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Variación inter e intraespecífica en la densidad y morfología de estomas y tricomas de Bromeliaceae epífitas creciendo en jardín común

TESIS
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Por

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Dedicatoria

A mis padres, Isabel y Miguel porque a pesar de la distancia siempre estuvieron apoyándome. A mis hermanos por ser parte de todo esto.

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Resumen

A pesar de que las bromeliáceas epífitas son capaces de colonizar una gran diversidad de hábitats climáticamente contrastantes, estudios recientes han demostrado que son altamente sensibles a la variación ambiental. Se determinó y comparó la variación morfológica en cinco especies de *Tillandsia* provenientes del matorral de duna costera, manglar chaparro, selva baja caducifolia y selva alta sub-perennifolia creciendo bajo condiciones de jardín común. Se determinó el crecimiento de las hojas nuevas (producidas en el jardín común), así como la densidad y morfología de estomas (tamaño del estoma y poro estomático) y tricomas (tamaño del tricoma y del disco central) en hojas preexistentes (que la planta poseía al momento de la colecta) y hojas nuevas; a partir de esto se calculó el índice de plasticidad morfológica de cada especie y con un análisis multivariado se determinó el grado de variación entre tipos de hoja, así como la similitud de respuesta morfológica por especie y sitio de colecta. Se encontró que plantas de *Tillandsia balbisiana* y *T. brachycaulos* provenientes de los sitios más húmedos presentaron mayor elongación en las hojas nuevas respecto a individuos de sitios más secos. La densidad de tricomas en la superficie adaxial fue mayor en las hojas nuevas respecto a las preexistentes, pero no en todos los casos, aunque sin diferencias significativas. El área del tricoma aumentó en individuos de *T. yucatana* procedentes del matorral de duna costera en las hojas nuevas respecto a las preexistentes; el tamaño del disco central del tricoma se redujo en la cara abaxial en individuos de *T. balbisiana* del matorral de duna costera en las hojas nuevas respecto a las preexistentes. En algunas especies se encontró que, la densidad estomática fue baja en hojas nuevas respecto a las hojas preexistentes; la densidad de tricomas fue mayor en individuos de *T. balbisiana* y *T. yucatana* en las hojas nuevas con respecto a hojas preexistentes de plantas procedentes del matorral de duna costera. A pesar de haber presentado una baja plasticidad (< 0.2) se encontró que existe variación entre las hojas nuevas respecto a las preexistentes y una similitud entre el grado de respuesta morfológica por especie, por sitio de colecta, lo que sugiere que las bromeliáceas epífitas responden morfológicamente a cambios en el ambiente.

Palabras clave: plasticidad morfológica, jardín común, microclima, hojas preexistentes, superficie de las hojas.

CAPÍTULO I

Introducción

Las epífitas son aquellas especies de plantas que crecen generalmente sobre otras plantas, en ramas o troncos de árboles y arbustos (comúnmente llamadas hospederos o forofitos) quienes les brindan soporte estructural, sin que éstas tengan contacto directo con los haces vasculares de los hospederos (Gentry y Dodson 1987). Las epífitas son elementos importantes para la biodiversidad de las comunidades vegetales boscosas, en dónde hasta un 60 % de las especies presentan esta forma de vida (Kress 1986; Gentry y Dodson 1987). En cuanto al número de especies epífitas la familia Bromeliaceae son el segundo grupo más importante, después de las orquídeas, ya que, de las 3,408 especies que conforman la familia, aproximadamente el 60 % son epífitas (Luther 2014; Zotz 2013). En ambientes como los bosques húmedos tropicales las bromeliáceas epífitas pueden representar hasta el 50 % de la biomasa foliar y un almacén de hasta el 84 % de los nutrientes, por lo que desempeñan un papel importante en la dinámica y flujo de nutrientes de los ecosistemas en los que se encuentran (Nadkarni 1983; Benzing 2000; Granados-Sánchez et al. 2004; De la Rosa-Manzano et al. 2014).

Las bromeliáceas se distribuyen, de manera natural, desde el sur de Estados Unidos de América hasta el norte de Argentina (con excepción de una especie que se distribuye en África) y están presentes en una gran diversidad de condiciones ambientales (Benzing 2000; Crayn et al. 2004). Lo anterior ha sido posible gracias al desarrollo de estrategias morfológicas y fisiológicas para superar condiciones ambientales adversas, entre las que se encuentra el desarrollo de fotosíntesis CAM, hojas suculentas, tricomas especializados para la protección de la luz ultravioleta y para la absorción de agua y nutrientes, así como el desarrollo de distintas formas de vida: tanque y atmosféricas (Kathleen y Craig 1996; Benzin 2000; Andrade et al. 2007).

