Ecophysiological specialisation: an explanation for the unimodal distribution of bryophytes on the Piton des Neiges gradient, La Réunion?

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ABSTRACT

Bryophytes along the altitudinal gradient of Piton des Neiges on Réunion Island display a unimodal pattern of species richness. This study tests the climatic favourability hypothesis to determine whether ecophysiological specialisation to humidity can be used to explain the unimodal distribution of bryophytes along this gradient. In order to test this hypothesis, climate data were analysed and changes in photosynthetic efficiency were tracked using chlorophyll fluorescence in dehydration experiments and subsequent rehydration experiments on bryophytes collected from this altitudinal gradient. The climate data showed that the mid-altitude site had the fewest dry days and dry spells, and no dry spells that lasted as long as seven days. However, the mean daily humidity was lowest at the low-altitude site. It was found that high altitude and widespread species did significantly better than low- and mid-altitude species when dehydrated and rehydrated. Although the results show an interesting pattern of separation between the specialisation of range-restricted species at each site and widespread species, ecophysiological specialisation to humidity does not fully explain the unimodal distribution of bryophytes along the gradient. Therefore, the interaction between humidity and other factors, such as temperature, needs to be considered. This study broadens our understanding of how bryophytes are influenced by climate and how this shapes their distribution, and how their distribution might change in a changing climate.

INTRODUCTION

There is a noticeable change in the environment with increasing altitude. In their voyages around the world, Darwin, Wallace and Von Humboldt were the first to write in-depth accounts of this phenomenon. Since then, elevational gradients, along with latitudinal gradients, have been the focus of considerable interest with regards to understanding species distributions (Lomolino, 2001).

The pattern of species richness along latitudinal gradients is one where richness decreases monotonically towards the poles. The pattern of species richness along altitudinal gradients was long thought to mirror this, because both gradients span a transition from warm to cold climatic conditions. However, Rahbek (1995, 2005) showed that this view was the result of an overemphasis on a few studies. In fact, three main patterns have emerged for different taxa in different areas: a uniform decline in richness with altitude (e.g. bacteria in the Rocky Mountains in Colorado; Bryant et al., 2008), a uniform increase in richness with altitude (e.g. salamanders in the Gomez Farias region; Martin, 1958), and a unimodal pattern where richness peaks at an intermediate position on the gradient (e.g. bryophytes on Réunion Island; Ah-Peng et al., 2012). This unimodal pattern is by far the most commonly reported and characterises a very wide range of organisms and localities (e.g. Nor, 2001; Vetaas & Grytnes, 2002; Bhattarai & Vetaas, 2003; Bhattarai et al., 2004; Rahbek, 2005; Grytnes & Beaman, 2006; Grau et al., 2007).

Studies have linked unimodal patterns of species richness to climate (e.g. Gradstein and Pócs, 1989; Wolf, 1993; Acebey et al., 2003; Grau et al., 2007). Altitudinal gradients are a useful context in which to study the relationship between species distribution and climate, as there is a large variation in climate within small geographical distances (MacArthur and Wilson, 1967). Altitudinal gradients are also important in the study of climate change, as global warming is pushing species towards higher elevations with the threat of mountaintop extinctions (Kelly and Goulden, 2008; Lenoir et al., 2008; Engler et al., 2009). This effect is likely to be exacerbated on islands, where climate change is expected to significantly influence species distributions (Kazakis et al., 2007; Petit, 2008), as island biotas experience barriers to dispersal (Lloret & González-Mancebo, 2011).

One example where these effects could have major consequences is the volcanic island of Réunion. Réunion has an area of 2 512km^2 and a maximum elevation of 3 069m (Ah-Peng et al., 2014), putting it at risk of both island and mountain effects of climate change. Thus, for

conservation purposes, it is important to broaden our understanding of what causes the patterns of species diversity along altitudinal gradients. Bryophytes on Réunion Island follow the classic unimodal pattern, with the diversity peak occurring at mid altitude in montane cloud forest at around 1 350m (Ah-Peng et al., 2012). The diversity hump, which encompasses a range of altitudes around 1 350m, is host to several endemic bryophytes and a high number of bryophyte species that are restricted to this range (Ah-Peng et al., 2012). Bryophytes on this gradient occur within certain altitudinal ranges. Widespread species occur along the whole gradient, while range-restricted species occur within smaller ranges, at low, mid and high altitudes (Wilding et al., 2012). Species with small range sizes generally have a higher risk of extinction, therefore, range-restricted species are of particular interest for biodiversity management.

Many explanations for the unimodal pattern of bryophytes have been proposed, but there is still no accepted general explanation. A key model is the Mid-Domain Effect as proposed by Colwell and Hurtt (1994). This model is based on the idea of geometric constraints and predicts the unimodal pattern of species richness due to the overlap of species ranges towards the middle of the gradient. Wilding et al. (2012) tested whether life history traits of corticolous bryophytes could be correlated with altitude, and whether this could explain the pattern of species richness along the gradient in Réunion. While research has been conducted in Réunion using both these hypotheses, it has failed to fully explain the unimodal pattern of species richness for bryophytes.

Another main class of explanations for this pattern, although one that has not been directly tested for bryophytes on Réunion, is climatic favourability. This hypothesis states that a peak in diversity occurs where the climate is most favourable. Bryophytes favour conditions where water is readily available in their environment, as they are poikilohydric and, therefore, rely heavily on water availability for sexual reproduction and vegetative growth (Wilding et al., 2012). Hosokawa (1964) found there was a direct correlation between the photosynthetic rate and relative humidity of several epiphytic bryophytes. Therefore, Grau et al. (2007), among others (Gradstein and Pócs, 1989; Wolf, 1993; Acebey et al., 2003), have suggested that the peak in diversity is probably due to the continuous supply of moisture to bryophytes in montane cloud forests. High photosynthetic activity is likely to be prevented on the gradient periphery due to high potential evapotranspiration (ecophysiological constraints) because of drier microhabitats and high temperatures. The decline in richness at the ends of the gradient is, therefore, attributed to lower available moisture, and significant propagule mortality

caused by the interaction between warmer temperatures and moderate humidity (Wilding et al., 2012). On the other hand, most bryophytes are desiccation tolerant and, once dried, can regain full metabolic function minutes or hours after rehydration (Csintalan et al., 1999).

I test the climatic favourability hypothesis directly in order to determine whether ecophysiological specialisation can explain the unimodal pattern of bryophyte richness on an altitudinal gradient on Réunion Island. Thus, the following questions were asked:

- Do species restricted to mid-altitudinal ranges have a lower drought tolerance than widespread species and species restricted to low- and high-altitudinal ranges?

- Do range-restricted and widespread species of bryophyte differ in their ecophysiological specialisation to dehydration, and can this be used to explain the unimodal distribution of species on an altitudinal gradient?

In order to answer these questions, climate data were analysed and changes in photosynthetic efficiency were tracked using chlorophyll fluorescence in dehydration, and subsequent rehydration, experiments on bryophytes collected from an altitudinal gradient on Réunion Island.

METHODS

Study Area

La Réunion Island (21°00′S; 55°39′E), together with Mauritius and Rodrigues, forms part of the Mascerene Archipelago, in the Western Indian Ocean. Réunion was largely formed by the dormant Piton des Neiges volcano (3 069 m), and has an area of 2 512 km^2, making it the largest and highest of the Mascarene Islands (Ah-Peng et al., 2014). The island has a tropical climate, with a summer rainfall period from November to April, and a cooler, drier period from June to September. Moist trade winds that act on the eastern side of Réunion provide the island with most of its rainfall. This eastern side has a higher mean annual precipitation, ranging from 1 500 mm yr^{-1} to over 8 000 mm yr^{-1} at high altitudes (Wilding et al., 2012). At 2 000 m a.s.l, there is a temperature inversion that causes the humid oceanic air to form clouds mainly between 1 400 m and 1 600 m (Ah-Peng et al., 2007). The mean annual temperature ranges from 24 °C to 12 °C at about 2 000 m a.s.l (Wilding et al., 2012). There is a temperature decrease of 0.7 °C to 0.8 °C every 100 m, presenting a steep temperature gradient with altitude (Ah-Peng et al., 2014).

