribution. In some cases, surrogate species were therefore used from other African localities, giving preference to those from other Indian Ocean Islands and taking traits from species of the same genus that occupy a similar habitat.

Hill et al. (2007) provide a comprehensive classification of various "attribute data" for British and Irish bryophytes. These attributes are numerous (28) and for the traits used in this study the following attribute data was imported: presence and number of vegetative structure types, life-form and life-strategy. These provided data for almost all Portuguese species, but not for Malagasy species, except for those species that are found in both Europe and Madagascar.

Specimen data

For Malagasy species, data from specimens was used and includes both freshly collected specimens (during this PhD's fieldwork) and 120 herbarium specimens (see Appendix A3.1, Table 3.21, p. 158 for list). Morphological traits were recorded from specimens as well as ecological and geographical data where available (older herbarium collections do not usually have accurate geographical or ecological data). Herbarium codes and names follows Index Herbariorum (Thiers, continuously updated). Different morphological traits were recorded depending on how many trait data were available from the literature for each species.

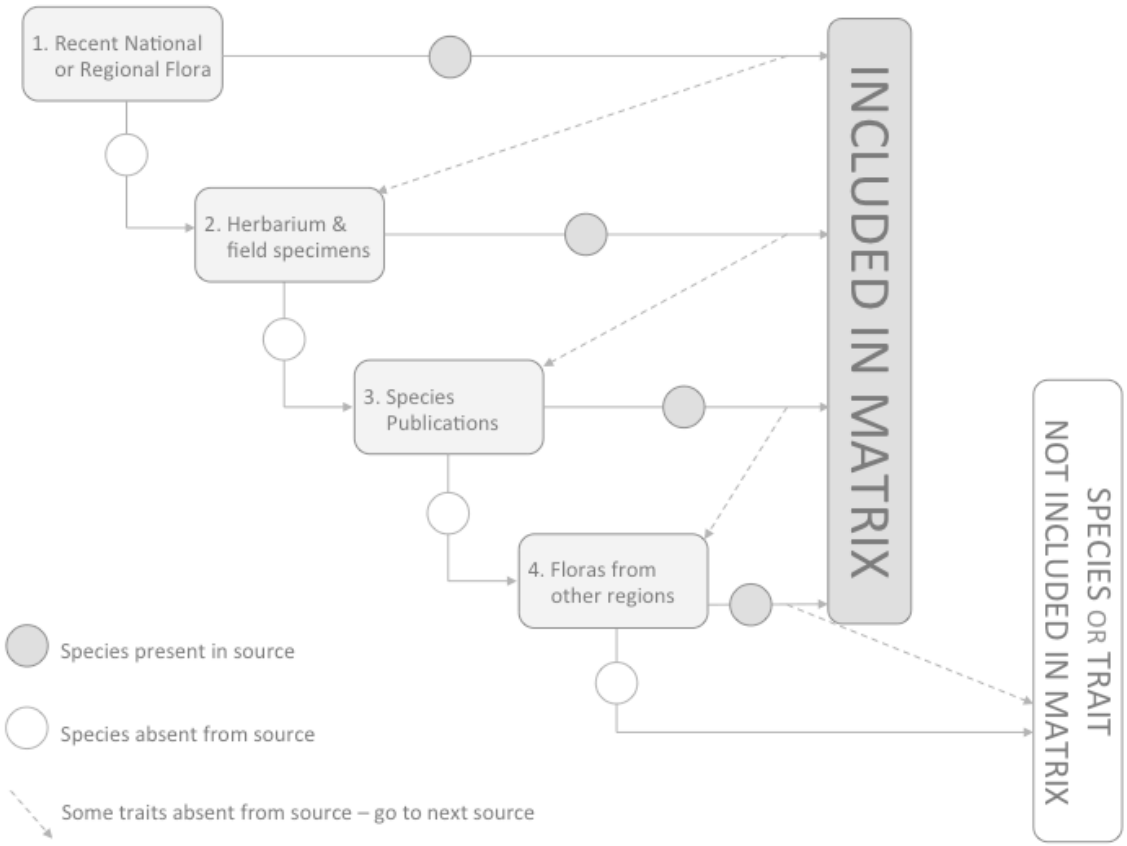


Figure 3.1 Prioritisation of sources used to collate species morphological traits. A species is searched for first in the national or regional flora (priority 1), if the species is present traits are entered into the matrix, any missing traits are then searched for in the next source type (priority 2 - herbarium and field specimens), and so on until all traits are complete, or as many traits can be found in the 4 types of sources. If a species is not present in the first type of source, then it is searched for in next source type, until it is found. The same process applies to any missing traits.

Taxonomic uncertainty

As the Malagasy bryoflora is understudied, there are likely to be misidentified taxa. A few taxonomic groups have had recent revisions, monographs or had type specimens reviewed such as the *Leucoloma* (La Farge, 2002a, 2002b, 2002c) and *Taxithelium* (Câmara, 2011). These taxa were therefore also prioritised during database building.

3.3.1.3 Recording and categorising bryophyte traits

Two terms are used when discussing traits: "state" refers to a term used to describe a trait (e.g. dendroid, fan, pendant, cushion, turf and tuft are six states within the life-form trait) and "category" refers to a grouping of states used for analyses in this work (e.g. "open" is a category grouping dendroid, fan and pendent states and "compact" is a category grouping cushion, turf and tuft states). The traits are divided into gametophyte, sporophyte and life-history traits as they are different types of traits: gametophyte traits are present throughout a bryophyte's life-cycle and so are those most responsive to environmental conditions; sporophyte traits are only present for a short period, if at all, and inform reproduction success; and life-history traits inform species phenology (Violle et al., 2007). Although some traits are found in all liverworts, mosses and hornworts, the morphological differences between these three groups (as described in Chapter 1, section 1.1.6, p. 12) mean that some types of traits are specific to each.

Usually when recording traits for a species, several specimens will be measured and an average taken to represent that species (Lavorel & Garnier, 2002; Kattge, Ogle, et al., 2011; Díaz et al., 2016). This was done for very few (19) species in this study due to time constraints and also simply the lack of availability of specimens for some species. However, this was not seen as reducing the data quality as data taken from floras are already representative of the species' morphology (except in the case of very rare species where only one or two specimens are known and so the degree of variation within a species is unknown). Where more than one source was consulted for a species, if there was conflicting information for a trait, the most recent publication was used or if it was a quantitative trait such as plant size or altitude, a combination of sources or range of values based on the sources was used.

All traits, unless otherwise indicated below, were recorded from the hydrated state as some traits vary depending on the hydration condition of the plant. This allows traits to be compared across species. For traits where subjectivity (qualitative estimates of trait characteristics: e.g. not shiny, shiny, very shiny) was unavoidable, problems were encountered or where only part of the trait was used in analyses the process is detailed in this section. Table 3.6, p. 106, provides a summary of all traits recorded, whether a trait is categorical nominal, categorical ordinal or continuous, its definition in this study, and all the trait's states and categories.

3.3.1.3.1 Gametophyte traits

Plant colour

When only herbarium specimens were available for a species, the colour was recorded but not included in analyses, as a dried plant's colour may not reflect their colour in their natural habitat; albeit bryophytes lose their colour to a lesser extent than vascular plants in herbaria – due to the previously discussed mechanism of metabolic shutdown in bryophytes. The full range of plant colours was inputted into the database. A column was automatically generated with all the colours present in a species coded to assign a colour code to each species e.g. if plant colour is yellowish green to brown colour is coded as YGBr (see Table 3.23, Appendix A3.5, p. 170). A few species exhibit a different colour on leaves close to the substrate. This colour was recorded in a separate column, as well as any colour listed as "occasional", but only the predominant colours were used in analyses.

Life-form

Species may exhibit more than one life-form, usually as a result of environmental differences or growth stage (e.g. young versus adult plants (La Farge, 2002a)). If a flora stated that a species occasionally had a certain life-form this was omitted from analyses and only the common life-form was used. If a flora stated that a species was found commonly in more than one life form then all these life-forms were recorded.

Some classifications also incorporate size into life-form categories (e.g. Tixier, 1966; Chuah-Petiot, 2003) as this has an effect on ecophysiology, but as size is recorded as a separate trait it was not necessary to include size with life-form. Following a search of several classifications used in both temperate and tropical bryophyte literature, the classification used in this study (Table 3.3) is a combination of the classifications from an African flora and a European trait database (Chuah-Petiot, 2003; Hill et al., 2007) so that the classification covers bryophyte life-forms present in temperate and tropical habitats. One type of life-form, aquatic-trailing, describes species that

have pendant or weft life-forms but that live in water (Hill et al., 2007) and so they are maintained in a separate category due to the different environment they occupy (Glime, 2013a).

Table 3.3 Life-form categories used in this study with their definitions and the source they are taken from.

Life-form with definition	Source
Aquatic trailing – attached to substrate	Hill et al. 2007
Cushion - numerous shoots very close together forming dome-shaped colonies	Chuah-Petiot, 2003 & Hill et al. 2007
Mat, rough - creeping, lateral branches erect	Hill et al. 2007
Mat, smooth - creeping, branches lying flat	Hill et al. 2007
Mat, thalloid - creeping, thalli forming a layer	Hill et al. 2007
Turf - vertical stems with little or no branching	Hill et al. 2007
Turf, protonemal - persistent protonema	Hill et al. 2007
Turf, scattered - scattered vertical shoots	Hill et al. 2007
Tuft - loose cushions, not dome-shaped	Hill et al. 2007
Dendroid - main stem erect with large leaves at top or many lateral shoots	Chuah-Petiot, 2003 & Hill et al. 2007
Fan - branches in plane on vertical substrate	Hill et al. 2007
Pendant - creeping stems on twigs with long secondary stems	Chuah-Petiot, 2003
Weft - intertwining branched layers	Hill et al. 2007

Plant size

Some floras indicate the exact size of species, either an average value (e.g. 3 cm) or the range of the most common sizes (e.g. 2-6 cm) whereas others provide categories instead (e.g. small or large). In the latter case other literature and herbarium specimens were consulted to obtain an exact size but this was not possible for all species. Therefore, to maximise the number of species available for analyses, and to ensure uniformity across species, species with exact sizes were classified into minute, small, medium, large and robust (Table 3.6, p. 106) based on classes used in the literature. For species whose size range varies across more than two categories (e.g. small to large), the median class was used. Although Crawford et al. (2009) suggest using the maximum size of a range in case measurements were taken from immature plants, floras use mature plants to base measurements on and so is not an issue here.

Because categorising a continuous variable is subjective and can remove information from the data and reduce variation (MacCallum et al., 2002), the exact size (in cm) was also retained in case the size categories did in fact reduce information in analyses.

Leaf characters

Leaf morphology can vary within an individual plant depending on its position on the plant: base, middle, apex, main stem or branches. This is particularly the case in pleurocarpous mosses (e.g. *Rigodium* genus (Zomlefer, 1993) (see Figure 3.19, p. 162) so care was taken to record traits from secondary branch leaves (in the case of pleurocarpous mosses where floras give leaf morphology for both stem and branch leaves) and when observing herbarium specimens from leaves in the middle of stems or branches. Branches interact more with the environment than the stem as the

latter is sheltered by the branches, and so I considered that using branch leaf morphology was valid in a study relating bryophytes to their environment.

Whereas all leaf traits were recorded in the hydrated condition, leaf orientation was recorded in both hydrated and dehydrated conditions (where available). This is to see if species whose leaf orientation changes most are associated with a particular environmental condition. For example, if a species has appressed leaves (closer to the stem: more closed) when dry, but spreading leaves (further away from the stem: more open) when wet, this could indicate that it is a species adapted to drier conditions compared to one whose leaf orientation changes little between dry or wet (Glime, 2015a). The main leaf orientations are shown in Figure 2.5, but combinations of two states are usually used in the literature, and these were all recorded in the database. A difficulty arises when species are listed as having two of the states, e.g. erect to spreading. One way to overcome this was to code each of the main states with a number, and then assign species with two states with a combination of the numerical value of those states (see Table 3.25 and Table 3.26, p. 171, Appendix A3.5). To analyse the difference between dry and wet leaf orientation, the orientation value when dry was subtracted from the value when wet.

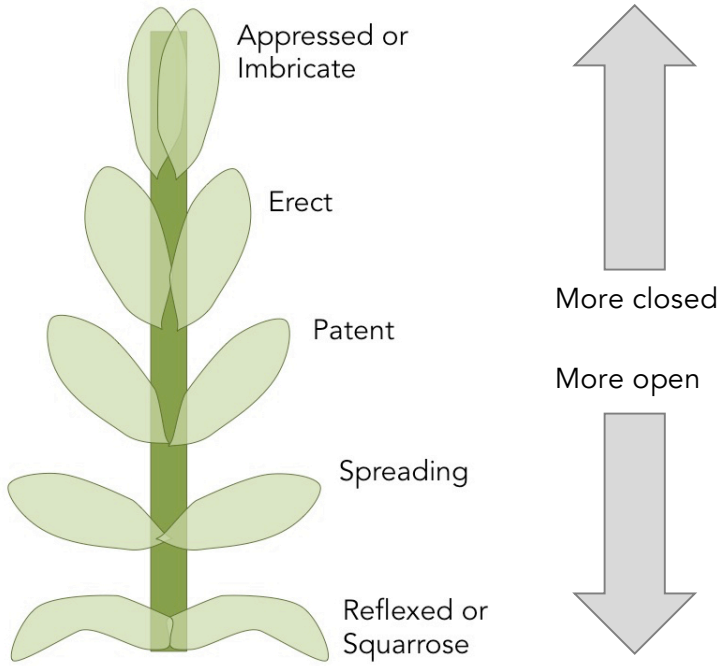


Figure 3.2 Schematic representation of the main leaf orientation states with the definition of "more closed" and "more open" represents in this study. Source: Sarah Stow.

In the case of cell wall shape, many species descriptions in floras stated whether species had weak or strong shapes (e.g. weakly nodulose; strongly sinuose) and so these were included as states resulting in seven states for cell wall shape (see Table 3.6, p. 106).

3.3.1.3.2 Sporophyte traits

Spores

Spore size was recorded as a continuous variable, and as spore size for a species is usually given as a range, the minimum and maximum was recorded and then the mean calculated from this. This yielded three continuous variables for spore size: minimum size in range, maximum size in range and mean spore size. Each of these three continuous variables was then also categorised into

"small" and "large", yielding an additional three categorical variables (Figure 3.3). The delimitation of small and large was based on the categories delimited by During (1992): small <20 µm and large ≥20 µm. For species that did not have a range of sizes, there is only one continuous variable and was recorded as the mean spore size.

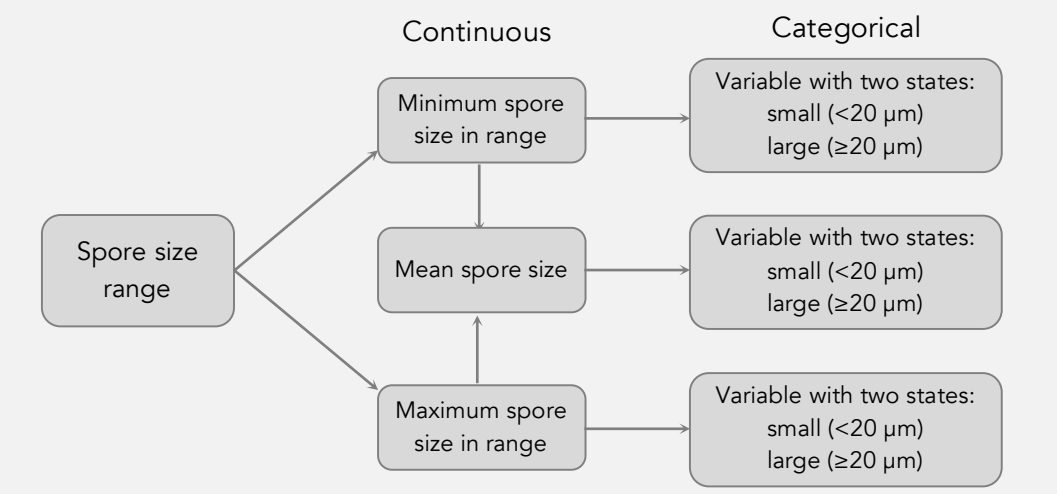


Figure 3.3 Categorisation of spore size from three continuous variables resulting in a further three categorical variables with two states each.

Capsule

Capsule shape has been simplified to 3 categories, though a variety exist (see Figure 3.20 p. 163, Appendix A3.3). Capsule orientation states, similarly to leaf orientation, were assigned a numerical value (Table 3.4) due to the existence of intermediate states (for full list of states see Table 3.27, p. 173, Appendix A3.5). Capsule exertence (how far above the perichaetial leaf the capsule is held) was categorised as immersed, emergent and exerted, Figure 3.5. Although the state "immersed" is part of the capsule exertence trait, it is also used as a state in the capsule orientation trait. Although immersed capsules have an "erect" orientation they are surrounded by the plant leaves and so interact differently with the environment than emergent and exerted erect capsules

Table 3.4 Numerical values assigned to capsule orientation states.

Orientation	Value assigned
immersed	0
erect	1
erect-inclined	1.5
inclined	2
horizontal	3
horizontal-pendulous	3.5
pendulous	4

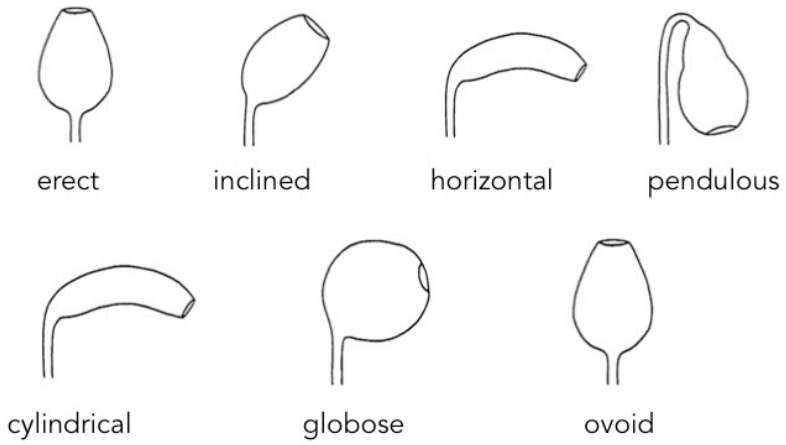


Figure 3.4 Capsule orientation (top) and shape (bottom) used in this study. Taken and adapted from Casas et al. (2006), fig. C, p. 331.

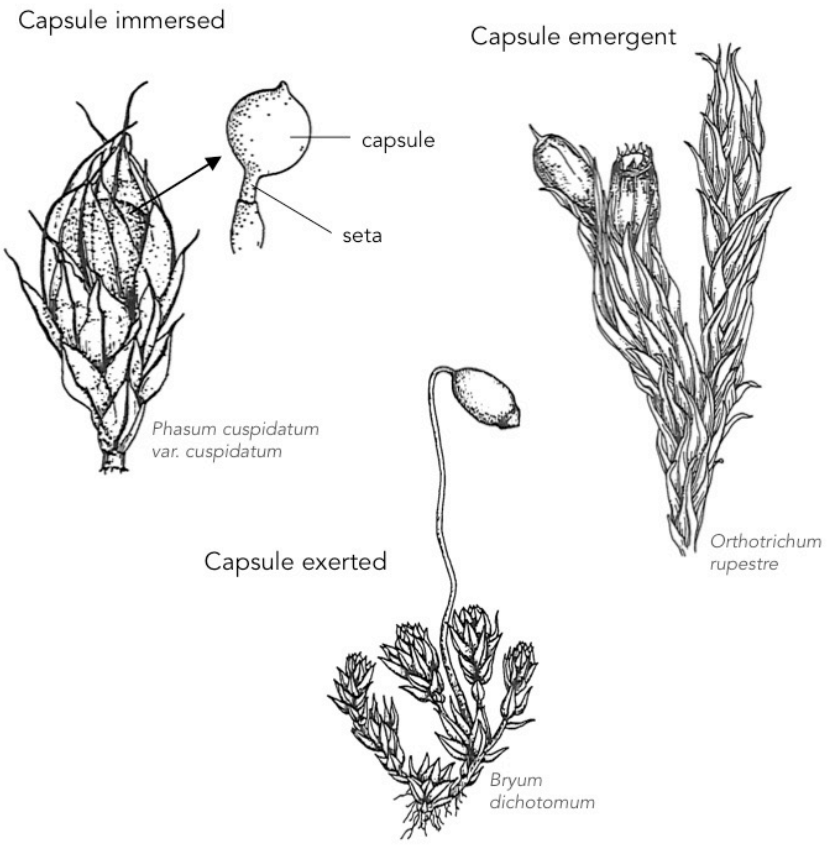


Figure 3.5 The three types of capsule exertence used in this study. Taken and adapted from Casas et al. 2006.

3.3.1.3.3 Life-history traits

Reproductive system

Studies that have looked at the trait of reproductive system have used a classification system with more than just two states: synoicous, paroicous, autoicous, and dioicous (e.g. Crawford et al.,

2009; Manyanga et al., 2011). However, in this study reproductive system was scored simply as monoicous, dioicous or both. Although this reduces information, previous studies have shown that this is an adequate grouping (e.g. Söderström & During, 2005; Kraichak, 2012).

Life strategy

Life-strategy was taken from Dierßen (2001) and the Portuguese Red Data Book (Sérgio et al., 2013). The life strategy categories detailed in Bates (2009) (based on During, 1992) were used in this study, and the categories used in Dierßen (2001) were re-categorised into the former (Table 3.5). Spores are considered "large" when they are greater than 20µm in diameter (Bates, 2009).

Table 3.5 Re-categorisation of Dierßen (2001) life strategy categories into Bates's (2009) categories (Table 8.2, p. 327, based on During, 1992).

Dierßen life-strategy	Life strategy used in this study	Determining characteristics		
		Life span	Spores	Reproductive effort
f	Fugitive	<1 year	Many small	High
c, ce, cp	Colonist	A few years	Many small	Medium
p, pc, ps	Perennial stayers	Many years	Many small	Low
a	Annual shuttle	<1 year	Few large	High
s, g	Medium shuttle	A few years	Few large	Medium
l, d	Dominant	Many years	Few large	Low

3.3.1.3.4 Summary

All traits were qualitative (categorical) except for plant size, underleaf size, spore size, capsule length and capsule width, which were all quantitative (continuous). Qualitative trait states were grouped into a limited number of categories in order to allow patterns to be seen as a large number of categories with narrow value ranges could prevent this e.g. life-form (Godefroid et al., 2014). Some quantitative traits (plant size, underleaf size and spore size) were also recorded as categorical variables. In some cases categorisation has been shown to yield significant relationships where quantitative values do not (e.g. spore size and sexual system (Crawford et al., 2009). When creating categories from numerical values attention was paid to not affect the relative weight each value of a trait may have in subsequent analyses (Wiens, 2001) e.g. spore size. As the nature of this work is not taxonomic, it was deemed appropriate to group similar trait states into the same category for analyses. It was shown by Hedenäs (2001) that this is a valid approach as traits with a high variability can lead to ambiguity in subsequent analyses. However, in the database the trait state was still recorded so that the information can be used in future for other purposes (e.g. taxonomic analyses and broader statistical analysis). Recording the full variability of a trait also means that this variation can be used as an explanatory factor in analyses if outliers or unexpected results occur. Although studies using plant trait data tend to use continuous variables (Díaz et al., 2016), the availability of this type of data is limited in bryophyte morphology and therefore most traits are categorical (ordinal and nominal). Previous bryophyte studies have used categorical variables and shown that it is a valid approach that yields meaningful results (Hedenäs et al., 2001).

Table 3.6 Gametophyte, sporophyte and life-history traits used in this study; the type of variable each trait is; the total number of species that had data for that trait; and the states or units (in the case of continuous traits) for each trait. States ordered by magnitude in the case of categorical ordinal traits. CatN – categorical nominal; CatO – categorical ordinal; Con – continuous. § Indicates liverwort-only trait or state; † indicates moss-only trait or state.

Trait	Variable type	Species number	Trait state or measurement unit		
Gametophyte traits					
Life-form	CatN	1155	Aquatic trailing (attached to substrate)		
			Cushion (dome-shaped colonies)		
			Dendroid (with stolons and erect shoots)		
			Fan (branches in plane on vertical substrate)		
			Pendant (creeping stems on twigs with long secondary stems)		
			Mat, rough (creeping, lateral branches erect)		
			Mat, smooth (creeping, branches lying flat)		
			Mat, thalloid (creeping, thalli forming a layer)		
			Turf (vertical stems with little or no branching)		
			Turf, protonemal (persistent protonema)		
			Turf, scattered (scattered vertical shoots)		
			Tuft (loose cushions, not dome-shaped)		
			Weft (intertwining branched layers)		
Plant colour	CatN	833	green, yellow, brown, red, purple, black, white		
Plant colour intensity	CatO		pale, medium, dark		
Plant colour number	CatO		total number of colours		
Plant shine	CatO	785	0 – none	1 – some shine	2 –shiny
Plant size	Con	860	centimetres		
Plant size category	CatO		minute	0.1-0.5 cm	
			small	0.51-1.5 cm	
			medium	1.51-4 cm	
			large	4.1-10 cm	
			robust	10.1-25 cm	

Trait	Variable type	Species number	Trait state or measurement unit		
Leaf orientation†	CatN	857	plane	erect	reflexed
Recorded wet and dry			appressed	erecto/patent	squarrose
			imbricate	patent	
			succubous§	incubbous§	
Leaf insertion§	CatN		oblique	horizontal	vertical
Transverse cross-section	CatN	825	plane	channelled	plicate
			concave	keel	
Longitudinal orientation	CatN	735	plane	secund	twisted
			flexuose	falcate	curved
			undulate	curled	
Leaf apex	CatN	982	round	hair-point – 200µm or more	
			acute	short hair-point - less than 200µm	
			apiculate	acuminate	cucullate
			lobed§	subulate	
Leaf surface	CatN	1007	smooth	papillose	scales§
Recorded in upper, middle, and basal regions			cilia	mamillose	
			hairs	prorate	
Papillose	CatN	1008	present	absent	
Papillosity level	CatO	993	0, 1, 2, 3		
			Based on papillae presence in upper, middle and basal regions		
Lamina thickness	CatN	963	unistratose	multistratose	thick cuticle§
			lamellae	wide nerve	partially bistratose
			bistratose		
Cell shape	CatN	985	undifferentiated	short	hyaline
Recorded in alar and basal regions			enlarged	elongate	enlarged & hyaline

Trait	Variable type	Species number	Trait state or measurement unit				
Cell wall shape	CatN	949	sinuose	sinuouse weak	nodulose	nodulose weak	
Upper, middle and basal			porose	porose weak	straight		
Cell wall thickness	CatO	1006	thin	medium	thick		
Leaf marginal cell shape	CatN	882	undifferentiated	smaller	opaque		
			enlarged	elongate	hyaline		
			thickened	narrow			
Leaf margin denticulation	CatN	864	entire	dentate	papillose		
			denticulate	crenulate-papillose			
Leaf margin curvature	CatN	820	plane	recurved	revolute		
				incurved	involute		
Leaf border	CatN	1288	present	absent			
Distinct alar region†	CatN	985	present	absent			
Leaf decurrence	CatO	867	0 – none	1 – short	2 – long		
Costa number	CatO	962	none, single, double				
Costa termination (length)	CatO	962	none, lower third, middle, upper third, apex or beyond				
Underleaves§	CatN	394	present	absent			
Underleaves size	CatO	129	minute, small, medium, large				
Water storage structures	CatN	1025	none	hyalocyst	enalarged cells	hydroid	hyaline cells
			sac§	leucocyst	sheathing base	petiolate	conduplicate
Oil bodies§	CatN	144	present	absent			
Oil bodies per cell	CatO	68	number per cell				
Oil body longevity	CatO	36	rapidly fugacious, fugacious, persistent				
Trigones§	CatN	74	present	absent			
Trigone size	CatO	74	minute, small, medium, large				
Vegetative propagules	CatN	904	present	absent	present/absent		

Trait	Variable type	Species number	Trait state or measurement unit		
Vegetative propagule type	CatN	873	gemmae	leaves	tubers
			bulbils	branches	
Number of types of vegetative propagules	CatO	873	total number of types		
Sporophyte traits					
Spore size	Con	783	diameter (µm) – minimum, maximum and mean		
Spore surface	CatN	93	smooth	papillose	verruca
			pilum	granular	
Seta length	Con	562	from base to capsule neck (mm)		
Capsule size	Con	436	from neck to tip (mm)		
	Con		widest part (mm)		
Capsule orientation	CatN	557	sub-erect	horizontal	inclined
			erect	pendulous	
Capsule shape	CatN		cylindrical	ovoid	globose
Capsule exertence	CatN	547	immersed	emergent	exerted
Stomata	CatN	290	present	absent	
Peristome	CatN	207	present	absent	
Life-history traits					
Reproduction system	CatN	973	Monoicous	Dioicous	Both
Life-strategy	CatN	737	Fugitive	Perennial stayers	Medium shuttle
			Colonist	Annual shuttle	Dominant

3.3.1.4 Recording and categorising environmental variables

Humidity, light, temperature and pH were taken from Dierßen (2001) who provides a classification for all European bryophytes (see Table 3.7 to Table 3.10). A numerical value was assigned to each humidity, light and temperature class to be able to apply statistical analyses and create an environmental index further on. Values range from 1 for the most humid, sheltered and cold to high values for the driest (9), most exposed (6) and hottest (7) – see Table 3.7, Table 3.8 and Table 3.10 for values assigned to humidity, light and temperature classes, respectively. Dierßen's environmental classes were used as it gives the range of ecological conditions a species occupies (e.g. hygrophyte to moderate xerophyte), whereas Ellenberg values (commonly used in plant studies) give only the most typical ecological conditions (e.g. on moist soils). As the aim is to select indicator species and as bryophytes have a high phenotypic plasticity it is important to record their ecological niche across their range, and not just in one part of it (Dierßen, 2001). If no data from Dierßen (2001) were available, then the value was assigned based on literature, herbarium specimens and expert knowledge, but only if these had sufficiently detailed information.

Table 3.7 Humidity classes in Dierßen (2001) and values assigned in this study to each class. e – extremely; h – highly; c – considerately; m – moderately.

Humidity class	Value	Humidity class definition
Rheophyte	1	in (fast) flowing water bodies
Limnophyte	1	in standing water bodies
Amphiphyte	1	temporarily submerged
Hydrophyte	1	adapted to tolerate inundation
e hygrophytic	2	extremely wet
h hygrophytic	3	very wet
c hrygrophytic	4	considerably wet
m hygrophytic	5	moderately wet
Mesophyte	6	moderately wet to moderately dry
m xerophytic	7	moderately dry
c xerophytic	8	considerably dry
h xerophytic	9	very dry

Table 3.8 Light classes in Dierßen (2001) and values assigned in this study to each class. h – highly; c – considerately; m – moderately.

Light classes	Value	Light class definition
h sciophytic	1	adapted to minimum light supply (<1/300 of the day light)
c sciophytic	2	considerably adapted to shade (<1/50 of the day light)
m sciophytic	3	moderately adapted to shade
m photophytic	4	in moderately illuminated habitats
c photophytic	5	in considerably illuminated sites
h photophytic	6	growing in full light

Table 3.9 Temperature classes in Dierßen (2001) and values assigned in this study to each class. h – highly; c – considerately; m – moderately.

Temperature class	Value	Temperature class definition
h cryophytic	1	distinctly adapted to cold microsites
c cryophytic	2	adapted to considerably cold microsites
m cryophytic	3	adapted to moderately cold microsites
mesothermophytic	4	intermediate between cold and warm microsites
m thermophytic	5	living on moderately-heated microsites
c thermophytic	6	living on considerably-heated microsites
h thermophytic	7	living on well-heated microsites

Table 3.10 Acidity classes in Dierßen (2001) and respective pH values. h – highly; c – considerately; m – moderately.

Acidity class	Acidity class definition
e acidophytic	pH <3.3 Extremely acidic
h acidophytic	pH 3.4 - 4.0 Highly acidic
c acidophytic	pH 4.1 - 4.8 Considerably acidic
m acidophytic	pH 4.9 - 5.6 Moderately acidic
subneutrophyte	pH 5.7 - 7.0 Subneutral
basiophyte	pH > 7.0 Basic

Some species are found in only one class, but many are found in a range of classes (e.g. high sciophyte to moderate photophyte). For these, the value assigned was the average of the maximum and minimum categories; for example, a species with a light range of high sciophyte to moderate photophyte is given a value of 2.5: this is the average of moderate photophyte (4) and high sciophyte (1):

$$(4 + 1)/2 = 2.5$$

Species that were classified in the humidity class "mesophyte" have a value of 6, as a mesophyte is defined as living in moderately wet (5) to moderately dry (7) conditions (Table 3.11), which therefore results in an average value of 6. However, for species that inhabit a range of categories whose lower or upper limit is the class "mesophyte", the humidity value was calculated using the values 5 or 7, not 6. The humidity value of species whose lower value (i.e. wetter) class is mesophyte was calculated with a value of 5 (e.g. mesophyte to considerable xerophyte). The humidity value of species whose higher value (i.e. drier) class is mesophyte was calculated with a value of 7 (e.g. moderate hygrophyte to mesophyte). If this was not done, then misleading humidity values would be calculated for species and subtle ecological differences missed, as shown in the example in Table 3.11 below.

Table 3.11 The effect on a species' humidity value when using the value 6 for the mesophyte class. The taxon *Orthotrichum cupulatum* is a moderate hygrophyte to high xerophyte, and so occupies environments ranging from moderately wet to very dry. *Lophocolea minor* is classified as a mesophyte to considerable xerophyte and so occupies environments ranging from moderately wet to considerably dry. Therefore, *O. cupulatum* can inhabit slightly drier environments than *L. minor*. If the class "mesophyte" is represented by its mean value of 6 when calculating the humidity numerical value for *L. minor*, this slight difference in environmental preference is hidden; however, if the class "mesophyte" is represented by its lower value, i.e. 5, then this difference is evidenced in the numerical humidity value.

Taxon and humidity classification		Wettest class = wettest environment	Driest class = driest environment	Numerical humidity value
Orthotrichum cupulatum	moderate hygrophyte to high xerophyte	moderate hygrophyte = moderately wet	high xerophyte = **very** dry	
	class numerical value	5	9	7
Lophocolea minor	mesophyte to considerable xerophyte	mesophyte = moderately wet	considerable xerophyte = **considerably** dry	
	individual class numerical value	5	8	6
	individual class **NOT** adjusting for mesophyte	mesophyte = **moderately wet to moderately dry**	considerable xerophyte = considerably dry	
	individual class numerical value **NOT** adjusting for mesophyte	**6**	8	7

3.3.1.5 Physiological parameters

Data on physiological parameters (water relations and photosynthetic activity) was collated from the literature. Names were checked for synonymy prior to inclusion of a species. Two main problems can arise when using data from other experiments: methodologies vary and so values may not be comparable between species and the conditions of a species may differ from the typical conditions of the species in the region of study, as well as the effect of different *field effects* – the conditions a species has been exposed to in the field prior to measuring DT (Stark et al., 2014). As data does not exist for most of the species on the database, surrogates from the same genus were used where possible. A problem with this is that a surrogate, though of the same genus, may occupy a different microhabitat. However, in some cases it has been found that the parameters are not significantly different between two species of the same genus (Song et al., 2015). To overcome these problems, where methods differ is recorded and the microhabitat of the species (when mentioned in the study) is also recorded: both substrate and habitat. For example, *boulder under tree canopy* is recorded as "rock" and "forest". Parameters recorded were: relative water content (RWC), water potential, maximum photosynthetic efficiency (PPFD), and light compensation point. Data from plasmolysis experiments was not used as they are considered to overestimate DT in bryophytes (Proctor, 2009).

3.3.2 Statistics

3.3.2.1 Species environmental preferences

To assess the desiccation tolerance of a species, the humidity and light conditions it is found in were used. Although a quantitative measure of DT (e.g. photosynthetic recovery) would accurately measure DT, the aim is to find a method to determine DT without need for physiological experiments. pH was not included as acidity reflects the substrate a species lives on (rock, bark, soil), and not the ambient environmental conditions. Temperature was also not included due to the lower amount of data available and also the fact that where data was available it might not reflect the field conditions as data is based on laboratory measurements (Dießen, 2001).

An environmental index (EI) from 0.1 (humid and sheltered) to 1 (dry and exposed) was calculated using moisture and light values, as both moisture and light affect the DT of bryophytes. The EI was calculated using the simple formula:

$$EI = \left(\frac{h}{9} + \frac{l}{6}\right) / 2$$

Where h is the moisture value and l is the light value. In order to calculate an average, both values must be relatable and on the same scale so as humidity has 9 classes and light has 6, it was necessary to divide each value by its respective maximum class value. No weighting was assigned to either environmental variable.

3.3.2.2 Physiology and environmental preference

Spearman correlation was used to test for a correlation between physiological DT parameters and the EI. Spearman was used rather than Pearson because the physiological parameters had a non-normal distribution and due to the presence of extreme values (e.g. the average PPFD value was 513 µmol m^{-2} s^{-1} but there were two outliers above 1000 µmol m^{-2} s^{-1}).

3.3.2.3 Trait and environment analyses

The traits were first individually analysed (with analysis of variance, ANOVA) to maximise the information available because if analyses were only conducted on all traits together, species with information missing in just one trait would be removed from these analyses. These single-trait analyses were carried out to also identify which trait state groupings are the most appropriate for subsequent matrix analyses in traits that have many states (analyses in Chapter 4). Although grouping states could be done based on knowledge alone, statistical tests were also used (ANOVA model simplification and comparison) in order to statistically test for non-significant differences between states, indicating that they can indeed be grouped together. Analyses were first carried out on all species together and then on the Bryophyta and Marchantiophyta separately to see if there are differences between the two phyla (which would be expected as liverworts tend to be less DT than mosses (Proctor, 2009) and the two phyla have different morphologies). Hornworts were excluded from analyses as there were only six species and so insufficient data for reliable analyses. Additionally, as mentioned in Chapter 2, hornworts are not known to be DT, except for one species. However, they have been maintained in the trait database to allow future analysis, either for DT or for taxonomic work.

In order to test the assumption that taxa from Portugal can be used to inform traits of Malagasy species, the univariate analyses for certain traits and environment were repeated for Malagasy and Portuguese mosses independently.

Analysis of variance

Forty-six traits were categorical (thirty-two nominal and fourteen ordinal) and five traits continuous (see Table 3.6, p. 106). Significant differences in the mean EI value for each state within a trait were tested using one-way analysis of variance (one-way ANOVA). Although some traits consist of presence and absence, and therefore two-sample t-tests would ordinarily be conducted on these, for simplicity in terms of statistical procedure and presentation of results, ANOVAs were used as it yields the same results (the ANOVA test statistic *F* is the t-test statistic, *t*, squared) (Sokal & Rohlf, 1995; Crawley, 2013). Ordinal categorical variables were ordered prior to analysis (e.g. plant size was ordered as: minute, small, medium, large and robust). Light and moisture values (used to create the EI) were also tested, but results are only reported if they yielded significant results where the EI did not, or where they yielded different results to the EI.

One of the core assumptions of an ANOVA is that there is constancy of variance, homoscedasticity, and therefore this must be tested before carrying out the analysis (Crawley, 2013). Although ANOVA is considered a robust analysis to small deviations from homoscedasticity, variance can be affected if there are very small sample numbers in a category (Quinn & Keough, 2002), particularly if the sample size is less than the number of levels in the factor (i.e. number of states in a trait), and if the study is highly unbalanced (Crawley, 2013). It was therefore particularly important to test for in this study due to the different sample numbers in the states of a trait; Levene's test was used in this study, a commonly used test that is robust to non-normality compared to other heteroscedasticity tests (e.g. Bartlett or Fligner-Killeen) (Quinn & Keough, 2002). For traits that failed the heteroscedasticity test (indicated in the results section), an ANOVA with Welch's correction was used. The interaction between life-form and plant size was tested using a two-way ANOVA (Type III, as samples are unbalanced (Quinn & Keough, 2002)).

For traits where ANOVAs showed significant differences, significant differences between the mean EI of groups, light or moisture values were identified using post-host multiple comparison tests (α=0.05). Although the ideal procedure is to specify which groups to contrast a priori, planned comparisons (Sokal & Rohlf, 1995; Crawley, 2005; Ruxton & Beauchamp, 2008), it is unknown how most traits relate to the EI and so unplanned comparisons between all groups were carried out (also known as a posteriori or multiple comparisons). To reduce the risk of increased Type I errors associated with multiple comparisons, the Games-Howell post-hoc test was used as it allows comparisons between groups with very different sample sizes, as is the case for many traits in this study (Quinn & Keough, 2002; Ruxton & Beauchamp, 2008). For the two-way ANOVA (life from and plant size) multiple contrasts of the least-square means were used as a post-hoc test as least-square means are adjusted for unbalanced samples (Quinn & Keough, 2002).

Grouping states via model simplification

In traits that had many states (e.g. colour), grouping of states was undertaken based on the difference in their mean EI and this difference being non-significant. Grouping states together was undertaken via model simplification using ANOVA to find the minimum adequate model and check for power lost with model simplification; i.e. comparing the ANOVA model with all states

and the ANOVA model with states grouped into new categories and checking the significance value and the number of degrees of freedom gained.

Normality of errors

Normality of errors was checked by inspecting diagnostic plots of the ANOVA models and undertaking a Shapiro-Wilk test of the model residuals. Although there were very small departures from normality for some traits, ANOVAs are robust to small deviations from normality, providing the variances are equal (Sokal & Rohlf, 1995; Crawley, 2013). No transformation of the EI was required for ANOVAs as it follows a normal distribution, for both mosses and liverworts (see Figure 3.22, p. 174 in Appendix A3.6). Although the Shapiro-Wilk test for non-normality indicates that both are non-normal (p<0.05), it is a very small deviation from normality: the slight positive skew in the moss histogram (0.026) is not significant (p=0.401); the slight negative (-0.258) and the slight platykurtosis (-0.442) in the liverwort histogram are also not significant (p=0.921 and p=0.887, respectively).

The car (Fox & Weisberg, 2011), multcomp (Hothorn et al., 2008), lsmeans (Lenth, 2016) and FSA (Ogle, 2016) packages in the statistical software R (version 3.3.2; R Development Core Team, 2016) were used.

3.4 Results

Six hundred and eighty seven species had data for all the morphological and reproduction traits (66% of all species). 80% of Portuguese species had data for all gametophyte traits and 57% had all sporophyte traits. This number is much lower for Malagasy species; 28% of Malagasy species had data for all gametophyte traits and only 15% had data for all sporophyte traits. As predicted, data availability is lower for sporophytic traits. Data completeness for gametophytic traits was on average 63% (minimum 9% for oil body longevity, maximum 100% for underleaves) and 49% for reproductive and sporophytic traits (minimum 9% for spore surface and capsule orientation, maximum 76% for spore size); see Figure 3.21, p. 169, Appendix A3.5. For Portuguese species the number traits missing per species was very low (an average of 93.9% completeness, lowest completion rate 75%, and highest 100%) but for the Malagasy species it was high, with 80 species having no trait data at all (all of them liverworts).

3.4.1 EI and physiological parameters

The EI is significantly correlated with both relative water content (Spearman correlation= -0.51, p<0.01) and photosynthetic recovery (Spearman correlation= 0.48, p<0.001) i.e. the higher the EI the better the photosynthetic recovery of a species therefore the more DT a species is.

3.4.2 Traits and environmental preferences

Analyses were carried out only for species with accurate environmental conditions available, meaning that a maximum of 730 taxa were available for the analyses relating traits to the environmental variables. This number varied between traits as there was not 100% completion for all traits (as mentioned above). Although significant differences were found in many traits when looking at mosses and liverworts combined, analyses carried out on liverworts alone did not yield significant results for most traits and so only results for mosses only are graphed below. The

number of species available per trait and summary statistics for ANOVAs are shown in Table 3.17, p. 136.

3.4.2.1 Gametophyte traits

Plant colour

As there was a high number of total colour states (39), and some were only represented by one species (see Table 3.24, p. 170, Appendix A3.5), no significant differences could be detected between different colour types ($F_{38,497}$=1.428, p>0.05). Therefore, states were grouped into 3 colour categories only: plants with only green colouring, plants with green and other colours, and plants with no green colouring (see Table 3.24, p. 170 for groupings). The mean EI was significantly different between the three groups (Figure 3.6 a); species with no green occupy significantly drier and more exposed environments (0.77 ±0.04SE) than both species with only green (0.59 ±0.02SE, p<0.001) and species that contain green and other colours (0.67 ±0.01SE, p<0.01) ($F_{2,5341}$=12.69, p<0.001).

When looking just at the number of colours present in a species, species with a larger diversity of colours occupy drier and more exposed habitats (Figure 3.6 b), though the only significant difference is between species with only one colour and those with two or three, and not between other colour numbers ($F_{1,531}$=13.15, p<0.001) likely due to the small sample sizes of these. Species with one colour have the lowest mean EI (0.060 ±0.02SE). The model can therefore be simplified to two groupings: whether species have only one colour or if they have more (no significant difference was found between the two ANOVA models (p=0.916) and 4 degrees of freedom were saved). It should be noted that the standard error for 4, 5 and 6 colours is large due to the small number of species in these categories.

There is no significant difference in species that are shiny or have some shine (scored as 2 and 1, respectively) appearance and so these two states were grouped into “present” and shine became a binary variable (Figure 3.6 c). Plants with a shiny appearance are found in significantly wetter and more sheltered environments (0.60 ±0.012, p<0.001).

Life-form

Nine species had more than one life-form and these were removed from analysis in order to be able to look at the effects of each life-form state on its own. This removal did not affect analysis power as they only made up 1.6% of the total species. When all twelve life-form categories are compared, the greatest differences in the EI are found between turfs and other life-forms, and tufts and other life forms (see Figure 3.23 and Table 3.28, p. 173). Following simplification of the initial ANOVA model, life-forms were grouped into six main categories: cushions, tufts, turfs, mats (smooth and rough mats), open (wefts, fan, dendroid), and aquatic trailing (Figure 3.6 d). An even simpler model would group together cushion, tufts and turfs, but as cushions and tufts are more densely arranged than turfs, and cushions are denser than tufts, it was decided that the more accurate representation of the morphology and ecology is to keep these states separate. There was also no significant difference between the mean EI value of mats and open forms, but again, as they represent very different plant forms they are kept as separate categories. Overall, closed life-forms (cushions, turfs and tufts) occupy drier and more exposed habitats than mats and open life-forms (Figure 3.6 d). Aquatic trailing life-forms had the lowest mean EI (0.44 ±0.08 SE) as expected as they are all species that live on the surface of water or submerged.

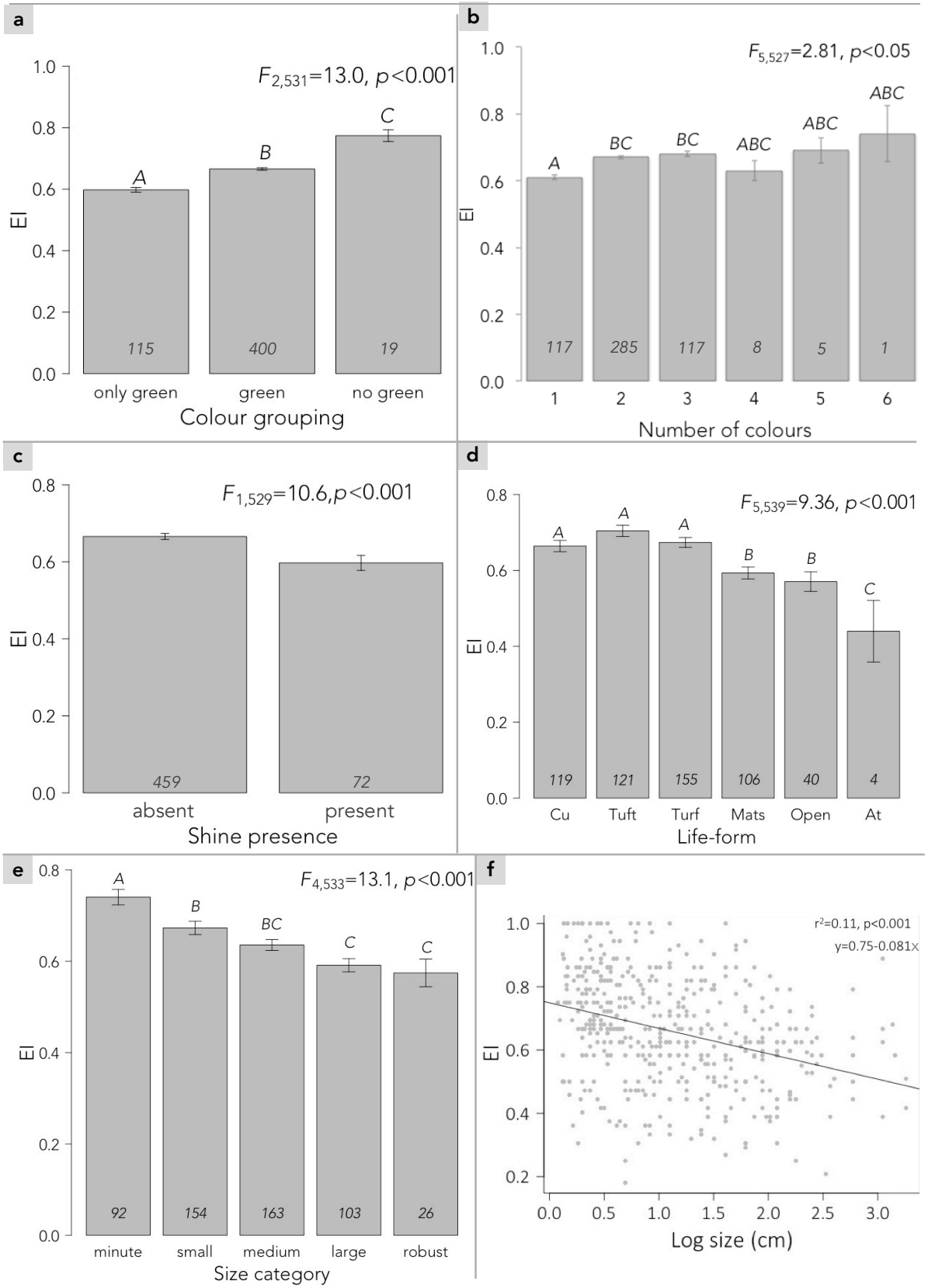


Figure 3.6 a) mean EI of species with different colour combinations (green refers to species with green and other colours); b) mean EI in different number of colours present; c) mean EI of species with or without shine; d) mean EI in the main life-form groups (Cu: cushion; Pe: pendant); e) mean EI in different size categories; f) linear regression of size in cm (size in cm was log transformed to meet normality assumptions). Sample sizes shown inside base of bars. Means with ±1SE and different letters indicate significant differences based on Games-Howell p<0.05.

Plant size

The mean EI is significantly different between different size categories; this was indicated by both the size categories and exact sizes (Figure 3.6 e & f) showing that the plant size categories did not mask differences in the EI. The smaller the plant the more likely it is to inhabit drier and more exposed habitats. There is no significant difference in the mean EI between the medium and the robust category, though the difference appears relatively large 0.06 (medium: 0.64 ±0.01SE and robust: 0.57 ±0.03SE), and this is likely due to a combination of small sample number and the presence of a 3 robust species that have a high EI (above 0.75): *Hypnum cupressiforme* var. *lacunosum, Racomitrium lanuginosum*, and *Squamidium brasiliense*. These were double-checked to ensure that the values were not erroneously recorded.

Life-form and plant size

Overall, minute life-forms occupy drier and more exposed habitats than other sizes but only within tufts and turfs was there a significant effect of size on the mean EI of a life-form (Table 3.12). Minute tufts are found in drier and more exposed habitats (0.82 ±0.03) than medium (0.67 ±0.02) or robust (0.30 ±0.09) tufts (p<0.05; Table 3.12). Medium sized open forms occupy wetter and more sheltered habitats than small and minute cushions, tufts and turfs as well as medium sized cushions and tufts. Size does not therefore have an overall effect on the environment occupied by life-forms (to see differences between all life-form sizes see Figure 3.24, p. 176, in Appendix A3.6).

Table 3.12 Differences in mean EI (±1SE) of life-forms with different sizes. Highlighted in bold are life-forms wuthin which size had a significant effect on mean EI. Two-way ANOVA with Tukey comparison test on least-sqaure means, p<0.05.

Life-form size comparison	Difference in mean EI	1 SE	df	t value	*p*
minute,Tuft - medium,Tuft	0.158	0.04	512	3.99	<0.05
minute,Tuft - robust,Tuft	0.526	0.11	512	4.61	<0.01
minute,Tuft - medium,Turf	0.202	0.04	512	4.91	<0.001
minute,Tuft - large,Turf	0.251	0.05	512	5.56	<0.0001
minute,Tuft - small,Mats	0.283	0.05	512	5.913	<0.0001
minute,Tuft - medium,Mats	0.207	0.04	512	5.19	<0.001
minute,Tuft - large,Mats	0.245	0.04	512	5.801	<0.0001
minute,Tuft - medium,Open	0.425	0.07	512	5.992	<0.0001
minute,Tuft - large,Open	0.234	0.05	512	5.12	<0.001
minute,Tuft - robust,At	0.386	0.09	512	4.07	<0.05
small,Tuft - small,Mats	0.172	0.04	512	3.93	<0.05
small,Tuft - medium,Open	0.314	0.07	512	4.6	<0.01
medium,Tuft - medium,Open	0.267	0.07	512	3.98	<0.05
robust,Tuft - minute,Turf	-0.455	0.11	512	-4.07	<0.05
minute,Turf - large,Turf	0.181	0.04	512	4.55	<0.01
minute,Turf - small,Mats	0.213	0.04	512	4.97	<0.001
minute,Turf - medium,Mats	0.137	0.03	512	4.06	<0.05
minute,Turf - large,Mats	0.175	0.04	512	4.8	<0.001
minute,Turf - medium,Open	0.354	0.07	512	5.25	<0.001
minute,Turf - large,Open	0.163	0.04	512	4.06	<0.05
small,Turf - medium,Open	0.300	0.07	512	4.46	<0.01

Life-form size comparison	Difference in mean EI	1 SE	df	t value	*p*
small,Cushion - minute,Tuft	-0.153	0.04	512	-3.97	<0.05
small,Cushion - medium,Open	0.272	0.07	512	4.09	<0.05
medium,Cushion - medium,Open	0.276	0.07	512	4.01	<0.05
large,Cushion - minute,Tuft	-0.238	0.06	512	-4.07	<0.05

Leaf orientation

Twenty-seven states were recorded for dry leaf orientation and thirty-two for wet leaf orientation meaning that, similarly with colour, there were states with very few species and so an *a priori* grouping of the states was necessary. Each state was numerically coded (see Table 3.25 and Table 3.26, p. 171, Appendix A3.5) and then grouped according to this value resulting in 8 categories (1, 1.5, 2, 2.5, 3, 3.5, 4, and 5). Species with appressed or imbricate leaves when dry occupy drier and more exposed habitats than species with more open leaf orientations (*Welch's* $F_{6,137.9}$=6.452, p<0.001), with the greatest difference being between species with appressed leaves and those with erecto/patent to patent leaves (the EI is 0.16 greater in appressed leaves, p<0.001). There was no significant difference between the mean EI of other orientation types. As the mean EI of species with patent and spreading leaves was the same (0.58 ±0.04SE) these two states were grouped into one category. Although there was no significant difference in the mean EI of species with appressed leaves and those with appressed to erect leaves, they were maintained as separate groups due to significant different EIs between the latter group and species with patent or spreading leaves. The final grouping consists of seven categories for dry leaf orientation (Figure 3.7 a). Leaf orientation when wet does not seem to have an effect on species' environmental preferences ($F_{7,514}$=1.368, p=0.217).

Each of the seven category groupings from above were assigned a number (1 to 7), for both leaf and wet leaf orientation, and then used to quantify the difference in leaf orientation between hydrated and dry. A negative value indicates that a species' leaves close when hydrated and there were few species in which this is the case (n=18, 4.4%). Many species (n=122, 30%) exhibit no change in their leaf orientation when hydrated although the majority of species (n=269, 66%) do open out their leaves when hydrated. Overall, species with leaves that open out more when hydrated occupy drier and more exposed environments Figure 3.7 c). Species with leaves that open out completely when hydrated (6) occupy significantly drier and more exposed envrionments that species with leaf orientations that do not change (0) or only open out slightly (1 to 3) when hydrated (Figure 3.7 c; p<0.05). Species with leaves that close slightly when wet (-1) occupy significantly wetter and more sheltered environments (0.58 ±0.04SE) than species with leaves that open out completely when hydrated (0.77 ±0.03SE, p<0.001).

Apex

As expected, species with hair-points occupy drier and more exposed habitats than those with any other apex type (except cucullate and subulate apices) ($F_{6,534}$=16.6, p<0.001) (Figure 3.7 b). Species with cucullate apices occupy the driest and most exposed environments (0.79 ±0.05), significantly more so than species with acute, acuminate and rounded apices; the latter three

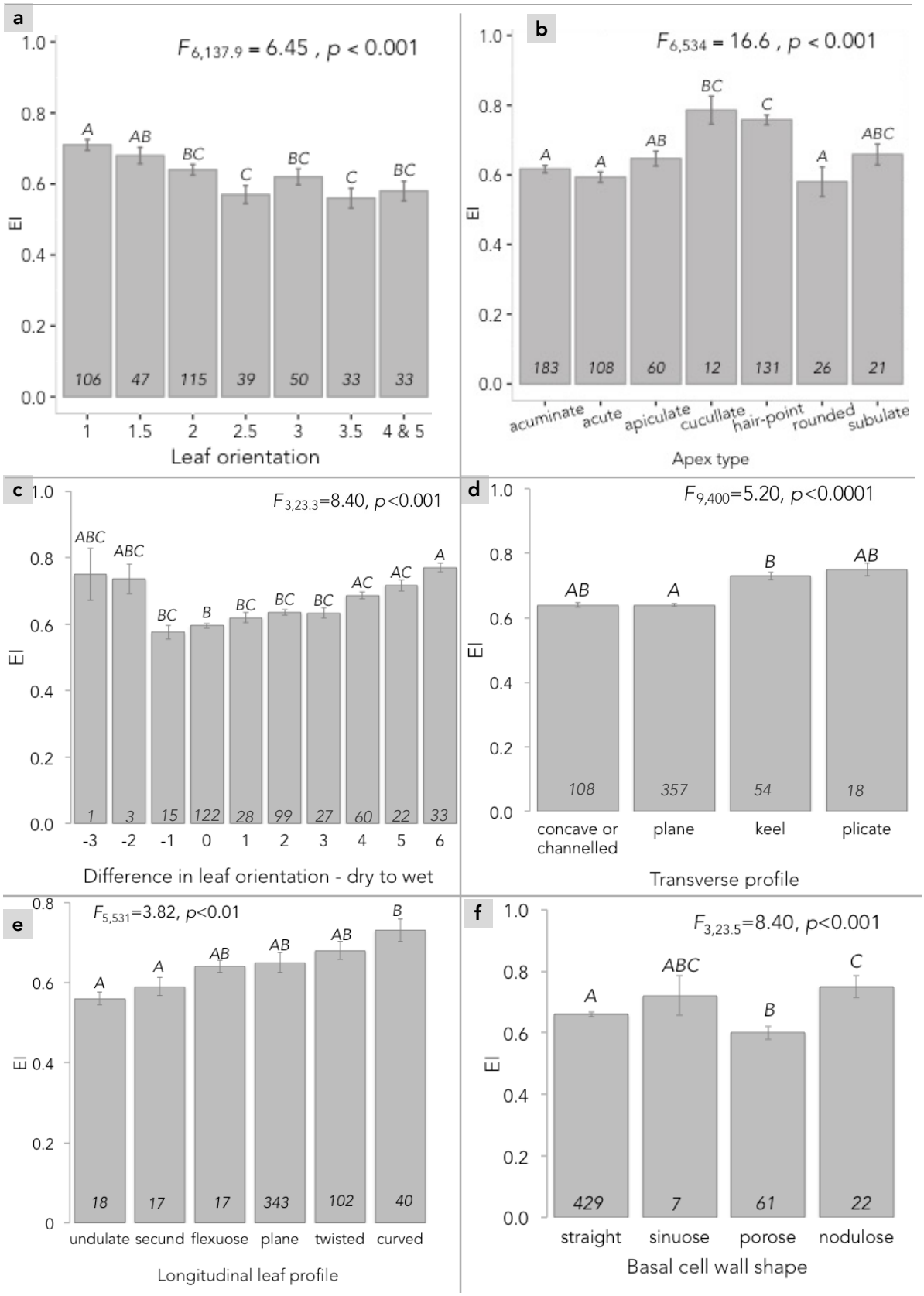


Figure 3.7 Mean EI (±1SE) in: a) leaf orientation when dry (1: appressed or imbricate; 1.5: appressed to erect; 2: erect; 2.5: appressed or imbricate to patent; 3: erecto/patent; 3.5: patent; 4 & 5: spreading or squarrose); b) leaf apex types; c) change from dry to wet leaf orientation (negative numbers indicate leaves close upon hydration; 0 indicates no change); d) transverse leaf profiles; e) longitudinal leaf profiles; f) basal cell wall shapes. Sample sizes shown at base of bars. Means with different letters are significantly different; ANOVA and Games-Howell *p*<0.05.

apex types occupying the wettest and most sheltered environments of all apex types (Figure 3.7 b). No significant difference was found in the mean EI between species with short (0.74 ±0.04) or long hair-points (0.78 ±0.02; p=0.90) so these two categories were grouped for subsequent analyses.

Leaf profiles

Species with a plane transverse profile occupy drier and more exposed habitats than species that have keeled leaves (0.63 ±0.01SE and 0.73 ±0.02SE, respectively, p<0.01) (Figure 3.7 d). Conversely, when looking at the longitudinal profile, species with a plane profile do not occupy different environmental conditions than species with other profile types (p>0.05, Figure 3.7 e). However, species that have flexuose or secund leaves occupy habitats that are significantly wetter and more sheltered than those with curved leaves (0.56 ±0.03 and 0.59 ±0.01 vs 0.73 ±0.03, respectively, p<0.01).

Cell wall

Suprisingly, cell wall thickeness had no effect on the environmental preference of species ($F_{2,538}$= 1.851, p=0.172; Table 3.17) and nor did the cell wall shape of the upper and mid cells ($F_{3,26.9}$=0.541, p=0.659; Table 3.17). However, species with nodulose basal cell walls occupy drier and more exposed environments (0.75 ±0.04SE) than species with straight or porose cell walls (0.66 ±0.01SE and 0.60 ±0.01SE, respectively, p<0.01). Species with porose cell walls occupy the wettest and most sheltered environments (Figure 3.7 f).

Whether the deviation from straight basal cell walls was weak or strong had no effect on the mean EI ($F_{2,516}$=0.454, p=0.635) and so these two states were grouped into a single state in subsequent analyses: porose, nodulose and sinuose.

Lamina

Species with pluristratose or subulate laminas seem to occupy drier and more exposed environments (Figure 3.8 a) but this difference is not significant ($F_{3,537}$=0.858, p=0.463). Species with lamellae inhabit drier and more exposed environments than those without ($F_{1,539}$=7.99, p<0.001) Figure 3.8 b).

Leaf surface

As expected, species with papillae occupy drier and more exposed environments than those without (0.69 ±0.03SE and 0.64 ±0.03SE, respectively, $F_{2,538}$=6.74, p<0.01; Figure 3.8 c). The more papillose the leaves of species the drier and more exposed the environment it occupies (Figure 3.8 d) although the only significant difference is between species with no papillae and those with two-thirds of their lamina with papillae (0.63 ±0.01SE and 0.71 ±0.03SE respectively, p<0.0001). Curiously, species that have papillae throughout the length of their leaves occupy similar environmental conditions to those with no papillae (a difference of only 0.01 ±0.05SE in EI) although the lack of statistical significance (t=-0.32, p=0.98) does not allow definite conclusions to be made.

To determine if a finer level of cell protuberance has an effect on mean EI, differences between papillose, mamillose, and prorate (the latter two classed as non-papillose in the previous analysis) were tested. Species with papillose cells in either the upper (Figure 3.8 e) or mid lamina

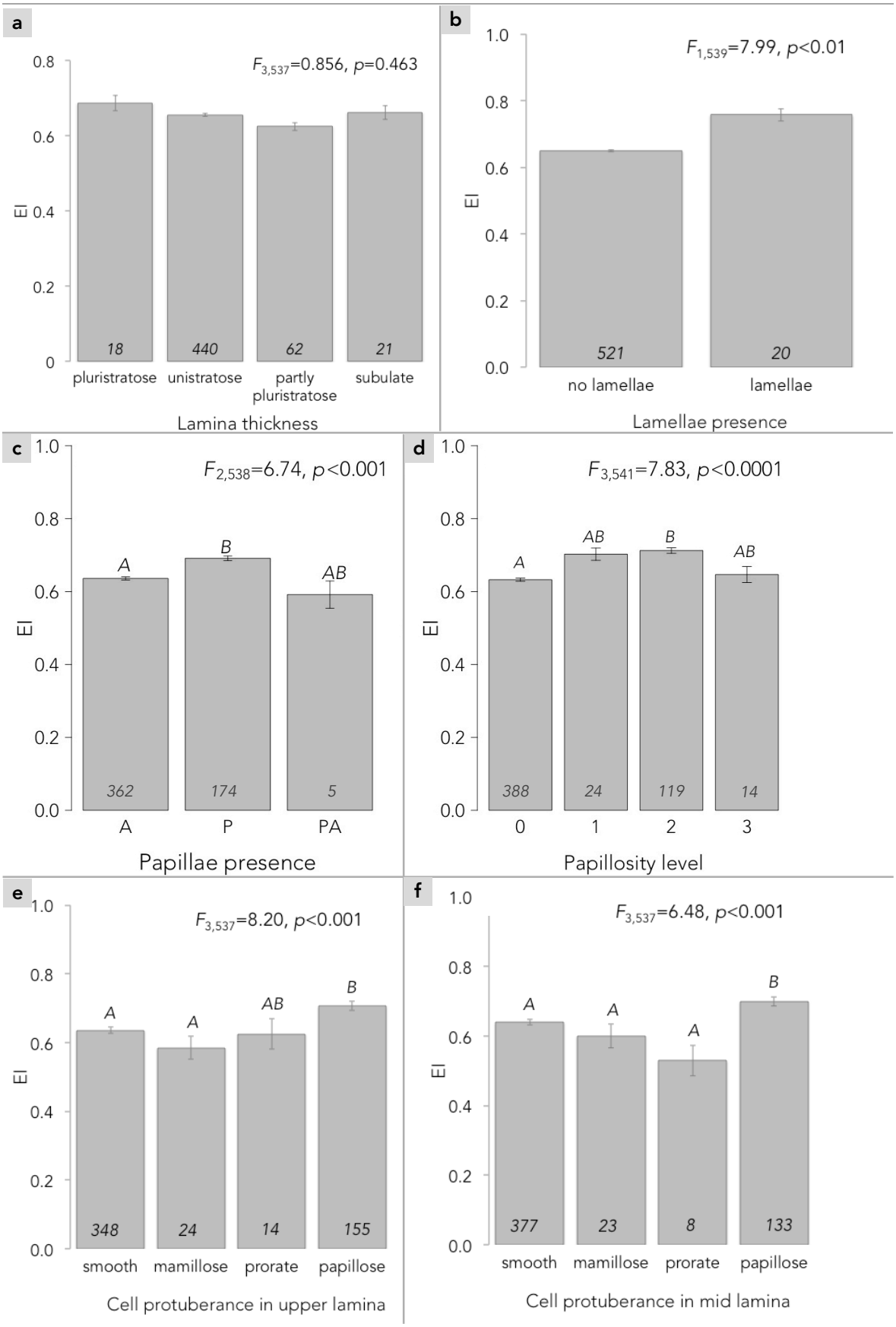


Figure 3.8 Mean EI (±1SE) in: a) different lamina thicknesses; b) species with and without lamellae; c) species with papillae (P), without papillae (A) and species where papillae may be present or absent in the species (AP); d) species with different levels of papillosity; e) species with different cell protuberances in the upper lamina; f) species with different cell protuberances in the mid lamina. Sample sizes shown at base of bars. Means with different letters are significantly different; ANOVA and Games-Howell *p*<0.05.

(Figure 3.8 f) again inhabit the driest and most exposed environments. Whether species had mamillose or smooth cells had no significant effect on the environment they occupy (a difference in mean EI of 0.05 ±0.04SE in both the upper and mid lamina, $p<0.05$). The same is true for species with prorate cells (Figure 3.8 f) indicating that the finer level of differentiation in cell protuberance does not determine a species' environmental preferences and so recording cell surface as merely being papillose or not is sufficient.

Leaf margins

Species with bordered margins occupy wetter and more shletered environments than those with no border (0.55 ±0.02SE, $p<0.001$; Figure 3.9 a). Although overall species with different margin cell shapes occupy different environments ($F_{16,521}=3.48$, $p<0.001$; Table 3.17, p. 136), only species that have bordered margins occupy significantly wetter and more sheltered environments (Table 3.13). Species with other cell margin shapes do not occupy different environments from each other which means the only margin morphology that affects the environment a species occupies is the presence of a border or not.

Table 3.13 Margin cell shapes that have significantly different mean EIs. Games-Howell post-hoc test , $p<0.05$.

Margin cell shape comparison	Difference in mean EI	t	df	*p*
bordered: bistratose	-0.38	9.80	20.4	<0.0001
bordered: thickened	-0.36	8.40	18.1	<0.0001
bordered: elongate	-0.35	8.20	15.6	<0.0001
bordered: smaller	-0.29	6.30	19.3	<0.001
bordered: narrow	-0.26	12.00	6.5	<0.001
bordered: partially bistratose	-0.31	7.30	12.9	<0.001
bordered: undifferentiated	-0.31	19.00	2	<0.05

There is no significant difference in the mean EI between species that have margin denticulation on part of their length (base, apex, lower half or upper half; $p>0.05$) so these states were grouped together. Similarly, there is no significant difference in the mean EI of species that are dentate or those that are denticulate (0.55 ±0.03SE and 0.58 ±0.02SE, respectively; $p=0.930$), so these two states were also grouped together. Three categories for denticulation are therefore used: entire, partial denticulation (species that are partly denticulate or dentate) and denticulation (denticulate or dentate from base to apex) (Figure 3.9 b). Species that have denticulation throughout their length are found in wetter and more sheltered environments than those that have no denticulation or those that have only partial denticulation (0.57 ±0.02SE vs. 0.70 ±0.01SE and 0.63 ±0.01SE, respectively; Figure 3.9 b).

A large number of margin curvature states were recorded (14 in total) but few significant differences could be found (Figure 3.9 c) and so grouping of states was undertaken (Figure 3.9 d). This grouping provided a better ANOVA model (saving 9 degrees of freedom) that showed more clearly the effect of margin curvature on species' environmental preferences. The more curved a species' margin the drier and more exposed environment it occupies (Figure 3.9 d). Species with involute and revolute margins are found in the driest and most exposed environments, whereas species with plane, partly incurved or recurved margins occupy wetter and more sheltered

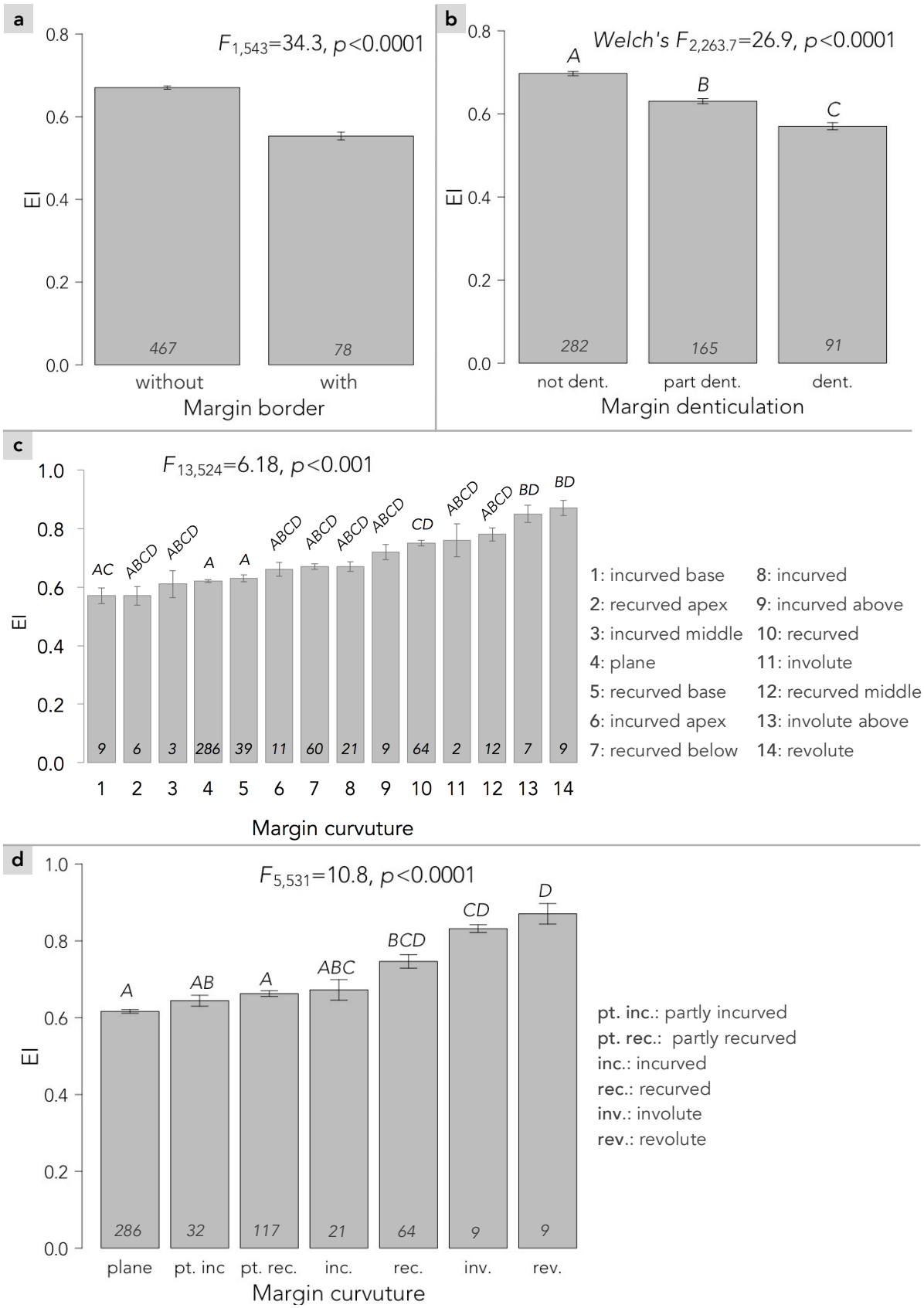


Figure 3.9 Mean EI (±1SE) in a) species without and with a margin border; b) species with no denticulation (not dent.), species with denticulation on part of their length (part dent.) and species with denticulation throughout their length (dent.); c) species with different margin curvatures; d) species with different margin curvatures following grouping of states into new categories. Sample sizes shown at base of bars. Means with different letters are significantly different: ANOVA and Games-Howell p<0.05.

environments (Figure 3.9 d). Species with recurved margins also occupy dry and exposed environments (0.75 ±0.02SE) compared to species with plane (0.62 ±0.01SE, p<0.001) or partly recurved margins (0.66 ±0.02SE, p<0.01).

Leaf decurrence

Species with longer leaf decurrence occupy wetter and more sheltered habitats (Figure 3.10 a) but there is no significant difference in the mean EI between short (1) and long (2) base decurrence (p=0.349)). Short and long base decurrence was therefore grouped into "present" and as there is a significant difference ($F_{2,535}$=6.43, p<0.01) in the mean EI of present and absence of leaf decurrence (and the simplified ANOVA model was not significantly worse, p>0.05) it was decided to score the decurrence trait as just present or absent in the matrix. This minimises the effects of subjectivity when classifying leaf base decurrence as short or long. Species with a decurrent base occupy wetter and more sheltered environments (0.60 ±0.02SE) than those without (0.67 ±0.01SE) (Figure 3.10 b). Species with sheathing bases seem to occupy drier and more exposed habitats than species with decurrent leaves (Figure 3.10 b) but this difference is not significantly different (difference in mean EI= 0.05, p=0.357). Although model simplification would suggest grouping these, sheathing bases are morphologically very different from decurrent bases so it was maintained as a separate trait state.

Basal region

Although species with a distinct alar region seem to occupy wetter and more sheltered environments than those without a differentiated alar region (0.63 ±0.02SE and 0.66 ±0.01SE, respectively) this difference was not significant ($F_{1,535=}$2.65, p=0.114; Figure 3.10 c). However, species with differentiated basal cells occupy slightly wetter and more sheltered environments than species with uniform cells throughout their leaves ($F_{1,531}$=11.5, p<0.001; Figure 3.10 d). When looking at the shape of basal cells, species with hyaline cells are found in the most dry and exposed environments (0.77 ±0.02SE), significantly more so than species with elongate, short or undifferentiated basal cells (p<0.001; Figure 3.10 e). In addition, no species with an EI below 0.4 have hyaline basal cells. Species with elongate cells are also found in drier and more exposed environments than those with undifferentiated basal cell shape (0.67 ±0.01SE and 0.62 ±0.01SE, respectively; Figure 3.10 e).

Costa

Species with a double costa are found in wetter and more sheltered environments (0.55 ±0.03SE) than species with no costa (0.64 ±0.02SE) or just a single costa (0.66 ±0.01SE) (Figure 3.11 a). The length of costa shows no clear pattern in relation to environmental preferences (Figure 3.11 b). Species with a costa that ends in the lower third of the leaf occupy the wettest and more sheltered habitats (Figure 3.11 b), but are only significantly lower than those whose costa extends to the apex (0.58 ±0.03SE and 0.66 ±0.01SE, respectively, p<0.001) (Figure 3.11 b). It therefore seems costa number rather than length has a greater effect on species' environmental preferences.

Water storage structures

Species with conduplicate leaves are found in significantly wetter and more sheltered environments (0.46 ±0.04SE) than species with no specialised water storage structures

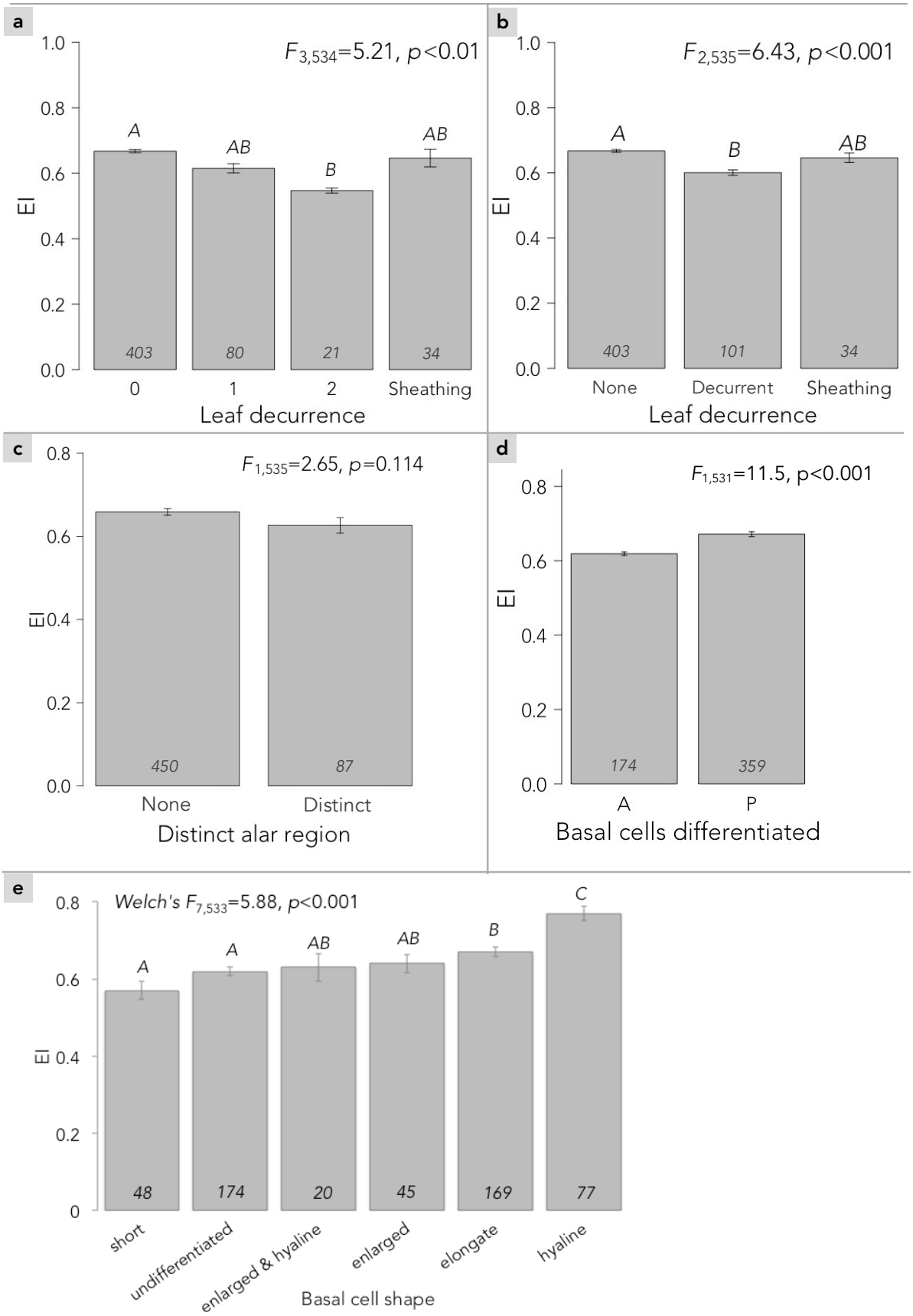


Figure 3.10 Mean EI (±1SE) in a) species with no decurrent base (0), with a short decurrent base (1), with a long decurrent base (2) and a sheathing base; b) species with or without a decurrent base and a sheathing base; c) species with a distinct alar region or not; d) species with or without basal cells differentiated from upper and mid laminal cells; e) different basal cell shapes. Sample sizes shown at base of bars. Means with different letters are significantly different: ANOVA and Games-Howell *p*<0.05.

