Environmental influence on the inter- and intraspecific variation in the density and morphology of stomata and trichomes of epiphytic bromeliads of the Yucatan Peninsula

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We set out to test whether trichome and stomata variation in epiphytic bromeliads in the field would support the hypothesis that trichomes confer protection from excess light, increase water absorption and protect the stomata. We characterized the intra- and interspecific plasticity in the morphology and density of trichomes and stomata of 14 epiphytic bromeliad species found in six contrasting vegetation types (coastal sand dune scrub, scrub mangrove, petén mangrove, and deciduous, semi-deciduous and semi-evergreen forest) on the Yucatan Peninsula along a precipitation gradient from 500 to 1500 mm (mean annual precipitation). We performed leaf impressions to determine trichome and stomatal density and to characterize morphology. Trichome morphology did not show variation between sites, but was distinctive for the species. Our data supported the existing hypothesis that trichome density and area were higher in the atmospheric species that inhabited the drier sites, compared with the wetter extreme, which may confer higher protection against photoinhibition and desiccation. Stomatal density increased with precipitation in tank species, and negatively correlated with vapour pressure deficit during the dry season. Fewer trichomes and greater numbers of stomata than expected by rainfall were found in mangroves that maintained low vapour pressure deficit during the dry season. © 2016 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2016

ADDITIONAL KEYWORDS: atmospheric species – Bromeliaceae – leaf structure morphology – leaf structure variability – tank species.

INTRODUCTION

The family Bromeliaceae, with 3352 species (Luther, 2012), has been very successful at colonizing the epiphytic environment, having a large number of angiosperm epiphytic species, only surpassed by the family Orchidaceae (Benzing, 2000). Success in the epiphytic habitat has been linked to a series of strategies, mainly to harvest and conserve water and nutrients (Crayn, Winter & Smith, 2004; Quezada &

Gianoli, 2011; Silvestro, Zizka & Schulte, 2014), which are provided in pulses during precipitation events (Reyes-García & Griffiths, 2009). In the Bromeliaceae family, leaves are the main component of the vegetative body, and processes that occur exclusively in roots in other plants operate by means of trichomes in the leaves of epiphytic members of this family. In early diverging lineages of terrestrial Bromeliaceae, the scale-like trichomes apparently function as structures for water repellency (Pierce *et al.*, 2001), whereas, in epiphytes, trichomes play a key role in the absorption of nutrients and water

from precipitation, and also in protecting the stomata in some species (Givnish *et al.*, 2007; Stefano, Papini & Brighigna, 2008). Within the Bromeliaceae, Tillandsioideae species have the most developed trichomes, which possess foot and dome cells embedded in the epidermis and a shield formed by a central disc consisting of four equal-sized cells surrounded by a ring of smaller cells; beyond the central disc lies a series of rings (1–4) followed by cells displayed as wings (Benzing, 2000).

In addition to their role in the absorption of water and nutrients, trichomes influence water and energy balance and may be involved in photoprotection. For instance, at high densities, trichomes act as a diffusive filter reflecting part of the incident light and reducing solar radiation on the leaf surface (Benzing & Renfrow, 1971; Lüttge *et al.*, 1986; Larcher, 2003). Light reflection by trichomes has been linked to reduced photoinhibition at saturating light (Pierce, 2007). In addition, trichome cover increases the boundary layer around stomata (Benz & Martin, 2006).

Other important adaptations in epiphytic bromeliads are the presence of crassulacean acid metabolism (CAM) and the differentiation in specialized life forms. The tank life form has wide, usually long leaves arranged in a rosette, which form a tank in which water is stored. The atmospheric life form has root systems that function primarily as holdfasts, lacks a tank and has narrow leaves, densely covered with trichomes (Adams & Martin, 1986; Benzing, 2000; Givnish *et al.*, 2007). In addition, the different life forms show differences in the distribution of trichomes along the leaf surface. Tank species usually have a higher trichome density at the leaf base, whereas, in atmospheric species, trichomes are relatively uniformly distributed (Benzing, 2000).

Epiphytic bromeliads are highly sensitive to changes in environmental conditions, such as water availability (as rain, dew or fog), the quantity of light they receive and the nutrients they obtain from organic decomposition within tanks or from precipitation, all of which vary among habitats, host tree species, canopy strata and seasons (Andrade, 2003; Graham & Andrade, 2004; Mondragón *et al.*, 2004; Zotz & Asshoff, 2010; Chilpa-Galván *et al.*, 2013; Cach-Pérez, Andrade & Reyes-García, 2014). In this sense, the environment influences both stomatal and trichome density; species from wetter environments tend to have reduced trichome densities and higher stomatal densities, whereas species from drier environments possess high trichome density, wide trichome shields and low stomatal densities (Benzing, 2000). The correlation between the environment and trichome and stomatal densities has been investigated using single-species approaches, but few have addressed this question using multiple species grown under natural conditions along an environmental gradient.

Our study site, the Yucatan Peninsula, Mexico, is characterized by a precipitation gradient that moves from a drier northwest region (less than 500 mm year^{-1}) to a wetter southern region (1600 mm year^{-1}) (Orellana *et al.*, 2009). Within this gradient, different environments are found, from mangroves and coastal sand dune scrubs to tropical forests (Cach-Pérez *et al.*, 2013). The association between the physiology of epiphytic bromeliads and environmental conditions has been well documented (Andrade, 2003; Cervantes, Graham & Andrade, 2005; González-Salvatierra *et al.*, 2010; Reyes-García, Mejia-Chang & Griffiths, 2012), but there is a lack of knowledge of the morphological associates.

The aim of this study was to test the existing hypothesis that bromeliad trichomes increase water use efficiency and photoprotection, and this confers advantages in water-limited and high-light environments. This hypothesis predicts that species found growing in the drier vegetation types will show higher trichome density, lower stomatal density and smaller stomata and trichomes than species of the wetter and denser vegetation types. We performed this test by comparing the intra- and interspecific plasticity in the morphology and density of trichomes and stomata of 14 epiphytic bromeliad species growing in different vegetation types.

MATERIAL AND METHODS

FIELD SITES

This study compared epiphytic bromeliads from six different vegetation types (Miranda & Hernández, 1963; Miranda, 1978) of the Yucatan Peninsula (Fig. 1): coastal sand dune scrub (21°19′N, 89°26′W; 500 mm mean annual precipitation), scrub and petén mangrove (20°51′N, 90°22′W; 675 mm mean annual precipitation), deciduous forest (21°05′N, 89°35′W; 900 mm mean annual precipitation), semi-deciduous forest (20°05′N, 89°32′W; 1150 mm mean annual precipitation) and semi-evergreen forest (18°06′N, 89°48′W; 1500 mm mean annual precipitation); the sites are described in detail in Cach-Pérez *et al.* (2013). In the Yucatan Peninsula, the petén mangrove vegetation is characterized by islands of vegetation formed by semi-deciduous forest trees that are embedded in a matrix of mangrove vegetation. This vegetation responds to the salinity gradient formed around a freshwater upwelling in central petén, which provides a suitable environment for the trees that protrude from the surrounding vegetation (Flores, Durán & Ortiz, 2010). The different sites

vary in canopy height, increasing with precipitation. The forests also show marked differences in the number of deciduous species, which influences the seasonal exposure of epiphytes (Cach-Pérez *et al.*, 2013). All epiphytic bromeliad species found were identified following Ramírez, Carnevali & Chi (2004).

Because ambient humidity may be very important in these species, vapour pressure deficit (VPD, kPa) and historical precipitation were considered when analysing the relationship between morphological traits and the environment. Measurements of VPD were made seasonally (rainy, early dry and dry seasons) between July 2009 and May 2011 for at least four consecutive days. Data were recorded in the stratum with higher epiphyte density according to Cach-Pérez *et al.* (2013). Temperature and humidity sensors (S-THB-002) connected to a data logger (HOBO micro station H21-002, station and sensors from Onset, Bourne, MA, USA) were used.