Las bromeliáceas tipo tanque (Fig. 1a) se caracterizan por una expansión de las bases de las hojas en forma de roseta formando un "tanque" lo que les permite almacenar agua, atrapar hojarasca y otras fuentes de material orgánico. Además, dentro del tanque se forma un ecosistema en miniatura donde habitan adultos y/o larvas de insectos y otros invertebrados, así como pequeños anfibios y reptiles (Granados-Sánchez et al. 2004; Pittendrigh 1948). Las bromeliáceas tipo atmosféricas (Fig. 1b) carecen de un tanque para el almacenamiento de agua, por lo que este recurso es obtenido directamente de la atmósfera, en forma de rocío, neblina y lluvia; sus hojas son angostas, y están densamente cubiertas por tricomas foliares (Gotsch et al. 2016; Pittendrigh 1948).

Figura 1. Imagen que ilustra (a) una bromeliácea epífita tipo tanque (*Tillandsia deppeana*) y (b) una especie tipo atmosférica (*Tillandsia schiedeana*). Fotografías: Manuel Cach.

En las Bromeliaceae epífitas las hojas comprenden el componente principal del cuerpo vegetativo de la planta y son la principal ruta de acceso al agua (Figura 1). A diferencia de otro tipo de plantas que dependen de sus raíces para la obtención y acumulación de nutrientes, las raíces de las bromeliáceas epífitas sirven principalmente de sostén, ya que estas plantas dependen de sus hojas para obtener todo lo que necesitan para su desarrollo (p. ej. nutrientes y agua), por lo que son un grupo altamente sensible y vulnerable a los cambios en el ambiente (Benzing 1990). Los tricomas que se encuentran en las hojas son estructuras complejas, que por lo general presentan formas similares a una escama y poseen la capacidad de absorción de agua y nutrientes, siendo una de las principales funciones en las bromeliáceas (Fig. 2), aunque también pueden jugar un papel importante para la fotoprotección de las plantas (Brighigna et al. 1997; Freschi et al. 2010; Benzing 2000).