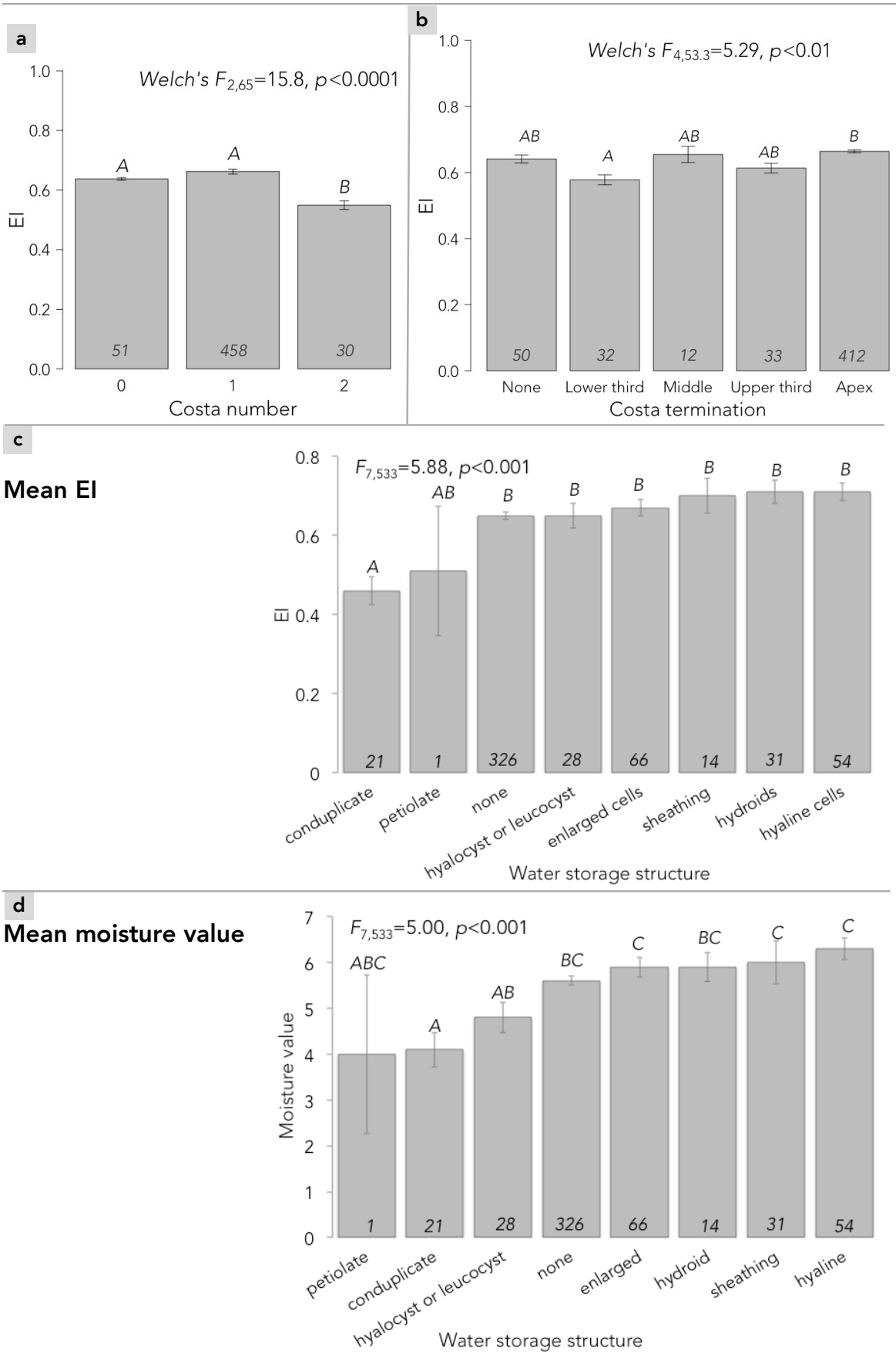


Figure 3.11 Mean EI (±1SE) in: a) species with no costa (0), with a single costa (1) and with a double costa (2); b) species with no costa, and species where the costa terminates in the lower third (including base), middle, upper third or apex; c) species with different water storage structures; d) mean moisture value (±1SE) in species with different water storage structures. Sample sizes shown at base of bars. Means with different letters are significantly different: ANOVA and Games-Howell p<0.05.

(0.65 ±0.01SE) as well as species with other types of structures (p<0.001; Figure 3.11 c). Suprisingly therefore, only conduplicate leaves as a water storage structure seem to affect a species'

environmental preferences. Only one species had petiolate leaves (a Malagasy epiphyte species, *Calymperes venezuelanum* (Mitt.) Pitt.) so although it seems to occupy a wetter and more sheltered environment (0.51 ±0.16SE) than species with no specialised water structures, it was not possible to make meaningful comparisons (p>0.05).

Because it is suprising that water storage structures seem to have no effect on a species' environmental preference, separate analyses were undertaken on light and moisture values individually (Table 3.19, p. 138). The same pattern was observed for mean light values (i.e. only species with conduplicate leaves inhabit significantly more sheltered environments), but water storage structures had a slightly different effect on species' moisture preferences (Figure 3.11 d). Species with hyalocysts or leucocysts prefer wetter environments (4.3 ±0.3SE) than species with hyaline cells (6.3 ±0.2SE), sheathing bases (6.0 ±0.5SE) or enlarged cells (5.9 ±0.2SE). Conduplicate species again occupy the wettest environment (4.1 ±0.4SE) followed by species with leucocysts or hyalocysts (4.8 ±0.03SE) (Figure 3.11 d). This indicates that water storage structures have a greater effect on species' moisture preferences than light preferences and that this is masked when using the EI.

When looking merely at the presence or absence of specialised water structures, no significant differences were found between the mean EI (0.66 ±0.01SE and 0.65 ±0.01SE, respectively, $F_{1,539}$=1.21, p=0.279; Table 3.17, p. 136), or between mean light or moisture values (Table 3.19, p. 138). Simply recording specialised water structures as present or absent does not therefore allow for the effect of water structures on species' environmental preferences to be seen.

Vegetative propagules

Species' environmental preferences were not significantly affected by either presence or absence of vegetative propagules ($F_{1,534}$=1.76, p=0.174) or by the number of vegetative propagules present ($F_{2,533}$=0.745, p=0.475). The same was true for light and moisture preferences individually (Table 3.19, p. 138). However, the type of vegetative propagules present did have an effect on species' environmental preferences (Figure 3.12 a). Species with tubers occupy the driest and most exposed environments (0.74 ±0.03), but only significantly more so than species with branches (0.61 ±0.04) or gemmae (0.62 ±0.02SE). Species with no vegetative propagules occupy significantly wetter and more sheltered environments (0.66 ±0.01SE) than species with tubers (p<0.05). Interestingly, when looking at the effect of propagules type on light and moisture preferences individually, there was no significant effect (Table 3.19, p. 138).

3.4.2.2 Sporophyte traits

The presence of stomata on the capsule had no effect on species' environmental preferences ($F_{1,207}$=3.48, p=0.064; Table 3.14) or on light preferences ($F_{1,207}$=0.007, p=0.934; Table 3.15) but species with stomata occupy wetter environments than those without (5.83 ±0.14SE and 4.74 ±0.36SE, respectively; Table 3.15).

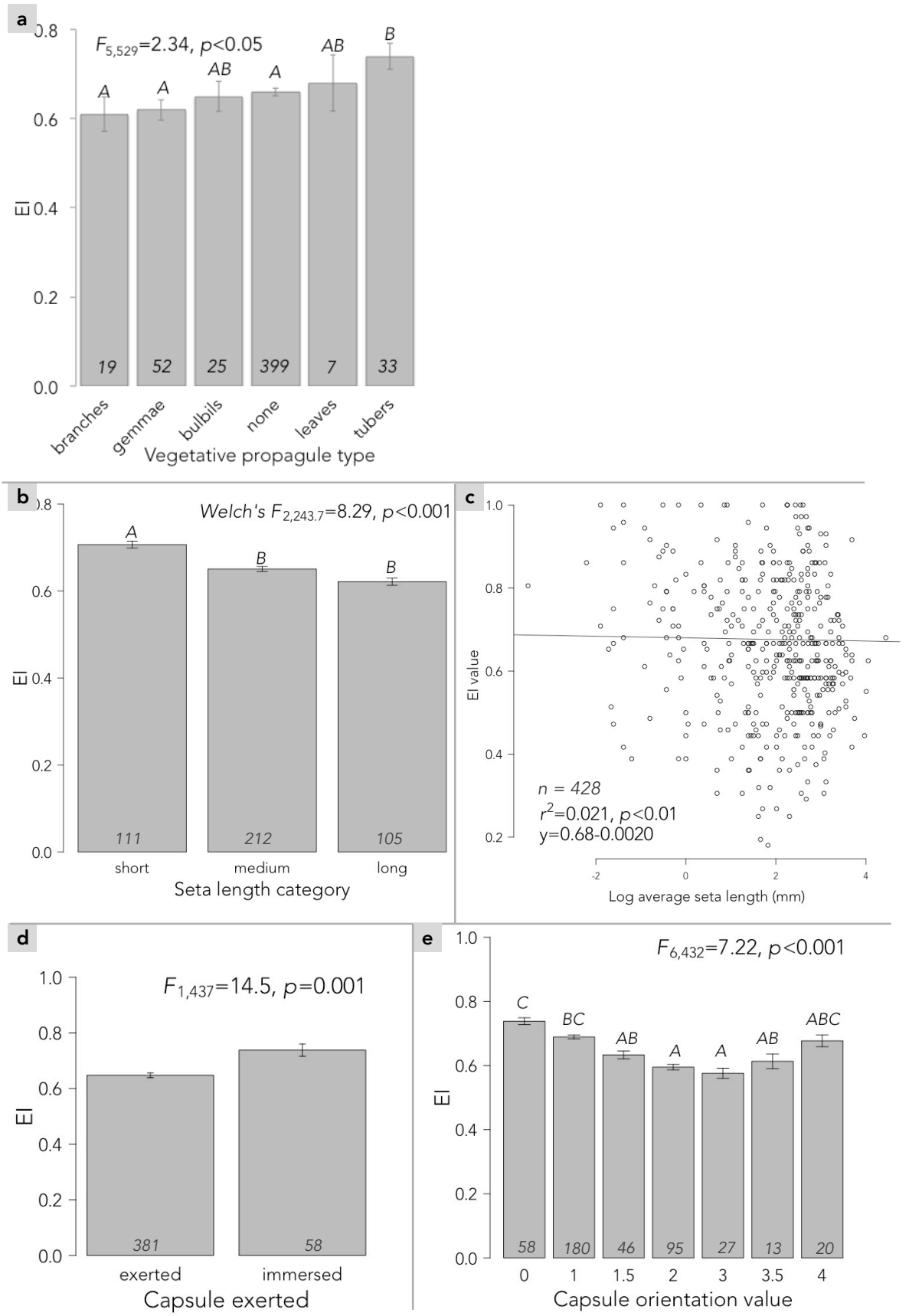


Figure 3.12 a) Mean EI (±1SE) in species with different types of vegetative propagules; b) mean EI (±1SE) in different seta length categories; c) continuous seta length in mm (log-transformed); d) mean EI (±1SE) in exerted and immersed capsules; e) mean EI (±1SE) in different capsule orientations (0: immersed; 1: erect; 1.5: erect-inclined; 2: inclined; 3: horizontal; 3.5: horizontal-pendulous; 4: pendulous). Sample sizes shown within bars. Means with different letters are significantly different: ANOVA and Games-Howell *p*<0.05.

Species with shorter setas occupy drier and more exposed environments (Figure 3.12 b and c). Although there was a significant effect of mean seta length on the EI (p<0.01) it was very small (r^2=0.021) and categorisation of seta length seemed to yield clearer results (Figure 3.12 b).

Species with immersed capsules occupy significantly drier and more exposed habitats than species whose capsules are exerted (0.74 ±0.02SE and 0.65 ±0.01SE, respectively) (Figure 3.12 d). There was no significant difference in mean EI between immersed and emergent so these were grouped into one category, called *immersed*, (0.73 ±0.03SE and 0.75 ±0.02SE, respectively, p=1.00). Capsule length did not have any effect on species' environmental, light or moisture preferences (Table 3.14 and Table 3.15).

When looking at capsule orientation, species with immersed capsules (value 0) are found in significantly drier and more exposed environments than species whose capsules range from inclined to sub-pendulous (values 2 to 3.5; Figure 3.12 e). Overall, as capsules approach a horizontal orientation (value 3; Figure 3.12 e) species are found in wetter and more sheltered environments although the only significant differences are between species with erect capsules (value 1; EI=0.69 ±0.01SE) and those that range from inclined to horizontal (values 2; EI=0.59 ±0.02SE and 3; EI=0.58 ±0.03SE, respectively).

Species with larger spores occupy significantly drier and more exposed environments although this relationship is not very strong (r^2= 0.002, p<0.05). When categorised into large and small, there is no significant effect of spore size on species' environmental preferences (p>0.05, see Table 3.14). However, there is a significant effect on mean light and moisture values as species with large spores occupy wetter but more exposed environments (Figure 3.13 and Table 3.16). This effect was found regardless of whether the spore categorisation was based on the minimum, maximum or mean range value.

Table 3.14 Summary statistics of moss sporophyte trait analyses (ANOVA) with the environmental index; significant effects in bold. ANOVAs with Welch's correction factor for heteroscedastic data indicated by §. Spore size category sample sizes differ due to some species not having a minimum and maximum spore size in the literature.

Trait	n	Test statistic		
Categorical		***F***	**df**	***p***
Stomata	209	3.48	1, 207	0.064
Capsule orientation	439	7.23	6, 432	**<0.001**
Capsule exertion	439	14.5	1, 437	**<0.001**
Peristome	69	0.332	1, 67	0.566
Seta length categorised	428	8.29	2, 243.7	**<0.001 §**
Spore size – categorised				
minimum	505	0.682	1, 503	0.409
maximum	497	0.618	1, 495	0.432
mean	506	0.054	1, 504	0.817
Continuous		r^2	**df**	***p***
Seta length – mean (mm)	428	0.021	1, 426	**<0.01**
Spore size – mean diameter (mm)	506	0.002	1, 504	**< 0.05**
Capsule length – mean length (mm)	343	-0.002	1, 341	0.618

Table 3.15 Summary statistics of moss sporophyte trait analyses (ANOVA) with light and moisture values; significant effects in bold. ANOVAs with Welch's correction factor for heteroscedastic data indicated by §. Spore size category sample sizes differ due to some species not having a minimum and maximum spore size in the literature.

		Light			Moisture		
	n	**F**	**df**	***p***	**F**	**df**	***p***
Stomata	208	0.007	1, 207	0.9341	5.50	1, 32.5	**<0.05§**
Capsule orientation	439	10.8	6, 432	**<0.001**	14.5	6, 432	**<0.001**
Capsule exertion	439	18.3	1, 437	**<0.001**	3.12	1, 437	0.078
Peristome	69	0.237	1, 67	0.628	0.147	1, 67	0.703
Seta length categorised	428	0.786	2, 425	0.456	2.59	2, 425	0.076
Spore size categorised							
minimum	505	11.4	1, 166.2	**<0.001§**	4	1, 503	**<0.05**
maximum	497	5.89	1, 495.0	**<0.05§**	15.6	1, 437.8	**<0.001§**
mean	506	9.48	1, 312.6	**<0.01§**	12	1, 250.6	**<0.001§**

Table 3.16 Mean light and moisture value (±1SE) in small (<20 µm) and large (≥20 µm) spore size categories; significant effects in bold. ANOVA p<0.05; § indicates ANOVA with Welch correction (due to heteroscedacity).

	Mean light or moisture per size ategory					
	Small	**1SE**	**Large**	**1SE**	F_{df}	***p***
Light						
Minimum range size	4.06	±0.06	4.51	±0.13	$11.4_{1,\ 166.2}$	**<0.001**
Maximum range size	4.01	±0.08	4.29	±0.09	$5.89_{1,\ 495.0}$	**<0.05§**
Mean size	4.04	±0.07	4.41	±0.11	$9.48_{1,\ 312.6}$	**<0.01§**
Moisture						
Minimum range size	5.72	±0.08	5.33	±0.18	$4_{1,\ 503}$	**<0.05§**
Maximum range size	5.92	±0.11	5.30	±0.12	$15.6_{1,\ 437.8}$	**<0.001§**
Mean size	5.81	±0.09	5.21	±0.14	$12_{1,\ 250.6}$	**<0.001§**

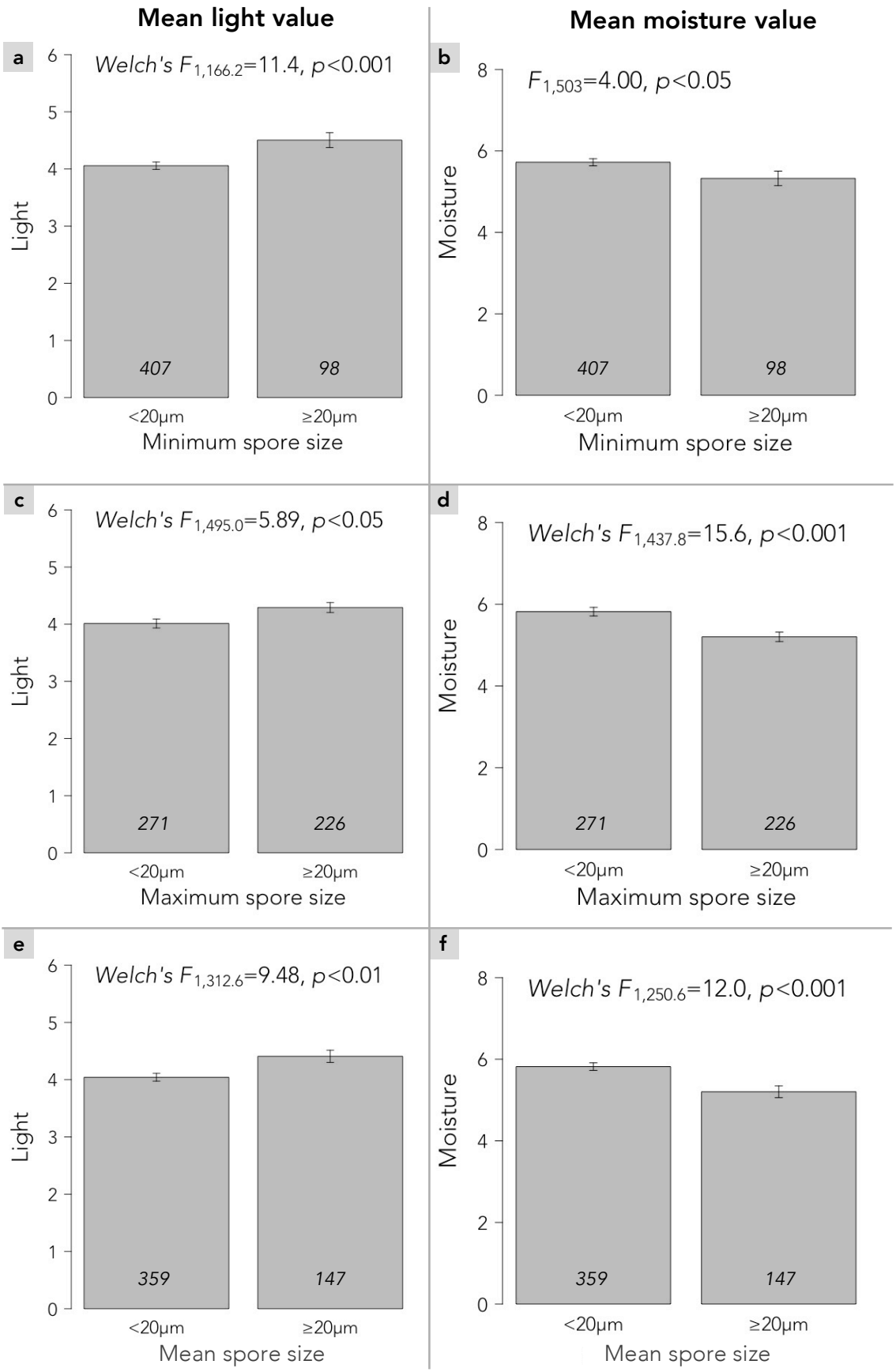


Figure 3.13 Mean light and moisture value (±1SE) of spore size categories: small (<20 µm) and large (≥20 µm). Sample sizes shown within base of bars. ANOVA, *p*<0. 05.

3.4.2.3 Life-history traits

Reproduction system - Monoicy or Dioicy

Although the ANOVA indicates that reproduction system affects a species' environmental preferences ($F_{2,532}$=3.47, p<0.05), with species that can be either monoicous or dioicous occupying wetter and more sheltered environments than others (0.59 ± 0.04SE), no significant differences between the different system types were found following post-hoc tests (Figure 3.14 a). This is due to the adjustment for small sample size that is undertaken in Games-Howell tests. There is also no significant effect on light preferences ($F_{2,532}$=1.53, p=0.218) but monoicous species are found in slightly drier environments than dioicous species (5.45 ±0.10SE and 5.94 ±0.12SE, respectively; Figure 3.14 b).

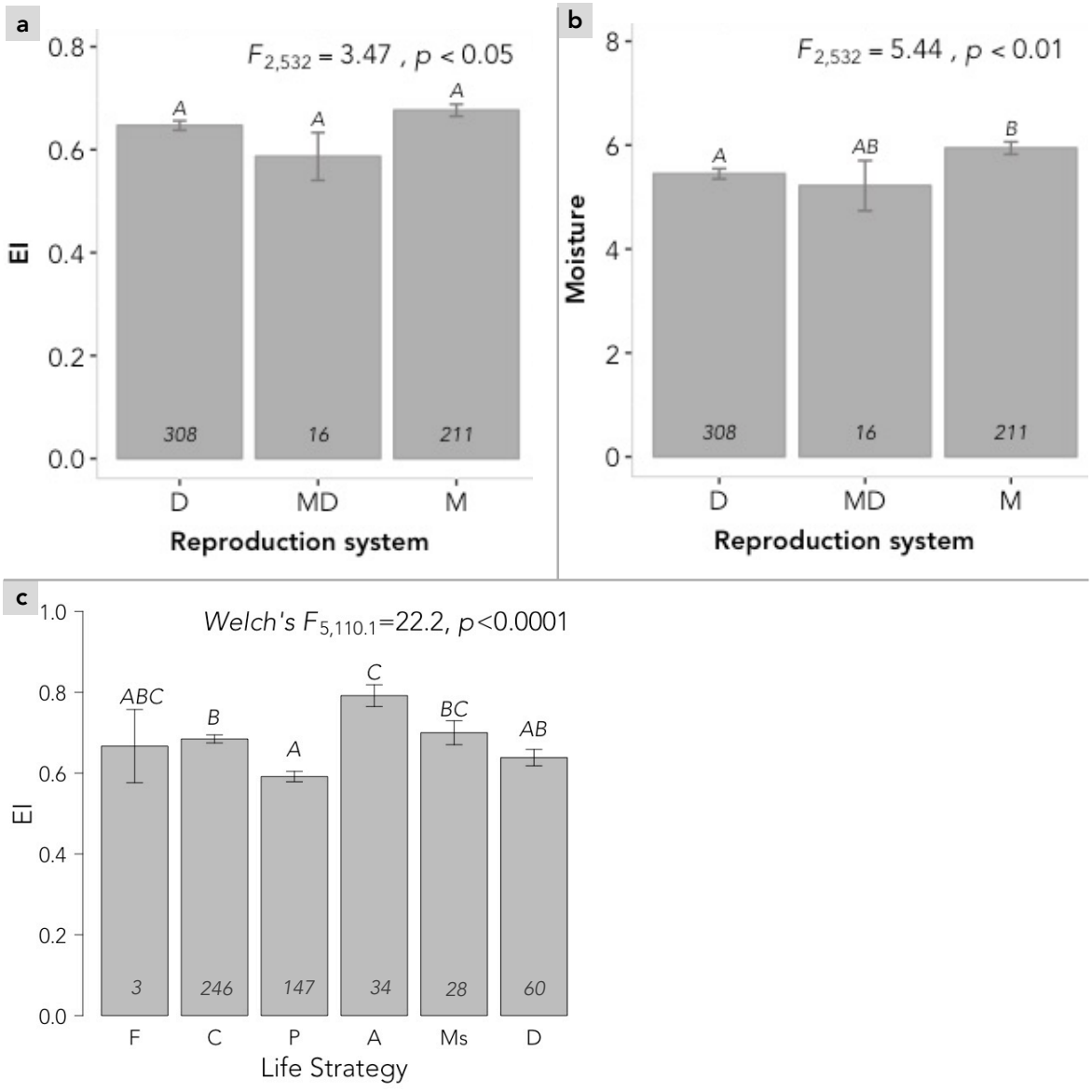


Figure 3.14 a) mean ±1SE moisture value in species with different reproduction strategies: D- dioicous; MD- monoicous and dioicous; M- monoicous; b) mean EI (±1SE) of species with different life-strategies. F- fugitive; C-colonist; P- pioneer; A- annual; Ms- medium shuttle; D- dominant. Sampe sizes shown within bars. Means with different letters are significantly different: ANOVA and Games-Howell p<0.05.

Life strategy

Annual species inhabit significantly drier and more exposed habitats (0.79 ±0.03SE) than colonist (0.68 ±0.01SE), perennial (0.59 ±0.01SE) and dominant species (0.64 ±0.02SE), with perennials

occupying more humid and sheltered environmental conditions than all other life-strategies (0.59 ±0.01SE, p<0.05) except dominant and fugitive species (due to non-significant differences).

3.4.2.4 Temperate vs. Tropical species

All but one of the traits tested in Malagasy and Portuguese species had the same effects on environmental preferences (Table 3.20, p. 139). Papillae presence was the only trait to respond differently in tropical taxa (Figure 3.15 and Table 3.20, p. 139), surprisingly, as species without papillae occupied drier and more exposed environments. This was not significant, however. Colour number and life-strategy also had no significant effect (Table 3.20, p. 139) although a similar pattern between the two regions occurred.

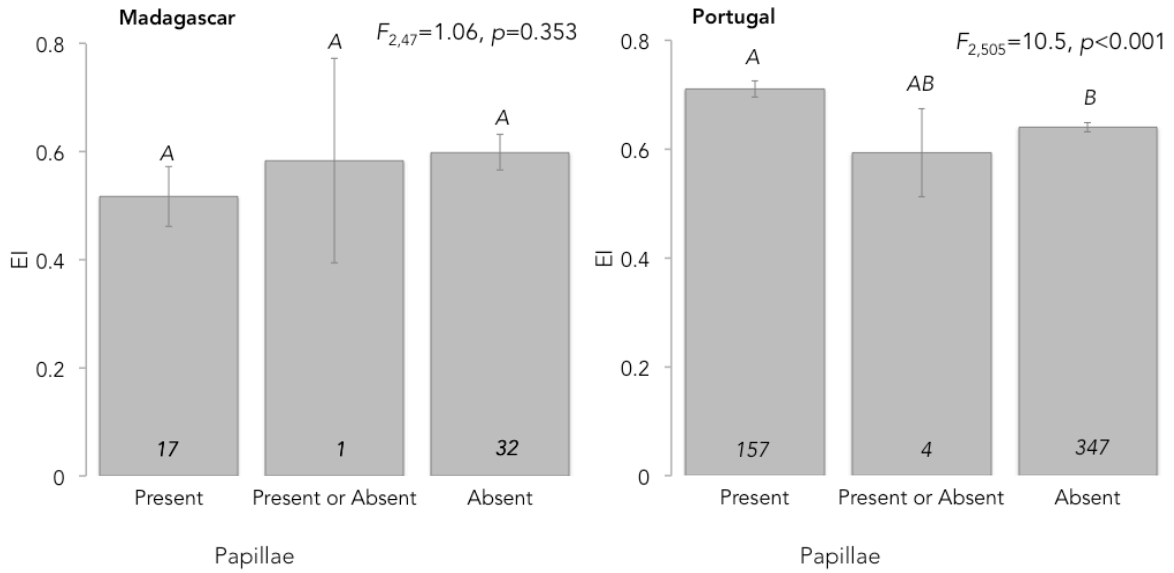


Figure 3.15 Mean EI (±1SE) in species with and without papillae in Malgasy and Portuguese species. Sample sizes shown at base of bars. Means with different letters are significantly different; ANOVA and Games-Howell p<0.05.

3.4.3 Summary

Most gametophyte traits recorded show an effect on the environments occupied by species, although this only applied to very few traits in liverworts (Table 3.17, p .136 and Table 3.18, p . 137). Species that have no green (i.e. species with yellow, orange, red or black) occupy drier and more exposed habitats than species with green in their colouration. Cushion and tuft life-forms, particularly minute ones (<0.51 cm), are found in the driest and most exposed environments, in contrast to open-forms (dendroid, weft and fan). Larger species tend to occupy wetter and more sheltered environments, both when using the exact size (cm) (Table 3.18, p. 137) and when using size classes. Leaf morphology had a significant effect on the environments occupied by species: species that had a smaller leaf area exposed (e.g. species with more closed leaves; recurved or revolute leaves) occupy drier and more exposed environments. Aditionally, species with leaves that have borders, dentation or papillae are also found in drier and more exposed environments. On the other hand, species with double costas, a shiny appearance and conduplicate leaves occupy wetter and more sheltered environments. Leaf cell shapes only had an effect on environmental preferences when the cells of the basal area are considered, with species that have hyaline or elongate basal cells found in drier and more exposed environments than those with short or undifferentiated cells. Suprisingly, cell wall thickness had no effect on species

environmental preferences but the shape of basal cell walls did: species with nodulose walls are found in drier and more exposed environments.

Because water storage structures are presumed to be closely related to a species' moisture preference, the effect of this trait on moisture and light individually was assessed. This interestingly reflected the results found when using the overall environmental preferences (EI): only species with conduplicate structures occupied wetter environments and more sheltered environments.

The type of vegetative propagules present in a species had an effect on environmental preferences, but not the number of different types of propagules nor merely the presence or absence. Species with tubers occupy drier and more exposed environments than species with no propagules or those with either gemmae or branches as propagules. Vegetative propagules traits had no effect on light and moisture individually (Table 3.19, p. 138)

Sporophyte traits showed a less strong effect on species' overall environmental preferences (Table 3.14, p. 131) although there was an effect on light and moisture individually (Table 3.15, p. 131). Species with short setas or immersed capsules, and species with erect capsules occupy drier and more exposed environments, both when looking at the overall environmental preferences (EI value) and light and moisture individually. Species with stomata in their capsules occupy wetter environments, but there was no effect on light preferences. Species with larger spores are found in overall drier and more exposed environments, although when looking at moisture individually, larger spores are found predominantly in species of wetter environments.

Life-history traits also had an effect on species' environmental preferences with diocious species found in wetter environments. Annual species occupy the driest and most exposed environments whereas perennial species occupy wetter and more sheltered environments.

Table 3.17 Summary statistics of single trait analyses (ANOVA) with the environmental index for all mosses and liverworts together, and for mosses and liverworts separately; significant results highlighted in bold. ANOVAs with Welch's correction factor for heteroscedastic data indicated by §.

	All				Mosses				Liverworts			
	n	*F*	*df*	*p*	*n*	*F*	*df*	*p*	*n*	*F*	*df*	*p*
Plant characters												
Life-form	714	40.2	4, 89.1	**<0.001 §**	539	11.2	4, 539	**<0.001**	169	0.273	4, 13.0	0.890 §
Colour	652	1.74	56, 595	**<0.01**	534	12.7	2, 531	**<0.001**	118	3.30	2, 115	**<0.05**
Colour number	651	1.85	6, 644	0.088	533	2.81	5, 527	**<0.05**	118	0.581	5, 112	0.715
Shine	599	16.5	1, 139.5	**<0.01 §**	531	10.6	1, 529	**<0.01**	67	1.294	3, 64	0.284
Size - categories	615	16.7	4, 610	**<0.001**	535	12.92	4, 530	**<0.001**	77	4.09	3, 73	**<0.01**
Leaf characters												
Leaf orientation - dry†	*na*	*na*	*na*	*na*	423	8.227	5, 94.1	**<0.001 §**	*na*	*na*	*na*	*na*
Leaf orientation – wet†	*na*	*na*	*na*	*na*	522	1.368	7, 514	0.217	*na*	*na*	*na*	*na*
Leaf orientation change - dry to wet†	*na*	*na*	*na*	*na*	410	8.69	5, 404	**<0.001**	*na*	*na*	*na*	*na*
Leaf transverse profile†	*na*	*na*	*na*	*na*	537	6.46	3, 533	**<0.001**	*na*	*na*	*na*	*na*
Leaf longitudinal profile†	*na*	*na*	*na*	*na*	537	3.82	5, 531	**<0.01**	*na*	*na*	*na*	*na*
Margin border†	*na*	*na*	*na*	*na*	544	5.86	1, 543	**<0.001**	*na*	*na*	*na*	*na*
Margin cell shape	635	2.40	33, 601	**<0.001**	538	3.48	16, 521	**<0.001**	97	1.93	14, 82	**<0.05**
Margin denticulation	607	6.54	4, 602	**<0.001 §**	538	27.0	2, 26.0	**<0.001 §**	69	1.73	4, 64	0.154
Margin curvature	604	4.01	29, 574	**<0.001**	538	10.8	6, 531	**<0.001**	66	2.72	4, 61	**<0.05**
Apex	644	4.97	29, 614	**<0.001**	541	16.6	6, 534	**<0.001**	103	1.22	21, 81	0.260
Nerve length	608	5.90	5, 57.4	**<0.001 §**	539	5.29	4, 53.3	**<0.01 §**	69	3.59	1, 67	0.063
Nerve number	608	19.0	2, 83.8	**<0.001 §**	536	4.96	3, 532	**<0.01**	69	3.59	1, 67	0.063
Papillae presence	641	10.6	2, 638	**<0.001**	541	6.74	2, 538	**<0.01**	100	0.56	2, 97	0.573
Papillosity level†	*na*	*na*	*na*	*na*	538	7.37	3, 534	**<0.001**	*na*	*na*	*na*	*na*

	All				Mosses				Liverworts			
	n	***F***	***df***	***p***	***n***	***F***	***df***	***p***	***n***	***F***	***df***	***p***
Basal cells differentiated	626	0.158	1, 621	0.691	531	14.82	1, 529	**<0.001**	95	2.70	1, 37.7	0.109
Leaf decurrence †	*na*	*na*	*na*	*na*	538	6.43	2, 535	**<0.01**	*na*	*na*	*na*	*na*
Basal cell shape	628	5.00	6, 18.7	**<0.01 §**	533	15.4	5, 116.3	**<0.001 §**	95	0.672	4, 90	0.613
Distinct alar region†	*na*	*na*	*na*	*na*	537	2.65	1, 535	0.104	*na*	*na*	*na*	*na*
Cell wall thickness†	*na*	*na*	*na*	*na*	541	1.77	2, 538	0.172	*na*	*na*	*na*	*na*
Upper & mid cell wall shape†	*na*	*na*	*na*	*na*	542	0.541	3, 26.9	0.659 §	*na*	*na*	*na*	*na*
Basal cell wall shape†	*na*	*na*	*na*	*na*	519	8.40	3, 23.3	**<0.001 §**	*na*	*na*	*na*	*na*
Water storage structures					541	5.88	7, 533	**<0.001**	185	9.87	2, 182	**<0.001**
Presence/absence					541	1.21	1, 539	0.279	185	18.2	1, 183	**<0.001**
Vegetative reproduction traits												
Vegetative propagule type					532	11.0	9, 10.4	**<0.001§**	183	14.2	4, 8.77	**<0.001§**
Presence/absence	702	2.37	1, 465.5	0.125 §	536	0.053	1, 534	0.818	183	4.77	1, 174.9	**<0.05**
Number					536	0.745	2,534	0.475	166			
Life-history traits												
Mono/dio	702	3.12	2, 699	<0.05	535	3.47	2, 532	**<0.05**	167	1.24	2, 164	0.293
Life strategy	701	36.3	3, 697	<0.001	518	18.9	3, 514	**<0.001**	183	0.81	4, 178	0.523

Table 3.18 Summary statistics of regression analysis of plant size (mean in cm) with the environmental index for all mosses and liverworts together, and for mosses and liverworts separately.

	All				Mosses				Liverworts			
	n	***r^2***	***df***	***p***	***n***	***r^2***	***df***	***p***	***n***	***r^2***	***df***	***p***
Plant characters												
Plant size – cm	547	-0.053	1, 545	**<0.001**	471	-0.081	1, 469	**<0.001**	76	-0.067	1, 74	**<0.001**

Table 3.19 Summary statistics of single trait analyses (ANOVA) with light and moisture for mosses; significant results highlighted in bold.

	n	Light			Moisture		
		F value	df	p	F value	df	p
Leaf characters							
Papillae cover	545	2.07	3, 541	0.103	11.4	2, 541	**<0.001**
Water storage structure type	541	4.37	7, 533	**<0.001**	5.00	7, 533	**<0.001**
Water storage structure PA	541	0.324	1, 449.7	0.569	1.35	1, 539	0.246
Vegetative propagule presence	536	0.428	1, 534	0.513	0.426	1, 534	0.514
Vegetative propagule number	536	0.72	2, 533	0.487	0.264	2, 533	0.768
Vegetative propagule type	532	1.18	9, 522	0.304	1.2	9, 522	0.294
Life-history traits							
Mono/dio	535	1.53	2, 532	0.218	5.44	2, 532	**<0.01**

Table 3.20 Summary statistics of single trait analyses (ANOVA) with the environmental index for Malagasy and Portuguese species separately.

	Madagascar				Portugal			
	n	***F***	***df***	***p***	***n***	***F***	***df***	***p***
Plant characters								
Life-form	50	5.62	3, 46	**<0.01**	509	10.4	5, 502	**<0.001**
Colour	44	3.89	2, 41	**<0.05**	507	11.6	2, 504	**<0.001**
Colour number	44	1.28	3, 39	0.296	507	2.57	5, 501	**<0.05**
Leaf characters								
Margin border†	50	6.55	1, 48	**<0.05**	508	25.9	1, 506	**<0.001**
Margin denticulation	50	2.65	4, 45	**<0.05**	508	2.74	4, 503	**<0.001**
Apex	50	2.89	5, 43	**<0.05**	508	8.63	6, 501	**<0.001**
Nerve number	48	3.98	2, 45	**<0.05**	508	5.35	2, 505	**<0.01**
Papillae presence	50	1.06	2, 47	0.353	508	0.56	2, 505	**<0.001**
Vegetative propagule type	50	4.12	8, 32	**<0.01**	508	2.03	9, 496	**<0.05**
Life-history traits								
Mono/dio	50	4.01	2, 47	**<0.05**	509	3.02	2, 506	**<0.05**
Life strategy	25	1.10	4, 20	0.383	506	12.3	5, 500	**<0.001**

3.5 Discussion

3.5.1 Environmental index and DT

As discussed in chapter 2, species with a higher photosynthetic recovery are more DT, and in this chapter photosynthetic recovery was found to be positively correlated with the EI. Therefore, the EI may be used as an indication of species' physiological performance and DT as the higher the EI the higher the DT of a species. Conversely, species with lower water contents, and therefore a higher DT level, have a higher EI; this correlation was less strong than photosynthetic recovery. Water content of bryophytes varies throughout the year (Dilks & Proctor, 1979) and so this will have an effect on the parameter comparison as measurements may have been made from plants collected at different times of year (Stark et al., 2014). It is important to note that most studies and measurements on bryophyte physiology are laboratory based, and so will not always accurately reflect the realities in the field as other factors come into play (Proctor, 2000a; Stark et al., 2014). When contextualising results of traits and environmental conditions this fact needs to be taken into account.

Although in most traits there was an effect on the EI, a few traits (papillae presence, reproduction system, stomata, and capsule exertion) only had an effect on either the species' humidity or light preferences. Because of this, subsequent analyses will also include humidity and light as individual variables, as well as the EI.

3.5.2 Mosses vs. liverworts

As no significant differences were found for most traits in liverworts, this shows the importance of taking into account taxonomy and ecology of target groups when carrying out large-scale analyses. Analyses where mosses and liverworts were analysed together showed significance, and if these results were then applied to all bryophytes it would be incorrect. However, it cannot be concluded that traits have no effect on the environmental preferences of liverworts. The non-significance is likely due to a combination of smaller sample size and the fact that there is a less variation in liverwort EI as a greater proportion of liverworts than mosses tend to be found in more humid and sheltered habitats (Vitt et al., 2014). Aditionally, many of the morphological traits chosen were more relevant to mosses, rather than liverworts for example: basal cell shape is highly variable in mosses but much less so in liverworts and the presence of a costa is absent in almost all liverwort species.

For a proper assessment of liverwort traits and environment, a selection of different traits may be needed (e.g. slime secretions or rhizomes (Vitt et al., 2014)), due to the differences between moss and liverwort morphology, as well as a larger sample size. Although some liverwort specific traits were included, oil bodies can be hard to observe in most species unless from fresh material, the likelihood therefore of underestimating oil body presence and number is high giving a large error for this trait. Continuing analyses using mosses only seems the most appropriate approach to minimise error. Although hornworts were not analysed due to the small number of species and lack of studies addressing DT in this phyla (Wood, 2007; Vitt et al., 2014), a separate study focussing solely on hornworts would be of merit as this group is understudied, not only in terms of DT but also their ecology.

3.5.3 Temperate and tropical taxa

Traits of Malagasy species had mostly the same effect on environmental preferences as results with Portuguese species. This therefore shows that different bryofloras can be used to inform upon each other, as suggested by other authors (e.g. Mateo et al., 2013). However, only 50 Malagasy species had complete trait data and environmental data, and so these analyses should be repeated once more trait data and environmental data are recorded for Malagasy species, to further confirm that traits from these two bryofloras respond similarly.

3.5.4 Morphological traits

Plant colour

It seems that the absence of green colour in a bryophyte can be used to indicate a species of dry and exposed environments whereas the presence of only green indicates species in humid and sheltered environments. Species with no green occupy drier and more exposed habitats as their mean EI is higher (0.77 ±0.06SE), as well as having a narrower environmental index range (0.6-1.0), whereas those with no other colour other than green are likely to be desiccation sensitive. Marschall and Proctor (2004) found that plants of more exposed habitats had high levels of chlorophyll, despite findings from a previous study Seel (1992a). Chlorophyll contents vary throughout the year (Glime, 2007). The error associated with the subjectiveness of categorising whether a plant is “shiny” or has ”some shine” is likely to explain the lack of significant difference in the mean EI.

Life form and plant size

Significant differences were found between more open life-forms and compact life-forms, as expected. The mean EI of aquatic trailing (At) species is indicative of their aquatic lifestyle and so they are not interacting with water in the same way as other species; they receive their moisture directly from water rather than from precipitation or water vapour. Althought morphologically they are an “open” life-form, ecologically they are very different and so were maintained in their own category.

No significant differences were found in the environmental preferences of cushion species of different sizes, but significant differences were found when looking at tufts and turfs of different sizes, which is what would be expected. This appears at first to be in contrast to previous findings where cushion size affects DT, however, Zotz et al. (2000) found that the relationship between size and recovery following rehydration is not clear-cut. Smaller cushions have lower surface:volume ratio meaning that they can metabolise for longer and so are more DT and so it would be expected that they inhabit drier and more exposed environments. However, larger cushions have a thicker boundary layer and lower evapotranspiration rates meaning they can remain hydrated for longer. Larger cushions also store more water per unit surface area (Zotz et al., 2000). However, photosynthetic and respiration rates are higher in smaller cushions but larger cushions can photosynthesize for longer following initial desiccation. Size therefore affects the physiology of bryophytes and must be taken into account when undertaking comparative studies (Zotz et al., 2000). Although size had some effect on the environment occupied by a life-form, the effect of life-form or size individually is greater. They are therefore maintained as separate traits in subsequent analyses.

Leaf orientation

There is no clear pattern between leaf orientation and the EI. Although species with appressed and erect leaves have a higher EI, once leaf orientation is patent to more open, there is no difference in environmental preferences. Species whose leaves open out once hydrated inhabit drier and more exposed habitats, as indicated by Stark et al. (2014). It was interesting that comparing the amount leaves' orientation changes between dry and wet yielded significant differences in the mean EI, with those that opened most occupying drier and more exposed habitats (these are the species whose leaves are the most closed when dry).

Another approach is to take the most-extreme state and ignore the other state(s) (i.e. if leaf orientation when dry is given as appressed to erect the appressed value is used) if leaf orientation when wet is appressed to erect the erect value is used.

Leaf profile

The lack of significant differences in mean EI between different longitudinal profiles is likely an effect of small sample size for some states as there did appear to be differences in the mean EI. The fact that no significant differences were found in the mean EI when profile was grouped into plane and not plane indicates that different types of deviation from plane have different effects on the environmental conditions preferred by the species. Could the curling or twisting of leaves also help retain water, either by physically creating a space or by reducing evaporative surface water loss? Also, plications sometimes only appear in species when they are dry (Glime, 2015a) so some level of uncertainty could have been introduced if a species' description in the literature only took into account profile in the hydrated state (though one of the assumptions is that species descriptions are based on the dry state).

Leaf apex

Species with hair-points and cucullate apices occupy drier and more exposed habitats than species with other apex types. This supports other studies that have indicated that hair-points can reduce the effects of desiccation on the plant by either creating an albedo effect or capture water vapour by creating condensation points (Proctor, 2009).

Leaf surface

The presence of papillae indicates that species are more likely to inhabit dry and exposed environments, except in species with papillae from base to apex cells. However, this was not found to be significant and could be due to the fact that only 14 species were assigned to this category. Similarly, only five species were classed as having papillae being present or absent explaining the non-significant difference.

There was no significant effect of lamina thickness on species' environmental preferences, in contrast to what was found by Vitt et al. (2014). However, the presence of lamellae is present in species that occupy drier and more exposed habitats, which are all species of the Polytrichaceae family (Proctor, 2005).

Leaf decurrence

Plants with sheathing bases did not occupy significantly drier and exposed environments than those with or without decurrent bases, and this is unexpected. It is widely established that

sheathing bases in mosses provide capillary space for water conduction and that these species therefore can occupy drier environments (Proctor et al., 1998). In fact, sheathing species did have a higher EI than other decurrence types, and the lack of significance is due to the low number of species that had sheathing bases (21) giving a large standard error.

Margin

Certain margin morphologies were significantly associated with species of dry and exposed environments: bordered margin, dentate margin, and curved margins. The shape of margin cells had no effect on species' environmental preferences, however. Margin borders and curvature play a role in capillary water transport by creating capillary spaces, as found by many authors (e.g. Tixier & Guého, 1997; Vitt et al., 2014) allowing any available water to be efficiently and quickly taken up by the bryophyte. Aditionally, margins that are revolute or involute can also provide physical protection to the leaf from excessive insolation (Glime, 2015a) or water storage. The presence of dentation could be related to photosynthetic activity (as found in a study on vascular plants; Royer & Wilf, 2006 *in* Glime, 2015b) by providing gas exchange sites that are free of water.

Nerve length

The length of nerve only showed a significant effect on environmental preferences if the nerve ended in the lower third of the leaf. The lack of significant difference could indicate that nerve length is not important for determining DT, but may be more for structural support, as suggested by (Vitt et al., 2014).

Basal cell shape

Basal cell shape had a significant effect onspecies' environmental preferences and this can be explained by basal cells acting as a storage for water (Vitt et al., 2014), especially as it was found that species with large hyaline basal cells occupy drier and more exposed habitats.

Cell wall

The lack of significant effect on environmental preferences among different cell wall thickness is unexpected as species of harsher environments tend to have thicker cell walls (Proctor, 2009; Vitt et al., 2014). However, some studies similarly found no relation between cell wall thickness and habitat dryness (Glime, 2015a). This result could be indicative that there are other traits that are more important than cell wall thickness in determining the habitat a species occupies and is supported by Proctor (1982) stating that species of dry habitats with papillae that have efficient ectohydric conduction actually have thin cell walls. On the other hand, the lack of effect could be due to the subjectiveness of the categories used. This could have been confounded by the fact that species may have thick cell walls in the upper cells, but thin cell wall in basal cells. The states thin, medium and thick are not exact measurements and so perhaps a better metric of cell wall thickness is cell wall to lumen ratio (Waite & Sack, 2010). Although not feasible within this study, as it requires observing specimens (wall:lumen ratio is not a characteristic used in floras), it would be feasible in a study focussing on a smaller number of species as it is an easily observable and quantifiable trait.

The small difference in mean EI and lack of significance in cell wall shape could be due to the small sample sizes in the weak states (less than 10). Although the sinuose state had no significant effect

on the mean EI, it was maintained as its own category because the sample size was small (12) and so no reliable conclusion can be made.

Vegetation propagules

Surprisingly, vegetative propagules had no significant effect on species' environmental preferences. Perhaps a better method to classify propagules would be into size as the size of propagules is a strategy to survive habitat characteristics (Rydin, 2009). However, aside from a few species where propagules size is a diagnostic characteristic in identification, there is very little data available on this. A more in-depth study, focussing on specific taxa (genera or families) could involve measuring the propagules of various specimens per taxa.

Sporophyte traits

The presence of stomata only had an effect on the moisture preferences of species, with species in wetter habitats tending to have stomata. This indicates that in habitats where moisture is not a limiting factor, sporophytes are able to use stomata as they are at lower desiccation risk, and therefore are likely to have a lower DT than species with no stomata.

Spore size (categorised) had no effect on the mean EI. This may be explained when looking at light and moisture individually: the EI is non-significant because the difference in individual values of light and moisture cancel each other out, yielding the same EI per size category. Spore size is therefore a good indicator of exposure or humidity, but not of the overall environmental conditions.

Interestingly, sporophyte traits do not seem to indicate the overall environmental preferences of a species as much as gametophyte traits, as indicated by the fact that almost all gametophyte traits affected the mean EI, but only four sporophyte trait did (capsule exertion, capsule orientation, seta length, and spore size). As expected, sporophyte traits are not as useful to indicate the overall environmental conditions of a species due to the fact that it is a part of the life-cycle that occurs only when particular environmental conditions are met (e.g. presence of water for fertilisation). The fact that larger spores are found in more exposed habitats is unsurprising as it has long been noted in the literature, however, they are also more likely to be found in wetter habitats. This demonstrates that they survive in habitats which are exposed and have long periods of drought, but that periodically will have abundant water.

Although this could indicate that the EI is masking the effects of the environmental conditions, this was only the case for sporophyte traits. Gametophyte traits where there was no significant differences in mean EI (namely, water storage structure presence/absence and vegetative propagules traits) were also test against moisture and light values separately and again yielded no significant differences. This indicates that the EI is appropriate for gametophyte traits, but may need re-calibration for sporophyte traits by, for example, weighting humidity and light differently.

3.5.5 Life-history traits

Reproduction system

Moisture had an effect on species' reproduction system, but the overall environmental conditions did not. This reflects the importance of moisture for the reproduction of bryophytes as they require water for fertilisation. Dioicous species are found in wetter environments than monoicous

ones, as the diflagellate sperm has futher to travel in dioicous species and therefore require water to do so.

Life-strategy

Annual and perennial species had the largest difference in environmenral preference, with annual species occupying drier and more exposed environments. This is expected as annual species complete their life-cycles when moisture is available and then rely on a spore bank for persistence during drought (Frahm, 2000; Vitt et al., 2014). Colonist species were found in drier and more exposed environments than perennials as colonists are often habitat pioneers of degraded habitats and so desiccation tolerant (Kürschner et al., 1999).

3.5.6 Methodology

Data completeness

Compared to studies looking at traits in other taxonomic groups (e.g. plants, birds, angiosperms, mammals) a sample size of 530 is small, but within bryophytes there have been very few studies with this many species (e.g. Hedenäs, 2001 looked at 439 mosses). Most studies looking at bryophyte traits have tended to be taxonomically focussed rather than geographically. Data completeness was much lower for Malagasy species as is the case with other tropical plant datasets (Schrodt et al., 2015). Although the dataset could of course be enlarged, it represents an important contribution to the study of bryophyte traits and their analyses. Additionally, this trait database makes available data that is currently not in electronic format and so not easily accessible to researchers, a fact noted by Hill et al. (2007). It provides a basis for future bryophyte trait databases, and adds significantly to tropical bryophyte trait data availability.

Quantitative vs. qualitative data

To have a more accurate idea of how traits vary among species, a study could be conducted by quantitatively measuring the traits using measurements from several individuals of each species. For example, instead of simply recording presence or absence of papillae, the length of papillae could be calculated by measuring 10 papillae on 10 individuals and calculating the mean papillae length for that species. Another trait that could be quantitatively measured is leaf orientation, by using the angle between the plant axis and the leaf (Figure 3.16). As mentioned above, it was not feasible to undertake this for the present study, but a next step could be to refine the species list to a particular taxonomic group (e.g. a particular family or bryophytes from a particular microhabitat) as has been done in previous studies (Hedenäs, 2001; Kürschner et al., 2007). This would make a bryophyte trait study more comparable to those undertaken on vascular plants, which often use continuous data (e.g. Lavorel & Garnier, 2002; Vazačová & Münzbergová, 2014).

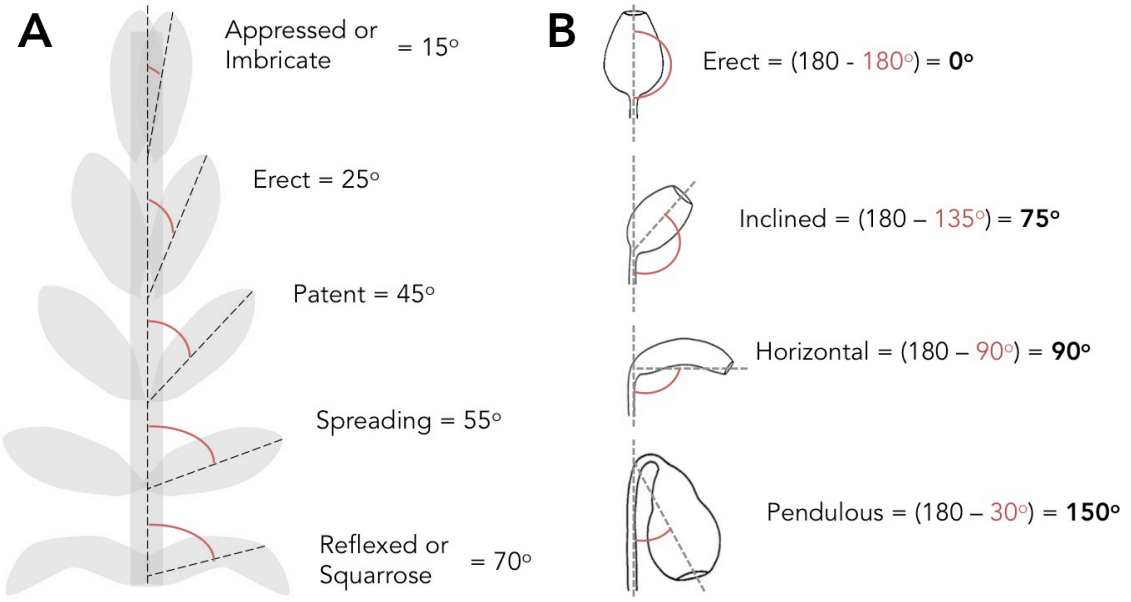


Figure 3.16 Schematic representation of a potential methodology for measuring: (A) the angle between stem and leaf central axis to obtain an exact value for leaf orientation and (B) the angle between stem and capsule central axis (from capsule base to operculum) to obtain an exact value for capsule inclination. Source: Sarah Stow.

In the case of leaf orientation, this categorical nominal trait was converted into a categorical ordinal one, which allowed for further analysis to be carried out on the trait (i.e. the change in leaf orientation between from dry to wet conditions) thus showing that assigning numerical values to quantitative traits may have greater range of applications. Based on the database created it would be possible to apply this technique to other traits such as margin curvature, leaf margin denticulation and capsule orientation.

As seen with spore size, the categorical variable did not yield any significant results with mean EI but when using continuous spore size (diameter in mm) a significant difference in mean EI was seen. Although different capsule orientations had significantly different mean EIs, measuring the precise angle of the capsule inclination (Figure 3.16) could yield clearer results. Although not feasible within the time-frame of this study, a future study focussing on a smaller number of species could include this measurement. However, when looking at humidity and light individually, categorical spore size did yield significant results.

Although many trait analyses use quantitative values for traits, and reducing states to a finite number of discrete categories could be seen as reducing the amount of information available, it has been shown to yield meaningful results, both in this study and others (Crawford et al., 2009; Kraichak, 2012). This suggests that exact information may not always be needed allowing a greater number of species to be included in analyses, including rare species that might have less information available. However, categorisation of a continuous trait should be done with caution and different category delimitations need to be tested. Categorisation of a qualitative variable will affect results as shown by longitudinal leaf profile where no differences were found when all original 27 and 32 states (dry and wet, respectively) were used, but when new categories were formed by grouping some of these states significant differences were found. Although statistically acceptable, grouping of certain traits may not make sense from a morphological or ecological perspective. Therefore, categorisation requires a combination of statistical technique with

taxonomic and ecological knowledge of the target taxa. In addition, categorising some traits may in fact yield a clearer result than using them as a continuous variable (e.g. seta length).

Qualitative traits tend to be less variable within a species and so facilitate analysis of large datasets by reducing uncertainty (Kattge, Ogle, et al., 2011). Aditionally, qualitative traits vary within species due to varying environmental conditions – the only way to account for this variation is to take several measurements of the trait from species in different habitats/environmental conditions in order to understand how the trait varies with environmental variables (Kattge, Ogle, et al., 2011). A mean trait value may be created from these measurements, although it is more appropriate for traits that have low variability (Kattge, Ogle, et al., 2011). Information from floras is in itself an aggregate of the variation in a trait as several specimens of a species will be observed to create the flora description.

Univariate analyses

As seen with plant colour, traits with high variability show no significant results and this is unlikely to be due to a true lack of significant difference in mean EI. What this more likely indicates is that univariate analyses are not the best type of analysis for these types of traits. Although grouping traits into three broad categories yielded significant differences in the mean EI, it was not possible to see the effect of individual colours on the mean EI. A multivariate analysis is therefore more useful in these instances. However, as part of a stepwise approach to rigorously analysing the data, univariate analysis was carried out on all trait groups prior to approaching the multi-variate analysis in the following chapter.

Where some trait states showed what appear to be large differences between their mean EI, but were not significantly different, this could be due to small sample size (therefore large standard error) or the conservativeness of post-hoc tests – which are inherently conservative when applied to unplanned comparison and unbalanced data (Sokal & Rohlf, 1995). For example, leaf transverse section only yielded a significant difference between plane and keel following the Games-Howell test, but a Tukey test yields three additional significant differences (concave or channelled and keel, concave or channelled and plicate, plane and plicate). The lack of significance must be accepted and stated (changing the test would be incorrect and misleading statistical procedure as it is not an appropriate one for the type of data), but the large observed difference in mean EI between other states suggests that greater sampling might yield significant differences. As mentioned above, continuous values for traits may also provide clearer results.

Correlation between traits is not addressed in the above univariate analyses although trait correlation in bryophytes may not be as prevalent as expected, and varies between studies. When looking at correlation between different life-history traits, one study found no significant relationship between diocy/monoicy and presence of vegetation propagules (Crawford et al., 2009) whereas previous authors found this to be significant (Rydin, 2009). Size has been shown in previous studies to affect the response of other traits to DT, and this was also found in this study when looking at life-form and plant size.

An extension of the analyses undertaken in this chapter is the experimental assessment of DT in selected species, following the protocol outlined in Wood (2007) (see A2.2, p. 90 for methodology), in order to further underpin the results of the univariate analyses. This would also

increase the data available on bryophyte DT as currently less than 1% of species have experimental data on DT (Wood, 2007).

Phylogenetic correction

Correction of phylogeny was not used in these analyses though it is a technique that has become commonly applied to studies of traits (Pagel, 1994), particularly those looking at evolution, including more recently in bryophytes (Crawford et al., 2009; Manyanga et al., 2011). This was not applied in this study because of the lack of uniform robust phylogenetic data available for bryophytes although combining trees has been shown to be acceptable (Crawford et al., 2009). Although some studies highlight the importance of using phylogenetic correction in order to determine if a trait is truly an adaptation to the environment, or due to shared evolutionary history (Kraichak, 2012), others have found that there is no effect of phylogeny for certain traits. These studies have used species within a taxonomic group (family or genus) and so correcting for phylogeny is more important in these studies than in the wider phylogenetic sample in this study. While the shared evolutionary history and consequent shared developmental pathways may lead to species with very different DTs possessing shared characteristics, a focus of this study, and the following analysis, is to identify a suite of (easy to identify) traits from the larger matrix that will indicate levels of DT, and also extinction risk based on presence or absence of these traits. As such, the effect of specific combinations of traits is more critical to this study than their evolutionary origins.

3.6 Conclusions

The trait database created in this study provides a significant contribution to the study of bryophyte traits, including tropical species. It also provides further evidence that a bryophyte flora from one region can be used to inform the bryoflora of another region. This is particularly important in the case of tropical bryofloras, which are critically understudied.

The lack of significant differences when certain traits were grouped into present or absent indicates that finer level of trait classification is required for analyses. There is a limit to this, however, as too many states of a trait will yield no significant differences due to small sample numbers per state. Results also indicate that while univariate tests can provide some level of insight into how traits relate to the environment, a multivariate approach is needed, particularly when looking at traits with substantial variation. Due to very few traits in liverworts having significant differences, subsequent multivariate analyses and distribution mapping will be carried out using mosses only.

The next chapter will build on the relationships found here and will analyse all traits together, rather than individually, in order to identify similarity between species based on their suite of traits, and find if particular trait profiles are associated with different environmental conditions.

Appendix 3 Trait Database

A3.1. Summary of sources used to create trait database

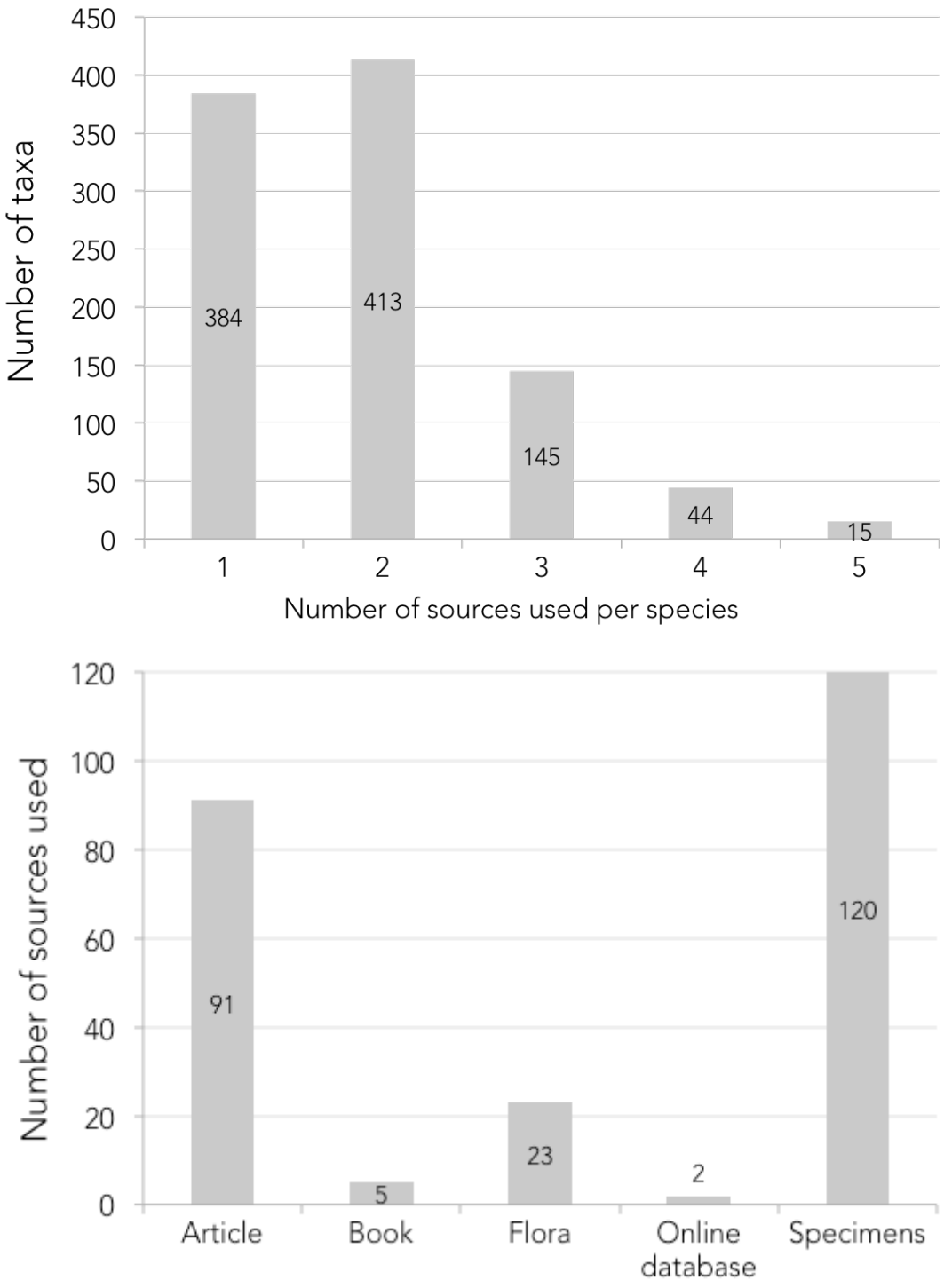


Figure 3.17 Sources used – literature and specimens – to compile trait database: top- number of different sources used per taxa; bottom- number of each type of source used. For the list of literature sources (articles, books, floras and online databases) see A3.2, p. 150 and for specimens consulted see Table 3.21, p. 158.

A3.2. Literature source reference list

Floras listed first, followed by books, online databases and lastly, articles. Sources listed alphabetically by authors and chronologically within same authors.

FLORAS

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Table 3.21 Specimens observed for trait recording in alphabetical order by species. BM- The Natural History Museum, London, UK; G- Conservatoire et Jardin botaniques de la Ville de Genève, Geneva, Switzerland; MO- Missouri Botanical Garden, Missouri, USA; PC - Muséum National d'Histoire Naturelle (Cryptogams), Paris, France; S- Swedish Museum of Natural History, Stockholm, Sweden (Thiers, continuously updated). Nomenclature follows Renzaglia *et al.* (2009) for hornworts, Crandall-Stotler *et al.* (2009) for liverworts and Goffinet *et al.* (2009) for mosses.

Specimen number	Taxon	Herbarium
PC0702578	*Barbella capillicaulis* var. *capillicaulis* (Renauld & Cardot) Cardot	PC
PC135842	*Brachythecium decurrens* Cardot	PC
PC0073340	*Breutelia madagassa* var. *madagassa* Thér.	PC
PC s.n.	*Bryum arachnoideum* Müll.Hal.	PC
PC0136971	*Bryum arachnoideum* Müll.Hal.	PC
PC0136972	*Bryum arachnoideum* Müll.Hal.	PC
PC0081531	*Bryum erythrocaulon* (Müll.Hal. ex Renauld) Cardot	PC
PC0092264	*Callicostella papillata* var. *brevifolia* M.Fleisch.	PC
PC0694334	*Callicostella papillata* var. *brevifolia* M.Fleisch.	PC
PC0116575	*Callicostella papillata* var. *papillata* (Mont.) Mitt.	PC
BM000097283	*Calymperes afzelii* Sw.	BM
BM000968474	*Calymperes afzelii* Sw.	BM
BM000518584	*Calymperes hispidum* Renauld & Cardot	BM
BM000855093	*Calymperes hispidum* Renauld & Cardot	BM
BM s.n.	*Calymperes loucoubense* Besch.	BM
MO-2800899	*Calymperes palisotii* Schwaegr.	MO
MO-2913958	*Calymperes pallidum* Mitt.	MO
PC0116578	*Calymperes tahitense* (Sull.) Mitt.	PC
MO-2914002	*Calymperes tenerum* Müll.Hal.	MO
PC0116576	*Calymperes venezuelanum* (Mitt.) Broth. *ex* Pittier	PC
PC0116577	*Calymperes venezuelanum* (Mitt.) Broth. *ex* Pittier	PC
PC0701867	*Cryphaea jamesonii* Taylor	PC
PC0116579	*Cryphaea rutenbergii* Müll.Hal.	PC
PC0116580	*Cryphaea rutenbergii* Müll.Hal.	PC
PC0091897	*Daltonia cardotii* Bizot & Onr.	PC
PC0091945	*Daltonia latimarginata* Besch.	PC
PC0091933	*Daltonia latimarginata* var. *madagassa* Renauld	PC
PC0092500	*Distichophyllum mascarenicum* Besch.	PC
PC0092490	*Distichophyllum rakotomariae* Crosby	PC
PC0105480	*Ectropothecium occultum* Renauld & Cardot	PC
PC0097062	*Ectropothecium ovalifolium* (Besch.) W.R. Buck	PC
PC0098410	*Ectropothecium perrieri* Thér.	PC
PC01311780	*Ectropothecium seychellarum* Besch.	PC
G00284077	*Ectropothecium tamatavense* Broth.	G
PC0101407	*Ectropothecium tamatavense* Broth.	PC
S-139403	*Ectropothecium tamatavense* Broth.	S

PC0703131	*Erpodium madagassum* Paris & Renauld	PC
PC0703136	*Erpodium madagassum* Paris & Renauld	PC
PC0105429	*Fabronia campenonii* Renauld & Cardot	PC
PC0080648	*Fabronia crassiretis* Renauld & Cardot	PC
PC0105465	*Fabronia fastigiata* Renauld & Cardot	PC
PC0054716	*Fabronia garnieri* (Paris & Renauld) Renauld & Paris	PC
PC0054738	*Fabronia lachenaudii* Renauld	PC
PC0105824	*Fabronia motelayi* Renauld & Cardot	PC
PC0054741	* *Fabronia perciliata* Müll.Hal.	PC
PC0054717	§ *Fabronia villaumii* Renauld & Cardot	PC
PC0054718	§ *Fabronia villaumii* Renauld & Cardot	PC
PC0032225	*Gammiella ceylonensis* (Broth.) B.C. Tan & W.R. Buck	PC
PC0105867	*Glossadelphus guineensis* (Broth. & Paris) Crosby, B.H. Allen & Magill	PC
PC0697953	*Glossadelphus semiscabrus* (Renauld & Cardot) Crosby, B.H.Allen & Magill	PC
PC0694422	*Helicodontium fabroniopsis* Müll.Hal.	PC
PC0105496	*Hookeriopsis diversifolia* (Renauld & Cardot) Broth. ex Cardot	PC
PC0105805	*Isopterygium ambreanum* Renauld & Cardot	PC
PC0105750	*Isopterygium argillicola* (Renauld & Cardot) Broth.	PC
PC0116591	*Isopterygium argyroleucum* Besch.	PC
PC0697397	*Isopterygium argyroleucum* Besch.	PC
PC0697400	*Isopterygium argyroleucum* Besch.	PC
PC0105499	*Isopterygium austrodenticulatum* (Renauld & Cardot) Broth.	PC
BM000850688	*Isopterygium combae* Besch.	BM
PC s.n.	*Isopterygium combae* Besch.	PC
PC0693420	*Isopterygium gracile* Renauld & Cardot	PC
PC0693426	*Isopterygium intortum* Renauld & Cardot	PC
PC0693427	*Isopterygium intortum* Renauld & Cardot	PC
PC0693422	*Isopterygium intortum* var. *chenagonii* Renauld & Cardot	PC
PC0693428	*Isopterygium leptoblastum* (Müll.Hal.) A. Jaeger	PC
PC0693437	*Isopterygium luteonitens* (Paris) Paris	PC
PC0693441	*Isopterygium meylanii* Cardot	PC
PC0097528	*Lepidopilum lastii* Mitt.	PC
PC00696870	*Lepidopilum verrucipes* Cardot	PC
BM s.n.	*Leucoloma brevioperculatum* Dixon	BM
BM s.n.	*Leucoloma fontinaloides* Dixon	BM
BM s.n.	*Leucoloma persecundum* var. *perrotii* Renauld	BM
BM s.n.	*Leucoloma thraustum* Besch.	BM
BM s.n.	*Leucoloma thuretii* Besch.	BM
BM000726127	*Leucophanes hildebrandtii* C.Muller	BM

BM s.n.	*Lopholejeunea lepidoscypha* Kiaer & Pearson	BM
BM000878680	*Mittenothamnium microthamnioides* (Müll.Hal.) Wijk. & Marg.	BM
PC0695040	*Neckeropsis boiviniana* (Besch.) Cardot	PC
PC0116587	*Neckeropsis disticha* (Hedw.) Kindb.	PC
PC0734120	*Neckeropsis disticha* (Hedw.) Kindb.	PC
PC0105047	*Octoblepharum africanum* (Broth.) Card.	PC
MO-2800822	*Octoblepharum albidum* Hedw.	MO
PC0146329	*Papillaria borchgrevinkii* Kiaer	PC
PC0721966	*Papillaria flaccidula* Cardot	PC
PC s.n.	*Pseudoleskea obtusiuscula* Renauld & Cardot	BM
PC0116588	*Rhizofabronia persoonii* var. *sphaerocarpa* (Dusén) Bizot ex Ochyra	PC
PC0116590	*Rhynchostegiella microtheca* (Renauld & Cardot) Broth.	PC
PC0105738	*Rhynchostegium angustifolium* Renauld & Cardot	PC
PC0098593	*Rhynchostegium pseudodistans* Cardot	PC
PC0106367	*Schimperella rhynchostegioides* Thér.	PC
PC0094867	*Syrrhopodon africanus* var. *africanus* (Mitt.) Paris	PC
MO s.n. (Magill – 9565)	*Syrrhopodon africanus* var. *mandrakensis* (Tixier) W.D.Reese	MO
MO-2689994	*Syrrhopodon asper* Mitt.	MO
MO-2753204	*Syrrhopodon asper* Mitt.	MO
PC0098859	*Syrrhopodon cuneifolius* Thér.	PC
PC0098861	*Syrrhopodon cuneifolius* Thér.	PC
PC0116585	*Syrrhopodon gardneri* (Hook.) Schwaegr.	PC
MO s.n. (Pócs - 90102/D)	*Syrrhopodon gaudichaudii* Mont.	MO
PC0105318	*Syrrhopodon graminifolius* Renauld & Cardot	PC
PC0116571	*Syrrhopodon graminifolius* Renauld & Cardot	PC
PC0116570	*Syrrhopodon involutus* Schwaegr.	PC
PC0099106	*Syrrhopodon parasiticus* (Sw. ex Brid.) Besch.	PC
PC0053355	*Taxithelium argyrophyllum* Renauld & Cardot	PC
PC0053361	*Taxithelium argyrophyllum* Renauld & Cardot	PC
PC0053413	*Taxithelium glaucophyllum* Besch.	PC
PC0053427	*Taxithelium hirtellum* Paris & Renauld	PC
PC0053602	*Taxithelium nepalense* (Schwägr.) Broth.	PC
PC0050356	*Taxithelium nossianum* Besch.	PC
PC0053595	*Taxithelium planulum* Besch.	PC
PC0053600	*Taxithelium planulum* Besch.	PC
PC0099087	*Thamnobryum malgachum* (Cardot) O'Shea	PC
PC0116592	*Trachyphyllum inflexum* (Harv.) Gepp.	PC
PC0116589	*Trachypus appendiculatus* (Renauld & Cardot) Broth.	PC
PC0116582	*Trichosteleum debettei* var. *laevisetum* Cardot	PC

PC0116583	*Trichosteleum debettei* var. *laevisetum* Cardot	PC
PC0128237	*Trichosteleum leviusculum* Renauld & Cardot	PC
PC0131765	*Trichosteleum microdontum* (Besch.) Renauld	PC
PC0131769	*Trichosteleum microdontum* var. *megapterum* Renauld & Cardot	PC
PC0105519	*Trichosteleum perrotii* Renauld & Cardot	PC
PC0105513	*Trichosteleum perrotii* var. *eurydictyon* Renauld & Cardot	PC

* This species is not listed in the Malagasy checklist (Marline et al., 2012) but is included in this study because the type specimens of *Fabronia lachenaudii* var. *latifolia* Renauld (PC0054741) and *Fabronia villaumi* Renauld & Cardot (PC0054717, PC0054718, PC0054719, PC0054720) were identified as *Fabronia perciliata* Renauld & Cardot in a revision by H. Matcham in May 2007.

§ The type specimen of this species was revised in May 2007 as *Fabronia perciliata* Renauld & Cardot by H. Matcham and so it is likely this species does not exist. The status of knowledge on *Fabronia villaumi* Renauld & Cardot was defined in the 1999 Checklist of Mosses (Crosby et al.) as "insufficiently known (…) for which no new information has been found in the post-1962 literature [the date Index Herbariorum was published]" (Crosby et al., 1999, p. 2). Although *F. villaumi* is listed in the 1983 (Crosby et al., 1983) and 2012 (Marline et al., 2012) Malagasy checklists, there is "(…) no new information about the species: we know nothing more about the nature of the species after the publication of the checklist (…) [as it is] merely relisted without additional specimens " (Crosby et al., 1999, p. 2). Because no new publications or specimens exist to confirm the status of this species and due to the revised identification of its type specimen as *F. perciliata*, *F. villaumi* is maintained in the database but removed from analyses to avoid duplicating the traits as they are the same for *F. villaumi* and *F. perciliata*.

A3.3. Recording traits.

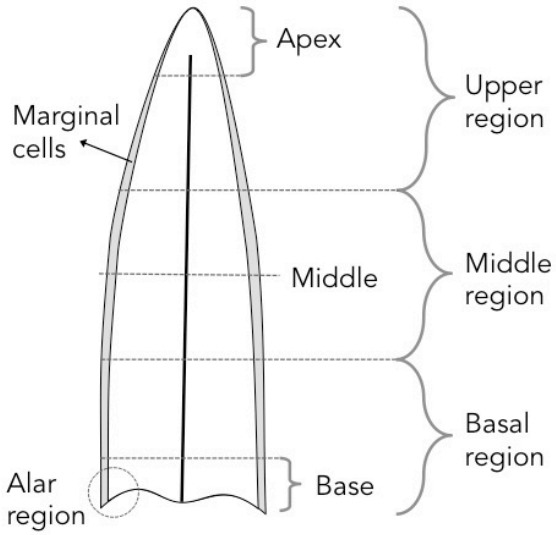


Figure 3.18 Delimitation of leaf areas used to record traits from in this study: leaf surface, cell wall, cell wall shape, costa length, and marginal cell traits. Source: Sarah Stow.

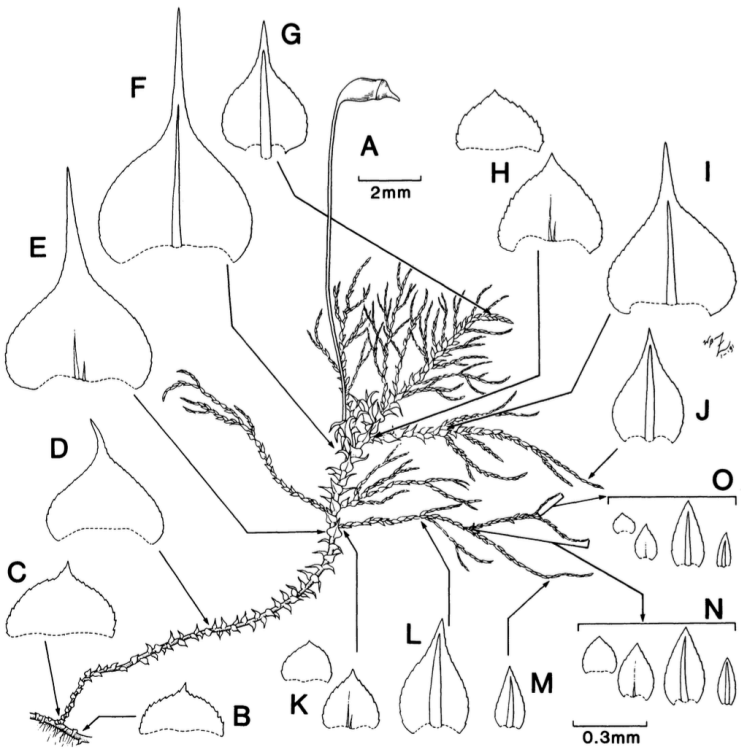


Figure 3.19 Variation in leaf morphology within an individual plant according to a leaf's position on the plant. A. Habit; B-E. Stem leaves; F-H. Upper stem leaves; I-O. Branch leaves. Taken from Zomlefer 1993, figure 3, p. 7.

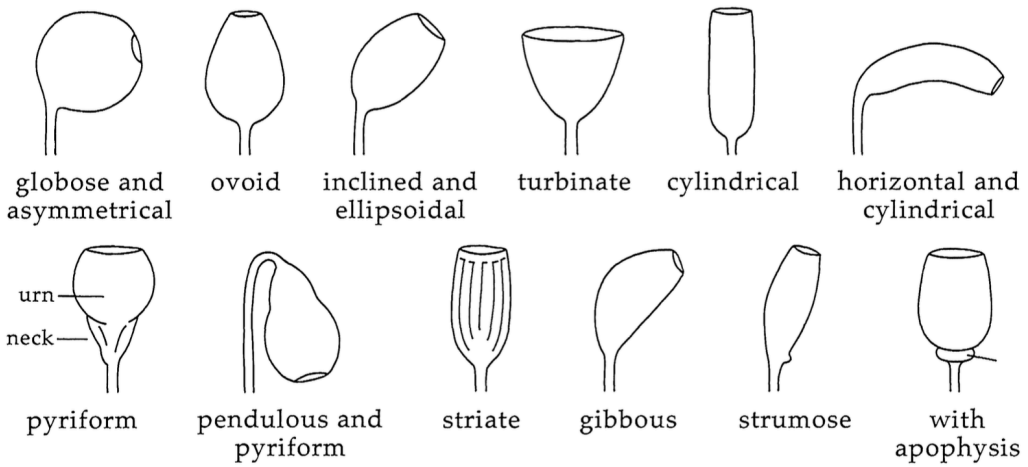


Figure 3.20 Variation in moss capsule shapes. Taken from Casas et al. 2006, fig. C, p. 331.

A3.4. SRLI species present in the trait database

Table 3.22 Malagasy and Portuguese species present in the SRLI, organised by class (mosses then liverworts), then in alphabetical order by species. In bold are species from the target families. * denotes a national endemic species; ** denotes a national endemic genus; § denotes a regional (Iberia or Indian Ocean) endemic species and §§ denotes a regional endemic genus. Red List status for Portugal taken from Sérgio *et al.*, 2013; Red List status for Madagascar taken from Reunion Red List (Ah-Peng, Bardat, et al., 2012), indicated by REU in the table. Nomenclature follows Renzaglia *et al.* (2009) for hornworts, Crandall-Stotler *et al.* (2009) for liverworts and Goffinet *et al.* (2009) for mosses.