Réunion Island has remarkably well-preserved biodiversity (Ah-Peng et al., 2007) and is part of one of 35 global biodiversity hotspots (Mittermeier et al., 2005). The island is home to 831 bryophyte species - 504 mosses, 322 liverworts, and 5 hornwort species (Ah-Peng et al., 2010; Ah-Peng et al., 2012), and has the richest bryophyte flora in the Mascarene Archipelago. The topography of the island makes urbanisation and agriculture unviable on a large proportion of the land, thus 32 % of Réunion's indigenous vegetation is well conserved (Ah-Peng et al., 2007). Since 2007, 42 % of the island has been under national park protection (Wilding et al., 2012).

Study Sites

Study sites were chosen at three altitudes along the Piton des Neiges gradient, which is the longest bioclimatic gradient on the Western Indian Ocean islands, ranging from 0 m a.s.l to 3 069 m a.s.l (Wilding et al., 2012). There were four study sites at the three altitudes: one site at low altitude (200-800 m), two sites at mid altitude (800-1 700 m) and one site at high altitude (1 700-2 200 m). The sites fell within three distinct vegetation types: tropical lowland forest at low altitude (0-800 m), tropical montane cloud forest at mid altitude (800-1 900 m), and alpine shrubland at high altitude (1 900-3 000 m) (Cadet, 1980).

Sampling

Climate data were recorded at 14 sites along the gradient using MadgeTech data loggers (RHTemp1000, Warner, USA). The loggers were set up at altitudinal intervals of 200m in May 2011. The loggers were placed 1 m above the ground on wooden poles. At hourly intervals, measures of relative humidity were logged. The data were collected using the MadgeTech 2.03 software for the period between June 2011 and May 2012.

Fieldwork was carried out on the 21st, 23rd, 24th and 26th of June 2016. At each site, between two and six replicate samples of several widespread and several range-restricted species of bryophytes were collected. The location and altitude of each sample was logged on a GPS. Each sample was placed in a re-sealable plastic packet to prevent dehydration, and labelled with the species code and location number. Samples were then transported to a temporary lab on Réunion where the experiments were done.

Table 1: Location and range of bryophyte species sampled along the Piton des Neiges gradient.

Species	Group	Site	Range
Atrichum androgynum (Müll. Hal.) A. Jaeger	Moss	Mid-altitude	Restricted
Calyptrochaeta asplenioides (Brid.) Crosby	Moss	Mid-altitude	Restricted
Dicranoloma billardierei (Brid. ex Anon.) Paris	Liverwort	Mid- and high-altitude	Widespread
Ectropothecium chenagonii Renauld & Cardot	Moss	Low-altitude	Restricted
Holomitrium borbonicum Hampe ex Besch.	Moss	Mid- and high-altitude	Widespread
Leptodontium flexifolium (Dicks.) Hampe	Moss	High-altitude	Restricted
Leucoloma longifolium (Brid.) Wijk & Margad.	Moss	Low-altitude	Restricted
Macromitrium serpens (Bruch ex Hook. & Grev.) Brid.	Moss	Mid-altitude	Restricted
Mastigophora diclados (Brid. ex F.Weber) Nees	Moss	Low-, mid- and high-altitude	Widespread
Porotrichum elongatum (Welw. & Duby) A.Gepp	Moss	Low-altitude	Restricted
Pyrrhobryum spiniforme (Hedw.) Mitt.	Moss	Low-, mid- and high-altitude	Widespread
Racomitrium membranaceum (Mitt.) Paris	Moss	High-altitude	Restricted
Schlotheimia badiella Besch.	Moss	Mid- and high-altitude	Widespread
Ulota fulva Brid.	Moss	High-altitude	Restricted

Chlorophyll-fluorescence measurements:

Chlorophyll fluorescence was used as a proxy for photosynthetic efficiency as it is quick and non-invasive (e.g. Csintalan et al., 1999; García et al., 2016; Proctor, 2003). Chlorophyll fluorescence was measured using a Portable Chlorophyll Fluorometer PAM-2100. Standard experiment 8.2 (Run 2: Determination of F_v/F_m) was run (Heinz Walz GmbH, 2003). All samples were dark adapted prior to measuring chlorophyll fluorescence, then measurements were taken under normal laboratory light ($< 8 \, \mu \, mol \, m^{-2} \, s^{-1}$), as per García et al. (2016).

Dehydration experiment

In order to determine how widespread and range-restricted bryophytes at each altitude responded to drying, a dehydration experiment was conducted.

The initial weight of each petri dish was measured (using an analytical balance) and recorded. Each sample was placed in a labelled petri dish and the initial fresh weight was recorded. Three chlorophyll-fluorescence readings were taken and the sample was weighed again. This was repeated for each sample. Three chlorophyll-fluorescence readings and a weight reading were taken for each sample at two-hour intervals until the samples were fully dehydrated (no change in mass) and no longer photosynthesising ($F_v/F_m < 0.010$). The dehydrated samples were then placed in paper bags and labelled. This was repeated for the sample sets at each site.

Rehydration experiment

In order to determine how widespread and range-restricted bryophytes at each altitude responded to being rehydrated after drying, a rehydration experiment was conducted.

A week after dehydration, samples were approximately halved and moved to two new petri dishes. One of these subsamples from each sample was rehydrated by spraying with de-ionised water, and two chlorophyll-fluorescence readings were taken after five minutes, one hour and 24 hours. In between measurements, the petri dishes were placed inside re-sealable plastic packets to prevent dehydration.

The process was followed for the other subsample, but rehydration was delayed by seven weeks.

Data Analysis

Water potential was calculated from the climate data using the following equation:

$\Psi_w = RT/V_w \ln(RH)$

Days where the maximum daily relative humidity fell below 95 % were considered dry days. This was chosen as a conservative estimate of a dry day, as with water potential well below -2 mPa, the water potential of the air is so low that bryophytes would not be able to gain moisture from it (Taiz et al., 2015).

Species were grouped into four categories: range-restricted species from the low-altitude site, range-restricted species from the mid-altitude site, range-restricted species from the high-altitude site and widespread species that occurred at all sites. These four groups are hereafter referred to as low-altitude, mid-altitude, high-altitude and widespread species, respectively. Curves of the form $y = a/(1 + e^{-b(x-c)})$ were fit to the dehydration data of photosynthetic efficiency (F_v/F_m) against water content (g H_2O g^{-1} dry weight) for each sample. Coefficients a, b and c were estimated for each curve using the nls (nonlinear least squares) function in R (R Development Core Team, 2016). To determine whether the dry-down response differed depending on species', range a Kruskal-Wallis test ("pgirmess" package in R; Giraudoux, P. 2016) was conducted, as the data were heteroskedastic and were not normally distributed ("gplots" package in R; Warnes et al., 2016). At each time point after rehydration, percent recovery was calculated using the average value of chlorophyll fluorescence (F_v/F_m) and the maximum chlorophyll fluorescence value for each sample. A Kruskal-Wallis test ("pgirmess" package in R; Giraudoux, P. 2016) also was used to determine whether rehydration response differed, depending on species' range, as the data were heteroskedastic and were not normally distributed ("gplots" package in R; Warnes et al., 2016). Data were analysed using R version 3.3.1 (R Development Core Team, 2016).

