

## Can we use the acrocarpous moss gametophyte length to assess microclimatic conditions in harsh environmental?

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Bryophytes are small non-vascular plants which are very sensitive to narrow variations in environmental conditions and can be considered to be useful bioindicators of local environmental conditions. Although their growth is encouraged by constant wetness, stem growth is a vigorous measure which can work as a good proxy for local microclimatic conditions. This is important when faced with the predicted climate change in the Caatinga. Our aim is to validate a trait which is easy to measure and which can act as a local condition proxy in monitoring changes in harsh environments. Based on the Generalized Linear Model (GLM), gametophyte length vs. soil depth, and descriptive statistics, we evaluated the vigor of length measurements as a proxy for local conditions in harsh environments. Soil depth varied as expected for both shallow and deep soil islands. Gametophyte's length is a trait with high variability in the moss under study and such variation is not correlated with soil depth. The distribution of length values was more aggregated in *Campylopus pilifer*, with stem length being higher than that seen in the literature for most of the selected gametophytes. We found that length is a poor predictor trait of local conditions in harsh environments. However, our results suggest that soil island can act as proof of harsh environments given that *C. pilifer*, a pioneer moss, possessed stems which were higher than expected.

### 1. Introduction

..... Bryophytes are small non-vascular plants which are very sensitive to narrow variations in environmental conditions (Delgadillo and Cárdenas, 1990). Nevertheless, bryophytes have a broad geographical distribution and contrary to the popular concept that moss growth is restricted to wet places, a great number of species are adapted to places such as dry and hot deserts (Frahm, 1996; Glime, 2015). In harsh habitats, they are typically small and may include acrocarpous perennial stayers (Glime, 2013). This information suggests that the occurrence of a given species in a specific location is related to microhabitat conditions such as humidity intervals. Considering that bryophytes are useful as bioindicators of local environmental conditions (Vanderpoorten and Gofinett, 2009) and that bryophyte growth is encouraged by constant wetness (Gradstein et al., 2001), stem growth is a vigorous measure which can function as a good proxy for local microclimatic conditions (see Stark et al., 2005).

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Plant density is influenced by microhabitat conditions such as wetness, temperature and light radiation in harsh environments (Smith and Stark, 2014). All of these environmental conditions interfere in the growth of bryophytes (Green and Lange, 1994; León-Vargas et al., 2006; Patiño et al., 2011; Santos et al. 2014). However, species in xeric habitats may exhibit internal variability as a response to environmental conditions – drought, intense light radiation – (Mason et al., 2005) and this is related to gametophyte length (Keever et al., 1951). Specifically, environmental conditions act as filters (de Bello et al., 2008) which influence community structure and species' maintenance (Chase and Meyers, 2011). Therefore, bryophytes living in harsh environments must possess a set of traits which allow for their survival (see Magdefräu, 1982; Kürschner, 2004; Kürschner and Parolly, 2005). In addition, investment in adaptive traits is assumed to mean less targeted energy for growth (Kappen and Valladares, 2007).

In order to test the above mentioned hypothesis, we used soil islands (SI) (patches of soil limited by a rocky matrix – Conceição et al., 2007) in rocky outcrops (RO) in the Caatinga. SIs are good models to test our hypothesis because they are of different sizes, depths, and climatic conditions (Porembski et al., 1998), as well as being distributed all over RO, in up to hundreds of units (Conceição et al., 2007), and represent the most species-rich environments on RO, contributing significantly to the dynamics of rocky ecosystems. In general, the versatility of new substrates for colonization (Delgadillo and Cárdenas, 1990) suggests that soil depth does not directly influence the chances of island colonization by acrocarpous mosses – which grow on vertical substrata –, but soil depth does influence bryophytes indirectly. For instance, the shallow soil typical of most SIs is the most limiting factor in the establishment of vascular plants in RO (Scarano, 2002), therefore leading to limited shading and consequent lower local moisture and higher temperatures, which are all important for bryophytes. Using a proxy which is easy to measure is important in the face of the rapid desertification predicted in the Caatinga over the next few years (Vieira et al., 2015).