	Species	Family	Red List Status	Country
Mosses				
	Acaulon fontiquerianum Casas & Sérgio	Pottiaceae	CR	Portugal
	Acaulon muticum (Hedw.) Müll.Hal.	Pottiaceae	LC	Portugal
*	*Acroporium stellatum* (Renauld & Cardot) Broth.	Sematophyllaceae		Madagascar
	Aerobryopsis capensis (Müll.Hal.) M.Fleisch	Meteoriaceae		Madagascar
	Aerolindigia capillacea (Hornsch.) M.Menzel	Brachytheciaceae		Madagascar
	Amblystegium serpens (Hedw.) Schimp.	Amblystegiaceae	DD	Portugal
	Amphidium mougeotii (Schimp.) Schimp.	Rhabdoweisiaceae	LC	Portugal
	Andreaea megistospora B.M.Murray	Andreaeaceae	LC	Portugal
	Andreaea rupestris Hedw.	Andreaeaceae	LC	Portugal
	Atractylocarpus madagascariensis (Thér.) Padberg & J.-P.Frahm	Leucobryaceae		Madagascar
*	*Barbula subobtusa* Thér.	Pottiaceae		Madagascar
	Brachymenium philonotula Broth.	Bryaceae		Madagascar

	Species	Family	Red List Status	Country
*	*Breutelia madagassa* Thér.	Bartramiaceae		Madagascar
	Bryum arachnoideum C. Müller	Bryaceae		Madagascar
	Bryum argenteum Hedw.	Bryaceae	LC	Madagascar and Portugal
	Bryum donianum Grev.	Bryaceae	LC	Portugal
	Bryum erythrocaulon (Schwägr.) Brid.	Bryaceae		Madagascar
	Bryum megalacrion Schwägr.	Bryaceae		Madagascar
	Bryum tenuisetum Limpr.	Bryaceae	DD-n	Portugal
	Calicostella lacerans (Müll.Hal.) A.Jaeger	Pilotrichaceae		**Madagascar**
	Callicostella fissidentella (Besch.) Kind.	Pilotrichaceae		**Madagascar**
	Callicostella seychellensis (Bescherelle) Renauld	Pilotrichaceae		Madagascar
	***Calymperes erosum* Müll.Hal.**	**Calymperaceae**		**Madagascar**
	***Calymperes pallidum* Mitt.**	**Calymperaceae**		**Madagascar**
	***Calymperes tahitense* (Sull.) Mitt.**	**Calymperaceae**		**Madagascar**
	Campylopus nivalis (Brid.) Brid.	Leucobryaceae		Madagascar
	Campylopus schmidii (Müll.Hal.) A.Jaeger	Leucobryaceae		Madagascar
	Campylopus trachyblepharon (Renauld & Cardot) J.-P.Frahm	Leucobryaceae		Madagascar
	Claopodium whippleanum (Sull.) Renauld & Cardot	Leskeaceae	LC-att	Portugal
	Climacium dendroides (Hedw.) F.Weber & D.Mohr	Climaciaceae	CR	Portugal
	***Cryphaea rutenbergii* Müll.Hal.**	**Cryphaeaceae**		**Madagascar**
	Dicranella madagassa Renauld	Dicranaceae		Madagascar
	Dicranella subulata (Hedw.) Schimp.	Dicranaceae	EN	Portugal
	Dicranoweisia cirrata (Hedw.) Lindb.	Rhabdoweisiaceae	LC	Portugal
	Dicranum tauricum Sapjegin	Dicranaceae	NT	Portugal
	Didymodon eckeliae R.H.Zander	Pottiaceae	DD-n	Portugal
	Didymodon rigidulus Hedw.	Pottiaceae	LC	Portugal
	Distichium capillaceum (Hedw.) Bruch, Schimp. & W.Gümbel	Ditrichaceae		Madagascar
*	*Entodon madagassus* Geh.	Entodontaceae		Madagascar
	Ephemerum recurvifolium (Dicks.) Boulay	Pottiaceae	EN	Portugal
*	*Fabronia lachenaudii* Renauld	Fabroniaceae		Madagascar

	Species	Family	Red List Status	Country
	Fissidens rivularis Bruch & Schimp.	Fissidentaceae	EN	Portugal
	Grimmia caespiticia (Brid.) Jur.	Grimmiaceae	CR	Portugal
	Grimmia laevigata (Brid.) Brid.	Grimmiaceae	LC	Portugal
	Grimmia orbicularis Bruch ex Wilson	Grimmiaceae	LC	Portugal
	Grimmia ramondii (Lam. & DC.) Margad.	Grimmiaceae	NT	Portugal
	Grimmia tergestina Tomm. ex Bruch & Schimp.	Grimmiaceae	DD-n	Portugal
	Holomitrium borbonicum Besch.	Dicranaceae		Madagascar
	Homaliodendron exiguum (Bosch & Sande Lac.) M.Fleisch.	Neckeraceae		Madagascar
	Homalothecium aureum H.Rob.	Brachytheciaceae	LC	Portugal
	Homalothecium sericeum (Hedw.) Schimp.	Brachytheciaceae	LC	Portugal
*	*Hymenostylium subcrispulum* Thér.	Pottiaceae		Madagascar
	Hyophila acuminata Broth. & P.de la Varde	Pottiaceae		Madagascar
	Isopterygium argyroleucum Besch.	Pylaisiadelphaceae		Madagascar
	Isopterygium gracile Renauld & Cardot	Pylaisiadelphaceae		Madagascar
*	*Isopterygium meylanii* Cardot	Pylaisiadelphaceae		Madagascar
	Isopterygium subleptoblastum Müll.Hal.	Pylaisiadelphaceae		Madagascar
	Kiaeria starkei (F.Weber & D.Mohr) I.Hagen	Rhabdoweisiaceae	VU	Portugal
	Lepidopilidium parvulum Cardot	Pilotrichaceae		Madagascar
	Leptodon smithii (Hedw.) F.Weber & D.Mohr	Leptodontaceae	LC	Portugal
	Leucobryum acutifolium (Mitt.) Cardot	Leucobryaceae		Madagascar
	Leucobryum mayottense Cardot	Leucobryaceae		Madagascar
*	*Leucoloma brevioperculatum* Dixon	Dicranaceae		Madagascar
	Leucoloma brotheri Renauld	Dicranaceae		Madagascar
*	*Leucoloma candidum* Broth.	Dicranaceae		Madagascar
	Leucoloma chrysobasilare (Müll.Hal.) A.Jaeger	Dicranaceae		Madagascar
	Leucoloma dichelymoides (Müll.Hal.) A.Jaeger	Dicranaceae		Madagascar
*	*Macromitrium adelphinum* Cardot	Orthotrichaceae		Madagascar
	Orthostichopsis subimbricata (Hampe) Broth.	Pterobryaceae		Madagascar
	Orthotrichum shawii Wilson	Orthotrichaceae	DD-n	Portugal

	Species	Family	Red List Status	Country
	Pelekium chenagonii (Müll.Hal. ex Renauld & Cardot) Touw	Thuidiaceae		Madagascar
*	*Philonotis byssiformis* Müll.Hal.	Bartramiaceae		Madagascar
	Plagiothecium cavifolium (Brid.) Z.Iwats.	Plagiotheciaceae	EN	Portugal
	Pohlia annotina (Hedw.) Lindb.	Mniaceae	LC	Portugal
	Pohlia melanodon (Brid.) A.J.Shaw	Mniaceae	LC	Portugal
	Polytrichum subpilosum P.Beauv.	Polytrichaceae		Madagascar
	Porotrichum usagarum Mitt.	Neckeraceae		Madagascar
	Pterigynandrum filiforme Hedw.	Pottiaceae	LC	Portugal
	Pyrrhobryum spiniforme (Hedw.) Mitt.	Rhizogoniaceae		Madagascar
	Racomitrium heterostichum (Hedw.) Brid.	Grimmiaceae	LC	Portugal
	Racopilum microdictyon Besch.	Racopilaceae		Madagascar
	Rhachithecium perpusillum (Thwaites & Mitt.) Broth.	Rhachitheciaceae		Madagascar
	Rhodobryum ontariense (Kindb.) Paris	Bryaceae		Madagascar
*	*Schlotheimia boiviniana* Besch.	Orthotrichaceae		Madagascar
	Schlotheimia perrotii Renauld & Cardot	Orthotrichaceae		Madagascar
	Seligeria acutifolia Lindb.	Seligeriaceae	DD-n	Portugal
	Sphagnum perichaetiale Hampe	Sphagnaceae		Madagascar
	Sphagnum tumidulum Besch.	Sphagnaceae		Madagascar
	Syntrichia virescens (De Not.) Ochyra	Pottiaceae	DD-n	Portugal
	Timmiella flexiseta (Bruch) Limpr.	Pottiaceae	EN	Portugal
	Tortella inflexa (Bruch) Broth.	Pottiaceae	LC	Portugal
*	*Tortula omissa* Thér.	Pottiaceae		Madagascar
	Trachypodopsis serrulata (P.Beauv.) M.Fleisch.	Trachypodaceae		Madagascar
	Trachypus appendiculatus (Renauld & Cardot) Broth.	Trachypodaceae		Madagascar
	Trachypus bicolor (Mitt.) Zanten	Trachypodaceae		Madagascar
	Trichodon cylindricus (Hedw.) Schimp.	Ditrichaceae	VU	Portugal
	Trichosteleum pervilleanum (Müll.Hal. ex Geh.) W.R.Buck	Sematophyllaceae		Madagascar
*	*Trichostomum sporaphyllum* (Renauld & Cardot) Cardot	Pottiaceae		Madagascar
*	*Trichostomum villaumei* Thér.	Pottiaceae		Madagascar

	Species	Family	Red List Status	Country
*	*Wijkia bessonii* (Renauld & Cardot) H.A.Crum	Pylaisiadelphaceae		Madagascar
§	*Zygodon catarinoi* C.Garcia, F.Lara, Sérgio & Sim-Sim	Orthotrichaceae	DD-n	Portugal
	Zygodon reinwardtii (Hornsch.) A.Braun	Orthotrichaceae		Madagascar
Liverworts				
	Acanthocoleus aberrans (Lindenb. et Gottsche) Kruijt	Lejeuneaceae		Madagascar
	Acanthocoleus madagascariensis (Steph.) Kruijt	Lejeuneaceae	NT-Reu	Madagascar
	Acrolejeunea emergens (Mitt.) Steph.	Lejeuneaceae		Madagascar
	Acrolejeunea pycnoclada (Taylor) Schiffn.	Lejeuneaceae		Madagascar
	Caudalejeunea recurvistipula (Gottsche) Schiffn.	Lejeuneaceae		Madagascar
*	*Cololejeunea ankaiana* Tixier	Lejeuneaceae		Madagascar
	Frullanoides tristis (Steph.) van Slageren	Lejeuneaceae	DD-Reu	Madagascar
	Jungermannia gracillima Sm.	Jungermanniaceae	LC	Portugal
	Lopholejeunea nigricans (Lindenb.) Schiffn.	Lejeuneaceae		Madagascar
	Lopholejeunea subfusca (Nees) Schiffn.	Lejeuneaceae		Madagascar
	Mastigolejeunea auriculata (Wilson) Schiffn.	Lejeuneaceae	DD-Reu	Madagascar
	Metalejeunea cucullata (Reinw. et al.) Grolle	Lejeuneaceae		Madagascar
	Mnioloma fuscum (Lehm.) R.M.Schust.	Calypogeiaceae		Madagascar
	Odontolejeunea lunulata (F.Weber) Schiffn.	Lejeuneaceae		Madagascar
	Plagiochila boryana Gottsche ex Steph.	Plagiochilaceae		Madagascar
	Scapania compacta (A.Roth) Dumort.	Scapaniaceae	LC	Portugal
	Scapania curta (Mart.) Dumort.	Scapaniaceae	EN	Portugal
	Scapania nemorea (L.) Grolle	Scapaniaceae	LC	Portugal
	Scapania scandica (Arnell et H.Buch) Macvicar	Scapaniaceae	EN	Portugal
	Scapania subalpina (Nees ex Lindenb.) Dumort.	Scapaniaceae	NT	Portugal
	Scapania undulata (L.) Dumort.	Scapaniaceae	LC	Portugal
*	*Symbiezidium barbiflorum* (Lindenb. et Gottsche) A.Evans	Lejeuneaceae		Madagascar

Species	Family	Red List Status	Country
Thysananthus spathulistipus (Reinw. et al.) Lindenb.	Lejeuneaceae	NT-Reu	Madagascar

A3.5. Trait database details

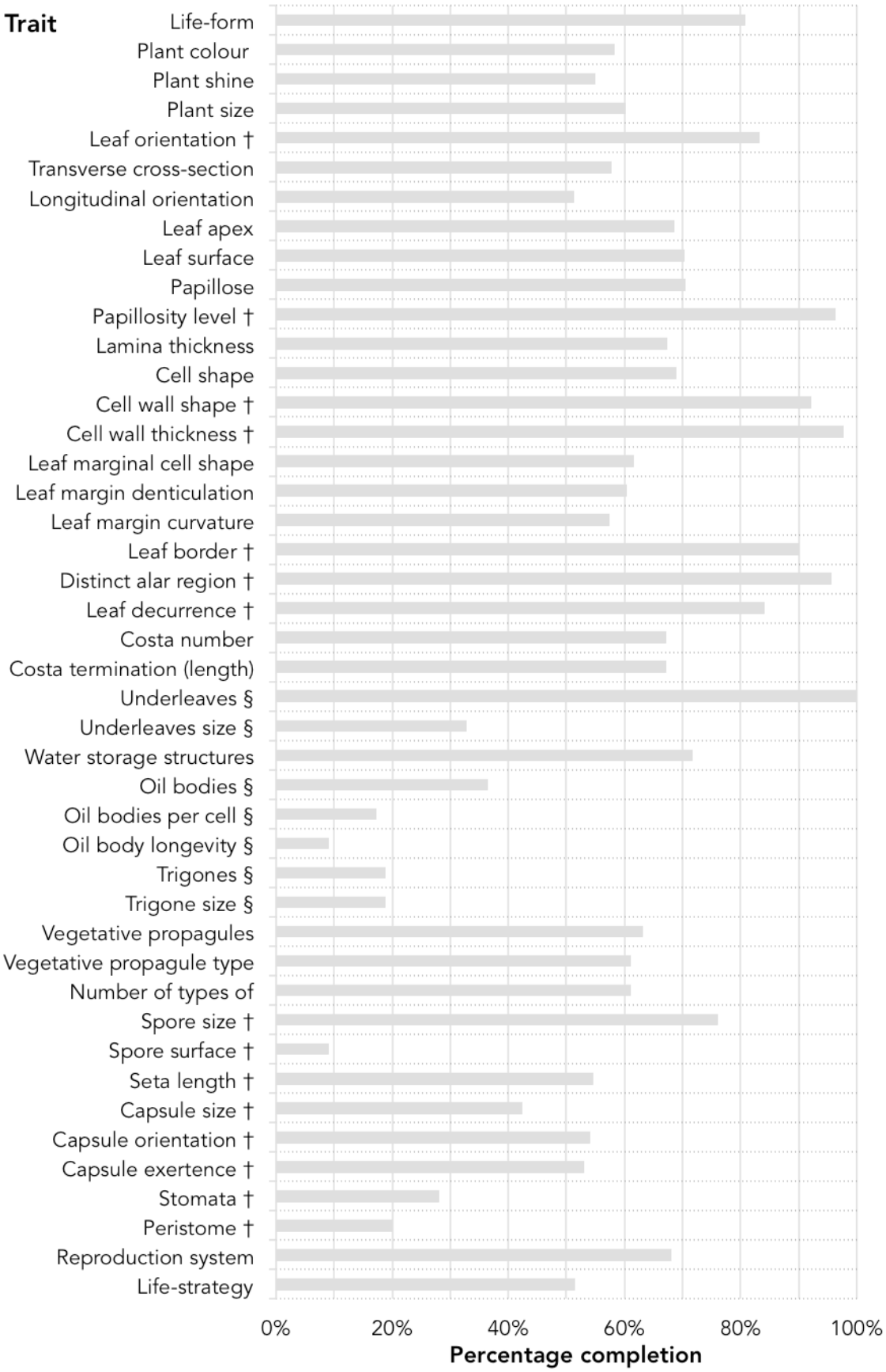


Figure 3.21 Completion rates for traits recorded in the database. † Trait only applies to mosses; § trait only applies to liverworts.

Table 3.23 Code letters used for colours to create colour codes for species (Table 3.24) ordered alphabetically.

Colour	Code letter
Black	B
Blue	Bl
Brown	Br
Green	G
Golden	Go
Orange	O
Pink	Pi
Purple	Pu
Red	R
White	W
Yellow	Y

Table 3.24 The groups assigned to 39 colour states with number of taxa per state, mean EI, standard error of the mean and variance. Listed by grouping and then alphabetically by colour code.

Colour code	Number of taxa	Group mean EI	Standard Error	Variance	Grouping
G	115	0.60	0.02	0.03	only green
BBr	4	0.80	0.08	0.0259	no green
BBrR	2	0.72	0.12	0.0047	no green
BrGo	1	0.79	0.16	NA	no green
BrR	2	0.83	0.12	0.0163	no green
BrW	1	0.85	0.16	NA	no green
W	1	0.79	0.16	NA	no green
Y	1	0.96	0.16	NA	no green
YBr	3	0.75	0.10	0.0123	no green
YBrGo	1	0.60	0.16	NA	no green
YBrO	1	0.64	0.16	NA	no green
YBrR	1	0.94	0.16	NA	no green
YRO	1	0.63	0.16	NA	no green
GB	24	0.62	0.03	0.0461	green
GBBr	8	0.61	0.06	0.0645	green
GBBrR	1	0.27	0.16	NA	green
GBlW	2	0.54	0.12	0.0062	green
GBR	1	0.68	0.02	NA	green
GBr	75	0.92	0.16	0.0274	green
GBrGo	2	0.53	0.12	0.0163	green
GBrO	3	0.52	0.10	0.0126	green
GBrR	23	0.74	0.03	0.0185	green
GBrW	2	0.49	0.12	0.0189	green
GBW	1	0.47	0.16	NA	green
GGo	8	0.67	0.06	0.012	green

Colour code	Number of taxa	Group mean EI	Standard Error	Variance	Grouping
Go	1	0.79	0.16	NA	green
GR	3	0.63	0.10	0.0039	green
GW	5	0.74	0.07	0.0331	green
GY	159	0.66	0.01	0.0241	green
GYB	1	0.32	0.16	NA	green
GYBr	62	0.69	0.02	0.0257	green
GYBrGo	1	0.64	0.16	NA	green
GYBrR	6	0.69	0.07	0.0445	green
GYBrRO	3	0.69	0.10	0.0028	green
GYBrRPu	2	0.68	0.12	0.0324	green
GYGo	4	0.69	0.08	0.0019	green
GYR	1	0.58	0.16	NA	green
GYROPiW	1	0.74	0.16	NA	green
GYW	1	0.57	0.16	NA	green

Table 3.25 Numerical coding for the 27 leaf orientation states when desiccated as well as number of taxa per state, mean EI, standard error of the mean, variance. In bold are the states commonly used in the literature. Ordered by the assigned numerical code, and then alphabetically by state name.

Leaf orientation	Number of taxa	Mean EI	Standard error	Variance	Numerical code
appressed	79	0.72	0.02	0.0356	**1**
imbricate	26	0.67	0.03	0.0373	**1**
imbricate-appressed	1	0.44	0.16	NA	**1**
appressed-erect	34	0.69	0.03	0.0222	**1.5**
imbricate-erect	7	0.74	0.06	0.0319	**1.5**
loose imbricate	6	0.56	0.06	0.0135	**1.5**
appressed-erecto/patent	10	0.73	0.05	0.0154	**2**
imbricate-erecto/patent	3	0.62	0.09	0.0298	**2**
erect	102	0.63	0.02	0.0229	**2**
appressed-patent	1	0.82	0.16	NA	**2.5**
erect-erecto/patent	38	0.57	0.03	0.0184	**2.5**
imbricate-spreading	3	0.6	0.09	0.0023	**3**
erect-patent	27	0.61	0.03	0.0106	**3**
erecto/patent	19	0.63	0.04	0.0199	**3**
erecto/patent-reflexed	1	0.42	0.16	NA	**4**
erecto/patent-spreading	8	0.64	0.06	0.0136	**4**
imbricate-squarrose	1	0.64	0.16	NA	**3**
loose imbricate-spreading	6	0.66	0.06	0.002	**3.5**
erecto/patent-patent	11	0.47	0.05	0.04	**3.5**
erecto/patent-recurved	1	0.36	0.16	NA	**4**
erecto/patent-squarrose	1	0.75	0.16	NA	**4**
erect-squarrose	2	0.62	0.11	0.0096	**4**
patent	3	0.44	0.09	0.0165	**4**

Leaf orientation	Number of taxa	Mean EI	Standard error	Variance	Numerical code
erect-spreading	16	0.59	0.04	0.0112	**4**
patent-spreading	5	0.59	0.07	0.0413	**5**
spreading	6	0.54	0.06	0.0259	**5**
squarrose	6	0.62	0.06	0.0244	**5**

Table 3.26 Numerical coding for the 32 leaf orientation states when hydrated as well as number of taxa per state, mean EI, standard error of the mean, variance. In bold are the single states commonly used in the literature. Ordered by the assigned numerical code, and then alphabetically by state name.

Leaf orientation	Number of taxa	Mean EI	Standard error	Variance	Numerical code
appressed	10	0.62	0.05	0.08389	**1**
imbricate	6	0.55	0.07	0.01987	**1**
appressed-erect	3	0.65	0.10	0.00084	**1.5**
appressed-erecto/patent	2	0.50	0.12	0.01388	**1.5**
erect-imbricate	1	1.00	0.17	NA	**1.5**
loose imbricate	3	0.66	0.10	0.00585	**1.5**
erect	20	0.69	0.04	0.03145	**2**
appressed-patent	1	0.53	0.17	NA	**2.5**
erect-erecto/patent	50	0.65	0.02	0.02987	**2.5**
erecto/patent	119	0.68	0.02	0.02304	**3**
erect-patent	51	0.66	0.02	0.03191	**3**
erect-spreading	33	0.62	0.03	0.02121	**3**
erecto/patent-secund	1	0.67	0.17	NA	**3**
imbricate-spreading	3	0.60	0.10	0.00232	**3**
imbricate-squarrose	1	0.64	0.17	NA	**3**
erecto/patent-patent	44	0.60	0.03	0.04012	**3.5**
loose imbricate-spreading	5	0.66	0.08	0.00237	**3.5**
erect-reflexed	2	0.56	0.12	0.03856	**4**
erect-squarrose	2	0.62	0.12	0.00963	**4**
erecto-secund	1	0.65	0.17	NA	**4**
erecto/patent-recurved	4	0.53	0.08	0.0182	**4**
erecto/patent-reflexed	1	0.82	0.17	NA	**4**
erecto/patent-spreading	43	0.67	0.03	0.03333	**4**
erecto/patent-squarrose	5	0.77	0.08	0.03228	**4**
patent	30	0.64	0.03	0.03362	**4**
patent-spreading	35	0.69	0.03	0.01855	**5**
patent-spreading/recurved	3	0.71	0.10	0.00334	**5**
patent-squarrose	1	0.79	0.17	NA	**5**
reflexed	1	0.68	0.17	NA	**5**
spreading	26	0.65	0.03	0.02515	**5**
spreading-squarrose	3	0.55	0.10	0.04367	**5**
squarrose	12	0.69	0.05	0.03295	**5**

Table 3.27 Numerical coding for the 16 capsule orientation states as well as number of taxa per state, mean EI, standard error of the mean and variance. In bold are the single states commonly used in the literature. Ordered by numerical code assigned.

Capsule orientation	Number of taxa	Mean EI	Standard error	Variance	Numerical code
immersed	48	0.73	0.02	0.0339	**0**
emergent	9	0.75	0.06	0.0151	**0**
shortly exert	1	0.82	0.17	NA	**0**
erect	180	0.69	0.01	0.0287	**1**
erect-curved	1	0.63	0.17	NA	**1.5**
erect-inclined	43	0.63	0.03	0.032	**1.5**
curved-horizontal	2	0.64	0.12	0.0062	**1.5**
erect-horizontal	8	0.57	0.06	0.0316	**2**
curved	4	0.59	0.08	0.0209	**2**
horizontal-inclined	41	0.60	0.03	0.0201	**2**
inclined	42	0.60	0.03	0.0286	**2**
horizontal	17	0.57	0.04	0.0274	**3**
inclined-pendulous	10	0.58	0.05	0.0183	**3**
horizontal-pendulous	13	0.61	0.05	0.0116	**3.5**
pendulous	19	0.68	0.04	0.0228	**4**
erect-pendulous	1	0.68	0.17	NA	**4**

A3.6. Analyses

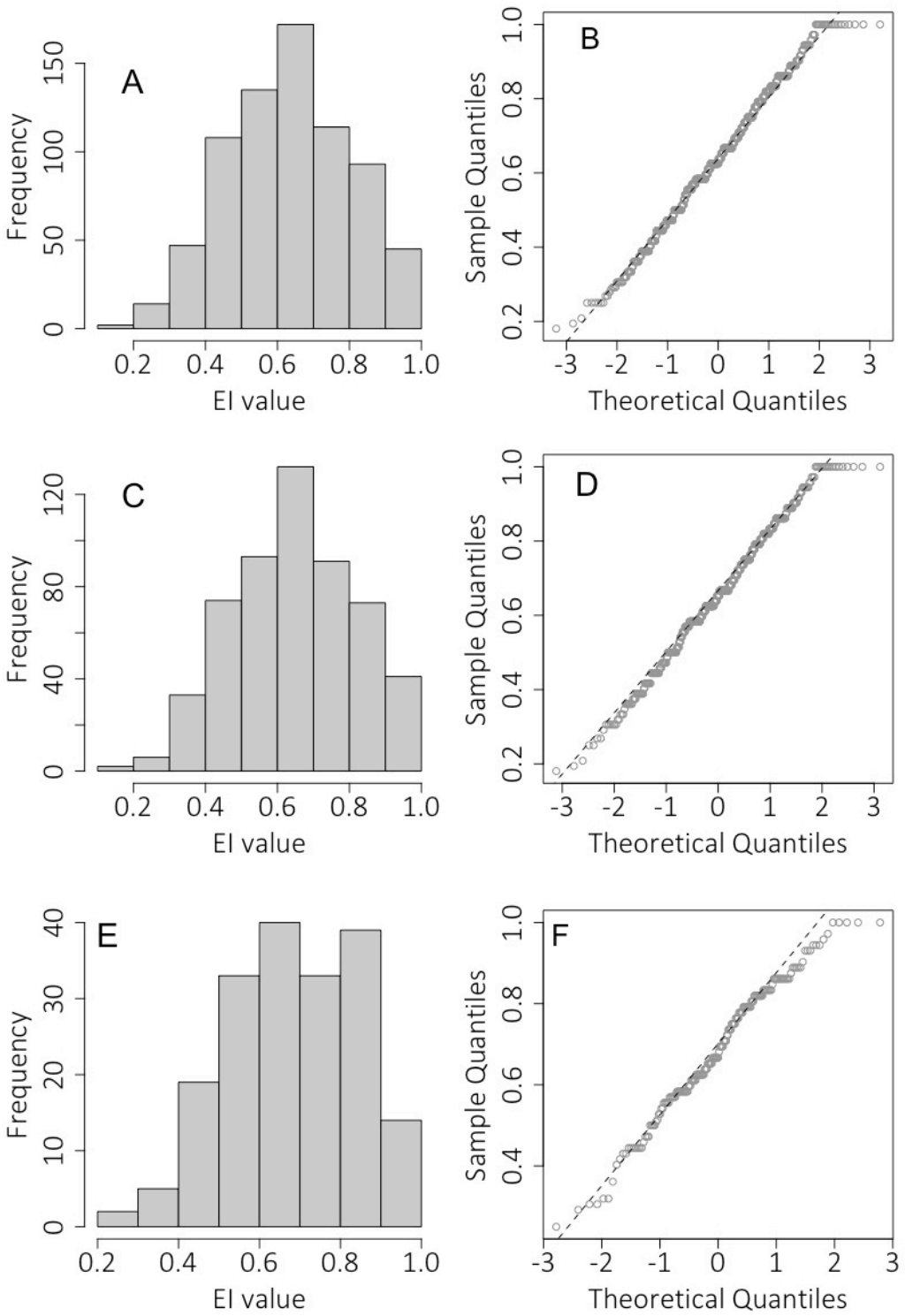


Figure 3.22 Normality plots of the Environmental Index (EI)- A) Histogram of EI for mosses and liverworts together; B) Quantiles normality plot of EI for mosses and liverworts together; C) Histogram of EI for mosses; D) Quantiles normality plot of EI for mosses; E) Histogram of EI for liverworts; F) Quantiles normality plot of EI liverworts. Although the Shapiro-Wilk test for non-normality indicates that the EI distribution in mosses and liverwort is non-normal ($p<0.05$), it is a very small deviation from normality: the slight positive skew (0.026) in the moss histogram (C) is not significant ($p=0.401$); the slight negative (-0.258) and the slight platykurtosis (-0.442) in the liverwort histogram (E) are also not significant ($p=0.921$ and $p=0.887$, respectively).

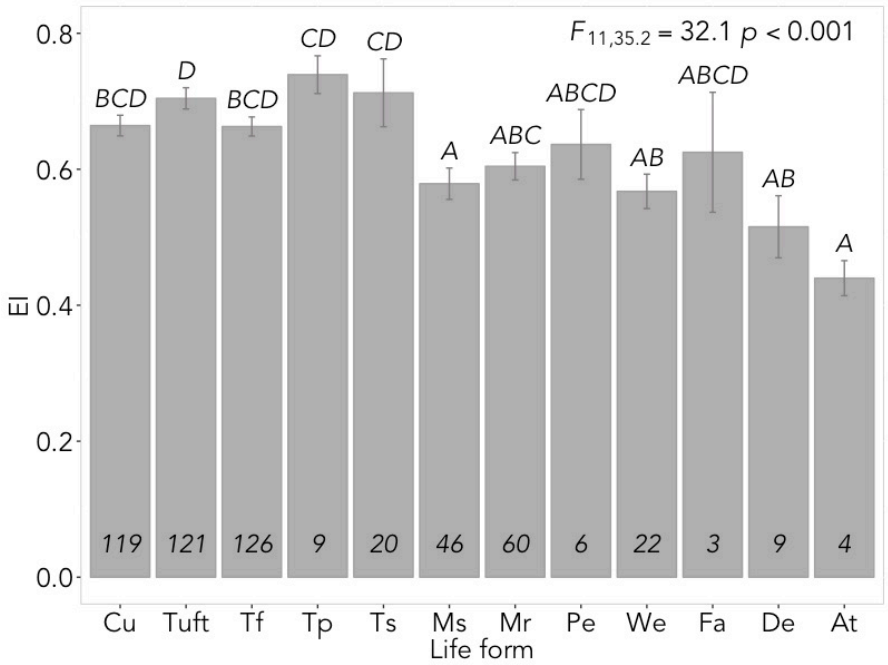


Figure 3.23 Mean EI (±1SE) in each life-form category. See Table 3.28 for significant differences in mean EI between groups.

Table 3.28 Life-form groups with significant differences between their mean EI (Games-Howell post-hoc test, p<0.05). Ordered alphabetically by life-form.

Comparison	Difference in mean EI between groups	t-value	df	p-value
Cu:At	-0.22	7.499	5.4	0.00787
Mr:At	-0.18	5.461	7.4	0.01700
Tuft:At	-0.26	8.77	5.6	0.00324
Tuft:Mr	-0.08	3.456	125.3	0.03460
Tuft:Ms	-0.12	4.503	88.9	0.00117
Tuft:We	-0.13	4.34	36.2	0.00534
Tf:At	-0.22	7.601	5	0.00960
Tp:At	-0.3	7.903	9.3	0.00062
Tp:De	-0.22	4.186	13.2	0.03108
Tp:Ms	-0.16	4.445	21.1	0.00915
Tp:We	-0.17	4.382	21.6	0.01010
Ts:At	-0.27	4.856	21	0.00369

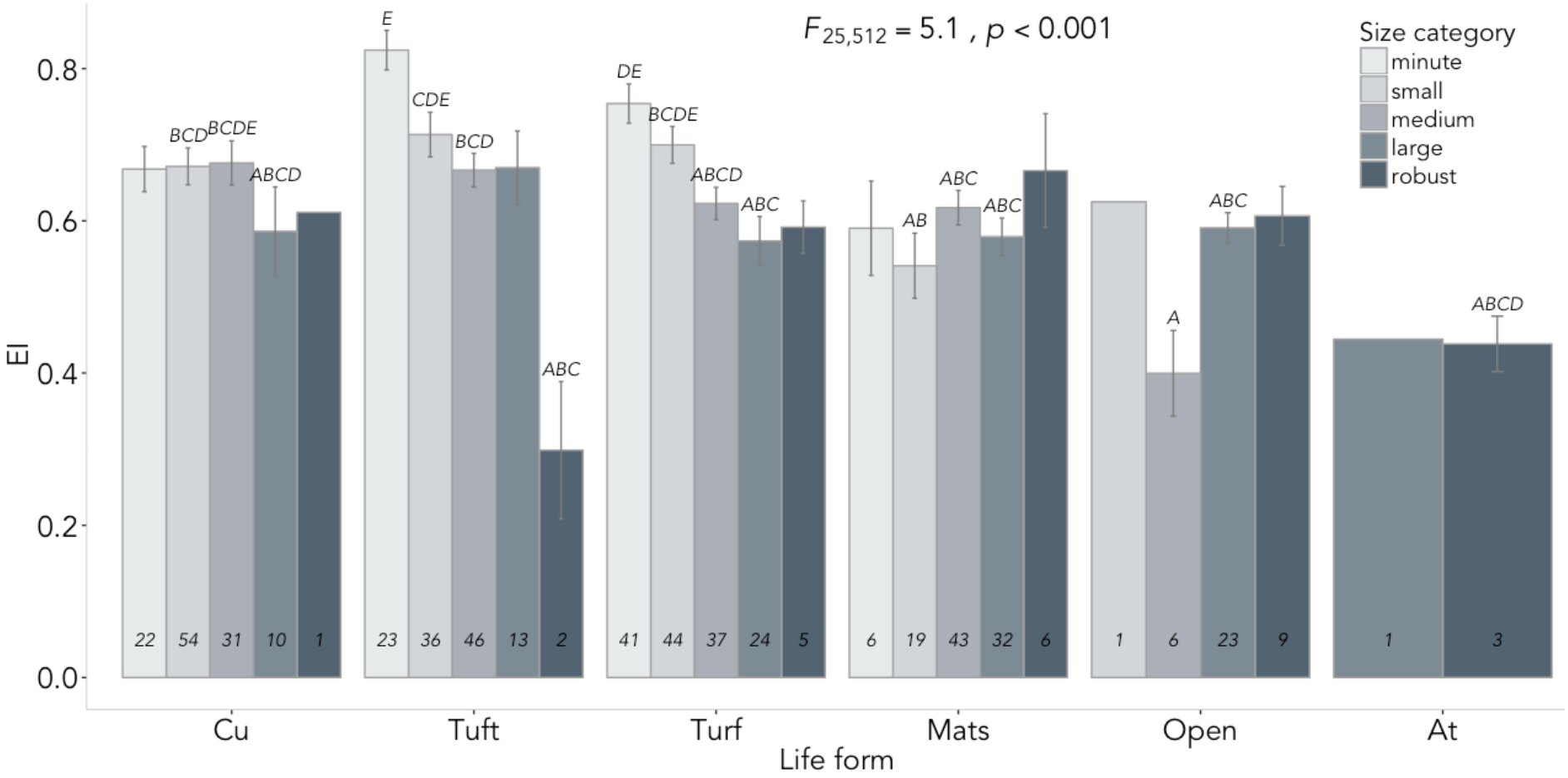


Figure 3.24 Mean EI (±1SE) of each life form category per size category (shaded in greys) in mosses – significantly different means are indicated by letters (p<0.05, Tukey multiple comparisons on least-square means), means with no letters are not significantly different from any other mean. Number of taxa per category are shown at base of bars

Chapter 4 Identifying bryophyte indicator taxa

Abstract

Ecological indicators are species, or groups of species, that provide insight into how a habitat, ecosystem, or landscape is affected as a result of anthropogenic change. They provide a cost-effective and rapid method of assessing biodiversity in order to inform conservation management decisions. Birds and invertebrates are popular indicators but bryophytes may also prove to be successful indicators, as they possess the key features of a suitable indicator. Bryophytes have been successfully used as environmental indicators (e.g. air pollution) but have also recently been proposed and used as ecological indicators albeit by only a few studies.

In forests, bryophyte reliance on microclimate and microhabitats make bryophytes particularly susceptible to disturbances due to a decrease in humidity and increase in incolation often associated with forest degradation. Species that are less desiccation tolerant, and therefore require more humid and sheltered conditions, will be more susceptible to forest degradation than more tolerant species. This means that they have the potential to indicate fine-level changes not detectable by other taxonomic groups. Added to the fact that bryophytes are understudied, especially in tropical forests, exploring their potential as indicators will add substantially to the current state of knowledge on the tropical bryoflora.

As seen in the previous chapters, bryophytes have varying degrees of desiccation tolerance which means bryophytes will respond differently to forest degradation. Using this, an indication index was created based on species' environmental preferences and desiccation tolerance. To create the index, the similarity of species based on their traits was determined using a principal component method – Multiple Correspondence Analysis (MCA). Following this, species were grouped through hierarchical clustering (with ward linkage), based on their trait similarity and their response to light and humidity (using their EI, and also their moisture and light values – assigned in Chapter 3). Species were allocated to two groups: a group of species that prefer humid and sheltered conditions, and another group that prefer dry and exposed environmental conditions. Two trait profiles were then determined based on the suite of traits (indicator traits) in each cluster that most characterise species from the two groups. An indication index was derived based on the group a species was allocated to and the proportion of indicator traits present in that species. Whereas the previous analyses (and those in Chapter 3) used only species that had environmental data, this step also included species that had missing environmental or trait data. An indicator value (IV) was also assigned to genera and families.

Species, genera and families were identified that indicate particular environmental conditions, and therefore can potentially indicate changes in forest integrity. Species that indicate humid and sheltered conditions are also those that have open life-forms and are large. Most epiphytic species are indicators of drier and more exposed conditions. The indicator index created therefore does reflect the different responses of bryophyte species. Based on these findings, the next chapter will validate species' IVs and the index through field sampling of bryophytes in a Malagasy forest.

4.1 Introduction

Indicators are used to detect changes in ecosystems, which can range from soil quality to habitat integrity, as a result of anthropogenic disturbances (Niemi & McDonald, 2004). Indicators are particularly useful in tropical research due to the high level of threat facing tropical biodiversity, the lack of data (which is particularly applicable to bryophytes (Hallingbäck & Hodgetts, 2000)), and the fact that research is often costly (Gardner et al., 2008). Using indicator species or assembleges of species as surrogates for biodiversity therefore provides a short-cut to assess biodiversity, either to provide forewarning to change, understand why the change is happening, predict potential ecosystem changes, or used to monitor biodiversity trends (Niemi & McDonald, 2004). Indicators can be used to assess abundances/diversity of other species, biodiversity patterns or changes in ecological integrity (Frego, 2007; Gardner et al., 2008). Because there is a wide literature on the subject of indicators, the focus here is on the tropics and bryophytes.

An ecological indicator must possess certain key features to be a useful, reliable and effective indicator of ecosystem changes (Table 4.1). The best indicators are: species with specific habitat and environmental requirements (Butler et al., 2012); species that can indicate whether degraded habitats can provide refuge for other taxa; suites of taxa that characterise undisturbed habitats; taxa that can be used as surrogates for other taxa (Gardner et al., 2008).

Table 4.1 Features of an ideal ecological indicator. Compiled from Frego, 2007; Gardner et al., 2008.

Feature
Ecological requirements
Respond to changes in the environment
Surrogate for other taxon groups
Ecological ranges known
Distribution known
Practical requirements
Easily sampled
Easily identified
Cost-effective
Widespread
Perennial

4.1.1 Bryophytes as indicators

Because bryophytes absorb water and nutrients directly through their leaves and are not able to regulate water uptake and gas exchange, they can accumulate large amounts of chemical compounds present in their surrounding environment (Vanderpoorten & Goffinet, 2009). As such, since the 1960s bryophytes have been used as successful indicators of air pollution (Winner & Bewley, 1978), heavy metal pollution (e.g. Burton & Peterson, 1979; Figueira et al., 2002) and water pollution (Heino et al., 2005), overwhelmingly in temperate regions of the world (Frahm, 2003). Researchers can either record the presence and abundance of species growing naturally within an area (and potentially monitor them over time) or place specific bryophyte species at particular locations to monitor the levels of pollutants by subsequently measuring the pollutant concentrations in the bryophyte's tissues (e.g. Meyer et al., 2012). The former method either

relies on creating indices based on species community composition and species abundance (e.g. (e.g. Aguiar et al., 2010; Delgado & Ederra, 2013) or pollutant concentrations can be measured from collected samples (e.g. Aceto et al., 2003).

Although bryophytes have been used as environmental indicators for several decades, only recently have they been put forward as ecological indicators (Salazar Allen et al., 1996) (Drehwald, 2005) with a few studies showing that they can be useful indicators of diversity levels in other organisms (e.g. Leal et al., 2010). A handful of studies have shown that bryophytes have great potential as indicators of habitat change (Drehwald, 2005; Frego, 2007) yet this important application remains under-studied, particularly in tropical Africa.

As described in Chapter 1, bryophytes are an important component of forests, particularly in the epiphytic layer (Frego, 2007; Wagner et al., 2014) and central to forest productivity. Frego (2007) provides a good overview of the criteria that make bryophytes suitable indicators of forest disturbance, highlighting that several studies have either directly used bryophytes as successful indicators of environmental change (particularly air and heavy metal pollution), or have tested their potential application. The main features which makes bryophytes suitable environmental indicators are: they are sensitive to changes in the forest ecosystem; they can indicate the species richness of other taxonomic groups at varying scales, from micro to macro; and their responses to changes in their habitat is varied between species (Hylander & Dynesius, 2006; Frego, 2007). There is a need for indicators as biodiversity as whole is difficult to measure and so there is value in exploring the use of bryophytes for such a task (Frego, 2007).

4.1.1.1 Features that make bryophytes suitable ecological indicators

Respond to changes in forest integrity

Gardner et al. (2008) identified that the most informative indicators were those that were most sensitive to habitat changes, not only large-scale changes but also within-habitat changes – bryophytes, due to their reliance on microhabitats, fit well within this criteria. The rationale behind the usefulness of bryophytes to indicate habitat change lies in their rapid responses to changes in insolation and relative humidity (Frahm & Gradstein, 1991; Sporn et al., 2009). In addition, as discussed in Chapter 2 (section 2.2.5, p. 80), different bryophyte species have different sensitivities to changes in their environment, which allows finer-scale habitat changes to be monitored or detected (Frego, 2007) by using a suite of species. Desiccation-intolerant shade epiphytes are particularly susceptible to increases in air circulation and solar radiation which result from anthropogenic habitat degradation (Acebey et al., 2003).

It is important to take into account the fact that many bryophyte species are locally rare (Birks et al., 1998; Frego, 2007) and so may be misleading when being used as indicators. A suite of bryophyte species would therefore be a more suitable approach than a single-taxon indicator.

Surrogate for other taxon groups

Several studies have found that bryophyte species richness can be used as surrogate for other taxon group richness as well as for habitat characteristics (Table 4.2). Scale is important with most studies finding that bryophyte surrogacy is strongest at smaller spatial scales (Frego, 2007), which may limit the use of bryophytes as indicators at a landscape level. Although these studies indicate that bryophytes may act as useful surrogates, there is variation among studies due to the scale of

the study, with some finding significant high surrogacy correlation rates and other studies finding no significant correlations (Table 4.2). Differences in sampling methodology and classification of forest degradation may be a factor in the variation in bryophyte surrogacy, as well as using different measures (e.g. species richness, species diversity, species richness/ha, bryophyte cover) (Frego, 2007; Karger et al., 2012). Nonetheless, bryophytes can provide insights into responses of biodiversity, as long as the study goal is clear and the sampling design explicit to avoid misinterpretations of results.

Table 4.2 Variation in surrogacy values for bryophytes. Compiled from Frego, 2007; (Karger et al., 2012). Significance levels: *ns*- not significant, * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

Bryophyte indicator metric	Surrogate	Correlation ranges (r^2)	Source
Taxon surrogacy			
species richness	vascular plants	0.76*** to 0.80***	Frego, 2007
	fungi	-0.52*ns* to 0.72*	Frego, 2007
	birds	0.64***	Frego, 2007
	lichens	0.47*** to 0.56***	Frego, 2007
	gastropods	0.55*** to 0.78***	Frego, 2007
	ants	0.59*	Frego, 2007
	spiders	-0.54*** to 0.39*ns*	Frego, 2007
	macroinvertebrates	0.43***	Heino et al., 2005
	fish	0.26**	Heino et al., 2005
Habitat and environmental surrogacy			
species diversity	canopy cover	0.71**	Frego, 2007
bryophyte cover	air humidity	0.36* to 0.62***	Karger et al., 2012
bryophyte cover	temperature	0.36*	Karger et al., 2012

Ecological ranges known

Some bryophytes display high substrate specificity but wide-ranging climate preferences – substratoid – whereas others are specific to a narrow climate conditions but a range of substrates – climatoid (Barkman, 1969). It is important to determine which category each species falls into as this will affect how effective it is as an indicator. Their reliance on microclimate and habitat means that the bryophyte community can inform habitat conditions, and variation within habitats (Dierßen, 2001). However, desiccation tolerance means that some bryophytes can subsist beyond their preferred ecological conditions and so survive for long periods of time after habitat disturbance; this can potentially confound results of indication studies (Fenton & Frego, 2005). However, there are bryophytes that will equally not survive disturbance and in fact will quickly respond to changes (Frego, 2007). Using a suite of indicator species that have varying responses to microclimatic changes (and therefore variation in DT) is the most appropriate approach for bryophytes. Although ecological ranges are relatively well established for temperate bryophytes (e.g. European bryophytes) this data is lacking for most tropical bryophytes and is therefore an important avenue for research.

Distribution known

Historically, bryophytes have been overlooked and so there is a lack of both historical data and distribution data. This affects the use of bryophytes when looking at temporal changes of habitat integrity (Frego, 2007). Bryophytes may not therefore be suitable for assessing historical changes in ecosystems. Geo-referencing and mapping historical collections (i.e. herbarium specimens) may help in redressing this lack of data.

Easily sampled

How easy it is to gather data on the taxa when in the field is also an important consideration when selecting indicators. Collecting bryophytes requires no particularly special techniques and necessary field equipment is simple: paper packets, hand-lens, and a small knife. This means they have great potential to be used in forest monitoring programs as training people is straightforward and equipment relatively inexpensive (for example in contrast with birds or insects). As a side note, this ease of collection can also allow bryophytes to be collected during studies that focus on other taxa – this historically was the case in many botanical expeditions where bryophytes were also incidentally collected. Although obviously this sampling strategy cannot provide an accurate depiction of bryophyte ecology or community dynamics, it can add to species lists.

Aditionally, bryophytes can be easily collected and taken back to the laboratory for identification. Although this is true of all plant groups, a further advantage of bryophytes is that they are small (therefore easy to transport and reducing transport costs) and their collection and storage is relatively straightforward. Whereas vascular plants require careful placement on sheets, pressing and drying prior to shipment to avoid damage to specimens, bryophytes can be easily dried and stored in envelopes and so require no special preservation equipment in the field or during transport. This allows more researcher time to be spent on collection and identification, increasing cost-efficiency by maximising time spent sampling during the fieldwork.

Easily identified

The key indicator feature where bryophytes fall short is in the availability of experts – both locally and globally – and existence of floras and identification guides. For Europe and North America this is less of an issue but other areas of the world, particularly Tropical Africa, there are fewer experts and no floras for many countries, Madagascar included. A way to circumvent this problem is to use easily recognisable/measurable bryophyte traits (e.g. life-form, cover) or limit identification to higher taxonomic levels (genus or family). However, Frego (2007) states that easy-to-identify bryophytes are usually of limited indicator use as is bryophyte cover and species richness although other studies have found that the latter two can be useful for certain forest integrity metrics, see Table 4.2 below. Using a suite of species in order to create similarity indices, on the other hand, has proven an effective indicator of biodiversity (Gardner et al., 2008). A similar approach could be used for bryophytes by looking at a suite of traits and their similarity across habitats and communities.

Cost-effective

A study on the cost-effectiveness of different taxon groups found that invertebrates are the most cost-effective in the field compared to vertebrates although more time is spent on identification post-field (Gardner et al., 2008). They additionally found that the most cost-effective ecological

indicators were among the cheapest to survey. Bryophytes are similar to vertebrates in this aspect as they are easy to collect in the field and require no specialist equipment to collect or survey. The cost-effectiveness of bryophytes is therefore likely to be high. Aditionally, taxa that can act as surrogates for a wide range of taxa are the most cost-effective (Gardner et al., 2008) and, as mentioned above, bryophyte response has been found to mirror that of a variety of taxonomic groups (see Table 4.2 below).

Widespread

Bryophytes are one of the most successful plant groups as they are found on every continent (except hornworts, which are not known from Antarctica) and all terrestrial habitats (Alpert, 2000a; Vanderpoorten & Goffinet, 2009; Tuba et al., 2011; Geffert et al., 2013). Due to their reliance on microhabitats, bryophytes tend to be locally rare but regionally or globally widespread as they have long dispersal ranges (Rydin, 2009; Gabriel et al., 2011). Common species may be useful as their widespread distribution means they can be included in indicator indices from different regions (Drehwald, 2005; Frego, 2007; Gaston & Fuller, 2008).

Perennial

An advantage of bryophytes is that, unlike most animals, they are present throughout the day and night and so collection and recording is not time-sensitive. They are also present year-round, making it logistically easy to plan fieldwork. However, the ideal time for bryophyte collection is during the wet season when the likelihood of sporophytes is greatest (as the identification of some species requires sporophytes).

4.1.1.2 Use of bryophytes as ecological indicators

Selecting the appropriate indicator species is vital to the successful prediction of forest disturbance (Butler et al., 2012). Little research exists on the selection of bryophyte species as indicators of forest disturbance. As such, selection of bryophyte indicator species in this study used a combination of methods for other taxonomic groups, mainly vascular plants and birds. The use of bryophytes as indicators of habitat change is relatively recent but they have proved to be useful (Mervin & Nadkarni, 2001). When a forest is disturbed, changes occur in the humidity and insolation levels, bryophytes are among the first species to be impacted as a result (Cordova & Del Castillo, 2001). Their sensitivity to ambient air humidity, due to the fact that they rely on water vapour or dew, means they respond to changes in forest structure. Frederick Clements (1874-1945), an American plant ecologist, noted that species are environmental indicators for documenting succession and stressful sites (Stohlgren, 2007).

Drehwald (2005) surveyed Neotropical epiphytic bryophytes and selected indicator species for different forest disturbance types. This was based on the forest disturbance each species occupied as well as the fact that they are not taxonomically problematic, are easy to identify and have recent flora descriptions. From this set of indicator species a computer program was developed in order to calculate a “naturalness index” for forest. This uses data from field surveys where the user inputs the species found.

As discussed above, bryophytes seem to fit the profile of suitable ecological indicators. However, there are naturally many interactions between bryophyte response to habitat change and biotic and abiotic variables, and so looking at bryophyte response in isolation from these variables may

confound results (Frego, 2007). Trait matrices are an important tool in plant ecology, used to investigate the relationship between a suite of traits and environmental variables, but have rarely been applied to bryophytes (Cornelissen et al., 2007). Neither have "formalized statistical morphometrics" of large datasets (Košnar & Kolář, 2009). Here, a matrix was used to investigate the relationship between morphological and reproductive traits and environmental (light (insolation) and moisture) and habitat variables.

4.1.2 Selecting indicators

The goal of the study at hand and the cost-effectiveness determines the type of indicator that is used (Niemi & McDonald, 2004; Frego, 2007; Gardner et al., 2008) as depending on the objective (e.g. measuring forest integrity, assessing the status of biodiversity in a habitat) different indicators will be more suitable. In the present study, identifying indicators that can indicate forest integrity is the aim. This can be defined as: "the capacity of an ecosystem to support and maintain a (…) community of organisms having a species composition (…) comparable to that of, and representing the full range of variability in, similar undisturbed ecosystems in the region (Frego, 2007, p. 67). Because of the numerous characteristics that define forest integrity (e.g. species diversity, productivity), indicators are of use as they allow integrity to be assessed without having to evaluate all the criteria (Frego, 2007). Initially, indicators tended to be taxon presence/absence and taxon richness (Niemi & McDonald, 2004) but several more indicator metrics have since been developed and used (Table 4.3). More recently, common species have been put forward as useful indicators. The disproportionate contribution of common species (a few species make up the largest proportion of biomass, function, spatial structure and number of individuals) means monitoring changes in their abundance can signal disturbances in the ecosystem as a whole (Gaston & Fuller, 2008). Often, indicators are selected but not empirically tested due to a lack of time and/or funding to spend on indicator research prior to a monitoring/assessment study (Frego, 2007; Gardner et al., 2008).

Table 4.3 Types of indicator metrics. Compiled from Frego, 2007; Gardner et al., 2008; Gaston & Fuller, 2008.

Type of indicator metric	
Single species presence/absence	
Single species abundance	Bryophyte cover can be used as a proxy for abundance
Species richness	
Multispecies	Multimetric indices – these can be either different species of the same taxonomic group or species from different taxonomic goups
Similarity in species composition between habitats	
Rare species	
Common species	

4.1.3 Forest bryophytes

Forest bryophytes inhabit several microhabitats within a forest, although most commonly they are epiphytic (Pócs, 1982; Vanderpoorten & Goffinet, 2009). The microclimate that surrounds an epiphytic bryophyte is determined by the forest habitat structure itself, the tree species and the

location of the bryophyte on the tree (Barkman, 1969). However, it is important not to forget ground-dwelling species as this is not only a physically different microhabitat but equally has different climatic conditions. Bryophyte species diversity is related to microhabitat heterogeneity as different microhabitats have different species, and rare species tend to be microhabitat-specific (Vanderpoorten & Engels, 2003).

Whereas vascular epiphytes tend to be more abundant in tropical forests, cryptogam (algae, lichen and bryophytes) epiphytes are found in abundance worldwide (Johansson, 1974), although in tropical areas there is a greater number of strictly epiphytic cryptogams (Barkman, 1969). Epiphytes evolved from plants growing in dark humid forests, from root climbers or those that lived in semi-desert conditions (Johansson, 1974). Vascular plant distribution is mostly dictated by edaphic and macro-climatic variables (Barkman, 1969), epiphytic bryophyte distribution is determined by microclimatic variables, predominantly moisture availability (Proctor et al., 2007; Pardow et al., 2012). As described in Chapter 1, light, humidity and temperature are the most important factors in determining bryophyte habitats – light is a consequence of the amount of sunlight that can penetrate the canopy and humidity is a consequence of this and evaporation rate (which is affected by wind) (Barkman, 1969).

Johansson (1974) states that for an epiphyte to be successful it must possess the following two characteristics: 1) produce spores/seeds that are able to establish on the host; and 2) be able to survive periods of drought. Seed and spore size and weight is therefore an important factor and so it is therefore not surprising that the groups with the most number of epiphytic species are orchids, ferns, lichens and bryophytes (orchids have very small seeds and the latter three all produce spores). Another important factor is the ability of the germinating plant to attach onto the substrate; in the case of bryophytes spores develop into protonema, a mesh-like structure which likely enables bryophytes to attach easily to the substrate. Vascular epiphytes have adapted to periods of drought by developing drought resistance through water storage capacity and rapid water uptake (Johansson, 1974) but bryophytes are desiccation-tolerant, allowing survival in more extremes of drought than other epiphytes. Johanssson & Benzing (p. 37) state that vascular epiphytes are either drought tolerant or drought avoidant – shape and texture of leaves often indicates to which group they belong. Drought avoidant species are often deciduous (e.g. *Davallia chaerophylloides* (fern), *Habenaria* (orchids) and *Liparis* (orchid)), while drought tolerant species reduced the number of leaves (e.g. *Microsorium punctatum* and *Angraecum distichum* (orchid)).

Within a forest habitat there are different climate scales that affect bryophytes: within different parts of a forest (edges and tree gaps) and within forest substrates (microhabitats). For bryophytes, the epiphytic niche is an ecosystem in itself. Along a tree, the humidity and light levels vary vertically, with more humid and sheltered conditions found at the tree base (Bader et al., 2013). This is important when thinking about DT of forest species, as a species found in the interior of a forest with dense canopy but high up the trunk or in the tree canopy can be as desiccation tolerant as a species that inhabits the forest edge (Bader et al., 2013). Bryophyte species will occupy particular parts of a tree: more desiccation tolerant species on the outer branches and less tolerant ones on the trunk (Bates, 2009). In European epiphytes, tree bark is an important determining factor as its pH, electric conductivity and roughness affects which tree species bryophytes can colonise (Hedenäs et al., 2004). Conversely, this has not been found to be the case in tropical epiphytic bryophytes (Frahm, 2003). In Mauritius, the determining factors for epiphytes' vertical distribution (of all taxonomic groups) was found to be light intensity and height

above ground (Tixier & Guého, 1997). Within a tropical forest different strata have different epiphyte communities and can be roughly divided into two types: the ground – mosaic formation and Top canopy – larger surface area than ground layer (Tixier & Guého, 1997)

Vascular epiphyte flora in Africa is mainly composed of ferns and orchids; in South America bromeliads and cacti; in Australasia also ferns and orchids as well as the angiosperm families Asclepiadeaceae and Rubiaceae (Johansson, 1974). Highest epiphyte densities are found where precipitation levels are high (Johansson, 1974). In a study looking at epiphyte vegetation in a tropical forest in Vietnam, Tixier (1966) found that mosses were more abundant than liverworts in high altitude rainforest and in fact more species rich than other epiphyte groups (cryptogams (including liverworts), orchids and other seed plants). Bryophytes make up one-third of the epiphyte species and can have the highest biomass of all epiphytic groups, particularly in tropical forests (Benzing, 2009; Wagner et al., 2014). According to Barkman (1969) the osmotic potential of epiphytes decreases in winter, when it is cold, but also when conditions are dry, suggesting that low osmotic potentials are an adaptation both to cold and drought.

As bark does not store water, and temperatures can be high in a forest, the epiphyte substrate can be viewed as a somewhat xeric habitat (Bader et al., 2013). This would mean that within a forest habitat, that is overall humid and sheltered, there are areas of higher insolation and lower humidity and so species will have different levels of DT. However, due to the lack of prolonged drought periods in forests (with the exception of dry forests) species are not under the same stress as those in dry and exposed habitats and so forest epiphyte species do not need the same level of desiccation tolerance.

4.2 Aim

The overall aim of this chapter is to identify taxa that can be used as indicators of particular environmental conditions, namely humid and sheltered, and dry and exposed, and so create a multi-species indicator to indicate forest degradation. This will be achieved through the following sub-aims:

1. Test if the EI varies significantly with ecological, habitat, distribution and conservation traits.
2. Identify trait profiles that represent species of different environments, namely: dry and exposed, and humid and sheltered.
3. Create an indicator index from the trait profiles identified and assign each taxa on the database with an indicator value.
4. Assign genera and families an IV in order to see if these taxonomic levels can be used as indicators.
5. Test if the indicator index varies significantly within certain easy-to-measure traits: life-form, plant size and presence of leaf papillae; and selected ecological, habitat, distribution and conservation traits.

4.3 Methods

This section begins by describing the methods used to record additional variables for species on the database and then describes the multivariate analyses and methods used in defining indicator species.

4.3.1 Recording and categorising habitat variables

Further to the morphological, reproductive, life-history and environmental traits recorded in chapter 3, additional ecological, distribution and conservation variables were recorded. The method for obtaining these is outlined below.

4.3.1.1 Species distribution

Due to the lack of habitat and distribution data available for Malagasy species, mapping of specimens was undertaken in order to be able to obtain this data for species. To map species distributions, publications and herbarium specimens were used. With the advent of digitisation of herbarium collections, a large amount of georeferenced data is available online. The main sources used in this study were Tropicos® (Missouri Botanical Garden's online herbarium database, (Missouri Botanical Garden, 2014)), Geneva Herbarium, Paris herbarium (PC) and GBIF (Global Biodiversity Information Facility). About 40% of Malagasy bryophyte specimens on GBIF have geographic coordinates, with around 80% of those coordinates being accurate.

To increase the amount of georeferenced species data available, manual georeferencing of herbarium material from BM and PC was undertaken, both from their online databases and the herbarium itself. Although the digital PC database is on GBIF, only 2% of PC specimens have geographic information (84 out of 4017). The data from GBIF was checked for accuracy and corrected wherever the geographic coordinates were incorrect. As locality data is scarce for Malagasy bryophytes, georeferencing of specimens was attempted whenever possible based on label information. Madagascar herbarium specimen data is distributed throughout several herbaria in the world, but most collections are at Antananarivo (Tsimbazaza Botanical Garden - TAN), Paris (PC), Geneve (G), Missouri Botanical garden (MO) and the Natural History Museum (BM). Most collections' locality information is too broad to georeferenced accurately and only those that had a specific location were used. For specimens that had missing locality data, but where the collector and precise date was known (day, month and year) it was possible to extrapolate the location from specimens collected on the same day by the same collector. While georeferencing, incorrect data was corrected including species names, locality description and geographic coordinates. In total 597 specimens were georeferenced (102 species from 37 families and 13 orders), mostly from herbarium specimens (from the Natural History Museum London, BM and Paris Natural History Museum, PC) but some also from online databases (TROPICOS and GBIF). Of these specimens, most are from the subhumid forests of northern and eastern Madagascar. This distribution also highlights some of the classical collection localities of bryophytes: in the northeast and from the centre to the east coast of Madagascar (Figure 4.1).

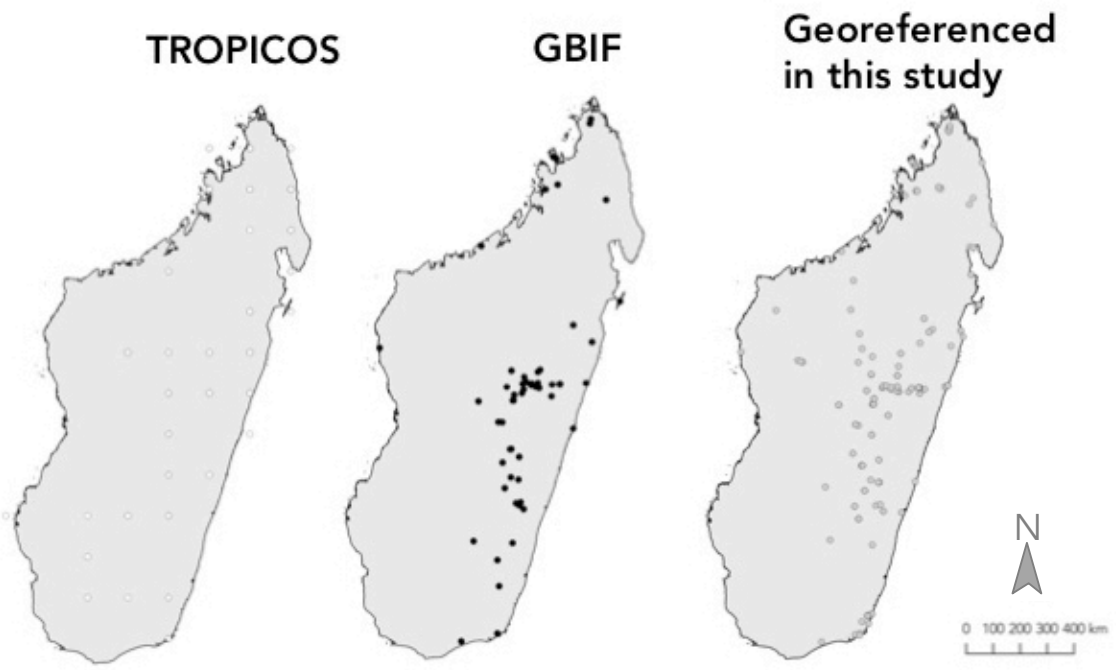


Figure 4.1 Distribution of specimens from different online repositories, TROPICOS and GBIF, and specimens manually georeferenced in this study. TROPICOS specimens are georeferenced to the nearest latitude and longitude intersection. The datasets on the middle and right-hand maps were used in this study.

Following geo-referencing, species locations were overlaid with habitat (Moat & Smith, 2007), elevation and protected area data (REBIOMA, 2012) and values for each layer extracted per specimen.

4.3.1.2 Ecological traits

Substrate

The type and number of substrates a species is found in was recorded. Substrate was simplified into 4 categories - epiphyte, saxicolous, terricolous and other- in order to account for the differing level of detail in different sources. Further to this, two traits were created: a binary trait indicating if a species is an epiphyte or not and a trait with the number of substrates a species is found in.

In texts where substrate was given to a detailed level (e.g. up to 11 in BRYOATT (Hill et al., 2007)) the grouping in Table 4.4 was used. This also allowed data from species to be included whose habitat requirements are less well known. Palms and tree ferns were considered epiphyte host (as opposed to epiphyll) but it was noted whether it was a palm or tree fern. When "other" the specific substrate was recorded. It is known that epiphytes that live in the canopy are more DT than those lower down the tree (lower branches and trunk), and so where available this information was recorded.

Table 4.4 Substrate categories used in this study with detailed substrate categories from the literature included in them.

Substrate category	Specific substrates included
Epiphyte	Trunk, branch, twigs, roots, tree ferns, palms
Saxicolous	Rock (hard & soft), walls, cliff
Terricolous	Soils, peat, gravel, sand, soil on rock, humus
Other	Bryophyte, dead or decaying vegetation (including logs), epiphyllous

As with the morphological and reproduction traits in Chapter 3, when recording from the literature, priority was given to region specific texts (e.g. Portuguese Red List or African floras). If data was not available from these, then other literature sources were used (e.g. Neotropical flora). When recording substrate from herbarium specimens, the main substrate was considered to be when at least 50% of specimens were found in that type of substrate (following (Reese, 2001)).

Species that occupied only one substrate type were classified as "specialists" and all other as "generalists".

Habitat type

Although the IUCN habitat classification is used in order to be in line with IUCN Red List categorisation, these are too broad for most of the Madagascar habitats and some of the Portuguese ones. Therefore the EUNIS habitat classification (European Environment Agency, 2012) and the vegetation classification from Moat & Smith (2007) for Malagasy species (Figure 4.2 and tables in Appendix 4, A4.1, p. 229 for list of habitat categories) are used in this study. Another alternative to achieve uniformity is to use the eco-regions outlined in Olson *et al*. (2001), but again, these provide a too broad classification for Malagasy habitats – seven versus sixteen in Moat & Smith (2007); see Figure 4.2. It would be a valuable system to use if looking at a global study of bryophytes, however, and it would allow such a study to be compared to studies on other taxa using the same classification system. Therefore this was also included (see 4.3.1.3 below). Similarly as with substrate above, two traits were created: a binary trait indicating if a species occupies a forest habitat or not and a trait with the number of habitats a species is found in.

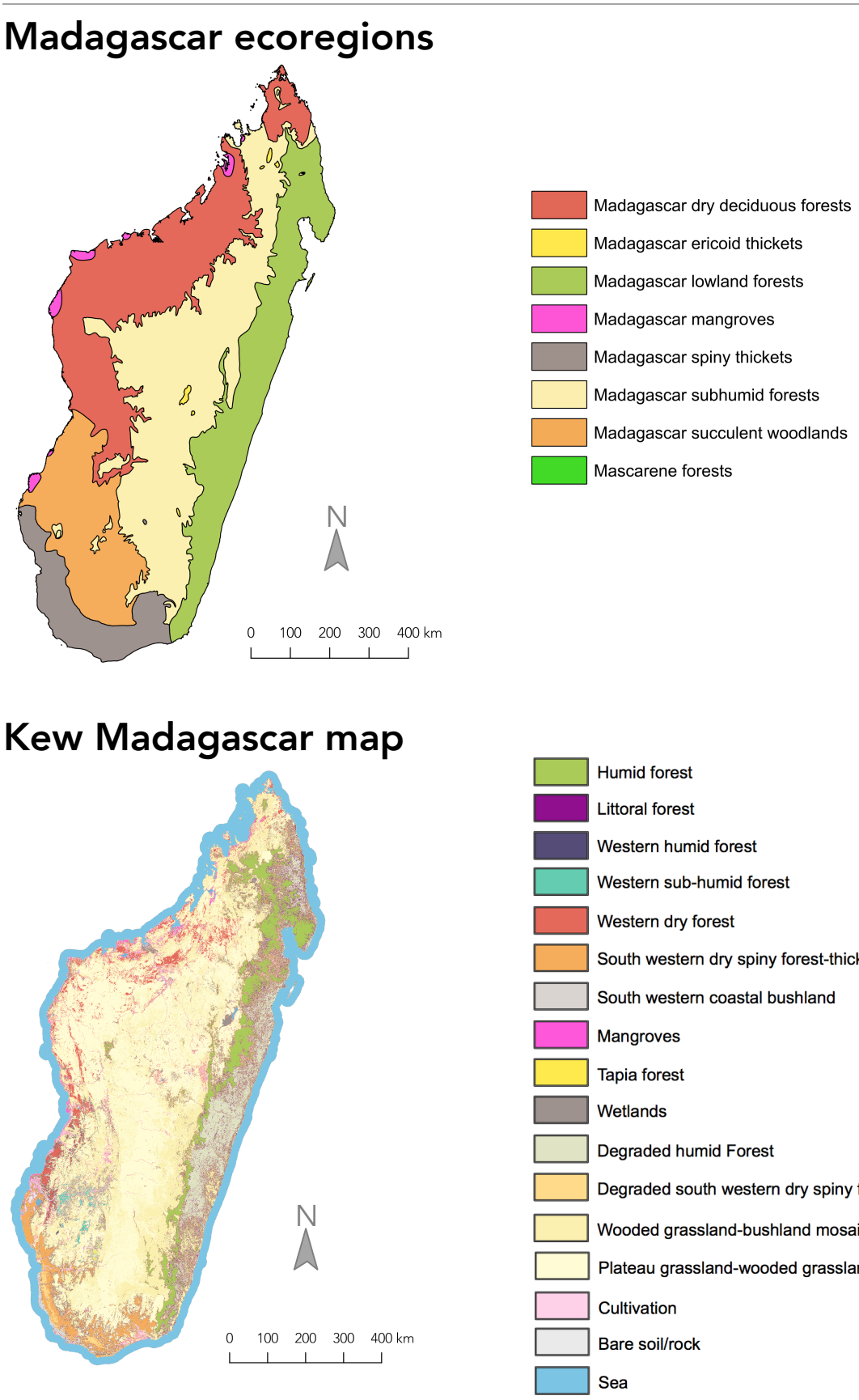


Figure 4.2 Madagascar habitat classification systems showing that the Kew classification provides a much greater level of detail than ecoregions. Maps created with data from Olson et al. (2009) and Moat & Smith (2007).

Habitat specialization

Species were classified into how much of a habitat specialist they are based on the number of broad EUNIS habitats they inhabit (Table 4.19, p. 231, Appendix A4.1). There are 10 broad EUNIS habitat categories.

Table 4.5 Habitat specialisation categories based on number of broad EUNIS habitats occupied.

Number of broad EUNIS habitats occupied - A to J	Habitat specialisation
1	specialist
2	narrow
3	narrow
4	widespread
5	widespread
6	widespread
7	widespread
8	very widespread
9	very widespread
10	very widespread

4.3.1.3 Distribution

Biogeographic realm

The biogeographic realms occupied by a species was recorded and an additional variable calculated from it: number of biogeographical realms. The distribution was recorded from Dierßen (2001) and also from floras, checklists and species publications, with the latest publications used if there was different information (particularly an issue in bryophytes due to the continuing exploration of bryoflora in previously understudied areas). The eight biogeographical realm classification from Olson et al. (2001) was used.

Table 4.6 Biogeographical realms used in this study (classification from Olson et al., 2001).

Biogeographical realm
Afrotropical
Indomalayan
Neotropical
Australian
Palearctic
Antarctic
Nearctic
Oceania

Altitude

Altitude was recorded as metres above sea level (asl) in four variables – minimum, maximum, median and range. Because many data on altitude are given as an approximation (by being rounded up or down), the median altitude was used in statistical analyses.

4.3.1.4 Conservation

Threat status

Threat status is the Red List status of a species; this is straightforward for Portugal due to the recently published Red Data Book (Sérgio et al., 2013), however, Madagascar data are much more scarce (Table 4.7) and a combination of sources were used: the Bryophyte World Red List (IUCN SSC Bryophyte Specialist Group, 2016); the Reunion Red List (Ah-Peng, Bardat, et al., 2012); and floras – Kenya and South African (Magill, 1981; Chuah-Petiot, 2003, respectively). Although these two floras do not have IUCN categories they sometimes indicate if a species is infrequent or common and so an infrequent species was considered VU (vulnerable) and common as LC (least concern). In Dierßen (2001) *Calymperes erosum* Müll.Hal. (a mostly tropical species found in Madagascar) is listed as VU, and so this information was also included. For IUCN Red List categories see Appendix 1, A1.2, p. 49.

The threat data for Madagascar must be viewed with caution, however, as it is taken from other countries and so a species may not be experiencing the same level of threat. It is a starting point for subsequent assessments of Malagasy species, but is not used in analysis in this study due to the unreliability of the data.

Table 4.7 Malagasy species with threat data available and the source it was taken from.

Species	Taxonomic division	Threat category	Source
Acanthocoleus madagascariensis	Marchantiophyta	NT	Ah-Peng, Bardat et al., 2012
Aneura pseudopinguis	Marchantiophyta	DD-n	Sérgio et al., 2013
Atrichum androgynum	Bryophyta	DD-n	Sérgio et al., 2013
Bryum alpinum	Bryophyta	LC	Sérgio et al., 2013
Bryum argenteum	Bryophyta	LC	Sérgio et al., 2013
Bryum caespiticium	Bryophyta	LC	Sérgio et al., 2013
Bryum capillare	Bryophyta	LC	Sérgio et al., 2013
Bryum erythrocaulon	Bryophyta	VU	Magill, 1981
Calymperes erosum	Bryophyta	VU	Dierßen, 2001
Calypogeia arguta	Marchantiophyta	LC	Sérgio et al., 2013
Campylopus flexuosus	Bryophyta	LC	Sérgio et al., 2013
Campylopus pilifer	Bryophyta	LC	Sérgio et al., 2013
Caudalejeunea grolleana	Marchantiophyta	EN	World Red List 2016
Caudalejeunea grolleana	Marchantiophyta	EN	World Red List 2016
Cephalozia connivens	Marchantiophyta	VU	Sérgio et al., 2013
Cyclodictyon laetevirens	Bryophyta	CR	Sérgio et al., 2013
Dumortiera hirsuta	Marchantiophyta	VU	Sérgio et al., 2013
Erpodium beccarii var. beccarii	Bryophyta	LC	Chuah-Petiot, 2003
Eurhynchium striatum	Bryophyta	LC	Sérgio et al., 2013
Fabronia garnieri	Bryophyta	LC	Chuah-Petiot, 2003
Fissidens asplenioides	Bryophyta	LC	Chuah-Petiot, 2003
Fissidens ovatus	Bryophyta	LC	Chuah-Petiot, 2003
Frullanoides tristis	Marchantiophyta	DD	Ah-Peng, Bardat et al., 2012
Hypnum jutlandicum	Bryophyta	LC	Sérgio et al., 2013
Hypopterygium tamarisci	Bryophyta	CR-int	Sérgio et al., 2013

Species	Taxonomic division	Threat category	Source
Leptobryum pyriforme	Bryophyta	EN	Sérgio et al., 2013
Macrocoma tenuis	Bryophyta	LC	Chuah-Petiot, 2003
Mastigolejeunea auriculata	Marchantiophyta	DD	Ah-Peng, Bardat et al., 2012
Mittenothamnium madagassum	Bryophyta	LC	Chuah-Petiot, 2003
Nogopterium gracile	Bryophyta	LC	Sérgio et al., 2013
Pohlia elongata	Bryophyta	LC	Sérgio et al., 2013
Polytrichastrum formosum	Bryophyta	LC	Sérgio et al., 2013
Polytrichum commune	Bryophyta	LC	Sérgio et al., 2013
Polytrichum piliferum	Bryophyta	LC	Sérgio et al., 2013
Racopilum africanum	Bryophyta	LC	Chuah-Petiot, 2003
Sphagnum cuspidatum	Bryophyta	LC	Sérgio et al., 2013
Symbiezidium madagascariensis	Marchantiophyta	EN	World Red List 2016
Thysananthus spathulistipus	Marchantiophyta	NT	Ah-Peng, Bardat et al., 2012

Rarity

As mentioned above, the Kenyan and South African floras (Magill, 1981; Chuah-Petiot, 2003, respectively) state how rare certain species are and so this was recorded in the case of Malagasy species. However, as with the threat categories above, because the knowledge of the Malagasy flora is poor, it is not possible to verify if a species that is rare in Kenya or South Africa is equally rare in Madagascar. This trait is therefore not used in analyses. Additionally, only data for ten species was available (Table 4.8).

Disturbance

In some species publications and floras the habitat disturbance a species is found in (primary, degraded, agriculture, anthropogenic) was stated and so this was included in the database. However, it was not used in analyses as only 29 species with disturbance data had accurate environmental data.

4.3.1.5 Summary

Fourteen traits on species' ecology, distribution and conservation were recorded (Table 4.8). All but one trait (altitude) are categorical and two traits (rarity and disturbance) are not included in subsequent analyses due to a lack of reliable data.

Table 4.8 Ecological, distribution and conservation traits recorded in this study; the type of variable each trait is; the total number of species that had data for that trait; and the states or units (in the case of continuous traits) for each trait. CatN – categorical nominal; CatO – categorical ordinal; Con – continuous.

Trait	Variable type	Species number	State
Substrate	CatN	1029	Epiphyte, Saxicolous, Terricolous, Other
Number of substrates occupied	CatO		1 to 4
Epiphyte	CatN		0 or 1
Substrate specialisation	CatO		Generalist or Specialist

Trait	Variable type	Species number	State
Habitat type	CatN	948	See Table 4.18 to Table 4.20 in Appendix A4.1, p. 229.
Number of habitats occupied	CatO		1 to 10
Forest species	CatN		0 or 1
Habitat specialisation	CatO		specialist, narrow, widespread, very widespread
Biogeographic realm	CatN	1012	Afrotropical, Indomalayan, Neotropical, Australian, Palearctic, Antarctic, Nearctic, Oceania
Number of realms occupied	CatO		1 to 8
Altitude	Con	822	metres above sea level Range, minimum, maximum and mean
Threat status	CatN	734	IUCN red list categories
Rarity	CatN	10	common, infrequent, rare
Disturbance	CatO	173	primary, degraded, agriculture, anthropogenic

4.3.2 DT categories and EI

Species were classified into a desiccation tolerance (DT) class based on their environmental indicator (EI) value. Values were ordered numerically and then categorical limits to delimit the groups based on the lower, 2nd, 3rd and upper quartiles. Each species was then assigned a DT category, which will be used in further analyses.

Desiccation tolerance category	DT index value
Very low desiccation tolerance	≤0.528
Low desiccation tolerance	≤0.639
Desiccation tolerant	≤0.750
Extremely desiccation tolerant	>0.750

For example, *Cyclodictyon laetvirens* (Hook. & Taylor) Mitt. has a humidity value of 4 (high to moderate hygrophyte) and a light value of 3 (high sciophyte).

$$EI = \left(\frac{4}{9} + \frac{3}{6}\right) / 2$$

$$EI = 0.306$$

It is therefore classified as a species with very low desiccation tolerance.

4.3.2.1 Environmental range

The environmental range for each environmental variable (light and moisture) was calculated using the range of light and moisture classes they are found in. First the range of light and

moisture for each species was calculated separately. For example, the epiphyte *Orthotrichum philibertii* is classified as a moderate xerophyte, giving it a humidity value of 7. Because it only occupies one humidity class it has a range of 0 making it humidity specific. Another epiphyte, *Orthotrichum cupulatum* is classified as a moderate hygrophyte to a high xerophyte, which also gives it a humidity value of 7 but it has a value range of 4; this means it has a very broad humidity range (Table 4.9). A range category was then assigned to each species based on the range value (Table 4.9). Acidity was not included as acidity reflects the substrate a species lives on (e.g. rock, bark, soil), and not the ambient environmental conditions.

Table 4.9 Light and humidity range values and associated range category.

Humidity or light range value	Humidity or light range category
0	Specific
1	Narrow
2	Medium
3	Broad
4 and above	Very broad

An overall environmental range was then calculated for each species by combining the range values of light and humidity. This was calculated simply as the product of the range value of humidity (*hs*) and light (*ls*) (with 1 added to both to allow multiplication of 0 values): $environmental\ range = (hs + 1) * (ls + 1)$. This value ranges from 1 (only found in one light and one moisture class) to 20 (found in the full range of light and moisture classes) and species were assigned to an environmental range category based on this value (Table 4.10). The cut-off values for categories were based not only on the overall environmental range value, but also took into account the individual environmental variable ranges – see Table 4.10 for description of each category.

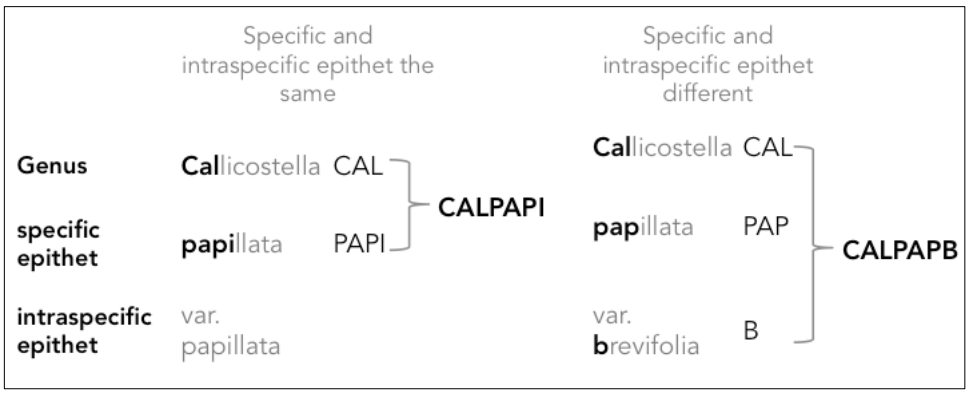
Table 4.10 Environmental range category assigned to species based on the range of environmental conditions they occur in.

Environmental range value	Environmental range category	Description
1	Specific	Species only found in one light and one humidity class.
2 3 4	Narrow	Species found in one light or humidity class, with the other variable having a narrow to broad range OR species found in a narrow range of both humidity and light.
5 6 8 9 10	Medium	Species found in one light or humidity class, with the other variable having a very broad range OR species found in a narrow range of humidity or light, with the other variable having a medium to very broad range OR species where both variables have a medium range.
12 15 16 20	Broad	Species where one variable has a medium range and the other a broad to very broad range OR both variables have a broad range OR one variable has a broad range and the other a very broad range.

4.3.3 Statistics

Following the results from Chapter 3, certain traits were re-categorised before proceeding onto multivariate analyses. Traits with missing values were interpolated based on the trait state of the other species in the same genus – this was done only for traits were there was no variability in that particular trait state within the genus and also for continuous traits were an average could be calculated (seta length and spore size).

To facilitate data analysis and visualisation, each taxon name was encoded using the first three letters of the genus and the first four letters of the specific epithet e.g. *Bryum argenteum* became BRYARG. For taxa with two or more varieties/subspecies, if the infraspecific epithet is the same as the specific epithet then it is encoded in the same manner as above (using the first three letters of the genus and the first four letters of the specific epithet e.g. *Callicostella papillata* var. *papillata* became CALPAPI); if the infraspecific epithet is different from the specific epithet then the first three letters of the genus, first three letters of the specific epithet and first letter of the infraspecific epithet was used e.g. *Callicostella papillata* var. *brevifolia* became CALPAPB (Box 4.1).



Box 4.1 Encoding system for taxa that have two or more varieties/subspecies.

Step 1. Trait database transformed into presence/absence matrix with species in rows and each trait state in columns (223 columns in total: 179 bryophyte trait states and 44 ancillary states; for list of states and associated codes see Table 4.23, p. 248, Appendix A4.3).

Step 2. Multiple Correspondence Analysis (MCA) undertaken on the presence/absence matrix – a covariance matrix is computed and eigenvalues calculated for trait states. These eigenvalues are then projected into a low-dimensional space (using euclidian distance) and patterns in the data can be seen and explored.

Step 3. Values from MCA are then used in a hierarchical clustering analysis (Euclidean distance with ward linkage) to group species according to similarity in traits and to relate these groupings to environmental, habitat and distribution traits.

Step 4. Three clusters were identified, one representative of dry and exposed environments, and another of humid and sheltered environments, named cluster 1 (C1) and cluster 3 (C3), respectively.

Step 5. Trait states that significantly characterise each cluster are tested using chi-squared tests.

Step 6. Trait states that are most represented in each cluster are identified by v-tests.

Step 7. With results from step 5 and 6, a suite of 15 trait states are selected to create a trait profile for each cluster.

Step 8. Each species is assigned a trait profile value for each cluster by adding all the profile traits and dividing by the number of traits present

Step 9. The value of cluster 1 (C1) is substracted from the value of cluster 3 (C3), to yield an indicator value (IV) – from -1 to 1, with negative values corresponding to indicators of wetter and more sheltered environments.

Step 10. Species are classified into four indicator groups based on whether or not they possess traits from both cluster trait profiles. If a species has a trait profile value of zero for one cluster, then it is classed as a "strict" indicator. The same method is applied to genera and families (Figure 4.3).

Step 11. Steps 9 and 10 are repeated to calculate indicator values (IVs) for genera using the mean IV of species within that genus.

Step 12. Steps 9 and 10 are repeated to calculate the IVs of families: the mean IV of all species within that family is calculated.

Step 13. Species can be further refined based on their environmental preference range with "narrow" species classified as the most precise indicators.

Full details on the rationale and methods are provided in the sections below.