ANATOMICAL MEASUREMENTS

Leaf samples of all the species found among the six vegetation types (Table 1) were collected between October 2011 and February 2012, and processed at Centro de Investigación Científica de Yucatán (CICY). Vouchers of representative samples were deposited at the CICY herbarium. A fully mature leaf of the mid-section of the rosette, of five individuals per species per site, was cut from the base, and transparent commercial nail polish was applied over the adaxial and abaxial surfaces. To observe the stomata, the leaf blade was shaved to remove the trichome wings before the nail polish was applied. Once dry, the nail polish was peeled off with watchmaker forceps; the impression was divided into three leaf sections of equal length: base, middle and tip. Each section was mounted on a microscope slide, observed and photographed using a light microscope (DME, Leica Microsystems GmbH, Weitzlar, Germany) with
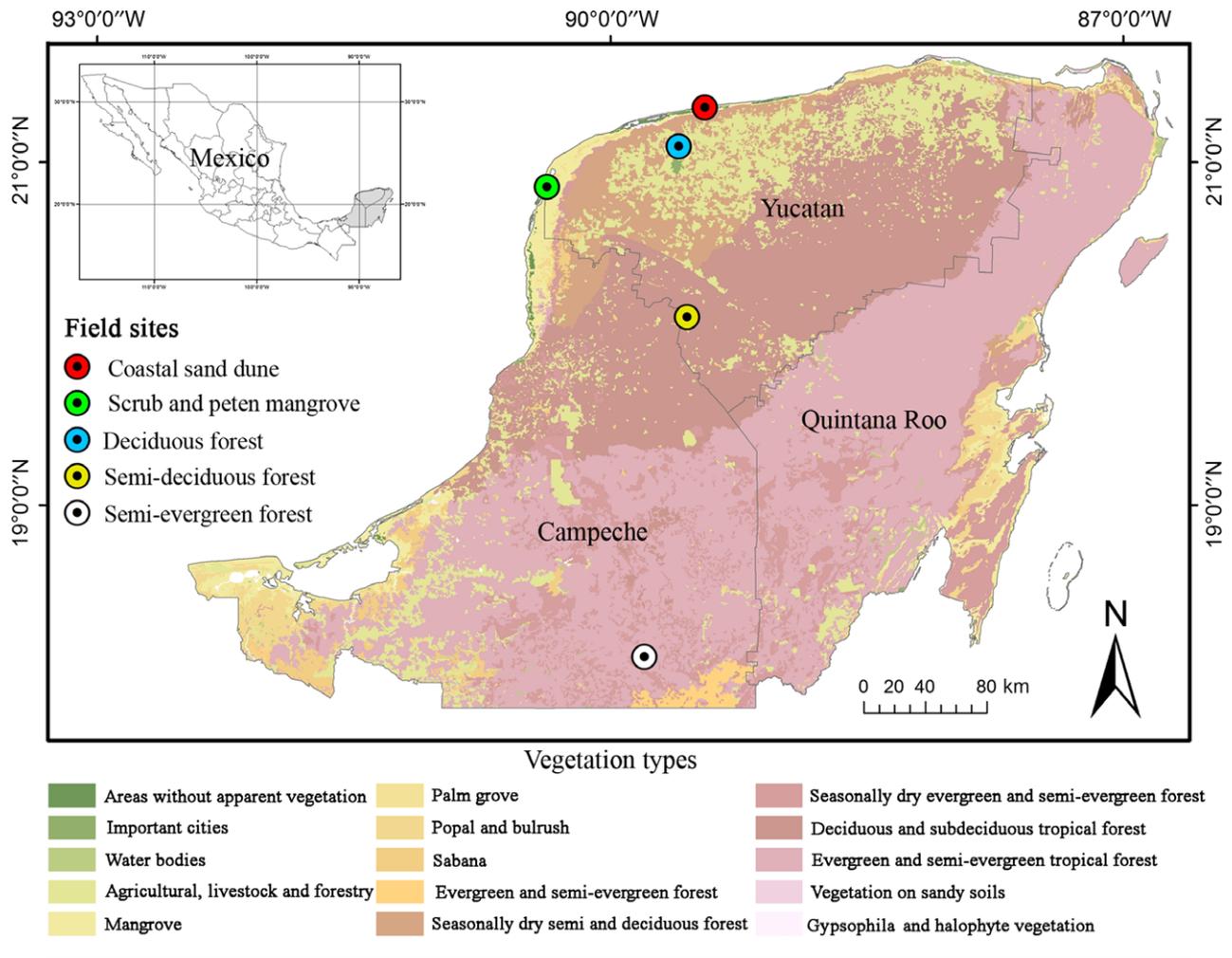
Figure 1. Location of field sites and vegetation types of the Yucatan Peninsula, Mexico. Projection of decimal coordinates over a fragment of the map of Mexican vegetation types (INEGI).

Table 1. Epiphytic bromeliad species found in six vegetation types of the Yucatan Peninsula. The sites refer to the vegetation in which the species were sampled

Epiphytic bromeliad species	Life form	Site
Aechmea bracteata Griseb.	Tank	DF, SE
Catopsis nutans Griseb.	Tank	SE
Tillandsia balbisiana Schult.f.	Atmospheric	CD, DF, SE
Tillandsia brachycaulos Schltdl.	Atmospheric	DF, SD, SE
Tillandsia dasyliriifolia Baker	Tank	CD, SM, SE
Tillandsia elongata Kunth var. *subimbricata* (Baker) L.B. Sm.	Tank	DF
Tillandsia fasciculata Sw.	Atmospheric	DF, SD, SE
Tillandsia juncea Poir	Atmospheric	SE
Tillandsia recurvata (L.) L.	Atmospheric	DF, SD, SE
Tillandsia schiedeana Steud.	Atmospheric	DF, SD, SE
Tillandsia streptophylla Schweid.	Atmospheric	PM
Tillandsia usneoides (L.) L.	Atmospheric	SE
Tillandsia utriculata L.	Tank	CD
Tillandsia yucatana Baker	Atmospheric	CD, SM, DF

CD, coastal sand dune; DF, deciduous forest; PM, petén mangrove; SD, semi-deciduous forest; SE, semi-evergreen forest; SM, scrub mangrove.

a $10\times$ objective and a digital camera (Moticam 2500, Motic China Group Co. Ltd., Hong Kong, China). The area covered by the photographs (0.3639 mm^2) was calibrated using a graduated measuring slide and the software Motic Image Plus (Motic China Group Co. Ltd.). A photograph per section per sample was taken.

The morphology of the stomata and trichomes was determined from the photographs of intact leaf samples, obtained using scanning electron microscopy. One square centimetre of the mid-section of each sampled leaf (except for *Tillandsia juncea* Poir for which the samples were narrower because the species has very narrow leaves, measuring less than 4 mm across) was progressively dehydrated in an ethanol series (20%, 40%, 60%, 80% and 100%; immersion lasted 20 min per concentration). Samples were dried to critical point in a SAMDRI 795 dryer (Tousimis, Rockville, MD, USA), fixed to copper specimen holders with double-sided tape and coated with gold in a Denton Vacuum Desk II metallizer (Denton Vacuum LLC, Moorestown, NJ, USA) for observation under a scanning electron microscope JEOL-JSM-6360LV (Jeol Ltd., Tokyo, Japan). Three haphazardly selected trichomes (on the abaxial face) and three stomata (from the adaxial face) were characterized, measuring the stomatal area, size of the stomatal pore and trichome area (base and shield) using CeleraScope V.1.0 software (Aurea Software, Merida, Mexico). We did not characterize the trichomes on the adaxial face because it was necessary to shave the leaf before fixation on the copper specimen holders to make the stomata visible. This did not affect the trichome counts on the abaxial face because trichome bases were visible. The density was calculated as the number of stomata (or trichomes) per square millimetre.

STATISTICAL ANALYSIS

We tested differences in trichome and stomatal density between leaf sections, life forms (tank vs. atmospheric species) and vegetation types (in species distributed in more than one habitat) using analysis of variance (ANOVA, $\alpha = 0.05$). The same test was used to compare differences among species. Tukey tests were carried out to discern specific differences among the data. The normality of the data was tested prior to each ANOVA; square root transformations were used to normalize the data when necessary. Statistica 8 software (Statsoft, Inc. 1984–2007, Tulsa, OK, USA) was used in all cases. A simple linear regression was performed to evaluate the correlations between trichome and stomatal densities, and to test the effect of mean annual precipitation on trichome and stomatal size and density. A multiple regression was used to evaluate the combined effect of VPD (dry season) and annual precipitation on trichome and stomatal densities. SigmaPlot 11 software (Systat Software, Inc., San Jose, CA, USA, 2008) was used for simple regression, and SPSS Statistics 17 (SPSS Inc., Chicago, IL, USA, 2007) was used for multiple regressions.

RESULTS

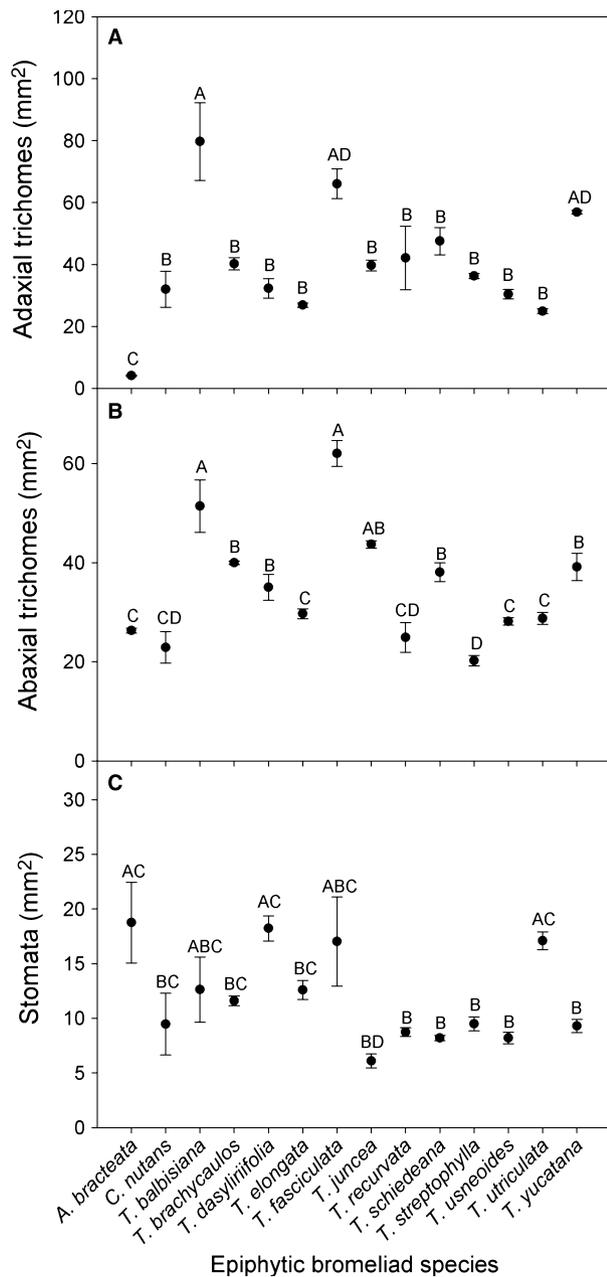## TRICHOME AND STOMATAL DENSITY

We found 14 epiphytic bromeliad species in the six vegetation types (Fig. 2); eight species were found in more than one vegetation type (Table 1). Trichome numbers were highly variable, both among species (Fig. 3) and within the same species in different habitats (Tables 2 and 3). The tank species *Aechmea bracteata* Griseb, from the dry forest, had the lowest trichome density, with mean values of 3.8 and 25.4 trichomes mm^{-2} on the adaxial and abaxial leaf surfaces, respectively. In contrast, the atmospheric species *Tillandsia balbisiana* Schult. f., from the coastal

Figure 2. Illustrations of 12 of the 14 epiphytic bromeliad species found in the precipitation gradient of the Yucatan Peninsula, Mexico. A, *Aechmea bracteata*; B, *Catopsis nutans*; C, *Tillandsia balbisiana*; D, *Tillandsia brachycaulos*; E, *Tillandsia dasyliriifolia*; F, *Tillandsia elongata* var. *subimbricata*; G, *Tillandsia fasciculata*; H, *Tillandsia recurvata*; I, *Tillandsia schiedeana*; J, *Tillandsia streptophylla*; K, *Tillandsia utriculata*; L, *Tillandsia yucatana*.

sand dune scrub, had the highest trichome density, with mean values of 101 and 57 trichomes mm^{-2} on the adaxial and abaxial leaf surfaces, respectively.