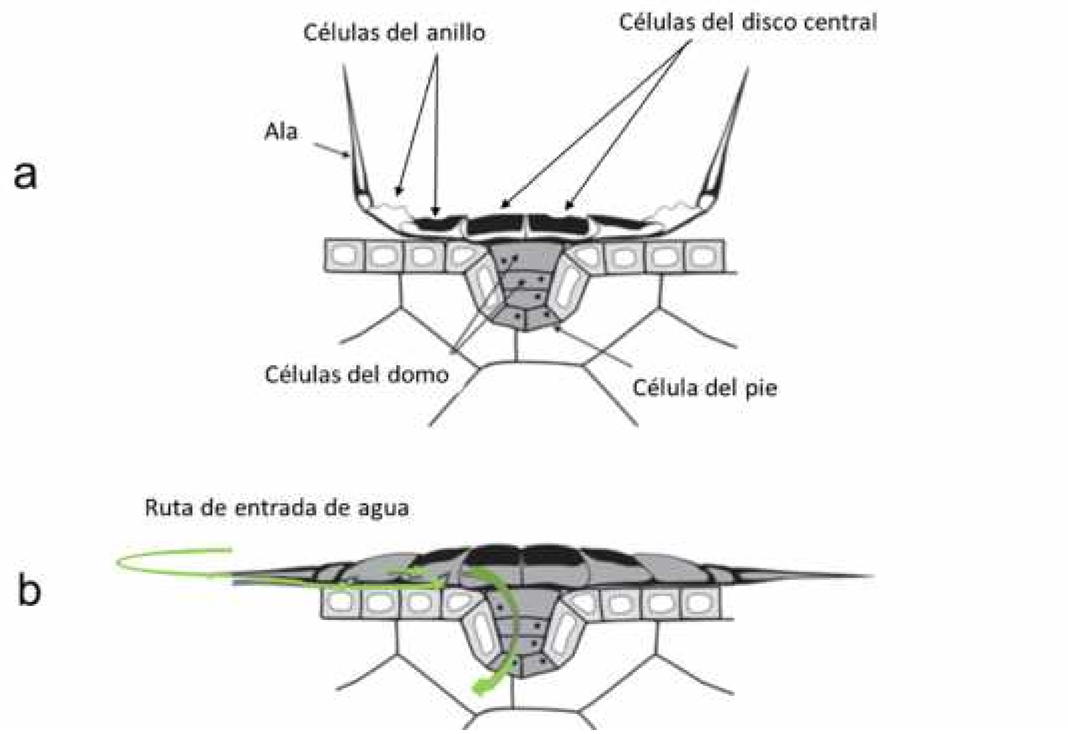


Figura 2. Vista transversal de un tricoma en una Bromeliaceae epífita en condiciones secas (**a**) y húmedas (**b**). En ausencia de agua el ala permanece erguida, exponiendo la superficie de la hoja subyacente (**a**). En contacto con el agua, las paredes celulares del anillo engrosadas diferencialmente dirigen el aumento de la presión para aplanar el ala contra la cutícula y crea una ruta de entrada de agua a las células vivas y mejora el movimiento capilar del agua hacia el disco central y paredes transversales de las células (**b**), permitiendo la absorción de agua por ósmosis o por transporte facilitado por acuaporinas (Ohrui et al. 2007; Pierce 2007). (Figura tomada y modificada de Pierce 2007).

En este sentido, la cantidad de luz que es reflejada por los tricomas no depende solo de su densidad como podría suponerse, sino también de su tamaño y forma ya que, a mayor tamaño, los tricomas actúan como un filtro difusivo, reflejando parte de la luz incidente y disminuyendo la radiación solar que llega a la superficie de las hojas (Benzing y Renfrow 1971; Benzing 1976; Pierce 2007). La variación en tamaño y forma en las diferentes especies que conforman la familia permite que respondan a cambios en los parámetros ambientales e influyen en gran parte en la respuesta fisiológica de las hojas (Pierce et al. 2001).

Se ha reportado que la densidad, morfología y arreglo espacial de los tricomas foliares varía en respuesta a diferentes condiciones ambientales; por ejemplo, Cach-Pérez et al. (2016) encontraron que la densidad y tamaño de los tricomas es mayor en especies que habitan en sitios secos en comparación con sitios húmedos. Por su parte, Chávez-Sahagún et al. (2019) encontraron que la densidad de tricomas se puede relacionar con las estrategias de adquisición de agua de las bromeliáceas epífitas, en donde una alta densidad de tricomas puede no favorecer la condensación de rocío, pero podría facilitar la absorción de agua en forma de neblina. Así mismo, Chávez (2020) sugiere que el papel que pudieran jugar los tricomas en la fotoprotección está asociado al tamaño del escudo o disco central del tricoma, que al tamaño total o densidad de estas estructuras.

Por otro lado, los estomas (Fig. 3) son estructuras que permiten el intercambio de gases entre el interior de la hoja y la atmósfera (p. ej. entrada de dióxido de carbono y liberación de oxígeno y agua), por lo que tienen una función importante en la regulación del movimiento del agua a través de la transpiración (Hetherington y Woodward 2003). Cuando existen altas temperatura o escases en la disponibilidad de agua en el ambiente, los estomas de las bromeliáceas epífitas se cierran reduciendo la pérdida de agua por transpiración (Stancato et al. 2001; Sperry et al. 2016). En este sentido, se ha reportado que la densidad estomática de las hojas es alta en plantas que se desarrollan en sitios con alta humedad y baja densidad estomática en sitios más secos y con un elevado déficit de presión de vapor de agua (DPV) (Willmer y Fricket 1996; Cach-Pérez et al. 2016).