RESULTS

Climatic data

Figure 1 shows the mean, minimum and maximum daily relative humidity at the low-, mid- and high-altitude sites along the Piton des Neiges gradient from June 2011 to May 2012. There is a trend of high relative humidity, with a large proportion of days having above 95 % relative humidity, with 100% relative humidity occurring at a high frequency. This trend can also be seen in the minimum daily relative humidity, meaning that the relative humidity remained 100% throughout the day.

C

400
300
200
100
0

2.5   27.5   52.5   77.5   97.5

400
300
200
100
0

2.5   27.5   52.5   77.5   97.5

400
300
200
100
0

2.5   27.5   52.5   77.5   97.5

B

Frequency (days)

400
300
200
100
0

2.5   27.5   52.5   77.5   97.5

400
300
200
100
0

2.5   27.5   52.5   77.5   97.5

400
300
200
100
0

2.5   27.5   52.5   77.5   97.5

A

400
300
200
100
0

2.5   27.5   52.5   77.5   97.5

400
300
200
100
0

2.5   27.5   52.5   77.5   97.5

400
300
200
100
0

2.5   27.5   52.5   77.5   97.5

Maximum Daily Relative Humidity (%)   Mean Daily Relative Humidity (%)   Minimum Daily Relative Humidity (%)
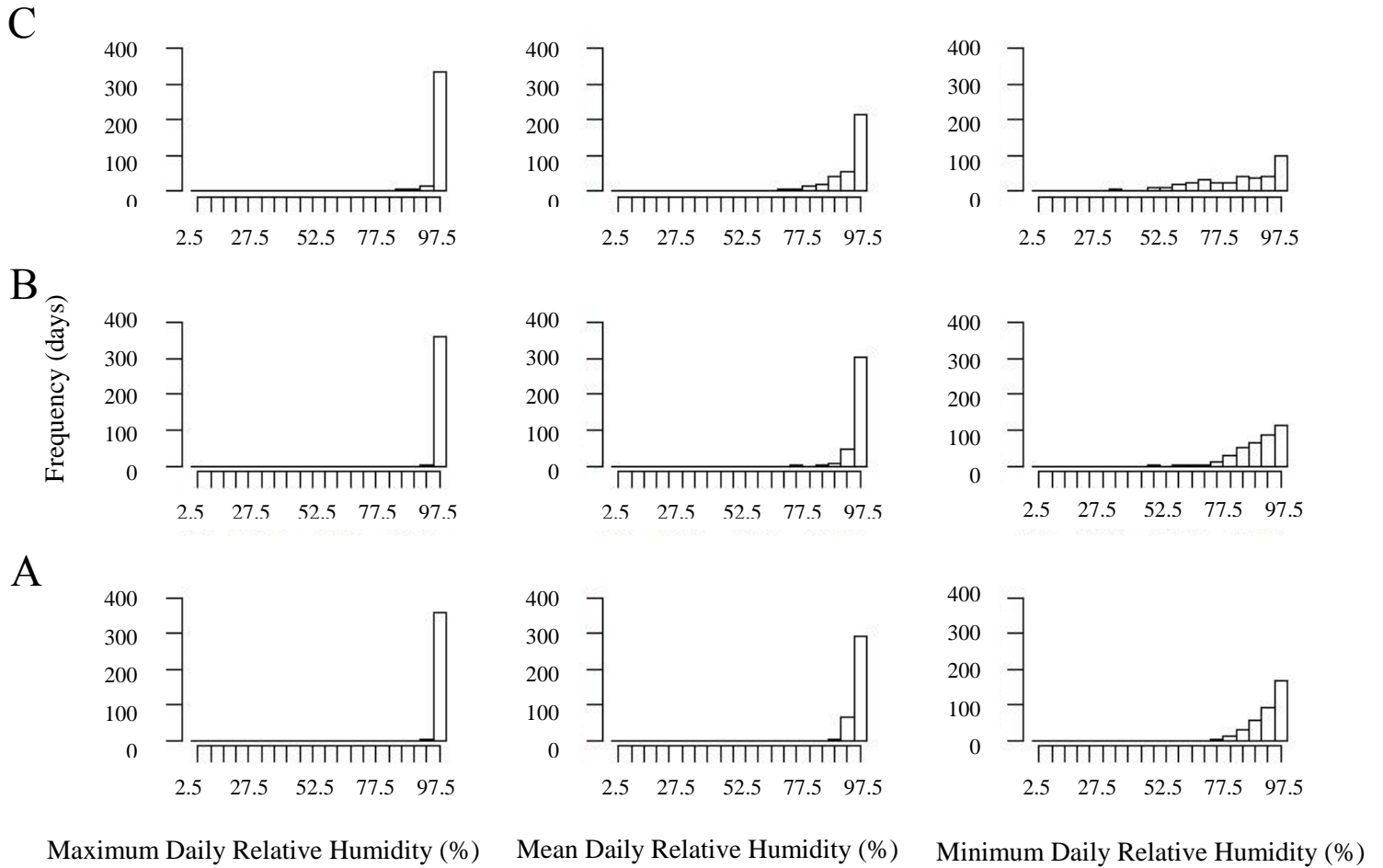
Figure 1: Mean, maximum and minimum daily relative humidity (%) from June 2011 to May 2012 at low- (A), mid- (B) and high-altitude (C) sites along the Piton des Neiges gradient.