The few tank species localized within the six sites, *A. bracteata*, *Catopsis nutans* Griseb, *Tillandsia dasyliriifolia* Baker, *Tillandsia elongata* Kunth var.

Figure 3. Stomatal and trichome densities between epiphytic bromeliad species. A, Adaxial trichome density. B, Abaxial trichome density. C, Stomatal density. Data are means ± SE. Different letters denote statistical differences.

subimbricata (Baker) L.B. Sm. and *Tillandsia utriculata* L., did not show differences in trichome number among leaf sections ($P > 0.05$), either on the abaxial or adaxial leaf surfaces (Tables 2 and 3). However, with the exception of *C. nutans*, tank species had higher trichome densities on the abaxial relative to the adaxial leaf surface. Individuals of *T. dasyliriifolia* had greater mean leaf trichome

density in the drier habitat than in the wettest one (Tables 2 and 3).

In contrast, the atmospheric species showed higher variation in trichome distribution, and those found in more than one vegetation type showed higher leaf surface trichome number in the driest habitat. The adaxial leaf surface was more variable than the abaxial surface among sites. In most cases, the atmospheric species showed either no significant differences between leaf sections or a significant decrease in trichome density from base to tip, whereas other arrangements, such as increased or decreased trichome density in the middle, were rare (Tables 2 and 3).

Stomatal density also showed high variation, both among species and within species in different habitats (Table 4). The lowest stomatal density was registered in *T. juncea* from the semi-evergreen forest (6.1 ± 1.1 stomata mm^{-2}), whereas the highest density was recorded in *A. bracteata* from the deciduous forest (25.7 ± 2.0 stomata mm^{-2}). There was low variation in stomatal density among leaf sections. None of the species had the greatest density on the leaf base; the tank species *T. elongata* and *T. utriculata*, and most atmospheric species, presented the highest stomatal density on the middle part of the leaf, whereas the tank species *A. bracteata* and *C. nutans* had the highest stomatal density on the leaf tip. Among the vegetation types, four atmospheric species (*T. balbisiana*, *Tillandsia fasciculata* Sw., *Tillandsia recurvata* (L.) L. and *T. schiedeana* Steud.) and two tank species (*A. bracteata* and *T. dasyliriifolia*) tended to have higher stomatal density in the drier vegetation types than in the wetter ones. *Tillandsia brachycaulos* Schltdl. was the species with the lowest variation in stomatal density, both among leaf sections and in different habitats, whereas *A. bracteata* was the species with the highest variation (Table 4).

Comparison between life forms (atmospheric vs. tank) showed that atmospheric epiphytic bromeliads had a mean of up to 32% more trichomes mm^{-2} than tank species, but tank species had a mean of up to 34% more stomata mm^{-2} than atmospheric species ($P < 0.05$ in both cases). Among genera, tank species of *Tillandsia* had 36.1% and 64.7% more trichomes than species of *Aechmea* and *Catopsis*, respectively, but *Aechmea* species had 12.7% and 49.5% more stomata than *Tillandsia* and *Catopsis* species. Overall, atmospheric bromeliad species presented higher trichome density on the leaf base (of both abaxial and adaxial surfaces) compared with tank species ($P = 0.0235$).

Trichome and stomatal density covaried in five epiphytic bromeliad species. The trichome and stomatal density showed a positive relationship in *T. balbisiana*, *T. dasyliriifolia*, *T. juncea* and *T. utriculata*.

Table 2. Number of trichomes mm^{-2} on the leaf adaxial surface for epiphytic bromeliads of six vegetation types (sites)

Bromeliad species	Life form	Site	Leaf base	Leaf middle	Leaf tip	Adaxial mean
Aechmea bracteata	T	DF	4.6 ± 0.5*	3.8 ± 0.9*	3.7 ± 0.5*	3.8 ± 0.5*
A. bracteata	T	SE	4.9 ± 0.9*	3.7 ± 0.5*	4.6 ± 1.0*	4.5 ± 0.8*
Catopsis nutans	T	SE	39.4 ± 9.7*	27.5 ± 2.7*	29.3 ± 3.7*	32 ± 10.8
Tillandsia balbisiana	A	CD	103.7 ± 8.7*	102.8 ± 7.4*	96.4 ± 8.0*	101 ± 4.2*
T. balbisiana	A	DF	89 ± 0.6*	75.2 ± 5.6*	77 ± 4.3*	80.4 ± 2.9†
T. balbisiana	A	SE	67.8 ± 5.6*	56.8 ± 3.9*	48.5 ± 1.8†	57.7 ± 3.6‡
Tillandsia brachycaulos	A	DF	45.2 ± 5.3*	36.8 ± 4.3*	38.82 ± 3.1*	40.3 ± 2.6*
T. brachycaulos	A	SD	33 ± 1.6†	40.3 ± 6.4*	36.7 ± 1.8*†	36.7 ± 2.2*
T. brachycaulos	A	SE	33.9 ± 8.8†	47.7 ± 0.9*	49.5 ± 5.7*	43.7 ± 3.9*
Tillandsia dasyliriifolia	T	CD	38.8 ± 8.4*	40.4 ± 5.1*	32.1 ± 5.6*	37.0 ± 3.5*
T. dasyliriifolia	T	SM	35 ± 5.20*	30.6 ± 2.7*	34.8 ± 3.6*	33.5 ± 2.1*
T. dasyliriifolia	T	SE	26.6 ± 0.9*	30.3 ± 4.2*	31.2 ± 31.9*	26.4 ± 2.0†
Tillandsia elongata	T	DF	27.6 ± 3.2*	26.6 ± 2.4*	26.6 ± 1.8*	26.9 ± 1.3
Tillandsia fasciculata	A	DF	70.5 ± 5.3*	77.1 ± 5.6*	67.7 ± 3.9*	71.7 ± 2.9*
T. fasciculata	A	SD	79.8 ± 6.4*	70.6 ± 2.4*	59.6 ± 5.6†	69.9 ± 3.9*
T. fasciculata	A	SE	61.4 ± 5.1*	50.4 ± 4.1*	57.8 ± 2.8*	56.5 ± 2.6†
Tillandsia juncea	A	SE	32.1 ± 3.9†	55.9 ± 4.6*	29.4 ± 4.8†	39.1 ± 4.8
Tillandsia recurvata	A	DF	59.8 ± 6.4*	45.3 ± 5.6*†	33.7 ± 3.8†	46.3 ± 4.3*
T. recurvata	A	SD	74.2 ± 3.2*	58.6 ± 5.1†	39.4 ± 2.4‡	57.4 ± 5.4*
T. recurvata	A	SE	19.2 ± 1.6*	26.3 ± 3.5*	22.7 ± 2.5*	22.7 ± 1.7†
Tillandsia schiedeana	A	DF	54.2 ± 2.4*	35.8 ± 3.2†	36.7 ± 2.4†	42.3 ± 3.3†
T. schiedeana	A	SD	47.8 ± 4.9*	46.8 ± 5.5*†	37.7 ± 3.3†	44.1 ± 2.8†
T. schiedeana	A	SE	56.1 ± 3.3*	59.7 ± 2.4*	53.3 ± 0.9*	56.3 ± 1.5*
Tillandsia streptophylla	A	PM	39.4 ± 3.9*	33 ± 1.6*	35.6 ± 2.8*	36.1 ± 1.7
Tillandsia usneoides	A	SE	37.6 ± 2.9*	26.6 ± 1.9*	26.6 ± 2.6*	30.3 ± 2.9
Tillandsia utriculata	T	CD	25.7 ± 2.4*	22.9 ± 3.3*	25.7 ± 3.7*	24.8 ± 1.7
Tillandsia yucatana	A	CD	51.4 ± 5.1†	63.4 ± 2.9*	54.2 ± 4.3*	56.3 ± 2.6*
T. yucatana	A	SM	70.9 ± 4.1*	45.5 ± 3.9†	52.4 ± 4.5†	56.3 ± 3.9*
T. yucatana	A	DF	64.1 ± 1.7*	59.9 ± 5.2*	50.3 ± 2.8†	58.1 ± 2.5*

Data are means ± SE. Different symbols denote significant differences ($P < 0.05$) between leaf sections; in the adaxial mean column, statistical differences among sites were tested. In species present in more than one vegetation type, the sites are listed in order of increasing precipitation.
A, atmospheric species; CD, coastal sand dune; DF, deciduous forest; PM, petén mangrove; SD, semi-deciduous forest; SE, semi-evergreen forest; SM, scrub mangrove; T, tank species.

In contrast, *Tillandsia streptophylla* Schweid showed a negative relationship between trichome and stomatal density (Fig. 4). *Aechmea bracteata, T. balbisiana* and *T. fasciculata* were the species that presented the greatest differences in trichome density with respect to the rest of the species. The first species had the lowest density in the adaxial leaf surface, whereas the other two had the highest density on both the abaxial and adaxial leaf surfaces. *Aechmea bracteata, T. dasyliriifolia, T. fasciculata* and *T. utriculata* presented the highest stomatal density, whereas, in the rest of the species, the densities of stomata were similar (Fig. 3).

When environmental variables were included, a negative relationship was found between mean annual precipitation and trichome shield area, trichome area, stomatal area and stomatal pore area of tank species (Fig. 5A–D). In atmospheric species, a negative correlation was found between mean annual precipitation and stomatal and stomatal pore area (Fig. 5C, D), whereas precipitation did not influence trichome area in these species.