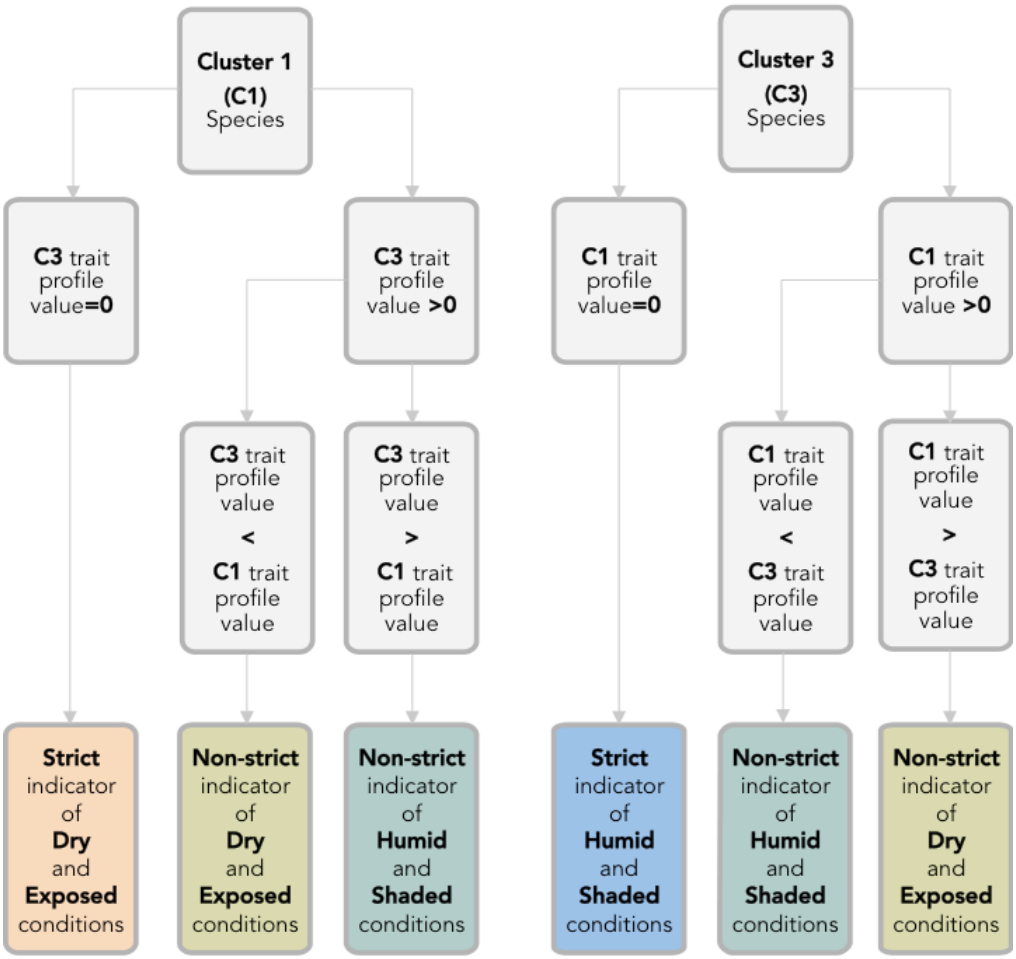


Figure 4.3 Process for assigning species to an indicator category.

4.3.3.1 Multiple Correspondence Analysis

Due to the large number of traits, a correspondence statistical method was used to identify patterns in traits. As the traits used in this study are categorical, and although there are techniques to recode categorical variables as numerical, these are not true continuous variables and so issues may arise during analysis. Rather than converting categorical variables into a continuous variable, multiple correspondence analysis (MCA) was used to identify suites of traits that indicate particular environmental conditions. Multiple correspondence analysis (MCA) is a type of principle component method (similar to Principal Components Analysis or Correspondence Analysis) but unlike these, which are for continuous variables, MCA is for categorical variables. Although a few states were assigned numerical values (e.g. leaf orientation, see Chapter 3), they are still non-equidistant ordinal variables, and therefore categorical (Pla et al., 2012). All individuals in the dataset (species in this case) have the same weight in an MCA analysis – this is of relevance here as there is no indication that some species are more important than others. Another advantage of MCA, is that different variables (traits in this case) can have different numbers of categories (trait states) without skewing the analysis results (Husson et al., 2011). The MCA eigenvalues are interpreted as the means of the squared correlation ratios and are used to validate the dimensions found – this is important when there are a large number of variables. Essentially, an MCA reduces the "noise" in the data (by calculating the principal components) and so provides stability to further analyses to be conducted on the data (Husson et al., 2011).

Variability is important in identifying how species can be grouped together – there is a balance between a trait with very high variability (where each species has a unique value) and one that has no variability (the value for the trait is the same in all species) (Husson et al., 2011). Trait selection is therefore an important consideration, as shown by results in chapter 3. Although continuous traits provide greater variation and so may group species better, sometimes they may mask difference (as discussed in Chapter 3). The continuous variables of seta length, spore size and plant size were categorised (using the categories defined in Chapter 3). Additionally, this provides an advantage in practical terms as species that lack exact data values (e.g. plant size) can be included in analyses.

The dataset is composed of two types of variables: morphological and reproduction traits, and environmental and habitat variables. In the MCA, the traits are specified as "active" and the environmental and habitat traits as "supplementary"; in other words, the MCA dimensions will be constructed using the trait data and the environmental and habitat traits can then be used to describe the patterns seen in the trait data plots and interpret the dimensions of the plots. The MCA will show how similar species are in terms of their trait composition and how the traits are related to the environment and habitat variables. An MCA transforms qualitative variables to quantitative ones by calculating their principle components so that further analyses can be done, such as clustering. MCA can be viewed as a "preprocessing step" for qualitative data (Husson et al., 2011) and clustering is usually the next step; in this case a clustering analysis will classify species based on their traits and response to the environment (see 4.3.3.2 below).

Prior to the MCA, the dataset was transformed into a complete disjunctive table: a presence (1)-absence(0) matrix with species in the rows and traits in the columns, with each column representing a state of a trait (e.g. life-form states: cushion, tuft, weft etc.) (Figure 4.4).

	A	B	C	CC	CD	CE	CF	CG	CH	CI	CJ
1	SpecieslD	Region	Species	ApexAcute	ApexRound	ApexApiculate	ApexAcuminate	ApexHair	ApexSubulate	ApexCucullate	XsectPlane
798	891	PT	DITSUBU	0	0	0	0	0	1	0	1
799	1076	PT	POGNANU	1	0	0	0	0	0	0	1
800	1253	PT	WEILONG	0	0	1	0	0	0	0	1
801	1225	PT	TORCUNE	1	0	0	0	0	0	0	0
802	736	PT	ULODONI	0	0	0	1	0	0	0	0
803	544	PT	ANDMEGI	0	0	0	1	0	0	0	1
804	1023	PT	MICDAVA	0	0	0	0	1	0	0	1
805	1072	PT	PLESUBU	0	0	0	0	0	1	0	1
806	1231	PT	TORMODI	0	0	0	0	1	0	0	1
807	797	PT	BARSTRI	0	0	0	1	0	0	0	1
808	1175	PT	SCOCIRC	0	0	0	1	0	0	0	0
809	899	PT	ENTCONV	0	0	1	0	0	0	0	0
810	919	PT	FISCRIS	0	0	1	0	0	0	0	1
811	588	PT	DIPFOLI	1	0	0	0	0	0	0	1
812	884	PT	DIDTOPH	1	0	0	0	0	0	0	1
813	1240	PT	TRICRIS	0	0	0	0	0	0	1	1
814	935	PT	FISVIRI	0	0	1	0	0	0	0	1
815	951	PT	FUNHYGR	0	0	1	0	0	0	0	0
816	1239	PT	TRIBRAC	0	0	0	0	1	0	0	1
817	733	PT	TORSUBU	0	0	0	0	1	0	0	0
818	911	PT	EURPULC	0	0	0	1	0	0	0	0
819	1217	PT	TORNITI	0	0	0	1	0	0	0	1
820	835	PT	CAMBREV	0	0	0	0	1	0	0	0
821	1177	PT	SCOSEND	0	0	0	1	0	0	0	1

Figure 4.4 Excerpt of the data matrix: each row is a different species (coded using first three letters of the genus name and first four letters of the species name) and each column is a state trait. This excerpt shows the apex type (*Apex*) and cross-section profile (*Xsect*) traits.

4.3.3.2 Clustering

Hierarchical clustering with ward linkage builds links between species, or groups of species. In this study clustering uses the similarity of species' traits and then finds variables that characterise each cluster - how traits relate to the environmental or habitat variables in this instance. Although Jaccard distances are often used in ecology due to categorical data usually being presence and absence data, because the clustering is using results from an MCA, the Euclidean distance was chosen, as it is the same measure used in the MCA. Usually initial rough partitioning is applied in a clustering of many individuals (Husson et al., 2011) however, this did not actually improve the clustering of the data in this study. Therefore, no prior partitioning was applied. The choice of number of dimensions to use from the MCA was chosen based on the percentage variance explained by each dimension; the first 50 dimensions were chosen as they explain 71% of the variation. Although the MCA plot can indicate that species are similar if they are close to one another, it only shows this along the 1st and 2nd dimensions, and we cannot know if these species are equally close along all the other dimensions (as being close on dimensions 1 and 2 does not mean that on other dimensions they are equally close together) (Husson et al., 2010). This is where clustering comes in: by seeing how closely two species are on other dimensions (in this instance the first 50). If two species are in the same cluster then they are close to each other in the other dimensions as well as dimensions 1 and 2.

The aim of the cluster analysis is to group species into a set number of clusters based on the similarity of their traits, as mentioned, and identify which trait states best characterise the clusters. Then, the traits that best describe the individuals within each cluster can be identified. We can also relate the clusters to the EI, light and moisture as well as habitat variables, because, as mentioned above, they are not used to calculate species similarity in the MCA. This will yield clusters with different environmental profiles. To test which trait states significantly characterize the clusters, chi-square tests are used. Then, to identify which trait states are most characteristic of each cluster, the proportion presence of a state within a certain cluster is compared with the proportion of the state in all individuals (the v-test).

MCA and clustering were undertaken in R using the FactoMiner (Husson et al., 2017) and factoextra (Kassambara & Mundt, 2017) packages.

4.3.3.3 Selecting indicator species

Using the clusters identified from the clustering analysis, a trait profile of 15 trait states was created for each cluster. Following selection of trait states and creation of the trait profile, a trait profile value was calculated for each species. This allowed species that had missing trait data (that were not used in the previous analyses and in the univariate analyses in Chapter 3) to be included in the indicator determination process. To determine whether to include a trait state in the trait profile, a combination of three values obtained from the clustering were used: the v-test value ($v>1.92$), the chi-squared test p-value ($p<0.01$) and the proportional representation of that state within a cluster (i.e. the proportion of species with that particular trait state occur in a cluster; >70% is the threshold used). If a trait state was present it was scored as 1, then the sum of these values for states was calculated and divided by the total number of states with non-missing data - to standardise values allowing species with traits missing to be included in the indicator pool (see Figure 4.25, p. 260, Appendix A4.4 for example).

This yields three values per species, one for each cluster trait profile (termed C1 and C3), and a third value (C1-C3) which will be used as the indicator value of that species. This indicator value is subsequently used to assign species to an indicator group. The more negative the value, the more likely a species is to indicate humid and sheltered environmental conditions. Species that had a value of zero for both C1 and C3 were determined to not be suitable indicators, as were those where C1=C3.

Indicator values were assigned to all species in the database (created in Chapter 3). First, species that are specific to either dry and exposed indicator group (C1) or the humid and sheltered indicator group (C3) are identified. This follows the rationale of Dufrêne & Legendre (1997) that an indicator species is one that is most characteristic of a group and usually found only in one group. These are considered to be strict indicators (Figure 4.3, p. 196). Species that were assigned to cluster 1 or 3, but that had some trait states that were found in the other cluster (i.e. had a positive C1 or C3 value) are delimited “non-strict” indicators. These species that had trait states found in both groups were assigned to a non-strict category based on their indicator value (C1-C3) (Figure 4.3, p. 196). Of the strict indicators, a further selection can be made based on their environmental specificity choosing those that have a narrow range as the best indicators. Species with more specific habitat and environmental requirements are more sensitive and so are the most useful indicators (Butler et al., 2012). Four indicator categories are defined: strict humid and sheltered indicator, strict wet and exposed indicator, non-strict humid and, sheltered indicator and non-strict dry and exposed indicator.

A list was also created of indicator genera. As most genera are spread out among the three clusters, the average trait profile value for that genus was calculated first and then the same method used for species was applied yielding a set of strict and non-strict indicators. In genera that have species that are both dry and wet indicators, these are classed as non-strict indicators and assigned a value based on the C1 – C3 trait profile values; this yields a value that indicates the proportion of dry or wet traits present, the more negative the value, the more likely the species is to indicate humid and sheltered conditions. For genera that had no species in the MCA and clustering analyses, due to missing environmental data, an indicator was still calculated for most

of these genera due to the presence of data on the traits. This was then repeated at the family level.

4.3.3.4 Univariate analyses

As in the univariate analyses in Chapter 3, ANOVAs were used to analyse differences in the mean EI and ecological, distribution and conservation traits, and for analyses with the IV and selected bryophyte traits. For traits with more than two states (IUCN threat category, life-form, plant size and papillosity) Games-Howell multicomparison post-hoc tests (α 0.05) were used to test for differences between states. See 3.3.2.3, p. 113, Chapter 3 for full details on ANOVA and Games-Howell.

4.4 Results

4.4.1 Relationship between the EI and habitat, distribution and conservation traits

4.4.1.1 Habitat & Distribution

When mosses and liverworts are analysed together, substrate specialists occupied slightly drier and more exposed environments than generalists (0.63±0.01SE and 0.65±0.01SE, respectively; Table 4.11). The range in EI was higher in generalist species. However, when looking within mosses and liverworts no effect on environmental preferences was found (p>0.05) (Table 4.11). This is in contrast to species' global distributions, as species with wider distributions have higher mean IVs (Table 4.11).

Epiphyte species occupied significantly more humid and sheltered habitats (p<0.001), albeit the difference between epiphyte and non-epiphytes was small, 0.065±0.01 (Table 4.11). There is also a significant difference in mean EI within epiphytic mosses but not liverworts. Forest species occupied wetter and more sheltered environments than non-forest species. The same was true for mosses but no effect was found in liverworts (Table 4.11).

4.4.1.2 Conservation

Introduced species occupy significantly drier and more exposed environments than threatened species (mean EI is 0.93±SE 0.05 and 0.62±0.01SE, respectively p<0.05) (Figure 4.5). Least concern (LC) species occupy slightly drier and more exposed habitats (0.67±0.01SE) than species in a threatened category (CR, EN or VU) (p<0.01). There was no significant difference between data-deficient (DD) and threatened species or new (DD-n) species and threatened species (p>0.05); as DD and New species have insufficient data to be assessed they cannot be compared anyway. Although for model simplification these categories should be grouped, ecologically it would not make sense to group a species that is LC with one that is DD, as the latter are species that have insufficient data to be assessed using the IUCN criteria.

Table 4.11 Summary statistics of ANOVAs of mean EI in substrate specialists, epiphytes, forest species and biogeographical realm within all bryophytes, within mosses and within liverworts.

Substrate specialism	*n*	Generalist	Specialist	Difference	*F*	DF	*p*
All	709	0.626	0.652	-0.026	4.127	1,705	**<0.05**
Mosses	543	0.652	0.649	0.003	0.052	1,541	0.820
Liverworts	166	0.689	0.652	0.037	2.092	1,164	0.150
Epiphyte substrate		**Not epiphyte**	**Epiphyte**				
All	730	0.662	0.598	0.064	24.230	1, 728	**<0.001**
Mosses	545	0.667	0.652	0.015	6.742	1, 543	**<0.001**
Liverworts	185	0.069	0.037	0.032	1.237	1, 183	0.268
Forest species		**Not in forest**	**Forest**				
All	730	0.648	0.524	0.123	23.070	1, 728	**<0.001**
Mosses	545	0.662	0.533	0.129	20.950	1, 543	**<0.001**
Liverworts	185	0.689	0.625	0.064	1.319	1, 183	0.252
Realms occupied		**1 to 2**	**3 or more**				
All	721	0.618	0.671	0.0528	18.1	1, 720	**<0.001**
Mosses	542	0.63	0.68	0.05	14.0	1, 539.1	**<0.001**
Liverworts	179	0.590	0.625	0.035	2.89	1, 130.5	0.091

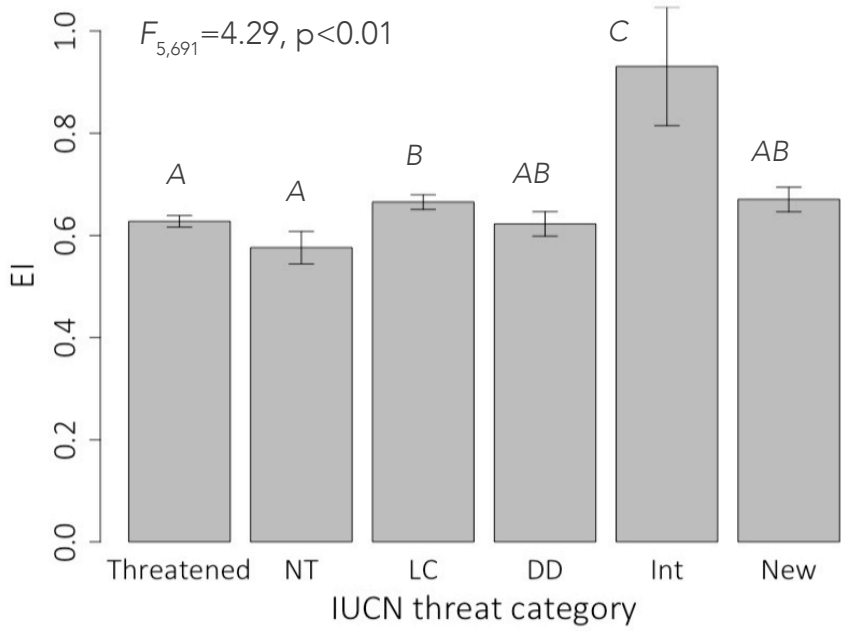


Figure 4.5 Mean EI (±1SE) of species in different threat categories. Means with different letters indicate significant differences; ANOVA and Games-Howell *p*<0.05.

4.4.2 Indicator species

4.4.2.1 MCA

The percentage of variance explained within the MCA was highest in the first 3 dimensions (Figure 4.18, Appendix IV). The variance explained by the first two dimensions is small (4.43% and 2.3%) and the contribution of each individual species to the dimensions is small, but this is expected due to the large number of species in the dataset (Husson et al., 2011). Most species were well projected on the dimensions (their cosine^2 values were close to 0). The MCA analysis showed the EI was significantly negatively associated with the first dimension (-0.40, p<0.001) as was moisture (-0.33, p<0.001) and light (-0.28, p<0.001). Average altitude was slightly positive associated with the first dimension (0.15, p<0.01) and third dimensions (0.12, p<0.05). The environmental specificity was not associated with any of the first 3 dimensions. EI, moisture and light can therefore be used to interpret the first dimension and hence how traits or species along it relate to the EI, moisture or light.

Looking at a plot of just the species, there appears to be some grouping of species, though it is not very clear. The plot of trait states, individuals and, environmental and habitat variables does not lend itself to obvious interpretation at first glance and no obvious patterns can be seen (see Figure 4.19, Figure 4.20, Figure 4.21 and Figure 4.22, p. 254, Appendix IV)– the plot was therefore manipulated to facilitate interpretation by first visualising traits (Figure 4.6 and Figure 4.7). Those that were most correlated with dimension 1 are margin denticulation, papillosity basal cell shape and basal cell differentiation. The traits that are most correlated with the 2nd dimension are type of vegetative propagules, number of vegetative propagules and presence of vegetative propagules. Many traits are not strongly linked (shown by the clustering of most around the origin). Capsule orientation and life-form are correlated with both dimensions, though more strongly with the 1st dimension. Looking within the traits, the 20 trait states that contribute the most to the construction of the plot, and are most dissimilar from each other are shown in Figure 4.7. Nodulose basal cells (nodulose), immersed capsules, keeled leaves (Xsect_keel), recurved margins (MarCurv_recurved) and the presence of papillae (Papillose_P) are associated with drier and more exposed conditions (to the left of dimension 1). On the opposite end, mats (LF_Mr and LF_Ms), a long seta (SetaAvg_long), a costa that terminates in the upper half (CostaLen_4) and undifferentiated basal cells (BasShape_0) are associated with more humid and sheltered conditions. This plot shows that vegetation propagules traits are more closely associated with dimension 2 than 1, suggesting that these traits are not as useful indicating environmental conditions. In order to visualise the distribution of species in the plot better, the 20 individuals that are best represented along with the 10 trait states and environmental values that are most important in the creation of the plot are highlighted in Figure 4.8. This plot suggests there are three species groupings, two along the 1st dimension, and one along the 2nd. Desiccation tolerance level is associated with the first axis, being negatively correlated with it as the category “Extreme DT” is found on the left-hand side of the plots, and “Low DT” on the right (Figure 4.8). Dimension 2 may be associated with species environmental range as the moisture range 0 (narrow) is at the top of the plot and light range 4 (broad) is at the bottom of the plot as shown in Figure 4.8 (but see Figure 4.22, p. 257, Appendix IV, for the location of all the environmental and habitat variables in the plot). The life-form trait is mostly partitioned along the 1st dimension, with closed life forms (cushions (LF_Cu) and tufts (LF_Tuft)) on the left-hand side and more open forms on the right (Figure 4.9).

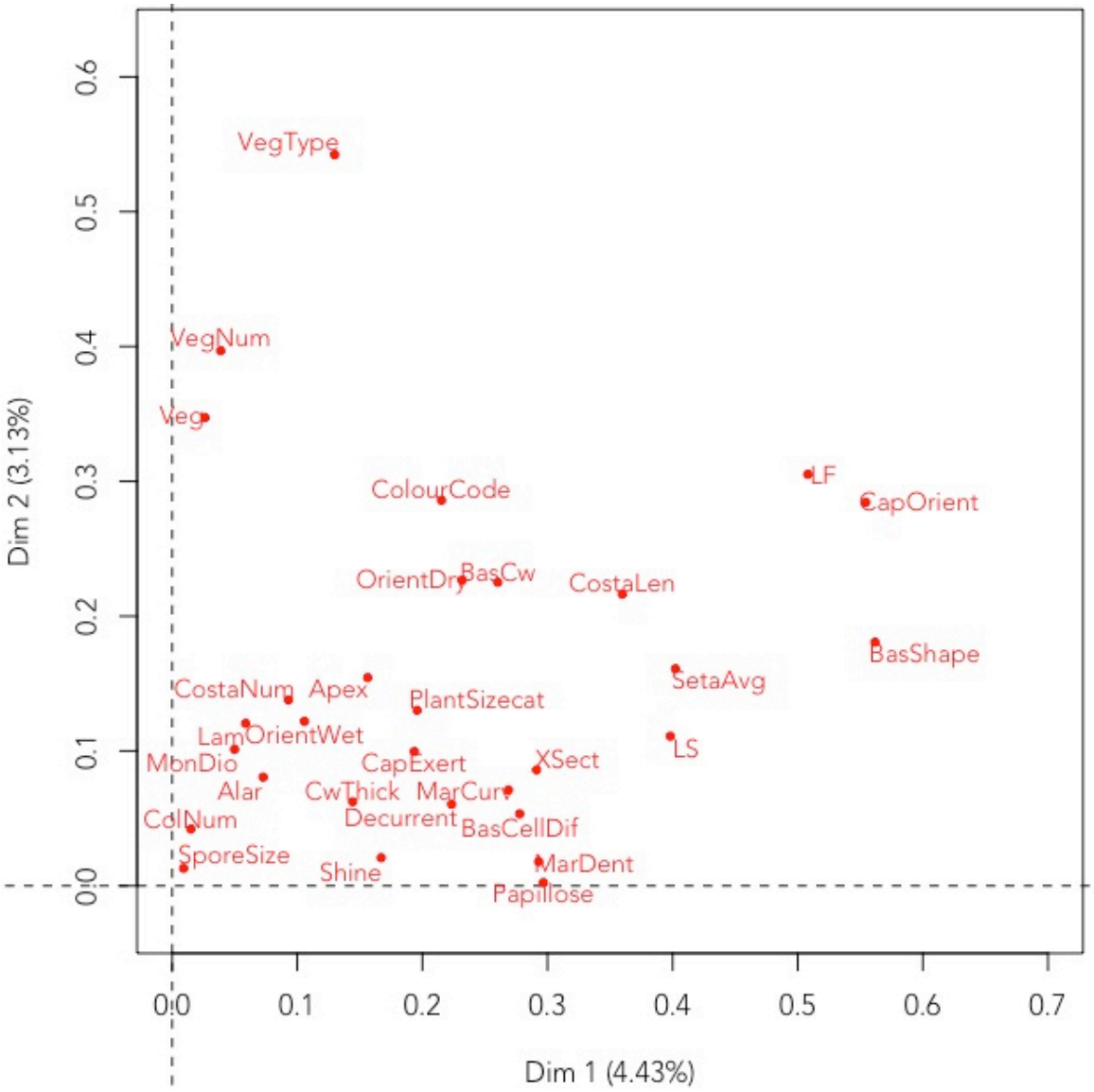


Figure 4.6 MCA plot showing the association of each trait with the 1st and 2nd dimensions. Margin denticulation, papillosity basal cell shape and basal cell differentiation are the most correlated with the 1st dimension. Vegetative propagules, number of vegetative propagules and presence of vegetative propagules are the most correlated with the 2nd dimension. Many traits are not strongly linked as shown by the clustering of many around the origin. Trait names are coded to simplify viewing, for code explanations see Table 4.23, p. 248, Appendix A4.3.

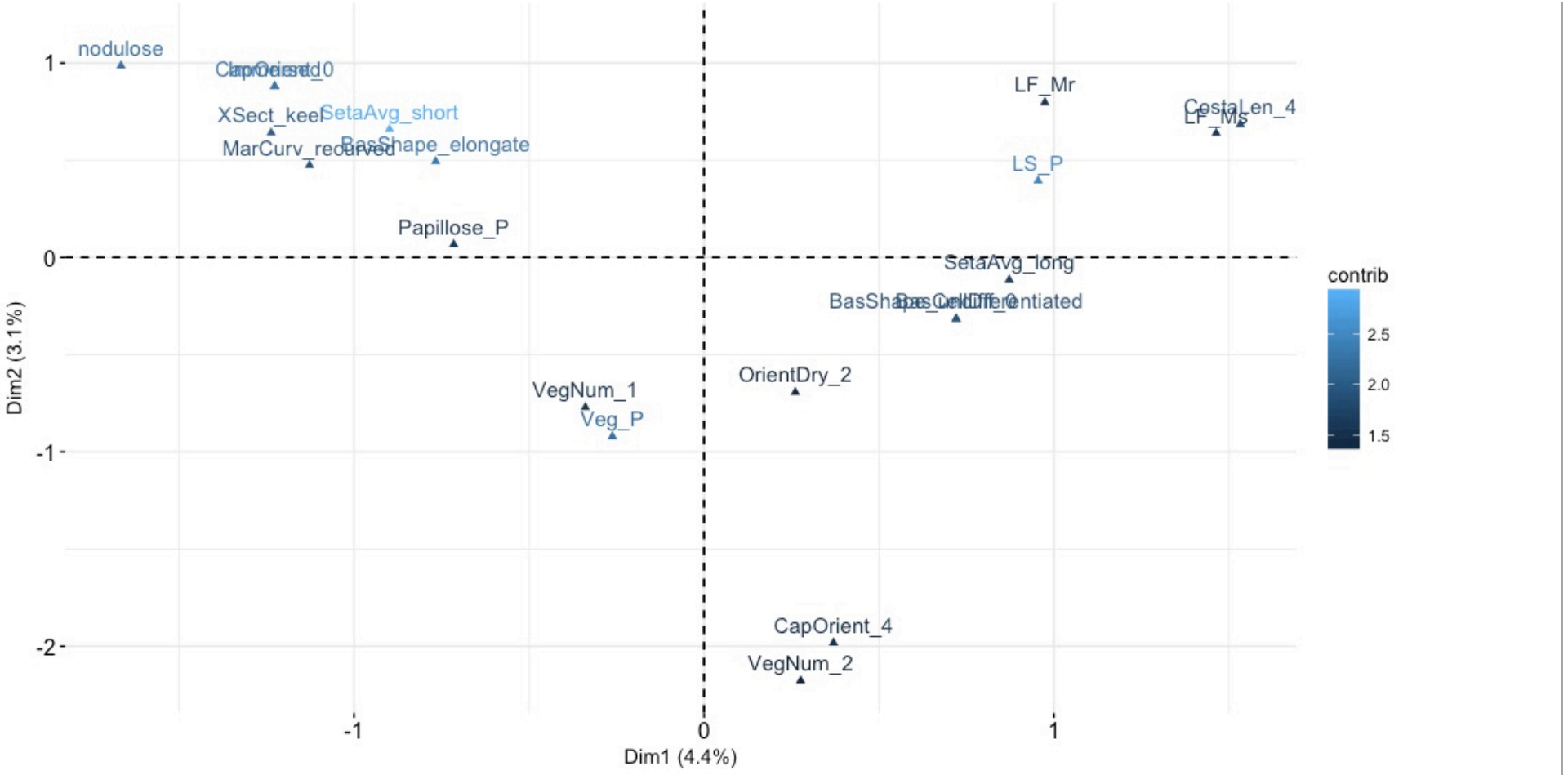


Figure 4.7 The 20 trait states that most contribute to the MCA plot construction, shaded according to their percentage contribution (contrib). Nodulose basal cells (nodulose), immersed capsules, keeled leaves (Xsect_keel), recurved margins (MarCurv_recurved) and the presence of papillae (Papillose_P) are associated with drier and more exposed environments (to the left of dimension 1). On the opposite end, mats (LF_Mr and LF_Ms), a long seta (SetaAvg_long), a costa that terminates in the upper half (CostaLen_4) and undifferentiated basal cells (BasShape_0) are associated with more humid and sheltered environments. Trait state names are coded to simplify viewing, for code explanations see Table 4.23, p. 248, Appendix A4.3.

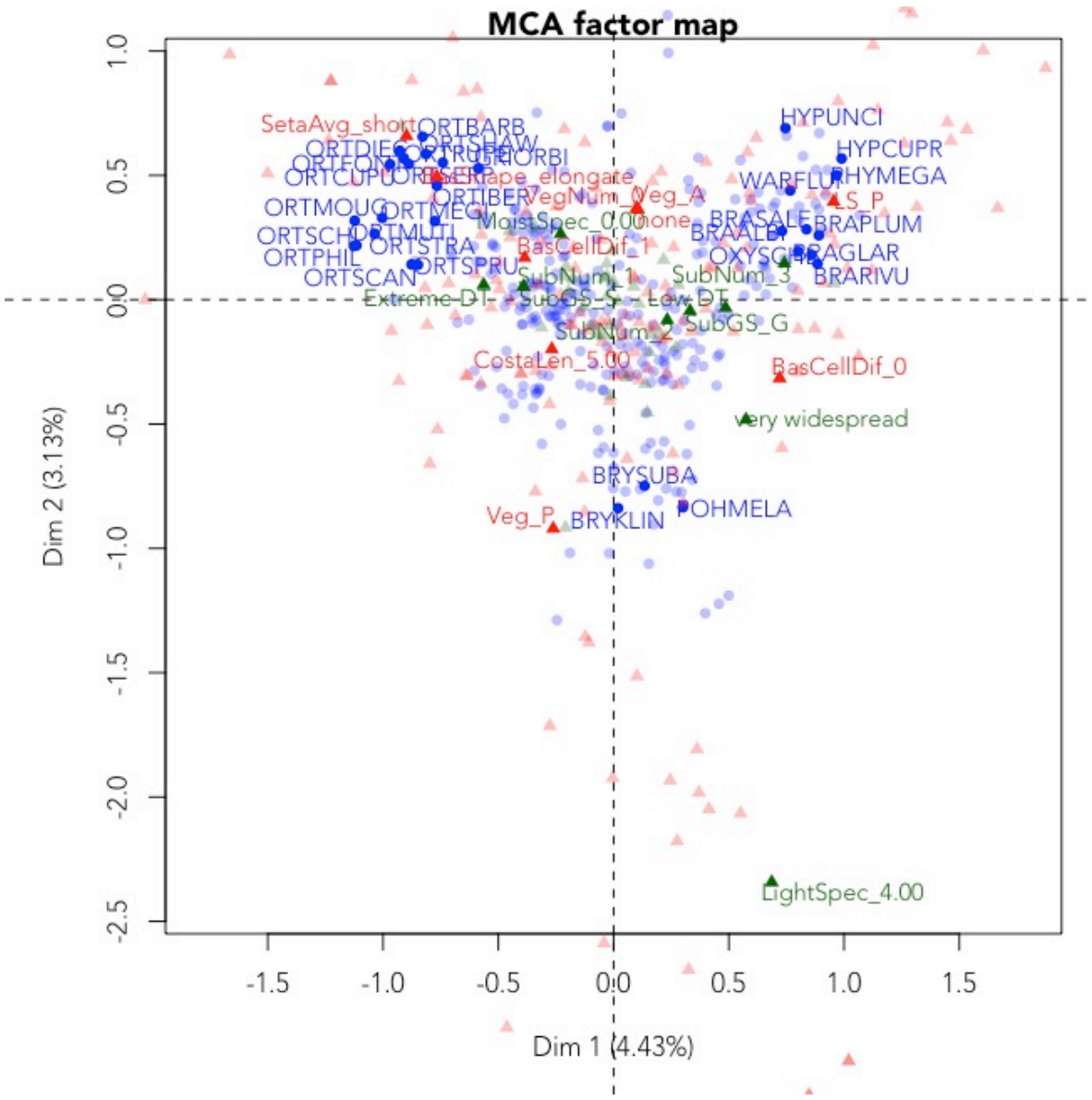


Figure 4.8 MCA plot with the 30 taxa that are best represented labelled (blue circles), along with the 10 trait states (red triangles) and environmental values (green triangles) that are most important in the creation of the plot highlighted (darker red and green triangles). This plot suggests there are three species groupings, two along the 1st dimension, and one along the 2nd. Desiccation tolerance level is negatively correlated with the first axis, as the category "Extreme DT" is found on the left-hand side of the plots, and "Low DT" on the right. All other species, traits and variables are shown by colour faded points. For species names see Table 4.21, p. 234, Appendix A4.2. Trait state names are coded to simplify viewing, for code explanations see Table 4.23, p. 248, Appendix A4.3.

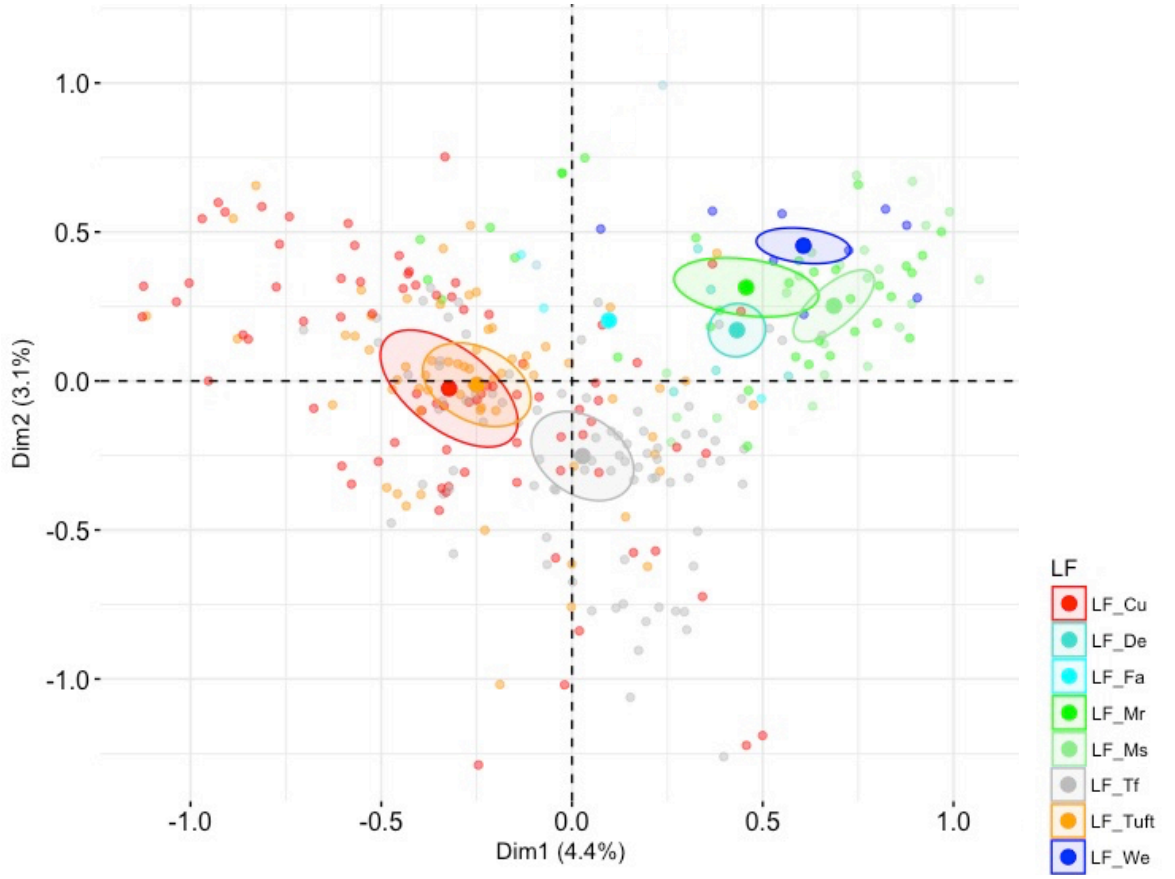


Figure 4.9 MCA plot showing clustering of individuals according to life-form – ellipses that do not overlap indicate these trait states are significantly different (95% confidence level). Cushions (red) and Tufts (orange) are separated from more open life-forms (blues and greens). LF_Cu: cushion; LF_De: dendroid; LF-Fa: fan; LF_Mr: mat rough; LF_Ms: mat smooth; LF_Open: open; LF_Tf: turf; LF_Tuft: tuft; LF_We: weft.

4.4.2.2 Clustering

The MCA showed that the 1st and 2nd dimensions are the best representation of individuals in the dimensional space, but the clustering allows us to investigate what is happening in the other dimensions. This is useful in this case to determine which dimensions partition cluster 2 from the other two clusters, and then which environmental or habitat variables are associated with those dimensions. Hierarchical clustering (Euclidean distance with ward linkage) on the results of an MCA with all traits, habitat and environmental variables created 3 main clusters (Figure 4.10). The number of clusters to use was decided based on the length of the branches and the loss of inertia between cluster numbers (e.g. moving from 4 to 3 clusters lost 0.003 so they can be grouped together, but moving from 3 to 2 loses 0.03, quite a large loss of inertia so they were kept separate). This number (three clusters) also fits with the MCA plot, which seemed to indicate three groupings of species (Figure 4.8, p. 204 above). Another possible cluster number would be six, based on the branch lengths of the dendogram (Figure 4.23, Appendix IV) but this yielded less defined groups along the 1st dimension (Figure 4.24, Appendix IV), which is the dimension of interest in this study as it relates to the environmental conditions.

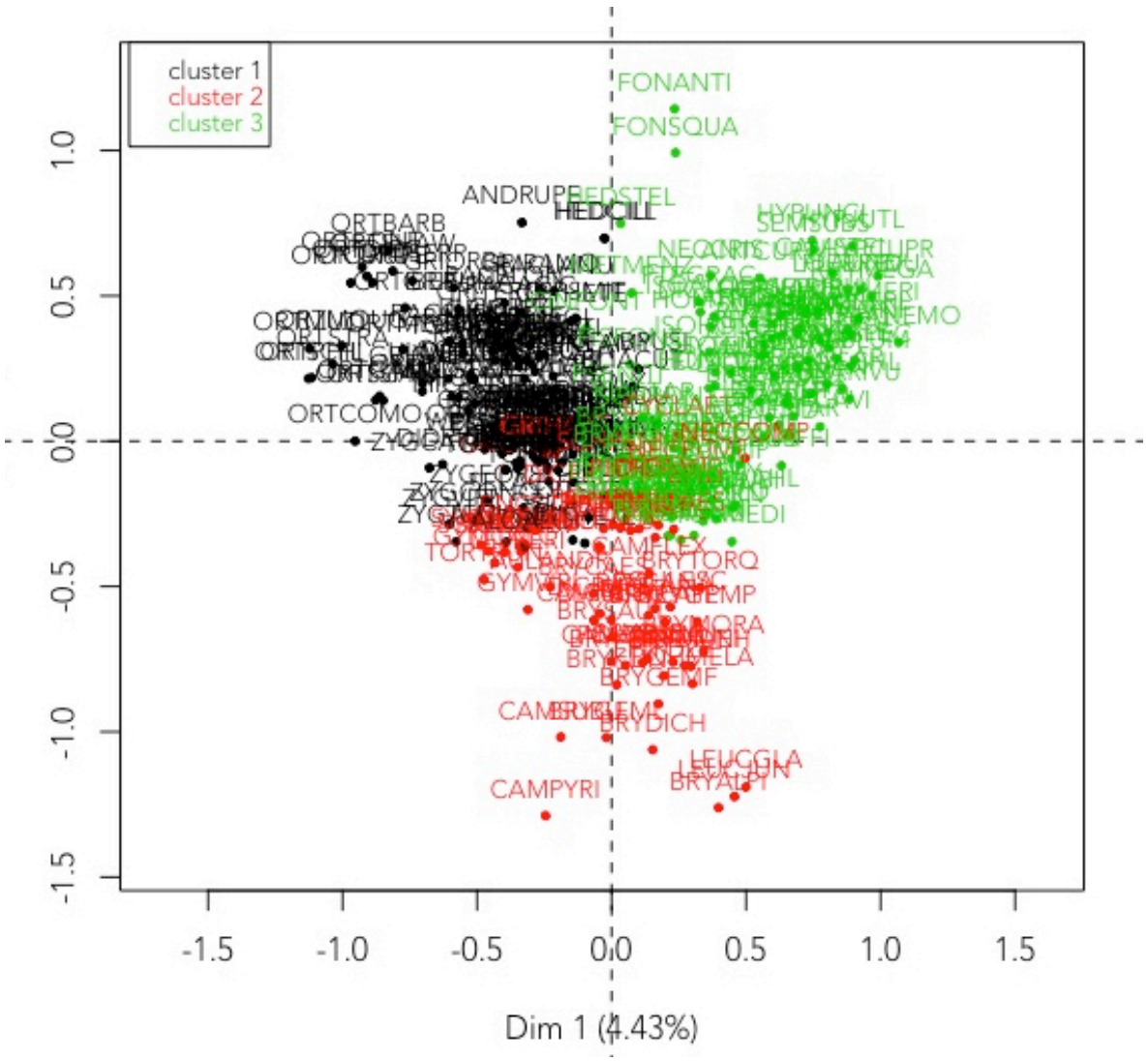


Figure 4.10 Parition of taxa in the three resultant groups from the clustering analysis. Cluster 1 (black) and 3 (green) are partitioned along the 1st dimension, and cluster 3 (red) is partitioned from clusters 1 and 3 along the 2nd dimension. Cluster 1 (black) are species that prefer drier and more exposed environmental conditions; cluster 3 (green) species are species that prefer more wetter and more sheltered environmental conditions; cluster 2 (red) are species not characterised by environmental preferences but by habitat and environmental specificity. For species names see corresponding codes in Table 4.21, p. 234, Appendix A4.2.

The first and third clusters are partitioned along the 1st dimension, which is associated with the environmental conditions (i.e. species to the right have low EI values and species to the left have high EI values) (Figure 4.10). However, the second cluster is not partitioned by the 1st dimension, and so the environmental variables do not explain this cluster (as mentioned above, the second dimension seems to be related to habitat and environmental ranges). This is confirmed when looking at the mean values of the environmental traits of each cluster (Table 4.12). Species from cluster 1 have above average EI values (indicated by the v-test), and also above average moisture and light values (drier and more exposed); species from cluster 3 have a below average EI, as well as below average moisture and light values (more humid and sheltered); and the species in cluster 2 are not characterised by the environmental variables. Therefore, to select species based on their environmental preferences using their traits, two trait profiles were defined based on clusters 1 and 3. Species of the first cluster can be said to be ones that are high DT as they occupy

dry and exposed habitats (C1 species), and those of cluster 3 are low DT as they inhabit humid and sheltered habitats (C3 species). None of the habitat variables characterised these clusters.

It is important to acknowledge that direction of the EI response will be the same as that of the moisture and light values, as it is calculated from these two variables. However, the latter two traits were included in the analyses to check the response of light and moisture: if species in one cluster are characterised by *above* average light values but also by *below* average moisture values it would indicate that the EI was not appropriate. This would be a cluster with species that occupy more humid but *less* exposed environments than those in another cluster, and this would not be indicated by the EI as it is an index from humid and sheltered to dry and exposed conditions (a low moisture value means it is a species of more humid environments, a low light value means it is a species of sheltered environments, therefore a low EI indicates more humid and sheltered environments, as defined in Chapter 3).

Table 4.12 Variables that are significantly associated with the clusters.

Variable	Cluster 1 Mean±1SE	v.test	p value	Cluster 2	Cluster 3 Mean±1SE	v.test	p value
EI	0.76±0.12	6.71	<0.001	Not significant	0.58±0.13	-6.43	p<0.001
Moisture	6.39±1.15	6.43	<0.001	Not significant	5.05±1.55	-5.04	p<0.001
Light	4.42±1.13	4.40	<0.001	Not significant	3.55±1.39	-5.19	p<0.001

4.4.2.3 Trait profiles of species indicating high and low EI

As outlined above, several trait states are involved in the characterisation of the clusters. From these, a trait profile was created using those trait states which best categorise a cluster (Table 4.13). Although statistically speaking (in terms of v-value and significance level), all significant trait states could be included, I wanted a balance between how well a trait state represents species of a particular cluster (high DT or low DT) and how easy to measure a trait is. For example, monoicous species were strongly represented in cluster 1 (proportion=63.3%, p<0.001, v=5.96) and species that can be both monoicous and dioicous characterise cluster 3 (proportion=84.6, p<0.001, v=3.59). However, this trait is not easy to measure and data in the literature are not always available for this trait. States were chosen based on their representation in the cluster, size of the v-value and minimising these values in the other cluster; if a state had 100% representation in a cluster it was used, even if its v-value was not as high as for other states that characterise the same cluster. Conversely, a state that had less representation (60-80%) but a high v-value, and the v-value of the same state in the other cluster was highly negative or the state did not characterise the other cluster at all, this trait was included. The states included were also those that were not found in cluster 2, or had negative representation (indicated by a negative v-value). Below I outline the most important trait states and the justification for including or omitting them in the trait profile creation in order to make the process transparent and not seem biased. The values quoted after trait states indicate the representation of species with that trait in the cluster (prop=x%), the v-test value (v) and the significance level.

Life-form characterised the different clusters well, with species with open life-form (dendroid, fan, weft and pendant) only found in cluster 3 (prop=100%). Out of these, weft, fan and dendroid are the most useful, as pendant species did not characterise the cluster as well as the other open life-forms (v=2.01, p>0.01). Conversely, more closed life-forms were characteristic of cluster 1, with cushions and tufts being most characteristic (prop=65%, v=4.51, p<0.001 and prop=68%, v=4.57, p<0.001, respectively).

Plant size is involved in the characterisation of clusters, with the most useful being robust size in characterising cluster 3 (prop=88%, v=2.90, p<0.01) and minute plants characterising cluster 1 (prop=66%, v=3.55, p<0.001). Sizes intermediate between these also characterise cluster 2, so only the extreme size categories were be used in the trait profile.

Plants with a strong shine characterised cluster 3 (prop=79.0%, v=3.90, p<0.001) and there were no species with an obvious shine in cluster 1. This could therefore be a useful trait to assign species to a cluster, even though species considered to have some shine are found in cluster 3. However, this representation is low and negative, prop=12%, v=-2.77. Due to the subjectiveness of judging the level of shininess only the state “very shiny” was used.

Leaf orientation when dry characterised well both clusters 1 and 3. Species whose leaves are more open when dry (patent to spreading/squarrose) were only found in cluster 3 (prop=100%, v=3.79, p<0.001) so this state can be used to characterise species with low DT. In cluster 1, species with appressed/imbricate (prop=67%, v=4.15, p<0.001) to suberect leaves (prop=69%, v=5.07, p<0.001) were well represented and characterised this cluster well. However, species with sub erect leaves were also found in cluster 2 (though they were under represented, v=-2.58). The trait state of patent to spreading leaves when dry was therefore be used to assign species to cluster 3.

Wet leaf orientation states also played a role in the clustering, though only spreading leaves (4.5) and imbricate to erect leaves (1.5), with the former characterising cluster 1 (prop=71%, v=3.22, p<0.01) and the latter cluster 3 (prop=100%, v=2.79, p<0.01). These are good states to use as none characterise cluster 2.

Species with a costa that terminates in the upper half of the leaf (but not in the apex, value 4) were all represented in cluster 3 (prop=100%, v=6.38, p<0.001) as were species with costa termination in the middle (prop=100%, v=3.11, p<0.01). Species with a costa that terminates in the apex (value 5) characterised cluster 1 well (prop=51%, v=6.28, p<0.001), but also characterised cluster 2 (prop=24%, v=2.90, p<0.01) and so it is not a useful trait to assign species to a cluster. Additionally, species with costas that terminate in the apex are also those that have hair points, so this may confound analyses. The absence of a costa characterised species of cluster 3 relatively well but species with a costa were similarly represented in clusters 1 and 3 (32.8% and 45.8%, respectively).

Plants with a keel characterised cluster 1 (prop=87%, v=5.71, p<0.001) whereas species with concave leaves characterised cluster 3 (prop=59%, v=3.20, p<0.001). Presence of keel was therefore included in the trait profile of cluster 1 species, but not concave because of the relatively low proportion of concave species in cluster 3.

The presence of papillae characterised well species from cluster 1 (prop=72%, v=7.55, p<0.01) and conversely, the absence of papillae characterised cluster 3 (prop=51%, v=8.15, p<0.001). However, species with no papillae were also found in cluster 1 (27% of species with no papillae were found in cluster 1) and so this state may not be so appropriate for assigning species to a cluster; therefore only the presence of papillae was be used in the trait profile. Species with thick cell walls characterised cluster 1 (prop=62%, v=4.86, p<0.001) and species with thin walls were more represented in cluster 3 (prop=55%, v=4.01, p<0.001) but as the proportional representation is relatively low this trait was not included in the trait profiles.

Species with some level of leaf decurrence (short or long) characterised cluster 3. Species with long decurrence were all found in cluster 3 (prop=100%, p<0.001, v=3.42) so this was deemed a good trait to use. Species with a thick lamina (subulate or bistratose) were characteristic of cluster 1, having no representation in cluster 3, but subulate species were also found in cluster 2. Bistratose species were all represented in cluster 1 (prop=100%, p<0.01, v=3.01) so this trait state was included in the trait profile. Although species with alar differentiation were more characteristic of species of cluster 1 (prop=59%, v=3.65, p<0.001), they were also found in cluster 3 so this trait was not used (prop=27%, v=-2.60, p<0.01). Species with hair-points were more characteristic of cluster 1 (prop=63%, v=3.02, p<0.01).

Margin denticulation was also involved in characterising the clusters, with species with entire margins mostly found in cluster 1 (prop=61%, v=6.98, p<0.001) and species with dentate margins characterised cluster 3 (prop=89%, v=4.72, p<0.001). Species with denticulate margins also characterised cluster 3 (prop=66%, v=3.61, p<0.001), but as species with part of their margin denticulate were also found in cluster 1 (albeit they are under-represented, v=-3.61), denticulation may not assign species accurately to a DT group.

Margin curvature seems to be a very useful trait to characterise species as all species with revolute and involute margins were found in cluster 1 (prop=100%, v=2.11 (involute), v=2.44 (revolute) p>0.01) and most species with recurved margins were also found in cluster 1 (prop=84.1%, v=5.94, p<0.001). Species with revolute and involute margins were only significant at α=0.05 due to the small number of species that represent these states (19), but as they were all found in cluster 1 I decided to make an exception on the significance level for these two trait states and include them in the trait profile.

Vegetative propagules traits (presence/absence, number and type) characterised all three cluster with cluster 2 characterised by presence of propagules and number of propagules. Vegetative propagules were therefore not suitable for indicating species of clusters 1 or 3.

Perennial species characterised cluster 3 (v=8.32, p<0.001, prop=74.1%) and medium shuttles characterised cluster 1 (v=3.87, p<0.001, prop=85.0%) This is therefore a good trait to use to assign species to a cluster, but in terms of data availability this is scare in tropical species and was therefore not included in the trait profile.

Capsule orientation is interesting as species with erect capsules were found mostly in cluster 1 and characterised this cluster well (prop=72.5%, v=8.32, p<0.001), and species with sub-pendulous were mostly found in cluster 3 (prop=75%, v=2.74, p<0.01) but species with pendulous capsules were well represented in cluster 2 (prop=84.6%, v=4.88, p<0.001). Species with short

setas were characteristic of cluster 1 (prop=85.2%, v=9.01, *p*<0.001), whereas species with long setas characterised cluster 3 (prop=65.8%, v=6.21, *p*<0.01).

The final trait profiles used are shown in Table 4.13 below. In the next step, the trait profile for cluster 1 species is referred to as C1 and the cluster 3 trait profile as C3; species will be referred to as C1 and C3 species.

Table 4.13 Trait profile of cluster 1 (C1) and cluster 3 (C3) species, with associated v-test value, significance value and percentage of species with this trait state present in cluster (prop %).

Cluster 1				Cluster 3			
Trait state	v.test	p	prop (%)	Trait state	v.test	p	prop (%)
Apex cucullate	2.12	<0.05	100	Basal cells not differentiated	8.98	<0.0001	69
Basal cells differentiated	9.84	<0.0001	63	Basal cell walls porose	3.68	0.0002	78
Basal cell walls nodulose	5.75	<0.0001	100	Basal cells short	5.01	<0.0001	86
Basal cells elongate	9.56	<0.0001	82	Basal cells undifferentiated	8.98	<0.0001	69
Capsule immersed	5.02	<0.0001	83	Capsule inclined to horizontal	4.55	<0.0001	73
Capsule erect	8.33	<0.0001	73	Costa absent	2.71	<0.01	71
Lamina bistratose	3.01	<0.01	100	Longly decurrent leaf	3.42	<0.001	100
Cushion life-form	4.51	<0.0001	65	Dendroid life-form	3.12	<0.05	100
Tuft life-form	4.57	<0.0001	69	Smooth mat life-form	5.01	<0.0001	86
Margin involute	2.12	<0.05	100	Weft life-form	3.97	<0.0001	100
Margin revolute	2.44	<0.05	100	Leaves erecto/patent-patent when dry	3.70	<0.001	100
Margin entire	6.98	<0.0001	61	Leaves spreading when dry	3.12	<0.01	100
Leave appressed/imbricate when dry	4.15	<0.0001	67	Leaves appressed/imbricate to erect when wet	2.79	<0.01	100
Leaves papillose	7.55	<0.0001	72	Plant size robust	2.90	<0.01	88
Lamina keeled	5.71	<0.0001	86	Plant very shiny	3.90	<0.0001	79

The absence of shine was not used to characterise species into cluster 1, but the presence of shine was used to assign species to cluster 3. This is because the proportion representation of species with no shine in cluster 1 and 3 was low and these proportions were not very different from each other (48% and 31%, respectively). Although costa length characterised well cluster 1 and 3, the subjective nature of classifying nerve length, and the variation that can occur means it may not be a suitable trait to include. A more useful trait is the presence or absence of a costa,

but similarly to shine, only the absence of costa was used to characterise species into cluster 3, and the presence was not used to assign species to a classification. Again, with margin denticulation, it is the absence of any denticulation (entire margin) that was used to characterise cluster 3 species.

4.4.2.4 Assigning taxa an indicator value

Using the trait profile created in the previous section, each species in the database was assigned two scores based on what proportion of trait states characteristic of C1 are present in it, and what proportion of states characteristic of C3 are in it. Whereas previous analyses (MCA and clustering) were only able to use species that had complete trait data, this allows us to use species with incomplete data.

A graphical representation of these genera and families is provided below. Tropical genera and families are shown in separate graphs, as these will be used in the next chapter, which concerns the distribution of bryophytes in a tropical lowland forest in Madagascar.

Species

The group of strict dry and exposed indicator species is mostly composed of either epiphytic species (*Cryphaea heteromalla*, *Orthotrichum* spp., *Ulota* spp. and *Zygodon* spp.) or saxicolous species (Table 4.14). Strict humid and sheltered indicators are mostly large pleurocarpous species in contrast to dry and exposed indicator species, which are all acrocarpous (Table 4.14). A similar pattern is seen in non-strict indicators (Table 4.15)

Table 4.14 Strict indicator species with those that have narrow environmental conditions in bold. Ordered alphabetically.

Strict dry and exposed indicator species	Strict humid and sheltered indicator species
Atrichum undulatum	***Brachythecium dieckii***
Bartramia pomiformis	*Brachythecium plumosum*
Cinclidotus riparius	*Brachythecium populeum*
Cryphaea heteromalla	*Brachythecium rivulare*
Cynodontium bruntonii	*Brachythecium rutabulum*
Cynodontium jenneri	*Brachythecium salebrosum*
Dialytrichia fragilifolia	***Brachythecium velutinum***
Dialytrichia mucronata	*Fissidens dubius*
Ditrichum heteromallum	*Fissidens taxifolius*
Dichodontium pellucidum	*Hygroamblystegium varium*
Encalypta vulgaris	*Hypnum cupressiforme*
Entosthodon fascicularis	*Mnium hornum*
Eucladium verticillatum	*Oxyrrhynchium hians*
Grimmia decipiens	*Oxyrrhynchium pumilum*
Grimmia laevigata	***Oxyrrhynchium schleicheri***
Grimmia pulvinata	*Oxyrrhynchium speciosum*
Grimmia tergestina	*Plagiomnium affine*
Kiaeria blyttii	*Pohlia wahlenbergii*
Leskea polycarpa	*Polytrichum commune*
Orthotrichum comosum	
Orthotrichum consimile	

Strict dry and exposed indicator species	Strict humid and sheltered indicator species
Orthotrichum lyellii	
Orthotrichum tenellum	
Pleuridium subulatum	
Ptychomitrium polyphyllum	
Racomitrium aciculare	
Racomitrium affine	
Racomitrium aquaticum	
Racomitrium elongatum	
Racomitrium heterostichum	
Rhabdoweisia fugax	
Seligeria acutifolia	
Syntrichia papillosa	
Syntrichia princeps	
Ulota calvescens	
Ulota crispula	
Ulota hutchinsiae	
Zygodon forsteri	
Zygodon viridissimus	

Table 4.15 Non-strict indicators and their associated indicator value (C1 - C3), ordered according to their indicator value: from most dry and exposed for dry and exposed indicators; from most humid and sheltered for humid and sheltered indicators.

Non-strict dry and exposed indicator species		Non-strict humid and sheltered indicator species	
Species	**Indicator value**	**Species**	**Indicator value**
Orthotrichum acuminatum	0.533	*Hypnum jutlandicum*	-0.400
Orthotrichum affine	0.533	*Hypnum uncinulatum*	-0.333
Orthotrichum philibertii	0.533	*Plagiothecium nemorale*	-0.333
Orthotrichum schimperi	0.533	*Sematophyllum substrumulosum*	-0.267
Orthotrichum diaphanum	0.467	*Fissidens pusillus*	-0.200
Orthotrichum ibericum	0.467	*Homomallium incurvatum*	-0.200
Orthotrichum shawii	0.467	*Isothecium myosuroides*	-0.200
Orthotrichum speciosum	0.467	*Neckera crispa*	-0.200
Orthotrichum stramineum	0.467	*Rhynchostegiella teneriffae*	-0.200
Orthotrichum striatum	0.467	*Brachythecium glareosum*	-0.133
Zygodon catarinoi	0.467	*Brachythecium mildeanum*	-0.133
Amphidium mougeotii	0.400	*Claopodium whippleanum*	-0.133
Didymodon fallax	0.400	*Fissidens viridulus*	-0.133
Microbryum davallianum	0.400	*Loeskeobryum brevirostre*	-0.133
Orthotrichum scanicum	0.400	*Polytrichum piliferum*	-0.133
Orthotrichum sprucei	0.400	*Pseudephemerum nitidum*	-0.133
Tortula muralis	0.400	*Rhynchostegiella curviseta*	-0.133
Weissia controversa	0.400	*Antitrichia curtipendula*	-0.133

Non-strict dry and exposed indicator species		Non-strict humid and sheltered indicator species	
Species	**Indicator value**	**Species**	**Indicator value**
Barbula unguiculata	0.333	*Homalothecium meridionale*	-0.133
Microbryum starckeanum	0.333	*Homalothecium sericeum*	-0.133
Syntrichia subpapillosissima	0.333	*Hookeria lucens*	-0.133
Tortula subulata	0.333	*Hypopterygium tamarisci*	-0.133
Trichostomum crispulum	0.333	*Plagiothecium undulatum*	-0.133
Ulota bruchii	0.333	*Campylium stellatum*	-0.067
Ulota crispa	0.333	*Climacium dendroides*	-0.067
Zygodon conoideus	0.333	*Fissidens bryoides*	-0.067
Zygodon rupestris	0.333	*Pseudoscleropodium purum*	-0.067
Brachydontium trichodes	0.267	*Rhynchostegiella litorea*	-0.067
Leptodon smithii	0.267	*Rhynchostegiella tenella*	-0.067
Orthotrichum rivulare	0.267	*Thamnobryum alopecurum*	-0.067
Seligeria pusilla	0.267	*Cratoneuron filicinum*	-0.067
Tortula marginata	0.267	*Dicranum crassifolium*	-0.067
Trichostomum brachydontium	0.267	*Entosthodon attenuatus*	-0.067
Archidium alternifolium	0.200	*Fissidens adianthoides*	-0.067
Blindia acuta	0.200	*Heterocladium wulfsbergii*	-0.067
Tortella humilis	0.200	*Pogonatum aloides*	-0.067
Didymodon luridus	0.133	*Pogonatum nanum*	-0.067
Acaulon muticum	0.067	*Rhizomnium punctatum*	-0.067
Seligeria calycina	0.067	*Sanionia uncinata*	-0.067
Pleuridium acuminatum	0.067		

Genera

Similarly to species, strict wet and sheltered genera indicators are all pleurocarpous (Table 4.16), with the exception of *Mnium,* which is acrocarpous. Three epiphytic genera are strict indicators of dry and exposed conditions (*Cryphaea, Dialytrichia* and *Leskea*). Overall, genera with pleurocarpous species have the lowest IVs (Figure 4.11) and predominantly epiphytic genera have among the highest IVs, including in tropical genera (Figure 4.11 and Figure 4.12). An exception are the Tropical epiphytic genera *Pinatella, Rhacopilopsis* and *Rigodium* which have negative indicator values (from -0.14 to -0.17; Figure 4.12 and see

Table 4.26, p. 265, Appendix A4.4 for values) and are therefore strict indicators of humid and sheltered environments (Table 4.16).

Table 4.16 Strict indicator genera; epiphytic genera indicated by §. Ordered alphabetically.

Strict dry and exposed indicator genera	Strict wet and sheltered indicator genera
Atractylocarpus	*Brachytheciastrum*
Bartramia	*Hygroamblystegium*
Cinclidotus	*Lepidopilum*
Cryphaea §	*Mnium*
Cynodontium	*Oxyrrhynchium*
Dialytrichia §	*Pinnatella §*
Ditrichum	*Plagiomnium*
Eucladium	*Rhacopilopsis §*
Kiaeria	*Rigodium §*
Leskea §	
Macromitrium §	
Mitthyridium §	
Phyllodon §	
Prionodon §	
Ptychomitrium	
Racomitrium	
Racopilum §	
Rhabdoweisia	
Schlotheimia §	
Trachypodopsis §	

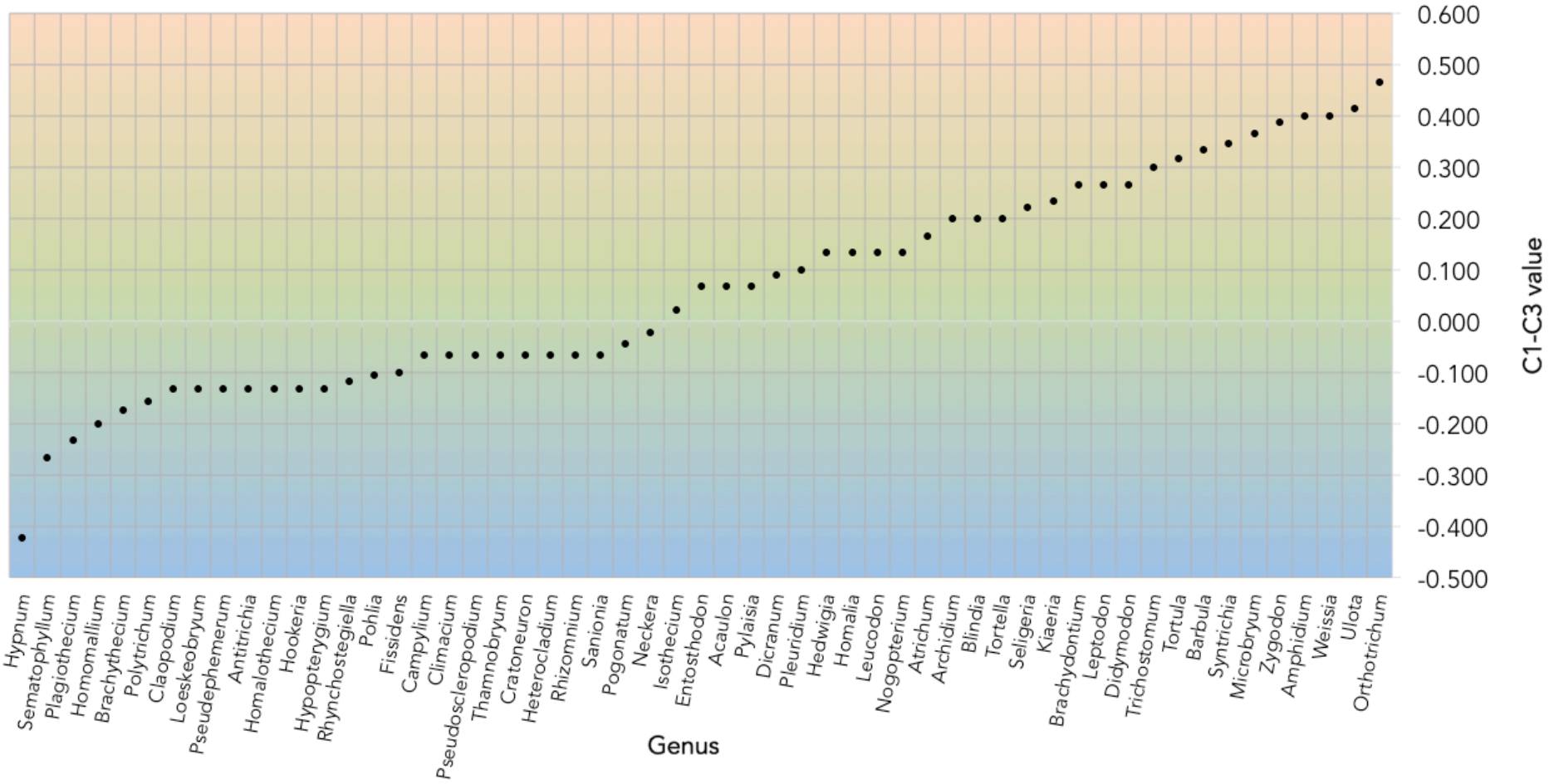


Figure 4.11 Indicator value of non-strict indicator genera. An increasing C1-C3 value means this genus indicates drier and more exposed conditions. See, Table 4.26, p. 265, Appendix A4.4 for values.

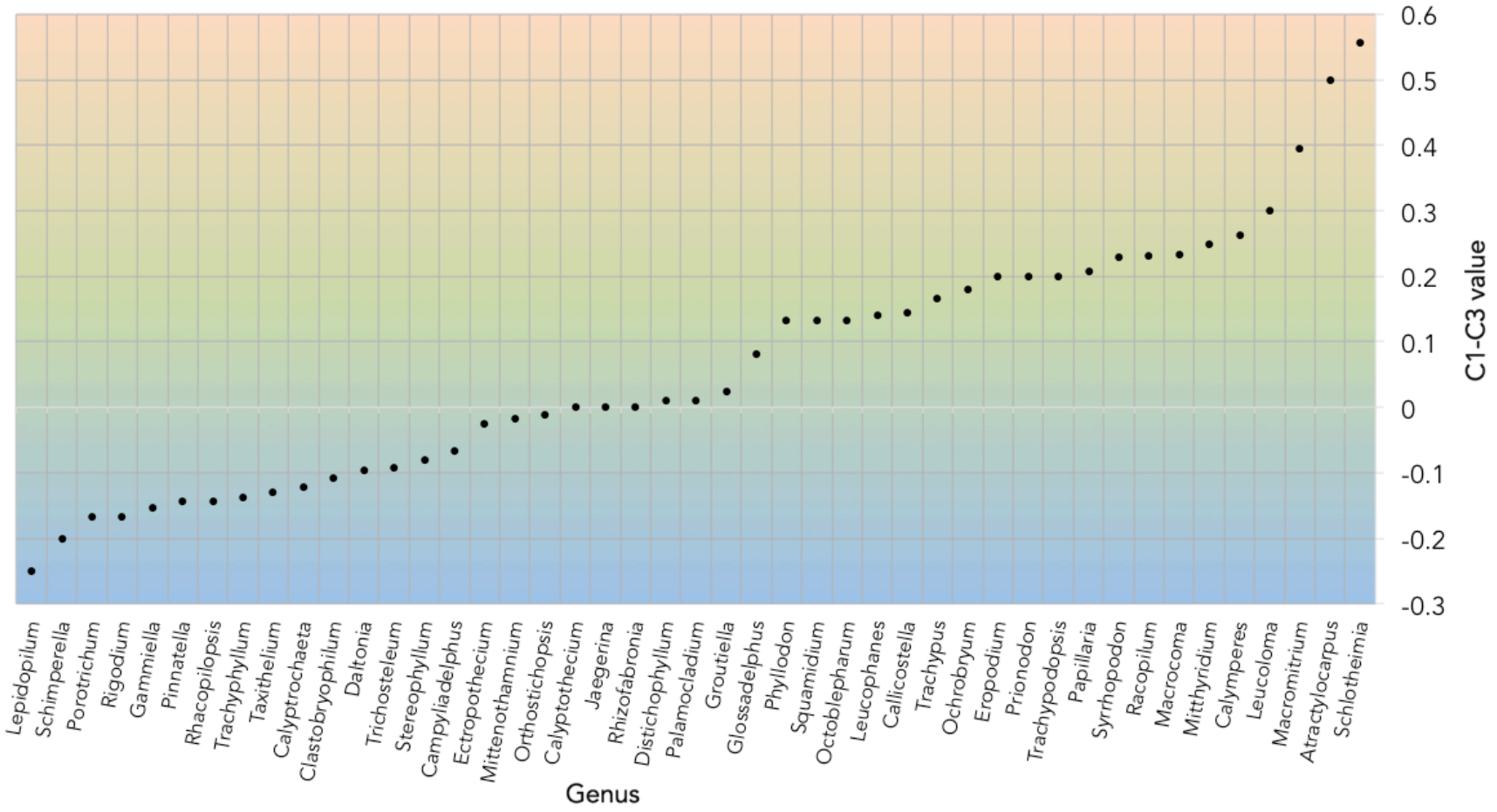


Figure 4.12 Tropical forest genera and their indicator value. An increasing C1-C3 value means this genus indicates drier and more exposed conditions. See Table 4.26, p. 265, Appendix A4.4 for values.

Families

As before, humid and sheltered indicator families are predominantly pleurocarpic Figure 4.13. Within families, species are more spread between the two clusters, as would be expected, and so there is only one strict indicator family Table 4.17. There is a clear delimitation in tropical forest families (Figure 4.14): almost all predominantly epiphytic familes are dry and exposed indicators, with the exception of the tropical epiphytic family Daltoniaceae which is an indicator of humid and sheltered environments.

Table 4.17 Families and their indicator category; epiphytic families indicated by §. Ordered by category and alphabetically within the category by family name.

Indicator category	Family
Strict dry and exposed indicator	Cryphaeaceae §
	Encalyptaceae
	Grimmiaceae
	Lekeaceae §
	Ptychomitraceae
	Racopilaceae §
Non-strict dry and exposed indicator	Aulacomniaceae
	Bartramiaceae
	Calymperaceae §
	Ditrichaceae
	Funariaceae
	Hedwigiaceae
	Lembophyllaceae §
	Leptodontaceae §
	Leucodontaceae §
	Neckeracea §
	Orthotrichaceae §
	Pottiaceae
	Rhabdoweisiaceae
	Seligeriaceae
Can indicate both	Dicranaceae
	Polytrichaceae
Non-strict humid and sheltered indicator	Amblystegiaceae
	Anomodontaceae
	Brachytheciaceae
	Daltoniaceae §
	Fissidentaceae
	Hookeriaceae
	Hylocomiaceae
	Hypnaceae
	Hypopterygiaceae
	Mniaceae
	Plagiotheciaceae
	Pterygynandraceae

Indicator category	Family
	Pylaisiadelphaceae
	Sematophyllaceae
	Stereophyllaceae
Strict humid and sheltered indicator	Rigodiaceaae

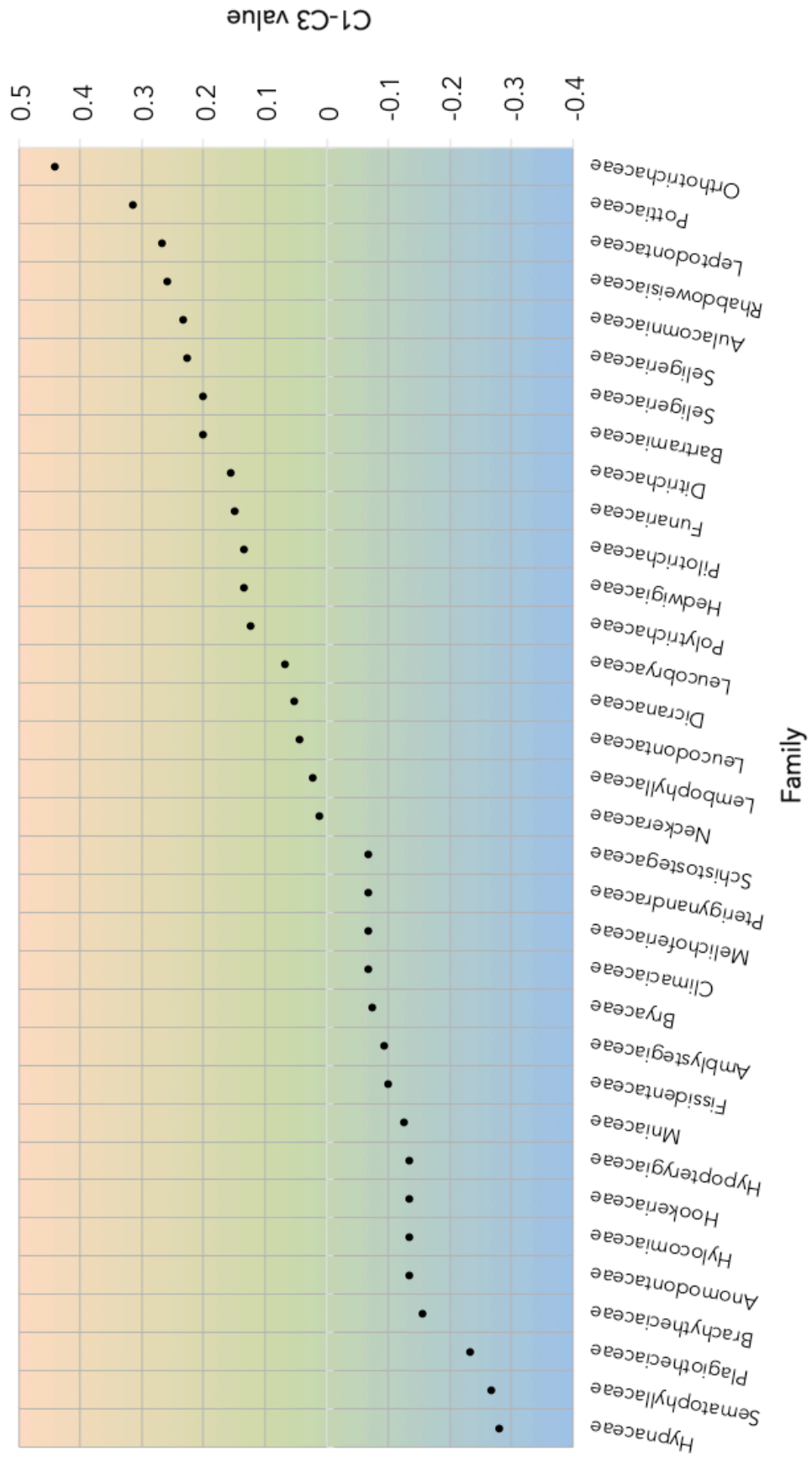


Figure 4.13 Indicator value of non-strict indicator families. An increasing C1-C3 value means this family indicates drier and more exposed conditions.

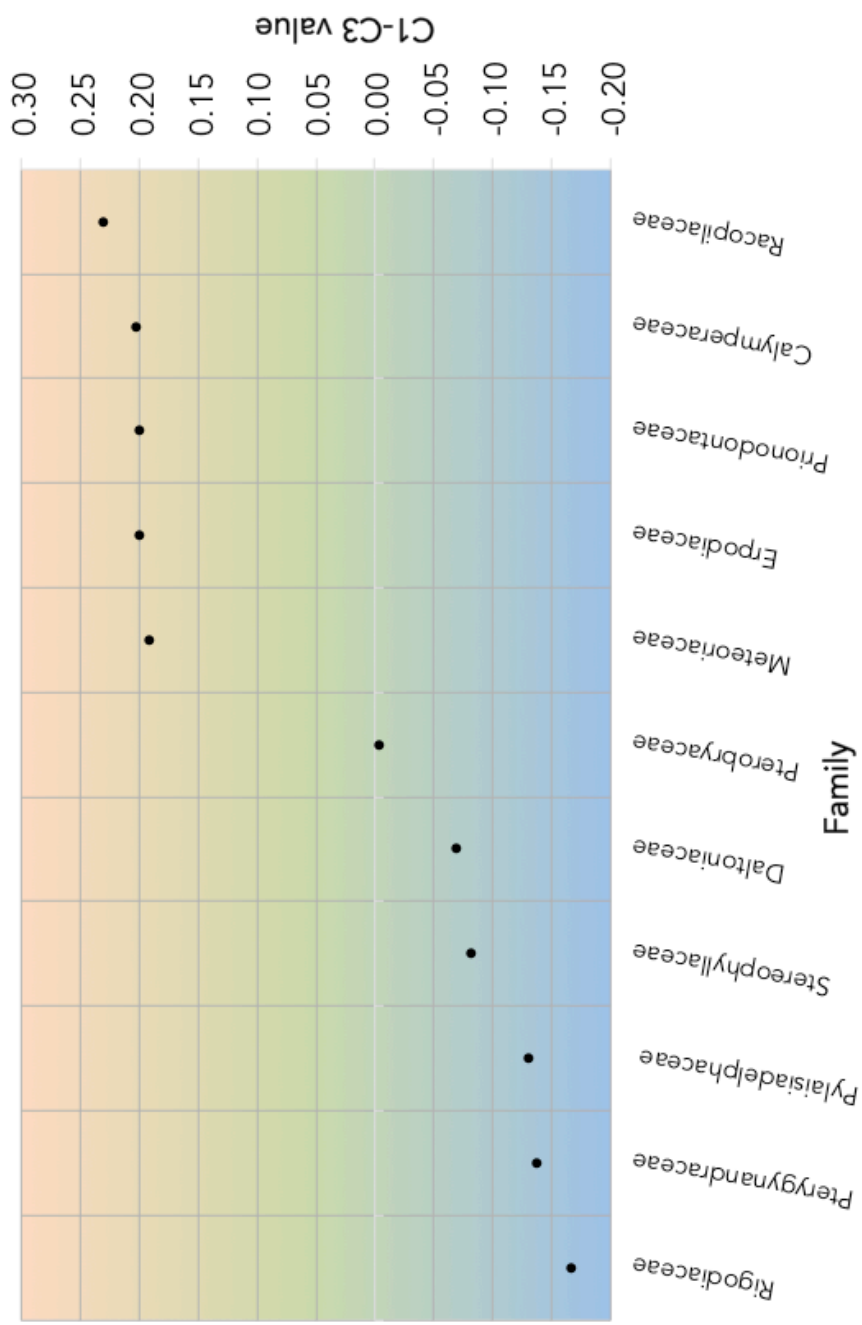


Figure 4.14 Tropical forest families and their indicator value. An increasing C1-C3 value means this genus indicates drier and more exposed conditions.

4.4.3 IV and traits

Substrate specialists had a more positive IV value (Figure 4.15 a) as did species that do not inhabit forest habitats (Figure 4.15 b). The global distribution of species had no effect on the IV (Figure 4.15 c); either when looking at the full range of biogeographical realms occupied (1 to 8) or when grouping them into 1-2 realms and 3 or more, as was done with the EI. The IV is the same across all IUCN threat categories and although the Critically Endangered introduced (CR-int) category has a negative IV (-0.143) this was not significant due to only being represented by one species (Figure 4.16 a).

Tuft and cushion species have significantly more positive IVs whereas wefts and dendroid life-forms have more negative IVs (Figure 4.16 b). There is a clear trend of decreasing IV with plant size (Figure 4.17 a) with robust species having significantly lower IVs than all other sizes. Species with papillae have significantly higher IVs than those without (0.29 ±0.01 and 0.07 ±0.01, respectively, Figure 4.17 b).

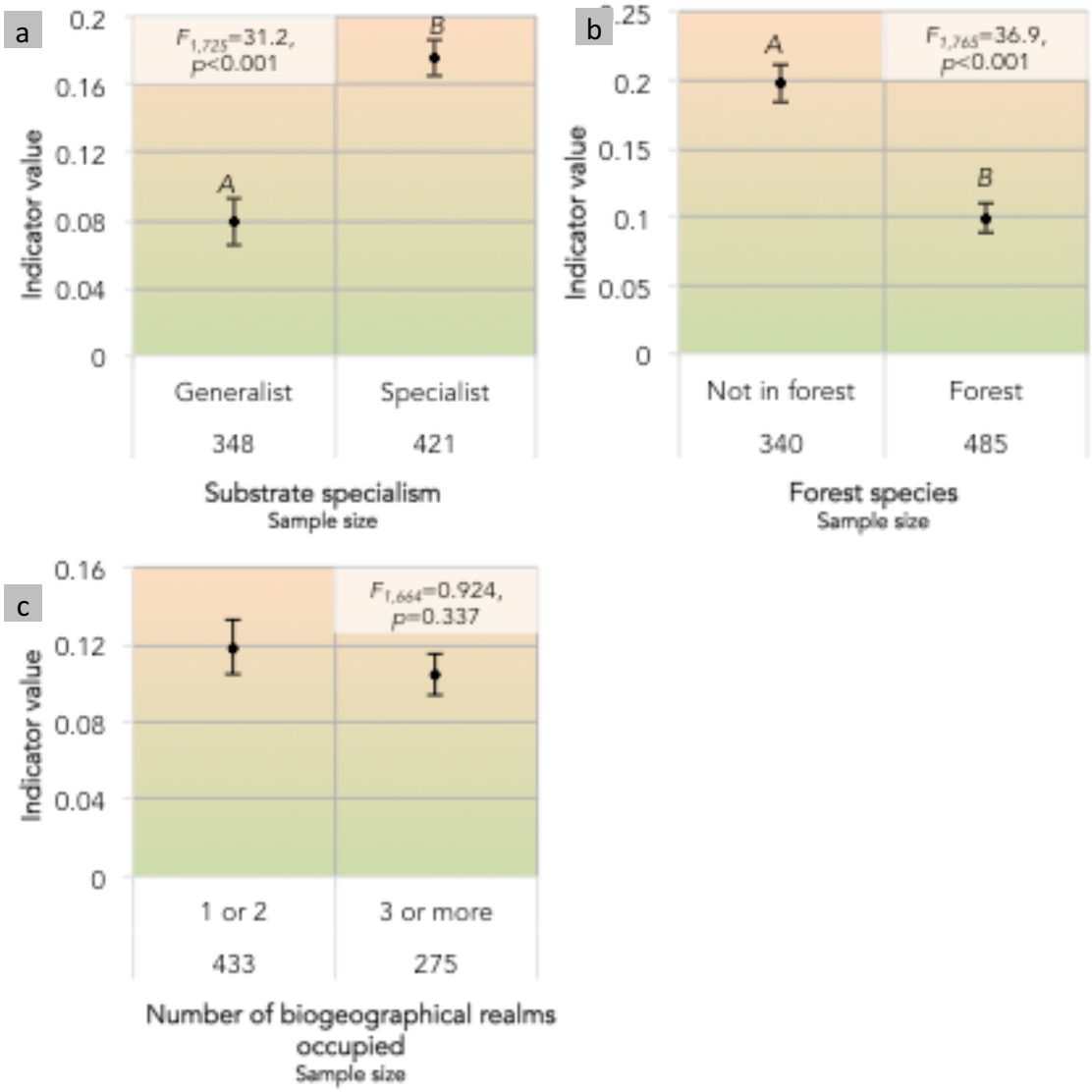


Figure 4.15 Mean IV (±1SE) in a) species with different life-forms; b) species of different sizes (category). Sample sizes shown under x-axis labels. Means with different letters indicate significant differences; ANOVA p<0.05.