By using acrocarpous moss and SI in a xeric environment as models to assess local conditions, we aimed to answer the following question: 1. Can the relationship between soil depth and gametophyte length be considered a proxy for microhabitat conditions? In order to answer this question we measured the gametophyte lengths of the acrocarpous mosses which are the most common species on these SI, namely, *Gemmabryum exile* (Bryaceae), *Fissidens lagenarius* var. *lagenarius* (Fissidentaceae), and *Campylopus pilifer* (Leucobryaceae). We expected to observe shorter gametophyte lengths in xeric environments when compared to those normally reported in the literature, and we assumed that this measurement would work as a good proxy for local conditions in harsh environments.

## 2. Materials and Methods

### 2.1 Study Areas

This study was conducted in four RO in northeastern Brazil (Figure 1). These RO were selected owing to the number of SI ( $\geq 60$ ) and the presence of vegetation coverage on top, and which was checked using data from Google Earth version 2012 (<http://www.softonic.com.br/s/google-earth-2012>). Rocky outcrops are impacted by tourism (RO 1, 2, and 3) and fire produced by religious rituals (RO 1 and 4) and, according to Wilby (2008), the dry season normally lasts at least six months. The vegetation consists mainly of open scrub forest. Rocky outcrops were characterized as in Silva et al. 2014 (Table 1). We did not correlate outcrop environmental variables with moss gametophyte length because their measures are on a macro scale (Hijimans et al., 2005). Here, we used outcrop environmental variables as a proxy to assert that soil islands are xeric environments.

### 2.2 Sampling Design

We mapped all the soil island on outcrops and randomly selected 15 to 20 (larger than or equal to 10 cm<sup>2</sup>) SI in each RO (cf. Silva et al. 2014). Only SI colonized by bryophytes were considered for this study, resulting in a total of 59 patches. A minimal distance of 20 m between SI was adopted in order to make sure that each SI was statistically independent from each other. We collected all the moss species living on soil for a period of one year and chose the most common acrocarpous mosses (i.e. vertically growing bryophytes with, generally, a terminal sporangia and possessing gametophytes which are easily individualized—Glime, 2007) found in at least 10% of the SI surface. We used the techniques for collection and herbarium preservation of bryophytes described in Frahm (2003). We estimated the average depth using a ruler buried in the SI for each one of the 59 SI. The depth of SI, with sizes up to 100 cm<sup>2</sup>, was estimated with measures taken at their center. Islands larger than 100 cm<sup>2</sup> had at least three points measured (edge-interior-edge), from which the average was estimated.

We randomly selected ten individuals from the three most common species, totaling 700 individuals: 480 *Campylopus pilifer*; 70 *Fissidens lagenarius* var. *lagenarius*; and 150 *Gemmabryum exile*. We considered highly frequent species which occurred in more than 10% of soil islands. Fertile or sexually expressive individuals were discarded at the time of specimens' selection. Gametophytes were dissected in order to prevent the interference of leaves in measuring the length and a digital caliper ruler was used for measurements (Figure 2). This methodology for measuring linear dimensions in plants was adapted from Stark et al. (2005). Measured Gametophyte length was then compared to the maximum length found in the literature (Frahm (2002)—*C. pilifer*; Bordin (2011)—*F. lagenarius* var. *lagenarius*; Spence and Ramsay (2012)—*G. exile*, Table 2).

### 2.3 Species ecology

*Gemmabryum exile* (Dozy and Molk.) J.R. Spence and H.P. Ramsay is a dioecious species with very small gametophytes. It is 4–10 mm tall, and grows in tufts, which are dense, yellowish or brownish, green, and distinctly glossy. (Spence and Ramsay, 2012). The leaves are imbricate and slightly folded inwards along costa, and are slightly concave. This aspect confers desiccation resistance (Kürschner, 2004; Kürschner and Parolly, 2005). *G. exile* is usually found in soil or on rocks. In this study, specimens often possessed rhizoidal tubers which are a type of asexual reproduction which aids the maintenance of populations in xeric environments (Glime, 2007).