Figure 2 shows the dry days for each site, as well as days where the mean and minimum daily relative humidity fell below 95 %. The dry days at the low site were spread relatively evenly throughout the year, however, those at the high site were spread over a shorter period from June 2011 to February 2012, with a cluster of dry days occurring from October to November. The mid site experienced only four dry days at the end of October and early November. However, the low-altitude site had observably fewer days where daily mean and minimum relative humidity values were below 95 %.
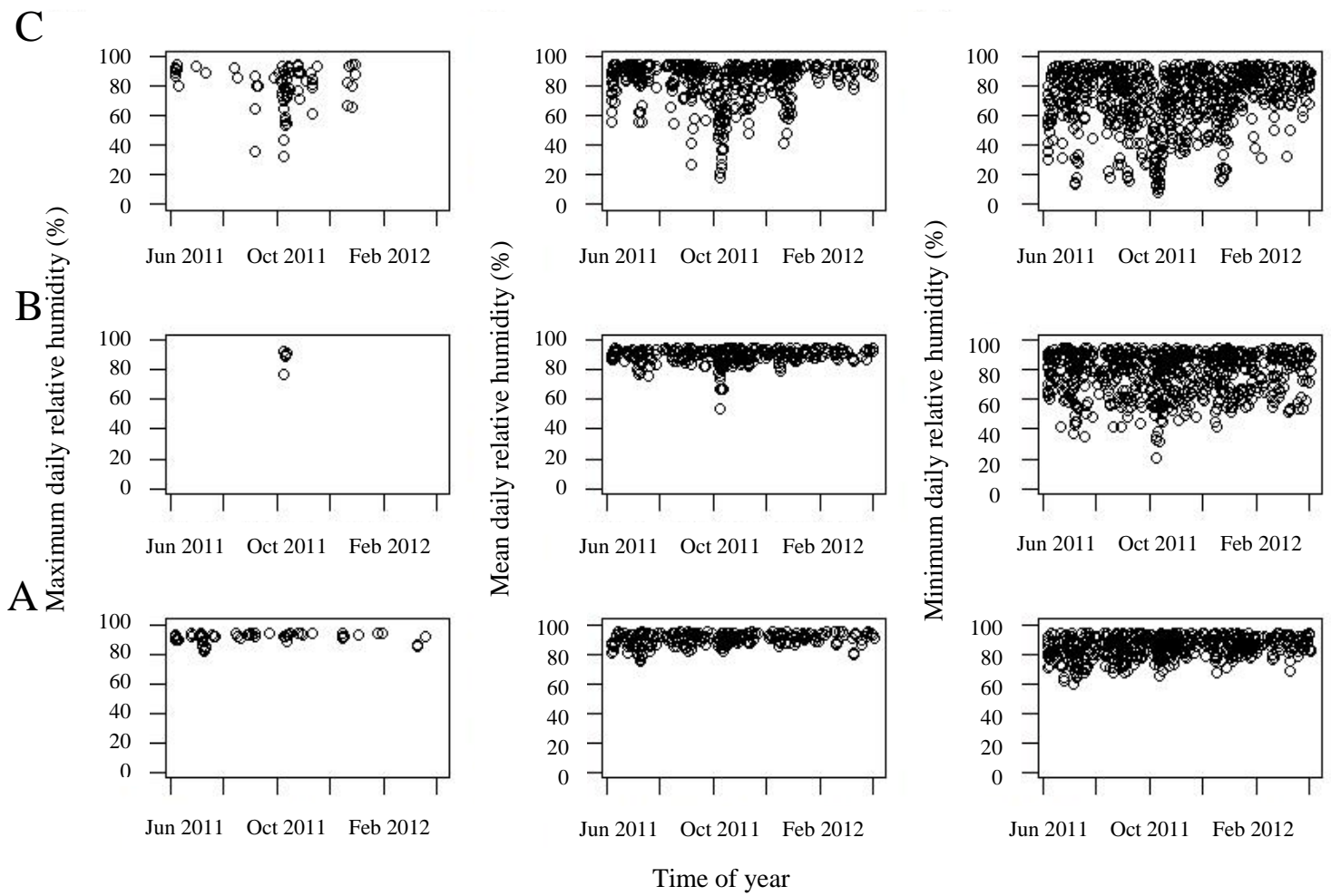
Figure 2: Dry days (maximum daily relative humidity < 95 %) and days where the mean and minimum daily relative humidity fall below 95 % from June 2011 to May 2012 at low- (A), mid- (B) and high-altitude (C) sites along the Piton des Neiges gradient.

Figure 3 shows the consecutive dry days, or dry spells, from June 2011 to May 2012, with the red line marking 7 days. The low- and high-altitude sites had more and longer dry spells than the mid-altitude site. The low- and high-altitude sites each only had one dry spell lasting longer than 1 week, while the mid-altitude site did not have any dry spells lasting longer than 1 week.
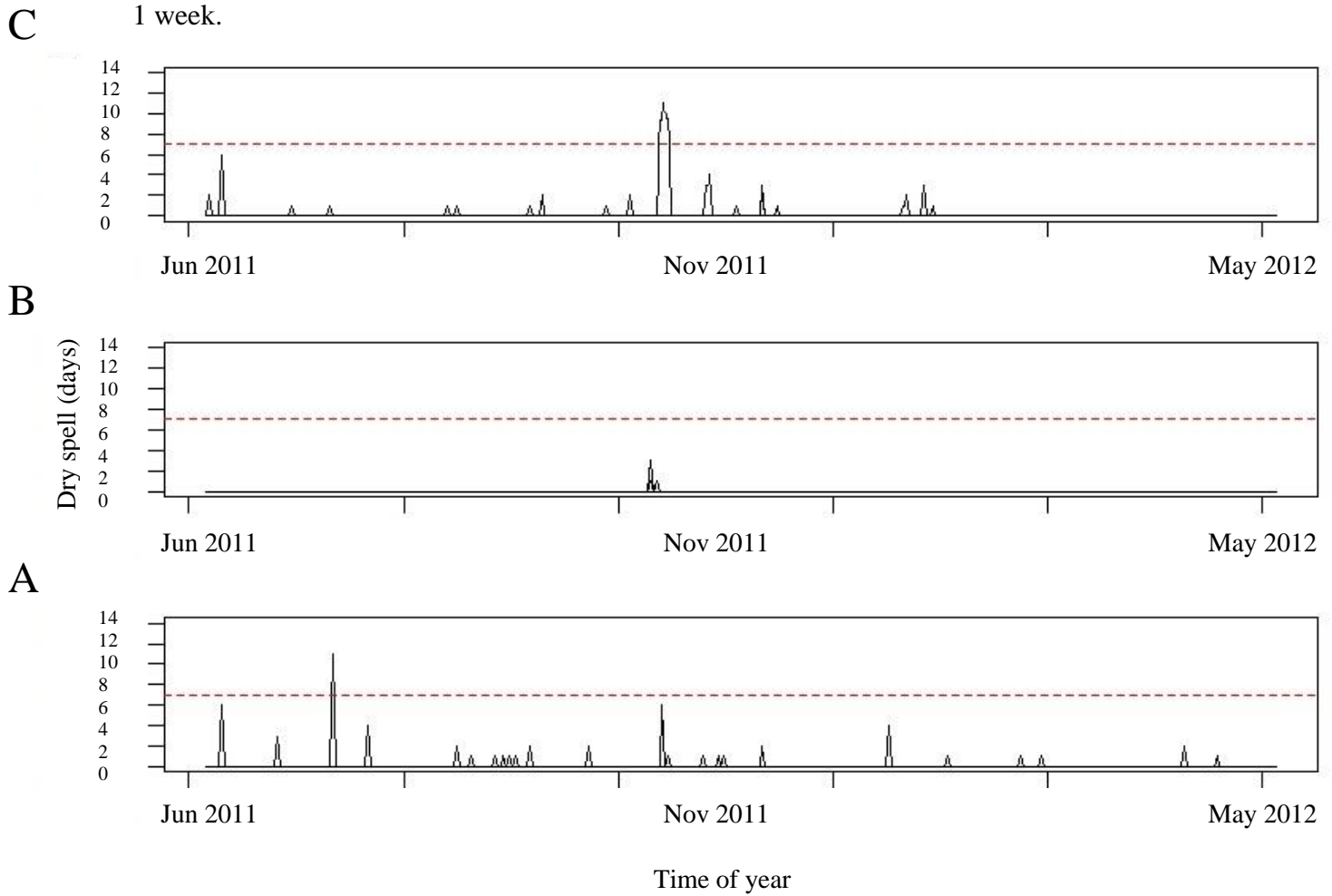
C

B

A

Time of year

Figure 3: Dry spells (consecutive dry days) from June 2011 to May 2012 at low- (A), mid- (B) and high-altitude (C) sites along the Piton des Neiges gradient.

Figure 4 and 5 show the dry-down curves for range-restricted species at each site and widespread species with the form $y=a/(1 + e^{-b\,(x-c)})$ which is, empirically, a good fit to the data averaged from each sample. Coefficient b (the slope) was significantly lower for low-altitude species than high-altitude and widespread species ($K = 16.14$, $df = 3$, $p < 0.05$). Coefficient c (the x-value of the sigmoid's midpoint) was significantly higher for low-altitude and mid-altitude species than high-altitude species ($K = 16.26$, $df = 3$, $p < 0.05$).

Table 2: Results of Kruskal-Wallis test comparing differences between range-restricted species at each site and widespread species within coefficient b and c; different letters represent significant differences between ranges ($p < 0.05$).