There were significant differences in the mean annual VPD among sites (Table 5), but differences were marked in each season. During the rainy season, the petén mangrove showed the highest VPD, whereas, in the dry season, the scrub and petén mangroves maintained a lower VPD compared with the other vegetation types.

For tank species, there was a negative relationship between precipitation and VPD during the rainy season and trichome density, whereas there was a positive relationship with VPD during the dry season (Fig. 6A; $r^2 = 0.103$; $P < 0.01$; figure legend

Table 3. Number of trichomes mm^{-2} on the leaf abaxial surface for epiphytic bromeliads of six vegetation types (sites)

Bromeliad species	Site	Leaf base	Leaf middle	Leaf tip	Abaxial mean
Aechmea bracteata	DF	22.9 ± 1.4*	26.6 ± 1.4*	26.6 ± 1.9*	25.4 ± 1.5*
A. bracteata	SE	26.6 ± 1.9*	32.0 ± 2.3*	22.9 ± 0.6*	27.2 ± 2.0*
Catopsis nutans	SE	25.6 ± 1.8*	24.7 ± 4.1*	19.2 ± 1.6*	23.2 ± 5.1
Tillandsia balbisiana	CD	55.0 ± 1.63†	51.3 ± 3.3†	66.9 ± 3.2*	57.6 ± 2.7*
T. balbisiana	DF	55.0 ± 4.2*	55.0 ± 1.5*	56.9 ± 0.9*	55.7 ± 1.3*
T. balbisiana	SE	33.9 ± 3.3†	49.5 ± 1.6*	39.4 ± 2.4†	40.9 ± 2.6†
Tillandsia brachycaulos	DF	39.1 ± 1.5*†	35.7 ± 1.9†	43.6 ± 2.2*	39.5 ± 1.3*
T. brachycaulos	SD	40.3 ± 3.9*	41.2 ± 1.6*	37.6 ± 2.5*	39.7 ± 1.5*
T. brachycaulos	SE	42.2 ± 3.4*	39.5 ± 2.4*	40.4 ± 3.3*	40.7 ± 1.4*
Tillandsia dasyliriifolia	CD	34.9 ± 2.9*	42.3 ± 3.3*	39.5 ± 2.4*	38.9 ± 1.7*
T. dasyliriifolia	SM	35.8 ± 1.6*	36.7 ± 0.9*	36 ± 0.25*	36.2 ± 0.6*
T. dasyliriifolia	SE	31.3 ± 0.9*	30.3 ± 1.6*	28.5 ± 1.8*	30.0 ± 0.9†
Tillandsia elongata	DF	26.6 ± 1.8*	34.9 ± 3.3*	27.6 ± 3.1*	29.7 ± 1.9
Tillandsia fasciculata	DF	66.6 ± 3.6*	72.1 ± 3.1*	60.5 ± 3.1*	66.4 ± 2.2[a]
T. fasciculata	SD	61.7 ± 3.6*	60.9 ± 2.7*	64.2 ± 3.7*	62.3 ± 1.8[a]
T. fasciculata	SE	56.8 ± 0.9*	53.2 ± 3.3*	62.3 ± 3.3*	57.4 ± 1.9†
Tillandsia juncea	SE	44.9 ± 2.4*	43.1 ± 1.8*	43.1 ± 3.3*	43.7 ± 1.3
Tillandsia recurvata	DF	37.6 ± 2.4*	32.9 ± 3.2*	20.2 ± 2.4†	30.2 ± 2.9*
T. recurvata	SD	22.9 ± 2.4*	23.8 ± 2.4*	27.9 ± 1.6*	24.7 ± 1.3†
T. recurvata	SE	19.2 ± 2.7*	21.1 ± 1.8*	19.2 ± 2.7*	19.8 ± 1.2‡
Tillandsia schiedeana	DF	47.8 ± 2.4*	35.8 ± 3.2†	37.7 ± 2.4*	40.1 ± 2.3*
T. schiedeana	SD	35.5 ± 1.3*	33.5 ± 1.3*	33.9 ± 1.2*	34.3 ± 0.7†
T. schiedeana	SE	32.1 ± 0.9†	45.9 ± 2.4*	41.8 ± 3.1*	39.8 ± 2.3*
Tillandsia streptophylla	PM	20.2 ± 2.4*	16.5 ± 1.6*	22.9 ± 0.9*	19.9 ± 1.3
Tillandsia usneoides	SE	29.3 ± 1.1*	28.4 ± 1.1*	26.6 ± 1.9*	28.1 ± 1.3
Tillandsia utriculata	CD	32.1 ± 3.3*	29.4 ± 2.4*	25.7 ± 2.4*	29.1 ± 1.6
Tillandsia yucatana	CD	34.9 ± 3.3*	33.1 ± 2.8*	35.8 ± 3.2*	34.6 ± 1.6‡
T. yucatana	SM	45.9 ± 1.8*	43.2 ± 3.3*	43.2 ± 1.8*	44.1 ± 1.3*
T. yucatana	DF	40.2 ± 0.9*	38.2 ± 1.7*	37.8 ± 1.8*	38.7 ± 0.9†

Data are means ± SE. Different symbols denote significant differences ($P < 0.05$) between leaf sections; in the adaxial mean column, for each species, comparison was made only for species in different vegetation types. In species present in more than one vegetation type, the sites are listed in order of increasing precipitation.
CD, coastal sand dune; DF, deciduous forest; PM, petén mangrove; SD, semi-deciduous forest; SE, semi-evergreen forest; SM, scrub mangrove.

shows the regression formulae). That is, trichome density increased in the drier sites, but was lower than expected from precipitation values in the mangrove, which has very low precipitation but maintains low VPD values in the dry season. In atmospheric species, when only precipitation and VDP during the dry season were plotted, both environmental factors were negatively correlated with trichome density (Fig. 6B). However, when VPD during the rainy season was introduced, the relationship changed; precipitation showed a positive interaction with trichomes, as the sites with lowest precipitation tended to have higher VPD values during the wet season; this equation had a higher r^2 value ($r^2 = 0.332$; $P < 0.001$; full equation in Fig. 6 caption). Thus, it appears that the effect of VPD overrides the effect of precipitation.

For stomata density in tank species, precipitation and VPD during the rainy season had a positive effect on density, which increased with precipitation, but showed abnormally large numbers in the mangrove, which maintained the moderate to low VPD values throughout the year (Fig. 6C; $r^2 = 0.225$; $P < 0.001$). For atmospheric species, precipitation and VPD in the dry season correlated negatively with stomata density, whereas VPD in the wet season correlated positively (Fig. 6D; $r^2 = 0.09$; $P < 0.01$).

MORPHOLOGICAL DESCRIPTION AND CHARACTERIZATION OF TRICHOMES AND STOMATA

The trichomes of *A. bracteata* had a central disc consisting of four cells in the centre of the shield and a rudimentary series of four rings, each formed by

Table 4. Number of stomata mm^{-2} on the leaf abaxial surface for epiphytic bromeliads of six vegetation types (sites)

Bromeliad species	Site	Leaf base	Leaf middle	Leaf tip	Mean
Aechmea bracteata	DF	23.7 ± 1.9*	25.7 ± 2.3*	27.5 ± 2.4*	25.7 ± 2.0*
A. bracteata	SE	6.8 ± 0.6‡	10.9 ± 0.9†	21.4 ± 0.3*	13.1 ± 2.2†
Catopsis nutans	SE	5.5 ± 1.6†	10.1 ± 1.8*†	13.7 ± 1.6*	9.8 ± 4.4
Tillandsia balbisiana	CD	16.5 ± 1.6*	13.6 ± 1.6*	15.6 ± 2.4*	15.3 ± 1.1*
T. balbisiana	DF	16.5 ± 1.6*	19.3 ± 1.6*	18.4 ± 2.4*	15.9 ± 1.3*
T. balbisiana	SE	5.5 ± 1.6*	6.4 ± 0.9*	8.2 ± 1.6*	6.7 ± 0.8†
Tillandsia brachycaulos	DF	12.1 ± 0.8*	9.6 ± 0.7*	11.3 ± 1.2*	11.0 ± 0.6†
T. brachycaulos	SD	12.8 ± 2.4*	12.8 ± 0.9*	11.9 ± 0.9*	12.5 ± 0.8*
T. brachycaulos	SE	10.1 ± 0.9*	11.9 ± 1.8*	11.9 ± 0.9*	11.3 ± 0.7†
Tillandsia dasyliriifolia	CD	18.4 ± 1.8*	23.9 ± 1.8*	19.3 ± 1.6*	20.5 ± 1.2*
T. dasyliriifolia	SM	16.5 ± 1.6*	16.2 ± 1.3*	17.7 ± 0.8*	16.8 ± 0.7†
T. dasyliriifolia	SE	20.2 ± 1.8*	18.4 ± 0.9*	13.8 ± 1.6*	17.4 ± 1.2†
Tillandsia elongata	DF	8.3 ± 1.6†	17.5 ± 0.9*	11.9 ± 1.8†	12.6 ± 1.5
Tillandsia fasciculata	DF	23.1 ± 2.2*	21.5 ± 2.5*	29.7 ± 2.8*	24.8 ± 1.7*
T. fasciculata	SD	11.9 ± 2.4†	20.2 ± 2.4*	13.7 ± 1.6†	15.3 ± 1.7†
T. fasciculata	SE	6.4 ± 1.8†	15.6 ± 1.8*	11.1 ± 1.6†	11 ± 1.6‡
Tillandsia juncea	SE	4.6 ± 0.9*	9.2 ± 2.4*	4.6 ± 0.9*	6.1 ± 1.1
Tillandsia recurvata	DF	4.6 ± 0.9†	11.9 ± 1.8*	9.2 ± 1.8*	8.5 ± 1.3*
T. recurvata	SD	10.1 ± 0.9*	9.2 ± 2.4*	9.2 ± 2.4*	9.5 ± 1.1*
T. recurvata	SE	6.4 ± 0.9*	8.2 ± 1.6*	10.1 ± 1.8*	8.2 ± 0.9*
Tillandsia schiedeana	DF	8.3 ± 1.6*	10.1 ± 0.9*	7.4 ± 1.8*	8.6 ± 0.9*
T. schiedeana	SD	6.6 ± 0.6*	9.2 ± 1.8*	8.9 ± 0.9*	8.2 ± 0.7*
T. schiedeana	SE	6.4 ± 0.9*	8.3 ± 1.6*	8.6 ± 1.1*	7.8 ± 0.7*
Tillandsia streptophylla	PM	7.3 ± 0.9†	12.8 ± 1.8*	8.3 ± 1.6†	9.5 ± 1.1
Tillandsia usneoides	SE	6.4 ± 0.5*	9.2 ± 1.4*	9.2 ± 0.5*	8.2 ± 0.9
Tillandsia utriculata	CD	15.6 ± 3.3*	16.5 ± 1.6*	12.3 ± 2.8*	17.1 ± 1.4
Tillandsia yucatana	CD	5.5 ± 1.6*	9.2 ± 2.4*	10.1 ± 2.4*	8.3 ± 1.3*
T. yucatana	SM	8.3 ± 1.6†	13.8 ± 1.6*	9.2 ± 0.9*	10.4 ± 1.1*
T. yucatana	DF	8.3 ± 1.6*	10.1 ± 0.9*	9.1 ± 0.9*	9.2 ± 0.6*