*Fissidens lagenarius* Mitt. var. *lagenarius* are plants which are small to medium sized, being 3–8 mm. This species is changeable. For example, marginal cells are differentiated, with the larger

specimens possessing a salient papilla in individuals collected in harsh environments (Bordin, 2011). Papilla is an important trait which drives water conduction (Kürschner, 2004). Although *F. lagenarius* is prevalent in the Atlantic Forest of the southeast region of Brazil, it also occurs in areas of the Cerrado and Caatinga, usually growing on logs, at the base of trunks and the branches of trees, but also on termite colonies, rocks and decaying wood, from sea level up to 1840 m alt. (Bordin, 2011). In the present study, *F. lagenarius* was found only in soil despite the xeric conditions of soil islands and rocky outcrops.

*Campylopus pilifer* Brid. are plants which vary from small to robust. They are 0.5-3 cm long, and are found in dirty green, olive green or yellowish green tufts. They are equally foliate (Frahm, 2002). The leaves are lanceolate, and end in a straight, long and serrate hairpoint. The alar cells are not differentiated or highly developed. They are inflated, with the basal laminal cells being hyaline. The costa occupies 1/2-3/4 of width of the leaf and possesses adaxial hyalocysts and abaxial groups of stereids in transversed sections (Frahm, 2002). The abaxial lamellae of the leaves are 3-4 cells high. Specialized asexual reproduction occurs occasionally through deciduous stem tips. All of these specific traits optimize the plant's maintenance in harsh environments either through storing water (hyalocysts/hyaline cells – Frahm, 2000; 2003), avoiding water loss (wide costa; hairpoint – Watson, 1914), conducting water (stereids – Glime, 2015), which help maintain the plant. *C. pilifer* occurs in acidic sandy soil, acidic rocks (sandstone, granite), and rock crevices in exposed and dry habitats at altitudes from 50 to 1500 m (Frahm, 2002).

## 2.4 Data Analysis

We assumed the depth of soil islands to be a proxy for local conditions: deeper soil islands support more robust vascular plants. In turn, vascular plants minimize the intensity of solar radiation in the lower layer and decrease the incidence of desiccant winds, ensuring lower temperatures and evapotranspiration and higher humidity than those in shallower islands.

We checked the normality of each data set, comparing the length of gametophytes of each species and the depth of soil where they occurred. The data was not normally distributed, even after log-transformation. ( $\log_{10}$ : *G. exile* –  $W = 0.81$ ,  $P < 0.05$ ;  $W = 0.76$ ,  $P < 0.05$ ; *F. lagenarius* var. *lagenarius* –  $W = 0.81$ ,  $P < 0.05$ ;  $W = 0.60$ ,  $P < 0.05$ ; *C. pilifer* –  $W = 0.92$ ,  $P < 0.05$ ;  $W = 0.89$ ,  $P < 0.05$ ). We checked the normality using the Shapiro-Wilk test, which works for any n sample (Zar 2010). We then applied Generalized Linear Models (GLM) with a confidence level of 5% to evaluate the effect of soil depth over stem length. The test provides for an alternative model for nonlinear and unspecified relationships (McCullagh and Nelder, 1989). We applied Poisson distribution (log-linear) with the log connection method as a canonical function, which is suitable for qualitative data (see Hair et al., 2006).

The comparison between the gametophyte lengths of the three species was carried out using a confidence interval (95%) illustrated in a Box-plot graphic (Cox, 2009). The Box-plot is a type of exploratory graph that allows for interpretations of location, dispersion, asymmetry, tail length distribution and outliers, and is especially useful for large data sets (Tukey, 1977; Cox, 2009). We used the average coefficients of dispersion and variance in gametophyte height in order to evaluate the response of this variable to environmental conditions (Gotelli and Ellison, 2011).

## 3. Results

Soil depth varied between 0.5 and 20 cm. Most of the SI were shallow, being less than 10 cm deep. Gametophyte length displayed wider variation than that reported in the literature, except for *Gemmabryum exile* (Table 2). No outliers were identified (Figure 3). The distribution of length values was more aggregated in *C. pilifer* when compared to the other species ( $CD = 4.70$ ; Table 2).