Differences between ranges		
Range	Coefficient b	Coefficient c
Low-altitude species	a	a
Mid-altitude species	ab	a
High-altitude species	b	b
Widespread species	b	ab

Coefficient b was significantly lower for the widespread species found at low altitude than the widespread species found at mid and high altitude ($K= 12.12$, $df = 2$, $p < 0.05$). Coefficient c was significantly lower for the widespread species found at high altitude than the widespread species found at low and mid altitude ($K= 9.28$, $df = 2$, $p < 0.05$).

Table 3: Results of Kruskal-Wallis test comparing differences between widespread species at each site within coefficient b and c; different letters represent significant differences between sites ($p < 0.05$).

Widespread species: Differences between sites		
Site	Coefficient b	Coefficient c
Low-altitude	a	a
Mid-altitude	b	a
High-altitude	b	b

A

Ectropothecium chenagonii

Leucoloma longifolium

Porotrichum elongatum

B

Atrichum androgynum

Calyptrochaeta asplenioides

Macromitrium serpens

C

Leptodontium flexifolium

Racomitrium membranaceum

Ulota fulva

D

Dicranoloma billardierei

Holomitrium borbonicum

Mastigophora diclados

Pyrrhobryum spiniforme

Schlotheimia badiella

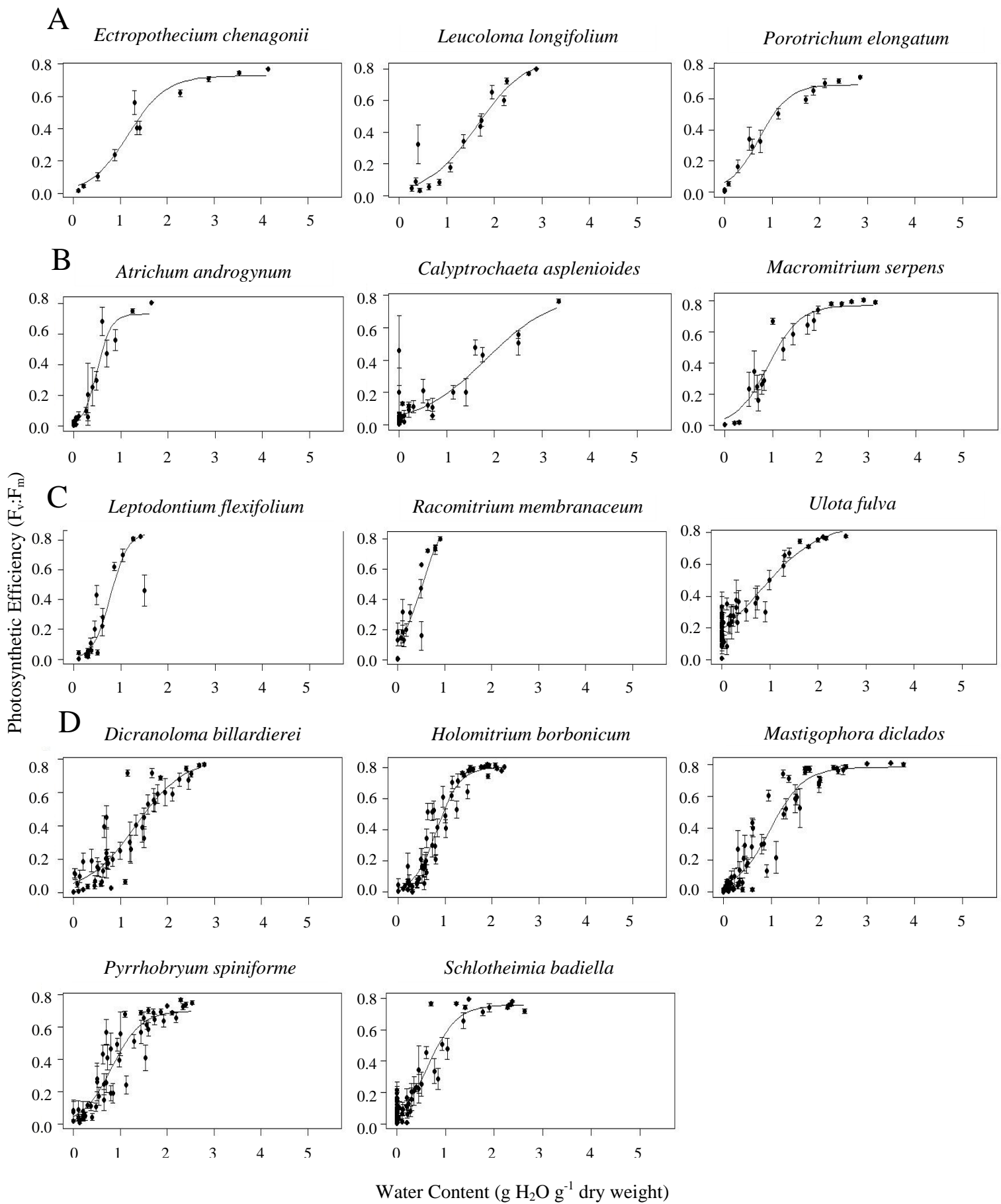Photosynthetic Efficiency (F_v:F_m)

Water Content (g H_2O g^{-1} dry weight)

Figure 4: Dry-down curves showing the relationship between chlorophyll fluorescence (F_v/F_m) and water content (g H_2O g^{-1} dry weight) for range-restricted bryophyte species sampled at low- (A), mid- (B) and high-altitude (C) sites and widespread (D) bryophyte species (mean ± SE).

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Figure 5: Average dry-down curves showing the relationship between chlorophyll fluorescence (F_v/F_m) and water content (g H_2O g^{-1} dry weight) for range-restricted bryophyte species sampled at low- (A), mid- (B) and high-altitude (C) sites and widespread (D) bryophyte species (mean ± SE).
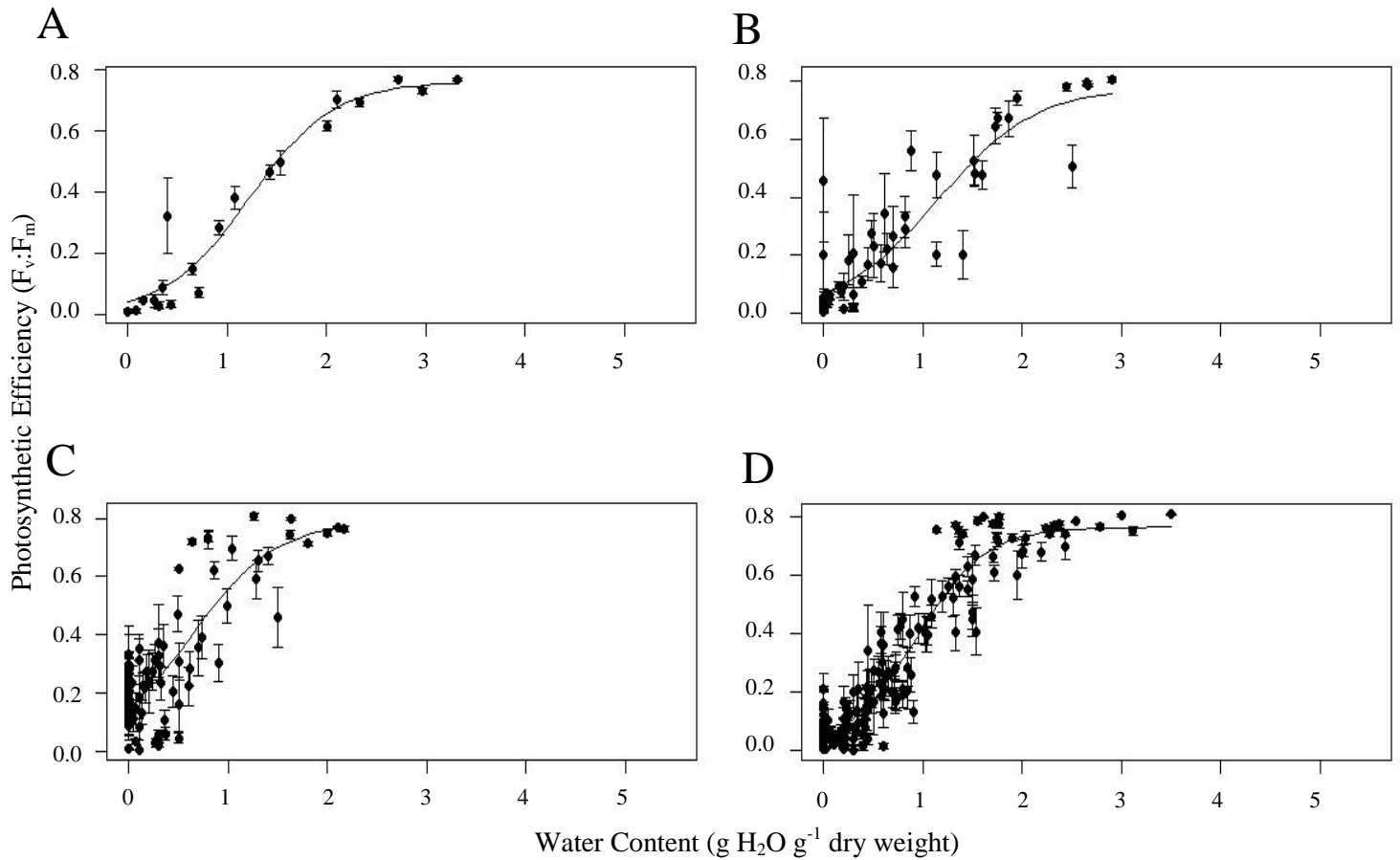
Figure 6 and 7 show the percent recovery of photosynthetic efficiency at each site 5 minutes, 1 hour and 24 hours after rehydration, following one week and seven weeks of dehydration, respectively.