Data are means ± SE. Different symbols denote significant differences ($P < 0.05$) between leaf sections; total leaf means are compared in cases in which a species is found in more than one vegetation type. In species present in more than one vegetation type, the sites are listed in order of increasing precipitation.
CD, coastal sand dune; DF, deciduous forest; PM, petén mangrove; SD, semi-deciduous forest; SE, semi-evergreen forest; SM, scrub mangrove.

sunken cells with thick walls (Fig. 7A). The outermost series of ring cells (fifth ring) constituted very small wings (Fig. 7B, C). Trichomes in *Tillandsia* (Fig. 7D, E) consisted of a central disc formed by four equal-sized and wedge-shaped cells surrounded by rings of cells. The number of cell rings varied from one (as in *T. dasyliriifolia* and *T. fasciculata*) to two (as in *T. balbisiana*, *T. brachycaulos*, *T. elongata*, *T. juncea*, *T. recurvata*, *T. schiedeana* and *T. streptophylla*); the wings were attached to the outermost ring in all cases and had an elliptical shape. The trichome structure in *C. nutans* also had a central four-celled disc; it was surrounded by only one ring of cells bearing small wings (Fig. 7F).

Wings of *T. balbisiana*, *T. juncea*, *T. recurvata*, *T. schiedeana*, *T. streptophylla* and *Tillandsia yucatana* Baker were smooth with thick walls which formed canals from the top of the wing to the central disc of the trichome (Fig. 8A–C). In *T. brachycaulos*, wings also showed canals, but they had a reticulated pattern (Fig 8D). Similar reticulated wings were found in *T. dasyliriifolia*, *T. fasciculata* and *T. utriculata*.

Aechmea bracteata showed sunken stomata with two large subsidiary cells clearly surrounding the guard cells (Fig. 9A). *Catopsis nutans* had slightly sunken stomata with subsidiary cells and thin-walled guard cells that were larger than those of *A. bracteata* (Fig. 8B). All the *Tillandsia* species observed (Fig. 9C) showed stomata aligned with the rest of the epidermis, having four subsidiary cells surrounding thicker walled guard cells. It was also possible to observe the substomatal chamber behind the ostiole (Fig. 9D).
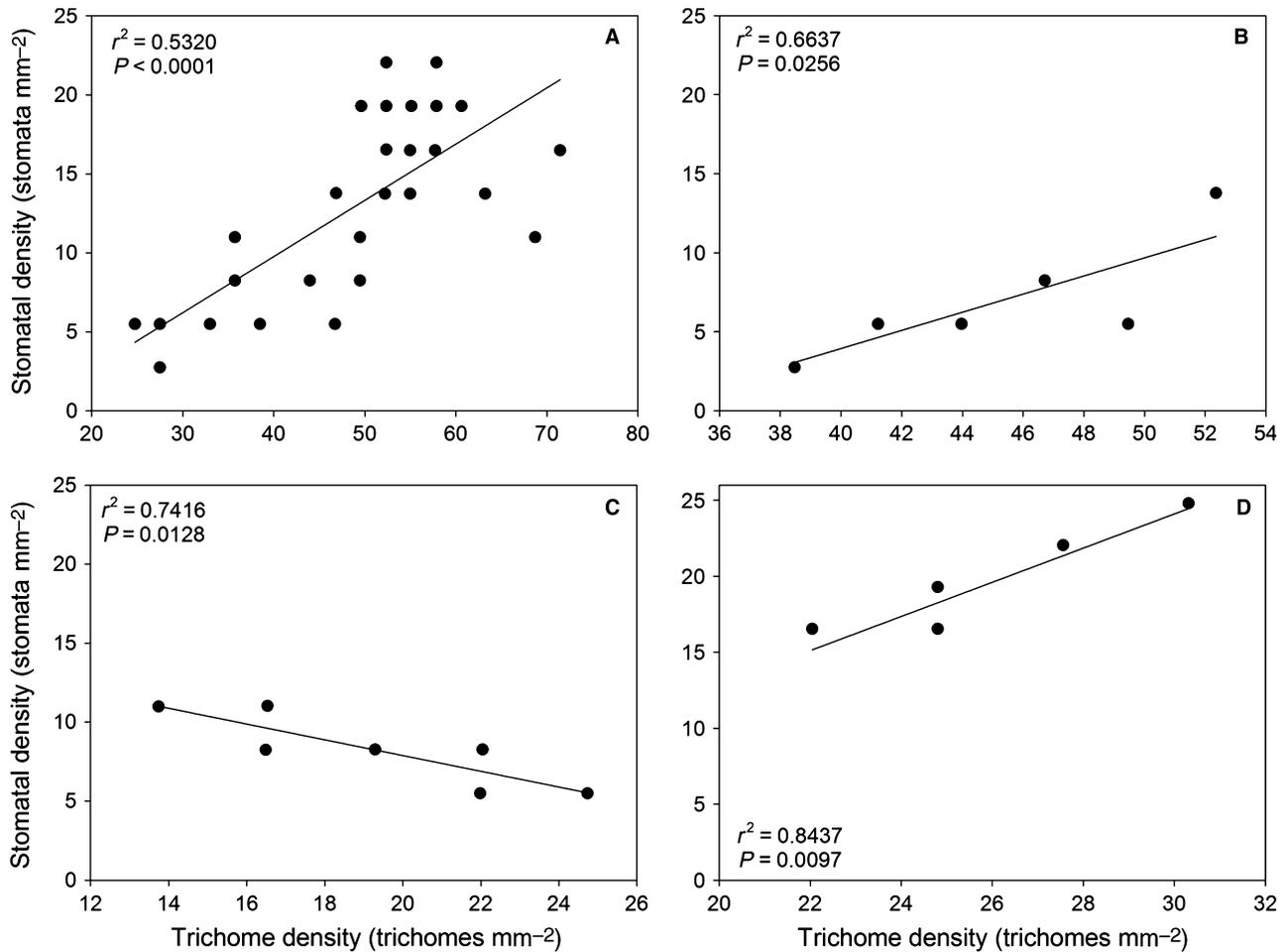
Figure 4. Relationship between stomatal density and trichome density in *Tillandsia balbisiana* (A), *Tillandsia juncea* (B), *Tillandsia streptophylla* (C) and *Tillandsia utriculata* (D). Note the differences in scale among the plots.

Catopsis nutans showed the smallest trichomes of all the observed species, being 55%–86% smaller (including wings) than those of the *Tillandsia* species (Table 6). *Tillandsia elongata* and *T. dasyliriifolia* from the scrub mangrove had the smallest central discs (3833 ± 34 and 4194 ± 25 μm^2, respectively), whereas populations of *T. recurvata* and *T. schiedeana* had the largest ($10\,407 \pm 48$ and $13\,170 \pm 49$ μm^2, respectively).

For some species present in more than one vegetation type, the trichome central discs were larger in wetter environments ($P < 0.05$; Table 6). Only *T. balbisiana* and *T. recurvata* maintained the same total trichome size across all habitats, whereas the rest of the species showed intraspecific variations ($P < 0.05$).

Tillandsia juncea had the smallest stomata (489 ± 6 μm^2) and some individuals of *T. schiedeana* had the largest stomata (1339 ± 24 μm^2; Table 6). The species *T. brachycaulos*, *T. dasyliriifolia* and

T. yucatana presented variation in both stomata and stomatal pore size between vegetation types, having larger stomata in the drier compared with the wetter sites; whereas *T. recurvata* showed the opposite trend.

There were no significant differences in stomatal size between tank and atmospheric types ($P > 0.05$). However, the stomatal pore was significantly larger in tank than in atmospheric species ($P < 0.05$).