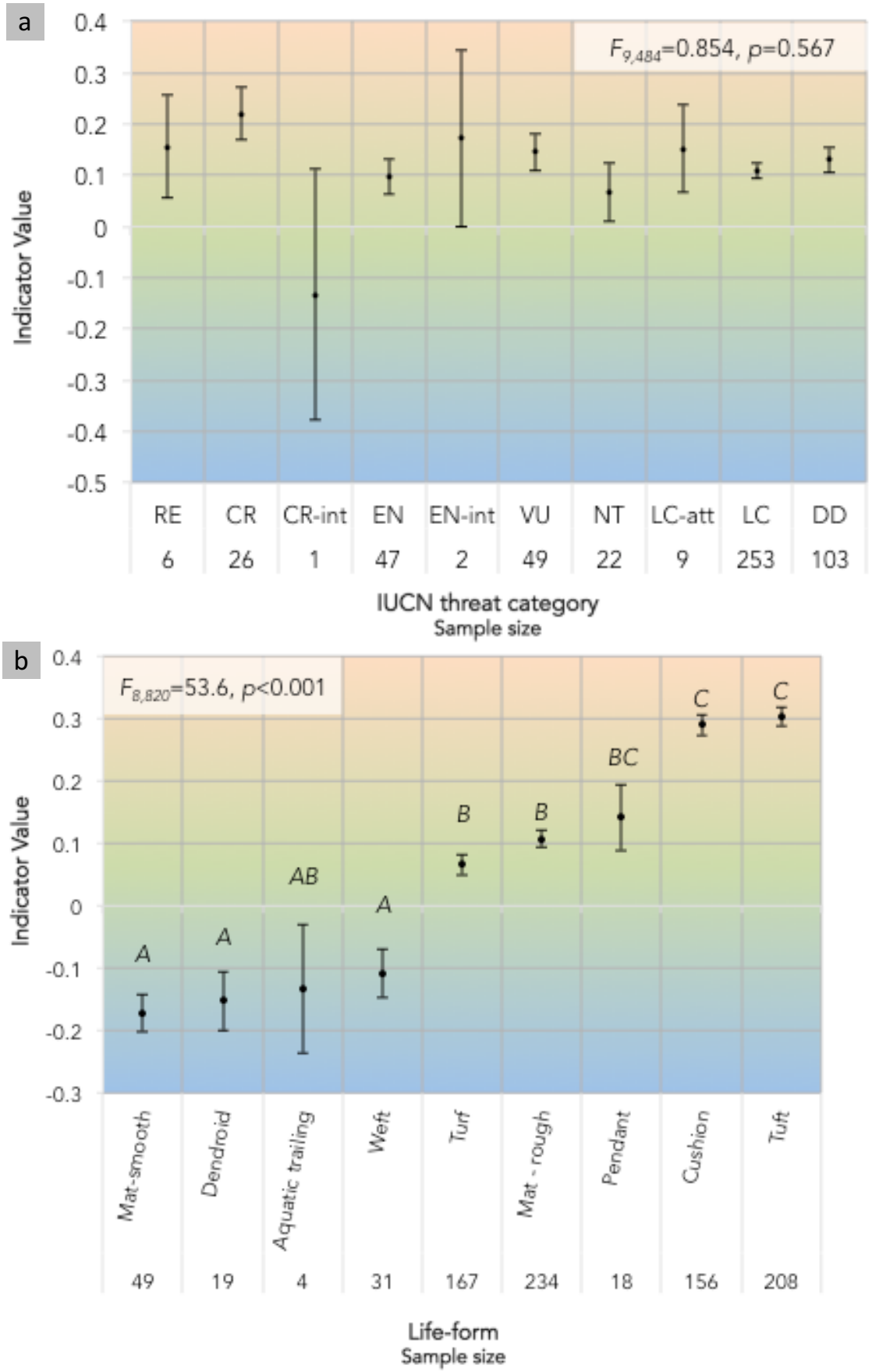


Figure 4.16 Mean IV (±1SE) in a) different IUCN threat categories; b) different life-form categories. Sample sizes shown under x-axis labels. Means with different letters indicate significant differences; ANOVA and Games-Howell p<0.05.

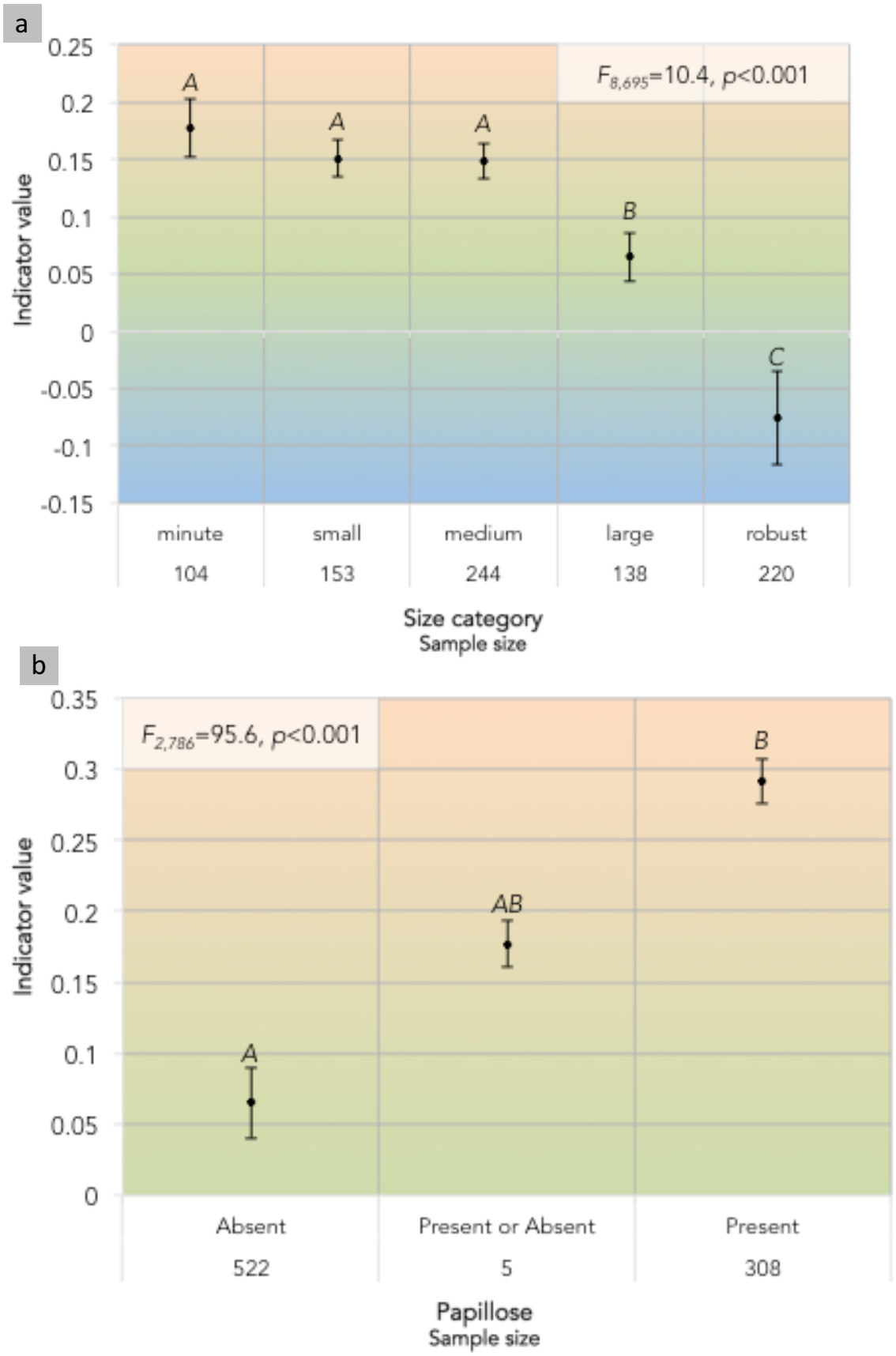


Figure 4.17 Mean IV (±1SE) in a) different plant sizes (categories); b) species with or without papillae. Sample sizes shown under x-axis labels. Means with different letters indicate significant differences; ANOVA and Games-Howell p<0.05.

4.5 Discussion

4.5.1 Habitat and environmental variables

Although epiphyte species had a significantly lower EI (and so greater affinity to humid and sheltered environments), this difference was very small but the mean EI difference was large between forest and non-forest species. This reinforces the point that even in the more humid and sheltered habitat that is forest, there are variations in microclimate meaning that species of varying DT and environmental preferences will be found in this habitat. At an even smaller scale, because the mean EI difference between epiphyte and non-epiphyte species was very small and their ranges were similar, it indicates that epiphytic bryophytes could be useful indicators as they occupy a range of environmental conditions and therefore have varying DT levels. From the multivariate analyses, no relationship was found between the habitat variables (epiphyte or not, forest species or not) and the species groupings (from MCA and clustering). Habitat variables are likely to be associated with other dimensions but as the aim is to identify indicators of dry and exposed vs. humid and sheltered environments, it was not of relevance at this stage to investigate further species groupings based on habitat variables.

The second dimension of the MCA seems to be related to habitat and environmental range, and with vegetative propagules traits. This indicates that the presence of vegetative propagules is related to the breadth of habitats, substrates and environmental conditions a species can inhabit, as vegetative propagules allow species to survive drought periods better as well as disperse (Rydin, 2009). As it was not relevant to the aim of this study, it was not investigated further but suggests that it would be a worthwhile to do so in order to ascertain the importance of vegetative propagules to species' ecology.

Temperature could not be used in analyses due to the low amount of data availability and its unreliability; data was taken from laboratory measurements (Dierßen, 2001) and so may not reflect field environmental preferences. It could be possible to obtain the temperature preferences of species using georeferenced specimen records and climate data. However, data available is not at a resolution fine enough to accurately reflect temperatures experienced by bryophytes.

4.5.2 Indicator species, genera and families

Renauld & Cardot (1915) stated that the dense humid forests in eastern Madagascar contained numerous bryophytes due to the low penetration of sunlight which diminishes evaporation and so maintains the high humidity ideal to many bryophyte species. These forests are characterised by species that have low DT such as species of the *Leucoloma*, *Neckera* and *Hookeria* genera. From the indicator values assigned in this study, these genera do have lower indicator values, but *Leucoloma* indicates drier and more exposed conditions. In contrast, those present in the grasslands and shrubland include desiccation tolerant species from the Pottiaceae and Dicranaeceae families. Curiously, Fissidentaceae species, which have low DT, are also found in these habitats. This could be explained by the presence of conduplicate leaves that act as water stores enabling *Fissidens* spp. to continue metabolic activity once water is no longer available in the surrounding environment. It also highlights the that the presence of microhabitats is extremely important for bryophytes allowing seemingly low DT species to inhabit relatively dry habitats.

Epiphytic species are mostly indicators of dry and exposed habitats, and although at first it seems unexpected as they are usually forest species (and therefore a usually humid and sheltered habitat), they occupy relatively drier microhabitats than other species (such as ground bryophytes).

A next step will be to correlate the IV of tropical species with the Red List assessments that are currently being undertaken for 1500 bryophytes (global, not only tropical) as part of the Sampled Red List Index project (SRLI). This will provide a large-scale global IV threat status, which will be useful to infer the status of species that have not been assessed.

This study identified species, genera and even one family of strict indicator taxa. These are likely to be the most reliable indicators as they do no occupy a wide range of environmental conditions; once their preferred microclimate is disturbed, they will not survive.

4.5.3 Indicator value

The indicator value (IV) seems to be a better metric to differentiate between species traits than the EI alone. The difference in mean IV of generalist and specialist species is much greater than the difference in EI; the same applies to forest and non-forest species. This suggests that rather than using data on species' environmental preferences, further work to select bryophyte indicators can be done based on the trait profile of species (and subsequently assign an IV).

The lack of differences between IUCN threat categories in both the EI and IV indicates that neither can be used to predict species' threat or rarity. They are therefore more suitable as indicators of ecology and habitat than extinction risk. It also shows that threat of extinction is due to a multitude of factors and not merely based on species' intrinsic environmental preferences.

As the IV varied significantly with different life-forms, plant size and the presence of papillae, these traits could be a useful indicator – they are easy to measure and so could be recorded by non-specialists making a studies cost-effective (Gardner et al., 2008).

Due to the lack of knowledge on tropical bryophyte ecological tolerances, the IV created here can be used as a proxy in lieu of experimental data. The corrobation of the IV in the next chapter will provide further evidence as to whether this is possible or not.

4.5.4 Methodology

The clustering analysis revealed clusters of trait profiles that indicate environmental preference of species. However, within these clusters there were traits that characterised the cluster's grouping better than others. While statistically those traits that best characterise a cluster have v-test values higher than 1.96 (outputting approximately 40 trait states per cluster from a total of 105) These were then refined to include those trait states with a high representation (percentage of species) within the cluster and with v-test values above 3. This threshold was chosen arbitrarily, using expert knowledge, in order to select a suite of traits that best characterise species of the two different environment preference groups. Expert knowledge of bryophyte ecology and morphology was necessary here in selecting trait states that can both be readily determined (easy to measure) and which are less subjective or ambiguous in their determination. This again shows the importance of combining statistical analysis with knowledge of the taxonomy, morphology and ecology of species.

4.5.4.1 Species selection

Following the assignment of indicator values to species, genera and families a manual check was made to ensure there were no taxa clearly erroneously allocated (based on knowledge of their ecology). Prior to implementation of these indicators, it would be necessary for the indicator list to be independently reviewed by taxonomic experts of particular groups. A future study could refine the indicator list further by focussing on those taxa that have been suggested as the most useful indicators. This would involve gathering data on all species of that taxa (genus or family), focussing on the suite of traits in the trait profiles defined – epiphyte species would be a good starting point.

4.5.4.2 Habitat traits

When recording the substrate a species occupies, the whole range of substrates may have been overlooked as mostly floras and articles were consulted, as opposed to as many herbarium specimens as possible. As an example, most species of the Calymperaceae family are assumed to be exclusively epiphytic and from the trait matrix most are. However, when Reese (2001) conducted a study on Calymperaceae and substrate preferences, using herbarium specimens, he found that although many species were epiphytic, some species were equally found on rock or soil with only a few species being obligate epiphytes.

Species that do not occur in forest habitats have a lower mean EI value than forest species, and this could be potentially due to the inclusion of species that occupy freshwater habitats and peat bogs that have very low moisture values (low moisture value corresponds to wetter habitats).

4.6 Conclusions

This chapter built on the previous chapter's analysis of individual traits' relationship to their environment. With an understanding of the individual traits most linked to desiccation tolerance the EI (Environmental Index) was formulated. The EI represents a species moisture and light preferences, with a lower value indicating species of humid and sheltered habitats, and a species with a higher value preferring dry and exposed habitats. However, while looking at how single traits relate to the EI is informative, traits must be considered in the context of the suite of traits of an individual in order to identify suitable indicator species. To do this multivariate MCA analysis was carried out on all species (303 spp.) in the trait database with complete trait profiles (30 traits). This analysis grouped species into clusters based on the similarity of their trait profiles.

Using these results, indicator values were assigned to all species (1011 spp.) including those with missing data, using average trait profile scores. This analysis identified groups of traits (rather than individual traits and trait states) that are similarly related to environmental conditions. Trait profiles were then used to assign species an environmental indicator value, and subsequently species were categorised into four indicator classes; strict humid and sheltered indicator, strict dry and exposed indicator, non-strict humid and sheltered, and non-strict dry and exposed indicator.

From results in this chapter, bryophytes do seem to have value as indicators as different groups of species have different IVs and therefore will respond differently to changes in forest structure. Because the trait life-form varied significantly with IV, it also suggests that bryophytes fulfil

another indicator pre-requisite that they are easy to survey: life-form is a very easy trait to record in the field, by eye.

Applying these findings, the average trait profile values (C1 and C3) of species within genera and family were calculated to assign genera and family an indicator value (C1-C3). Ranked by their indicator values we can see from Figures 4.13 to 4.15 that genus and family could be used as indicators of environmental preference of the taxa. Although using this taxonomic level may have a greater margin of error (due to the increasing variability with increasing taxonomic level), it is a useful first approach for taxa that are difficult to identify to the species level – as is the case of some tropical bryophytes.

Among the traits that can be used to assign species to an environmental class (humid and, sheltered *or* dry and exposed), life-form was found to successfully partition species, with more closed life-forms found in dry and exposed conditions, and more open life forms found in humid and sheltered. As this trait is one that is easy to measure it is a particularly useful trait because it allows species for which trait and environmental data is scarce (as in the case of most tropical Malagasy species) to be used. The next chapter will use life-form and the indicator values assigned to species, genera and families in this chapter to test how life-form relates to forest degradation and if the indicator values assigned reflect the reality in the field.

Appendix 4 Multivariate analyses

A4.1. Habitat types

Table 4.18 Habitat types according to the IUCN classification, version 3.1.

IUCN Habitats
1 Forest
1.1 Boreal Forest
1.2 Subarctic Forest
1.3 Subantarctic Forest
1.4 Temperate Forest
1.5 Subtropical/Tropical Dry Forest
1.6 Subtropical/Tropical Moist Lowland Forest
1.7 Subtropical/Tropical Mangrove Forest Vegetation Above High Tide Level
1.8 Subtropical/Tropical Swamp Forest
1.9 Subtropical/Tropical Moist Montane Forest
2 Savanna
2.1 Dry Savanna
2.2 Moist Savana
3 Shrubland
3.1 Subarctic Shrubland
3.2 Subantarctic Shrubland
3.3 Boreal Shrubland
3.4 Temperate Shrubland
3.5 Subtropical/Tropical Dry Shrubland
3.6 Subtropical/Tropical Moist Shrubland
3.7 Subtropical/Tropical High Altitude Shrubland
3.8 Mediterranean-type Shrubby Vegetation
4 Grassland
4.1 Tundra
4.2 Subarctic Grassland
4.3 Subantarctic Grassland
4.4 Temperate Grassland
4.5 Subtropical/Tropical Dry Lowland Grassland
4.6 Subtropical/Tropical Seasonally Wet/Flooded Lowland Grassland
4.7 Subtropical/Tropical High Altitude Grassland
5 Wetlands (inland)
5.1 Permanent Rivers, Streams, Creeks [includes waterfalls]
5.2 Seasonal/Intermittent/Irregular Rivers, Streams, Creeks
5.3 Shrub Dominated Wetlands
5.4 Bogs, Marshes, Swamps, Fens, Peatlands [generally over 8 ha]

5.5 Permanent Freshwater Lakes [over 8 ha]
5.6 Seasonal/Intermittent Freshwater Lakes [over 8 ha]
5.7 Permanent Freshwater Marshes/Pools [under 8 ha]
5.8 Seasonal/Intermittent Freshwater Marshes/Pools [under 8 ha]
5.9 Freshwater Springs and Oases
5.10 Tundra Wetlands [includes pools and temporary waters from snowmelt]
5.11 Alpine Wetlands [includes temporary waters from snowmelt]
5.12 Geothermal Wetlands
5.13 Permanent Inland Deltas
5.14 Permanent Saline, Brackish or Alkaline Lakes
5.15 Seasonal/Intermittent Saline, Brackish or Alkaline Lakes and Flats
5.16 Permanent Saline, Brackish or Alkaline Marshes/Pools
5.17 Seasonal/Intermittent Saline, Brackish or Alkaline Marshes/Pools
5.18 Karst and Other Subterranean Inland Aquatic Systems
6 Rocky Areas [e.g. inland cliffs, mountain peaks]
7 Caves and Subterranean Habitats (non-aquatic)
7.1 Caves
7.2 Other Subterranean Habitats
8 Desert
8.1 Hot
8.2 Temperate
8.3 Cold
9 Marine Neritic (Submergent Nearshore Continental Shelf or Oceanic Island)
9.1 Pelagic
9.2 Subtidal Rock and Rocky Reefs
9.3 Subtidal Loose Rock/Pebble/Gravel
9.4 Subtidal Sandy
9.5 Subtidal Sandy-Mud
9.6 Subtidal Muddy
9.7 Macroalgal/Kelp
9.8 Coral Reef
9.8.1 Outer Reef Channel
9.8.2 Back Slope
9.8.3 Foreslope (Outer Reef Slope)
9.8.4 Lagoon
9.8.5 Inter-Reef Soft Substrate
9.8.6 Inter-Reef Rubble Substrate
9.9 Seagrass (Submerged)
9.10 Estuaries
12 Marine Intertidal
12.1 Rocky Shoreline
12.2 Sandy Shoreline and/or Beaches, Sand Bars, Spits, etc.
12.3 Shingle and/or Pebble Shoreline and/or Beaches
12.4 Mud Shoreline and Intertidal Mud Flats
12.5 Salt Marshes (Emergent Grasses)

12.6 Tidepools
12.7 Mangrove Submerged Roots
13 Marine Coastal/Supratidal
13.1 Sea Cliffs and Rocky Offshore Islands
13.2 Coastal Caves/Karst
13.3 Coastal Sand Dunes
13.4 Coastal Brackish/Saline Lagoons/Marine Lakes
13.5 Coastal Freshwater Lakes
14 Artificial - Terrestrial
14.1 Arable Land
14.2 Pastureland
14.3 Plantations
14.4 Rural Gardens
14.5 Urban Areas
14.6 Subtropical/Tropical Heavily Degraded Former Forest
15 Artificial - Aquatic
15.1 Water Storage Areas [over 8 ha]
15.2 Ponds [below 8 ha]
15.3 Aquaculture Ponds
15.4 Salt Exploitation Sites
15.5 Excavations (open)
15.6 Wastewater Treatment Areas
15.7 Irrigated Land [includes irrigation channels]
15.8 Seasonally Flooded Agricultural Land
15.9 Canals and Drainage Channels, Ditches
15.10 Karst and Other Subterranean Hydrological Systems [human-made]
15.11 Marine Anthropogenic Structures
15.12 Mariculture Cages
15.13 Mari/Brackish-culture Ponds
16 Introduced Vegetation
17 Other
18 Unknown

Table 4.19 Habitat types according to the EUNIS classification,.

EUNIS habitat	
A	**Marine habitats**
A1	Littoral rock and other hard substrata
A2	Littoral sediment
A3	Infralittoral rock and other hard substrata
A4	Circalittoral rock and other hard substrata
A5	Sublittoral sediment
A6	Deep-sea bed
A7	Pelagic water column

A8 Ice-associated marine habitats

B Coastal habitats

B1 Coastal dunes and sandy shores

B2 Coastal shingle

B3 Rock cliffs, ledges and shores, including the supralittoral

C Inland surface waters

C1 Surface standing waters

C2 Surface running waters

C3 Littoral zone of inland surface waterbodies

D Mires, bogs and fens

D1 Raised and blanket bogs

D2 Valley mires, poor fens and transition mires

D3 Aapa, palsa and polygon mires

D4 Base-rich fens and calcareous spring mires

D5 Sedge and reedbeds, normally without free-standing water

D6 Inland saline and brackish marshes and reedbeds

E Grasslands and lands dominated by forbs, mosses or lichens

E1 Dry grasslands

E2 Mesic grasslands

E3 Seasonally wet and wet grasslands

E4 Alpine and subalpine grasslands

E5 Woodland fringes and clearings and tall forb stands

E6 Inland salt steppes

E7 Sparsely wooded grasslands

F Heathland, scrub and tundra

F1 Tundra

F2 Arctic, alpine and subalpine scrub

F3 Temperate and mediterranean-montane scrub

F4 Temperate shrub heathland

F5 Maquis, arborescent matorral and thermo-Mediterranean brushes

F6 Garrigue

F7 Spiny Mediterranean heaths (phrygana, hedgehog-heaths and related coastal cliff vegetation)

F8 Thermo-Atlantic xerophytic scrub

F9 Riverine and fen scrubs

FA Hedgerows

FB Shrub plantations

G Woodland, forest and other wooded land

G1 Broadleaved deciduous woodland

G2 Broadleaved evergreen woodland

G3 Coniferous woodland

G4 Mixed deciduous and coniferous woodland

G5 Lines of trees, small anthropogenic woodlands, recently felled woodland, early-stage woodland and coppice

H Inland unvegetated or sparsely vegetated habitats

H1	Terrestrial underground caves, cave systems, passages and waterbodies
H2	Screes
H3	Inland cliffs, rock pavements and outcrops
H4	Snow or ice-dominated habitats
H5	Miscellaneous inland habitats with very sparse or no vegetation
H6	Recent volcanic features
I	**Regularly or recently cultivated agricultural, horticultural and domestic habitats**
I1	Arable land and market gardens
I2	Cultivated areas of gardens and parks
J	**Constructed, industrial and other artificial habitats**
J1	Buildings of cities, towns and villages
J2	Low density buildings
J3	Extractive industrial sites
J4	Transport networks and other constructed hard-surfaced areas
J5	Highly artificial man-made waters and associated structures
J6	Waste deposits

Table 4.20 Habitat types in Madagascar according to the classification by Moat & Smith, 2007.

Madagascar Habitats
Humid forest
Littoral forest
Western humid forest
Western sub-humid forest
Western dry forest
South western dry spiny forest-thicket
South western coastal busland
Mangroves
Tapia forest
Wetlands
Degraded humid forest
Degraded south western dry spiny forest
Wooded grassland-bushland mosaic
Plateau grassland-woodland grassland mosaic
Cultivation
Bare soil/rock

A4.2. Species codes

Table 4.21 Taxon codes used in database and analyses, listed alphabetically by taxon name. Nomenclature follows Goffinet *et al.* (2009).

Taxon Code	Taxon	Order	Family
ACAFONT	*Acaulon fontiquerianum*	Pottiales	Pottiaceae
ACAMEDI	*Acaulon mediterraneum*	Pottiales	Pottiaceae
ACAMUTI	*Acaulon muticum*	Pottiales	Pottiaceae
ACATRIQ	*Acaulon triquetrum*	Pottiales	Pottiaceae
ALOALOI	*Aloina aloides*	Pottiales	Pottiaceae
ALOAMBI	*Aloina ambigua*	Pottiales	Pottiaceae
ALORIGI	*Aloina rigida*	Pottiales	Pottiaceae
AMBHUMI	*Hygroamblystegium humile*	Hypnales	Amblystegiaceae
AMBSERP	*Amblystegium serpens*	Hypnales	Amblystegiaceae
AMPMOUG	*Amphidium mougeotii*	Dicranales	Rhabdoweisiaceae
ANAWEBB	*Anacolia webbii*	Bryales	Bartramiaceae
ANDFRIG	*Andreaea frigida*	Andreaeales	Andreaeaceae
ANDHEIC	*Andreaea heinemannii* subsp. *crassifolia*	Andreaeales	Andreaeaceae
ANDHEIN	*Andreaea heinemannii* subsp. *heinemannii*	Andreaeales	Andreaeaceae
ANDMEGI	*Andreaea megistospora*	Andreaeales	Andreaeaceae
ANDROTF	*Andreaea rothii* subsp. *falcata*	Andreaeales	Andreaeaceae
ANDROTH	*Andreaea rothii* subsp. *rothii*	Andreaeales	Andreaeaceae
ANDRUPE	*Andreaea rupestris*	Andreaeales	Andreaeaceae
ANOJULA	*Anomobryum julaceum*	Bryales	Bryaceae
ANOLUSI	*Anomobryum lusitanicum*	Bryales	Bryaceae
ANOVITI	*Anomodon viticulosus*	Leucodontales	Anomodontaceae
ANTCALI	*Antitrichia californica*	Leucodontales	Leucodontaceae
ANTCURT	*Antitrichia curtipendula*	Leucodontales	Leucodontaceae
ARCALTE	*Archidium alternifolium*	Archidales	Archidiaceae
ASCCARN	*Aschisma carniolicum*	Pottiales	Pottiaceae
ATRANDR	*Atrichum androgynum*	Polytrichales	Polytrichaceae
ATRANGU	*Atrichum angustatum*	Polytrichales	Polytrichaceae
ATRUNDU	*Atrichum undulatum*	Polytrichales	Polytrichaceae
AULANDR	*Aulacomnium androgynum*	Bryales	Aulacomniaceae
AULPALU	*Aulacomnium palustre*	Bryales	Aulacomniaceae
BARBOLL	*Barbula bolleana*	Pottiales	Pottiaceae
BARCONV	*Barbula convoluta*	Pottiales	Pottiaceae
BARITHY	*Bartramia ithyphylla*	Bryales	Bartramiaceae
BARPOMI	*Bartramia pomiformis*	Bryales	Bartramiaceae
BARSTRI	*Bartramia stricta*	Bryales	Bartramiaceae
BARUNGU	*Barbula unguiculata*	Pottiales	Pottiaceae
BLIACUT	*Blindia acuta*	Seligerales	Seligeriaceae
BRAALBI	*Brachythecium albicans*	Hypnales	Brachytheciaceae
BRADIEC	*Brachythecium dieckii*	Hypnales	Brachytheciaceae

Taxon Code	Taxon	Order	Family
BRAGLAR	*Brachythecium glareosum*	Hypnales	Brachytheciaceae
BRAMILD	*Brachythecium mildeanum*	Hypnales	Brachytheciaceae
BRAOLYM	*Brachythecium olympicum*	Hypnales	Brachytheciaceae
BRAPLUM	*Brachythecium plumosum*	Hypnales	Brachytheciaceae
BRARIVU	*Brachythecium rivulare*	Hypnales	Brachytheciaceae
BRARUTA	*Brachythecium rutabulum*	Hypnales	Brachytheciaceae
BRASALE	*Brachythecium salebrosum*	Hypnales	Brachytheciaceae
BRASALI	*Brachythecium velutinum*	Hypnales	Brachytheciaceae
BRASECU	*Braunia secunda*	Hedwigiales	Hedwigiaceae
BRATRIC	*Brachydontium trichodes*	Seligerales	Seligeriaceae
BRUVOGE	*Bruchia vogesiaca*	Dicranales	Bruchiaceae
BRYALPI	*Bryum alpinum*	Bryales	Bryaceae
BRYARAC	*Bryum arachnoideum*	Bryales	Bryaceae
BRYARGE	*Bryum argenteum*	Bryales	Bryaceae
BRYCAES	*Bryum caespiticium*	Bryales	Bryaceae
BRYCAMP	*Bryoerythrophyllum campylocarpum*	Pottiales	Pottiaceae
BRYCANA	*Bryum canariense*	Bryales	Bryaceae
BRYCAPP	*Bryum capillare*	Bryales	Bryaceae
BRYDICH	*Bryum dichotomum*	Bryales	Bryaceae
BRYDONI	*Bryum donianum*	Bryales	Bryaceae
BRYELEG	*Bryum elegans*	Bryales	Bryaceae
BRYGEMF	*Bryum gemmiferum*	Bryales	Bryaceae
BRYGEML	*Bryum gemmilucens*	Bryales	Bryaceae
BRYGEMP	*Bryum gemmiparum*	Bryales	Bryaceae
BRYKLIN	*Bryum klinggraeffii*	Bryales	Bryaceae
BRYKUNZ	*Bryum kunzei*	Bryales	Bryaceae
BRYMILD	*Bryum mildeanum*	Bryales	Bryaceae
BRYMINI	*Bryum minii*	Bryales	Bryaceae
BRYMORA	*Bryum moravicum*	Bryales	Bryaceae
BRYMUEH	*Bryum muehlenbeckii*	Bryales	Bryaceae
BRYPALL	*Bryum pallescens*	Bryales	Bryaceae
BRYPSEU	*Bryum pseudotriquetrum*	Bryales	Bryaceae
BRYRADI	*Bryum radiculosum*	Bryales	Bryaceae
BRYRUBE	*Bryum rubens*	Bryales	Bryaceae
BRYRUDE	*Bryum ruderale*	Bryales	Bryaceae
BRYSAUT	*Bryum sauteri*	Bryales	Bryaceae
BRYSCHL	*Bryum schleicheri*	Bryales	Bryaceae
BRYSUBA	*Bryum subapiculatum*	Bryales	Bryaceae
BRYTENU	*Bryum tenuisetum*	Bryales	Bryaceae
BRYTORQ	*Bryum torquescens*	Bryales	Bryaceae
BRYVALP	*Bryum valparaisense*	Bryales	Bryaceae
CALCUSP	*Calliergonella cuspidata*	Hypnales	Hypnaceae
CALEROS	*Calymperes erosum*	Dicranales	Calymperaceae

Taxon Code	Taxon	Order	Family
CALPALL	*Calymperes pallidum*	Dicranales	Calymperaceae
CALPAPI	*Callicostella papillata* var. *papillata*	Hookeriales	Pilotrichaceae
CALTAHI	*Calymperes tahitense*	Dicranales	Calymperaceae
CALVENE	*Calymperes venezuelanum*	Dicranales	Calymperaceae
CAMBREV	*Campylopus brevipilus*	Dicranales	Leucobryaceae
CAMCHRY	*Campyliadelphus chrysophyllus*	Hypnales	Amblystegiaceae
CAMFLEX	*Campylopus flexuosus*	Dicranales	Leucobryaceae
CAMFRAG	*Campylopus fragilis*	Dicranales	Leucobryaceae
CAMINTR	*Campylopus introflexus*	Dicranales	Leucobryaceae
CAMPILI	*Campylopus pilifer*	Dicranales	Leucobryaceae
CAMPITA	*Campylostelium pitardii*	Grimmiales	Ptychomitriaceae
CAMPYRI	*Campylopus pyriformis*	Dicranales	Leucobryaceae
CAMSTEL	*Campylium stellatum*	Hypnales	Amblystegiaceae
CAMSTRI	*Campylostelium strictum*	Grimmiales	Ptychomitriaceae
CAMSUBU	*Campylopus subulatus*	Dicranales	Leucobryaceae
CERPURP	*Ceratodon purpureus* subsp. *purpureus*	Dicranales	Ditrichaceae
CHECHLO	*Cheilothela chloropus*	Dicranales	Ditrichaceae
CINAQUA	*Cinclidotus aquaticus*	Pottiales	Pottiaceae
CINFONT	*Cinclidotus fontinaloides*	Pottiales	Pottiaceae
CINRIPA	*Cinclidotus riparius*	Pottiales	Pottiaceae
CIRCRAS	*Cirriphyllum crassinervium*	Hypnales	Brachytheciaceae
CLABOGO	*Clastobryophilum bogoricum*	Hypnales	Sematophyllaceae
CLAWHIP	*Claopodium whippleanum*	Leucodontales	Anomodontaceae
CLIDEND	*Climacium dendroides*	Leucodontales	Climaciaceae
COSCRIB	*Coscinodon cribrosus*	Grimmiales	Grimmiaceae
CRAFILI	*Cratoneuron filicinum*	Hypnales	Amblystegiaceae
CROCRAS	*Crossidium crassinerve*	Pottiales	Pottiaceae
CROSQUA	*Crossidium squamiferum*	Pottiales	Pottiaceae
CRYHETE	*Cryphaea heteromalla*	Leucodontales	Cryphaeaceae
CTEMOLL	*Ctenidium molluscum*	Hypnales	Hylocomiaceae
CYCLAET	*Cyclodictyon laetevirens*	Hookeriales	Pilotrichaceae
CYNBRUN	*Cynodontium bruntonii*	Dicranales	Rhabdoweisiaceae
CYNGRAC	*Cynodontium gracilescens*	Dicranales	Rhabdoweisiaceae
CYNJENN	*Cynodontium jenneri*	Dicranales	Rhabdoweisiaceae
CYNPOLY	*Cynodontium polycarpon*	Dicranales	Rhabdoweisiaceae
DENLAMY	*Dendrocryphaea lamyana*	Leucodontales	Cryphaeaceae
DIAFRAG	*Dialytrichia fragilifolia*	Pottiales	Pottiaceae
DIAMUCR	*Dialytrichia mucronata*	Pottiales	Pottiaceae
DICCIRR	*Dicranoweisia cirrata*	Dicranales	Rhabdoweisiaceae
DICCRAS	*Dicranum crassifolium*	Dicranales	Dicranaceae
DICHETE	*Dicranella heteromalla*	Dicranales	Dicranaceae
DICHOWE	*Dicranella howei*	Dicranales	Dicranaceae
DICPELL	*Dichodontium pellucidum*	Dicranales	Rhabdoweisiaceae

Taxon Code	Taxon	Order	Family
DICRUFE	*Dicranella rufescens*	Dicranales	Dicranaceae
DICSCOP	*Dicranum scoparium*	Dicranales	Dicranaceae
DICSUBU	*Dicranella subulata*	Dicranales	Dicranaceae
DICTAUR	*Dicranum tauricum*	Dicranales	Dicranaceae
DICVARI	*Dicranella varia*	Dicranales	Dicranaceae
DIDACUT	*Didymodon acutus*	Pottiales	Pottiaceae
DIDAUST	*Didymodon australasiae*	Pottiales	Pottiaceae
DIDBIST	*Didymodon bistratosus*	Pottiales	Pottiaceae
DIDECKE	*Didymodon eckeliae*	Pottiales	Pottiaceae
DIDEROS	*Didymodon erosus*	Pottiales	Pottiaceae
DIDFALL	*Didymodon fallax*	Pottiales	Pottiaceae
DIDINSU	*Didymodon insulanus*	Pottiales	Pottiaceae
DIDLURI	*Didymodon luridus*	Pottiales	Pottiaceae
DIDNICH	*Didymodon nicholsonii*	Pottiales	Pottiaceae
DIDRIGI	*Didymodon rigidulus*	Pottiales	Pottiaceae
DIDSICC	*Didymodon sicculus*	Pottiales	Pottiaceae
DIDSINU	*Didymodon sinuosus*	Pottiales	Pottiaceae
DIDSPAD	*Didymodon spadiceus*	Pottiales	Pottiaceae
DIDTOPH	*Didymodon tophaceus*	Pottiales	Pottiaceae
DIDUMBR	*Didymodon umbrosus*	Pottiales	Pottiaceae
DIDVINE	*Didymodon vinealis*	Pottiales	Pottiaceae
DIPFOLI	*Diphyscium foliosum*	Diphysciales	Diphysciaceae
DISCAPI	*Distichium capillaceum*	Dicranales	Ditrichaceae
DITHETE	*Ditrichum heteromallum*	Dicranales	Ditrichaceae
DITSUBU	*Ditrichum subulatum*	Dicranales	Ditrichaceae
DREADUN	*Drepanocladus aduncus*	Hypnales	Amblystegiaceae
DREPOLY	*Drepanocladus polygamus*	Hypnales	Amblystegiaceae
ECTCHEN	*Ectropothecium chenagonii*	Hypnales	Hypnaceae
ENCCILI	*Encalypta ciliata*	Encalyptales	Encalyptaceae
ENCSTRE	*Encalypta streptocarpa*	Encalyptales	Encalyptaceae
ENCVULG	*Encalypta vulgaris*	Encalyptales	Encalyptaceae
ENTATTE	*Entosthodon attenuatus*	Funariales	Funariaceae
ENTCONV	*Entosthodon convexus*	Funariales	Funariaceae
ENTFASC	*Entosthodon fascicularis*	Funariales	Funariaceae
ENTMOUR	*Entosthodon mouretii*	Funariales	Funariaceae
ENTOBTU	*Entosthodon obtusus*	Funariales	Funariaceae
ENTPULC	*Entosthodon pulchellus*	Funariales	Funariaceae
ENTSCHI	*Entosthodon schimperi*	Funariales	Funariaceae
EPHMINU	*Ephemerum minutissimum*	Funariales	Ephemeraceae
EPHRECU	*Ephemerum recurvifolium*	Funariales	Ephemeraceae
EPHSERR	*Ephemerum serratum*	Funariales	Ephemeraceae
EPHSESS	*Ephemerum sessile*	Funariales	Ephemeraceae
EPHSTEL	*Ephemerum stellatum*	Funariales	Ephemeraceae

Taxon Code	Taxon	Order	Family
EPITOZE	*Epipterygium tozeri*	Bryales	Melichoferiaceae
EUCVERT	*Eucladium verticillatum*	Pottiales	Pottiaceae
EURPULC	*Eurhynchiastrum pulchellum*	Hypnales	Brachytheciaceae
EURSTRI	*Eurhynchium striatum*	Hypnales	Brachytheciaceae
FABLACH	*Fabronia lachenaudii*	Hypnales	Fabroniaceae
FABMOTE	*Fabronia motelayi*	Hypnales	Fabroniaceae
FABPUSI	*Fabronia pusilla*	Hypnales	Fabroniaceae
FISADIA	*Fissidens adianthoides*	Fissidentales	Fissidentaceae
FISBRYC	*Fissidens bryoides* var. *caespitans*	Fissidentales	Fissidentaceae
FISBRYO	*Fissidens bryoides*	Fissidentales	Fissidentaceae
FISCRAS	*Fissidens crassipes*	Fissidentales	Fissidentaceae
FISCRIS	*Fissidens crispus*	Fissidentales	Fissidentaceae
FISCURV	*Fissidens curvatus*	Fissidentales	Fissidentaceae
FISDUBI	*Fissidens dubius*	Fissidentales	Fissidentaceae
FISEXIL	*Fissidens exilis*	Fissidentales	Fissidentaceae
FISFONT	*Fissidens fontanus*	Fissidentales	Fissidentaceae
FISGRAC	*Fissidens gracilifolius*	Fissidentales	Fissidentaceae
FISGRAN	*Fissidens grandifrons*	Fissidentales	Fissidentaceae
FISJANS	*Fissidens jansenii*	Fissidentales	Fissidentaceae
FISMONG	*Fissidens monguillonii*	Fissidentales	Fissidentaceae
FISOSMU	*Fissidens osmundoides*	Fissidentales	Fissidentaceae
FISOVTI	*Fissidens ovatifolius*	Fissidentales	Fissidentaceae
FISPOLY	*Fissidens polyphyllus*	Fissidentales	Fissidentaceae
FISPUSI	*Fissidens pusillus*	Fissidentales	Fissidentaceae
FISRIVU	*Fissidens rivularis*	Fissidentales	Fissidentaceae
FISSERR	*Fissidens serrulatus*	Fissidentales	Fissidentaceae
FISTAXI	*Fissidens taxifolius*	Fissidentales	Fissidentaceae
FISVIRI	*Fissidens viridulus*	Fissidentales	Fissidentaceae
FONANTI	*Fontinalis antipyretica*	Leucodontales	Fontinalaceae
FONHYPN	*Fontinalis hypnoides*	Leucodontales	Fontinalaceae
FONSQUA	*Fontinalis squamosa*	Leucodontales	Fontinalaceae
FUNCURV	*Funariella curviseta*	Funariales	Funariaceae
FUNHYGR	*Funaria hygrometrica*	Funariales	Funariaceae
GAMCEYL	*Gammiella ceylonensis*	Hypnales	Hypnaceae
GRICAES	*Grimmia caespiticia*	Grimmiales	Grimmiaceae
GRIDECI	*Grimmia decipiens*	Grimmiales	Grimmiaceae
GRIDISS	*Grimmia dissimulata*	Grimmiales	Grimmiaceae
GRIDONN	*Grimmia donniana*	Grimmiales	Grimmiaceae
GRIFUNA	*Grimmia funalis*	Grimmiales	Grimmiaceae
GRIHART	*Grimmia hartmanii*	Grimmiales	Grimmiaceae
GRIHORR	*Grimmia horrida*	Grimmiales	Grimmiaceae
GRILAEV	*Grimmia laevigata*	Grimmiales	Grimmiaceae
GRILISA	*Grimmia lisae*	Grimmiales	Grimmiaceae

Taxon Code	Taxon	Order	Family
GRIMONT	*Grimmia montana*	Grimmiales	Grimmiaceae
GRIORBI	*Grimmia orbicularis*	Grimmiales	Grimmiaceae
GRIOVAL	*Grimmia ovalis*	Grimmiales	Grimmiaceae
GRIPULV	*Grimmia pulvinata*	Grimmiales	Grimmiaceae
GRIRAMO	*Grimmia ramondii*	Grimmiales	Grimmiaceae
GRIREFL	*Grimmia reflexidens*	Grimmiales	Grimmiaceae
GRITERG	*Grimmia tergestina*	Grimmiales	Grimmiaceae
GRITORQ	*Grimmia torquata*	Grimmiales	Grimmiaceae
GRITRIC	*Grimmia trichophylla*	Grimmiales	Grimmiaceae
GYMCALC	*Gymnostomum calcareum*	Pottiales	Pottiaceae
GYMVIRI	*Gymnostomum viridulum*	Pottiales	Pottiaceae
GYRREFL	*Gyroweisia reflexa*	Pottiales	Pottiaceae
GYRTENU	*Gyroweisia tenuis*	Pottiales	Pottiaceae
HABPERP	*Habrodon perpusillus*	Hypnales	Pterigynandraceae
HEDCILI	*Hedwigia ciliata*	Hedwigiales	Hedwigiaceae
HEDCILL	*Hedwigia ciliata* var. *leucophaea*	Hedwigiales	Hedwigiaceae
HEDSTEL	*Hedwigia stellata*	Hedwigiales	Hedwigiaceae
HEDSTRI	*Hedwigia striata*	Hedwigiales	Hedwigiaceae
HETHETE	*Heterocladium heteropterum*	Hypnales	Pterigynandraceae
HETWULF	*Heterocladium wulfsbergii*	Hypnales	Pterigynandraceae
HOMAURE	*Homalothecium aureum*	Hypnales	Brachytheciaceae
HOMINCU	*Homomallium incurvatum*	Hypnales	Hypnaceae
HOMLUSI	*Homalia lusitanica*	Hypnales	Neckeraceae
HOMLUTE	*Homalothecium lutescens*	Hypnales	Brachytheciaceae
HOMMERI	*Homalothecium meridionale*	Hypnales	Brachytheciaceae
HOMSERI	*Homalothecium sericeum*	Hypnales	Brachytheciaceae
HOMTRIC	*Homalia trichomanoides*	Hypnales	Neckeraceae
HOOLUCE	*Hookeria lucens*	Hookeriales	Hookeriaceae
HYGOCHR	*Hygrohypnum ochraceum*	Hypnales	Amblystegiaceae
HYGTENA	*Hygroamblystegium tenax*	Hypnales	Amblystegiaceae
HYGVARI	*Hygroamblystegium varium*	Hypnales	Amblystegiaceae
HYLSPLE	*Hylocomium splendens*	Hypnales	Hylocomiaceae
HYOACUM	*Hyophila acuminata*	Pottiales	Pottiaceae
HYOARMO	*Hyocomium armoricum*	Hypnales	Hypnaceae
HYPANDO	*Hypnum andoi*	Hypnales	Hypnaceae
HYPCUPF	*Hypnum cupressiforme* var. *filiforme*	Hypnales	Hypnaceae
HYPCUPL	*Hypnum cupressiforme* var. *lancunosum*	Hypnales	Hypnaceae
HYPCUPP	*Hypnum cupressiforme* var. *resupinatum*	Hypnales	Hypnaceae
HYPCUPR	*Hypnum cupressiforme*	Hypnales	Hypnaceae
HYPIMPO	*Hypnum imponens*	Hypnales	Hypnaceae
HYPJUTL	*Hypnum jutlandicum*	Hypnales	Hypnaceae
HYPTAMA	*Hypopterygium tamarisci*	Hookeriales	Hypopterygiaceae
HYPUNCI	*Hypnum uncinulatum*	Hypnales	Hypnaceae

Taxon Code	Taxon	Order	Family
ISOALGA	*Isothecium algarvicum*	Hypnales	Lembophyllaceae
ISOALOP	*Isothecium alopecuroides*	Hypnales	Lembophyllaceae
ISOHOLT	*Isothecium holtii*	Hypnales	Lembophyllaceae
ISOMYOS	*Isothecium myosuroides*	Hypnales	Lembophyllaceae
ISOPULC	*Isopterygiopsis pulchella*	Hypnales	Hypnaceae
KIABLYT	*Kiaeria blyttii*	Dicranales	Rhabdoweisiaceae
KIASTAR	*Kiaeria starkei*	Dicranales	Rhabdoweisiaceae
KINPRAE	*Kindbergia praelonga*	Hypnales	Brachytheciaceae
LEPBERI	*Leptobarbula berica*	Pottiales	Pottiaceae
LEPFLEX	*Leptodontium flexifolium*	Pottiales	Pottiaceae
LEPLEPT	*Leptophascum leptophyllum*	Pottiales	Pottiaceae
LEPPYRI	*Leptobryum pyriforme*	Splachnales	Meesiaceae
LEPRIPA	*Leptodictyum riparium*	Hypnales	Amblystegiaceae
LEPSMIT	*Leptodon smithii*	Leucodontales	Leptodontaceae
LESPOLY	*Leskea polycarpa*	Hypnales	Leskeaceae
LEUCGLA	*Leucobryum glaucum*	Dicranales	Leucobryaceae
LEUCHRY	*Leucoloma chrysobasilare* var. *chrysobasilare*	Dicranales	Dicranaceae
LEUCJUN	*Leucobryum juniperoideum*	Dicranales	Leucobryaceae
LEUCMAD	*Leucobryum madagassum*	Dicranales	Leucobryaceae
LEUDICH	*Leucoloma dichelymoides*	Dicranales	Dicranaceae
LEUGRAN	*Leucoloma grandidieri*	Dicranales	Dicranaceae
LEULEPE	*Leucoloma lepervancheri*	Dicranales	Dicranaceae
LEUMADA	*Leucoloma madagascariense*	Dicranales	Dicranaceae
LEUSANC	*Leucoloma sanctae-mariae*	Dicranales	Dicranaceae
LEUSCIU	*Leucodon sciuroides*	Leucodontales	Leucodontaceae
LEUSEYC	*Leucoloma seychellense*	Dicranales	Dicranaceae
LEUSUBC	*Leucoloma subchrysobasilare*	Dicranales	Dicranaceae
LEUTALA	*Leucoloma talazaccii*	Dicranales	Dicranaceae
LOEBREV	*Loeskeobryum brevirostre*	Hypnales	Hylocomiaceae
METMENZ	*Metaneckera menziesii*	Hypnales	Neckeraceae
MICDAVA	*Microbryum davallianum*	Pottiales	Pottiaceae
MICFOSB	*Microbryum fosbergii*	Pottiales	Pottiaceae
MICRECT	*Microbryum rectum*	Pottiales	Pottiaceae
MICSTAR	*Microbryum starckeanum*	Pottiales	Pottiaceae
MICTENE	*Micromitrium tenerum*	Funariales	Ephemeraceae
MIEMIEL	*Mielichhoferia mielichhoferiana*	Bryales	Melichoferiaceae
MNIHORN	*Mnium hornum*	Bryales	Mniaceae
MNISTEL	*Mnium stellare*	Bryales	Mniaceae
NECCOMP	*Neckera complanata*	Hypnales	Neckeraceae
NECCRIS	*Neckera crispa*	Hypnales	Neckeraceae
NECPUMI	*Neckera pumila*	Hypnales	Neckeraceae
NECPUMP	*Neckera pumila* var. *pilifera*	Hypnales	Neckeraceae
ORTBARB	*Orthotrichum speciosum*	Orthotrichales	Orthotrichaceae

Taxon Code	Taxon	Order	Family
ORTCANA	*Orthotrichum tenellum*	Orthotrichales	Orthotrichaceae
ORTCOMO	*Orthotrichum comosum*	Orthotrichales	Orthotrichaceae
ORTCONS	*Orthotrichum consimile*	Orthotrichales	Orthotrichaceae
ORTCUPU	*Orthotrichum cupulatum*	Orthotrichales	Orthotrichaceae
ORTDIEC	*Orthotrichum striatum*	Orthotrichales	Orthotrichaceae
ORTFONT	*Orthotrichum acuminatum*	Orthotrichales	Orthotrichaceae
ORTIBER	*Orthotrichum ibericum*	Orthotrichales	Orthotrichaceae
ORTMEGI	*Orthotrichum lyellii*	Orthotrichales	Orthotrichaceae
ORTMOUG	*Orthotrichum diaphanum*	Orthotrichales	Orthotrichaceae
ORTMUTI	*Orthotrichum affine*	Orthotrichales	Orthotrichaceae
ORTPHIL	*Orthotrichum philibertii*	Orthotrichales	Orthotrichaceae
ORTRIVU	*Orthotrichum rivulare*	Orthotrichales	Orthotrichaceae
ORTRUPE	*Orthotrichum rupestre*	Orthotrichales	Orthotrichaceae
ORTSCAN	*Orthotrichum scanicum*	Orthotrichales	Orthotrichaceae
ORTSCHI	*Orthotrichum schimperi*	Orthotrichales	Orthotrichaceae
ORTSERP	*Orthotrichum anomalum*	Orthotrichales	Orthotrichaceae
ORTSHAW	*Orthotrichum shawii*	Orthotrichales	Orthotrichaceae
ORTSPRU	*Orthotrichum sprucei*	Orthotrichales	Orthotrichaceae
ORTSTRA	*Orthotrichum stramineum*	Orthotrichales	Orthotrichaceae
OXYHIAN	*Oxyrrhynchium hians*	Hypnales	Brachytheciaceae
OXYPUMI	*Oxyrrhynchium pumilum*	Hypnales	Brachytheciaceae
OXYSCHL	*Oxyrrhynchium schleicheri*	Hypnales	Brachytheciaceae
OXYSPEC	*Oxyrrhynchium speciosum*	Hypnales	Brachytheciaceae
PALFALC	*Palustriella falcata*	Hypnales	Amblystegiaceae
PHIARNE	*Philonotis arnellii*	Bartramiales	Bartramiaceae
PHICAES	*Philonotis caespitosa*	Bartramiales	Bartramiaceae
PHICALC	*Philonotis calcarea*	Bartramiales	Bartramiaceae
PHIFONT	*Philonotis fontana*	Bartramiales	Bartramiaceae
PHIMARC	*Philonotis marchica*	Bartramiales	Bartramiaceae
PHIRIGI	*Philonotis rigida*	Bartramiales	Bartramiaceae
PHISERI	*Philonotis seriata*	Bartramiales	Bartramiaceae
PHITOME	*Philonotis tomentella*	Bartramiales	Bartramiaceae
PHYPYRI	*Physcomitrium pyriforme*	Funariales	Funariaceae
PHYREAD	*Physcomitrella readeri*	Funariales	Funariaceae
PLAAFFI	*Plagiomnium affine*	Bryales	Mniaceae
PLACAVI	*Plagiothecium cavifolium*	Hypnales	Plagiotheciaceae
PLADENT	*Plagiothecium denticulatum*	Hypnales	Plagiotheciaceae
PLALAET	*Plagiothecium laetum*	Hypnales	Plagiotheciaceae
PLALATE	*Plagiothecium latebricola*	Hypnales	Plagiotheciaceae
PLALUSI	*Platyhypnidium lusitanicum*	Hypnales	Brachytheciaceae
PLAMEDI	*Plagiomnium medium*	Bryales	Mniaceae
PLAMERI	*Plasteurhynchium meridionale*	Hypnales	Brachytheciaceae
PLANEMO	*Plagiothecium nemorale*	Hypnales	Plagiotheciaceae

Taxon Code	Taxon	Order	Family
PLAPILI	*Plagiothecium piliferum*	Hypnales	Plagiotheciaceae
PLARIPA	*Platyhypnidium riparioides*	Hypnales	Brachytheciaceae
PLAROST	*Plagiomnium rostratum*	Bryales	Mniaceae
PLASTRL	*Plasteurhynchium striatulum*	Hypnales	Brachytheciaceae
PLASUCC	*Plagiothecium succulentum*	Hypnales	Plagiotheciaceae
PLAUNDL	*Plagiomnium undulatum*	Bryales	Mniaceae
PLAUNDM	*Plagiomnium undulatum* var. *madeirense*	Bryales	Mniaceae
PLAUNDU	*Plagiothecium undulatum*	Hypnales	Plagiotheciaceae
PLEACUM	*Pleuridium acuminatum*	Dicranales	Ditrichaceae
PLESCHR	*Pleurozium schreberi*	Hypnales	Hylocomiaceae
PLESQUA	*Pleurochaete squarrosa*	Pottiales	Pottiaceae
PLESUBU	*Pleuridium subulatum*	Dicranales	Ditrichaceae
POGALOI	*Pogonatum aloides*	Polytrichales	Polytrichaceae
POGNANU	*Pogonatum nanum*	Polytrichales	Polytrichaceae
POGURNI	*Pogonatum urnigerum*	Polytrichales	Polytrichaceae
POHANDA	*Pohlia andalusica*	Bryales	Mniaceae
POHANNO	*Pohlia annotina*	Bryales	Mniaceae
POHBOLA	*Pohlia bolanderi*	Bryales	Mniaceae
POHCRUD	*Pohlia cruda*	Bryales	Mniaceae
POHELOA	*Pohlia elongata* var. *acuminata*	Bryales	Mniaceae
POHELOG	*Pohlia elongata* var. *greenii*	Bryales	Mniaceae
POHELON	*Pohlia elongata*	Bryales	Mniaceae
POHFILU	*Pohlia filum*	Bryales	Mniaceae
POHLESC	*Pohlia lescuriana*	Bryales	Mniaceae
POHLONG	*Pohlia longicolla*	Bryales	Mniaceae
POHMELA	*Pohlia melanodon*	Bryales	Mniaceae
POHNUTA	*Pohlia nutans*	Bryales	Mniaceae
POHPROL	*Pohlia proligera*	Bryales	Mniaceae
POHWAHL	*Pohlia wahlenbergii*	Bryales	Mniaceae
POLALPI	*Polytrichastrum alpinum*	Polytrichales	Polytrichaceae
POLCOMM	*Polytrichum commune*	Polytrichales	Polytrichaceae
POLFORM	*Polytrichastrum formosum*	Polytrichales	Polytrichaceae
POLJUNI	*Polytrichum juniperinum*	Polytrichales	Polytrichaceae
POLPILI	*Polytrichum piliferum*	Polytrichales	Polytrichaceae
PSEDURI	*Pseudorhynchostegiella duriaei*	Hypnales	Brachytheciaceae
PSEELEG	*Pseudotaxiphyllum elegans*	Hypnales	Hypnaceae
PSEHORN	*Pseudocrossidium hornschuchianum*	Pottiales	Pottiaceae
PSEINCU	*Pseudoleskea incurvata*	Hypnales	Leskeaceae
PSELAET	*Pseudotaxiphyllum laetevirens*	Hypnales	Hypnaceae
PSENITI	*Pseudephemerum nitidum*	Dicranales	Dicranaceae
PSEPATE	*Pseudoleskea patens*	Hypnales	Leskeaceae
PSEPURU	*Pseudoscleropodium purum*	Hypnales	Brachytheciaceae
PSEREVO	*Pseudocrossidium revolutum*	Pottiales	Pottiaceae

Taxon Code	Taxon	Order	Family
PTEFILI	*Pterigynandrum filiforme*	Hypnales	Pterigynandraceae
PTEGRAC	*Nogopterium gracile*	Leucodontales	Leucodontaceae
PTEGRAM	*Pterogonium gracile* var. *madagassum*	Hypnales	Leucodontaceae
PTESAMP	*Pterygoneurum sampaianum*	Pottiales	Pottiaceae
PTYNIGR	*Ptychomitrium nigrescens*	Grimmiales	Ptychomitriaceae
PTYPOLY	*Ptychomitrium polyphyllum*	Grimmiales	Ptychomitriaceae
PYLPOLY	*Pylaisia polyantha*	Hypnales	Hypnaceae
PYRTETR	*Pyramidula tetragona*	Funariales	Funariaceae
RACACIC	*Racomitrium aciculare*	Grimmiales	Grimmiaceae
RACAFFI	*Racomitrium affine*	Grimmiales	Grimmiaceae
RACAQUA	*Racomitrium aquaticum*	Grimmiales	Grimmiaceae
RACELON	*Racomitrium elongatum*	Grimmiales	Grimmiaceae
RACHESP	*Racomitrium hespericum*	Grimmiales	Grimmiaceae
RACHETE	*Racomitrium heterostichum*	Grimmiales	Grimmiaceae
RACLAMP	*Racomitrium lamprocarpum*	Grimmiales	Grimmiaceae
RACLANU	*Racomitrium lanuginosum*	Grimmiales	Grimmiaceae
RACLUSI	*Racomitrium lusitanicum*	Grimmiales	Grimmiaceae
RACMACA	*Racomitrium macounii* subsp. *alpinum*	Grimmiales	Grimmiaceae
RACMACO	*Racomitrium macounii* subsp. *macounii*	Grimmiales	Grimmiaceae
RACOBTU	*Racomitrium obtusum*	Grimmiales	Grimmiaceae
RACSUDE	*Racomitrium sudeticum*	Grimmiales	Grimmiaceae
RHAFUGA	*Rhabdoweisia fugax*	Dicranales	Rhabdoweisiaceae
RHAPURP	*Rhamphidium purpuratum*	Dicranales	Ditrichaceae
RHIMAGN	*Rhizomnium magnifolium*	Bryales	Mniaceae
RHIPERS	*Rhizofabronia persoonii* var. *sphaerocarpa*	Hypnales	Fabroniaceae
RHIPUNC	*Rhizomnium punctatum*	Bryales	Mniaceae
RHOONTA	*Rhodobryum ontariense*	Bryales	Bryaceae
RHYCONF	*Rhynchostegium confertum*	Hypnales	Brachytheciaceae
RHYCURV	*Rhynchostegiella curviseta*	Hypnales	Brachytheciaceae
RHYLITO	*Rhynchostegiella litorea*	Hypnales	Brachytheciaceae
RHYLORE	*Rhytidiadelphus loreus*	Hypnales	Hylocomiaceae
RHYMEGA	*Rhynchostegium megapolitanum*	Hypnales	Brachytheciaceae
RHYMURA	*Rhynchostegium murale*	Hypnales	Brachytheciaceae
RHYSQUA	*Rhytidiadelphus squarrosus*	Hypnales	Hylocomiaceae
RHYTENE	*Rhynchostegiella teneriffae*	Hypnales	Brachytheciaceae
RHYTENL	*Rhynchostegiella tenella*	Hypnales	Brachytheciaceae
RHYTRIQ	*Rhytidiadelphus triquetrus*	Hypnales	Hylocomiaceae
SANUNCI	*Sanionia uncinata*	Hypnales	Amblystegiaceae
SAREXAN	*Sarmentypnum exannulatum*	Hypnales	Calliergonaceae
SCHAGAS	*Schistidium agassizii*	Grimmiales	Grimmiaceae
SCHAPOC	*Schistidium apocarpum*	Grimmiales	Grimmiaceae
SCHBRUN	*Schistidium brunnescens* subsp. *brunnescens*	Grimmiales	Grimmiaceae
SCHCONF	*Schistidium confertum*	Grimmiales	Grimmiaceae

Taxon Code	Taxon	Order	Family
SCHCRAS	*Schistidium crassipilum*	Grimmiales	Grimmiaceae
SCHELEG	*Schistidium elegantulum* subsp. *wilsonii*	Grimmiales	Grimmiaceae
SCHFLAC	*Schistidium flaccidum*	Grimmiales	Grimmiaceae
SCHHELV	*Schistidium helveticum*	Grimmiales	Grimmiaceae
SCHPENN	*Schistostega pennata*	Schistotegales	Schistostegaceae
SCHPONT	*Schizymenium pontevedrensis*	Bryales	Mniaceae
SCHRIVU	*Schistidium rivulare*	Grimmiales	Grimmiaceae
SCIPOPU	*Brachythecium populeum*	Hypnales	Brachytheciaceae
SCISTAR	*Brachythecium starkei*	Hypnales	Brachytheciaceae
SCLCESP	*Scleropodium cespitans*	Hypnales	Brachytheciaceae
SCLTOUR	*Scleropodium touretii*	Hypnales	Brachytheciaceae
SCOCIRC	*Scorpiurium circinatum*	Hypnales	Brachytheciaceae
SCODEFL	*Scorpiurium deflexifolium*	Hypnales	Brachytheciaceae
SCOSEND	*Scorpiurium sendtneri*	Hypnales	Brachytheciaceae
SELACUT	*Seligeria acutifolia*	Seligerales	Seligeriaceae
SELCALY	*Seligeria calycina*	Seligerales	Seligeriaceae
SELPUSI	*Seligeria pusilla*	Seligerales	Seligeriaceae
SEMSUBP	*Sematophyllum subpinnatum*	Hypnales	Sematophyllaceae
SEMSUBS	*Sematophyllum substrumulosum*	Hypnales	Sematophyllaceae
SPHANGU	*Sphagnum angustifolium*	Sphagnales	Sphagnaceae
SPHAURI	*Sphagnum denticulatum*	Sphagnales	Sphagnaceae
SPHCAPI	*Sphagnum capillifolium*	Sphagnales	Sphagnaceae
SPHCENT	*Sphagnum centrale*	Sphagnales	Sphagnaceae
SPHCOMP	*Sphagnum compactum*	Sphagnales	Sphagnaceae
SPHCUSP	*Sphagnum cuspidatum*	Sphagnales	Sphagnaceae
SPHFALL	*Sphagnum fallax*	Sphagnales	Sphagnaceae
SPHFLEX	*Sphagnum flexuosum*	Sphagnales	Sphagnaceae
SPHGIRG	*Sphagnum girgensohnii*	Sphagnales	Sphagnaceae
SPHMOLL	*Sphagnum molle*	Sphagnales	Sphagnaceae
SPHPALU	*Sphagnum palustre*	Sphagnales	Sphagnaceae
SPHPAPI	*Sphagnum papillosum*	Sphagnales	Sphagnaceae
SPHPLAT	*Sphagnum platyphyllum*	Sphagnales	Sphagnaceae
SPHRUBE	*Sphagnum rubellum*	Sphagnales	Sphagnaceae
SPHRUSS	*Sphagnum russowii*	Sphagnales	Sphagnaceae
SPHSQUA	*Sphagnum squarrosum*	Sphagnales	Sphagnaceae
SPHSUBN	*Sphagnum subnitens*	Sphagnales	Sphagnaceae
SPHSUBS	*Sphagnum subsecundum*	Sphagnales	Sphagnaceae
SPHTENE	*Sphagnum tenellum*	Sphagnales	Sphagnaceae
SQUBRAS	*Squamidium brasiliense*	Hypnales	Brachytheciaceae
STRSTRA	*Straminergon stramineum*	Hypnales	Calliergonaceae
SYNCALC	*Syntrichia calcicola*	Pottiales	Pottiaceae
SYNLAEV	*Syntrichia laevipila*	Pottiales	Pottiaceae
SYNLATI	*Syntrichia latifolia*	Pottiales	Pottiaceae

Taxon Code	Taxon	Order	Family
SYNMONT	*Syntrichia montana*	Pottiales	Pottiaceae
SYNPAPI	*Syntrichia papillosa*	Pottiales	Pottiaceae
SYNPAPS	*Syntrichia papillosissima*	Pottiales	Pottiaceae
SYNPRIN	*Syntrichia princeps*	Pottiales	Pottiaceae
SYNRURA	*Syntrichia ruralis*	Pottiales	Pottiaceae
SYNRURR	*Syntrichia ruralis* var. *ruraliformis*	Pottiales	Pottiaceae
SYNSUBP	*Syntrichia subpapillosissima*	Pottiales	Pottiaceae
SYNVIRE	*Syntrichia virescens*	Pottiales	Pottiaceae
SYRALBI	*Syrrhopodon albidus* var. *integrifolium*	Dicranales	Calymperaceae
SYRDIMO	*Syrrhopodon dimorphophyllus*	Dicranales	Calymperaceae
SYRSPIR	*Syrrhopodon spiralis*	Dicranales	Calymperaceae
THAALOP	*Thamnobryum alopecurum*	Hypnales	Neckeraceae
THAMADE	*Thamnobryum maderense*	Hypnales	Neckeraceae
THUTAMA	*Thuidium tamariscinum*	Hypnales	Thuidiaceae
TIMBARB	*Timmiella barbuloides*	Pottiales	Pottiaceae
TIMFLEX	*Timmiella flexiseta*	Pottiales	Pottiaceae
TORACAU	*Tortula acaulon*	Pottiales	Pottiaceae
TORACPA	*Tortula acaulon* var. *papillosa*	Pottiales	Pottiaceae
TORACPI	*Tortula acaulon* var. *pilifera*	Pottiales	Pottiaceae
TORATRO	*Tortula atrovirens*	Pottiales	Pottiaceae
TORBOLA	*Tortula bolanderi*	Pottiales	Pottiaceae
TORBREV	*Tortula brevissima*	Pottiales	Pottiaceae
TORCANE	*Tortula canescens*	Pottiales	Pottiaceae
TORCUNE	*Tortula cuneifolia*	Pottiales	Pottiaceae
TORFLAG	*Tortella flavovirens* var. *glareicola*	Pottiales	Pottiaceae
TORFLAV	*Tortella flavovirens*	Pottiales	Pottiaceae
TORFREI	*Tortula freibergii*	Pottiales	Pottiaceae
TORGUEP	*Tortula guepinii*	Pottiales	Pottiaceae
TORHUMI	*Tortella humilis*	Pottiales	Pottiaceae
TORINCL	*Tortella inclinata* var. *inclinata*	Pottiales	Pottiaceae
TORINER	*Tortula inermis*	Pottiales	Pottiaceae
TORINFL	*Tortella inflexa*	Pottiales	Pottiaceae
TORISRA	*Tortula israelis*	Pottiales	Pottiaceae
TORLANC	*Tortula lanceolata*	Pottiales	Pottiaceae
TORMARG	*Tortula marginata*	Pottiales	Pottiaceae
TORMODI	*Tortula modica*	Pottiales	Pottiaceae
TORMURA	*Tortula muralis*	Pottiales	Pottiaceae
TORNITI	*Tortella nitida*	Pottiales	Pottiaceae
TORPALL	*Tortula pallida*	Pottiales	Pottiaceae
TORSOLM	*Tortula solmsii*	Pottiales	Pottiaceae
TORSUBU	*Tortula subulata*	Pottiales	Pottiaceae
TORTORT	*Tortella tortuosa* var. *tortuosa*	Pottiales	Pottiaceae
TORTRUN	*Tortula truncata*	Pottiales	Pottiaceae

Taxon Code	Taxon	Order	Family
TORVAHL	*Tortula vahliana*	Pottiales	Pottiaceae
TORWILS	*Tortula wilsonii*	Pottiales	Pottiaceae
TRIARAP	*Triquetrella arapilensis*	Pottiales	Pottiaceae
TRIBRAC	*Trichostomum brachydontium*	Pottiales	Pottiaceae
TRICRIS	*Trichostomum crispulum*	Pottiales	Pottiaceae
TRICYLI	*Trichodon cylindricus*	Dicranales	Ditrichaceae
TRITENU	*Trichostomum tenuirostre*	Pottiales	Pottiaceae
TRITRIU	*Trichostomum triumphans*	Pottiales	Pottiaceae
ULOCALV	*Ulota calvescens*	Orthotrichales	Orthotrichaceae
ULOCRIS	*Ulota crispa*	Orthotrichales	Orthotrichaceae
ULOCRPU	*Ulota crispula*	Orthotrichales	Orthotrichaceae
ULODONI	*Ulota bruchii*	Orthotrichales	Orthotrichaceae
ULOHUTC	*Ulota hutchinsiae*	Orthotrichales	Orthotrichaceae
WARFLUI	*Warnstorfia fluitans*	Hypnales	Calliergonaceae
WEIBRAC	*Weissia brachycarpa*	Pottiales	Pottiaceae
WEICONA	*Weissia condensa* var. *armata*	Pottiales	Pottiaceae
WEICOND	*Weissia condensa*	Pottiales	Pottiaceae
WEICONT	*Weissia controversa*	Pottiales	Pottiaceae
WEILEVI	*Weissia levieri*	Pottiales	Pottiaceae
WEILONG	*Weissia longifolia*	Pottiales	Pottiaceae
WEIWIMM	*Weissia wimmeriana*	Pottiales	Pottiaceae
ZYGCATA	*Zygodon catarinoi*	Orthotrichales	Orthotrichaceae
ZYGCONO	*Zygodon conoideus*	Orthotrichales	Orthotrichaceae
ZYGFORS	*Zygodon forsteri*	Orthotrichales	Orthotrichaceae
ZYGTENU	*Zygodon rupestris*	Orthotrichales	Orthotrichaceae
ZYGVIRI	*Zygodon viridissimus*	Orthotrichales	Orthotrichaceae

A4.3. Auxillary plots and trait codes used in analyses

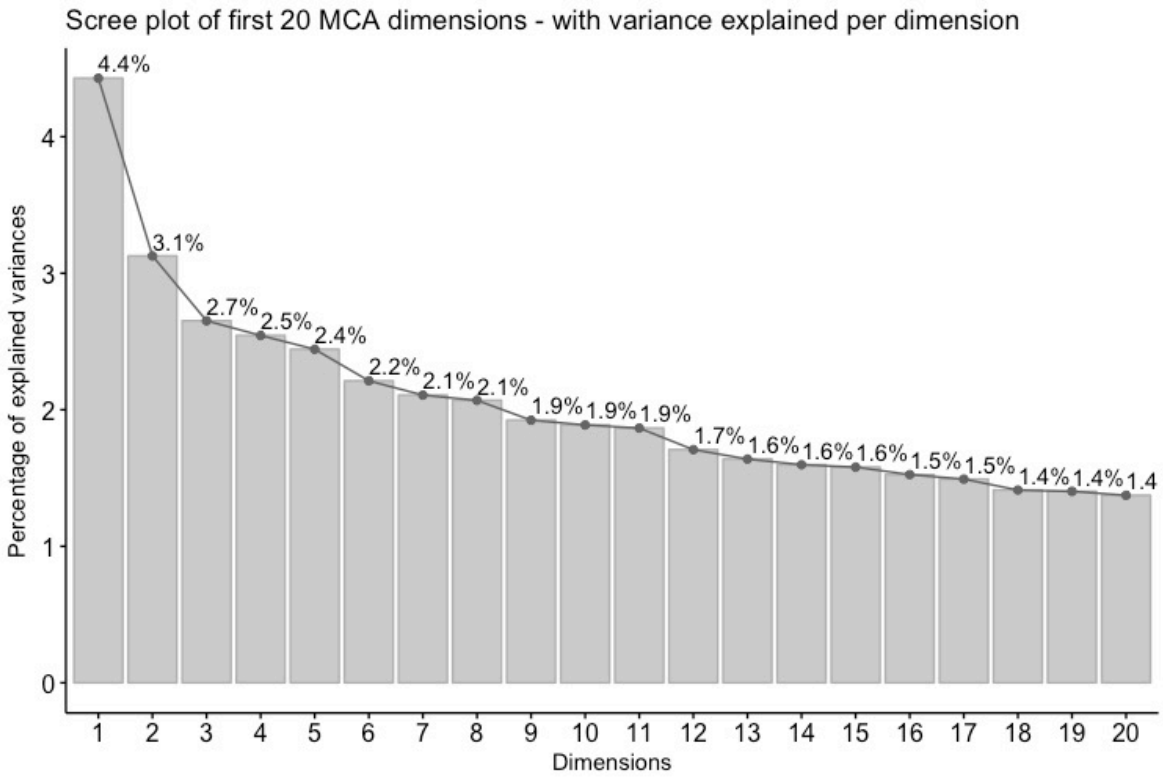


Figure 4.18 Scree plot showing percentage of variance explained in the first 20 dimensions. Amount of variance is low due to the large number of variables in the dataset.

Table 4.22 Codes used for traits in MCA and clustering analyses. Ordered alphabetically by trait code.

Trait code	Trait
Alar	Alar cells diferentiated
Apex	Apex type
BasCellDif	Basal cells differentiated
BasCw	Basal cell wall shape
BasShape	Shape of basal cells
CapExert	Capsule exertence
CapOrient	Capsule orientation
ColNum	Number of colours in plant
ColourCode	Plant colour
CostaLen	Costa termination (length)
CostaNum	Costa number
CwThick	Cell wall thickness
Decurrent	Leaf decurrence
Lam	Lamina thickness
LF	Life-form
LS	Life strategy
MarCurv	Leaf margin curvuture
MarDent	Leaf margin denticulation

Trait code	Trait
MonDio	Reproduction strategy
OrientDry	Leaf orientation when dry
OrientWet	Leaf orientation when hydrated
Papillose	Papillose
PlantSizecat	Plant size (category)
SetaAvg	Seta length (mean)
Shine	Plant shine
SporeSize	Spore size (mean)
Veg	Presence of vegetative propagules
VegNum	Number of vegetative propagules
VegType	Type of vegetative propagules
XSect	Leaf cross-section shape
Environmental, habitat and distribution traits	
AltAvg	Mean altitude (m)
DTcat	Desiccation Tolerance category
DTval	Desiccation Tolerance value (EI)
EcoSpec	Environmental range value
EnvNiche	Environmental range category
HabNiche	Habitat range category
LightSpec	Light specificity value
LightVal	Light value
MoistSpec	Moisture specificity value
MoistVal	Moisture value
Realms	Number of biogeographical realms occupied
SubGS	Substrate specialism
SubNum	Substrate number
Threat	IUCN threat category

Table 4.23 Trait state codes used in MCA and clustering analyses; listed first are bryophyte traits and then ancillary traits (environmental, habitat and distribution). Ordered alphabetically by trait state code.