Low-altitude species

In the one-week rehydration experiment, a maximum of 22.7 % recovery was reached at 24 hours and in the seven-week rehydration experiment a maximum of 5.3 % recovery was reached at 5 minutes. However there was no significant difference in percent recovery at 5 minutes, 1 hour or 24 hours after rehydration in either the one-week (K = 1.42, df = 2, p > 0.05) or the seven-week rehydration experiment (K = 5.81, df = 2, p > 0.05). It was found that the percent recovery was significantly higher in the one-week rehydration experiment than in the seven-week rehydration experiment (K = 35.32, df = 1, p < 0.001).

Table 4: Results of Kruskal-Wallis test comparing differences between percent recovery of low-altitude species at different times within the one-week and seven-week rehydration experiments; different letters represent significant differences between times (p < 0.05).

Differences between percent recovery of low-altitude species at different times		
Time	One-week rehydration	Seven-week rehydration
5 minutes	a	a
1 hour	a	a
24 hours	a	a

Mid-altitude species

In the one-week rehydration experiment, a maximum of 42.9 % recovery was reached at 24 hours and in the seven-week rehydration experiment a maximum of 14.7 % recovery was reached at 24 hours. However, there was no significant difference in percent recovery at 5 minutes, 1 hour or 24 hours after rehydration in either the one-week ($K = 1.13$, df = 2, p > 0.05) or the seven-week rehydration experiment ($K = 0.36$, df = 2, p > 0.05).

The percent recovery was significantly higher in the one-week rehydration experiment than in the seven-week rehydration experiment ($K = 10.87$, df = 1, p < 0.001).

Table 5: Results of Kruskal-Wallis test comparing differences between percent recovery of mid-altitude species at different times within the one-week and seven-week rehydration experiments; different letters represent significant differences between times (p < 0.05).

Differences between percent recovery of mid-altitude species at different times		
Time	One-week rehydration	Seven-week rehydration
5 minutes	a	a
1 hour	a	a
24 hours	a	a

High-altitude species

In the one-week rehydration experiment the percent recovery was significantly higher at each time reading ($K = 38.12$, df = 2, p < 0.001); 47.3 % recovery at 5 minutes, 79.9 % recovery at 1 hour and 95.7 % recovery at 24 hours. In the seven-week rehydration experiment the percent recovery was significantly higher at 24 hours than at 5 minutes and one hour ($K = 30.52$, df = 2, p < 0.001); there was a minimum of 23.3 % recovery at 5 minutes and a

maximum of 76.5 % recovery at 24 hours.

It was found that the percent recovery was significantly higher in the one-week rehydration experiment than in the seven-week rehydration experiment (K = 21.94, df = 1, p < 0.001).

Table 6: Results of Kruskal-Wallis test comparing differences between percent recovery of high-altitude species at different times within the one-week and seven-week rehydration experiments; different letters represent significant differences between times (p < 0.05).

Differences between percent recovery of high-altitude species at different times		
Time	One-week rehydration	Seven-week rehydration
5 minutes	a	a
1 hour	b	a
24 hours	c	b

Widespread species

In the one-week rehydration experiment the percent recovery got significantly higher at each time reading (K = 97.09, df = 2, p < 0.001); 39.5 % at 5 minutes, 64.1 % at 1 hour and 81.9 % at 24 hours. There was no significant difference in percent recovery at 5 minutes, 1 hour and 24 hours after rehydration in the seven-week rehydration experiment (K = 4.80, df = 2, p > 0.05) and a maximum of 39.1 % recovery was reached at 1 hour.

It was found that the percent recovery was significantly higher in the one-week rehydration experiment than in the seven-week rehydration experiment (K = 134.62, df = 1, p < 0.001).

Table 7: Results of Kruskal-Wallis test comparing differences between percent recovery of widespread species at different times within the one-week and seven-week rehydration experiments; different letters represent significant differences between times (p < 0.05).

Differences between percent recovery of widespread species at different times		
Time	One-week rehydration	Seven-week rehydration
5 minutes	a	a
1 hour	a	a
24 hours	a	a

The percent recovery of the widespread species found at low altitude was significantly lower than that of the widespread species found at mid and high altitude in both the one-week (K = 27.00, df = 2, p < 0.001) and the seven-week (K = 45.85, df = 2, p < 0.001) rehydration experiments.

Table 8: Results of Kruskal-Wallis test comparing differences between percent recovery of widespread species at different altitudes within the one-week and seven-week rehydration experiments; different letters represent significant differences between times ($p < 0.05$).

Differences between percent recovery of widespread species at different ranges		
Range	One-week rehydration	Seven-week rehydration
Low-altitude	a	a
Mid-altitude	b	b
High-altitude	b	b

One-week rehydration experiment

The percent recovery of the low-and mid-altitude species were not significantly different from one another, but were significantly lower than the percent recovery of the high-altitude species and the widespread species. The percent recovery of the high-altitude species and the widespread species were not significantly different from one another ($K = 105.09$, df = 3, $p < 0.001$).

Seven-week rehydration experiment

The percent recovery of the low-and mid-altitude species were not significantly different from one another, but were significantly lower than the percent recovery of the high-altitude species and the widespread species. The percent recovery of the high-altitude species was significantly higher than the percent recovery of the widespread species ($K = 105.09$, df = 3, $p < 0.001$).

Table 9: Results of Kruskal-Wallis test comparing differences between percent recovery of range-restricted species at each site and widespread species within the one-week and seven-week rehydration experiments; different letters represent significant differences between ranges ($p < 0.05$).