DISCUSSION

Epiphytic bromeliad species were highly plastic with regard to the size of structures, such as stomatal pores and trichome wings, as well as the density of trichomes and stomata, and these traits varied in relation to the local annual precipitation and seasonal VPD. Consequently, these anatomical traits may be of limited taxonomic value, as the variation
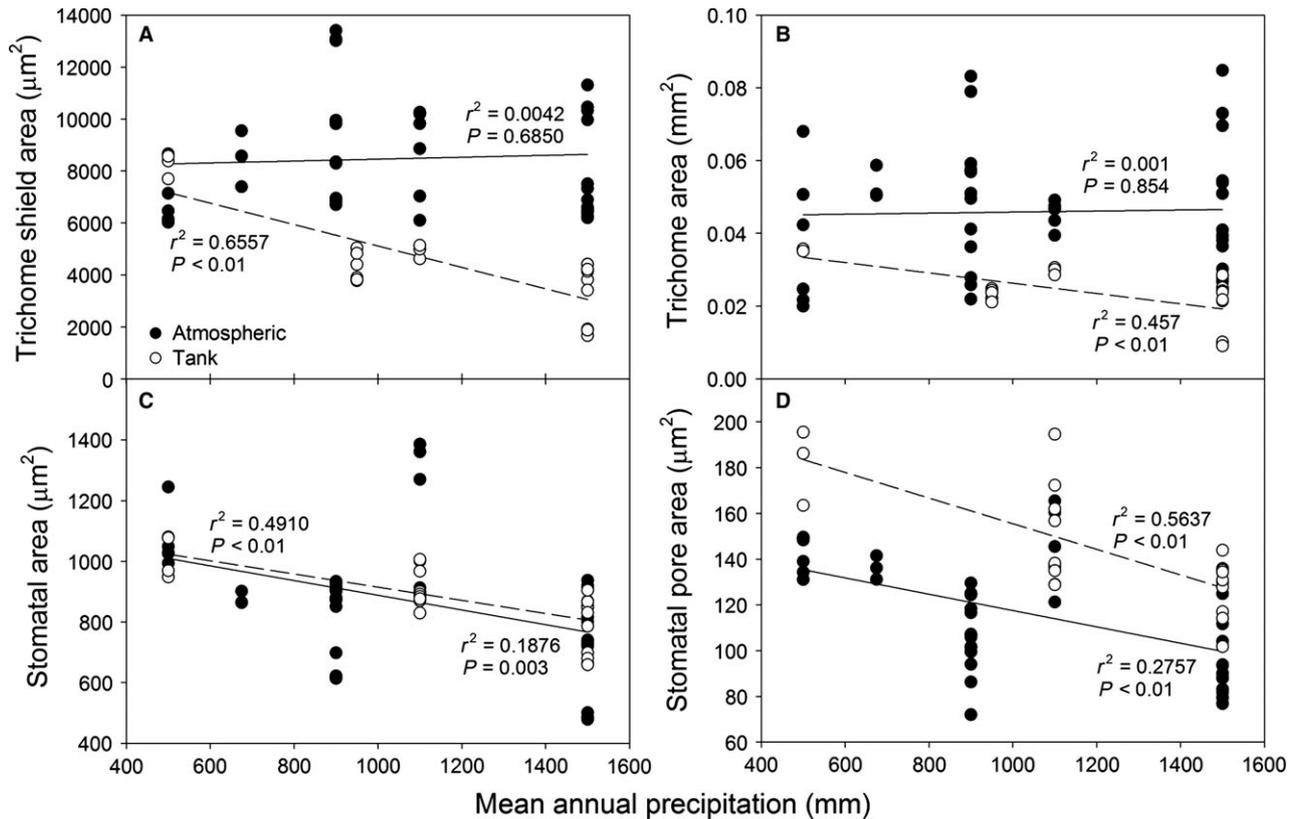
Figure 5. Trichome shield area (A), full trichome area, including wings (B), stomatal area (C) and stomatal pore area (D) in relation to mean annual precipitation of each vegetation type. Species are grouped into the atmospheric (closed circles, continuous line) and tank (open circles; dotted line) functional groups.

**Table 5.** Seasonal vapour pressure deficit (kPa) in the study sites

Site	Rainy season	Early dry	Dry	Annual mean
Coastal sand dune	0.87 ± 0.13†	0.88 ± 0.05†	1.74 ± 0.06¶	1.16 ± 0.29*
Scrub mangrove	0.95 ± 0.05†	1.03 ± 0.6*	1.08 ± 0.07‖	1.02 ± 0.06*
Petén mangrove	1.07 ± 0.06*	0.75 ± 0.4‡	1.49 ± 0.08§	1.11 ± 0.22*
Deciduous forest	0.47 ± 0.01§	0.88 ± 0.1†	2.59 ± 0.09*	1.65 ± 0.51*
Semi-deciduous forest	0.52 ± 0.02‡	0.24 ± 0.01§	1.96 ± 0.05‡	0.91 ± 0.53*
Semi-evergreen forest	0.44 ± 0.02§	0.31 ± 0.01¶	2.25 ± 0.08†	0.99 ± 0.62*

Different symbols denote significant differences ($P < 0.05$) within each column.

within species was sometimes as large as the variation between species.

Differences in trichome density and size between leaf surfaces, between the two life forms and among individuals of a species present in different habitats may reflect environmental pressures on the different potential ecophysiological role of trichomes. Differences between leaf surfaces may reveal the relevance of photoprotection (dissipating light from both leaf faces) (Benzing & Renfrow, 1971; Benzing, 1976;

Lüttge et al., 1986; Pierce, 2007) or the role of creating microenvironmental conditions suitable for gas exchange by protecting the stomata and increasing the boundary layer on the abaxial face, which influences water balance (Benzing, Seemann & Renfrow, 1978; Benz & Martin, 2006). Differences between life forms reflect differences in the strategy of the acquisition of water and nutrients (Benzing, 1976, 2000), whereas differences among sites might relate to a cluster of correlated environmental conditions, such

## Tank species

Atmospheric species
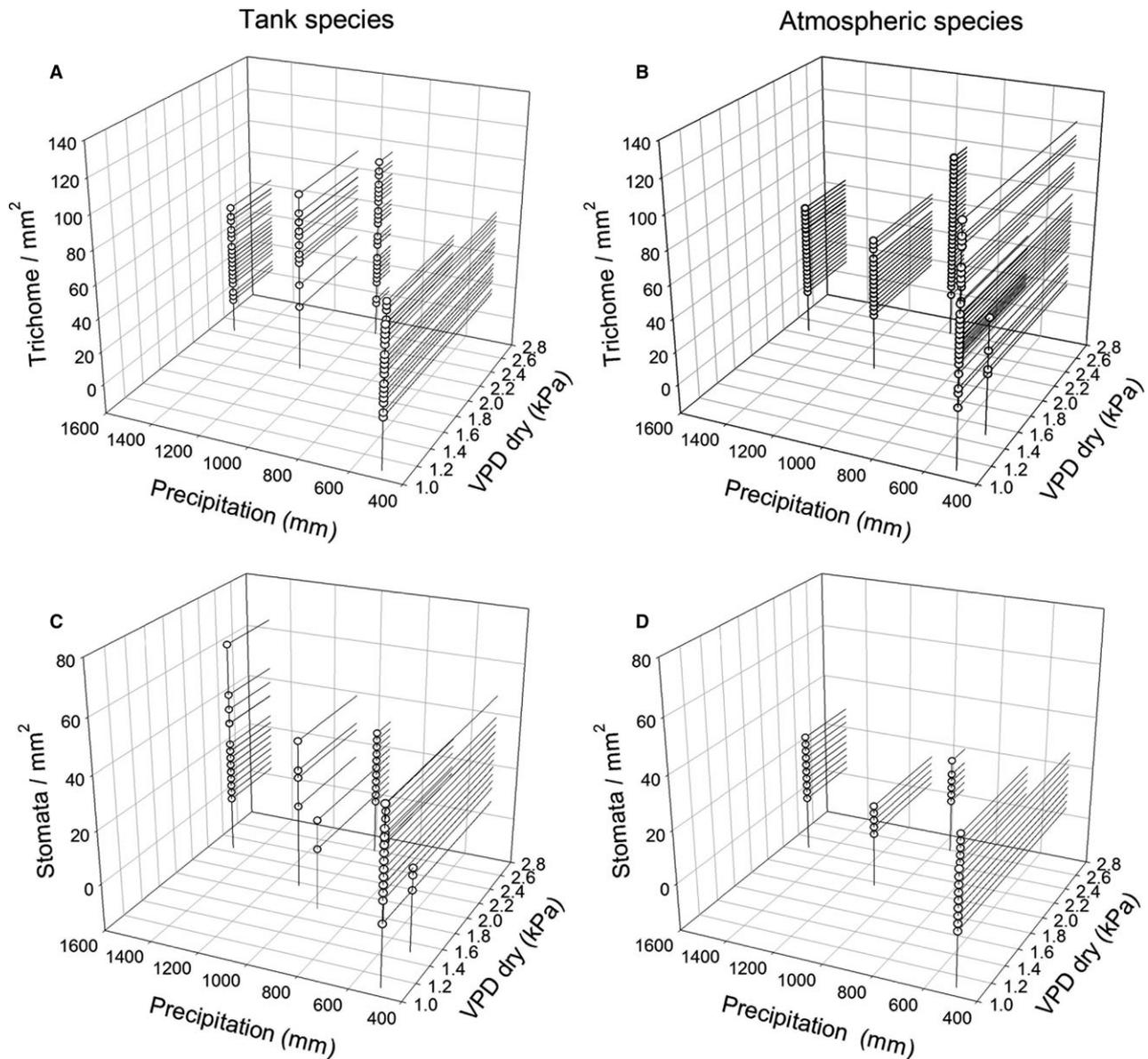
Figure 6. Relationship between mean annual precipitation, vapour pressure deficit (VPD) during the dry season and trichome (A, B) and stomatal (C, D) densities in tank (A, C) and atmospheric (B, D) species from an environmental gradient in the Yucatan Peninsula. Multiple regression equations for tank species: t.d. = 74.34 - 0.07Pp - 47.47VPDr + 41.11VPDd; and s.d. = 10.79 + 0.003Pp + 13.49VPDr - 3.67VPDd. For atmospheric species: t.d. = 44.6 + 0.07Pp - 6.39VPDd + 71.61VPDr; and s.d. = 11.7 - 0.003Pp + 1.21VPDr - 0.05VPDd. All regressions were significant at $P < 0.05$; r^2 values were 0.103 and 0.332 for trichome density for tank and atmospheric species, respectively, and 0.225 and 0.09 for stomatal density for tank and atmospheric species, respectively.

as precipitation, VPD and radiation, which influence trichome cover as a result of the combined roles of photoprotection, water use, gas interchange and water acquisition.