Trait State Code	Trait	State
Alar_0	Distinct alar region	absent
Alar_1	Distinct alar region	present
Apex_acuminate	Leaf apex	acuminate
Apex_acute	Leaf apex	acute
Apex_apiculate	Leaf apex	apiculate
Apex_cucullate	Leaf apex	cucullate
Apex_hair-point	Leaf apex	hair-point
Apex_rounded	Leaf apex	rounded
Apex_subulate	Leaf apex	subulate
BasCellDif_0	Basal cell shape differentiated	not differentiated
BasCellDif_1	Basal cell shape differentiated	differentiated
BasShape_elongate	Basal cell shape	elongate
BasShape_elongate&hyaline	Basal cell shape	elongate & hyaline

Trait State Code	Trait	State
BasShape_enlarged	Basal cell shape	enlarged
BasShape_enlarged&hyaline	Basal cell shape	enlarged & hyaline
BasShape_hyaline	Basal cell shape	hyaline
BasShape_short	Basal cell shape	short
BasShape_undifferentiated	Basal cell shape	undifferentiated
branches	Vegetative propagule type	branches
branches;leaves	Vegetative propagule type	branches and leaves
bulbils	Vegetative propagule type	bulbils
bulbils;branches	Vegetative propagule type	bulbils and branches
bulbils;gemmae	Vegetative propagule type	bulbils and gemmae
bulbils;leaves	Vegetative propagule type	bulbils and leaves
bulbils;tubers	Vegetative propagule type	bulbils and tubers
CapOrient_0	Capsule orientation	immersed
CapOrient_1	Capsule orientation	erect
CapOrient_1.5	Capsule orientation	erect-inclined
CapOrient_2	Capsule orientation	inclined
CapOrient_2.5	Capsule orientation	inclined-horizontal
CapOrient_3	Capsule orientation	horizontal
CapOrient_3.5	Capsule orientation	horizontal-pendulous
CapOrient_4	Capsule orientation	pendulous
ColNum_1	Number of colours	1
ColNum_2	Number of colours	2
ColNum_3	Number of colours	3
ColNum_4	Number of colours	4
ColNum_5	Number of colours	5
ColourCode_BBr	Plant colour	Black, Brown
ColourCode_BBrR	Plant colour	Black, Brown, Red
ColourCode_BrR	Plant colour	Brown, Red
ColourCode_G	Plant colour	Green
ColourCode_GB	Plant colour	Green, Black
ColourCode_GBBr	Plant colour	Green, Black, Brown
ColourCode_GBlW	Plant colour	Green, Blue, White
ColourCode_GBr	Plant colour	Green, Brown
ColourCode_GBR	Plant colour	Green, Brown, Red
ColourCode_GBrO	Plant colour	Green, Brown, Orange
ColourCode_GBrR	Plant colour	Green, Brown, Red
ColourCode_GBW	Plant colour	Green, Black, White
ColourCode_GGo	Plant colour	Green, Golden
ColourCode_GR	Plant colour	Green, Red

Trait State Code	Trait	State
ColourCode_GW	Plant colour	Green, White
ColourCode_GY	Plant colour	Green, Yellow
ColourCode_GYB	Plant colour	Green, Yellow, Black
ColourCode_GYBr	Plant colour	Green, Yellow, Brown
ColourCode_GYBrGo	Plant colour	Green, Yellow, Brown, Golden
ColourCode_GYBrR	Plant colour	Green, Yellow, Brown, Red
ColourCode_GYBrRPu	Plant colour	Green, Yellow, Brown, Red, Purple
ColourCode_GYGo	Plant colour	Green, Yellow, Golden
ColourCode_W	Plant colour	White
ColourCode_YBr	Plant colour	Yellow, Brown
ColourCode_YBrGo	Plant colour	Yellow, Brown, Golden
ColourCode_YBrR	Plant colour	Yellow, Brown, Red
ColourCode_YRO	Plant colour	Yellow, Red, Orange
CostaLen_0.00	Costa termination (length)	none
CostaLen_0.50	Costa termination (length)	base
CostaLen_1.00	Costa termination (length)	base
CostaLen_2.00	Costa termination (length)	lower third
CostaLen_3.00	Costa termination (length)	middle
CostaLen_4.00	Costa termination (length)	upper third
CostaLen_5.00	Costa termination (length)	apex
CostaNum_0	Costa number	none
CostaNum_1	Costa number	single
CostaNum_2	Costa number	double
CwThick_medium	Cell wall thickness	medium
CwThick_thick	Cell wall thickness	thick
CwThick_thin	Cell wall thickness	thin
D	Reproduction system	dioicous
Decurrent_0	Leaf decurrence	not decurrent
Decurrent_1	Leaf decurrence	short decurrent
Decurrent_2	Leaf decurrence	long decurrent
Decurrent_sheathing	Leaf decurrence	sheathing
dentate	Leaf margin denticulation	dentate
dentate part	Leaf margin denticulation	partly dentate
denticulate	Leaf margin denticulation	denticulate
denticulate part	Leaf margin denticulation	partly denticulate
entire	Leaf margin denticulation	entire
Exert	Capsule exertence	exert
gemmae	Vegetative propagule type	gemmae
Immersed	Capsule exertence	immersed
Lam_bistratose	Lamina thickness	bistratose

Trait State Code	Trait	State
Lam_lamellae	Lamina thickness	lamellae
Lam_partially bistratose	Lamina thickness	partially bistratose
Lam_subulate	Lamina thickness	subulate
Lam_unistratose	Lamina thickness	unistratose
leaves	Vegetative propagule type	leaves
LF_Cu	Life-form	cushion
LF_De	Life-form	dendroid
LF_Mr	Life-form	mat rough
LF_Ms	Life-form	mat smooth
LF_Open	Life-form	open
LF_Tf	Life-form	turf
LF_Tuft	Life-form	tuft
LF_We	Life-form	weft
LF_Fa	Life-form	fan
LS_A	Life strategy	annual
LS_C	Life strategy	colonist
LS_L	Life strategy	dominant
LS_Ms	Life strategy	medium shuttle
LS_P	Life strategy	perennial
LS_F	Life strategy	fugitive
M	Reproduction system	monoicous
MarCurv_involute	Leaf margin curvature	involute
MarCurv_plane	Leaf margin curvature	plane
MarCurv_recurved	Leaf margin curvature	recurved
MarCurv_recurved part	Leaf margin curvature	partly recurved
MarCurv_revolute	Leaf margin curvature	revolute
MarCurv_incurved	Leaf margin curvature	incurved
MarCurv_incurved part	Leaf margin curvature	partly incurved
MD	Reproduction system	monoicous or dioicous
nodulose	Basal cell wall shape	nodulose
none	Vegetative propagule type	none
OrientDry_1.00	Leaf orientation dry	appressed/imbricate
OrientDry_1.50	Leaf orientation dry	appressed-erect
OrientDry_2.00	Leaf orientation dry	erect
OrientDry_2.50	Leaf orientation dry	appressed-patent
OrientDry_3.00	Leaf orientation dry	erect-patent
OrientDry_3.50	Leaf orientation dry	erecto/patent-patent
OrientDry_5.00	Leaf orientation dry	spreading/squarrose
OrientDry_4.00	Leaf orientation dry	patent
OrientDry_4.50	Leaf orientation dry	patent-spreading
OrientWet_1.50	Leaf orientation wet	appressed-erect

Trait State Code	Trait	State
OrientWet_2.50	Leaf orientation wet	appressed-patent
OrientWet_3.00	Leaf orientation wet	erect-patent
OrientWet_4.50	Leaf orientation wet	patent-spreading
OrientWet_1.50	Leaf orientation wet	appressed-erect
OrientWet_2.00	Leaf orientation wet	erect
OrientWet_3.50	Leaf orientation wet	erecto/patent-patent
OrientWet_4.00	Leaf orientation wet	patent
OrientWet_5.00	Leaf orientation wet	spreading/squarrose
Papillose_A	Papillose	absent
Papillose_P	Papillose	present
Papillose_PA	Papillose	present or absent
PlantSizecat_large	Plant size	large
PlantSizecat_minute	Plant size	minute
PlantSizecat_robust	Plant size	robust
PlantSizecat_small	Plant size	small
PlantSizecat_medium	Plant size	medium
porose	Basal cell wall shape	porose
SetaAvg_long	Seta length (mean)	long
SetaAvg_medium	Seta length (mean)	medium
SetaAvg_short	Seta length (mean)	short
Shine_0	Plant shine	none
Shine_1	Plant shine	some shine
Shine_2	Plant shine	shiny
sinuose-porose	Basal cell wall shape	sinuose-porose
sinuose	Basal cell wall shape	sinuose
SporeSize_large	Spore size category	large >20µm
SporeSize_small	Spore size category	small <20µm
straight	Basal cell wall shape	straight
tubers	Vegetative propagule type	tubers
tubers;gemmae	Vegetative propagule type	tubers and gemmae
Veg_A	Vegetative propagule presence	absent
Veg_P	Vegetative propagule presence	present
Veg_PA	Vegetative propagule presence	present or absent
VegNum_0	Number of types of vegetative	none
VegNum_1	Number of types of vegetative	1
VegNum_2	Number of types of vegetative	2
XSect_channelled	Transverse cross-section	channelled
XSect_concave	Transverse cross-section	concave
XSect_keel	Transverse cross-section	keel
XSect_keel part	Transverse cross-section	partly keeled
XSect_plane	Transverse cross-section	plane

Trait State Code	Trait	State
XSect_plane-concave	Transverse cross-section	plane to concave
XSect_plicate	Transverse cross-section	plicate
Environmental, habitat and distribution traits		
Broad	Environmental niche range	broad
CR	Threat category	Critically Endangered
DD	Threat category	Data Deficient
DT	Desiccation tolerance category	DT
EN	Threat category	Endangered
Extreme DT	Desiccation tolerance category	Extreme DT
LC	Threat category	Least Concern
LC-att	Threat category	Least concern-
LightSpec_0.00	Light range	Specific
LightSpec_1.00	Light range	narrow
LightSpec_2.00	Light range	medium
LightSpec_3.00	Light range	broad
LightSpec_4.00	Light range	very broad
Low DT	Desiccation tolerance category	Low DT
Medium	Environmental niche range	medium
MoistSpec_0.00	Moisture range	Specific
MoistSpec_1.00	Moisture range	narrow
MoistSpec_2.00	Moisture range	medium
MoistSpec_2.50	Moisture range	
MoistSpec_3.00	Moisture range	broad
MoistSpec_4.00	Moisture range	very broad
narrow	Habitat range	narrow
Narrow	Environmental niche range	narrow
NT	Threat category	Near Threatened
Rare	Threat category	Rare
RE	Threat category	Regionally Extinct
Realms_1	Number of biogeographical realms occupied	1
Realms_2	Number of biogeographical realms occupied	2
Realms_3	Number of biogeographical realms occupied	3
Realms_4	Number of biogeographical realms occupied	4
Realms_5	Number of biogeographical realms occupied	5
Realms_6	Number of biogeographical realms occupied	6
Realms_8	Number of biogeographical realms occupied	8
specialist	Habitat range	specialist
Specific	Environmental niche range	specific
SubGS_G	Substrate specialisation	generalist
SubGS_S	Substrate specialisation	specialist
SubNum_1	Substrate number	1
SubNum_2	Substrate number	2
SubNum_3	Substrate number	3
SubNum_4	Substrate number	4
Very low DT	Desiccation tolerance category	Very Low DT
very widespread	Habitat range	very widespread
VU	Threat category	Vulnerable
widespread	Habitat specialisation	widespread

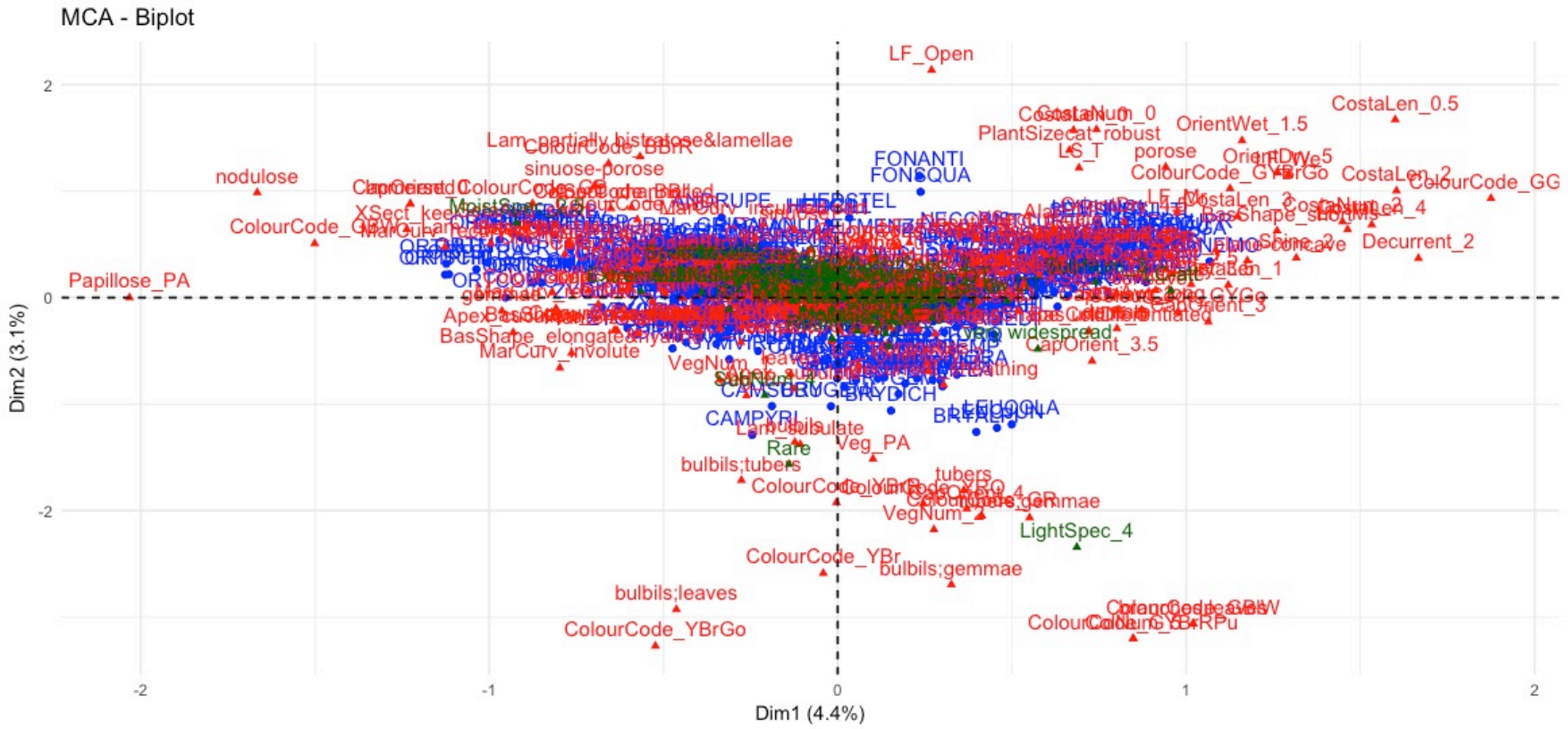


Figure 4.19 MCA plot with all species, traits and environmental, ecological and habitat variables.

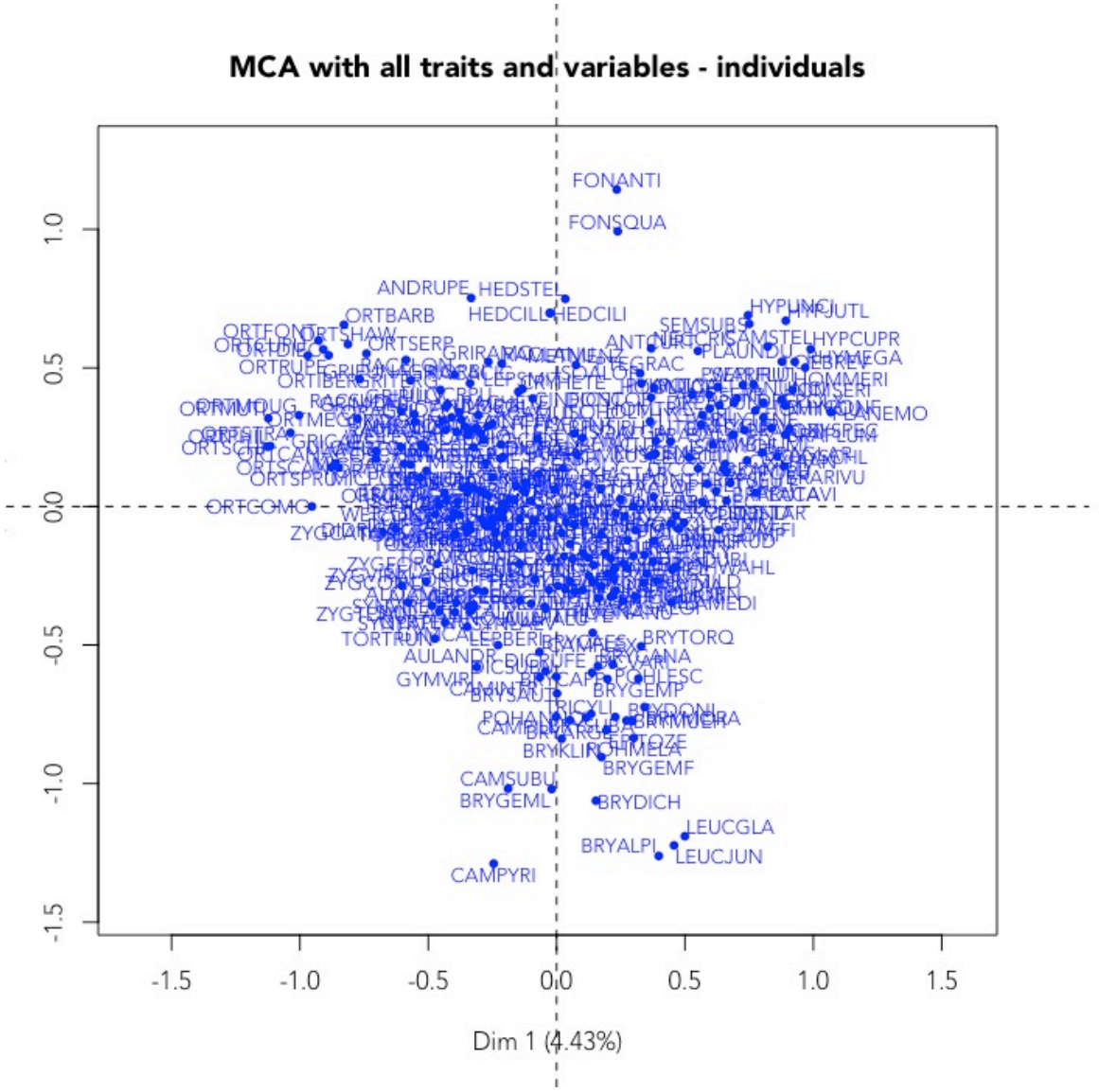


Figure 4.20 MCA plot showing the distribution of species along dimensions 1 and 2.

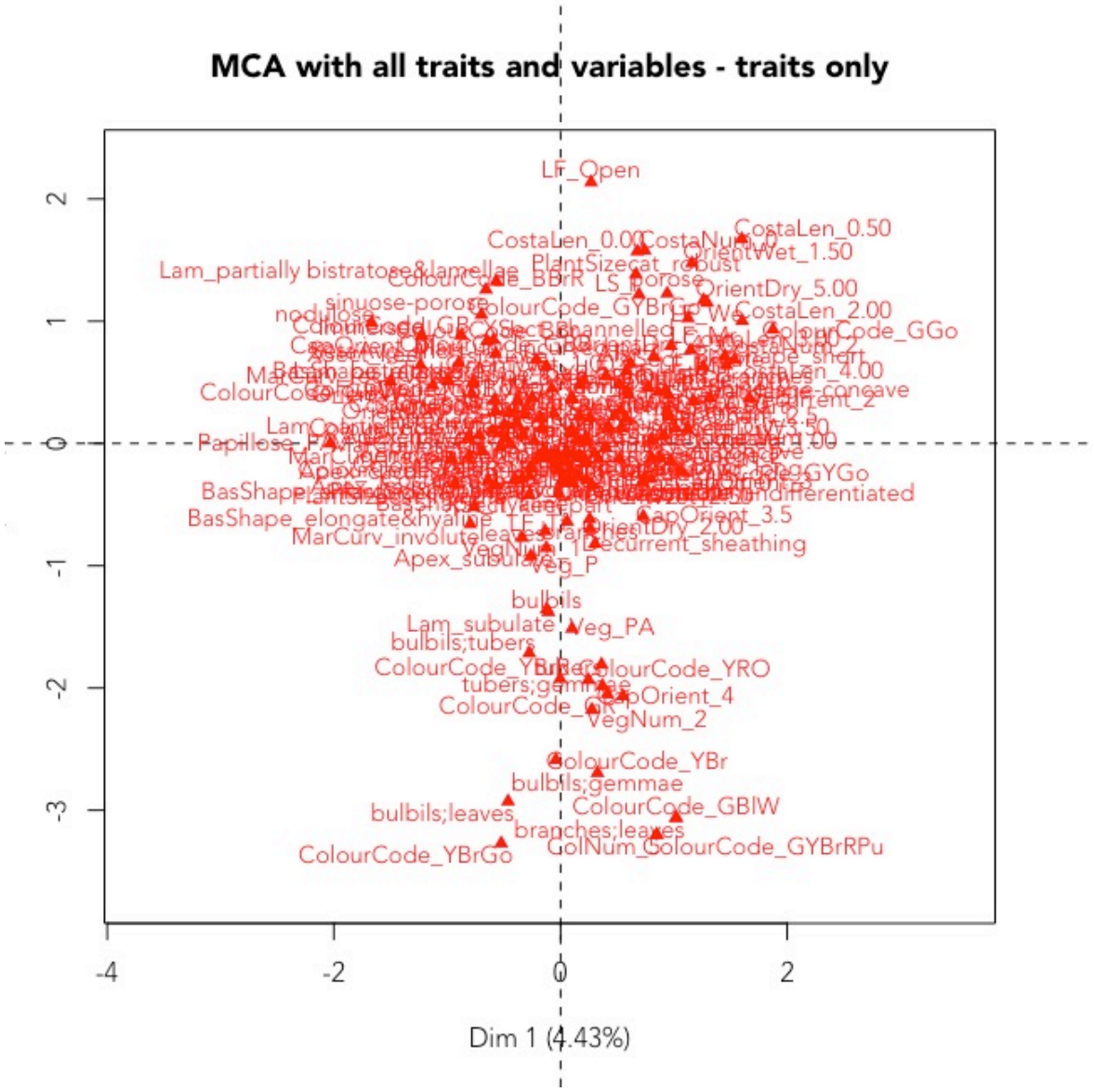


Figure 4.21 MCA showing distribution of all the trait states along dimensions 1 and 2.

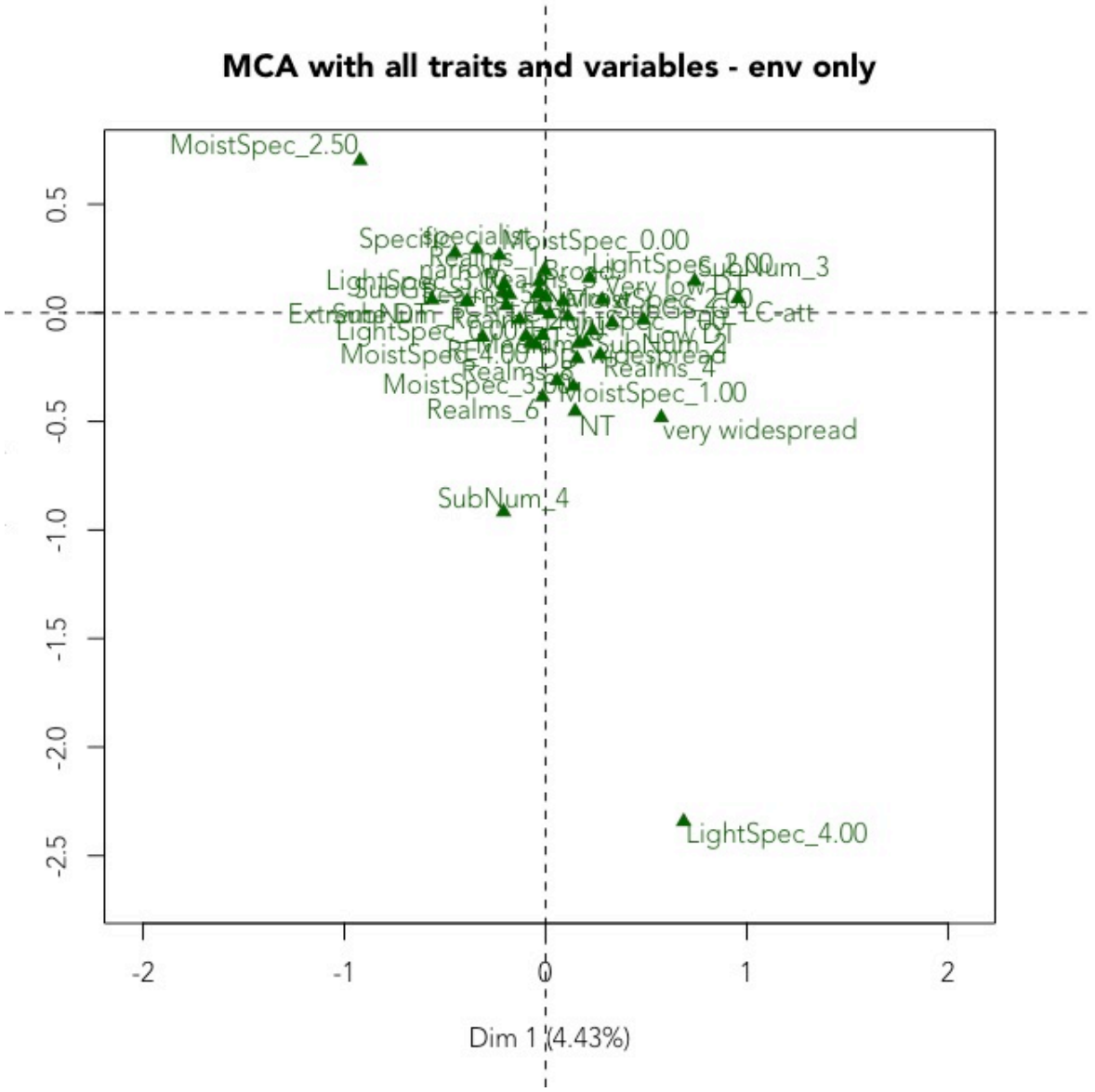


Figure 4.22 MCA of environmental, ecological and habitat variables along dimensions 1 and 2.

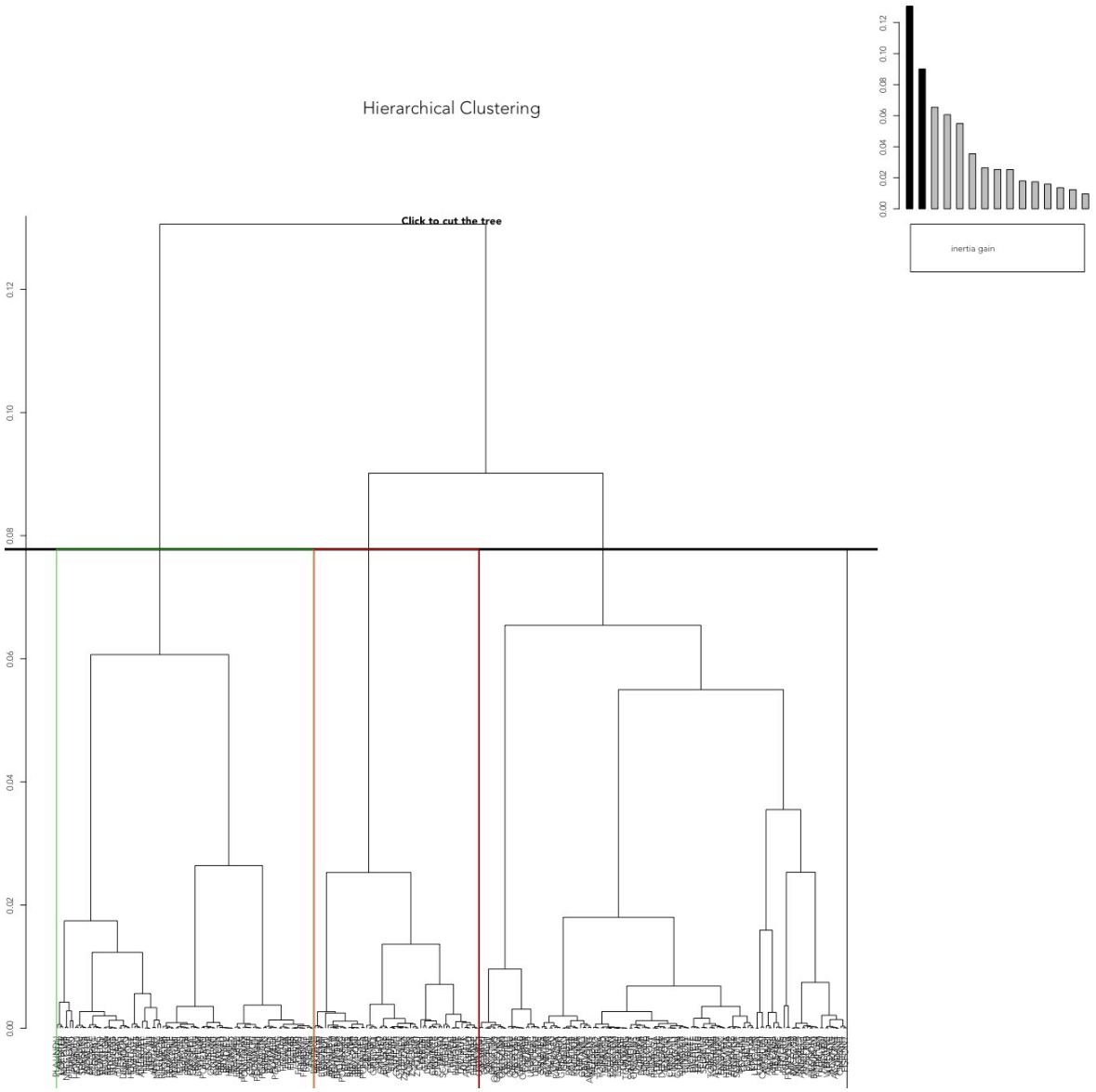


Figure 4.23 Hierarchical clustering dendrogram, outlining the three clusters used.

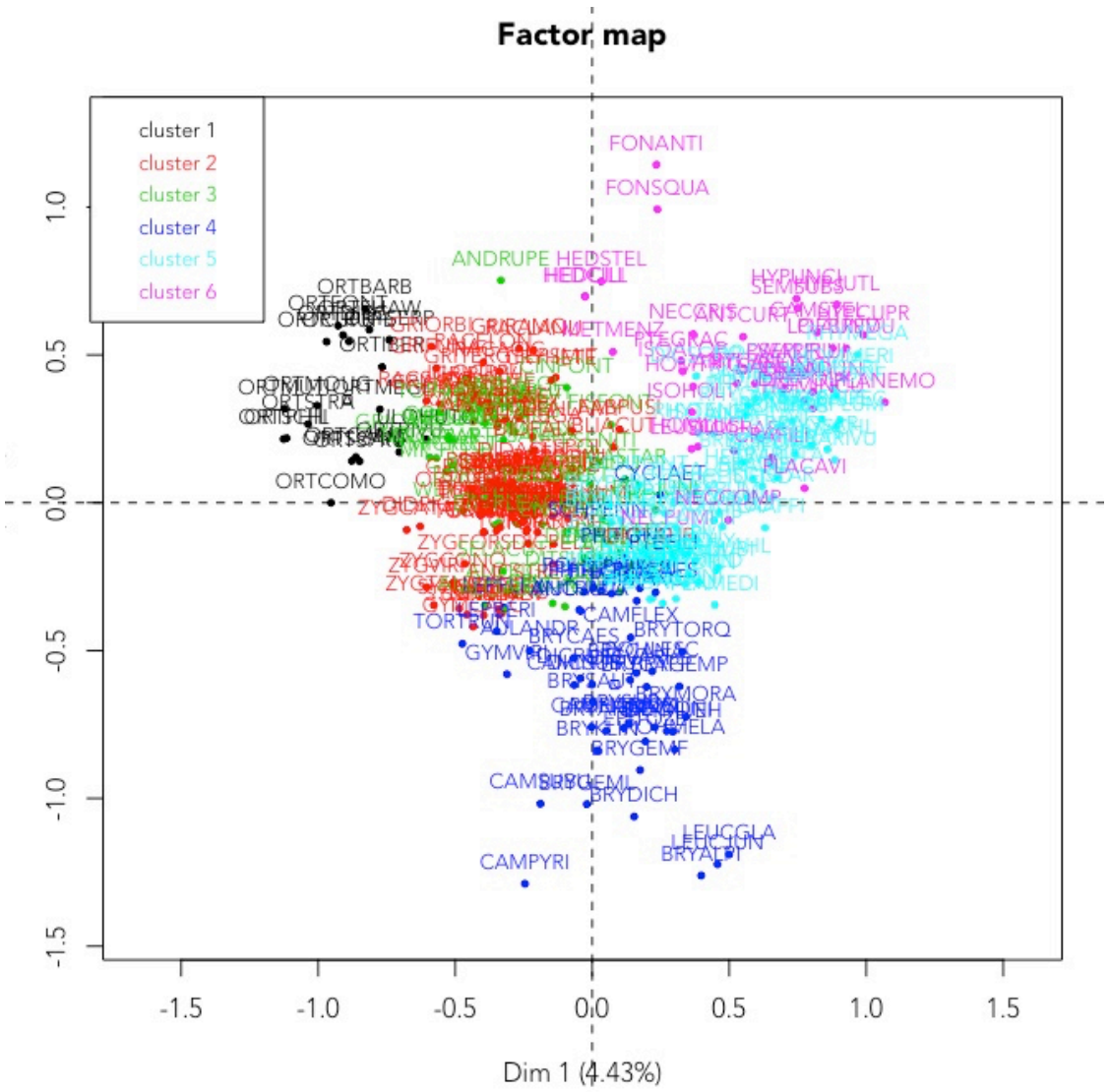


Figure 4.24 Clustering of six bryophyte groups, showing loss of group definitions along the 1st dimension.

A4.4. Indicator value of species and genera

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	U
1	Species code	mar entire	bascell dif 1	lf CU	lf tuft	oreintdry 1	papillose p	caporient 1	bashsape elongate	capexert immersed	xsect keel	apex cucullate	bascw nodulose	Lam=Lam_ bistratose	MarCurv= MarCurv_ involute	MarCurv= MarCurv_ revolute	Traits with missing data	Traits with complete data	Sum of traits present	Cluster 1 value	
2	ZYGFORS	1	1	1	0	0	1	0	0	1	0	0	0	0	0	15	0	15-0= **15**	5.000	**5/15= 0.333**	
3	SPHSUBN	0	1	0	0	0	0	NA	1	NA	0	0	NA	0	0	0	3	15-3= **12**	2.000	**2/12= 0.167**	

Figure 4.25 Process for calculating cluster values for species. If a trait state was present it was scored as 1, then the sum of these values for states was calculated (red) and divided by the total number of states with non-missing data - to standardise values allowing species with traits missing to be included in the indicator pool.

Table 4.24 Species found only in cluster 1 or 3, ordered alphabetically.

Cluster 1	Cluster 3
Acaulon fontiquerianum	*Amblystegium serpens*
Acaulon muticum	*Antitrichia californica*
Acaulon triquetrum	*Antitrichia curtipendula*
Aloina aloides	*Atrichum angustatum*
Aloina ambigua	*Brachythecium albicans*
Aloina rigida	*Brachythecium dieckii*
Amphidium mougeotii	*Brachythecium glareosum*
Andreaea frigida	*Brachythecium mildeanum*
Andreaea megistospora	*Brachythecium plumosum*
Andreaea rupestris	*Brachythecium populeum*
Archidium alternifolium	*Brachythecium rivulare*
Atrichum undulatum	*Brachythecium rutabulum*
Barbula unguiculata	*Brachythecium salebrosum*
Bartramia ithyphylla	*Brachythecium starkei*
Bartramia pomiformis	*Brachythecium velutinum*
Bartramia stricta	*Bryum elegans*
Blindia acuta	*Bryum kunzei*
Brachydontium trichodes	*Bryum mildeanum*
Cinclidotus riparius	*Bryum pallescens*
Coscinodon cribrosus	*Bryum pseudotriquetrum*
Cryphaea heteromalla	*Campylium stellatum*
Cynodontium bruntonii	*Cinclidotus fontinaloides*
Cynodontium jenneri	*Claopodium whippleanum*
Cynodontium polycarpon	*Climacium dendroides*
Dendrocryphaea lamyana	*Cratoneuron filicinum*
Dialytrichia fragilifolia	*Dicranum crassifolium*
Dialytrichia mucronata	*Dicranum scoparium*
Didymodon acutus	*Drepanocladus aduncus*
Didymodon fallax	*Drepanocladus polygamus*
Didymodon luridus	*Entosthodon attenuatus*
Didymodon nicholsonii	*Entosthodon obtusus*
Didymodon rigidulus	*Fissidens adianthoides*
Didymodon tophaceus	*Fissidens bryoides*
Didymodon vinealis	*Fissidens crassipes*
Ditrichum heteromallum	*Fissidens curvatus*
Ditrichum subulatum	*Fissidens dubius*
Encalypta ciliata	*Fissidens exilis*
Encalypta vulgaris	*Fissidens fontanus*
Entosthodon fascicularis	*Fissidens gracilifolius*
Eucladium verticillatum	*Fissidens monguillonii*
Fabronia pusilla	*Fissidens osmundoides*
Grimmia caespiticia	*Fissidens polyphyllus*
Grimmia decipiens	*Fissidens pusillus*
Grimmia donniana	*Fissidens rivularis*
Grimmia funalis	*Fissidens serrulatus*

Cluster 1	Cluster 3
Grimmia laevigata	*Fissidens taxifolius*
Grimmia montana	*Fissidens viridulus*
Grimmia orbicularis	*Fontinalis antipyretica*
Grimmia pulvinata	*Fontinalis squamosa*
Grimmia ramondii	*Funaria hygrometrica*
Grimmia tergestina	*Hedwigia stellata*
Grimmia torquata	*Heterocladium wulfsbergii*
Hedwigia ciliata	*Homalia lusitanica*
Hedwigia ciliata var. leucophaea	*Homalia trichomanoides*
Kiaeria blyttii	*Homalothecium meridionale*
Leptodon smithii	*Homalothecium sericeum*
Leskea polycarpa	*Homomallium incurvatum*
Microbryum davallianum	*Hookeria lucens*
Microbryum fosbergii	*Hygroamblystegium humile*
Microbryum rectum	*Hygroamblystegium varium*
Microbryum starckeanum	*Hypnum cupressiforme*
Orthotrichum acuminatum	*Hypnum jutlandicum*
Orthotrichum affine	*Hypnum uncinulatum*
Orthotrichum anomalum	*Hypopterygium tamarisci*
Orthotrichum comosum	*Isothecium algarvicum*
Orthotrichum consimile	*Isothecium alopecuroides*
Orthotrichum cupulatum	*Isothecium holtii*
Orthotrichum diaphanum	*Isothecium myosuroides*
Orthotrichum ibericum	*Kiaeria starkei*
Orthotrichum lyellii	*Leucodon sciuroides*
Orthotrichum philibertii	*Loeskeobryum brevirostre*
Orthotrichum rivulare	*Metaneckera menziesii*
Orthotrichum rupestre	*Mielichhoferia mielichhoferiana*
Orthotrichum scanicum	*Mnium hornum*
Orthotrichum schimperi	*Neckera crispa*
Orthotrichum shawii	*Nogopterium gracile*
Orthotrichum speciosum	*Oxyrrhynchium hians*
Orthotrichum sprucei	*Oxyrrhynchium pumilum*
Orthotrichum stramineum	*Oxyrrhynchium schleicheri*
Orthotrichum striatum	*Oxyrrhynchium speciosum*
Orthotrichum tenellum	*Plagiomnium affine*
Pleuridium acuminatum	*Plagiomnium medium*
Pleuridium subulatum	*Plagiothecium cavifolium*
Pseudocrossidium hornschuchianum	*Plagiothecium nemorale*
Pseudocrossidium revolutum	*Plagiothecium undulatum*
Pterygoneurum sampaianum	*Pogonatum aloides*
Ptychomitrium polyphyllum	*Pogonatum nanum*
Racomitrium aciculare	*Pogonatum urnigerum*
Racomitrium affine	*Pohlia cruda*
Racomitrium aquaticum	*Pohlia elongata*
Racomitrium elongatum	*Pohlia wahlenbergii*

Cluster 1	Cluster 3
Racomitrium heterostichum	*Polytrichum commune*
Racomitrium lanuginosum	*Polytrichum juniperinum*
Racomitrium sudeticum	*Polytrichum piliferum*
Rhabdoweisia fugax	*Pseudephemerum nitidum*
Seligeria acutifolia	*Pseudoscleropodium purum*
Seligeria calycina	*Pylaisia polyantha*
Seligeria pusilla	*Rhizomnium punctatum*
Syntrichia montana	*Rhynchostegiella curviseta*
Syntrichia papillosissima	*Rhynchostegiella litorea*
Syntrichia princeps	*Rhynchostegiella tenella*
Syntrichia ruralis	*Rhynchostegiella teneriffae*
Syntrichia ruralis var. ruraliformis	*Rhynchostegium confertum*
Syntrichia subpapillosissima	*Rhynchostegium megapolitanum*
Timmiella flexiseta	*Sanionia uncinata*
Tortella humilis	*Schizymenium pontevedrensis*
Tortula acaulon var. pilifera	*Sematophyllum substrumulosum*
Tortula atrovirens	*Thamnobryum alopecurum*
Tortula canescens	*Warnstorfia fluitans*
Tortula cuneifolia	
Tortula freibergii	
Tortula israelis	
Tortula marginata	
Tortula muralis	
Tortula solmsii	
Tortula subulata	
Tortula vahliana	
Tortula wilsonii	
Trichostomum brachydontium	
Trichostomum crispulum	
Ulota bruchii	
Ulota calvescens	
Ulota crispa	
Ulota crispula	
Ulota hutchinsiae	
Weissia controversa	
Weissia levieri	
Weissia longifolia	
Zygodon catarinoi	
Zygodon conoideus	
Zygodon forsteri	
Zygodon rupestris	
Zygodon viridissimus	

Table 4.25 Genera found only in cluster 1 or 3, ordered alphabetically.

Genera only found in cluster 1	Genera only found in cluster 3
Acaulon	*Amblystegium*
Aloina	*Brachytheciastrum*
Amphidium	*Brachythecium*
Andreaea	*Campylium*
Archidium	*Claopodium*
Barbula	*Climacium*
Bartramia	*Cratoneuron*
Blindia	*Drepanocladus*
Brachydontium	*Fissidens*
Coscinodon	*Fontinalis*
Cryphaea	*Funaria*
Cynodontium	*Heterocladium*
Dendrocryphaea	*Homalia*
Dialytrichia	*Homalothecium*
Didymodon	*Homomallium*
Ditrichum	*Hookeria*
Eucladium	*Hygroamblystegium*
Fabronia	*Hypnum*
Leptodon	*Hypopterygium*
Leskea	*Isothecium*
Microbryum	*Leucodon*
Orthotrichum	*Loeskeobryum*
Pleuridium	*Metaneckera*
Pseudocrossidium	*Mielichhoferia*
Pterygoneurum	*Mnium*
Ptychomitrium	*Nogopterium*
Racomitrium	*Oxyrrhynchium*
Rhabdoweisia	*Plagiomnium*
Seligeria	*Plagiothecium*
Timmiella	*Pogonatum*
Tortella	*Polytrichum*
Trichostomum	*Pseudephemerum*
Ulota	*Pseudoscleropodium*
Weissia	*Pylaisia*
Zygodon	*Rhizomnium*
	Rhynchostegiella
	Rhynchostegium
	Sanionia
	Schizymenium
	Sematophyllum
	Thamnobryum
	Warnstorfia

Table 4.26 Forest genera with their trait profile values (C1 and C3), their indicator value (C1-C3) and their indicator category; ordered alphabetically by genus. Colour coded by indicator category:

1- Strict humid and sheltered indicator	2- Non-strict humid and sheltered indicator	3- Non-strict dry and exposed indicator	4- Strict dry and exposed indicator

Genus	C1 value	C3 value	Indicator value	Indicator category
Acaulon	0.200	0.133	0.067	3
Amphidium	0.467	0.067	0.400	3
Antitrichia §	0.133	0.267	-0.134	2
Archidium	0.267	0.067	0.200	3
Atractylocarpus	0.500	0.000	0.500	4
Atrichum	0.233	0.067	0.166	3
Barbula	0.400	0.067	0.333	3
Blindia	0.267	0.067	0.200	3
Brachydontium	0.333	0.067	0.266	3
Brachythecium	0.017	0.192	-0.175	2
Callicostella §	0.167	0.022	0.145	3
Calymperes §	0.269	0.007	0.262	3
Calyptothecium	0.000	0.000	0.000	None
Calyptrochaeta	0.100	0.222	-0.122	2
Campyliadelphus	0.077	0.143	-0.066	2
Campylium	0.133	0.200	-0.067	2
Claopodium	0.067	0.200	-0.133	2
Clastobryophilum	0.091	0.200	-0.109	2
Climacium	0.133	0.200	-0.067	2
Cratoneuron	0.067	0.133	-0.066	2
Daltonia §	0.154	0.250	-0.096	2
Dicranum	0.200	0.111	0.089	3
Didymodon	0.367	0.100	0.267	3
Distichophyllum	0.154	0.143	0.011	3
Ectropothecium §	0.098	0.123	-0.025	2
Entosthodon	0.133	0.067	0.066	3
Eropodium §	0.333	0.133	0.200	3
Fabronia §	0.000	0.000	0.000	None
Fissidens	0.083	0.183	-0.100	2
Gammiella §	0.077	0.231	-0.154	2
Glossadelphus §	0.182	0.100	0.082	3
Groutiella §	0.167	0.143	0.024	3
Hedwigia	0.267	0.133	0.134	3
Heterocladium	0.067	0.133	-0.066	2
Homalia §	0.267	0.133	0.134	3
Homalothecium	0.133	0.267	-0.134	2
Homomallium	0.067	0.267	-0.200	2

Genus	C1 value	C3 value	Indicator value	Indicator category
Hookeria	0.133	0.267	-0.134	2
Hypnum	0.044	0.467	-0.423	2
Hypopterygium	0.133	0.267	-0.134	2
Isothecium	0.178	0.156	0.022	3
Jaegerina	0.167	0.167	0.000	None
Kiaeria	0.233	0.000	0.233	4
Lepidopilum	0.000	0.250	-0.250	1
Leptodon §	0.333	0.067	0.266	3
Leucodon §	0.267	0.133	0.134	3
Leucoloma §	0.371	0.071	0.300	3
Leucophanes §	0.188	0.048	0.140	3
Loeskeobryum	0.067	0.200	-0.133	2
Macrocoma §	0.333	0.100	0.233	3
Macromitrium §	0.394	0.000	0.394	4
Microbryum	0.433	0.067	0.366	3
Mittenothamnium §	0.119	0.137	-0.018	2
Mitthyridium §	0.249	0.000	0.249	4
Neckera §	0.200	0.222	-0.022	2
Nogopterium §	0.267	0.133	0.134	3
Ochrobryum §	0.333	0.154	0.179	3
Octoblepharum §	0.200	0.067	0.133	3
Orthostichopsis §	0.100	0.111	-0.011	2
Orthotrichum §	0.518	0.051	0.467	3
Palamocladium §	0.154	0.143	0.011	3
Papillaria §	0.244	0.036	0.208	3
Phyllodon §	0.133	0.000	0.133	4
Pinnatella §	0.000	0.143	-0.143	1
Plagiothecium	0.100	0.333	-0.233	2
Pleuridium	0.133	0.033	0.100	3
Pogonatum	0.089	0.133	-0.044	2
Pohlia	0.027	0.133	-0.106	2
Polytrichum	0.111	0.267	-0.156	2
Porotrichum	0.083	0.250	-0.167	2
Prionodon §	0.200	0.000	0.200	4
Pseudephemerum	0.067	0.200	-0.133	2
Pseudoscleropodium	0.133	0.200	-0.067	2
Pylaisia §	0.200	0.133	0.067	3
Racopilum §	0.231	0.000	0.231	4
Rhacopilopsis §	0.000	0.143	-0.143	1
Rhizofabronia §	0.133	0.133	0.000	None
Rhizomnium	0.067	0.133	-0.066	2
Rhynchostegiella	0.100	0.217	-0.117	2
Rigodium §	0.000	0.167	-0.167	1
Sanionia	0.067	0.133	-0.066	2

Genus	C1 value	C3 value	Indicator value	Indicator category
Schimperella §	0.133	0.333	-0.200	2
Schlotheimia §	0.556	0.000	0.556	4
Seligeria	0.289	0.067	0.222	3
Sematophyllum	0.133	0.400	-0.267	2
Squamidium §	0.267	0.133	0.134	3
Stereophyllum	0.133	0.214	-0.081	2
Syntrichia	0.360	0.013	0.347	3
Syrrhopodon §	0.241	0.012	0.229	3
Taxithelium §	0.093	0.224	-0.131	2
Thamnobryum	0.133	0.200	-0.067	2
Tortella	0.267	0.067	0.200	3
Tortula	0.383	0.067	0.316	3
Trachyphyllum	0.077	0.214	-0.137	2
Trachypodopsis §	0.200	0.000	0.200	4
Trachypus §	0.250	0.083	0.167	3
Trichosteleum §	0.103	0.194	-0.091	2
Trichostomum	0.367	0.067	0.300	3
Ulota §	0.440	0.027	0.413	3
Weissia	0.467	0.067	0.400	3
Zygodon §	0.427	0.040	0.387	3

Chapter 5 Identifying tropical bryophyte indicators

Abstract

Tropical forests are highly threatened worldwide making their conservation both a priority and urgent. Madagascar is no exception, and although a protected area network exists, threats are ongoing. Aditionally, not all components of the Malagasy are well studied, as is the case with bryophytes. This is a necessary field of research in order to predict how species abundance and diversity will change, or have changed, as a result of forest degradation or deforestation.

Sampling was undertaken in a lowland humid forest in February 2016, a forest type that is heavily degraded and does not have high levels of protection. Bryophytes were collected in a range of forest degradation plots, and in non-forest plots (cleared for shifting cultivation). To quantify disturbance, both discrete categories and an index of degradation were used. Degradation is difficult to define and studies often use different definitions, thus making comparisons between studies difficult or impossible.

The IV of taxa collected varied according to forest degradation, although there was no clear pattern of increasing IV with degradation. This would be expected as degraded forests have higher insolation and lower humidity and therefore bryophytes with low IVs should not be able to survive. The lack of trend indicates that the IV of bryophytes may not be the most suitable due to the fact that bryophytes, most having some level of desiccation tolerance, can persist for a certain period of time after disturbance has occurred. However, there are some methodological considerations that may affect the performance of the IV and further work will go into this in future. Life-form continues to be a realiable indicator, as open life-forms were not found in heavily degraded forest or non-forest plots. This means that bryophyte life-forms could be used as a quick, easy and cost-effective way to monitor forest degradation.

5.1 Introduction

Tropical humid forests are one of the richest ecosystems but also, historically, one of the least protected (Myers, 1981). In 1980, between 200 000 km^2 to 250 000 km^2 of tropical humid forests was estimated to have been degraded per year (Myers, 1981). Currently, it is estimated that more than 50% of all tropical habitats are degraded (Struebig et al., 2013). Madagascar is highly regarded for being a “biodiversity and endemism hotspot” (Mittermeier et al., 1998; Myers et al., 2000; Ganzhorn et al., 2001) but is also known for the significant human threats to its ecosystems (Gardner, 2011). No two tropical forests are alike – and Madagascar's flora has exceptionally high levels of endemism – so applying findings from one area to another should be done with caution (Primack & Corlett, 2005). However, the lack of bryophyte ecology studies in Madagascar means that most studies referenced here are inevitably from other regions, particularly the Neotropics where significant amounts of bryophyte ecological studies have been undertaken (Mervin & Nadkarni, 2001).

5.1.1 Madagascar

Madagascar is the fourth largest island in the world, at 58.7 million hectares the country is largely divided between the humid east and the dry west (Figure 5.3). Most of Madagascar’s 25 million

inhabitants live in extreme poverty, with GNI per capita in 2015 of $410 (World Bank, 2016). The majority of the population live rurally (66%) and most rural households rely on underproductive subsistence agriculture with a strong reliance on natural resources for their living requirements (World Bank, 2016).

As described in Chapter 1, over 80% of the island's 2984 vascular plants are endemic (Goodman & Benstead, 2003), including 90% of its orchid species, and it has more primate species than any other country (Cable, 2011). Endemism rates are lower among spore-bearing plants: ferns (45%; Goodman & Benstead, 2003) and bryophytes (28.7%; Marline et al., 2012) (see Table 1.10, p. 42, Chapter 1), althought the endemism rate of bryophytes is higher than in many other countries. It is one of the world's biodiversity hotspots and priority conservation areas (biodiversity hotspot, plant hotspot, EDGE (Myers et al., 2000)), and is globally important both in terms of biodiversity conservation and understanding evolutionary processes (Myers et al., 2000; Myers & Knoll, 2001 *in* Cable, 2011).

5.1.1.1 Geography & Geology

Madagascar is located in the Indian Ocean just over 400 km, at its closest point, from the coast of East Africa, between 11°57' and 25°39' latitude. It first separated from continental Africa 160 MYA, and drifted northwards as part of what is now the Indian sub-continent until it became separate from it 70-80 MYA. Its isolation has allowed distinct evolutionary lineages to evolve and radiate, subsequently many of these lineages became extinct on continental Africa & India (Goodman & Benstead, 2005).

Geologically, most of Madagascar is pre-cambrian crystalline basement with granite and gneiss which, through soil development over millennia, has created Madagascar's characteristic layer of nutrient poor red ferralitic soils with outcrops of gneiss, quartz and granite in some areas of the central plateau (Fischer & Theisen, 2000). The southwestern and southern soils are mainly unconsolidated sands on a basement of limestone. The southeast (study location) presents a mix of these with most remaining humid forests on gneiss and granite elements (under ferralitic soils) of the central mountain ridge and with littoral humid forests found on coastal sandy plains (Du Puy & Moat, 1996). The geomorphology and climate give rise to delimitations in the Malagasy vegetation (Renauld & Cardot, 1915), with humid forest to the east and north, dry deciduous forests in the west and north, spiny forest in the south (Figure 5.3, p. 277) and a central plateau with grassland, grassland mosaic, thickets and sclerophyllous forest. As several works provide good detailed descriptions of the Malagasy flora, such as Perrier de la Bâthie (1921, 1936), Koechlin (1974) and more recently in Moat & Smith (2007), I shall focus on describing the vegetation of the study region – lowland humid forest of the southeast.

5.1.1.2 Lowland humid forest

Lowland humid forest in Madagascar is found up to a maximum altitude of between 500 - 800m (Moat & Smith, 2007) and is defined as evergreen dense forest on the east coast of Madagascar receiving 1500-3000 mm annual precipitation (Goodman & Benstead, 2003). The lack of a strict altitude delimitation in forest type is due to the gradual transition between low- to mid-altitude forest through much of Madagascar's eastern humid forest corridor (Du Puy & Moat, 2003), although at the southern extent of this corridor, near the town of Fort Dauphin (Tolagnaro) the transition zone is narrow (Perrier de La Bâthie, 1921; Koechlin et al., 1974; Moat & Smith, 2007).

The eastern forest corridor stretches from northern to southern Madagascar (approximately 1400 km) in a narrow strip along the eastern escarpment which descends from the central highlands to the eastern coastal plain (Koechlin et al., 1974). Lower altitude humid forest receives more precipitation than at higher altitudes where water is in the form of vapour (Moat & Smith, 2007) and so this has implications for the vegetation type it can support. It is therefore to be expected that the bryoflora of the lowland forests may be different from higher altitude forests due the capacity for bryophytes to intake water as vapour.

Lowland humid forests in Madagascar are evergreen and characteristic canopy species include those from the Myristicaceae family and *Anthosema* genus (Perrier de La Bâthie, 1921; Koechlin et al., 1974; Moat & Smith, 2007). Canopy height is usually between 25-35 m at lower elevations and middle elevations (Koechlin et al., 1974; Moat & Smith, 2007). Trees are generally narrow with few measuring more than 80 cm to 1 m at DBH (Koechlin et al., 1974; Moat & Smith, 2007). *Ocotea* species, *Slonea rhodontha,* which can grow to 40 m, and *Canarium madagascariense,* which can reach two meters in DBH, are the largest trees but still smaller than other African humid forests (Koechlin et al., 1974). These forest trees are lower and narrower than dominant trees in other tropical forests worldwide.

This study takes place in a southeastern lowland humid forest, Tsitongambarika Forest (TGK) (Figure 5.3, p. 277). Until recently very little botanical or zoological research had been undertaken in TGK and only a handful of bryophyte collections are known from here. Adding bryophyte species to the forest's species list will enhance its profile as an important area to protect. Recent biodiversity studies were undertaken as part of an ilmenite mine's biodiversity offset measures, a Rio Tinto mine located near the town of Fort Dauphin (Tolagnaro). The first study was a survey of the herpetofauna in 1999 and again in 2005-2006 along with studies on vascular plants, birds, ants, bats and lemurs (BirdLife International, 2011). The results of these surveys showed that TGK contains a high level of biodiversity including globally threatened species and regionally endemic species, some known only from this forest. Vascular plant surveys conducted in 2005-2006 estimated that over 1000 plant species exist in Bemangidy-Ivohibe and around 20 new species to science, potentially 50 in total, were discovered during these inventories (Razakamalala et al., 2011).

5.1.1.3 Decline of forests

The deforestation in Madagascar is well documented (Goodman & Benstead, 2003; Cable, 2011) and its pattern and rate is the result of both historical and current social and economic factors. Until recently it was thought that the first human settlements of Madagascar occurred around 1800 years ago (Cable, 2011), however recent evidence has demonstrated a human presence from approximately 4000 years before present (Dewar et al., 2013). This earliest evidence of human occupation of Madagascar indicates a foraging culture rather than the agro-pastoral culture from the later evidence of human occupation of Madagascar – and so may account for the major Holocene extinction events and land use changes currently thought to be concurrent with the later waves of arrival approximately 1800 to 2000 years before present (Dewar et al., 2013). Most evidence for early habitation of Madagascar indicates first settling in the coastal areas (Figure 5.1). Today, approximately 21% of the land area of Madagascar remains forested (forest, and other woodland) (Cable, 2011; Food and Agriculture Organization of the United Nations (FAO), 2015), although debate exists as to whether the central plateau was as forested prior to human occupation. This debate remains current and controversial and was dealt with thoroughly

in McConnell & Kull (2014) citing strong evidence that the narrative of large-scale human induced deforestation of 80 to 90% of Madagascar is unsupported.

Latest estimates state that pristine humid forest in Madagascar covers an area of 47 737 km^2 (estimated at around 68 000 km^2 in 2003 – Du Puy & Moat, 2003) and degraded humid forest an area of 58 058 km^2 (Moat & Smith, 2007); in 1960 it was estimated to cover 61 320 km^2, a 33.4% decrease (Guichon, 1960 in Koechlin *et al.*, 1974).

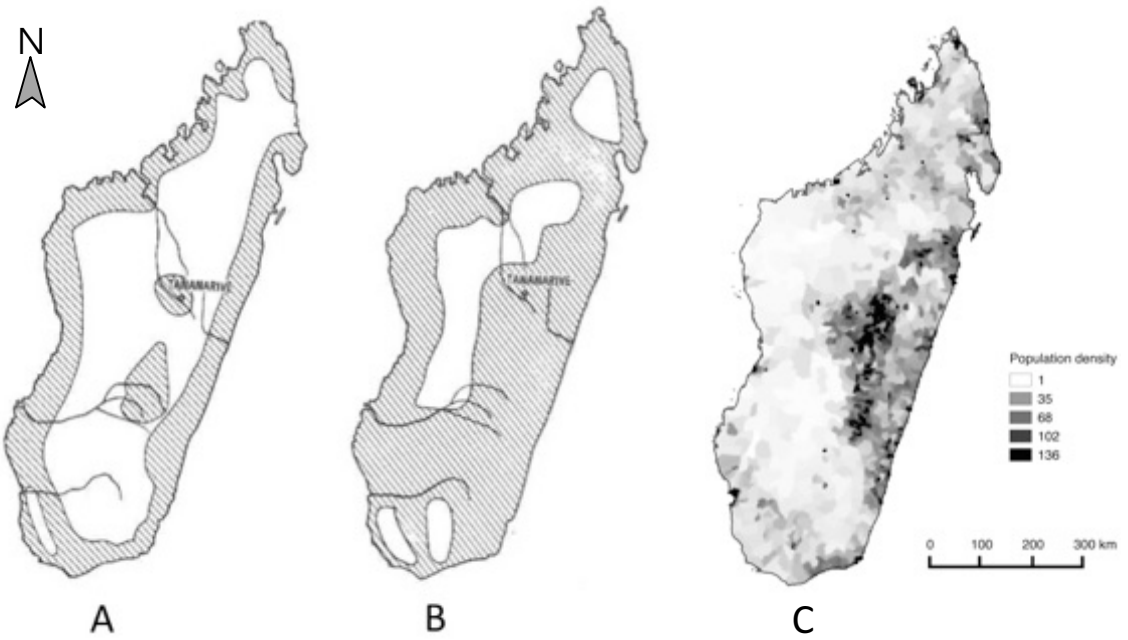


Figure 5.1 Areas with human settlement (hashed areas) circa (A) 1750 and (B) 1894; and (C) population density (persons/km^2) in 2000. 1750 and 1894 maps taken from Koechlin et al., 1974, p. 60 and 2000 map created using data published by NASA's Socioeconomic Data and Applications Center (Center for International Earth Science Information Network (CIESIN), Columbia University & Centro Internacional de Agricultura Tropical (CIAT), 2005).

In Réunion, M. Debette, a mining engineer, already in 1877 described how the forests had shown a rapid decrease in under 100 years as a result in rapid population growth and agriculture, namely sugar cane plantations (Bescherelle, 1880). In Southern Madagascar, large areas were cleared for Sisal plantations, lowland coffee production and commercial logging (personal communication, Director of Regional Forest Service – DREF). Significant commercial logging or deforestation for commercial agriculture no longer occurs in Madagascar at the scales seen in other tropical countries. These factors notwithstanding, as with other tropical forests in Africa the primary drivers of deforestation in Madagascar are the production of charcoal for fuel, both in rural areas and urban centres (Scales, 2014; Food and Agriculture Organization of the United Nations (FAO), 2015) and subsistence agriculture. As in most forests throughout Madagascar, TGK is under pressure from agriculture and timber extraction. Slash-and-burn and selective extraction (large trees for canoes and a creeper, *Flagellaria indica*, for lobster baskets) occurs in Ivohibe but is relatively low level compared to other forest areas in Tsitongambarika, such as periphery areas and the northern and southern limits, where very little or no lowland forest remains (Figure 5.2).

Quantification of revenues from such activities is difficult due to a lack of knowledge of value chains and distribution networks but there have been several studies attempting to quantify the economic value from forests. Within the study area (Tsitongambarika forest and surrounding

areas) agriculture is a main activity but is highly inefficient (due to lack of agricultural extension, improved seed availability and the low availability and high cost of fertilizers) and is mainly limited to rice and cassava crops with few cash crops (BirdLife International, 2011). Shifting cultivation by clearing areas of forest is widespread although in forest areas managed by a community association (CoBa) there has been some decrease in this practice (BirdLife International, 2011). Most produce harvested is for subsistence but some is sold at markets providing a small source of cash for households (BirdLife International, 2011). Forest resources are an important part of livelihoods (Table 5.1) – where GNI per capita is one of the lowest in the world: 400 US dollars, just over 1USD a day (World Bank, 2016). The Forest Resources Assessment of the FAO (2015) report over 97% of timber extractions from Madagascar's forests between 2010 and 2015 were for fuelwood. An alarming fact is that within 60 km of the town of Fort Dauphin (Tolagnaro), over 95% of remaining forest (primary and secondary) is within a protected area meaning that, inevitably, these forests will be used to source fuel and building materials (among other uses). The high population growth rate (2.8%) coupled with a lack of investment in improving agricultural production places ever-increasing pressure on Malagasy biodiversity and its resources.

Table 5.1 Financial revenue (Ariary & USD) from forest products in Tsitongambarika forest showing the significant contribution of forest products to daily income. Data compiled from Birdlife International, 2011. Percentage of daily income based on a GNI per capita of 420 USD for 2011, the year of the Birdlife International report.

Use	Amount	Malagasy Ariary	US dollar	% of daily income
Revenue for CoBa				
Timber for house	1 tree	500 - 1000	0.24 - 0.47	21 - 41%
Timber for boat	1 tree	3000 - 5000	1.42 - 2.36	123 - 205%
Fine for setting fire to forest		10 000	4.73	411%
Revenue for harvesters				
Lobster traps	1	200 - 500	0.10 - 0.24	8 - 20%
Branches for house walls	100	3000	1.42	123%
Leaves for roofing	100	3000 - 5000	1.42 - 2.36	123 - 205%
Charcoal	30kg bag	1100 - 2000	0.52 - 0.57	45 - 49%
Ebony plank	125 x 25 cm	6000	2.84	246%
Collared brown lemur	1	3000 - 15 000	1.42 - 7.09	123 - 617%
Finished boat – one month to construct	1	120 000 - 300 000	56.7 - 141.75	14 - 24% of yearly income

5.1.1.4 Forest conservation

Overall, forest management in Madagascar has evolved from a top-down approach to a mixed approach of state managed national parks (~25%) coupled with a much larger network of "New Protected Areas" which are typically co-managed by community forest management associations (CoBas & CoGe's) and national and international NGO's (~75%) (Raik, 2007) – see Table 5.2 for a summary of changes. There has also been a change from viewing forests purely as a resource to be exploited to a stronger focus on biodiversity conservation; a change that has been led by the Government of Madagascar and conservation organisations (national & international). The Madagascar Code of Protected Areas was established in 2001 and it outlines protected area creation and management (ANGAP, 2001). Further to this, in 2003 it was announced that

Madagascar would extend its protected area network to create 6 million ha of protected areas, The System of Protected Areas of Madagascar (SAPM), thus tripling the existing total protected area which was 1.7 million ha (Raik, 2007). Together with national and international conservation organisations, 7.1 million ha of protected areas have been created, 12% of Madagascar's area (Figure 5.3, p. 277) (Gardner et al., 2018). The efficacy of these protected areas is debatable, however (Gardner et al., 2018), with illegal logging, timber and animal harvesting still prevalent in many forests.

Of the vegetation types that have some of their area under high protection status (National Park, Special Reserves or Reserves Naturelles Integrales), low-altitude humid forests have one of the lowest levels of protection, about 5%, compared to others: evergreen sclerophyllous 16%, mid-altitude humid forests 10%, deciduous seasonally dry western forests 9%, lower montane humid forests 4% and deciduous dry southern forests and scrubland 2% (Figure 5.2) (Du Puy & Moat, 2003). Du Puy & Moat (2003) suggest that one of the areas "desirable for conservation", at least from the point of view of plants, are the low altitude evergreen humid forests between the towns of Fort Dauphin (Tolagnaro) and Vangaindrano. These correspond mostly to Tsitongambarika Forest, which, when this 2003 study was published, did not have any protected status. Since then, biodiversity surveys of Tsitongambarika Forest (TGK) have been undertaken and it has been given protected area status (Nouvelle Aire Protégé, IUCN Category V) under management by the NGO Asity Madagascar/Birdlife International (Figure 5.3). In 1996 it was included in Birdlife's Important Bird Area list and following the 2005-2006 surveys TGK was awarded official protected status as a New Protected Area (NAP, IUCN Category V) in 2008, which was formally gazetted in 2015. The protected area management management is overseen by Asity Madagascar, a BirdLife International partner, and the day-to-day management of forest resources is under the responsibility of about 60 community forest management associations (CoBas) under the umbrella of a co-management committee (COGE) based in or near the forest.

Table 5.2 Summary of the evolutions of forest policy in Madagascar. Taken from Raik, 2007, Table 1, p. 6.

Period		Dominant narrative	Policy	Role of government	Role of governed
Pre-Colonial until 1896)		Madagascar was once fully forested	Cutting live firewood forbidden	Create and enforce repressive forest policy (through banning deforestation)	Abide by centrally-created laws
		Deforestation resulted from human activity	Burning and settling in forests forbidden	Ensure forests (i.e., royal property) are preserved for the use of royals	
			Clearing the land for agriculture forbidden		
Colonial (1896-1961)		Madagascar's forest resources are for French use and to enrich France	Reforestation of fast growing species	Create and enforce repressive forest policy (through establishing conservation areas or banning deforestation)	Abide by centrally-created laws
		Malagasy are unable to manage forests	Hunting lemurs forbidden	Manage forests uni- laterally	Resist centrally-created laws by continuing
					tavy as a cultural practice
		Reforestation is needed for human consumption and development	Forest fires and deforest-ation forbidden		
			Logging concessions established		
Post-Colonial (1962-Present)	Early Independence (1962-1991)	The State is the only legal manager of forest resources	Deforestation forbidden	Create and enforce repressive forest policy	Abide by centrally- created laws
		Deforestation resulted from human activity	Hunting of several species forbidden	Manage forests unilaterally	Resist centrally-created laws by continuing tavy and burning as cultural practices
			Reforestation mandatory		

Period		Dominant narrative	Policy	Role of government	Role of governed
	National Environmental Action Plan Era (1992-Present)	Conservation is needed to save Malagasy bio-diversity	Integrated conservation and development projects	Create protected areas	Stop destructive forest practices
		Standardized models are appropriate	Fences and fines	Enforce laws	Use economic development activities as an alternative to resource extraction
				Provide economic development opportunities	
	Community-based Forest Management	Local people can manage and conserve forests	Decentralization of forest management	Transfer management rights and responsibilities to local people	Conserve and manage forests for long-term sustainability
		The state is ill-equipped to manage forests effec-tively everywhere	Empowerment of local forest users to make decisions regarding forests	Monitor and oversee local-level management decisions	Adhere to principles established by the government or third-party NGOs

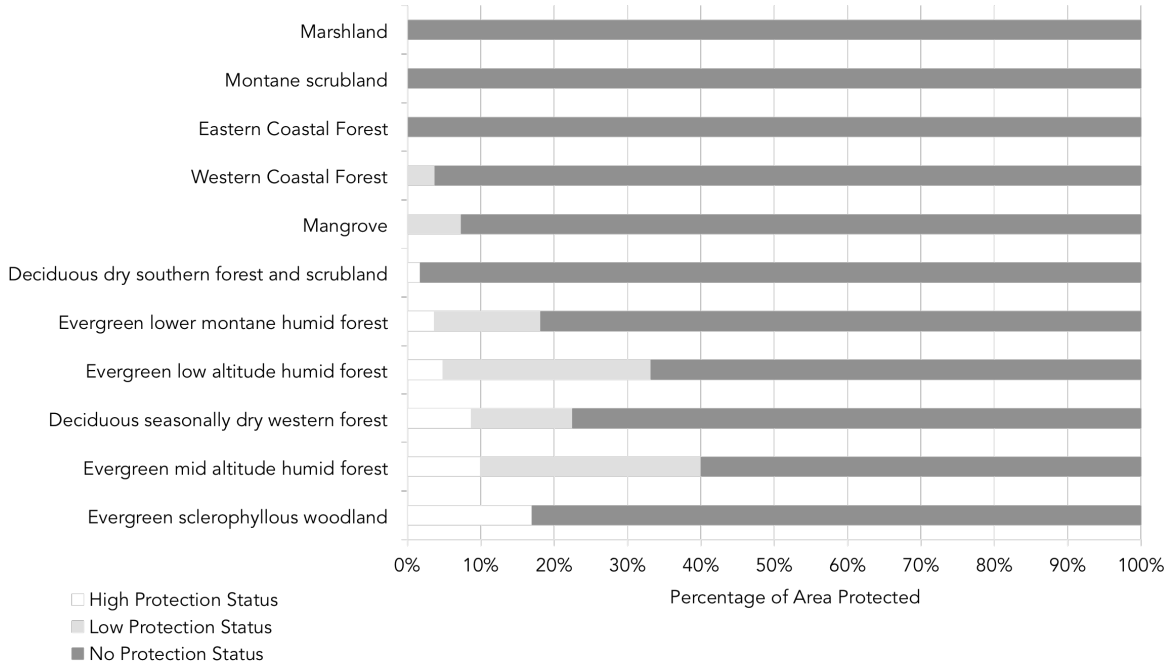


Figure 5.2 Percentage and level of protection status in primary vegetation types in Madagascar. Data is from 2003 as no more current data exist. Adapted from Du Puy & Moat, 2003, fig. 2.23, p. 59.

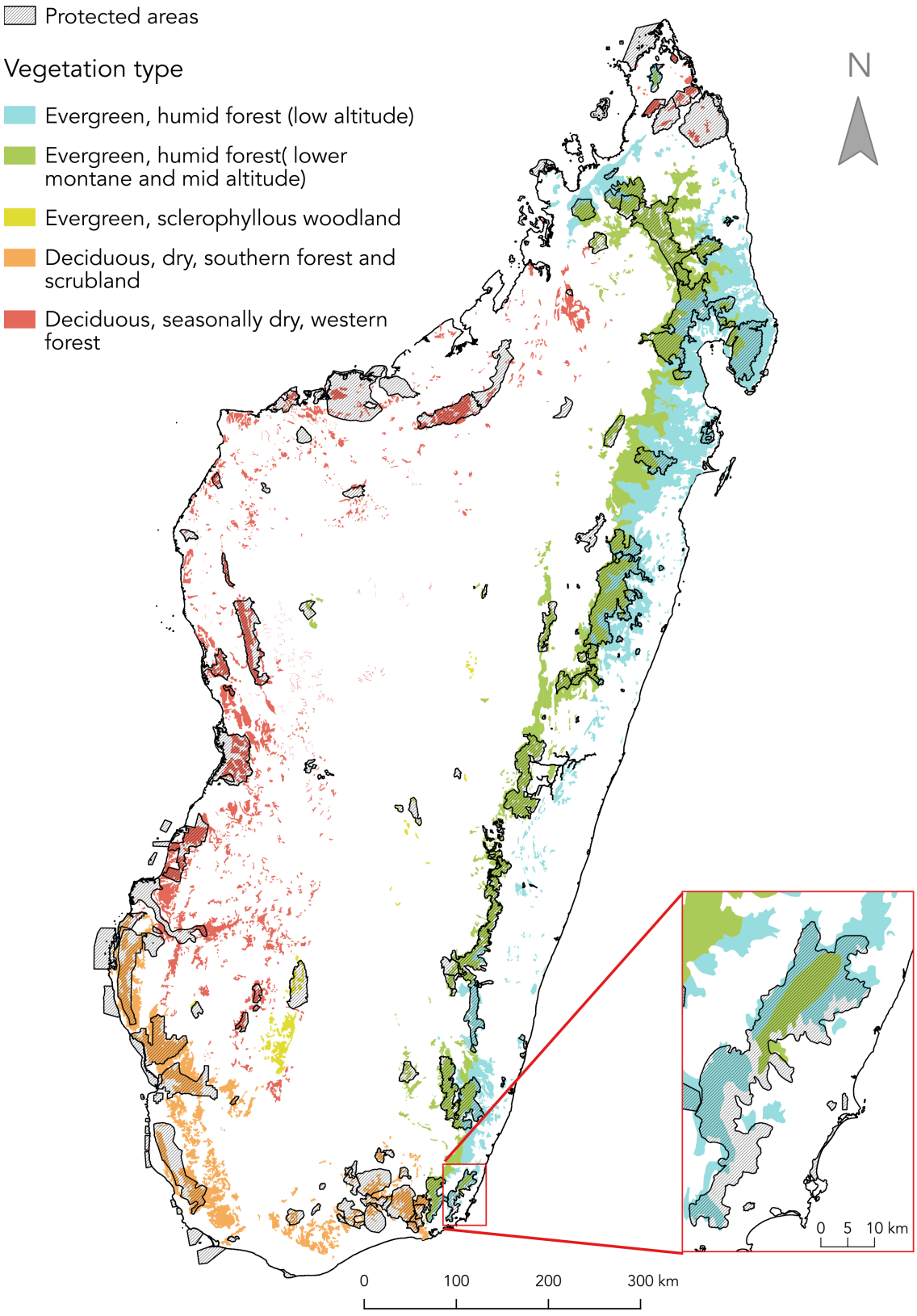


Figure 5.3 Main forest types in Madagascar and SAPM protected areas with inset map of Tsitongambarika Forest Protected Area. Map created with vegetation data from Moat & Smith, 2007 and SAPM protected area data from (REBIOMA, 2012).

5.1.2 Forest degradation

When forests (and other ecosystems) suffer disturbance, left behind is a matrix of different levels of degradation and types of land-use; the characteristics of this matrix will affect the response of

species to habitat loss and change (Ewers & Didham, 2006; Ruffell et al., 2017). This is because degradation is not always due to complete removal of forest cover (e.g. clear-cutting for agriculture) but also due to lower-impact actions that affect forest integrity differently and result in a different level of degradation (e.g. fuelwood collection) (Simula, 2009). The matrix can include land-uses such as shade-plantations, timber plantations, grazing land, cash-crop agriculture, and forest corridors. Within this matrix of land-uses, the border between these is an equally important concept, with "edge-effects" having a significant impact on the abundance of different species (Cardoso et al., 2013). Edge-effects are the effects of disturbance experienced by areas that are on the outskirts of a land-use type (Ewers & Didham, 2006). It has been proposed that managing this matrix by enhacing land-uses that have a lower impact on biodiversity may be a more efficient and speedier approach to conserve biodiversity than focussing on re-creating the lost habitat (Ruffell et al., 2017). However, if a landscape already contains a proportion of high-value land-use, then increasing this has limited effect, especially if the cover of native forest is high (Ruffell et al., 2017). Additionally, different groups of species (e.g. different functional guilds) may respond differently suggesting it is important to make management decisions based on the response of threatened or rare species. This matrix concept is applicable to bryophytes, not only at the landscape level, but at the habitat level due to the reliance of bryophytes on microhabitats (Vitt & Belland, 1997).

Because this is a relatively new concept, the effect of this matrix landscape on biodiversity and how to manage it is still not sufficiently known: how do economic and social factors impact conversion to higher-quality land-uses and how do different land-use qualities impact biodiversity (Ruffell et al., 2017). Aditionally, identifying how species and community compositions change along a gradient of disturbance is equally important in order to identify matrix management strategies that maximises biodiversity (Cardoso et al., 2013), especially as most areas of tropical forests are now degraded to some extent (Struebig et al., 2013). Within the SAPM plan, protected areas have two zones: a "strict centre [*noyau dur*]" and a "utilisation zone [*zone d'utilisation*]", a method of managing the matrix that applies the concept of edge-effects by maintaining an area of more pristine habitat, but allowing use of resources upon which many people living by forests rely on for income and subsistence (Gardner et al., 2018).

Defining forest degradation is complex, as exemplified by the various definition systems that exist (each based on different criteria), but necessary in order to inform conservation decisions (Simula, 2009). At a site-level, a common methodology for defining degradation is by measuring the structure and composition of a habitat such as: forest canopy percentage cover, tree species richness, species community composition, changes in forest cover (Simula, 2009). Within the bryophyte literature, most delimitations used in tropical studies are discrete classes of varying degradation: undisturbed primary, low disturbance primary, moderate disturbance primary, secondary, isolated trees, plantation, logged (e.g. Drehwald, 2005; Ariyanti et al., 2008; Gradstein & Sporn, 2009). At a landscape, regional or national level indicators used in defining degradation include biomass, carbon stocks, habitat connectivity and fragmentation (Simula, 2009) increasingly by using remote sensing data (Thompson et al., 2013).

Although forest degradation negatively impacts biodiversity overall, the response varies among different taxonomic groups – with recorded decreases ranging from 10% to 90% (Struebig et al., 2013). There is also variation within a taxonomic group, different groups of species will be more sensitive to degradation and there is also variation in response between different geographical

regions (Gradstein & Sporn, 2009). A comparison of studies looking at bryophyte diversity in different levels of forest degradation (Gradstein & Sporn, 2009) found that there were also differences in response between different tropical regions. Some of this variation can be attributed to genuine species reponses, but differences in methodological design and classifications of degradation also have an effect (Holz & Gradstein, 2005; Struebig et al., 2013). For example, many studies have looked at differences between primary and secondary forests, but the definition of secondary forest is a broad one and varies between studies. For bryophytes in particular, variables such as tree architecture (Johansson, 1974) and canopy cover affect distribution but there is usually no discerning between degrees of canopy cover when assigning study areas to the category "secondary forest" (Holz & Gradstein, 2005). This makes comparisons between studies difficult or misleading.

Four different levels of degradation are used in this study: two levels of forest degradation and two levels of non-forest (land deforested for shifting agriculture). A deforested area is defined as an area that has suffered a reduction in canopy cover and a degraded area is a forest that still has canopy cover but has suffered from some form of disturbance (FRA, 2015). A degraded forest can therefore still have canopy cover but "The conversion of forest to other land use or the permanent reduction of the tree canopy cover below the minimum 10 percent threshold."

Table 5.3 Land-use types in degraded forest landscapes.

	Land use	Definition	Level of degradation
Forest	Primary forest		
	Secondary forest	Forests regenerating following previous land-use – agriculture, logging	
	Shade-plantation		
	Old growth		
Non-forest	Young fallow	Former agriculture	
	Monocrop plantation	Usually tree species for harvesting: Eucalyptus, Rubber, Oil Palm	
	Intensively logged		
	Intensively managed agriculture		
	Urban areas		

5.2 Aim

Lowland humid forest is highly threatened and underprotected; can bryophytes be used as indicators of forest integrity?

The aim of this chapter is to determine if the indicator value calculated in Chapter 4 varies between different levels of forest degradation. Concurrently, I will also test if the life-form trait, determined to be useful in characterising species into indicator groups and to be associated with different environmental conditions (in Chapters 3 and 4), also varies between degradation levels. This provides two types of indicators: an indicator metric based on microclimatic preferences and an indicator metric based on species' functional group (i.e. life-form).

5.3 Methodology

It is essential that the data collected is appropriate to the research questions and the analyses that it will be subject to (Crawley, 2005; Sutherland, 2006). As each element of data collected will inform several analyses and have multiple applications - species distribution maps and assessing bryophyte indicator values - the collection methodology must be appropriate to this.

5.3.1 Field Sites

Fieldwork was undertaken in February 2016 in Tsitongambarika Forest (TGK) in the Anosy Region of south-eastern Madagascar (Figure 5.5), a 60 500 ha lowland to mid-altitude humid forest on the Vohimena mountain chain. Ivohibe forest which lies between 90 to 400 m, on Ivohibe Mountain on the North-East of TGK (Iabakoho commune) (Figure 5.5) was chosen as it holds lowland humid forest below 100 m, one of the most threatened tropical forest types due to its desirability for conversion to agriculture with much of the eastern humid lowland forest in Madagascar having been cleared in the last century (Harper et al., 2007), because it is exceptionally rich in plant species and because it contains areas of forest at different degradation levels: intact, secondary and heavily-degraded (mainly due to shifting agriculture) (Razakamalala et al., 2011). Average annual precipitation (1960-1990) for the study area is 1874 mm, with a wet season from November to March (Figure 5.4), and annual average daily temperature range is 24.4˚C (BirdLife International, 2011). Two seasons occur: a dry season from May to November, and a wet season from November to March/April. In 2013 the longest consecutive number of days without precipitation in the city of Fort Dauphin (Tolagnaro) (the nearest weather station to the field site) was 33 (29th August to 30th September). 1000-1100 mm/year of rain was recorded during 60-75 days in 1907-1910 for Fort Dauphin (Renauld & Cardot, 1915).

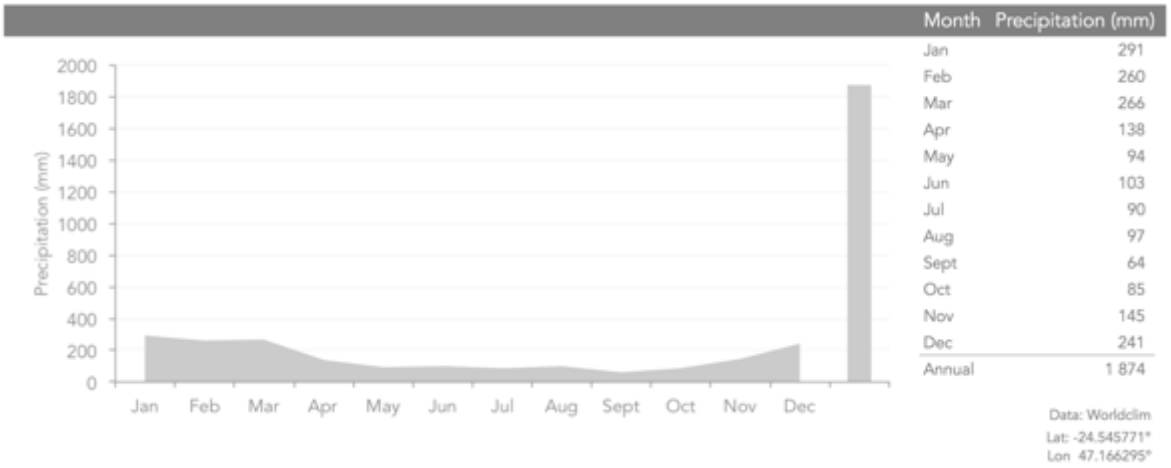


Figure 5.4 Average monthly and total annual precipitation (1960-1990) for Madagascar field study site. Data from WorldClim (www.worldclim.org; Hijmans et al., 2005).

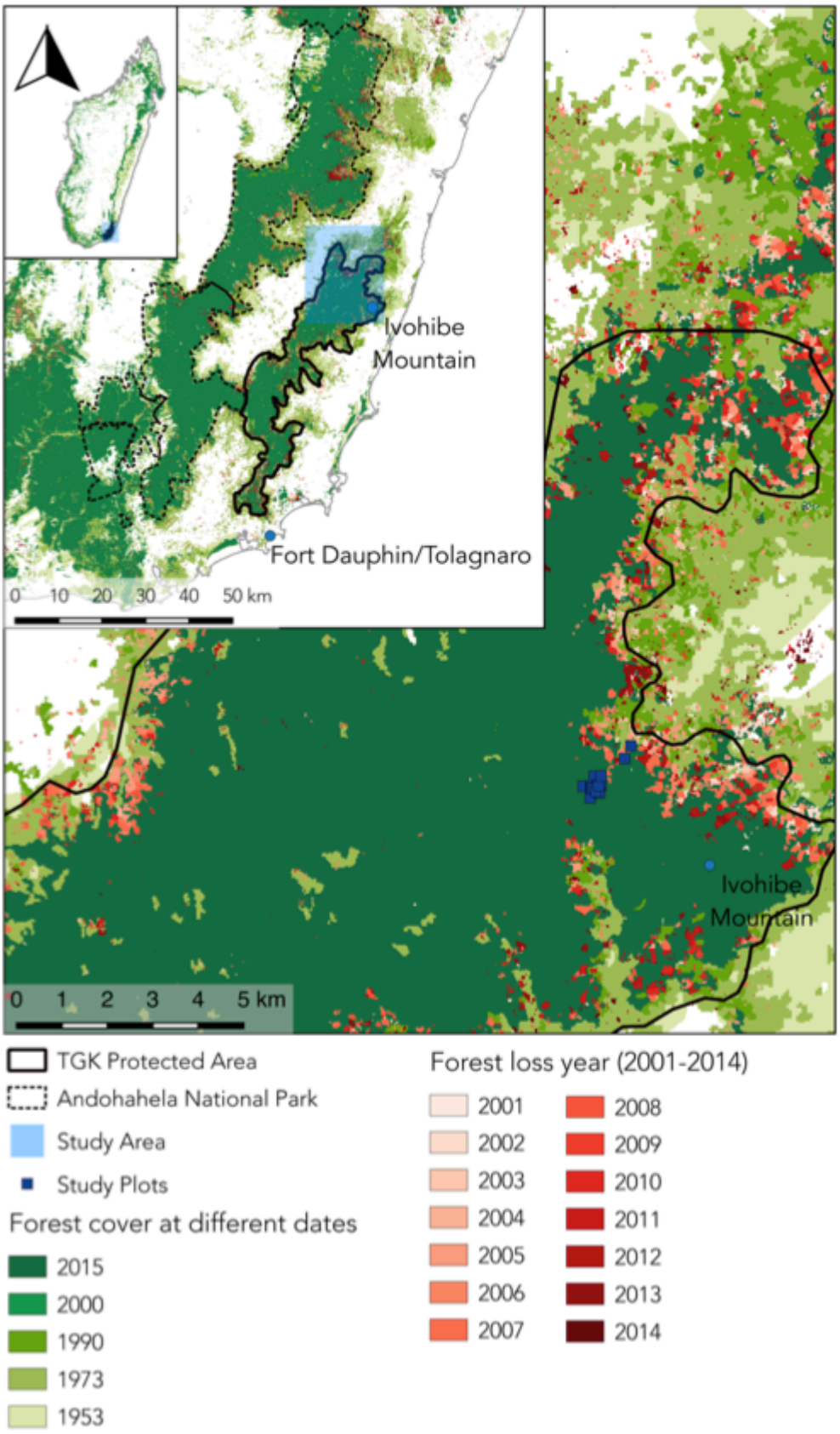


Figure 5.5 Location of Tstitongambarika Forest Protected Area within Madagascar and location of Ivohibe study site. Forest loss data from Hansen et al. (2013).