Furthermore, most of the gametophytes of *C. pilifer* reached the highest lengths, although the average was around ~12mm. *Fissidens lagenarius* var. *lagenarius* e *G. exile* had heights of mostly around 3.79 e 3.18, respectively, both inside the expected range of variation. Yet, variation is high for both species, especially *G. exile* (Table 2; Figure 1). Although gametophyte length is a trait which displays heterogeneity in all species (*C. pilifer* being the most heterogeneous – CV = 60.32%; Table 2; Figure 3), the correlation between length and soil depth was not observed for any of the species under study (*G. exile*:  $G = 0.14$ ;  $P = 0.7$ ; *F. lagenarius* var. *lagenarius*:  $G = 0.01$ ;  $P = 0.9$ ; *C. pilifer*:  $G = 0.03$ ;  $P = 0.8$ ). The failure for predictive models is 1.28, 1.47, and 2.46%, respectively.

#### 4. Discussion

.... Rocky outcrops are unique formations which often act as environmental filters (e.g. Frahm, 1996; Porembski et al., 1998; Sarthou et al., 2003). Nevertheless, to our knowledge, no study has yet proven the existence of these environmental barriers. The application of the gametophyte height of acrocarpous mosses would allow us to monitor areas along the Caatinga in an efficient manner, provided that its correlation with the microclimate is consistent. This monitoring is especially important given the rapid advance of desertification in the Caatinga areas in recent years (Vieira et al., 2015).

Deep islands are expected to offer a better support for robust vascular plants (see Scarano, 2002) and thus confer more shade (more humidity/lower temperature) and protection against desiccant winds (low evaporation) than shallow soils. These environmental conditions are important structuring agents for bryophyte communities at the local level on soil islands (Silva et al., 2014). If the height of the stem of acrocarpous mosses proved to be positively related to soil depth and its implied benefits, we would be able to infer the health of plant communities in harsh environments by measuring this attribute. In this sense, there is a "domino effect": pioneer bryophytes shape the environment and promote seed establishment (Scarano, 2002; Cornelissen et al., 2007). Then plant density promotes better moisture and temperature conditions in relation to its surroundings, and in turn, this optimizes investment in biomass on the part of bryophytes (as observed, for example, in epiphytic bryophytes,– Ah-Peng et al., 2007). In fact, bryophytes can offer services to the ecosystem such as maintaining local humidity (Chang et al., 2002; Pypker et al., 2006) promoting richness, diversity and maintenance of non-vascular and vascular plants. Nevertheless, our results show that the positive effects of soil depth and bryophyte coverage on vascular flora may not occur concomitantly, as soil depth does not result in taller bryophytes.

The lack of relationship between gametophyte length and soil depth might have two explanations. Firstly, most of the deeper islands (87.5%) were small areas (< 5 m<sup>2</sup>) (Silva, 2014), supporting fewer vascular plants, probably because these islands were formed by soil deposits in deep but small rock depressions. This is in contrast to other SI which were formed by soil deposits in shallow but big depressions (Silva JB., personal observation). On one hand, deep but small islands may support more robust vascular plants but of fewer species will be represented and therefore result in less shade and higher temperatures. In turn, larger but shallower islands offer support for less robust species, and fail to provide shading. On the other hand, deeper soil islands should retain more water content than shallower soil patches which dry more quickly due to insolation. Bryophytes have no true roots but instead possess rhizoids, whose main function is to fix the plant to the substrate. Additionally, rhizoids can only reach a few centimeters in length (Glime, 2007). Yet, contrary to expectation, when Silva et al. (in prep.) performed a survey on the humidity of soil islands, they found that there is an inverse correlation of soil humidity and soil depth when assessing the humidity in the first 5 cm ( $\tau = -0.23$ ;  $P = 0.02$ ). An explanation for this finding is that water must percolate downwards until the rocky surface below the soil of the deepest islands,

while water remains closer to the soil surface in shallower islands, under the same climatic conditions.

The second explanation for the lack of relationship between gametophyte length and soil depth is that potential adaptive traits of the species under study would allow them to achieve optimal growth. Mosses displayed traits for water storage (*e.g.*, concave, twisted and tabulate leaves, the presence of hyalocists and lamellae on leaves, and tuft life forms – Kürschner and Parolly, 2005; Glime, 2007) which optimize their survival prospects in harsh environments. Some species displayed a better performance in rain forests instead of xeric habitats, although they are registered in both of them (*e.g.* Polytrichaceae spp.–Kappen and Valladares, 2007). For example, *F. lagenarius* var. *lagenarius* is found in forests, but it is also found in hotter environments (Bordin 2011), where *G. exile* and *C. pilifer* are easily found (Frahm, 2002; Spence and Ramsay, 2012). Thus, we suggest that future studies focus on elucidating questions about bryophytes' adaptive traits to soil islands and how we can assess and monitor microclimate using those traits.