In contrast with the results reported for wet forests, tank species did not show significant differences in trichome density along the length of the leaf on either leaf surface. Tank species from wet forests typically show higher trichome density on the base of the leaf (Pittendrigh, 1948; Stefano *et al.*, 2008; Casañas & Jáuergui, 2011). These trichomes may be submerged in tank fluids and absorb moisture and nutrients from them. In atmospheric species, however, absorption by trichomes occurs along the whole leaf (Benzing, 2000). The lack of this typical trichome arrangement in tank species has been reported
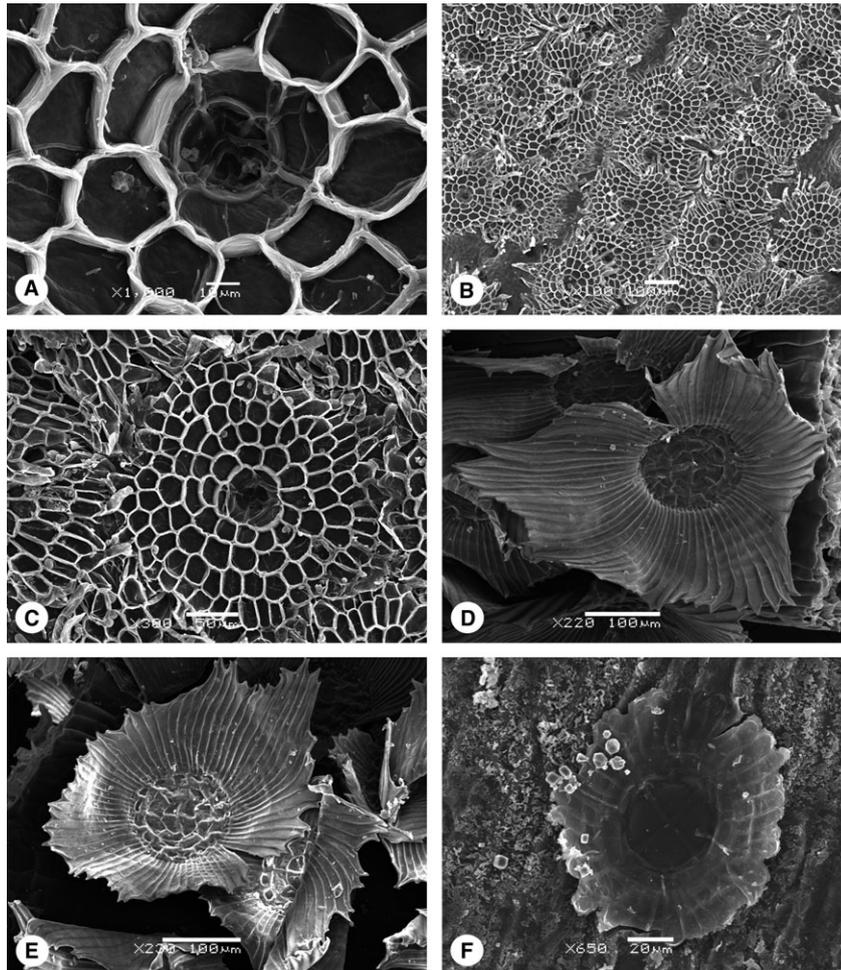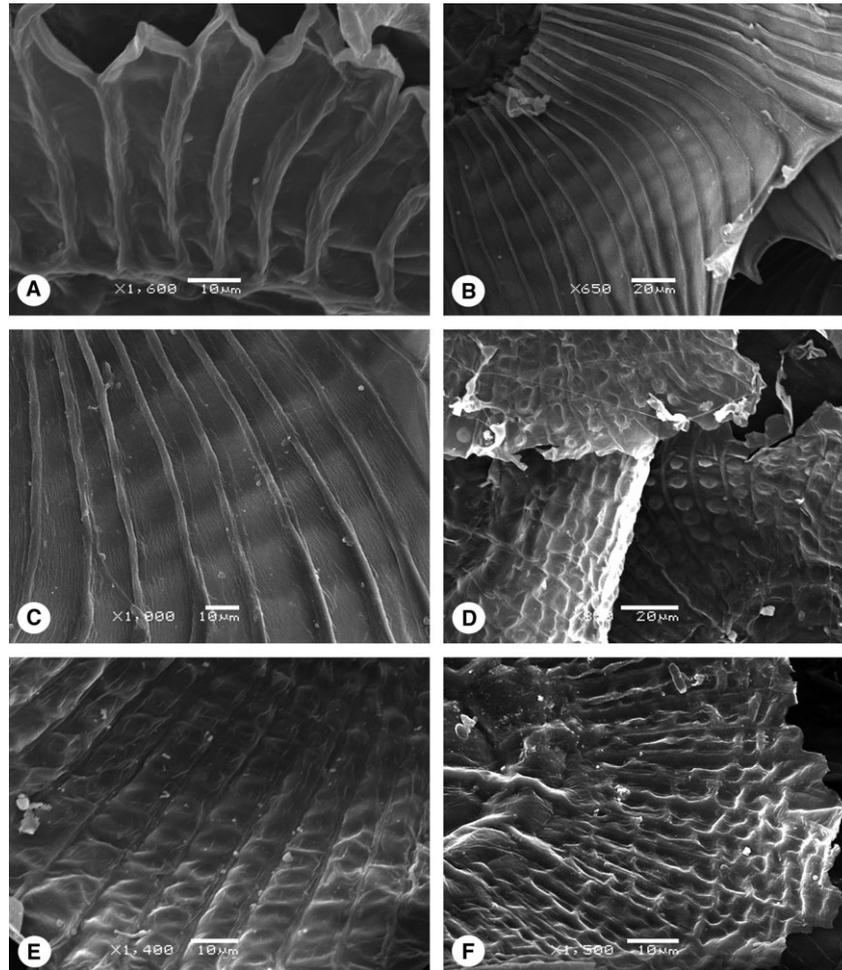
Figure 7. Scanning electron micrographs of leaf trichomes. A, Nucleus of trichome of *Aechmea bracteata* (reference bar, 10 μm). B, *Aechmea bracteata* (reference bar, 100 μm). C, Maximization of *A. bracteata* (reference bar, 50 μm). D, *Tillandsia recurvata* (reference bar, 100 μm). E, *Tillandsia schiedeana* (reference bar, 100 μm). F, *Catopsis nutans* (reference bar, 20 μm).

previously in one *Tillandsia* species from a dry forest (Reyes-García *et al.*, 2008), and has been related to the increased absorption of small precipitation pulses, dew and fog, which may contribute substantially to the annual water budget of epiphytes in water-limited environments, and which takes place along the whole leaf length (Andrade, 2003; Reyes-García & Griffiths, 2009; Reyes-García *et al.*, 2012).

Most tank species presented lower trichome density on the adaxial relative to the abaxial leaf surface, whereas most atmospheric species did not differ in trichome density between leaf surfaces. This difference may relate to either higher water absorption capacity or higher light dissipation in atmospheric species. Atmospheric species may have leaves displayed at vertical angles and individuals are not always positioned in an upright manner, as can be found for tank species, which can leave abaxial surfaces highly exposed to sunlight. Furthermore, many of these species tend to have thick and almost cylindrical leaves (Benzing, 2000); therefore, it is expected that there would be great similarity between the morphology and anatomy of the upper and lower surfaces of the leaf. The increased trichome cover on the abaxial surface has been shown to increase the boundary layer (Benz & Martin, 2006), which is known to decrease the transpiration rates under xeric conditions (Nobel, 2009). Yet, the only study performed to examine gas exchange in bromeliads to explore the role of this boundary layer change in plant water use found that it was not affected significantly (Benz & Martin, 2006). Therefore, further studies are needed to assess the physiological relevance of the higher abaxial trichome cover.

Strehl (1983) proposed that adaxial trichomes in Bromeliaceae exhibit greater organization than those

Figure 8. Scanning electron micrographs of the wings of leaf trichomes. A, *Tillandsia balbisiana*. B, C, *Tillandsia recurvata*. D, E, *Tillandsia brachycaulos*. F, *Tillandsia fasciculata*. A, reference bar, 10 micrometers; B, reference bar, 20 micrometers; C, reference bar, 10 micrometers; D, reference bar, 20 micrometers; E, reference bar, 10 micrometers; F, reference bar, 10 micrometers.

of the abaxial side of the leaf, but we did not observe this in the species in our study. Differences in organization were only found among the species (presence of canals or depressions in the trichome wings), or among subfamilies; there was a clear differentiation in the trichome structure of Bromelioideae and Tillandsioideae species. Gardner (1984) described trichome traits similar to those reported here for some species of *Tillandsia* from Mexico; in particular, the author found similar features on the surface of the wings of trichomes for *T. utriculata*.

Atmospheric species showed higher variation in trichome density than tank species, exhibiting differences along the length of the leaf that were variable depending on the habitat; for example, *T. yucatana* showed lower trichome cover on the base of the leaf compared with other leaf sections in the coastal sand dune scrub, but showed the opposite pattern in the

dry forest. Tank species may be buffered from environmental pressures because they possess a relatively more permanent water reservoir (Woods, Cardelús & DeWalt, 2015).

Plasticity in trichome density was lower on the abaxial surface which may result from a more fixed arrangement of the trichomes around the stomata (exclusively abaxial). In contrast, the trichomes in the adaxial surface may vary as a response to light, water and VPD more freely. However, further common garden studies focusing on the effect of environmental factors are needed to assess their influence more clearly.