5.3.2 Sampling Strategy

The aim of fieldwork was to determine which bryophyte taxa are specific to which habitat quality types and how each species responds to different environmental variables (particularly forest quality, insolation and relative humidity). When studying bryophytes, two sampling scales exist: the substrate level and the habitat level. Sample size (bryophyte quadrats and habitat plots) was chosen based on the trade-off between capturing a representative sample of bryophyte species and time available.

Pilot field surveys were carried out in Madagascar in March to April 2014 to test the sampling and collection methodology and adapt them accordingly. Bryophytes are known to be sensitive to atmospheric pollution and used as indicators for this purpose (e.g. Figueira et al., 2009). In order to remove the effect of pollution on the bryoflora sampled, study sites were located far enough away from pollution sources (urban areas, factories and heavily used roads) so that atmospheric pollution effects are negligible; the closest pollution source is the town of Fort Dauphin/Tolagnaro 60 km away. Aditionally, elevation is known to have a significant effect on bryophyte distribution and abundance and so plots were all located at similar altitudes to each other to avoid confounding effects.

5.3.2.1 Habitat sampling

A systemized sampling methodology was used rather than random sampling as a representative sample of the bryoflora was needed in each forest degradation type. Randomised sampling was avoided due to the fact that as vegetation is naturally aggregated in distribution it could lead to one bryophyte family being sampled a disproportionate number of times (Daubenmire, 1968). Initially it was planned to sample 10 X 10m forest plots placed along six transects of 100m with each plot in a different forest degradation type. This sampling strategy and the location of transects was chosen after visiting forest areas in March and April 2014.

However, when returning to the field in January 2016, the forest area used in pilot studies had suffered significant deforestation and the survey area had to be relocated to an area nearby (previously selected from satellite images as a backup to the original site if the latter became degraded or inaccessible). As such, only three 100 m transects with three 10 X 10m plots each (Figure 5.6) were undertaken and a further eight individual 10 X 10m plots were placed in each habitat degradation type (Figure 5.7). In total there were six plots in undisturbed forest, six in degraded forest and five in cleared-forest (shifting agirciculture, two of these under current agriculture). This did not affect the sampling effort, just the method in which sampling was made.

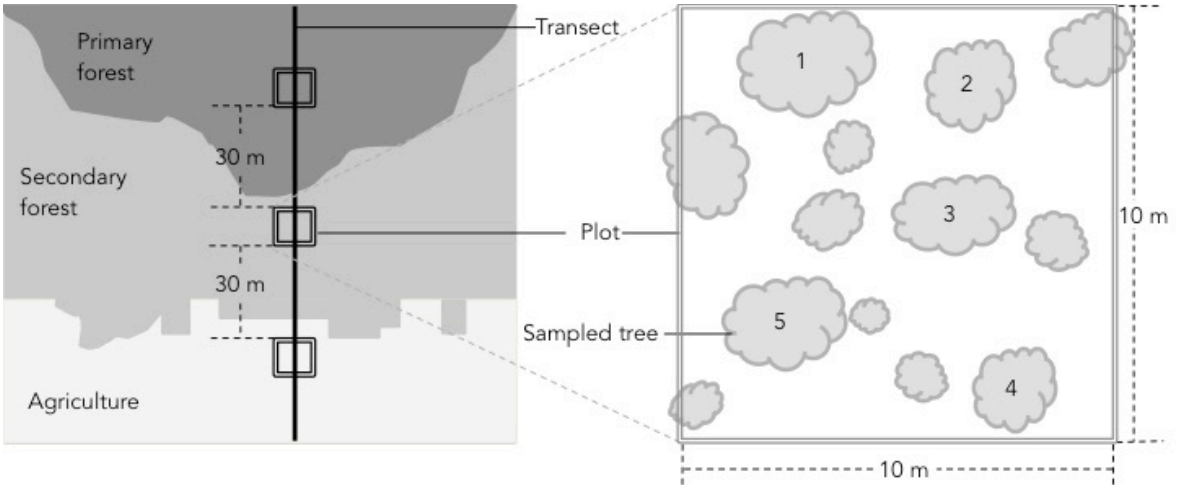


Figure 5.6 Systematic sampling design with plots at 30 m intervals along a transect, each plots in a different degradation type.

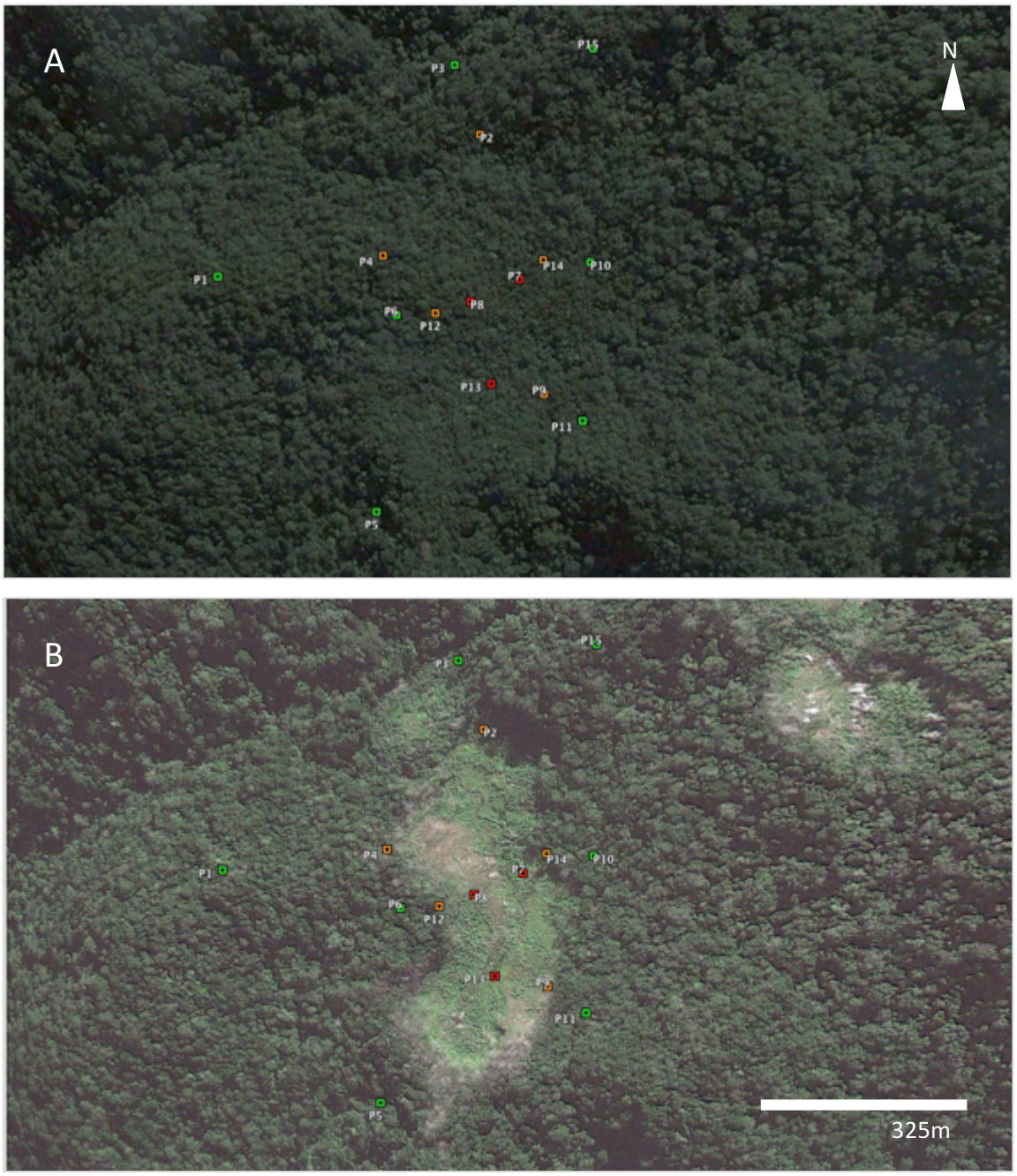


Figure 5.7 Satellite imagery of study area taken at two different dates showing the area before and after deforestation, A) 14th June 2003; B) 12th June 2009. Also shown is location of field plots: green indicates plots in primary forest, orange plots in secondary forest and red plots in degraded forest. Source: Google Earth Pro v 7.1.5.1557. Iabokoho, Madagascar. S24.5484° E47.1636° Eye alt 763m. Aquired 29 April, 2016.

Variables

In each plot, geographic location, altitude (metres above sea level – WGS84 ellipsoid), aspect, slope, habitat type, canopy cover, shade index, canopy height, ground cover, bare ground % cover, leaf litter % cover, stem density, insolation and humidity were recorded. Insolation was recorded as lux which is a measure of illumination, not insolation, but can then be converted to insolation (Watts per area: Wm^{-2}) by multiplying by 0.00402 (Thimijan & Heins, 1983). Both insolation and humidity (relative humidity) were recorded at each site with an environment meter (Lutron LM-8000A). Measurements were taken at the same time of day in each plot to avoid variation due to time of day. The meter was placed in the middle of each plot at 50 cm above

ground. This height was chosen due to the fact that most bryophytes are found below 1m above the forest floor (following modification of the method used in (Rice & Schneider, 2004). The meter was used over a period of 10 minutes where the minimum and maximum of each variable were recorded and an average of the two calculated.

Canopy cover was measured by taking a photograph of the canopy from 1m above the ground and calculating the percentage of sky visible in photographic software (ImageJ, see Figure 5.31, Appendix A5.4, p. **Error! Bookmark not defined.**). Shade index was assessed visually and classified into one of seven categories (see Table 5.13, Appendix A5.2, p. 319) as used in the UK bryophyte survey (BRECOG, 2011). Canopy cover and shade index were recorded as another measure of light availability in the habitat; as none of the plots were on a slope, it can be assumed that irradiance incidence is equal across sites and therefore canopy cpver and amount of shade can be used. Ground cover was also measured using a photograph of an area of ground representative of the plot's ground cover and processed similarly to canopy cover (see Figure 5.31, Appendix A5.4, p. 321).

Degradation

The degradation of each site as a measure of forest integrity was recorded by classifying plots into four degradation and land-use classes (Figure 5.8 & Figure 5.9) using the criteria shown in Table 5.4. These criteria were decided upon based on the literature (Drehwald, 2005; Gradstein & Sporn, 2009; Simula, 2009; Struebig et al., 2013). Degradation in the study area is a result of shifting agriculture, with some small-level selective timber extraction. Aditionally, any disturbances, both anthropogenic and natural, were recorded using the categories in Table 5.5. The number of logs, stumps and dead standing trees in each site were recorded as measures of disturbance as they are evidence of past disturbance such as selective logging. The absence of any disturbance was also recorded. Historical data was used to confirm land-use changes using remote sensing (Hansen et al., 2013) and historical satellite images, (Figure 5.5 and Figure 5.7, respectively) as well as local land records and information from forest guides and the local community forest management association (CoBa). This data and information was used to confirm that currently degraded forest did indeed used to be forest – this is in order to be make valid conclusions on the changes of the forest bryoflora (Crawley, 2005).

Table 5.4 Forest degradation and land-use classes used in this study and the criteria used to define them.

Degradation indicator	Forest		Non-forest	
	Primary undisturbed to slightly degraded (PU)	Primary moderately to considerably degraded (PD)	Agriculture – former (Af)	Agriculture – current (A)
Canopy cover (%)	High (>85%)	Medium (<75%)	Low (<25%)	None
Ground vegetation cover (%)	Low (0-25%)	Medium (>25% - 50%)	High (> 50%)	Low
Leaf litter (%)	High (>75%)	Medium 25-75%)	Low (<25%)	None
Bare ground (%)	Low (0-10%)	Medium (>10% - 25%)	High (>25%)	High (>50%)
Number of stumps	Very few	Some to many	Depends on land-use	Depends on land-use
Number of logs	Very few	Some to many	Depends on	Depends on

			land-use	land-use

Table 5.5 Disturbance types recorded in this study and definition.

Disturbance type	Definition
Logging	selective, clear-cut, slash-and-burn, firewood
Domesticated animals	goats, pigs, cows
Settlement	current or abandoned
Agriculture	current – open pasture, agroforestry, crops, open tillage or abandoned
Fire	man-made or natural
Natural tree fall	
Tarmac road, dirt road, trail	
None	

Using the disturbance data collected, a composite measure of degradation was calculated (Cardoso et al., 2013) to be used in subsequent analyses. The advantage of using a continuous variable rather than discrete categories is that it can reflect the reality of disturbance more accurately (Cardoso et al., 2013; Struegib et al., 2013) and reduces ambiguity associated with assigning categories. A continuous measure can also avoid pseudoreplication by allowing sites to be viewed as occupying a gradient of degradation rather than discrete degradation classes (Ramage et al., 2013). As this study is concerned with human-induced disturbance, natural tree fall is not included in the index creation. Following a combination of the methods in Cardoso et al. (2013) and Mitchell & Schaab (2008), a degradation index (DI) was calculated as:

$$DI = \frac{\sum\ (logs, stumps, standing\ trees, total\ disturbance\ types) \quad * bare\ ground\ cover}{(canopy\ cover + 1)}$$

Bare ground was weighted more heavily as it is highly indicative of disturbance.

Both degradation metrics (categorical and continuous) are used in subsequent analyses. Using categorical degradation will allow for comparison with results from other bryophyte studies.

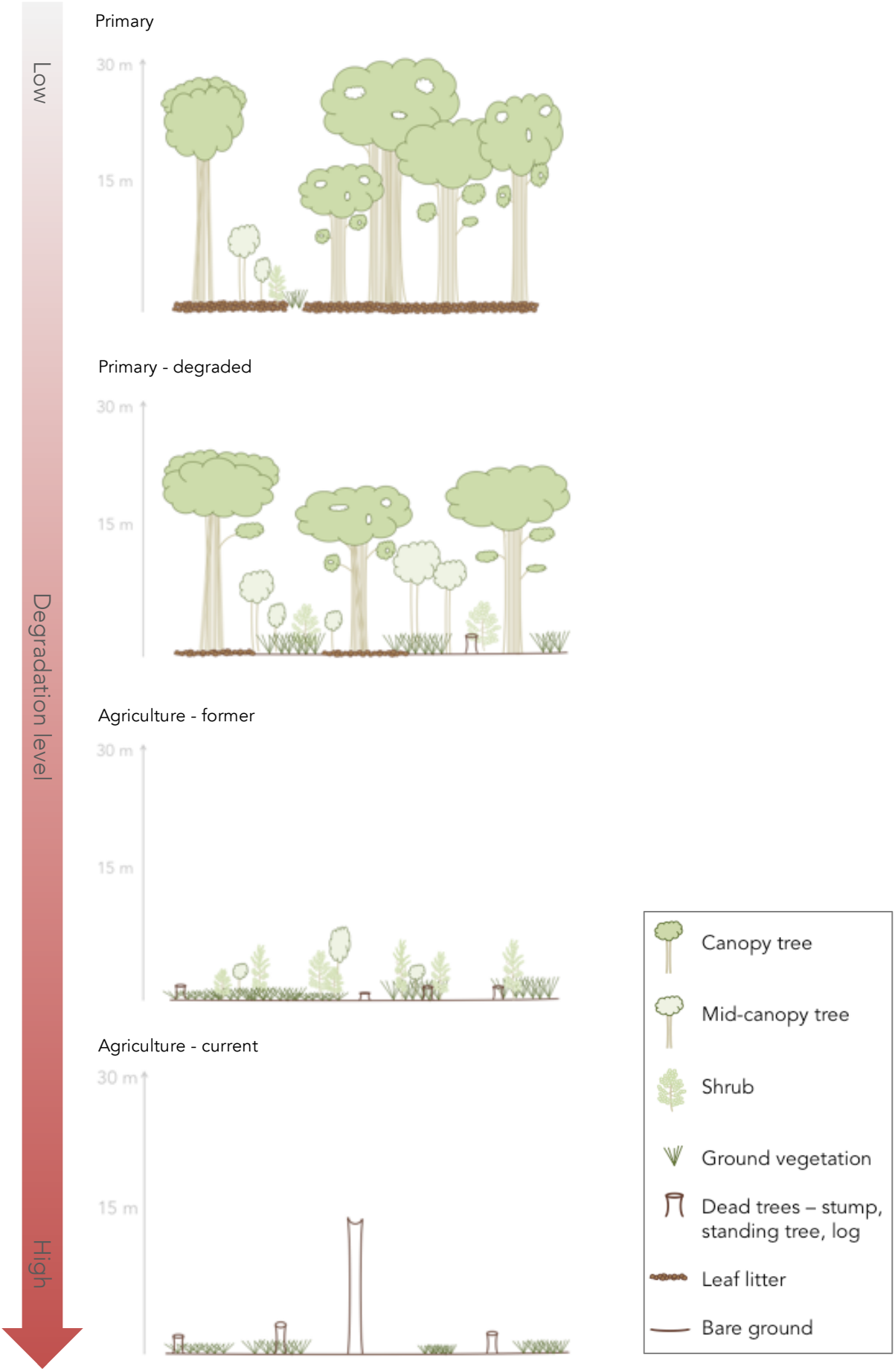


Figure 5.8. Schematic representation of forest degradation and agriculture classes delimited in this study.

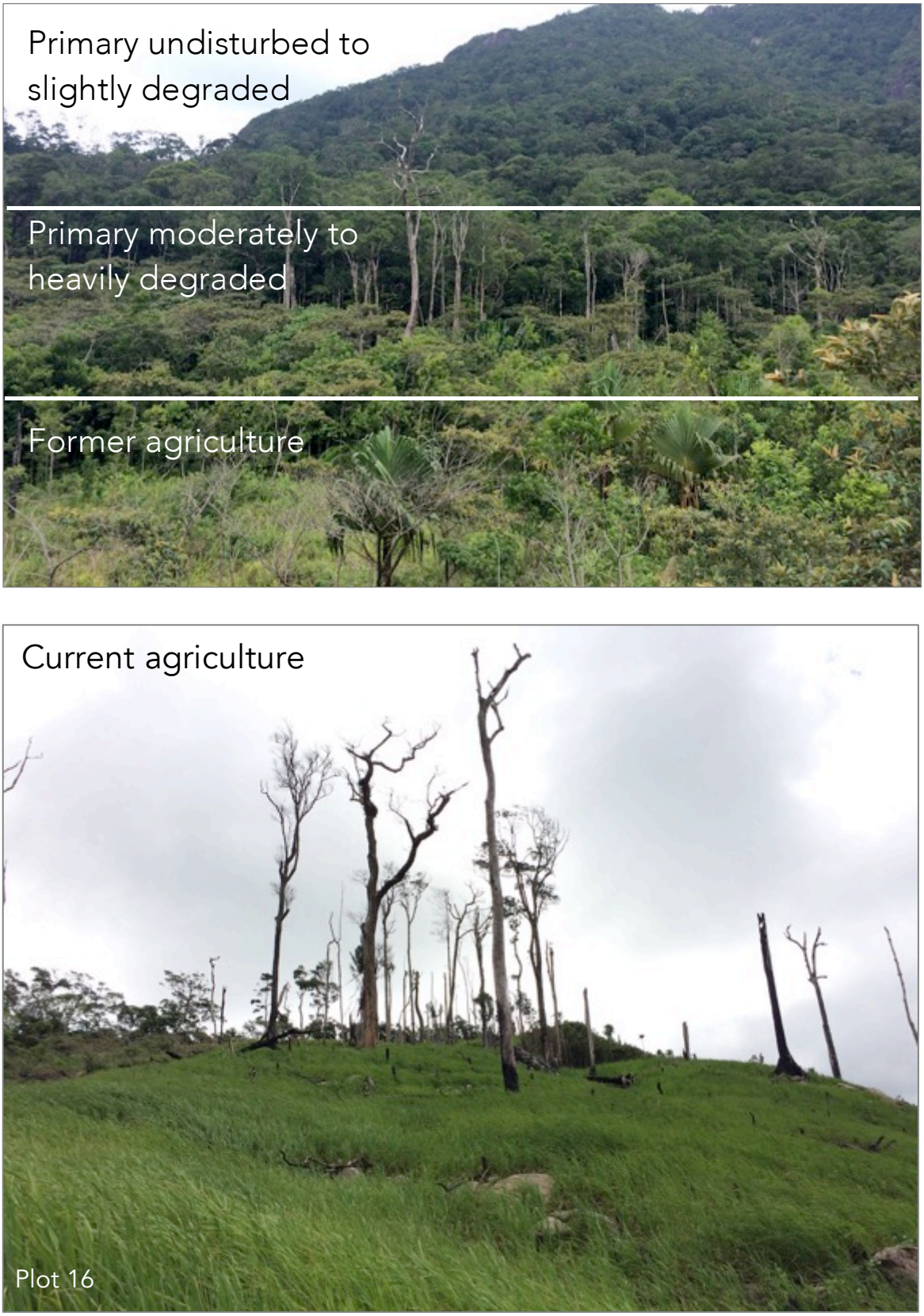


Figure 5.9 Photographs of study area showing degraded forest types and agriculture sampled. Source: Sarah Stow.

5.3.2.2 Bryoflora sampling

As it is not feasible to sample a whole forest, a representative sample must be taken allowing extrapolation to areas not sampled (Daubenmire, 1968; Mueller-Dombois & Ellenberg, 2003). Ideally, plots sampled need to be homogenous which, in a heterogenous habitat, as in the case of the forest habitat of bryophytes, will lead to smaller plot sizes being used. This is so that each plot has the same environmental characteristics throughout, enabling a direct relatation of the vegetation to the environment (Daubenmire, 1968). Three microhabitats per plot were sampled: epiphyte, saxicolous and terricolous, in order to have a more complete idea of the bryoflora. The epiphyllous microhabitat was not sampled as only one epiphyll was found in the study sites.

For bryophyte sampling, single-scale sampling with quadrats is used and varying quadrat sizes have been applied: Daubenmire (1968) suggests 0.25 m^2 for small mosses of drier regions and 0.1 m^2 for larger mosses; Barkman (1969) states that 0.25 m^2 quadrats capture sufficient cryptogam epiphyte diversity to infer community relationships but that 0.1 m^2 is too small; Gradstein et al. (2003) suggest 0.6 m^2 (30 X 20 cm) quadrats and in UK habitat surveys (BRECOG, 2011) 1.25 m^2 (25 X 50 cm) quadrats are used. During pilot studies quadrats of different sizes were used, 25 X 50 cm and 10 X 25 cm. It was decided to use the smaller quadrat on three different zones of the trunk as this seemed to capture the greatest diversity whilst maximising time available. This sampling unit is not strictly a *quadrat* as it is rectangular in shape (Daubenmire, 1968) but for simplicity it shall be referred to as such. The rectangular shape is better suited than a square as it has been shown to capture a larger proportion of the flora meaning less sampling plots are needed (Daubenmire, 1968) and it can also fit trees of varying girths. Barkman (1969) discusses the use of varying quadrat sizes for different vegetation types but as this study was only looking at humid lowland forest I considered that it was not necessary to have different quadrat sizes. A 10 X 25 cm quadrat (0.25 m^2) was therefore used (see Figure 5.29, Appendix A5.3, p. 321).

When sampling, it was ensured that not all of one species was removed (about 10% were collected) and that no bare trunk was exposed. Although in some studies the whole quadrat is collected and identified afterwards allowing all species to be recorded (Medina et al., 2014), I thought it inappropriate in this study as it would likely lead to the indiscriminate collection of rare and potentially threatened species. As the conservation status of most Malagasy bryophytes is unknown, it is always best to act with caution when collecting.

Variables

A reliable index is preferable to an unreliable count (Greenwood & Robinson, 2006). As already mentioned, it would be unfeasible in the time available to record the number of each species present (as well as the fact that there is no accepted definition of "individual" in bryophytes). As such, percentage cover was used as an index of abundance. Overall hornwort, liverwort and moss percentage cover was estimated within the quadrat whilst in the field. When it was not possible to distinguish a patch it was recorded as "microscopic epiphyte" and a sample taken – this were usually cyanobacteria, algae or protonema (Cordova & Del Castillo, 2001). To obtain a reliable estimate, accuracy and precision were ensured (vital when recording an index (Greenwood & Robinson, 2006) by having a 2 X 2.5 cm grid inside the quadrat (Figure 5.29) as each grid square corresponds to 5% cover. It was not possible to record percentage cover of different species in each quadrat because most species could not be identified in the field. For an ecological study this

would be an important measure to have, but as this study is looking at presence or absence of species I did not consider the lack of information on individual species cover as a drawback. Also, species frequency is considered a more objective measure than percentage cover and they have been found to be directly proportional in studies of epiphytic cryptogams (Barkman, 1969). For an index of abundance, the number of times a species was collected was used as a measure of abundance, with the assumption that all species on the microhabitat were collected – this is an acceptable assumption as sampling strategy was chosen to maximise species diversity capture (see above). Presence or absence of fertile species for each bryophyte phyla was recorded. Fertility of bryophyte species is an indication of environmental quality: when quality is low then bryophytes will not produce sporophytes (Rao, 1982).

5.3.2.3 Microhabitat sampling

Trees

As trees are heterogenous in shape, size and surface a clear sampling strategy was defined to allow sampling of bryophytes in environmental homogenous quadrats. As microhabitats on a tree trunk vary more latitudinally than longitudinally and forest bryophyte communities have been found to be more similar at a particular trunk section across trees than the community within a tree itself (Pardow & Lakatos, 2013), an elongate quadrat will capture more diversity. This is explained by the environmental conditions being different at different heights of the tree e.g. the base of the tree has higher humidity than areas higher up, especially in areas that get flooded, or be more exposed to dust in arid areas as well as different temperatures along the trunk (Barkman, 1969; Song et al., 2015).

As such, the sampling quadrat placement needs to be consistent. As sampling the whole length of the tree would take time not available a sub-division of the tree was studied (Johansson, 1974). Following observation of trees it was decided to sample the trunk up to 2 m above ground by placing quadrats in three places: between 0-50 cm, between 0.5-1 m and above 1 m. The greatest diversity in epiphytes on the lower trunk (below 5 m) seems to be from the ground up to 1 m. This allows a larger area to be sampled but without being as time-consuming as sampling the whole trunk fully. Other studies (Drehwald, 2005; Pardow et al., 2012) have sampled the whole length of the trunk, from 0 to 200 cm, and although this allows a more complete capture of the epiphytic bryoflora, it is time-consuming and the purpose is not to characterise the bryophytes flora of each forest type but to discover which species are specific to which forest quality type and so sampling needs to be as environmentally homogenous as possible. Tixier (1966) divided into 5 levels: ground, 50 cm, 1 m, 1.5 m, 2 m. Also, a height of 60 cm above the ground allows for close observation and more accurate recording of the bryoflora as it captures the transition between basal and upper epiphytes – this relates to the elongate quadrat reasoning (Johansson, 1974). (Kürschner et al., 1999) studied the trunk between 1-3 m (sometimes 5). Tixier (1966) observed the greatest species richness of bryophytes in montane and low altitude rainforest on the forest floor, but in mid-altitude rainforest it was highest at mid-trunk (1 m). Johansson (1974) states that the sampling area size should vary according to the tree size to allow comparison and Gradstein (2003) suggests following the Johansson tree height brackets.

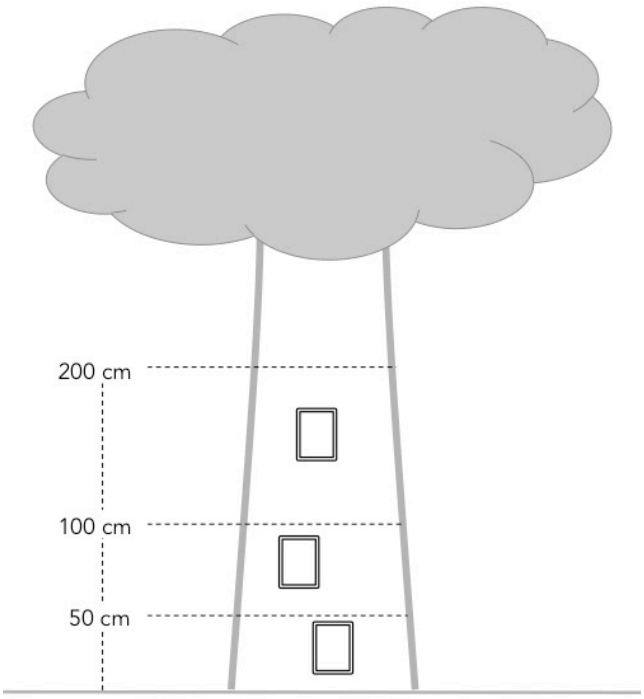


Figure 5.10 Quadrat placement for epiphytic bryophytes with sampling in base (between 0-50 cm), lower trunk (between 50 and 100 cm) and mid-trunk (between 100 and 200 cm).

Only the trunk itself was sampled as the branches, upper trunk (above 2 m) and canopy are less sensitive to changes in habitat as well as having a different bryoflora community (Drehwald, 2005). It has also been shown that understorey species are more desiccation sensitive than canopy species (Pardow & Lakatos, 2013) suggesting that they are better indicators of changes in their habitat. This can be explained by the fact that higher up the trunk the higher the insolation and lower the relative humidity (Song et al., 2015) meaning that there is less desiccation in the lower trunk, as well as on ground-level microhabitats (e.g. rocks and tree roots).

Species-area curves for bryophytes in neo-tropical forests indicate that five trees capture about 80% of the bryophyte diversity (Gradstein et al., 2003). Although randomisation is necessary for subsequent statistical analyses, the only way to choose trees in a truly random manner is to number all the trees in the study area and then randomly generate numbers and the corresponding trees (Crawley, 2005), which is not feasible time-wise or logistically in this case. As a compromise to true randomisation, five trees in each site were sampled (down from 10 in the pilot studies). It was always attempted to sample mature trees with a DBH greater than 25 cm that were representative of the habitat's epiphyte bryoflora in order to capture as much bryophyte diversity as possible (modification of the UK Bryophytes Habitat Survey method (BRECOG, 2011) and (Drehwald, 2005; Pardow & Lakatos, 2013). This was done on the basis of knowledge and observation.

It was always attempted to sample trunks with an inclination of 90^{o} (i.e. completely vertical) as significant inclination can affect the distribution of species as well as the species composition (Barkman, 1969). Eleven had a slight inclination of ±5^{o} and the rest were 90^{o}. Four trees had buttresses (*Canarium* sp. and *Mammea* sp.) and one had stilt roots (*Uapaca* sp.); in these cases a micro-quadrat was made on an area at the trunk just above the buttress or stilt roots and another quadrat on the buttress or stilt root itself. Sampling crevices was avoided as this is another tree

microhabitat and would not allow for comparison between trees and sites if some quadrats were placed on crevices and others not.

Variables

Since a forest habitat is particularly heterogeneous for bryophytes due to the variety of microhabitats available to them, even within a tree, it was important to record characteristics of each tree sampled: tree species, slope of tree at quadrat, aspect of sampling quadrat, girth at 1.3 m and tree architecture which was classified into one of 8 categories (Figure 5.11). Slope of the tree provides an indication of water run-off (Giordani et al., 2014) and so this was recorded. Tree architecture is important for epiphyte distribution as it affects the amount of sunlight and precipitation that reach the lower trunk as well as the amount of surface area available for colonisation (see Johansson, 1974). I had planned to place a quadrat on the south and north aspect of the tree, but after the pilot studies, the reduced size of the quadrat made this unfeasible but also from observations it was found that there was no clear difference between the aspects.

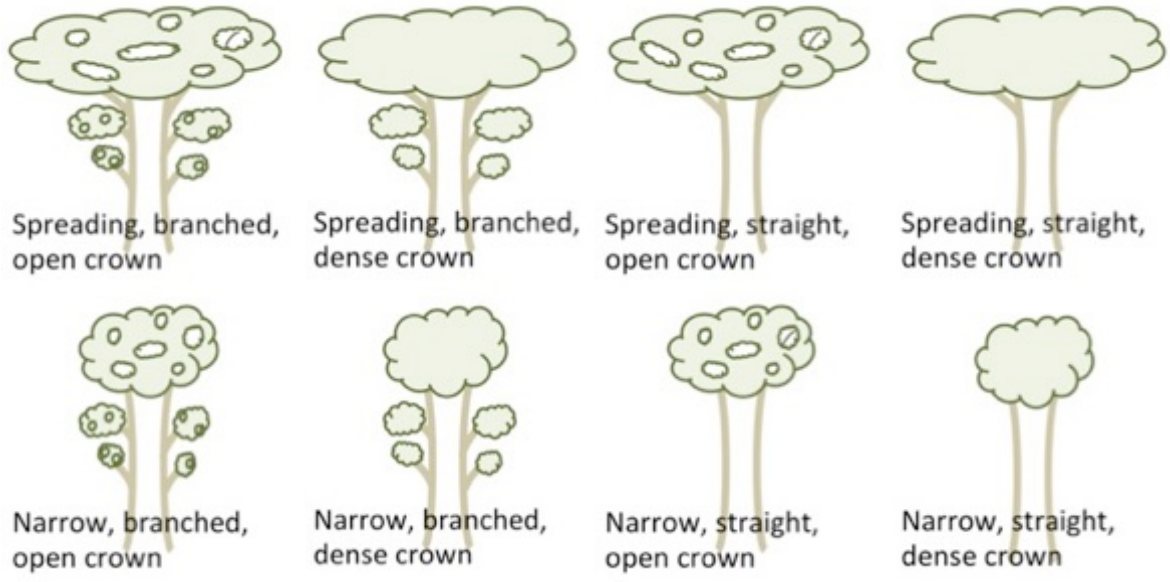


Figure 5.11 Schematic representation of overall tree architecture categories

Bryophytes' reliance on microhabitats means that bark type plays a key role in determining bryophyte distribution (Hedenäs et al., 2004; Bates, 2009). Bark type was recorded and classified into six qualitative categories (adapted from Frahm, 2003; Hedenäs et al., 2004) based on the bark's hardness and texture: soft flaky, soft smooth, soft rough, firm flaky, firm smooth and firm rough. Although more objective methods exist to quantify bark roughness (Glitzenstein & Harcombe, 1979; Rosabal et al., 2013), the method trialled during pilot studies (see Figure 5.28, p. 320, Appendix 5) was deemed too time consuming for the level of detail that is required in this study.

For many cryptogam species, particularly lichens, bark pH determines which tree species they will colonise (Barkman, 1969). In temperate areas bark pH plays an important role in determining bryophyte distribution (Hedenäs et al., 2004) but is not so important in tropical regions due to the lack of basic barks in the tropics (Frahm, 2003) and Rosabal *et al.* (2013) report pHs of between 4.6-5.8. As such, pH was not measured in this study though samples were taken for future ecological analyses that are beyond the scope of this study.

In order to have an understanding of the overall epiphyte flora of the tree, data on other epiphytes was also recorded. In vascular vegetation studies, sward height is often measured as an indication of succession and this was adapted here by measuring epiphyte layer thickness. A wire probe was made with 1 mm markings and pushed into the deepest and median depth area of the quadrat. Percentage cover of bare trunk and other epiphyte groups - lichen, fungi, ferns and vascular plants - was recorded in the same manner as for bryophytes. Whether or not these were fertile and the percentage cover of fertile plants was also recorded.

Other microhabitats

Stump tops were sampled (some studies have observed differences in the epiphyte flora due to the varying pH in the outer and inner rings (Barkman, 1969)). For rock and soil microhabitat variables collected on these substrates were limited to rock type, soil type, soil depth, micro-quadrat aspect and slope.

In addition to the targeted microhabitats (tree trunk, soil, rock and dead ligneous vegetation), further microhabitats were also included, although these will not be used in analyses for this study as they are highly underrepresented (see Table 5.8, p. 297). They were sampled in order to be able to create a future checklist of the bryophytes of Tsitongambarika National Park.

5.3.3 Statistical analyses

Environmental parameters and bryophyte richness and abundance were compared across habitat degradation types using ANOVAs and post-hoc Tukey HSD tests. As the sampling strategy was nested (microquadrats within microhabitat within a plot) linear mixed effect models (LME) were used to account for this pseudoreplication (Quinn & Keough, 2002; Crawley, 2013; Ramage et al., 2013). Site was fitted as a random effect and microhabitat and degradation level as fixed effects within the LME. Percentage cover data was arcsine root-transformed prior to analyses in order to meet normality asusmptions. All statistical analyses were carried out in statistical software R (version 3.1.1) and the lme4 package (Bates et al., 2015) was used for LME models.

5.4 Results

5.4.1 Degradation characterisation

Insolation was strongly related to the canopy cover at a plot (r^2=0.921, *df*=15, p-value<0.001) but humidity much less so (r^2=0.159, *df*=15, p-value>0.05) (Figure 5.12 a). However, it shows that insolation increases with decreasing canopy cover. There was a clear increase in insolation along the degradation index (r^2=0.769, *df*=15, p-value<0.001) (Figure 5.12 b) and a slight decrease in relative humidity, though not significant (r^2=0.364, *df*=15, p-value>0.05).

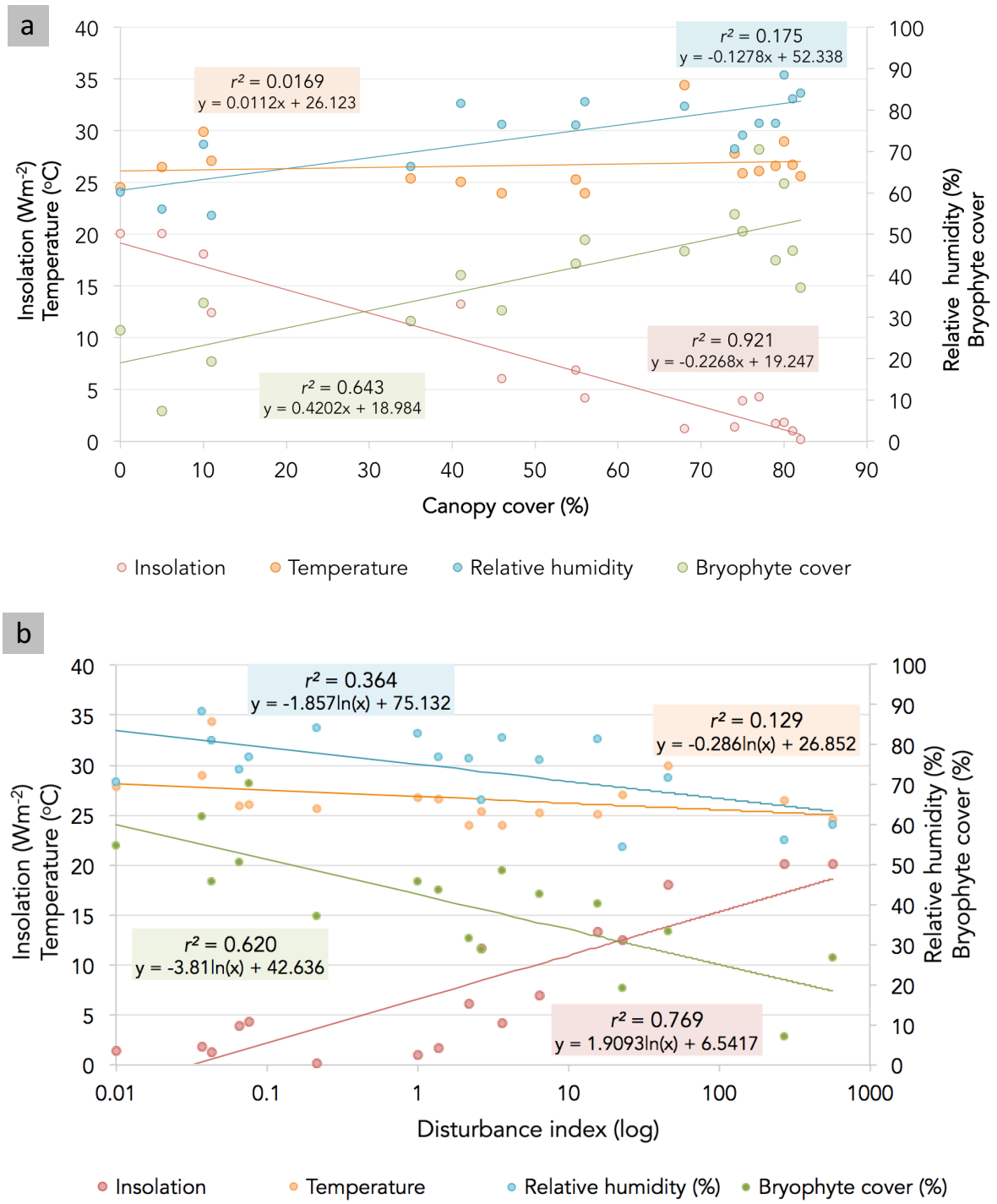


Figure 5.12 Insolation (Watts per m^2), temperature (^oC), relative humidity (%) and bryophyte cover (%) in a) different canopy covers (%); b) along the disturbance index. When humidity was recorded in two plots it was raining and so values are not comparable to those of other plots.

Undisturbed and moderately disturbed study plots vary significantly in their canopy cover and insolation but are not significantly different in terms of the other habitat variables (Table 5.6). Similarly, heavily disturbed and agriculture plots are similar, but vary significantly in bare ground cover and stem density. Undisturbed forest plots vary significantly from degraded plots in most habitat variables, except microhabitat number, bare ground cover and stem density (Table 5.6).

All degradation types surveyed are found within an altitude range of 185 to 285m (Table 5.7), all within the altitude range characterising lowland humid forest. Plots that were classed as "agriculture – former (Af)" are characteristic of Malagasy "savoka" which are secondary thickets that occur once cultivated land (created from clear-cutting forest) is abandoned (Irwin et al., 2010).

Table 5.6 Mean values of habitat variables in different forest degradation and land-use classes based on sampling of habitat variables at the study plots. Significantly different values between degradation types indicated by different letters (ANOVA and Tukey HSD test p<0.05).

	Primary undisturbed to slightly degraded		Primary moderately degraded		Agriculture - former		Agriculture - current	
	Mean	1SE	Mean	1SE	Mean	1SE	Mean	1SE
Relative humidity*	56.1	3.88	55.2	7.40	49.5	6.14	60.2	2.04
Insolation	1.66 [a]	1.29	6.82 [b]	1.30	15.5 [c]	1.84	19.9 [c]	2.25
Canopy height (m)	22.5 [a]	2.85	18.6 [a]	2.85	4.50 [b]	1.00	0 [b]	0.00
Canopy cover (%)	77.5 [a]	4.66	54.8 [b]	4.66	12.3 [c]	6.59	2.50 [c]	8.01
Stem density	15.0 [a]	3.19	20.3 [a]	3.19	15.7 [a]	4.51	0 [c]	0.00
Ground vegetation cover (%)	22.0 [a]	18.7	31.7 [a]	17.1	47.0 [b]	11.2	25.0 [a]	13.7
Leaf litter (%)	70.5 [a]	8.60	62.5 [a]	8.59	29.7 [b]	12.2	0 [c]	0.00
Bare ground (%)	9.17 [a]	5.44	5.83 [a]	5.42	23.3 [a]	7.69	65.0 [b]	9.42
Number of stumps	1.83 [a]	2.18	3.66 [ab]	2.18	5.00 [b]	3.08	12.5 [b]	3.78
Number of logs	2.00 [a]	1.16	6.33 [ab]	1.15	8.67 [b]	1.64	3.50 [ab]	2.00
Number of microhabitats	6.66 [a]	1.69	7.50 [a]	1.69	8.67 [a]	1.89	5.00 [a]	1.46

* Relative humidity values cannot be used: when humidity was recorded in two plots it was raining and so values are not comparable to those of other plots.

Table 5.7 Mean, minimum and maximum altitude for the different forest degradation types

Altitude (m)	Primary	Secondary	Degraded	Agriculture
Mean	266	248	246	231
Minimum	236	223	244	185
Maximum	289	259	248	245

5.4.2 Bryoflora

In total, 384 micro-quadrats were studied in 21 microhabitats, within 17 forest and non-forest plots (Table 5.8 & Table 5.9). In addition to the targeted microhabitats (tree trunk, soil, rock and dead ligneous vegetation), further microhabitats were also included, although these will not be used in analyses for this study (Table 5.8 & Table 5.9). In total, 898 specimens were collected and identification to species level for some specimens is still underway, although all specimens have been identified to genus level – 51 genera in 31 families, 19% and 34% of all Malagasy genera and families, respectively. No bryophyte flora exists for Madagascar, which hinders identification to species level; typically various floras need to be consulted which prolonged identification times beyond the scope of this study. Some identification was achieved with the help of expert bryologists (namely the Calymperaceae and Orthotrichaceae families) and in order to identify all

species, specimen samples will need to be sent to experts for identification or confirmation. Analyses in this chapter are at the genus and family level, which ties in with the results from Chapter 4 where indicator values were assigned to genera and family.

Table 5.8. Microhabitats sampled in this study per degradation and land-use class. The unbalanced number of microhabitats both within and between different degradation classes is due to the lower number of those microhabitats present. Dead ligneous vegetation includes logs, stumps and dead standing trees.

Main microhabitat	Primary undisturbed	Primary degraded	Agriculture - former	Agriculture - current
Epiphyte on trunk base (0-0.5 m above ground)	30	30	8	6
Epiphyte on tree trunk (between 0.5-1 m above ground)	30	30	8	6
Epiphyte on tree trunk (between 1-2 m above ground)	30	30	8	6
On mineral soil	6	6	3	3
On soil with decaying vegetation	6	6	3	3
On rock	12	12	3	7
Decaying ligneous vegetation	15	45	17	15
Further quadrats not included in analyses of current study				
Recently fallen tree or branch (not decaying)		1	4	
On exposed tree root	6			
In tree crevice 60 cm above ground	1			
On tree buttress	4			
On a liana	2	1		
Epiphyllous		1		
On termite mound - soil	1			

Gamiella and *Glossadelphus* were found only in forest with very little degradation and three further genera were found only in primary forest, *Acanthorrhynchium, Neckera, Pyrrhobryum* and *Hookeria* (Figure 5.13). Only one genus was only found in non-forest plots, *Philonotis*, but there were some associated only with non-forest and heavily degraded forest: *Dicranella, Eropodium, Funaria, Phyllodon, Pohlia* and *Trichosteleum.*

Table 5.9 Microhabitats sampled in this study per plot, with disturbance index value (to 3 significant figures) and degradation class of each plot. The unbalanced number of microhabitats is due to the lower number of those microhabitats present. Dead ligneous vegetation includes logs, stumps and dead standing trees.

Plot number	**P5**	**P15**	**P1**	**P10**	**P6**	**P11**	**P3**	**P2**	**P14**	**P12**	**P9**	**P4**	**P13**	**P8**	**P7**	**P17**	**P16**
Degradation class	**PU**	**PU**	**PU**	**PU**	**PD**	**PU**	**PU**	**PD***	**PD***	**PD***	**PD***	**PD***	**Af**	**Af**	**Af**	**A**	**A**
Degradation index value	0.00	0.037	0.043	0.066	0.077	0.22	1.00	1.38	2.17	2.67	3.67	6.48	15.6	22.9	45.8	272	567
Main microhabitat																	
Epiphyte on trunk base (0-0.5 m above ground)	5	5	5	5	5	5	5	5	5	5	5	5	4	2	5	1	1
Epiphyte on mid tree trunk (between 0.5-1 m above ground)	5	5	5	5	5	5	5	5	5	5	5	5	4	2	5	1	1
Epiphyte on upper tree trunk (between 1-2 m above ground)	5	5	5	5	5	5	5	5	5	5	5	5	4	2	5	1	1
On mineral soil	1	1	1	1	1	1	1	1	1	1	1	1	1	1	2	1	1
On soil with decaying vegetation	1	2	1		1	1	1	1	1	1	1	1	1		3	1	1
On rock	1	4	1	1	1	4	1	2	1	3	3	2	1	2	1	3	3
Decaying ligneous vegetation	1	6	2	11	8	8	2	4	3	7	2	6	2	6	5	12	7
Further microquadrats not included in analyses of current study																	
Recently fallen tree or branch (not decaying)		1	4														
On exposed tree root	3	2		1													
In tree crevice 60 cm above ground						1											
On tree buttress		3				1											
On a liana		1				1		1									
Epiphyllous											1						
On termite mound - soil		1															

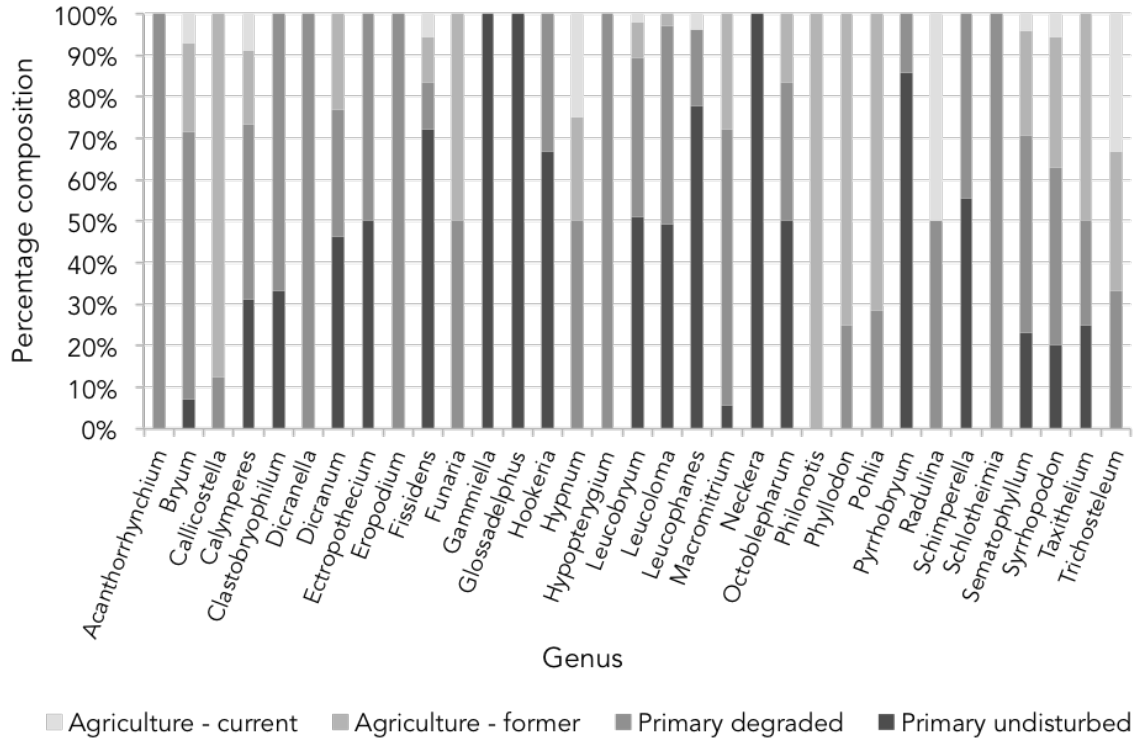


Figure 5.13 Distribution of moss genera in each degradation and land-use class.

5.4.2.1 Taxon richness

Primary forest that is moderately degraded had the highest genus richness (25) whilst current agriculture plots, unsurprisingly, had the lowest (10) (Figure 5.14 a). Liverwort genus richness decreases steadily from undisturbed primary plots to current agriculture plots (Figure 5.14 a). When using the disturbance index, these trends were not evident as no clear pattern in genus richness could be seen in mosses as there is (Figure 5.14 b). The highest moss genus richness (14) was found in a moderately disturbed primary plot but the second highest (13) was found in both a site with very low degradation (P15) and one with the third highest degradation value, a former agriculture plot (P7). There appeared to be an increase in moss genus richness from slightly disturbed plots to moderately disturbed, with a peak in an edge plot (P2, DI= 1.38).

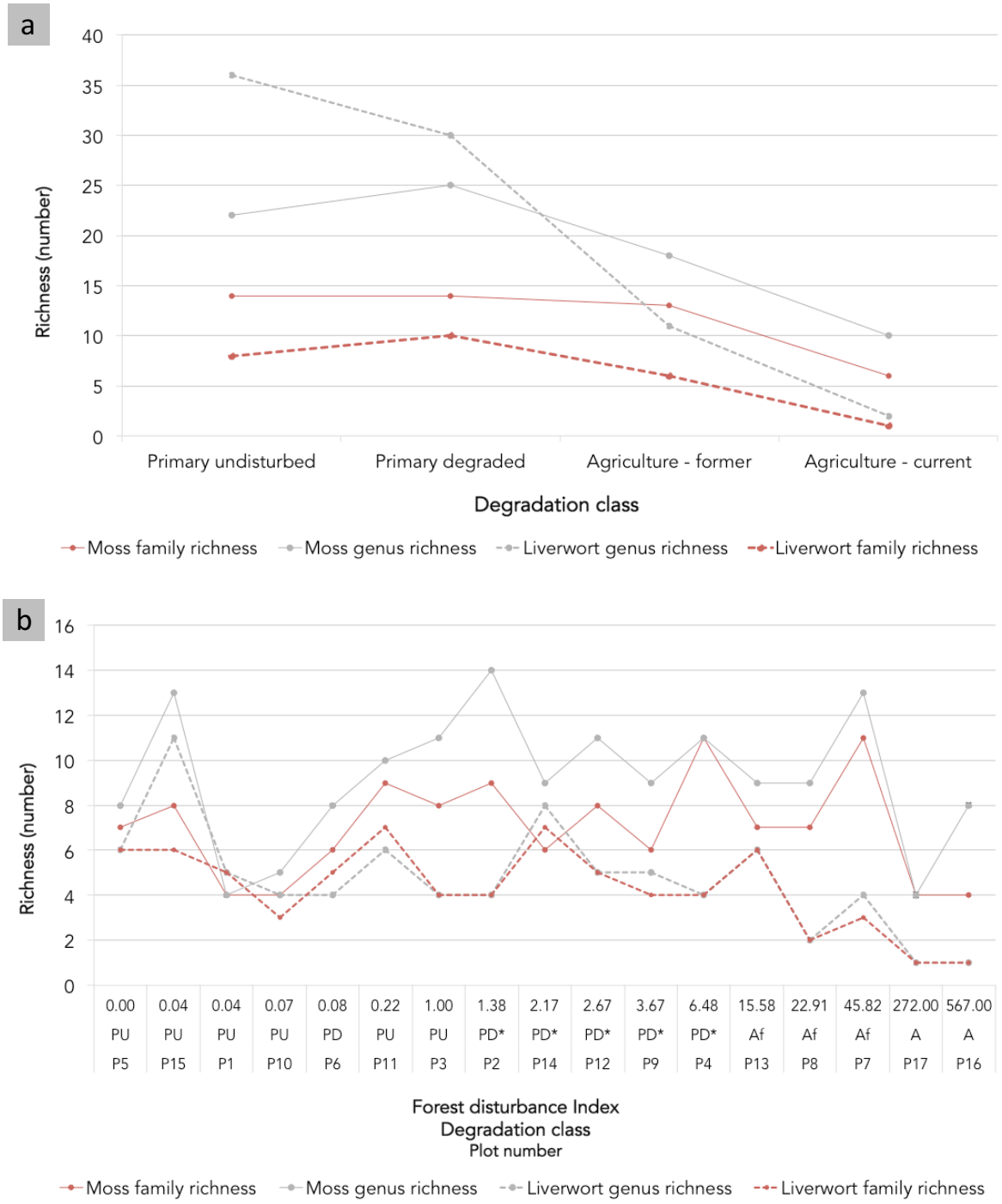


Figure 5.14 Genus and family richness in a) different degradation and land-use classes and b) along the forest degradation gradient. * indicates a plot located on the forest edge.

5.4.2.2 Abundance

Bryophyte cover decreased with overall degradation, both when looking at the discrete degradation and land-use classes (Figure 5.15 a) and the degradation index values (r^2=0.620, p<0.01) (Figure 5.15 b). The plot with the highest specimen number was one with relatively low degradation (P6, DI=0.077), but which was classed as "moderately disturbed primary forest" when using discrete degradation categories. Non-forest plots had the lowest number of total specimens, with current agriculture having significantly less than all other classes (Figure 5.16 a). Moderately degraded primary forest had the highest specimen number, although not significantly different from undisturbed primary forest. Total specimen number was greatest in moderately

degraded forest plots (corresponding mostly to edge plots, Figure 5.16 b), decreasing towards less degraded forest plots and towards agriculture plots. There were two exceptions, with the highest specimen richness found in an undisturbed plot (P15, DI=0.037) and an agriculture plot (P7, DI=45.8), 108 and 82 specimens, respectively.

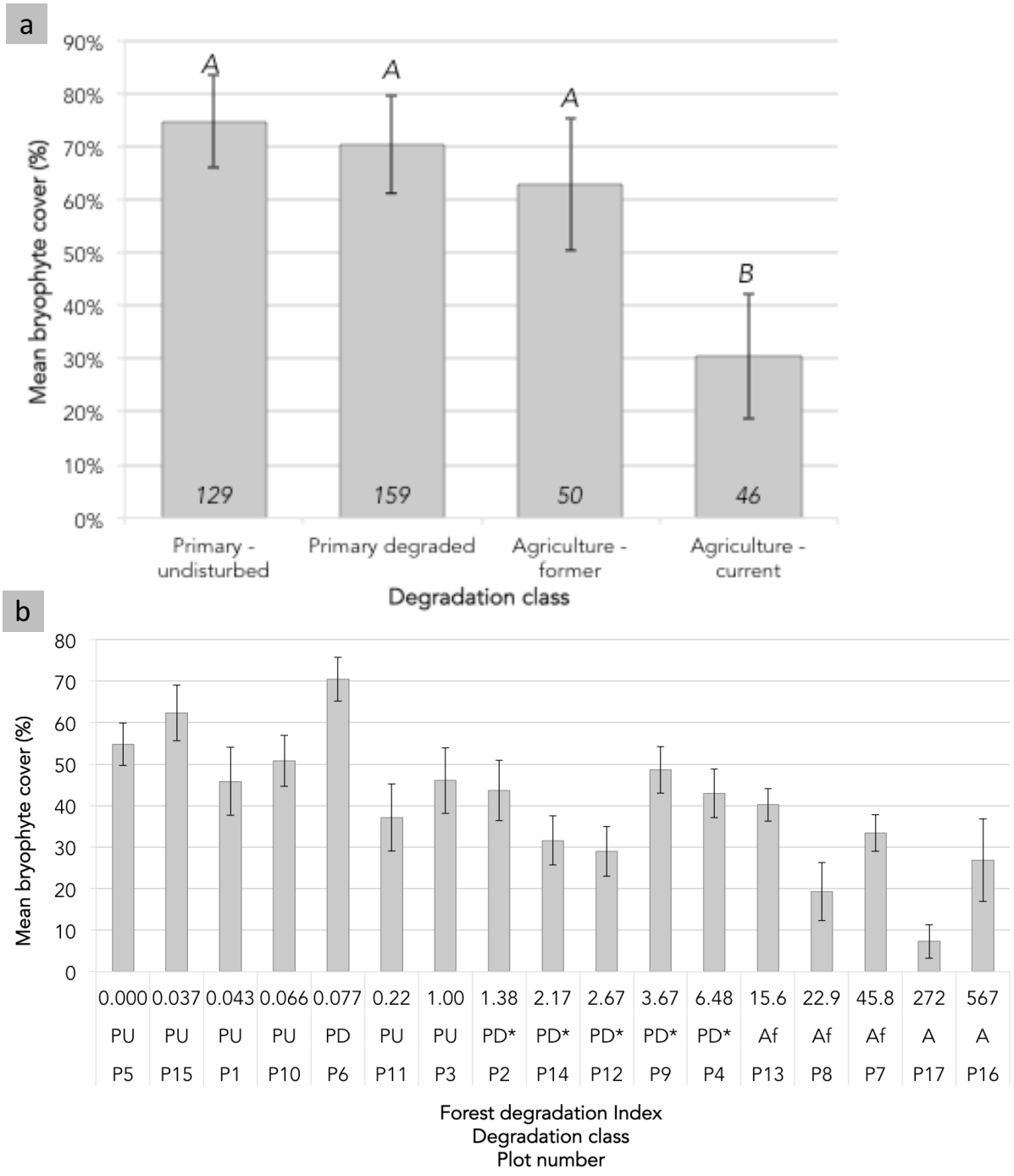


Figure 5.15 Mean bryophyte cover (±1SE) in a) different degradation and land-use classes and b) along the forest degradation gradient. * indicates a plot located on the forest edge.

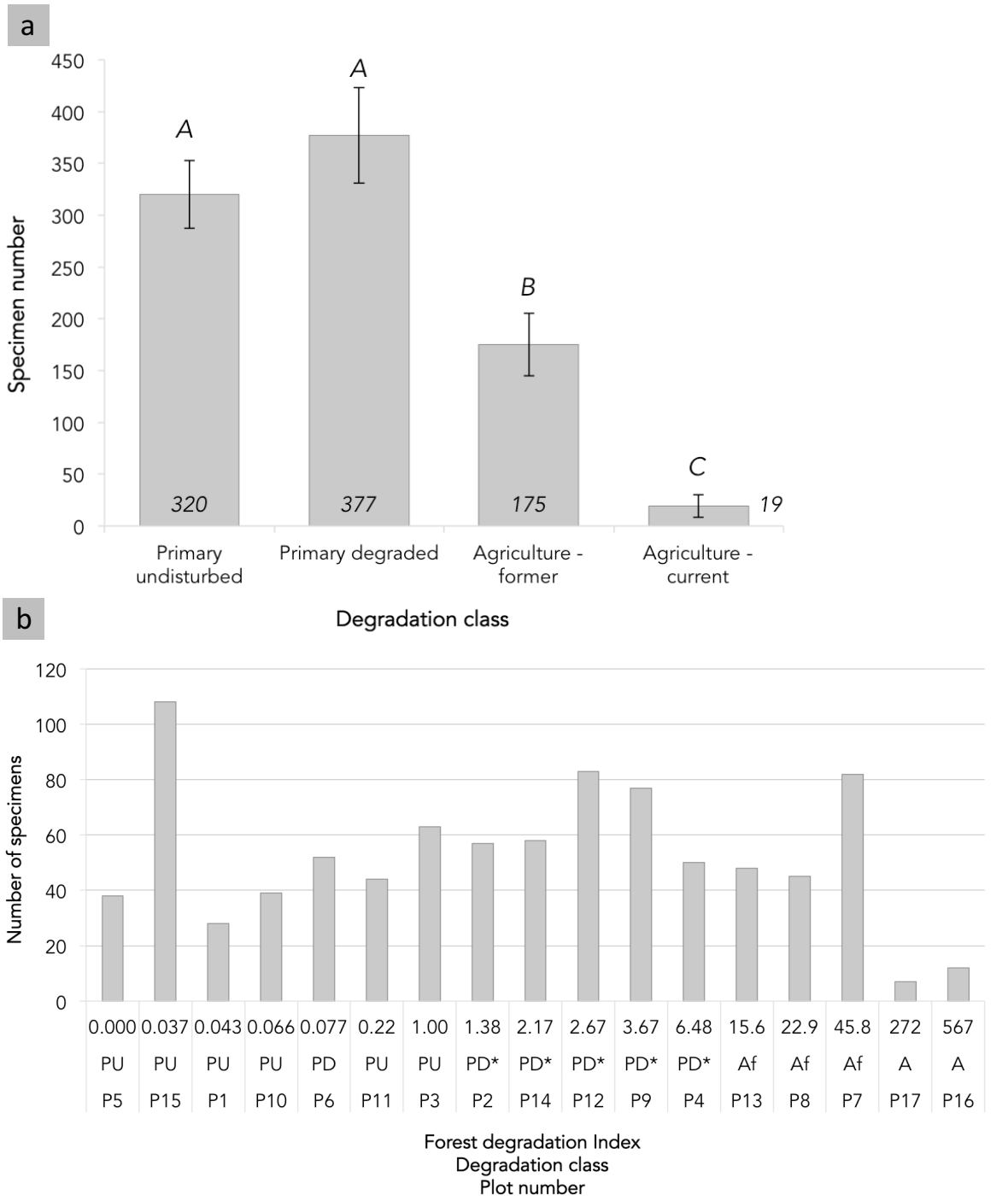


Figure 5.16 Total species richness in a) different degradation and land-use classes (±1SE) and b) along the degradation gradient. * indicates a plot located on the forest edge.

5.4.2.3 Life-form

The cushion life-form (identified in Chapter 4 as a trait state that characterises species of dry and exposed habitats) is predominantly found in degraded and agriculture areas, whereas wefts and dendroids (identified as traits that characterise species of humid and sheltered habitats) are only found in primary or secondary forest, with a greater proportion in primary (chi^2=143.4, p<0.001) (Figure 5.17). Tufts, another life-form identified to characterise more dry and exposed habitats, was found in degraded and agriculture areas, but equally in secondary forest (Figure 5.18). In all degradation types, mats (rough and smooth) and turfs make up the greatest proportion of life-forms (Figure 5.18). Primary and secondary forest had more life-form types (9 each) than degraded or agriculture areas (6) (Figure 5.17). Total bryophyte cover was significantly greater in

primary than in degraded or agriculture (74% ±8 versus 31% ±12, t=-3.75, p<0.01). There was a significant difference between secondary and agriculture and slight significant difference in bryophyte cover between agricultural and degraded areas (p>0.05).

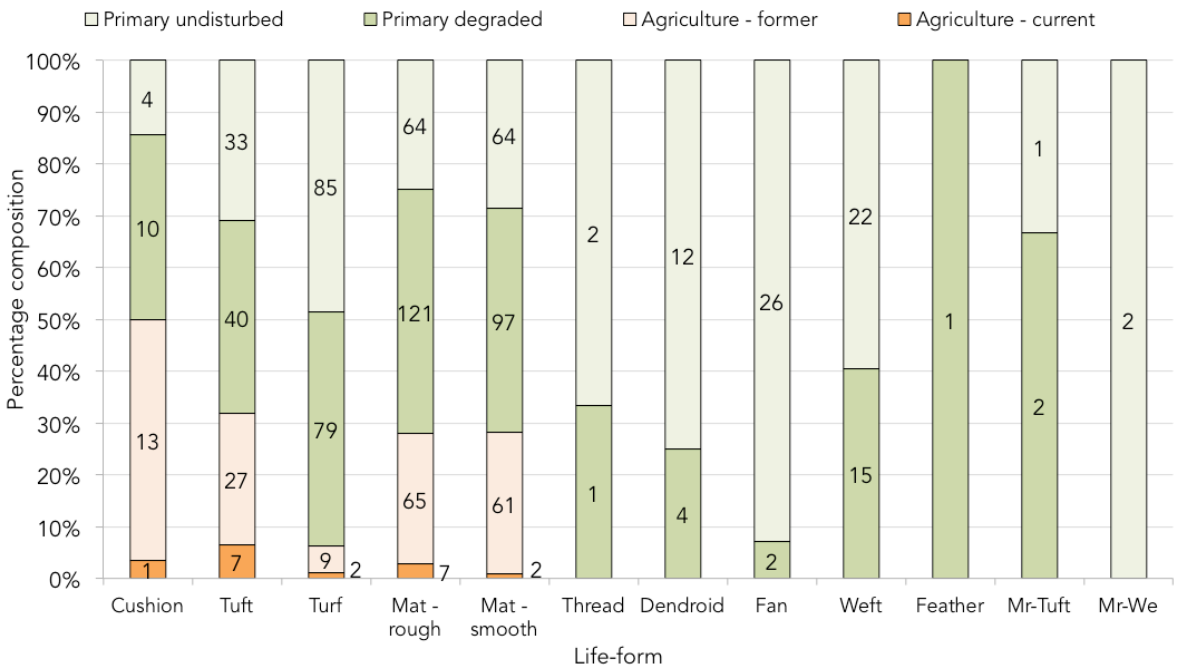


Figure 5.17 Distribution of life-forms in each degradation and land-use class; numbers of specimens per life-form shown on bars.

Table 5.10 Distribution of life-forms along the degradation index. § denotes an open life-form.

Plot number	P5	P15	P1	P10	P6	P11	P3	P2	P14	P12	P9	P4	P13	P8	P7	P17	P16
Degradation class	**PU**	**PU**	**PU**	**PU**	**PD**	**PU**	**PU**	**PD***	**PD***	**PD***	**PD***	**PD***	**Af**	**Af**	**Af**	**A**	**A**
Degradation Index	**0**	**0.037**	**0.043**	**0.066**	**0.077**	**0.217**	**1.00**	**1.38**	**2.17**	**2.67**	**3.67**	**6.48**	**15.6**	**22.9**	**45.8**	**272**	**567**
Cushion		3		1				2	2	1	3	2		4	9		1
Tuft	11	6	2	6	4	4	4	3	6	5	12	10	2	8	17	3	4
Turf	4	37	7	12	11	12	13	9	18	22	11	8	2	4	3	1	1
Mat - rough	16	13	5	10	18	11	9	22	11	23	31	16	21	22	22	2	5
Mat - smooth	3	26	11	5	9	7	12	14	18	30	13	13	23	7	31	1	1
§Thread		1		1						1							
§Dendroid		7			3		5				1						
§Fan	1	5				6	14	1			1						
§Weft	3	10	1	1	6	2	5	3	3	1	2						
§Feather					1												
Mr-Tuft			1								2						
Mr-We				1		1											

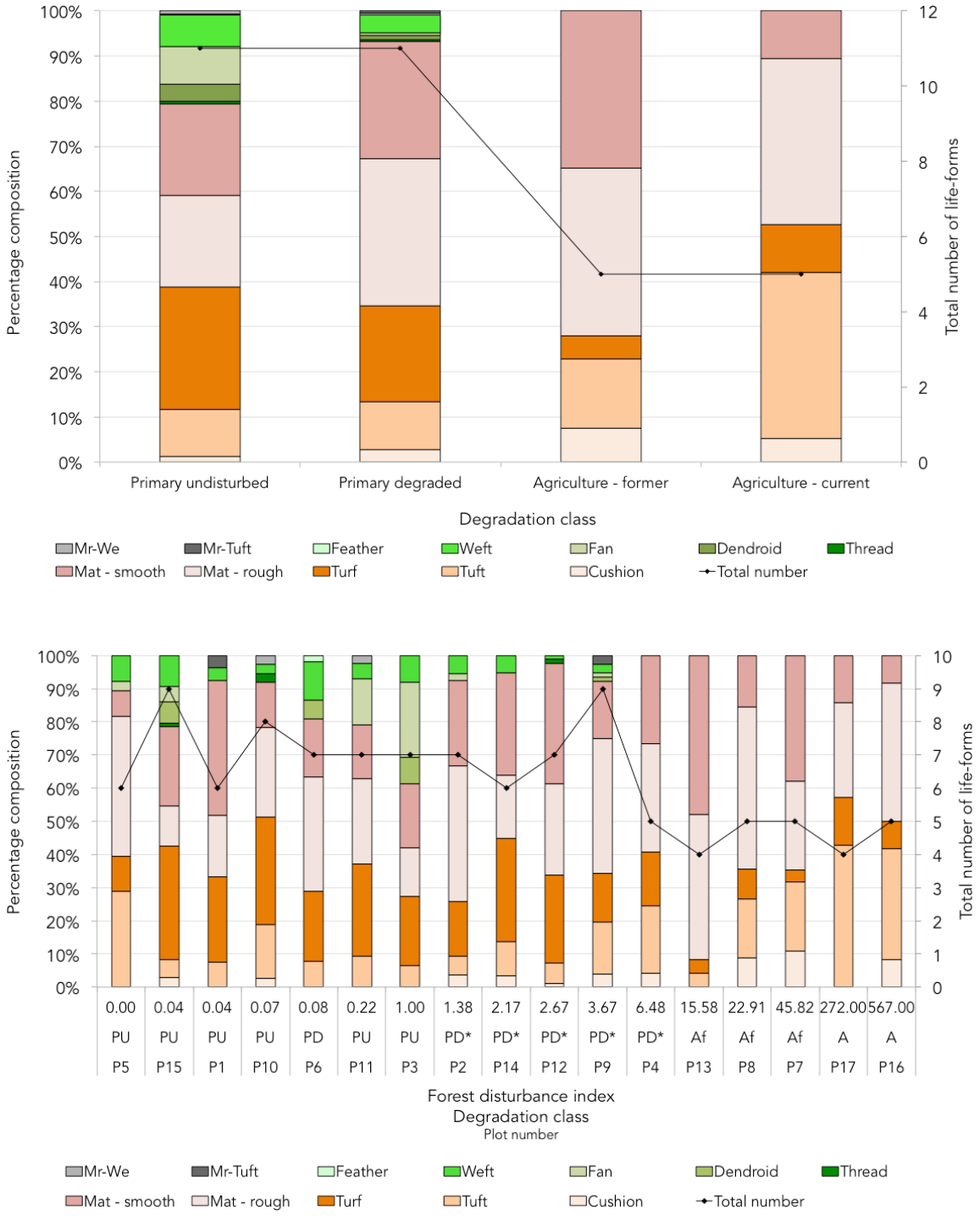


Figure 5.18 Life-form composition and total number of life-forms (black line): a) in each degradation and land-use class; b) along the forest degradation gradient.

5.4.3 Indicator values of different forest land-use types

The mean genus and family indicator values (IVs) are not significantly different between degradation levels (p>0.05) (Figure 5.19); there is a very small increasing IV from primary to secondary (0.004) and secondary to degraded (0.003) (0.007 difference between primary and degraded). There is a significant difference between agriculture and all the other forest degradation types (p<0.05), with the mean IV being much lower in agriculture than in the other forest degradation levels. The lack of difference between degradation types can be explained when looking at the IVs using the disturbance values (Figure 5.20) which show no pattern along the degradation index.

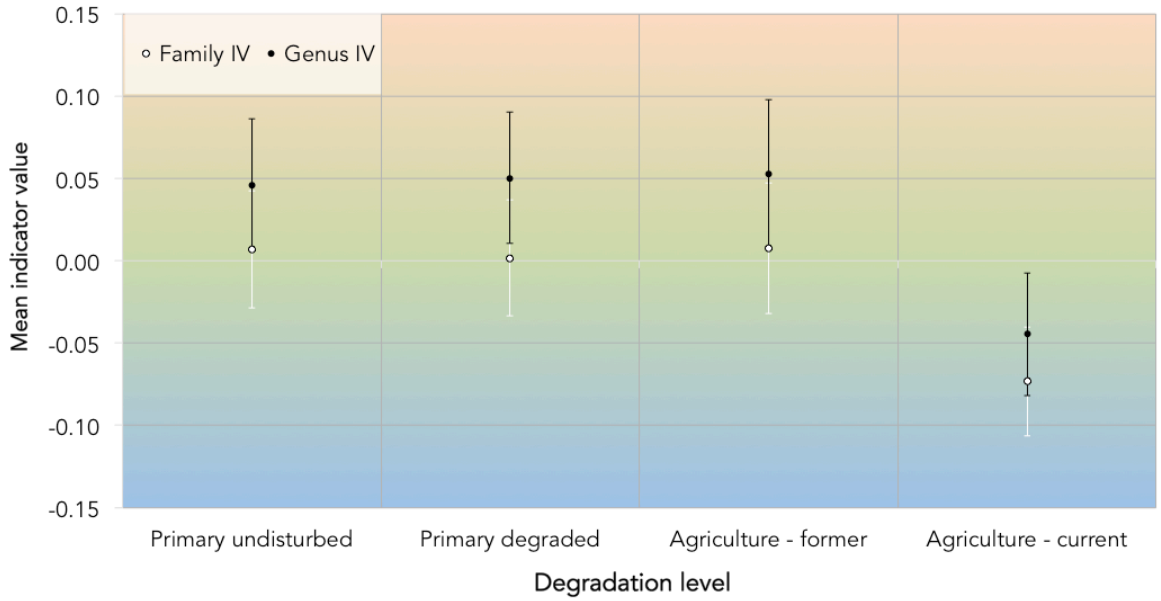


Figure 5.19 Mean genus and family indicator values (± 1SE) in different forest degradation types.

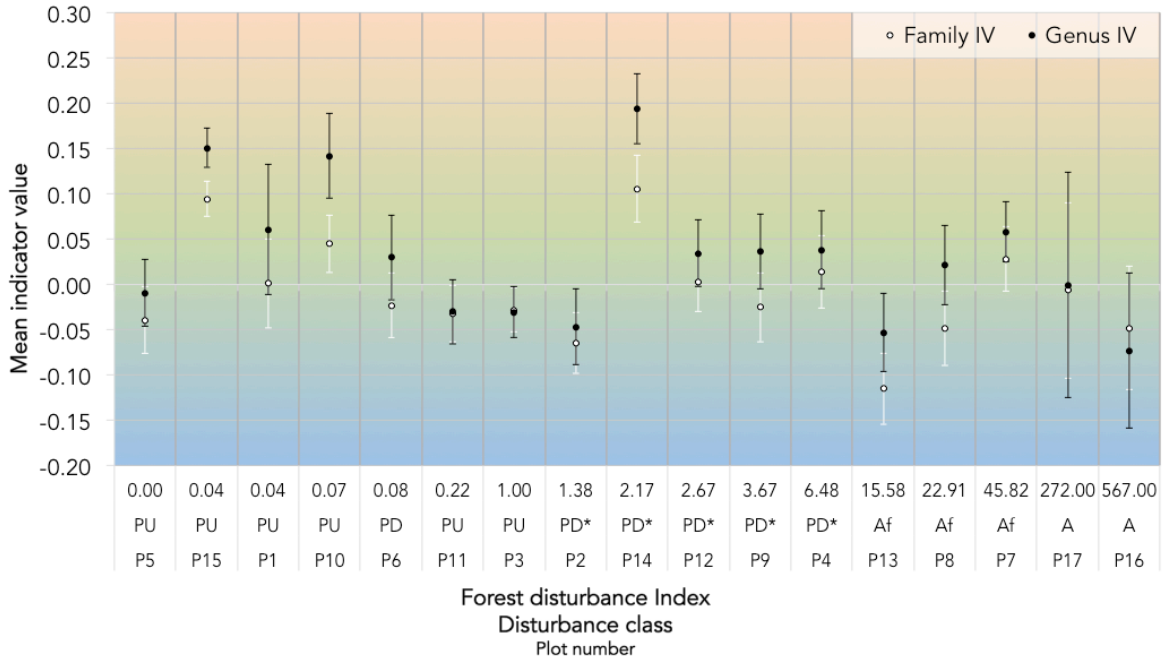


Figure 5.20 Mean genus and family indicator values (±1SE) along the disturbance value gradient. Edge plots are denoted by *. PU - primary undisturbed/slightly distrubed; PD - primary moderately degraded; Af - former agriculture; A - current agriculture.

5.4.4 Indicator values of microhabitats

Different microhabitats have significantly different genus indicator values (IV) (Figure 5.21). Looking at family IVs first, rock (C), soil (B1 and B2), stump bases (D3) and logs (D1) had a negative IV – all ground-level microhabitats. The microhabitat with the lowest family IV is the top of stumps (D5). Those with the highest IVs are the upper trunk of both living (A3) and dead standing trees (D6) as well as the side of stumps (above 50 cm from ground level, D4). Genus IVs are similar to the family IVs, except the upper trunk microhabitat (A3) has an IV much closer to 0 (0.007). Due to the large standard error, the only significant differences are between the genus IVs of stump tops and rocks, and tree trunks (mid and upper) (p>0.05). There are no significant differences between the mean genus and family IVs (p>0.05).

When looking within different forest degradations, the microhabitat IVs vary, with secondary and degraded forest having a higher IV (both genus and family) than primary forest in the case of epiphytic microhabitats (Figure 5.22). In ground-level microhabitats, the pattern is similar when looking at genus IVs (Figure 5.23), except in humic soil (B2), which has the lowest IV in secondary forest and highest in primary forest and in the rock (C) microhabitat where the genus IV is highest in secondary forest with very little difference between primary and degraded (0.014). The pattern is different when looking at family IVs where in mineral soil (B1) the IV increases from primary to secondary, but then decreases in degraded. The greatest difference in family IV is between humic soil (B2) of primary and secondary forest (0.072 less in secondary). In decaying ligneous vegetation (logs, stumps and dead standing trees) there is a smaller difference between the mean IV values of genus and family (Figure 5.24). There seems to be an opposite pattern to those found in the other microhabitat groups, with a decreasing mean IV from primary to secondary to degraded forest. The IV is both the highest and lowest in fallen branches (D2), the highest in primary, the lowest in degraded, and this difference is significant (p<0.001). However, fallen branch microhabitat should not be compared to the others, as it is not an understorey microhabitat and was not sampled strategically as the other microhabitats were. Dead standing trees were only found in secondary forest plots and so no comparison can be made with other degradation levels. It is important to note that microhabitats in agricultural disturbance had lower mean IVs (both genus and family) than their counterparts in more intact forest areas.

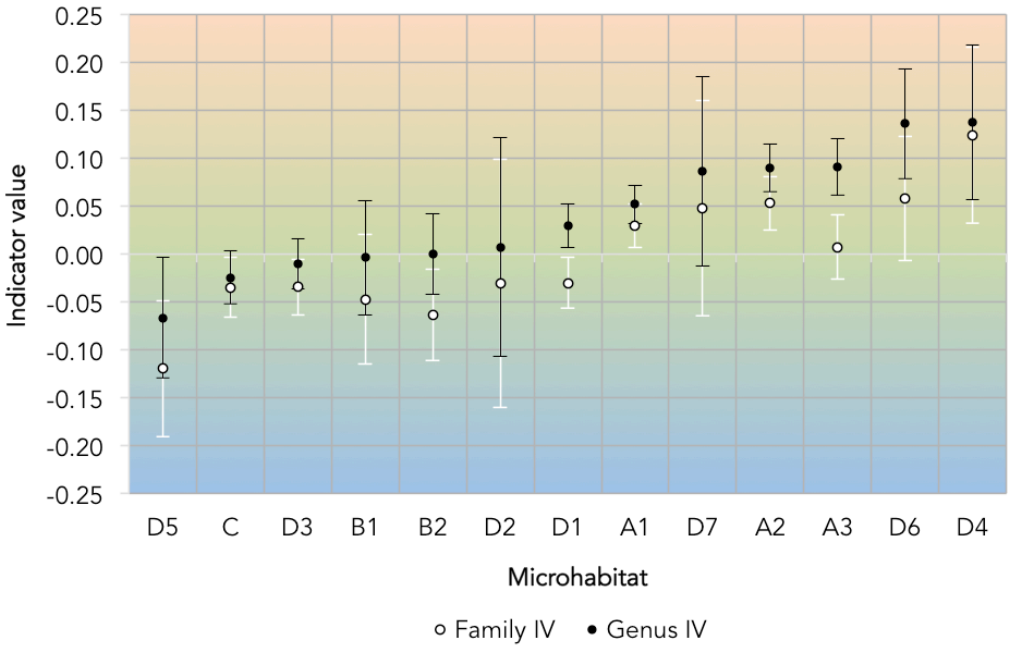


Figure 5.21 Mean family and genus IV (± 1SE) in different microhabitats.

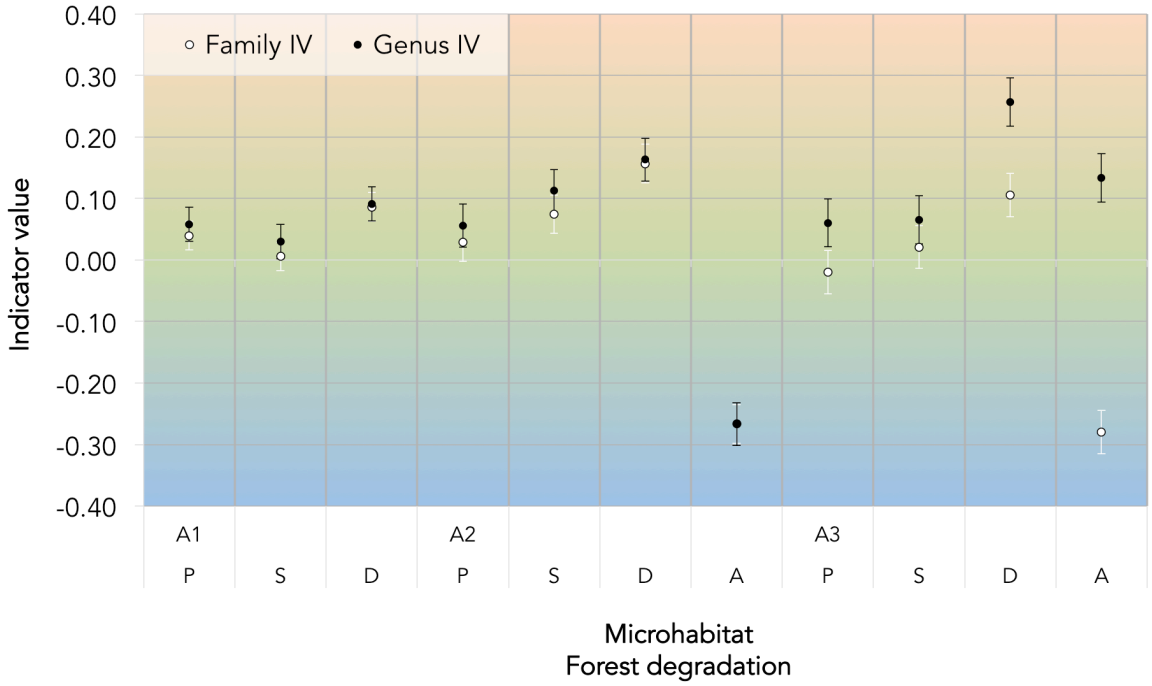


Figure 5.22 Family and genus IV (± 1SE) of epiphyte microhabitats within different forest degradation types. Where no white circles are visible, the IV of family and genus are the same.