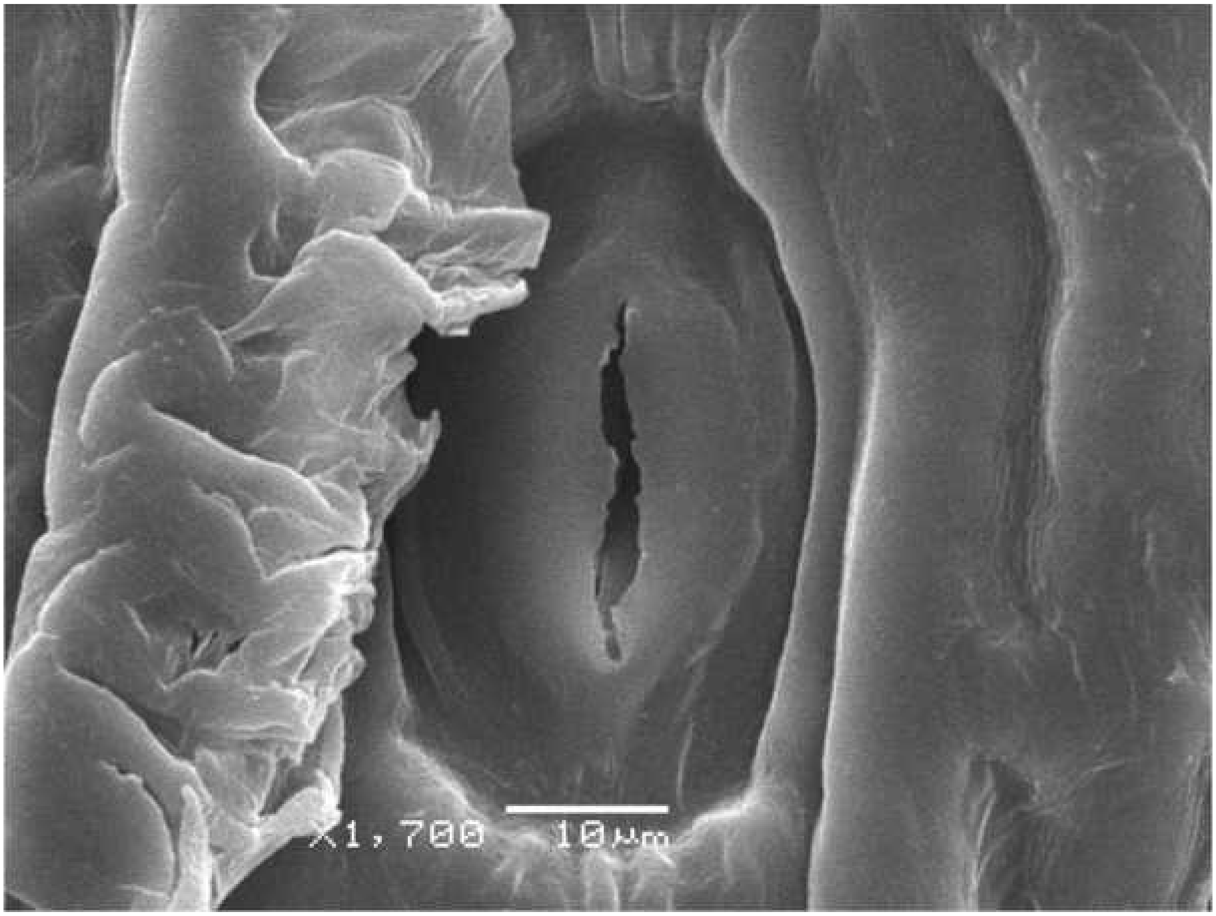


Figura 3. Fotografía al microscopio electrónico de barrido que ilustra un estoma semi-abierto de *Tillandsia balbisiana* (barra de referencia = 10µm). Fotografía: Lilia Can.

La respuesta morfológica de los estomas a factores ambientales es compleja, puesto que puede variar en función de las condiciones ambientales particulares (p. ej. radiación solar, temperatura o déficit de presión de vapor de agua atmosférica) bajo las cuales se forman las hojas. Por ejemplo, la cantidad de luz influye en la densidad y tamaño de los estomas, es decir, que plantas creciendo bajo condiciones con alta intensidad de luz presentan un número reducido de estomas a diferencia de plantas creciendo con baja intensidad lumínica (Ciha y Brun 1975; Cach-Pérez 2008; Salazar-Tortosa et al. 2018).

Una buena aproximación para determinar el grado de respuesta de las plantas ante la variación ambiental, desde el punto de vista morfológico o fisiológico, por ejemplo, es el cálculo de la plasticidad. La plasticidad se refiere a la capacidad de las plantas para enfrentar las condiciones en las que habitan, así como para sobrellevar, adaptarse y tener la capacidad de responder ante la variación climática (Valladares et al. 2006; Gentry y Dodson 1987; Murren et al. 2015; Cach-Pérez et al. 2018). En los últimos años, se ha presentado un creciente interés en la plasticidad como una medida para elaborar predicciones certeras sobre la respuesta de las especies ante fenómenos como el cambio climático (Valladares et al. 2014; Turcotte y Levine 2016).