As expected, we observed that most of the species that were found in three different sites (*T. balbisiana*, *T. dasyliriifolia*, *T. fasciculata* and *T. recurvata*) had consistently higher trichome density on both leaf surfaces in the drier, more exposed, sites.
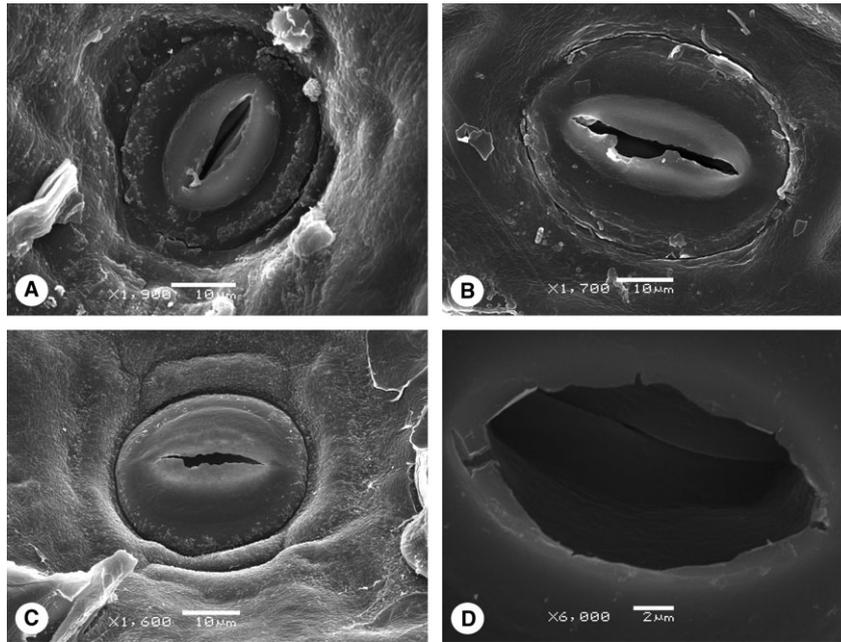
Figure 9. Scanning electron micrographs of stomata. A, *Aechmea bracteata* (reference bar, 10 micrometers). B, *Catopsis nutans* (reference bar, 10 micrometers). C, *Tillandsia balbisiana* (reference bar, 10 micrometers). D, Ostiole of *Tillandsia streptophylla* (reference bar, 2 micrometers).

In these environments, the capacity of denser trichomes to enhance water uptake and photoprotection would be strongly selected. Pierce (2007) found that trichomes of atmospheric species can play a significant role in photoprotection: the presence of trichomes can increase leaf reflectivity by 18%–40% compared with plants without these structures. The author also proposed that the morphology of the trichomes may represent a trade-off for epiphytic bromeliads between water acquisition and light reflectance, as ornamented, large trichomes, such as those found in *T. brachycaulos*, *T. fasciculata* and *T. utriculata*, are better at reflecting light, but smaller, elongated trichomes can facilitate leaf ventilation and the condensation of water vapour on the cooler cuticle, with liquid water subsequently enveloping the trichomes.

There was an interesting interplay between precipitation and VPD in relation to trichome and stomatal density. In tank species, the regressions predicted higher trichome densities in drier sites, as expected. This is consistent with the need for higher photoprotection, water absorption and an increased boundary layer to reduce water loss. However, for atmospheric species, VPD during both the dry and rainy seasons played a more important role, and the negative relationship between precipitation and trichomes became positive when VPD values were introduced into the regression. The sites with lower precipitation maintained low VPD values during the dry season.

Because VPD is calculated from both relative humidity and temperature, low VPD values encompass both heightened relative humidity, which may lead to higher condensation of water drops on the leaves (Andrade, 2003; Pierce, 2007), and the reduced need for photoprotection because of more moderate temperatures (Takahashi & Murata, 2008.). Thus, atmospheric species may be more dependent on water condensation under higher humidity. The smaller leaves of the atmospheric species may also be subjected to higher temperature fluctuations under high VPD. Species inhabiting scrub and petén mangrove forests, with both the tank and atmospheric life forms, had fewer trichomes than expected from the low annual precipitation of the site, but this apparent abnormality was explained by the low VPD values during the dry season.

Trichome area was reduced in drier sites, consistent with the observation of Pierce (2007) that smaller, skewed trichomes promote water condensation on the leaf. These smaller trichomes cool the leaf surface, but do not cover it completely, allowing for better circulation of the droplets (Pierce, 2007), and probably reducing boundary layer resistance, which increases the interception of the droplets (Martorell & Ezcurra, 2007).

As seen for trichomes, the relationship between stomata and precipitation was affected by local VPD values, with higher stomatal densities being found in wetter sites, but also unexpectedly high stomatal

Table 6. Trichome and stomatal size (area) for epiphytic bromeliads in six vegetation types

Bromeliad species	Site	Trichome area		Stomatal area	
		Central disc (μm^2)	With wings (μm^2)	Stomata (μm^2)	Stomatal pore (μm^2)
Aechmea bracteata	SE	–	–	840 ± 34	127 ± 8
Catopsis nutans	SE	1821 ± 67	9755 ± 334	1438 ± 47	301 ± 7
Tillandsia balbisiana	CD	6087 ± 35‡	22113 ± 1373*	1117 ± 65[a]	135 ± 2*
T. balbisiana	DF	6837 ± 61†	25190 ± 1734*	839 ± 41†	119 ± 2†
T. balbisiana	SE	7243 ± 32*	26286 ± 871*	793 ± 34†	130 ± 3[a]
Tillandsia brachycaulos	DF	8326 ± 14*	42343 ± 3883*	914 ± 8[a]	124 ± 3[a]
T. brachycaulos	SD	7366 ± 47†	47853 ± 655*	892 ± 10*	129 ± 4*
T. brachycaulos	SE	6298 ± 62‡	32270 ± 3003†	767 ± 28†	84 ± 3†
Tillandsia dasyliriifolia	CD	8286 ± 48[a]	35483 ± 191*	998 ± 39[a]	182 ± 7[a]
T. dasyliriifolia	SM	4194 ± 25†	24990 ± 2052†	679 ± 11†	119 ± 4†
Tillandsia elongata	DF	3833 ± 34	23566 ± 772	992 ± 12	176 ± 6
Tillandsia fasciculata	DF	4759 ± 57*	23026 ± 977†	866 ± 20[a]	141 ± 8*
T. fasciculata	SD	4921 ± 49*	29733 ± 602*	884 ± 4[a]	136 ± 1[a]
T. fasciculata	SE	4347 ± 65†	23246 ± 751†	861 ± 6*	133 ± 1*
Tillandsia juncea	SE	6655 ± 34	38913 ± 1301	489 ± 6	83 ± 4
Tillandsia recurvata	DF	9894 ± 38†	73203 ± 7970[a]	618 ± 2†	84 ± 1†
T. recurvata	SE	10407 ± 48*	75790 ± 4608[a]	740 ± 13*	103 ± 5*
Tillandsia schiedeana	DF	13170 ± 49*	55690 ± 2425*	908 ± 17†	105 ± 3†
T. schiedeana	SD	10095 ± 39†	43206 ± 2081†	1339 ± 24*	159 ± 2*
T. schiedeana	SE	10250 ± 12†	53073 ± 1084[a]	924 ± 6†	90 ± 2‡
Tillandsia streptophylla	PM	8506 ± 56	53296 ± 2694	877 ± 12	136 ± 3
Tillandsia utriculata	CD	8023 ± 32	42014 ± 741	823 ± 17	132 ± 2
Tillandsia yucatana	CD	5517 ± 45†	30163 ± 3672†	1023 ± 16*	149 ± 1*
T. yucatana	DF	8486 ± 32*	70395 ± 2029*	879 ± 16†	103 ± 1†

Data are means ± SE. Different symbols denote significant differences ($P < 0.05$); comparisons were made only for species in different vegetation types. In species present in more than one vegetation type, the sites are listed in order of increasing precipitation.
CD, coastal sand dune; DF, deciduous forest; PM, petén mangrove; SD, semi-deciduous forest; SE, semi-evergreen forest; SM, scrub mangrove.

density in mangrove forests. The low VPD values in mangroves allow for higher gas exchange rates to occur in leaves with high stomatal density, but limit gross water loss, which follows a decreasing humidity gradient from inside the leaf to the environment (Reyes-García *et al.*, 2008). On the contrary, results from our environmental gradient are consistent, either grouping all the species together (Fig. 5) or observing differences within the same species in different sites (Table 3). Stomata are larger in the drier sites. This may compensate for the reduced density, increasing potential photosynthetic rates. This is supported by physiological data, as species from the drier sites also showed higher photosynthetic rates than those found for species in the wetter extreme (M. j. Cach-Pérez, J. L. Andrade & C. Reyes-García, unpubl. data).

In order to determine whether trichome and stomatal densities are heritable (ecotypes) or environmentally controlled, reciprocal transplants and germination experiments under contrasting environments would be relevant. However, trichome and stomatal densities in leaves of *Tillandsia heterophylla* E. Morren (an epiphytic bromeliad species of montane forest) respond to different light treatments (M. j. Cach-Perez, unpubl. data).

Surprisingly, one of the species that was more abundant along the entire environmental gradient, *T. brachycaulos*, showed the lowest variation in trichome and stomatal density. This suggests that morphological plasticity may not be the only strategy contributing to local adaptation. Indeed, physiological studies have found high plasticity in this species in different microenvironments (Graham & Andrade, 2004; Cervantes *et al.*, 2005; González-Salvatierra *et al.*, 2010; M. j. Cach-Pérez, unpubl. data), which may be responsible for its wide distribution.

In conclusion, our results highlight the lack of variation in trichome and stomatal structure within species included in our study, and the high variability in their size and density among species of the Yucatan Peninsula. The size and density of

trichomes and stomata may differ among parts of the leaf, between leaf surfaces and between environments. In tank species, trichome density correlated negatively with precipitation and positively with VPD during the dry season, supporting the hypothesis that they play an important role in photoprotection as, in drier, more exposed environments, higher trichome densities may reflect more light. However, in atmospheric species, VPD seemed to be a more important factor than precipitation. The implications of this may be the heightened importance of water absorption of condensed moisture for this life form. Furthermore, trichomes tended to be smaller in drier sites, which may enhance water condensation on leaf surfaces. In tank species, stomatal density showed a positive correlation with precipitation and VPD during the rainy season, but a negative one with VPD during the dry season; thus, reduced stomatal densities in dry sites protect the plant from desiccation. However, this was not observed for atmospheric species, which showed a positive correlation between precipitation and stomatal density, and may be prone to increasing photosynthetic rates with higher exposure in the drier sites. The high plasticity may be a key to the ability of these species to be successful within a variety of sites, and the plasticity appears to be a more relevant strategy in the atmospheric life forms. However, plasticity did not necessarily correlate with the species range (limited or wide) within the studied climatic gradient.

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