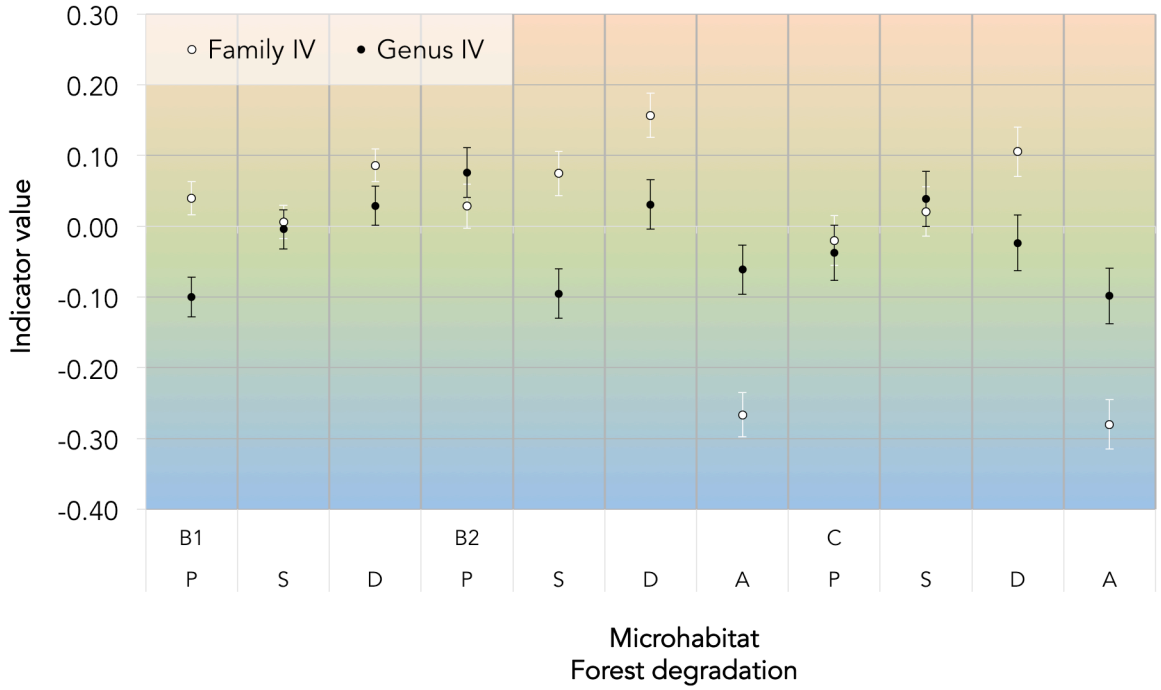


Figure 5.23 Family and genus IV (± 1SE) of soil (B1 and B2) and rock (C) microhabitats within different forest degradation types. Where no white circles are visible, the IV of family and genus are the same.

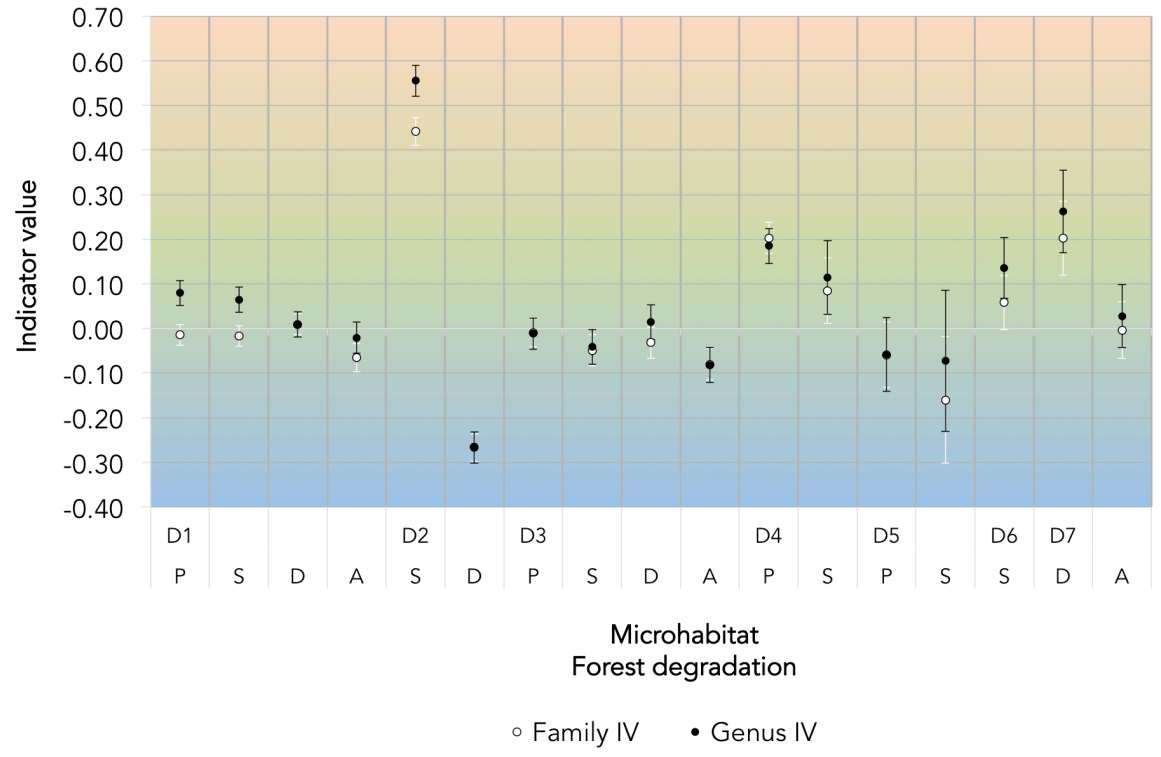


Figure 5.24 Family and genus IV (± 1SE) of decaying ligneous vegetation microhabitats within different forest degradation types. Where no white circles are visible, the IV of family and genus are the same.

5.4.5 Bryophyte indicator values and habitat type

The mean IV of mangroves is significantly lower than that of other ecoregions (p<0.05, Figure 5.25), which is to be expected as it is a wet habitat. On the other hand, sub-humid and lowland

forests have a lower mean IV (both family and genus IV) than the other ecoregions (with the exception of the genus IV of ericoid thickets), which is unexpected as these ecoregions are more humid than spiny thickets and dry deciduous forests. The accuracy of genus versus family IV has not been tested, but due to the greater number of species within the family than genus taxonomic level, it would be expected that there is greater error associated with family IV values. The habitat bias in collections can be seen in Figure 5.26 with almost all specimens collected from humid forests (lowland and subhumid).

Table 5.11 Distribution of accurately georeferenced specimens within Madagascar ecoregions and associated IV.

Ecoregion	Number of specimens	Mean family IV	1SE	Mean genus IV	1SE
dry deciduous forests	6	-0.076	0.059	-0.002	0.062
ericoid thickets	2	-0.007	0.042	0.404	0.000
lowland forests	656*	0.017	0.008	0.088	0.009
mangroves	1	-0.280	0.000	-0.025	0.000
spiny thickets	1	-0.073	0.000	-0.038	0.000
subhumid forests	401	0.043	0.010	0.091	0.011

*This number includes identified field specimens collected during this PhD's fieldwork.

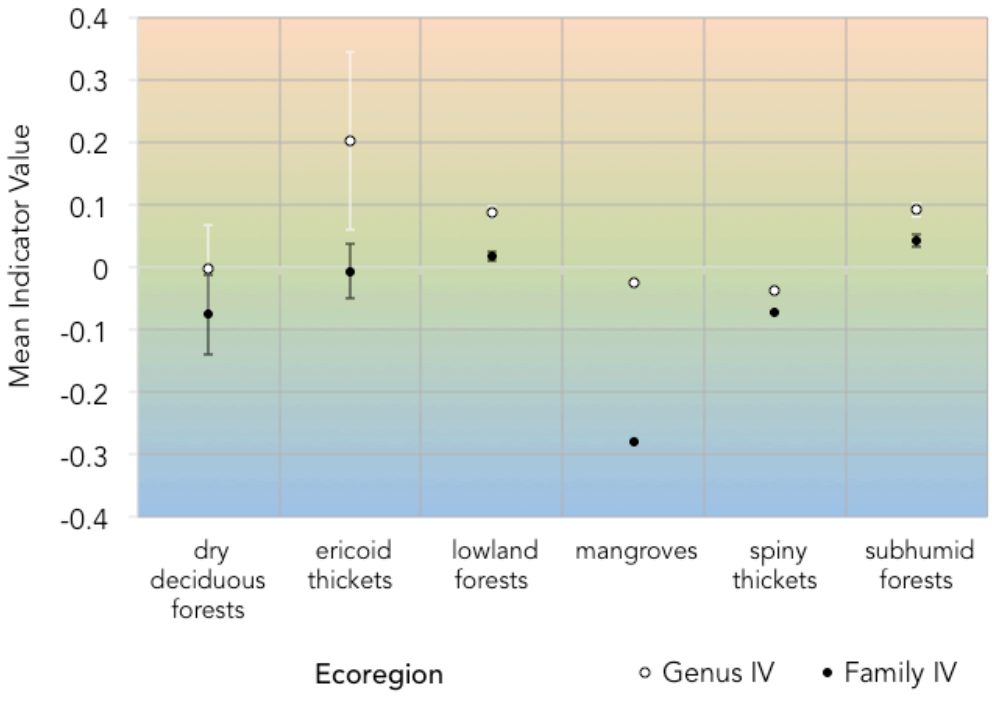


Figure 5.25 Mean IV (±1SE) in different ecoregions of Madagascar.

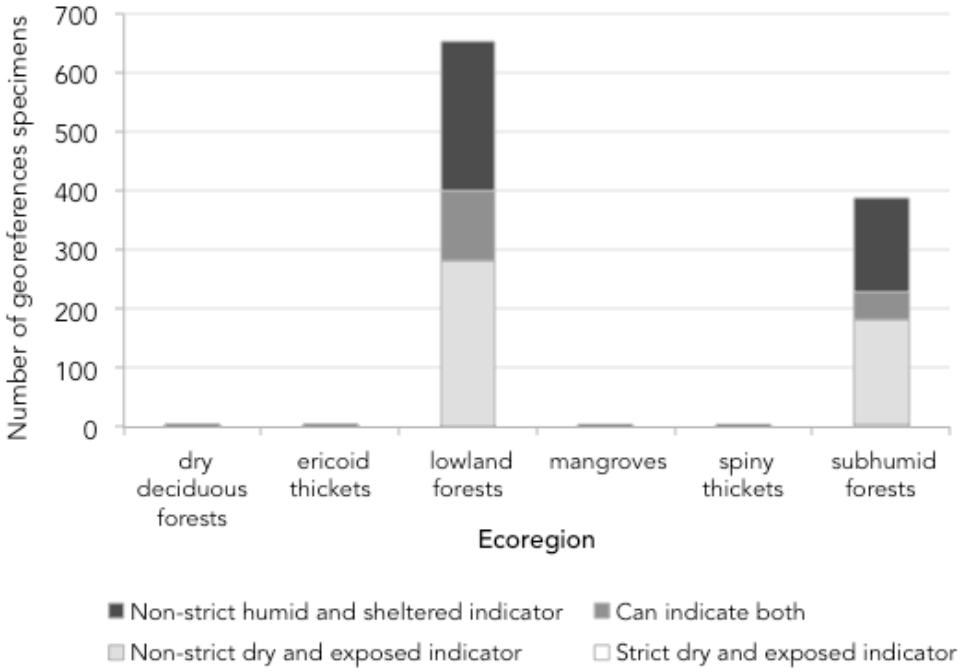


Figure 5.26 Number of different indicator types (genus level) present in the ecoregions of Madagascar showing the disproportionately large number of specimens from lowland and subhumid forests.

5.5 Discussion

5.5.1 Bryoflora

Taxon richness and abundance

The lack of trend in genus or family richness across the gradient of degradation reflects what has been found in other studies that richness alone is not the best indicator of forest integrity and that different species groups (e.g. feeding guilds) vary in their response to degradation (Dufrêne & Legendre, 1997; Struebig et al., 2013). It also highlights the issue of classifying disturbance into discrete categories, rather than using a finer-level gradient of degradation (Struebig et al., 2013). When looking simply at undisturbed and moderately degraded it seems that moderately degraded forest has a higher genus richness, however, this is clearly not the case when viewing degradation at a finer level.

When looking at total specimen number, the highest number appears in degraded primary forest, according to the discrete degradation categorisation. However, the highest specimen richness is in a forest plot that has relatively low DI, comparable to plots classed as undisturbed primary forest, but that in the discrete categorisation has been classified as moderately degraded forest. This again highlights the problem with categorising degradation in broad classes, rather than using an index, as it could lead to misinterpretation of the repsonses of bryophytes to forest degradation.

Plots that were moderately to heavily degraded had among the highest genus richness which can be attributed to edge-effects as all but one of these plots were at the edge of the forested area. This is commonly found in studies of species richness, although there are exceptions (Ewers & Didham, 2006). It would be expected that these edge habitats would have a more positive

indicator value (IV) than interior forest plots as species are more exposed to desiccation due to higher insolation and wind at these edges. No pattern was seen and this could either be due to methodological reasons or the fact that bryophytes are overall more tolerant of this modified environment.

Liverworts had a more marked pattern of decreasing genus richness with degradation suggesting they are more sensitive to habitat change and therefore potentially more suitable indicators than mosses. It also shows the importance of differentiating between similar groups of species rather than grouping them together as a single entity and therefore interpreting their responses as one and the same.

The spike in taxon richness in a heavily degraded plot (P7 – former agriculture) is likely due to the fact that the site had flooded and experiences periodical flooding (pers. comm. M. Denis, CoBa president). Moisture availability is therefore much greater compared to other plots (forest and non-forest) allowing a greater number of species to survive. Additionally, five epiphytic microquadrats were sampled which is greater than in other non-forest plots. This accounts for the fact that despite being a wet environment, the mean genus and family IV was positive, as the epiphyte microclimate is a realively dry one compared to ground microhabitat; epiphyte microhabitats were found to have a significantly higher IV value.

Forest degradation does not, therefore, seem to affect bryophyte taxon richness and abundance as has been found in other studies (e.g. Holz & Gradstein, 2005; Struebig et al., 2013) but non-forest land-uses do (in this case clear-cutting for agriculture). Forest than had been clear-cut and then left fallow had a higher taxon richness and abundance indicating that bryophytes are able to re-colonise areas that have been converted to non-forest and then abandoned, as found by Gradstein & Sporn (2009) in Indonesia. In this study, a likely reason is due to the fact that the former agriculture plots were surrounded by forest, which provides a population source. This is not universally the case in bryophytes, as studies from other geographical regions found that forest bryophytes did not colonise fallow areas (e.g. Indonesia, Gradstein & Sporn, 2009).

Life-form

When looking at bryophyte life-form, however, a clear pattern could be seen. Open life-forms were only found in undisturbed primary plots. Wefts and dendroid life-forms were found exclusively in undisturbed and moderately degraded primary forest. Mats and turfs are the most predominant life-form found during the study, and these were found to be associated with drier and more exposed habitats in Chapter 3. Although in the analysis in Chapter 3 turfs were found to be associated with species of dry and exposed habitats, in this study they were most abundant in undisturbed and moderately degraded primary forest. This can be explained by the fact that epiphytic species were almost all turf species, as the epiphytic microhabitat is relatively drier than soil microhabitats (Johansson, 1979; Bader et al., 2013). This indicates that life-form is a better indicator of forest degradation and disturbance than simply bryophyte richness, reflecting other studies that have found that functional groups or species with similar life histories have a better response to degradation than when looking at species individually (Ewers & Didham, 2006; Struebig et al., 2013).

The proportion of cushions, tufts and turfs remained similar across the various degradation levels, but open life-forms clearly decreased with increasing degradation suggesting they are more

sensitive to degradation. This fits with results from studies on other taxonomic groups that different functional groups respond differently (e.g. Bregman et al., 2016).

The genera found only in agriculture or degraded habitats are all ones a high IV value, and classed as non-strict indicators of dry and exposed conditions, suggesting bryophytes and the IV assigned to them have potential to be successfully used as an indication method.

Almost all the species included in the statistical analyses to define the trait profile and subsequently the indicator values were Portuguese bryophytes (due to data completeness). The life-form trait was shown to respond similarly in Malagasy species and this indicates that it is possible to use trait data from species in one region to predict responses of species in another. This is in contrast to a study that compared vascular plant indicator species and found that they could not be used in different areas (Godefroid & Dana, 2007). However, vascular plants are likely to vary more within a region than bryophytes due to the larger dispersal ranges of bryophytes.

5.5.2 IV and microhabitat

There is no significant difference in the mean IV values between primary, secondary and degraded forest, but there is between agriculture and the latter three. However, it was expected that a highly disturbed area would have a positive IV value, as it has higher insolation and lower humidity than a more intact area. In fact, even though different microhabitats within forest disturbance types showed that there were significant differences, microhabitats in agriculture have lower IV values than their counterparts in more intact forest areas. No environmental data was recorded at the microhabitat level; this could potentially help to elucidate differences found. A possible explanation for the low IV could be that as there is a lack of canopy in agricultural areas, the soil is wetter and so species bryophyte taxa that are found here are actually ones that do well in wet habitats, such as species of the genus *Fissidens* that were found in this habitat. This will need to be confirmed further when all specimen identification is complete to species level. Specimens for this study were identified to genus and family level in order to collect a sufficient sample size for analysis. Due to the complexity of identifying species from an understudied bryoflora this was not within the scope of this study.

When looking at microhabitats overall, it was found that ground-level microhabitats had lower IVs than those that are on trunks or dead standing trees. This is explained by the fact that the microclimate in the lower level of the forest habitat is more humid and sheltered, due to less wind penetration and less insolation reaching the ground. Although the microhabitat “fallen branch” (D2) cannot be compared with other microhabitats (as it was opportunistically sampled in order to be able to produce a checklist of bryophytes for the study area), it provides an indication that bryophytes in different forest strata are exposed to different environmental conditions, as found by several studies comparing bryophytes across all forest strata (e.g. Holz & Gradstein, 2005). The differences in microhabitat shown by bryophytes shows that they are able to respond to fine-scale changes within a habitat, an important feature of the best indicators (Gardner et al., 2008).

Dead trees have different characteristics to live trees such as bark texture (sometimes no bark) and higher sunlight exposure (due to lack of foliage) (Johansson, 1974) meaning the bryophyte flora will be different and so can confuse species-habitat relationships (Drehwald, 2005). The higher level of family and genus IV found in this micro-habitat confirms this. Moisture

accumulates on flatter microhabitats such as flat-topped stumps (Barkman, 1969) and this could explain the low IV values found in this microhabitat. Bark texture determines microhabitat availability for cryptogams, bark with fissures provides a sheltered and moist environment and also a more stable environment than scaly bark (Barkman, 1969) for the development of protonema and consequently gametophytes.

5.5.3 IV and forest degradation

The lack of difference in IVs when viewing plots as belonging to discrete degradation categories highlights a common problem of pseudoreplication in tropical studies (Ramage et al., 2013). The six plots assigned to primary undisturbed/slightly degraded and primary moderately degraded are not true replicates as they are all located within a small distance of each other and therefore can be seen as samples of the same forest fragment. However, the location of plots was chosen to minimize environmental and stochastic effects, such as altitude, which could cause effects on species that are not due to forest degradation. The proximity of plots occurred so that there would be no effect of altitude on the bryoflora, as altitude has significant effect on bryophyte distribution and abundance (Bader et al., 2013; Wagner et al., 2014). Using a disturbance index can circumvent this issue of pseudoreplication, as shown by Struebig et al., 2013.

Five of the undisturbed primary plots had tree-fall and as bryophytes are known to be similar between tree-fall areas and forest edges (Bader et al., 2013) this could explain the lack of pattern between the IV and the disturbance index. Aditionally, the impact of edge-effects on bryophytes is not clear-cut, with few studies looking specifically at edge-effects on bryophytes – this is an interesting area of future research.

Although there is a lack of clearn pattern in mean IVs and forest degradation and land-use, forest plots had a wider range if IV values reflecting that they are home to a more diverse range of bryophytes. The lack of clear trend in IVs and richness with varying disturbance results exemplify the complexity of defining forest degradation and the interaction of different factors (Ewers & Didham, 2006; Simula, 2009). Bryophytes may also be too sensitive in that they rely on microclimate and so if a suitable microhabitat is present in a degraded habitat a sensitive species may occupy it – therefore and indicator value based on micrclimate preferences, such as the index created here, may be more appropriate.

Some information is lost when creating a composite measure and finer-level differences are masked (Simula, 2009). The composite measure created for disturbance may not be the most appropriate, and further refinement is needed by, for example, weighting variables. Natural tree fall was not included in the disturbance value (as this study focuses on human-induced disturbance) but likely had an impact on the species of bryophytes recorded.

It could also be argued that no fragmentation has taken place in the study area, and therefore there is continuity between different sites (Ewers & Didham, 2006). None of the forest sites were located in an “island” of forest surrounded by heavily degraded or non-forest habitats. Sites that were heavily degraded had been so within the previous 10 years, and therefore some sensitive species could still be present due to the high persistence of some bryophyte taxa, and effects of degradation can overall take decades to be evidenced (Ewers & Didham, 2006). The distance to the nearest forest edge of the agriculture plots should be included within the disturbance index.

5.5.4 IV and ecoregion

The higher IV in lowland and subhumid forests than in dry deciduous forest is curious. This could be an indication that the genus and family IV levels are too broad to indicate habitat preference of the respective taxa. However, most of the georeferenced specimens in Madagascar are from either lowland or subhumid forest (Figure 5.26), and so it is not possible to make comparison between the ecoregions with statistical confidence. Inclusion of more georeferenced specimens from other habitats and ecoregions is needed; this can be achieved through further georeferencing of herbarium specimens but will also require fieldwork in understudied habitats. Another factor to take into account when looking at the IV value in the forest overall, is that in lowland forests water evaporation rates are higher than in forests at higher altitude leading to a thinner epiphyte layer (Frahm, 1990).

5.5.5 Species Dessication Tolerance

A next step (following this study) is to measure physiologically the DT of bryophytes collected. The most accurate methods of measuring DT are through photosynthetic recovery experiments and so this will be used. Additionally, a method that is easy to carry out and requires minimal equipment so that it can be carried out by researchers in Madagascar will be developed. This is based on measuring the water content of hydrated plants at set intervals producing a water release curve, as undertaken by Song et al. (2015). Slow drying rather than rapid drying was used to more closely resemble the environmental conditions encountered in the field. An accurate physiological measure of DT can then be used to further explore the IV and calibrate it.

As mentioned in chapters 2 & 3, physiological measurements in the laboratory may not always reflect the true field conditions (Proctor, 2000a) and comparative studies are hindered due to the effect of different "field effects" – the conditions a species has been exposed to in the field prior to measuring DT (Stark et al., 2014). The former problem can be minimised by drying bryophytes slowly, in an open environment, rather than in desiccation chambers. A common technique used to overcome field effects is to grow new shoots of collected species under laboratory controlled environmental conditions (Stark et al., 2014). This could be an interesting and useful physiology experiment, particularly as there are relatively few studies on DT of tropical bryophytes.

Another measurement that could be made is the colour of plants when freshly collected versus when dry. This would be a simple method to undertake. Several leaves could be prepared on microscope slides and photographs taken with the same settings (ISO, aperture and exposure time) and then compared quantitatively using photographic software (such as ImageJ) (similar to the method used by Stark et al. (2015).

A classical ecological study of vertical zonation, from base to crown, would be interesting to do, as most data for this comes from temperate studies

5.5.6 Indicator values

The IVs within microhabitats and degradation types were overall positive, which is unexpected. A likely explanation for this is that lowland forest is generally drier than other forest types (Kürschner et al., 1999) and so bryophytes living here will naturally be more adapted to longer periods of drought and therefore be more DT. A comparison of this study with other forest types in Madagascar would therefore be interesting.

In order to further substantiate the usefulness of bryophytes as indicators, a cost-efficiency analysis, following the method in Gardner et al. (2008), could be undertaken using data form this study.

5.5.7 Methodology

5.5.7.1 Environmental variables

Fog was observed in the early morning in the field and this has implications for bryophyte distribution (Song et al., 2015). This is of particular interest as lowland forests are drier than forest at higher altitude and so bryophytes will make the most of water available. The measurements of environmental variables (temperature and humidity) were not reliable, particulary in the case of humidity. To record environmental variables more accurately, measurements should be taken at each micro-quadrat but time-constraints made this impossible. Another option to circumvent the unreliability of the environmental data would be to use climate data for the study area (e.g. data available on WorldClim; www.worldclim.org, Hijmans et al., 2005). However, the resolution of this data is too coarse: 1 X 1 km is the highest resolution currently available meaning all study plots are contained within three 1 X 1 km squares only. This means there is insufficient differentiation in the environmental variables between the study plots, and so would not have an effect in analyses. For example, the annual mean temperature for the sites only ranges from 21.4 ^{o}C to 22.6^{o}C: six sites have a temperature of 21.9 ^{o}C, nine sites have 21.4 ^{o}C and two sites have 22.6 ^{o}C (WorldClim data, Hijmans et al., 2005). The same level of variation applies to other environmental variables.

Ideally, data loggers should be used over a series of months in order to accurately capture humidity values so that a finer-scale analysis of ecological preferences of different bryophyte species can be undertaken, in conjunction with physiological DT measurements (Pardow et al., 2012). Due to the logistics on the ground, it was not feasible to place data-loggers during this study due to difficulties in retrieving the data and also likelihood of accidental vandalism. The sites originally targeted for sampling (those visited in 2014) were subject to deforestation and so any data loggers placed there would likely have been lost. However, the establishment of a long-term collaboration between the CoBa and the NGO Asity (following the fieldwork) will make it feasible to place data-loggers for future work.

5.5.7.2 Data collection

The disparity in the number of microhabitats sampled between plots, particularly between forest and agriculture (both former and current) epiphytic microhabitats, could have had an effect on results of taxon richness. However, all the available trees in the agriculture plots were surveyed and so the epiphytic diversity recorded is a reflection of the bryoflora in these plots. The same is true of soil and rock microhabitats, even within plots

Future sampling should focus on targeted species based on results from this study. This technique of selective sampling has been used in bryophyte research and proved successful (Hedenäs et al., 2004). It also allows to record true absences of species which is important for identifying plant associations and modelling distributions (Stohlgren, 2007).

Pseudoreplication is a significant issue in ecological studies, especially in tropical areas (Ramage et al., 2013). By creating a disturbance index, and by using mixed-effects models, this study has

attempted to reduce erroneous conclusions as a result of pseudoreplication. True replication requires plots of different degradation levels and/or land-use types to be interspersed among each other (systematic design) (Hurlbert, 1984). This is possible with experimental studies but much more difficult in measurement studies (i.e. field sampling) as researchers often have no control over where different degradations and/or land-use types are located (a notable exception is the Biological Dynamics of Forest Fragments Project (BDFFP) in Brazil (Lovejoy et al., 1986) and the Stability of Altered Forest Ecosystems (SAFE) project in Borneo (Ewers et al., 2011)).

5.5.7.3 Bryophyte population data

Population processes impact the presence and abundance of species and it is important to take these into account when looking at species composition and distribution. Bryophyte population dynamics and patterns is an understudied area (Rydin, 2009) with very few field experiments on this subject and focussed on temperate regions (Frego, 2007; Rydin, 2009). Because of this, it is not possible to make definite conclusions as to how the results of field studies are affected by population dynamics, such as recruitment and interspecific competition. From studies that have been undertaken, it is known that spore or propagules banks are a determinant factor in recolonisation following disturbance (Rydin, 2009). Certain groups of species, such as leafy liverworts, have been found to have poor recruitment rates and therefore fail to re-colonise when habitats recover (Frego, 2007). This has clear implications when selecting bryophyte indicator species and as a first step to understanding population dynamics, Malagasy bryophytes could be classified into During's (1992) life strategies based on the trait data recorded in the present study. Further research into tropical bryophyte population dynamics based on field experiments is therefore an important avenue for research, especially at forest edges where it is known that species competition dynamics can change (Ewers & Didham, 2006).

5.5.7.4 Quantifying disturbance

An extension of the work carried out here is to undertake spatial analyses: quantify disturbance of each site in the landscape context (using deforestation data [Hansen et al., 2013], socioeconomic data, biomass data); similarity values using species-level data to analyse spatial variation and species turn-over (Ramage et al., 2013). This will allow comparison with bryophytes collected in other parts of Madagascar (Mitchell & Schaab, 2008) such as the recent collections by MBG which have accurate georeferencing data and habitat information. Including a wider range of forest degradation indicators allows the creation of a disturbance value that is in line with international policies on quantifying forest degradation. Once all specimens have been identified to species-level, similarity comparisons between degradation intensities can be conducted (e.g. by following the method in Struebig et al, 2013). These will provide further insight into bryophyte assemblages and which species are most affected by degradation. Aditionally, a true comparison of the effect of degradation would be to return to any forest plots with none to low degradation that have since suffered moderate to heavy degradation.

5.6 Conclusion

A key output from this chapter is that it was shown that the IV varies between microhabitats and different forest degradation types. Further refinement of the IV is needed and it remains to be determined confidently if the IV is a reliable predictor of environmental conditions and habitat degradation. However, it provides a useful first step in determining how bryophyte traits can be

used to select species whose presence or absence can be a reliable indicator of habitat quality. The IV produced here reflects to a certain extent the distribution of bryophytes in the field study site, but further testing is required. Due to the lack of identification of all specimens to species level, it was not possible to look at the species IV values, but this would be likely to lead to more robust associations with species and degradation. The ability for bryophytes to survive periods of drought will become increasingly important in the light of climate change (Song et al., 2015).

Using an index of degradation, rather than discrete categories, provides further insights into the responses of bryophytes to degradation. Subtle changes in bryophyte compostion (i.e. life-form and richness) will have an effect on associated species, such as invertebrates by limiting habitat availability. Further exploring this index of degradation, and refining it, would be of merit for future bryophyte studies. Valid comparisons of study plots would be possible if degradation is quantified, as in the index created here. This would help resolve some of the disparities seen in bryophyte and other taxa responses (Frego, 2007; Streubig et al., 2013) and so contribute to realiable data interpretations and its application in conservation management.

Preliminary spatial analyses of the IV values of genera and families across ecoregions of Madagascar show that they differ within these, although the pattern is unexpected. This may merit further examination and highlights the disparity in data availability between ecosystems and forest types in Madagascar. What it also shows is the value of historical collections in herbaria, four hundred of which contributed to this analysis. The georeferencing of historical collections, their compilation to one resource (and future publications based on this work) will remain ongoing as a followup to this study, the use of which will contribute to future bryological field studies in Madagascar.

Appendix 5 Field methodology

A5.1. Field sites

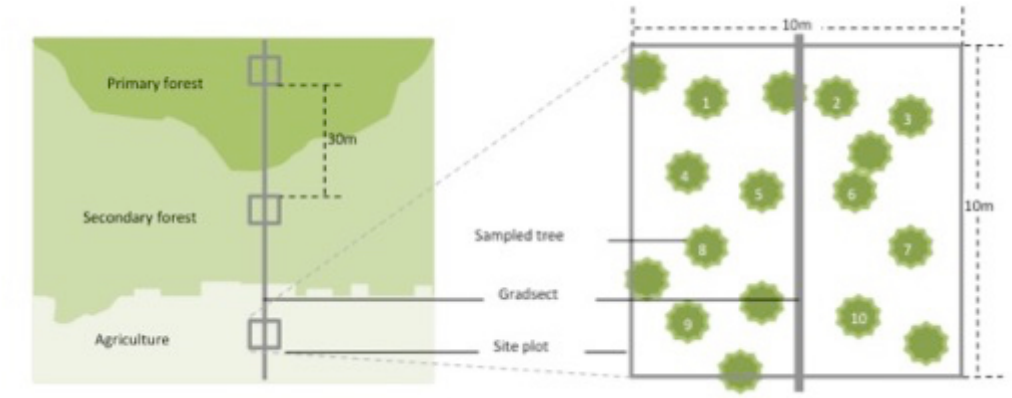


Figure 5.27 Original sampling strategy for field plots.

A5.2. Collection variables

Table 5.12 List of Madagascar habitats – from Moat & Smith, 2007.

Madagascar habitat type
Humid forest
Littoral forest
Western humid forest
Western sub-humid forest
Western dry forest
South western dry spiny forest-thicket
South western coastal bushland
Mangroves
Tapia forest
Wetlands
Wooded grassland-bushland mosaic
Plateau grassland-woodland grassland mosaic
Cultivation
Bare soil/rock

Table 5.13 Shade index categories and definition - taken from BRECOG methodology (Bates, 2011).

Category	Definition
1	fully exposed to sunlight at all times
2	shaded from direct sunlight for up to half the day
3	receiving significant direct sunlight but for less than half the day

4	moderately shaded from direct sunlight
5	permanently shaded from direct sunlight but otherwise open to the sky
6	in deep woodland shade with no sunflecks
7	in perpetual very deep shade as in a cave entrance

Measuring bark roughness

To measure bark texture quantitatively a "roughness coefficient" can be derived. A piece of string is pinned in the middle of the left side of the quadrat and laid across the bark to the opposite side by pressing it into all the crevices (Figure 5.28). The length of string is then measured and divided by the width of the quadrat to provide a ratio of roughness; modified from: (Glitzenstein & Harcombe, 1979; Rosabal et al., 2012). The smoother the bark the closer the value will be to 1.

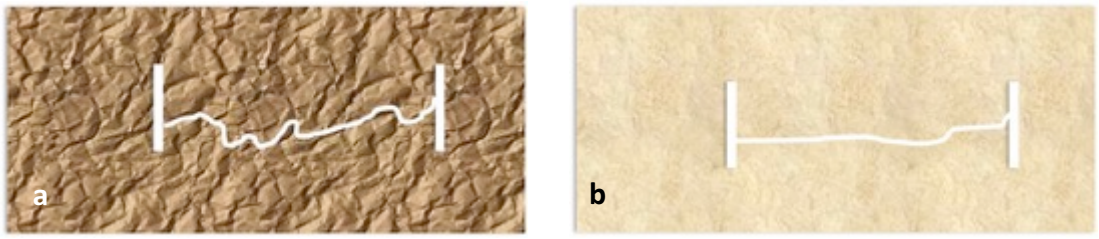


Figure 5.28 Quantitatively measuring bark texture using string on a) rough and b) smooth bark

Bark pH

A small scrape sample of bark was taken from each tree using a cheese-grater that was cleaned between trees. Care was taken not to expose live wood. The scrapings were placed in separate paper envelopes for each tree and transported back to the field laboratory for pH measurements. pH measurements follow the BRECOG method as it is straightforward and easy to reproduce (BRECOG, 2011).

A5.3. Field equipment

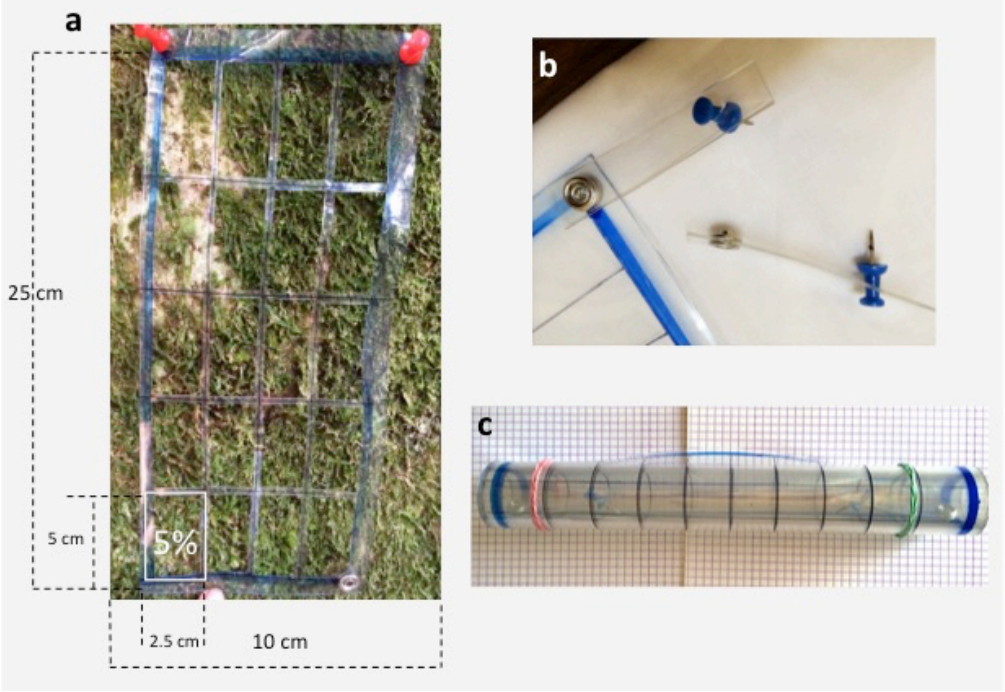


Figure 5.29 Sampling quadrat with 5% grid for estimating percentage cover. a) The quadrat is made out of transparent vinyl with a 5% grid marked out in permanent pen. b) Pins are attached to the quadrat with poppers so that they are not lost, to allow the quadrat to be quickly placed on a tree and allows the pins to be placed in the tree in a range of places which can be useful if want to avoid pinning a particular spot on the tree. c) The quadrat can be easily rolled-up and carried in the field.

A5.4. Data recording

Specimens

When collecting samples from the field it is vital that all the appropriate information is written on the packets in order to be able to identify the specimen packets on return from the field. To facilitate collection in the field, bryologists have developed several methods such as creating pre-written packets (Glime, 2013b). In this study each packet was marked with a unique identification code representing the plot number, the microhabitat type and the sample number of that substrate in that plot (Table 5.14); for example: the second micro-quadrat on a tree base in plot 13 would be coded P13.A1.2. This was modified from a system used by Ah-Peng in Reunion (2007). All dates, both written and digital, use the alphabet month instead of numeric month (i.e. 01/Feb/2016 instead of 01/01/2016), to avoid confusion in future between American and European date writing convention (Glime, 2013b).

Information on specimens collected (date collected, survey point, specimen species/genus if known, collector, substrate) was handwritten into a researcher's field notebook at the end of each day and also input into an excel spreadsheet. Specimens were placed in field packets made from A4 printer paper (non-acidic) and then transferred to clean herbarium packets once dry and back from the field. Duplicates of specimens were left at the national herbarium in Madagascar (Tzimbazaza, TAN).

Table 5.14 Microhabitat codes used to label field packets.

Code	Microhabitat
A1	Epiphyte on trunk base, between 0-0.5m above ground
A2	Epiphyte on tree trunk, between 0.5-1m above ground
A3	Epiphyte on tree trunk, between 1-2m above ground
A4	On exposed tree root
A5	In tree crevice
A6	On tree buttress
A7	On a liana
B1	On mineral soil
B2	On soil with rotting vegetation
B3	On termite mound (soil
C	On rock
D1	On decaying fallen log
D2	Recently fallen tree or branch (not decaying)
D3	On stump base (<50 cm)
D4	On stump trunk (above 50 cm)
D5	On stump top.
D6	On dead standing tree.

Data forms

Field data was collected digitally using Open Data Kit (ODK 1.4.14) on an electronic tablet (Asus Nexus 7 running Android 4.2), which saved time by avoiding manual entry of the field data onto a computer. It also allowed automatic backups of the data to be made instantly on the tablet, as

well as backups on the computer at the end of each field day. During the pilot study two different data entry softwares were tested: ODK and EpiCollect and also the traditional paper data collection forms. Electronic data collection reduced the amount of time needed in the field and ODK was the most flexible in terms of form creation and usability. Electronic data collection techniques will also facilitate future implementation of monitoring as a conservation tool.

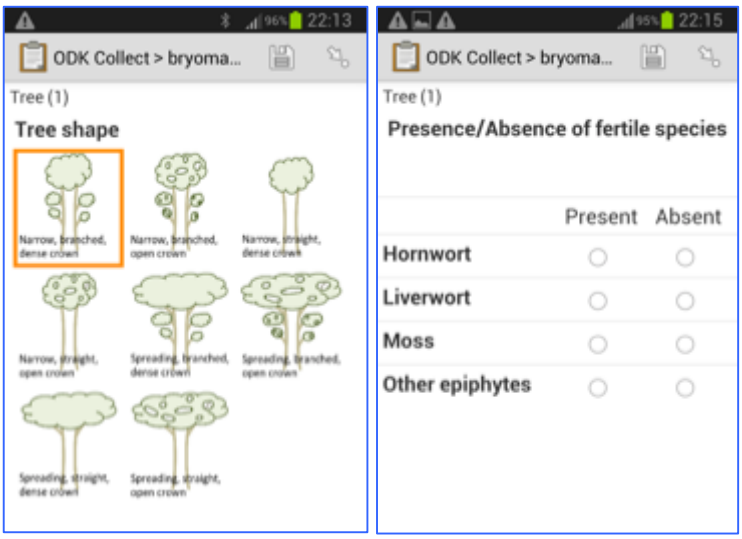


Figure 5.30 Tree architecture question in Open Data Kit (ODK) questionnaire.

Photographic record

Keeping a photographic record of field research is highly valuable for a variety of reasons such as purely illustrative purposes in publications and communications (Frahm, 2003) and double-checking recorded field-data (e.g. bryophyte cover or canopy cover). At every site a photograph was taken of the north, east, south and west aspect; of the canopy and ground cover (as mentioned in section 5.3.2.1, p. 283); of every tree sampled; of every micro-quadrat; and of any interesting feature at the site. Each photograph was databased and named according to the site, and then with either the aspect, canopy, ground or micro-quadrat number e.g. a north aspect photograph of site 12 was labelled as “P.12_north” and a micro-quadrat on the base of the third tree at site 10 was labelled as “P.10_A3.3” (the same coding convention as for specimen packets above).

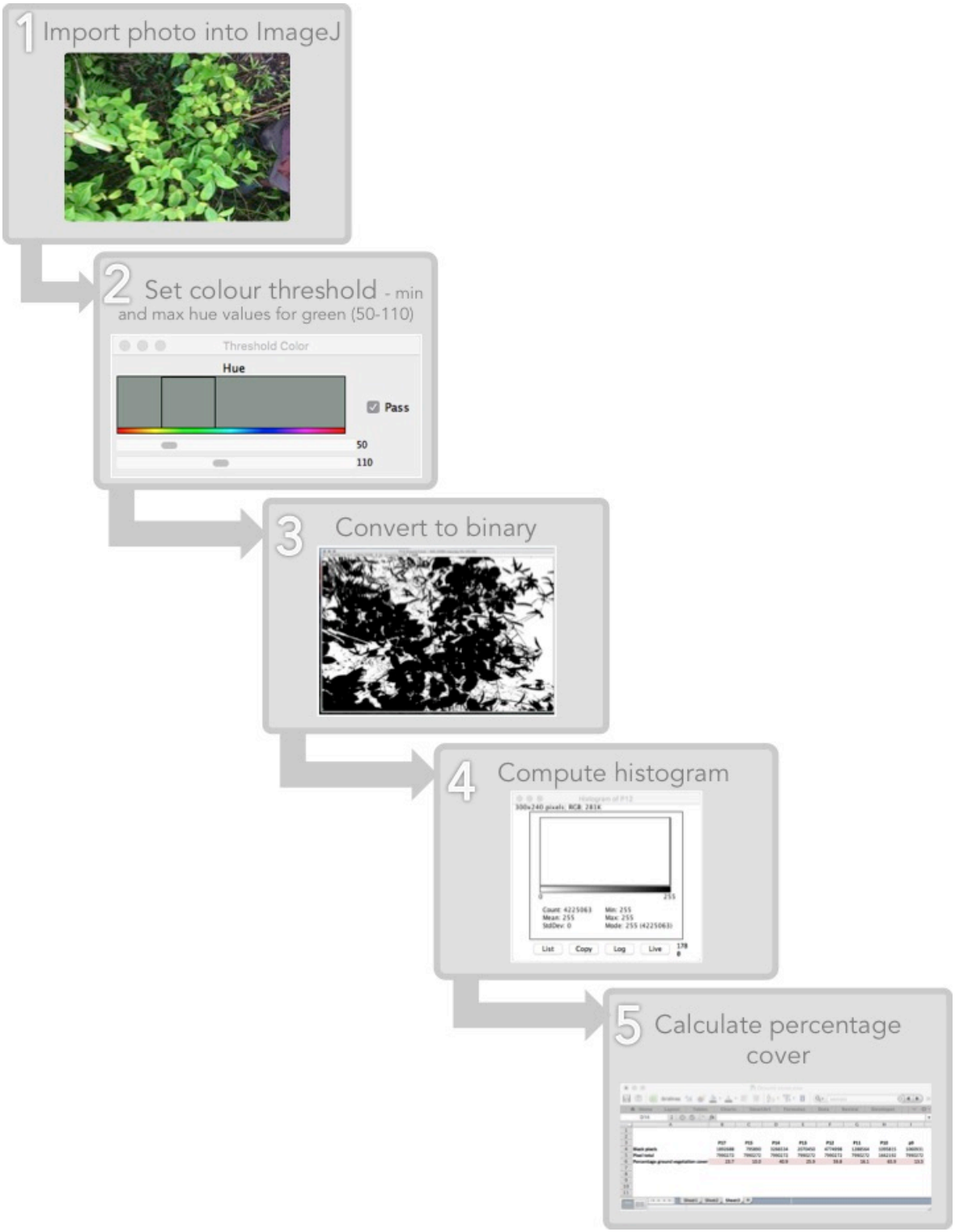


Figure 5.31 Process for calculating canopy and ground percentage cover.

Chapter 6 Conclusions

6.1 Thesis summary

Bryophytes are a morphologically diverse group of plants that inhabit a wide range of ecosystems and habitats; they are arguably one of the most successful plant groups, by the measures of longevity, species richness and distribution. The starting point for this thesis was the fact that bryophytes of different habitats and environmental conditions have different traits – a feature of all living organisms that is long established, and has been studied and discussed widely, including in bryophytes. Together with the fact that bryophytes are micro-climate specific, and are sensitive to changes in their environment, the idea to use them as indicators of habitat change based on the relationship between environmental preferences and traits emerged.

The first step in this study was to summarise knowledge on how different bryophyte traits respond to the environment; how well established the relationship between particular traits and environmental conditions is; and how bryophyte traits can be measured. The basis for the study of bryophyte traits in this thesis is the feature of vegetative desiccation tolerance (DT), which is (in its strict sense) a survival mechanism almost exclusively unique to bryophytes. DT allows bryophytes to survive periods of drought by losing almost all their cellular water without suffering irreversible damage, suspending their metabolic function and then resuming it once water is available once more. Bryophytes are also able to utilise water in forms that most other land plants are not, namely fog and dew, due to their poikilohydry, allowing them to equilibrate their water content with the ambient air humidity. These two features, DT and poikilohydry, confer them with competitive advantage over the other larger terrestrial plant groups, allowing them to inhabit both physical and climatic conditions that are unavailable to the latter (Proctor & Tuba, 2002). However, the trade-off with being able to suspend metabolism means that when water is not available bryophytes are not able to grow or reproduce.

Another important first step was establishing that there is a variation within this DT. Establishing this variation was important, as if there was no variation in DT then it would not be possible to associate particular trait states to different levels of DT, and therefore find indicators of different environmental conditions. Habitat degradation is associated with a change in microclimatic conditions and so it was hypothesised that bryophytes that are sensitive to desiccation and have a low DT level will be good indicators of habitat change. There is a wide range in DT levels with some bryophytes being able to survive long periods of drought at very low relative humidity (5 to 10%) and others not recovering after exposure to much less intense humidity. This has been experimentally demonstrated by many studies, though almost all of these have concerned temperate species, with very few on tropical bryophytes, although this is an emerging field. To date, the level of DT has only been quantitatively categorised in two studies (Wood, 2007; Pardow & Lakatos, 2013), using physiological measurements of DT, with the latter study including tropical forest bryophytes.

Following a review of which bryophyte traits are related to particular environmental conditions, either dry and exposed, or wet and sheltered, the compilation of a trait database was begun. Many trait databases exist in the study of plants, and it is a fertile field with several databases publically available and concerted global efforts exist to create a standardised global databases

for plants (Kattge, Ogle, et al., 2011). However, in bryology it remains an understudied methodology. To the best of my knowledge, there are only two publically available bryophyte trait databases, one for UK bryophytes, BRYOATT (Hill et al., 2007), and very recently (in April 2017) a study was published (Henriques et al., 2017) describing a bryophyte trait database of Azorean bryophytes, showing that this is a methodology gaining popularity in bryology. There have been studies relating traits to the environment (e.g. Hedenäs, 2001) although not within the context of a trait database.

Although the aim is to study Malagasy bryophytes, due to the lack of knowledge of this bryoflora and the level of threat they face, exactly because of this lack of knowledge, compiling a database solely with Malagasy bryophytes would be beyond the time-frame of this PhD as there would be insufficient species for robust analyses. I therefore decided to create the trait database with Portuguese species as well because it is a relatively well known flora and one that I am familiar with and so I was able to use expert knowledge during the compilation. Malagasy species were also scored for traits, although the level of missing data is much greater for these. In total, 42 morphological and reproductive traits were recorded, as well as nine habitat and environmental variables in 1011 species (although the level of trait completeness varies). Over 100 literature sources were used to compile the database, and additionally over 100 herbarium specimens. The importance of historical collections is highlighted as for many Malagasy species the only source for trait data was herbarium specimens, due to the lack of taxonomic publications or revisions on most Malagasy bryophytes. Concurrently, georeferencing of herbarium specimens was undertaken, again showing the importance and relevance of herbarium collections in modern ecological and conservation studies as ecological and habitat data available from specimen labels was also recorded. This will also serve as a resource for future bryological studies in Madagascar.

In order to be able to relate species traits to their environmental preferences, and environmental index (EI) value was assigned to each species based on a combination of their humidity and light preferences. Analyses of the trait database showed that different traits and trait states do vary significantly according to the environmental conditions of a species. From these results, a subset of traits were selected and were used in a multivariate analysis to group species based on their morphological trait similarity. Analyses were successful in grouping species into two groups, one that comprises species of humid and sheltered environments, and the other dry and exposed environments. Extracted from this was a trait profile that defines the two groups of species. All other species in the database were then assigned a trait profile value based on the proportion of presence of traits within each trait profile. This maximised the data availability in the database, which is particularly useful for species where data is lacking or is hard to obtain. An indicator value (IV) was created and this is the basis for determining which species, genera and family could be used as suitable indicators, with species that have narrow environmental preferences highlighted as likely to be most useful. As well as species-level, genus and family level IVs were calculated to test if the IV can be successfully scaled. These results show how taxonomic characters which are commonly used in species identification and revision studies, can be applied successfully to other fields in biology. It reinforces the need for taxonomists and taxonomic study as many of the sources used to create the trait database were taxonomic publications, as well as the methods for assessing species trait states.

The next step was to ascertain if the traits selected and the IV obtained using literature and specimen data reflected the distribution of bryophytes in the field. Field research was conducted

in Madagascar, within lowland forest habitat, in different forest degradation types where sampling of bryophytes was undertaken in different microhabitats (namely epiphyte, soil and rock). The trait life-form was shown to be significantly related to different forest degradation types, showing that it is a useful trait to use as an indication of forest degradation.

The IV also varied between forest degradation and land-use types, and in different habitat types (humid and dry forest), but further testing is required. The response obtained when looking at life-form in Malagasy field specimens reflects what was expected, based on univariate analyses from Chapter 3. This indicates that it is possible to use trait data from species in one region to predict responses of species in another, but more detailed analyses of both the field data and the trait data will be required to establish this.

6.2 The trait database

One of the main outputs from this PhD is the creation of a trait database (Figure 6.1) that includes tropical bryophyte species, the first of its kind. This PhD study was necessarily broad as there was no basis for the study of Malagasy bryophyte traits, and no publically available database on bryophyte traits associated with desiccation tolerance or species environmental preferences. It was therefore necessary to consult many different data sources for Malagasy species. In order to ensure there was a sufficient number of species with data within the time-frame of this PhD, data was collected for Portuguese species, as this is a relatively well-known bryoflora that is actively being studied (Garcia et al., 2013; Cacciatori et al., 2015). Additionally, Portugal's location at the southwestern tip of Europe means its bryoflora has some subtropical affinities (Sérgio et al., 2013) and so reducing the disparity between European and Malagasy species in terms of trait responses to the environment allowing data from Portuguese bryophytes to be used for Malagasy bryophytes.

Although the trait analyses included 530 species (as these were the species which had 100% completeness in all traits as well as environmental, habitat and distribution variables), which by vascular plant trait analyses is not a large dataset (Díaz et al., 2016), it represents an important contribution to the study of bryophyte traits and their relationship with the environment. The large number of traits (Figure 6.1) can also provide data for studies of a different nature. The database itself has 1430 species, of which 731 are from Madagascar, but data is still missing for many Malagasy species. It was possible within the scope of this PhD to gather substantial data on traits for tropical forest families (e.g. Calymperaceae and Orthotricaceae).

As the trait database compiled in this study was produced from the ground-up, it provided the opportunity to add data tailored to the needs of this study, as well as allow me to directly verify data quality. It is the starting point for a full trait database for Malagasy species and provide a baseline for other tropical bryofloras.

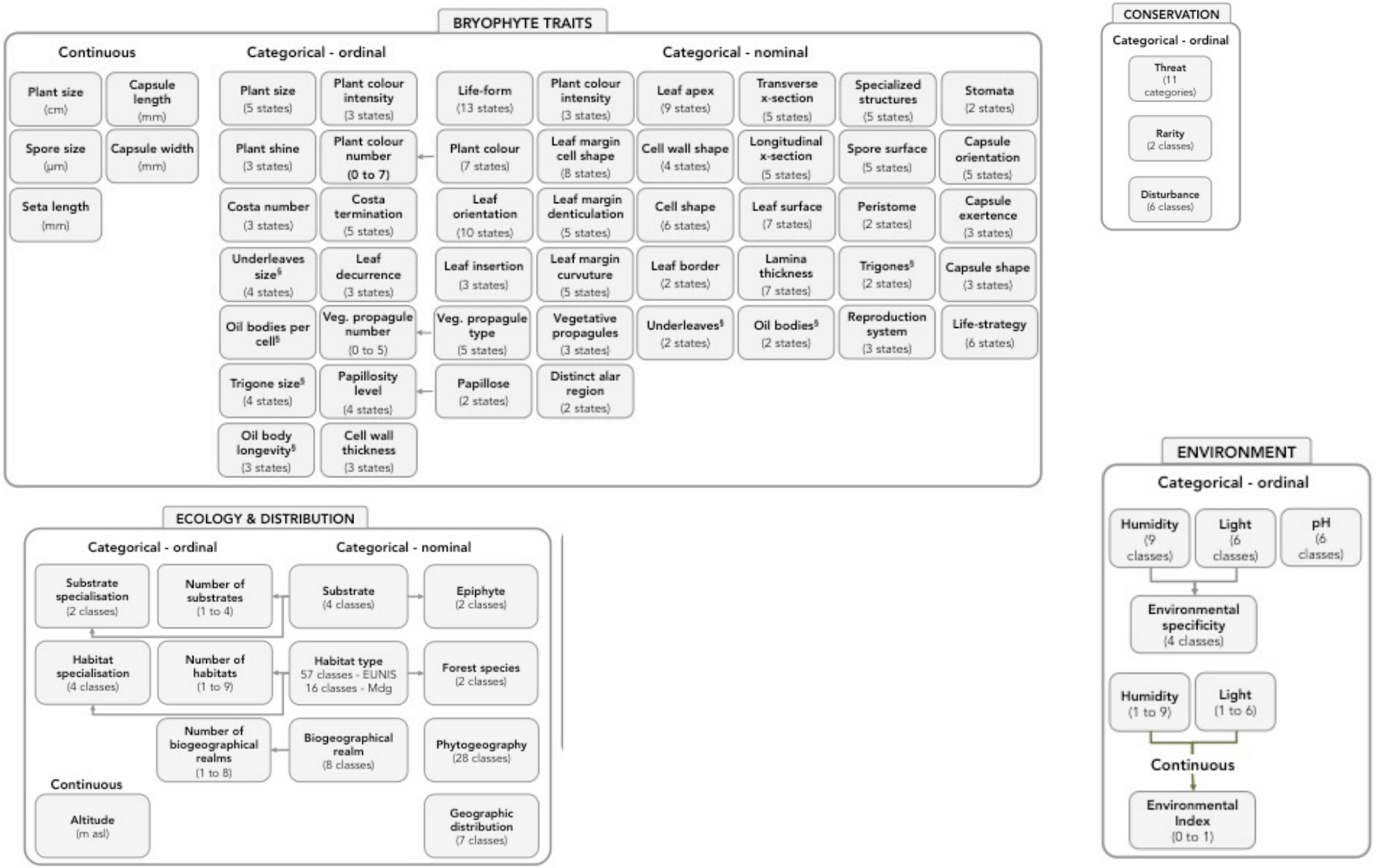


Figure 6.1 Summary of traits recorded in the bryophyte database, and ancillary environmental, ecological, distribution and conservation traits. Arrows indicate where traits were derived from.

6.3 Conservation applicability

The relatively low bryophyte research effort in Madagascar when compared to other tropical regions, even within Africa, means that the specimens collected during this PhD will greatly add to bryofloristic knowledge in Madagascar. The level of threat of Malagasy ecosystems means there is urgency in doing this. At a local level, the creation of a bryophyte checklist for Tsitongambarika Forest National Park will highlight the importance of conserving this forest to policy makers, and show the value of protected areas in attracting researchers. Another important contribution of this study is that it provides a baseline of species data, which is lacking in bryophytes, and is vital for effective biodiversity monitoring.

An additional result from the field data is that the differences found between microhabitats shows that it is sufficient to sample understorey microhabitats; this has a practical implication for conservation methods as surveying the upper canopy is more logistically and time intensive. This makes bryophytes cost-effective, a vital feature of indicators, particularly in the tropics (Gardner et al., 2008). Life-form is a useful trait to use as an indication of forest degradation as open life-forms (dendroid, fans and wefts) were only found in forest sites that had moderate degradation. This provides a method of using indicators that requires no specialist knowledge and little training.

While the indicator value calculated in this study showed some variation between forest degradation types and microhabitats, another application could be as indicators of other biodiversity components. Bryophytes have been shown to indicate diversity in other taxonomic groups, both in tropical and temperate areas (Leal et al., 2010). Life-form continues to be a realiable indicator, as open life-forms were not found in heavily degraded forest or non-forest plots. This means that bryophyte life-forms could be used as a quick, easy and cost-effective way to monitor forest degradation.

The difficulty of bryophyte identification was highlighted in this study, as most specimens were not yet identified to species-level, thus limiting the usefulness of bryophytes as indicators. However, bryophyte identification in other areas of the world (particularly temperate and neotropical) is easier and so bryophytes still have potential as indicators. Aditionally, it was found that easy-to-measure traits, such as life-form and size, varied with the indicator value and so could be a way of circumventing the identification problem.

Another important conclusion from this study is the usefulness of creating an index to quantify forest degradation. Whilst the index in this study was a first step, and refining is needed, it nonetheless evidenced that is provides greater insights into species' responses to degradation. Aditionally, valid comparisons of study plots would be possible if degradation is quantified, as in the index created here. This would help resolve some of the disparities seen in bryophyte and other taxa responses (Frego, 2007; Streubig et al., 2013) and so contribute to realiable data interpretations and its application in conservation management.

6.4 Limitations

Once a bryophyte is established in a habitat, it may persist even once the microclimatic conditions have changed due to their ability to survive drought. This would mean that a degraded habitat may have species that are normally associated with a less degraded habitat. Bryophyte longevity comes into play here (Rydin, 2009), and a further refinement of bryophyte indicators could include those that have annual life-cycles, rather than those that are perennials. A new trait could be easily created as life-strategy data is already included in the database. Henriques et al. (2017) suggest grouping the bryophyte life-strategies into K- and R- strategists (fugitives, colonists and annual shuttles in the former, and medium shuttles, perennial stayers and dominants in the latter).

Because each species is only represented once on the trait database there is no intraspecific trait variation. A further refinement in assigning species an indicator value would be to take into account their morphological variation. This is important as a species that has high plasticity may not be suitable as an indicator because it is able to adapt its morphology and survive in changing environmental (or habitat) conditions (Vitt et al., 2014; Stark & Brinda, 2015a). However, as this is a more data-intensive process, and hence more time-consuming, it should be undertaken after a preliminary selection of species, and focus on those alone.

As indicated, bryophyte identification is not straight-forward, which is one of the main requirements for suitable indicators (Butler et al., 2012). This is particularly the case for tropical bryophytes, at least in Madagascar, where not only there is no bryophyte flora, but also very few taxonomists focussing on Madagascar. Therefore, how useful would bryophyte indicators be in the tropics? Previous tropical bryophyte research has suggested that they make good indicators (Drehwald, 2005) due to their sensitivity, and this study found that they do vary according to habitat degradation, therefore from an ecological perspective they have potential for successful indicators. In practical conservation terms, however, in a country where there are no resident bryologists or where bryological study is limited, creating species lists of indicators has limited use. Using bryophyte traits could be a way to overcome this, such as life-form – a very easy to observe trait, that does not require specialist equipment (i.e. a microscope). Another parameter could be bryophyte cover or abundance, which would be useful in monitoring forest health as shown in other studies (Frego, 2007) and it was shown in this study to vary significantly between different forest disturbance levels.

6.5 Future research

As has been discussed, the byroflora of Madagascar is critically understudied. Considering how intrinsically linked the study of plant traits and plant taxonomy are – this work may also be used for creating a flora of the Malagasy Bryophytes. Even with the lower rates of speciation typically seen in bryophytes it is certain that there remains a much to discover about this interesting flora. An indication of this is the fact that within the specimens collected during this PhD's fieldwork a new subspecies of moss (*Syrrhophodon* sp.) has been found (pers comm. Len Ellis).

One the indicator index is refined, creating maps of indicator bryophyte species will be useful to assess the status of other bryophytes, and also potentially vascular plants. Using the specimens

georeferenced in this study as a starting point, ecological niche modelling coupled with the indicator index could provide a method of assessing the impacts of forest degradation on bryophytes, and other taxa.

As mentioned, the IV needs refining. Following identification of all specimens to species level, which will involve sending some specimens to expert bryologists for identification or confirmation, physiological measurements of DT of field specimens will be undertaken focussing on species from different habitat disturbance types, including those found exclusively in one type of habitat. This will fit in with similar research that has looked at how species with different life-forms in tropical forests have varying DT (Proctor, 2002; Song et al., 2015). In addition, further analyses will then be performed – fourth-corner and RLQ multivariate analyses (Dray et al., 2014), which will combine environmental and habitat data collected from the field, species trait data and species abundance. This will provide an in-depth look at how traits vary across habitat types and feed into further refining and calibrating the indicator selection method.

Because life-form consistently showed a relationship with both environmental conditions and forest degradation, this is a line of research worth pursuing. Establishing a forest monitoring protocol based on recording bryophyte life-forms in understorey microhabitats would be a a simple and cost-effective way to monitor biodiversity. Future studies should look into the correlation of bryophyte life-forms with other taxa and forest degradation indicators.

While a complete body of work in itself, this study has been the starting point for further trait research on tropical bryophytes. The next step is to focus collection of trait data on certain species, including bryophytes that are on the Sampled Red List Index, as this project is currently assessing the Red List Status of 1500 bryophytes globally (Brummitt et al., 2015), including tropical ones. To further refine the trait analyses, focus will be given to those traits that were found to be significantly associated with different environmental conditions and bryophyte desiccation tolerance.

List of Acronyms

ABA	Abscisic acid
Af	Agriculture - former
A	Agriculture - current
BM	Herbarium of the London Natural History Museum, formerly at the British Museum, UK.
C1	Cluster 1 – a cluster of species that indicate dry and exposed environments.
C3	Cluster 3 – a cluster of species that indicate wet and sheltered environments.
CoBas	Village community associations in Madagascar who manage and ensure protection and sustainable use of the forest.
CoGEs	Co-management associations in Madagascar who manage protected areas. Similar to CoBas, but at a higher administrative level.
DBH	Diameter at breast height measured at 1.3m above ground.
DI	Degradation Index
DT	Desiccation tolerance
EI	Environmental Index
GNI	Gross National Income
IUCN	International Union for the Conservation of Nature
IV	Indicator Value
LISU	Herbarium of Lisbon Natural History and Science Museum, Portugal.
MCA	Multiple Correspondence Analysis - a type of principle component method (similar to Principal Components Analysis or Correspondence Analysis) but unlike these, which use continuous variables, it uses categorical variables.
NAP	New Protected Area - IUCN Category V
PC	Cryptogamic Herbarium of the National Museum of Natural History in Paris, France.
PD	Moderately to heavily degraded Primary forest

PPFD	Photosynthetic Photon Flux Density
PU	Undisturbed or slightly disturbed Primary forest
RH	Relative Humidity
RLI	Red List Index
RWC	Relative Water Content
SAPM	The System of Protected Areas of Madagascar
sp. | spp. (pl.)	Abbreviation for species or multiple species (plural)
SRLI	Sampled Red List Index
TAN	Herbarium of Parc de Tsimbazaza, Antananarivo, Madagascar
TGK	Tsitongambarika Forest Protected Area
Tukey HSD	Tukey Honest Significant Difference post-hoc multiple comparisons test

Glossary

Acrocarp | acrocarpous (adj.) gametophyte is unbranched, sporophytes develop at the end of the stem and they tend to grow upright; applies to mosses. [Figure 1.11 L, p. 16]

Angiosperm flowering, seed-producing tracheophytes (land plants).

Antheridium | antheridia (pl.) male sex organ. [Figure 1.5, p. 10]

Anthocerophyta one of the three phyla of small terrestrial plants that are referred to as "bryophytes"; they are dark green in appearance and leafless (thalloid). To date there are 14 genera distributed among 5 families. Commonly known as hornworts. See also *Hornwort, Bryophyte.*

Archegonium | archegonia (pl.) female sex organ [Figure 1.5, p. 10]

Autoicous a monoicous bryophyte with antheridia and archegonia on separate branches on the same plant (Casas et al., 2006, p. 321). See also *Paroicous, Synoicous, Monoicous.* [Figure 1.6, p. 10]

Bryophyta one of the three phyla of small terrestrial plants that are referred to as "bryophytes"; they have leaves arranged around a main stem that may be branched or not. Commonly known as mosses. See also *Moss, Bryophyte.*

Bryophyte a group of small terrestrial plants that uniquely have a life-cycle with a dominant gametophyte (haploid) generation and produce spores. This group is composed of the following taxonomic phyla: Bryophyta (mosses), Marchantiophyta (liverworts) and Anthocerophyta (hornworts).

Bryophyte group refers to the taxonomic rank "phylum": Bryophyta, Marchantiophyta or Anthocerophyta.

Bulbil a specialized organ of asexual reproduction shaped like a small bulb (Paton, 1999, p. 606; Casas et al., 2006, p. 322). [Figure 2.13, p. 77]

Calyptra — a group of tissues that cover the capsule as it develops thought to be involved in protecting the capsule. In some species it is visible as a "hat" on the mature capsule [Figure 1.11 M, p. 16].

Capsule — a usually cylindrical or globose structure at the end of the sporophyte where spores develop and from which they are released when mature [Figure 1.11 F & M, p. 16]

Cilium | cilia (pl.) — a slender, simple extension of a margin or apex consisting of a row of 2-6 cells in length or more (Paton, 1999, p. 606). [Figure 2.10, p. 73]

Conduplicate — a folded part of the leaf creating a pocket-like structure on the leaf.

Coprophile — a species that grows upon dead animals or dung.

Costa — multistratose median region of a leaf that provides structural support to moss leaves and transports water. See also *Midrib.*

Cryptogams — generic term for a group that encompasses ferns, algae, bryophytes, lichen and fungi; essentially non-flowering plants and fungi.

Cushion — a bryophyte life-form: numerous shoots very close together forming dome-shaped colonies (Chuah-Petiot, 2003 & Hill et al., 2007). [Figure 2.4, p. 4]

Degradation — "the reduction of the capacity of a forest to provide goods and services" (FRA, 2012, p. 26). This includes soil erosion, nutrient depletion and disturbances in biological cycle. See also *Deforestation, Forest integrity, Disturbance.*

Deforestation — "the conversion of forest to other land use or the permanent reduction of the tree canopy cover below the minimum 10 percent threshold" (FAO, 2012, p. 5).

Dendroid — a bryophyte life-form: main stem erect with large leaves at top or many lateral shoots (Chuah-Petiot, 2003 & Hill et al., 2007). [Figure 2.4, p. 4]

Desiccation tolerance	a strategy that has enabled plants to adapt to life on the relative dry terrestrial environment by avoiding damage from lack of water availability: the "ability to reach equilibrium with air that is moderately to extremely dry and then regain normal function after rehydration" (Alpert, 2005, p. 686). DT mechanisms can be classified as constitutively DT (CDT) - they can survive rapid drying with minimal damage - or inducibly DT (IDT) – they require slow drying in order to minimise damage and can be considered to go through a hardening process (Stark et al., 2014; Stark & Brinda, 2015). Put simply, CDT species recover fast following desiccation as the mechanisms are already in place, whereas IDT species recover slowly.
Dioicy | Dioicous (adj.)	a plant species that bears male and female reproductive organs on separate individual plants. See also *Monoicous.*
Diploid	cells that have a double set of chromosomes "2n"; the sporophyte is composed of diploid cells. See also *Haploid, Sporophyte.*
Disturbance	"environmental fluctuation and destructive event that affects forest health, structure, and/or changes resource or physical environment at any spatial or temporal scale" (Simula, 2009, p. 30). In this thesis it refers to disturbance form human-related activities. See also *Degradation, Forest integrity.*
Division	refers to a high level taxonomic rank and is synonymous with *phylum* (McNeill et al., 2012, Article 3.1).
Ecophysiology	the physiological ecology of species – how a species' physiology relates to environmental conditions.
Ectohydric	a plant where water conduction takes place in its external capillary spaces - the case in bryophytes.
Epiphyll	a species that grows on leaf surfaces.
Epiphyte	a species that grows on bark.
Epixylic	a species that grows on logs and stumps.
Fan	a bryophyte life-form: branches in plane on vertical substrate (Hill et al., 2007, p. 11). [Figure 2.4, p. 4]

Forest integrity — "the capacity of an ecosystem to support and maintain a (…) community of organisms having a species composition (…) comparable to that of, and representing the full range of variability in, similar undisturbed ecosystems in the region (Frego, 2007, p. 67). See also *Degradation.*

Gametophyte — multicellular haploid stage developing the sex organs; (…) it forms the dominant vegetative phase of the life cycle of bryophytes (Vanderpoorten & Goffinet, 2009, p. 260). [Figure 1.5, p. 10]

Gemmae | gemmae (pl.) — a unicellular or multicellular specialized body mainly borne on leaves and thalli and capable of asexual reproduction (Paton, 1999, p. 609). [Figure 2.13, p. 77]

Growth-form — the "(…) positions of [a plant's] growing points, its mode of branching, leaf orientation, etc." (Bates, 1998, p. 224)

Gymnosperm — non-flowering, seed-producing tracheophytes such as conifers.

Haploid — cells that have a single set of chromosomes, "n"; the gametophyte is composed of haploid cells. See also *Diploid, Gametophyte.*

Hornwort — one of the three phyla of small terrestrial plants that are referred to as "bryophytes"; they are dark green in appearance and leafless. To date there are 14 genera distributed among 5 families. See also *Anthocerophyta, Bryophyte.*

Humidity — water vapour present in air

Hyalocyst — large hyaline cell that acts as reservoir for water in bryophytes allowing them to maintain their metabolic functions for longer when the environment becomes drier (Frahm, 2000; Proctor, 2009).

Hydroid — specialised cells that conduct water; only present in species of the Polytrichaceae and Mniaceae families (Bryophyta).

Insolation — the amount of solar irradiation reaching a particular point on earth. Also known as the incident solar radiation. See also *Light.*

Lamella | lamellae (pl.) — ridges or plates along a leaf blade or nerve (Casas et al., 2006, p. 324). [Figure 2.8, p. 72]

Lamina | laminae (pl.) "the flat blade of a leaf not including the nerve" (Casas et al., 2006, p. 324).

Life-form life-form is "(…) the assembly of [plants'] shoots into colonies" (Bates,1998, p. 224). See also growth-form. [Figure 2.4, p. 4]

Life-strategy a concept that brings together different aspects of bryophytes' morphology and life-history: life-span, reproductive effort, reproduction type, age of first reproduction, spore size, longevity and growth-form (During, 1979). Life-strategy is a useful concept as it helps explain and determine bryophyte distribution and aspects of their ecology (Bates, 2009).

Light used in this study to refer to the amount of insolation that is available for photosynthesis and measured as the amount of photons: Photosynthetic Photon Flux Density (PPFD, µmol m-2 s-1). See also *Insolation.*

Litter species a species that grows on non-ligneous substrates, such as leaf litter.

Liverwort one of the three phyla of small terrestrial plants that are referred to as "bryophytes"; they have a flattened appearance (Crandall-Stotler et al., 2009) and either have leaves arranged around a stem or are leafless and thalloid. See also *Marchantiophyta, Bryophyte.*

Lobule the small, plane, convex or helmet-shaped ventral part of folded liverwort lobes (Paton, 1999, p. 610). [Figure 2.12, p. 76]

Lowland tropical rainforest evergreen dense forest on the East coast of Madagascar at an altitude of up to 400m receiving 2000-3000 mm annual precipitation. Note that lowland tropical forest in other regions of the world can be up to 1000m in altitude - the definition in this thesis is in the context of Malagasy forests.

Marchantiophyta one of the three phyla of small terrestrial plants that are referred to as "bryophytes"; they have a flattened appearance (Crandall-Stotler et al., 2009) and either have leaves arranged around a stem or are leafless and thalloid. Commonly known as liverworts. See also *Liverwort, Bryophyte.*

Mat, rough a bryophyte life-form: creeping, lateral branches erect (Hill et al., 2007, p. 11). [Figure 2.4, p. 4]

Mat, smooth — a bryophyte life-form: creeping, branches lying flat (Hill et al., 2007, p. 11). [Figure 2.4, p. 4]

Mat, thalloid — a bryophyte life-form: creeping, thalli forming a layer (Hill et al., 2007, p. 11). [Figure 2.4, p. 4]

Midrib — multistratose median region of a thallus (Paton, 1999, p. 606) which provides structural support in liverworts. See also *Costa.*

Monoicy | Monoicous (adj.) — a plant species that bears male and female sex organs on the same individual plant. See also *Dioicious.*

Moss — colloquially the term moss is applied to any bryophyte, but it is used in this thesis to refer solely to the Bryophyta phylum. One of the three phyla of small terrestrial plants that are referred to as "bryophytes"; they have leaves arranged around a main stem that may be branched or not. See also *Bryophyta, Bryophyte.*

Neotropics — tropical America.

Oil body — membrane-bound organelle that contains terpenoid oils and aromatic compounds (Crandall-Stotler & Crandall-Stotler, 2000).

Operculum — opening at the end of moss capsules with lid-like structure - this is absent in fourmoss genera: Andraeae and Acrochisma (4 longitudinal slits); Takakia (spiral slit); Andreobryum (various longitudinal slits). [Figure 1.11 M, p. 16]

Osmotic potential — the potential of water to move from a solute with high water concentration to a solute with lower water concentration.

Paleotropics — tropical Africa and Asia.

Pappila | pappilae (pl.) — small protuberance of a cell by a local thickening of the cell wall (Casas et al., 2006, p. 325). [Figure 2.10, p. 73]

Paraphyllium | paraphyllia (pl.) — photosynthetic filamentous or foliose appendages in mosses (Vanderpoorten & Goffinet, 2009, p. 263).

Paroicous — a monoicous plant with the antheridia just below the archegonia (Casas et al., 2006, p. 325). See also A*utoicous*, S*ynoicous*, *Monoicous.* [Figure 1.6, p. 10]

Pendant — a bryophyte life-form: creeping stems on twigs with long secondary stems (Chuah-Petiot, 2003). [Figure 2.4, p. 4]

Perichaetium | perichaetial (adj.)	the archegonia from where the sporophyte develops; perichaetial leaves are leaves that surround the archegonia and eventually the sporophyte.
Peristome	a ring of filaments surrounding the capsule operculum of mosses thought to regulate the release of spores.
Photosynthetic Photon Flux Density	(PPFD) the amount of photons available for photosynthesis, with the units µmol m-2 s-1.
Phylum | phyla (pl.)	the second-highest taxonomic classification rank, after "kingdom" (e.g. Plant) and before "class" (e.g. Bryopsida – a class of mosses).
Plant group	an informal grouping of plant phyla with similar characteristics as used in the Plant List (2013).
Pleurocarp | pleurocarpous (adj.)	gametophyte is branched, sporophytes develop on the stem/branch and they tend to grow horizontally along the substrate; applies to mosses. [Figure 1.11 J, p. 16]
Poikilohydric	an organism that is unable to actively regulate its water content, as is the case with bryophytes.
Primary forest	naturally regenerated forest of native species, where there are no clearly visible indications of human activities and the ecological processes are not significantly disturbed (FAO, 2012, p. 7).
Pteridophyte	spore-producing tracheophytes such as ferns.
Pyrenoid	protein structure which contains high concentrations of the photosynthetic enzyme RuBisCO, unlike in other land plant chloroplasts where RuBisCO is found on starch grains (Renzaglia et al., 2009). This fundamental difference in the chloroplasts is shared with algae and it is not fully understood what physiological purpose it serves (Villarreal & Renzaglia, 2015).
Red List Index	an index created to track changes and trends in species' threat (Butchart et al., 2004). A set of species is repeatedly assessed at set intervals using the IUCN Red List Criteria (IUCN, 2012) and an index is then calculated based on the threat category the species are in; by comparing the index between assessments, changes in extinction risk can be tracked (Butchart et al., 2004, 2007). The lower the index value, the more threatened the species or group of species.

Relative Water Content	the amount of water a plant can uptake. It is used to measure the plant's water capacity when dry relative to its capacity at full turgor (maximum water capacity) (Proctor et al., 1998).
Rhizoid	branched root-like, slender filaments that arise from the stem and usually anchor the gametophyte to the substrate (Casas et al., 2006, p. 326).
Sampled Red List Index	an index which calculates the RLI for a sample of species from an animal or plant group that has few species assessed on the Red List. That sample is then used to monitor trends for the group overall (Baillie et al., 2008; Brummitt et al., 2015).
Saxicole | saxicolous (adj.)	a species that grows on rock surfaces.
Seta	a stalk that emerges from the bryophyte and supports the capsule. It can be elongated or very short. [Figure 3.5, p. 104]
Sporangium | sporangia (pl.)	specialized region of the sporophyte (…) within which spore mother-cells are formed and undergo meiosis to yield haploid spores (Vanderpoorten & Goffinet, 2009, p. 265). [Figure 1.5, p. 10]
Spore	a unicellular, haploid reproductive body produced in the sporangium as a result of meiosis (Casas et al., 2006, p. 327). They are usually spherical with a surface that can be smooth, rough or winged. [Figure 1.5, p. 10]
Sporophyte	multicellular stage of the life-cycle of plants characterised by a diploid genome and producing spores in specialized tissue (Vanderpoorten & Goffinet, 2009, p. 265). [Figure 1.5, p. 10]
Stoma | stomata (pl.)	minute, epidermal opening of the capsule, usually at base, surrounded by two kidney-shaped cells (Casas et al, 2006, p. 327).
Synoicous	a monoicous plant with the antheridia and archegonia mixed (Casas et al., 2006, p. 327). See also *Paroicous, Autoicous, Monoicous.* [Figure 1.6, p. 10]
Taxon | taxa (pl.)	specifically in this thesis, it refers to an organism that is at the taxonomic rank of species, subspecies or variety e.g. the species *Polytrichum commune* has three varieties: *Polytrichum commune* var. *commune* Hedw., *Polytrichum commune* var. *humile* Sw., *Polytrichum commune* var. *perigoniale* (Michx.) Hampe; they are three taxa.

Temperate	regoins of the world where the climate is mild, there are no extremes of temperature or precipitation. Located between latitudes of 23°N/S to 66°N/S.
Teniolae	intra-marginal, elongate and hyaline cells present in some Calymperaceae species.
Terricole | terricolous (adj.)	a species that grows on soil.
Thallus | thalli (pl.)	loosely differentiated fleshy lobes, which can be arranged in rosettes or be spreading, present in hornworts and in some liverwort species [Figure 1.11 C, D & P, p. 16]
Tomentum | tomentose (adj.)	felt-like covering made up of abundant rhizoids on the stem of mosses (Casas et al., 2006, p. 328; Vanderpoorten & Goffinet, 2009, p. 265).
Tracheophyte	land plants that possess a vascular system, xylem and phloem, for the transport of nutrients and water. Used in this thesis to refer to any terrestrial plant that is not a bryophyte.
Trait	any morphological, physiological or phenological feature measurable at the individual level, from the cell to the whole-organism level (…)" (Violle et al., 2007, p. 884).
Trait state	refers to a term used to describe a trait e.g. dendroid, fan, pendant, cushion, turf and tuft are six states within the life-form trait.
Trigones	thickenings at the corner of cells where thin or somewhat thickened walls meet (Paton, 1999, p. 615).
Tropical	broadly defined as any region of the world where the climate is always hot and the dry season is short or absent (Primack & Corlett, 2005, p. 3). See also: *Neotropical* and *Paleotropical*.
Tuber	a subterranean globose, ellipsoidal or reniform body produced on margins and ventral side of thalli capable of asexual reproduction (Paton, 1999, p. 615; Casas et al., 2009, p. 165). [Figure 2.13, p. 77]
Tuft	a bryophyte life-form: loose cushions, not dome-shaped (Hill et al., 2007, p. 11). [Figure 2.4, p. 4]
Turf	a bryophyte life-form: vertical stems with little or no branching (Hill et al., 2007, p. 11). [Figure 2.4, p. 4]

Turf, protonemal — a bryophyte life-form: persistent protonema (Hill et al., 2007, p. 11).

Turf, scattered — a bryophyte life-form: scattered vertical shoots (Hill et al., 2007, p. 11). [Figure 2.4, p. 4]

Underleaves — a third row of leaves along the ventral side of a stem or branch, present in some liverworts; they are usually smaller than the other stem leaves (Paton, 1999, p. 615; Casas et al., 2009, p. 165). [Figure 2.12, p. 76]

Vegetative propagules — specialized structures produced by bryophytes that allow asexual reproduction. See also *Bulbil, Gemma, Tubers.* [Figure 2.13, p. 77]

Water sac — a lobule that is helmet-shaped in liverworts species of the Porrelales and is used for water storage. [Figure 2.12, p. 76]

Weft — a bryophyte life-form: intertwining branched layers (Hill et al., 2007, p. 11). [Figure 2.4, p. 4]

Wing — the lamina of a thallus (Casas et al., 2009, p. 165).

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