## 5. Conclusion

Despite being the richest habitats for plants on outcrops, soil islands have been insufficiently researched. The wide variability verified for stem length in all studied species and the lack of correlation with soil depth indicates that gametophyte length is a poor predictor and should not be used as a proxy to evaluate local conditions once that species seems adapted to the local conditions. On the other hand, the remarkable development of *Campylopus pilifer* in reaching heights higher than those described in the literature, suggests that soil islands are xeric environments. The rationale is that this pioneer species, common in dry environments (Scarano, 2002), is expected to develop best under drier conditions. To our knowledge, this is the first experimental study which suggests that soil islands are xeric environments.

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Table 1. Bioclimatic variables for each one rock outcrops. The variables were measured in macroscale.

Bioclimatic variables	RO1	RO2	RO3	RO4
Temperature Seasonality (°C) *	132.5	137.3	152.8	169.7
Precipitation of Driest Quarter (mm)**	43	27	8	44
Distance from Sea (Km)	118.53	122.2	212.89	192.17
Annual Precipitation (mm)	623	466	393	609

\*Temperature Seasonality = indicates temperature fluctuation mean along the year

\*\*Precipitation of Driest Quarter = indicates precipitation on driest bimester over year.

Table 2. Descriptive analysis about the variation of the gametophytes length of the three moss species studied.

Family/Species	Expected variation (literature) (mm)	Observed variation (mm)	s	s <sup>2</sup>	Ȳ	Med	CV (%)	CD
<b>Bryaceae</b>								
<i>Gemmabryum exile</i> (Dozy and Molk.) J.R. Spence and H.P. Ramsay	4 – 10	0.88 – 5.63	1.13	1.28	3.21	3.18	35.20	0.39
<b>Fissidentaceae</b>								
<i>Fissidens lagenarius</i> Mitt. var. <i>lagenarius</i>	3 – 8	1.01 – 9.1	1.69	2.86	3.53	3.79	44.59	0.75
<b>Leucobryaceae</b>								
<i>Campylopus pilifer</i> Brid.	5 – 30	1.00 – 39.5	7.8	60.82	12.93	11.39	60.32	4.70

s = Standard deviation; s<sup>2</sup> = Variance; Ȳ = Mean; Med = Median; CV = Coefficient of Variance; CD = Coefficient of dispersion.

Symbols and abbreviations are according to Gotelli and Ellison (2011).

Figure 1. Location of rocky outcrops in northeastern Brazil, in the Caatinga. RO = Rocky outcrop; number after acronyms identifies each outcrop. Superior pictures represent RO and lateral pictures, soil islands.