Differences between ranges		
Range	One-week rehydration	Seven-week rehydration
Low-altitude species	a	a
Mid-altitude species	a	a
High-altitude species	b	b
Widespread species	b	c

Table 10: Results of Kruskal-Wallis test comparing differences between percent recovery between the one-week and seven-week rehydration experiments within range-restricted species at each site and widespread species; different letters represent significant differences between rehydration experiments (p < 0.05).

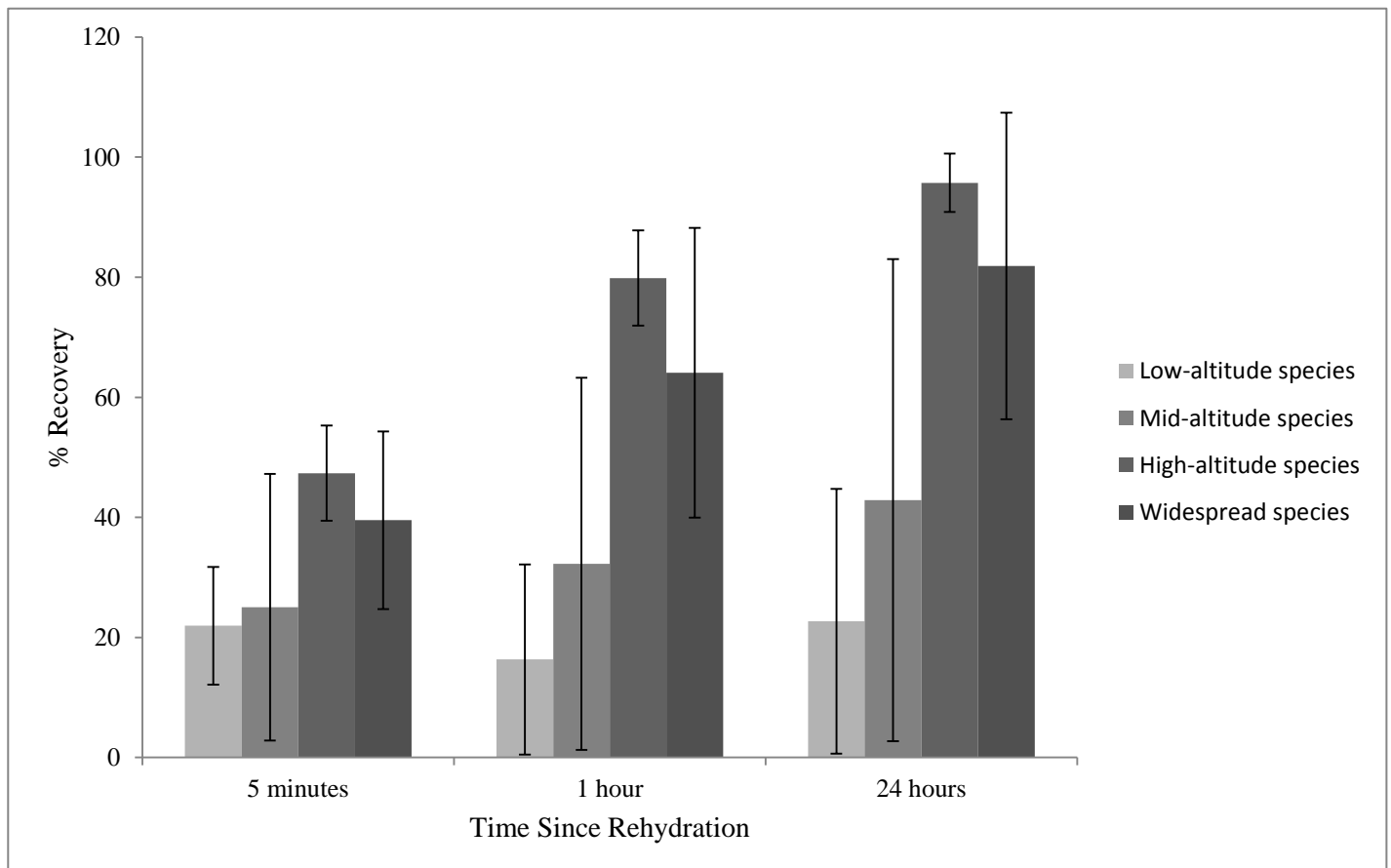| Differences between one-week and seven-week rehydration experiment				
Experiment	Low-altitude species	Mid-altitude species	High-altitude species	Widespread species
One-week rehydration	a	a	a	a
Seven-week rehydration	b	b	b	b

Figure 6: Percent recovery of widespread bryophyte and range-restricted bryophyte photosynthetic efficiency 5 minutes, 1 hour and 24 hours after rehydration following 1 week of dehydration (mean ± SD).
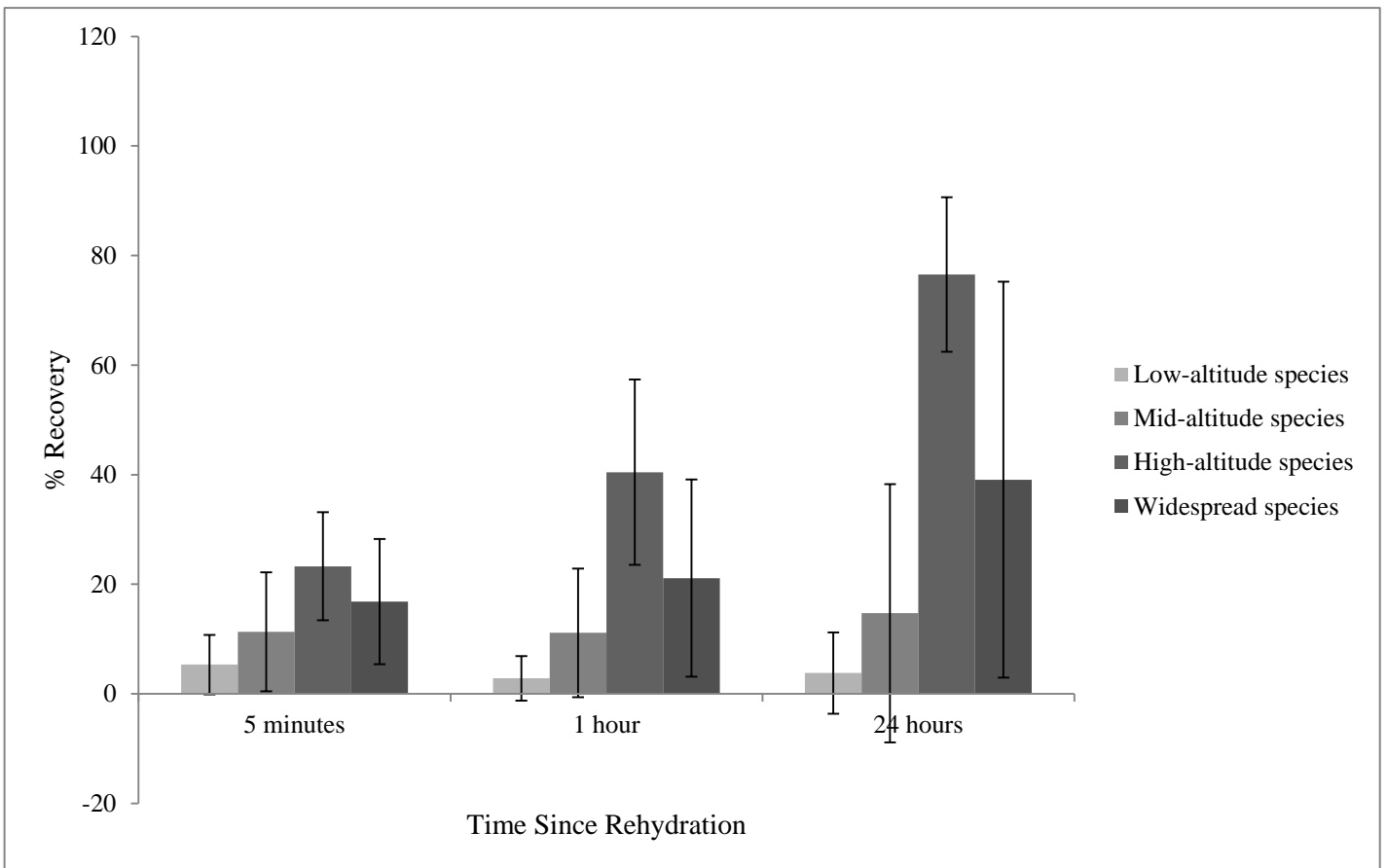
Figure 7: Percent recovery of widespread bryophyte and range-restricted bryophyte photosynthetic efficiency at each site (low-, mid-and high-altitude) 5 minutes, 1 hour and 24 hours after rehydration following 7 weeks of dehydration (mean ± SD).