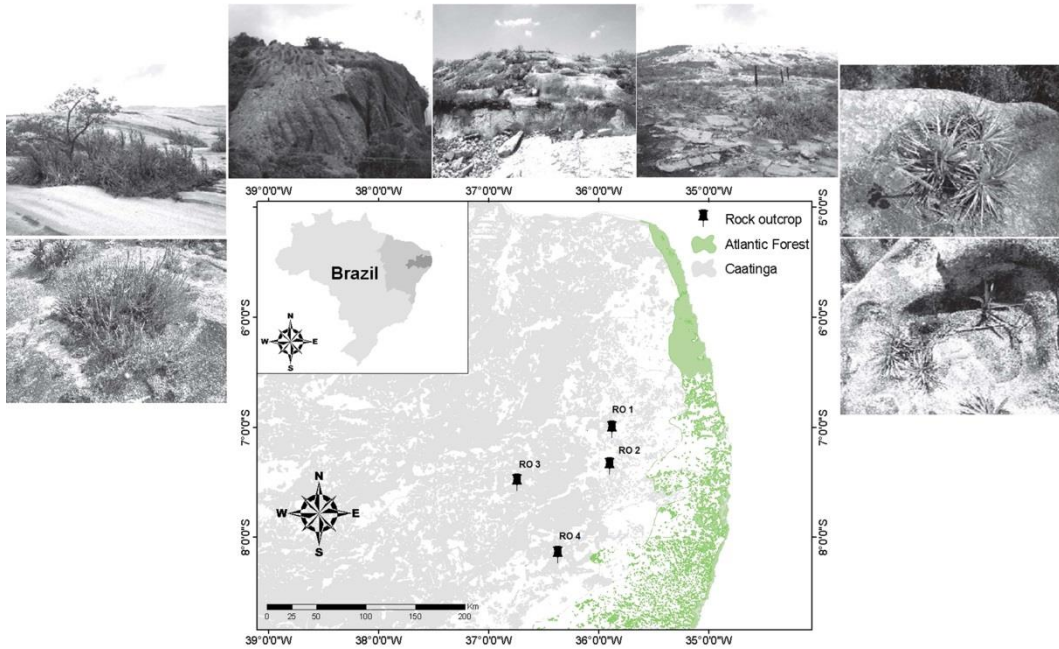




Figure 2. Representation of fieldwork and laboratory procedure. We randomly selected soil islands on rocky outcrops, measured their depth, and collected acrocarpous mosses. We randomly selected ten individuals of each population of the most frequent mosses. Then, we dissected and measured gametophytes.

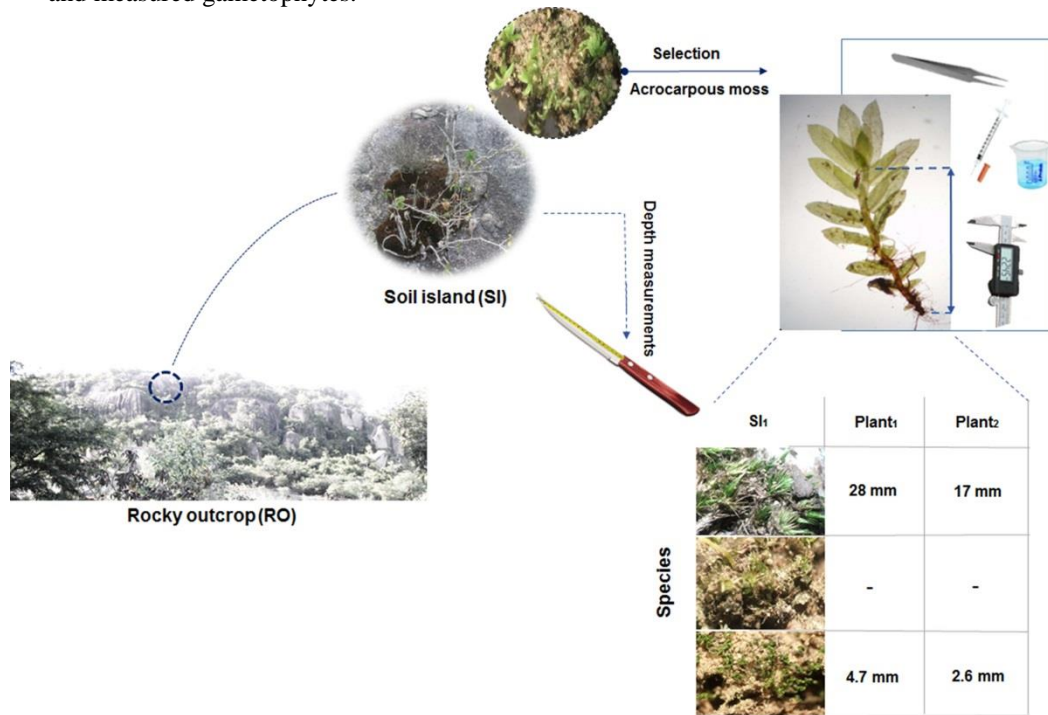




Figure 3. Dispersion of stem length over maximum and minimum measures observed. The majority of *C. pilifer* (*Campylopus pilifer*) gametophytes possessed a length close to or above that expected; *F. lagenarius* (*Fissidens lagenarius* var. *lagenarius*) and *G. exile* (*Gemmabryum exile*) display measures scattered between maximum and minimum ranges.