DISCUSSION

Due to the temperature inversion causing cloud formation between 1 400 -1 600 m (Barcelo, 1996; Ah-Peng et al., 2007), and the comparitively high rainfall at mid altitude (12 000 mm p.a.) (Barcelo, 1996), the pattern of dry day occurrence along the Piton des Neiges gradient was unsurprising, with many dry days experienced at the low- and high-altitude sites and very few dry days at the mid-altitude site. Given this pattern of moisture availability, it was expected that the mid-altitude bryophyte species would be less drought tolerant than the species at the other sites and the widespread species (Bader et al., 2013).

In the dry-down experiment, the photosynthetic efficiency of the high-altitude and widespread species was found to decrease at a higher rate than that of the low-altitude species as water content dropped. However, the high-altitude and widespread species generally started out at a lower water content. They were, thus, able to maintain full photosynthetic efficiency at a water content where the low-altitude species were only photosynthesising at

half capacity or lower (Figure 4). This was further illustrated by the fact that both low- and mid-altitude species reached half photosynthetic efficiency at a water content significantly higher than that of the high-altitude species. This finding is similar to that of Pardow and Lakatos (2012), who found that tropical lowland understory species are less tolerant to low humidity.

Most bryophyte species can tolerate vegetative desiccation, but this is an uncommon trait in vascular plants (Oliver at al., 2000). Species sensitive to drying experience cell death due to loss of cellular membrane integrity when dehydrated (Oliver & Bewly, 1984). Bryophytes prevent cell death during dehydration by means of protective mechanisms when drying and reparative mechanisms during rehydration (Bewly & Krochko, 1982). The results of the dry-down experiments indicate that the low- and mid-altitude species were more physiologically stressed by drying than the high-altitude species, probably due to differences in damage to cell structure when drying (García et al., 2016). This suggests that the low- and mid-altitude species are, in fact, less drought tolerant than the high-altitude species, with the widespread species having an intermediate tolerance.

Throughout the recorded year, the mid-altitude site did not experience any week-long dry spells. The low- and high-altitude sites had many more dry spells, but each had only one dry spell lasting longer than a week, and none lasting as long as seven weeks. Desiccation tolerance in bryophytes generally mirrors their environmental moisture availability (Proctor 2001). Thus, the low-altitude, high-altitude and widespread species were expected to regain full photosynthetic efficiency when rehydrated after a week of dehydration, but were expected to do worse after seven weeks of dehydration.

In the one-week rehydration experiment the high-altitude and widespread species slowly regained photosynthetic efficiency, while both the low- and mid-altitude species recovered low levels of photosynthetic efficiency immediately, but did not recover significantly more after that. As expected, the extent of recovery of all species was lower after seven weeks of dehydration than after one week of dehydration. This was especially true for the widespread species as they, along with the low- and mid-altitude species, recovered only low levels of photosynthetic efficiency immediately, and did not recover significantly after that. The high-altitude species slowly recovered normal levels of photosynthetic efficiency. Despite the fact that they took a while to recover fully, the high-altitude and widespread species did significantly better than the low- and mid-altitude species after rehydration. However, in the

seven-week rehydration experiment, the high-altitude species did significantly better than the widespread species. Their ability to recover is probably due to a combination of differences in damage to cell structure when drying (García et al., 2016) and differences in capacity to repair when rehydrated.

The overall pattern seen in both the dry-down experiments and the rehydration experiments is that the low- and mid-altitude species are less drought tolerant than the high-altitude and widespread species. The low drought tolerance of the low-altitude species appears anomalous when considering the pattern of dry days along the gradient. However, if the criterion for a dry day is considered to be when the mean relative humidity falls below 95 % (figure 2), a pattern of increasing dry days from low to high altitude emerges. This pattern is then consistent with the pattern of drought tolerance along the gradient.

The overall pattern observed shows a marked difference between the ecophysiological specialisation to humidity of the range-restricted species at each site and the widespread species. The resilience of the widespread species when water stressed illustrates an ability to tolerate the conditions of humidity all along the gradient. The widespread species showed some variation between sites, indicating that they are locally adapted to different altitudes. This adaptability could explain their presence along the whole gradient. Moreover, analyses suggest that the mid-altitude species can't survive in the drier climate of the high altitude, and perhaps this is why most species are restricted to mid altitude. However, while there is separation between each altitude, this does not fully explain the unimodal distribution of bryophytes along the gradient, as there is no evidence indicating that the species restricted to mid altitude are restrained by the climate and could not survive at low altitude. Thus, if we are to attribute the unimodal pattern to ecophysiological specialisation, there must be other factors at play.

The interaction between temperature and humidity has been found to be an important factor in bryophyte distribution (García et al., 2016; Wilding et al., 2012) and bryophytes are expected to be thermal specialists (Chan et al., 2016). There is a steep decrease in temperature with an increase in altitude, with every 100 m marking a 0.7 °C to 0.8 °C decrease in temperature (Barcelo, 1996). Therefore, the reason the low-altitude site has lower species richness, despite the moderate humidity, could be due to the interaction with high temperatures at the low site causing faster decomposition and increased propagule mortality (Wilding et al., 2012).

Furthermore, low-altitude bryophytes are not substrate-limited, but the smooth bark of the trees common at low altitude might prevent many species from establishing here. In addition, at very high altitudes bryophytes face challenging conditions, including low temperatures, frost, fewer trees (smaller habitat) and high levels of UV (Wilding et al., 2012). Therefore, looking only at humidity might be reductionist, and further research should consider the interaction between other variables to explain the distribution of bryophytes along the gradient. The use of statistical models, such as in García et al.'s (2016) paper, might be useful in determining the most important drivers of bryophyte distribution.

This study gives us interesting insight into the ecophysiological specialisation of bryophytes to humidity. Bryophytes are sensitive to environmental changes and thus have the potential to be used as indicators of climate change (Gignac, 2011) and can act as an early-warning system for other species. In order to use them as indicators, we need to understand the relationship between bryophytes and climate. The pattern revealed in this study is an important finding as it broadens our understanding of how bryophytes are influenced by climate and how this shapes their distribution. There is already some evidence of bryophyte distribution changing in response to climatic factors (Tuba et al., 2011) and this study adds to our knowledge of how they might respond to predicted climate change (Bader, 2013). Such knowledge is useful for biodiversity management, especially of range-restricted species, in a biodiversity hotspot vulnerable to island effects and mountaintop extinctions caused by climate change (Kazakis et al., 2007; Kelly and Goulden, 2008; Lenoir et al., 2008; Petit, 2008; Engler et al., 2009). Furthermore, there is potential for this kind of study to be done on a larger scale, examining species distributions along latitudinal gradients.

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