Estudios de plasticidad en bromeliáceas epífitas, sobre todo en especies del género *Tillandsia*, han mostrado una mayor plasticidad en las variables fisiológicas respecto a las variables morfológicas (Cach-Pérez et al. 2018), es decir, que, ante condiciones de variación ambiental, las plantas modifican en mayor medida su fisiología respecto a su morfología. Algunos estudios se han llevado a cabo en especies que provienen de sitios que presentan diferentes condiciones ambientales (Cach-Pérez et al. 2016), mientras que otros se han realizado bajo condiciones ambientales controladas, por ejemplo, en un experimento con individuos de *Tillandsia utriculata* procedentes de dos diferentes sitios con un marcado contraste ambiental (matorral de duna costera y selva alta perennifolia); los individuos procedentes del matorral de duna costera se establecieron en un bosque nuboso y los de la selva alta perennifolia en el matorral de duna costera. Las plantas se aclimataron durante 45 días, después de lo cual, fueron trasladadas a un vivero semi-controlado en el que solo incidía la luz y temperatura local. Una vez en el vivero, fueron sometidas a una sequía de 20 días, los resultados mostraron una rápida aclimatación fisiológica (Rosado-Calderón et al. 2020), indicando una gran sensibilidad a la variación ambiental en su entorno, así como una alta capacidad de respuesta a las condiciones del ambiente, sin embargo, hasta el momento, ningún estudio ha documentado respuestas similares en rasgos morfológicos en plantas de una misma especie, procedentes de distintos sitios con ambientes contrastantes.

En general, entre los mecanismos y estrategias de aclimatación que las bromeliáceas usan en respuesta a la variación climática se pueden encontrar: la optimización del uso de agua y de la asimilación de carbono (Rosado-Calderón et al. 2020). Bajo limitaciones de agua presentan una fotorrespiración reducida (Smith 1989). La inducción de C_3 a CAM es probablemente una estrategia de supervivencia en comparación con su contribución al crecimiento y productividad (Griffiths 1992).

Determinar la plasticidad y las respuestas morfológicas como la densidad y la morfología de los estomas y los tricomas foliares de bromeliáceas epífitas, procedentes de ambientes contrastantes creciendo en condiciones de jardín común (vivero), puede ayudar a comprender el comportamiento y las estrategias de aclimatación ante la variación climática y, esto podría ser un elemento importante que nos permita discernir qué especies son más sensibles a los cambios en el ambiente y su capacidad para sobrevivir ante cambios ambientales; al mismo tiempo nos aportara información para diseñar planes y estrategias que contribuyan para su conservación ante fenómenos como el cambio climático y el cambio de uso del suelo.

Objetivo general

Determinar y comparar la plasticidad morfológica, así como la densidad y morfología de estomas y tricomas de cinco especies de bromeliáceas epifitas provenientes de cuatro tipos de vegetación, climática y estructuralmente contrastantes creciendo bajo condiciones de jardín común.

Objetivos particulares

Comparar, entre individuos de la misma especie procedentes de distintos sitios de colecta, el crecimiento en hojas nuevas bajo condiciones de jardín común.

Comparar la densidad de estomas y tricomas foliares en hojas preexistentes (formadas previo a la colecta de las plantas), hojas nuevas, así como entre ellas, por especie y sitio de colecta.

Caracterizar y comparar el tamaño (área) de los estomas, poro estomático, área y tamaño del disco central del tricoma de hojas preexistentes y nuevas en cinco especies de bromeliáceas epífitas establecidas en jardín común.

Determinar y comparar la plasticidad morfológica de cinco especies de bromeliáceas epífitas a partir de la densidad y morfología de estomas y tricomas de plantas colectadas en diferentes tipos de vegetación creciendo en jardín común.

Hipótesis

El agua es el factor limitante para el crecimiento y desarrollo de las epífitas, sin embargo, en el jardín común siempre estuvo presente a través de la lluvia local o aplicación de riego, entonces:

H1. Las especies procedentes de los sitios de colecta más secos presentarán un mayor crecimiento foliar respecto a las especies procedentes de los sitios más húmedos.

H2. Las hojas presentaran menor densidad de tricomas y mayor densidad de estomas en hojas nuevas (producidas en el jardín común) en comparación con las hojas prexistentes como respuesta a menor estrés hídrico, particularmente en las especies procedentes de los sitios de colecta más secos.

H.3 Los estomas y tricomas de las especies de bromeliáceas provenientes de sitios con periodos de sequias más largos como el matorral de duna costera, manglar chaparro y selva baja caducifolia, tendrán cambios más drásticos en los rasgos morfológicos en estomas y tricomas en comparación con los sitios que ofrecen mayor disponibilidad de agua a las plantas, como la selva alta sub-perennifolia.

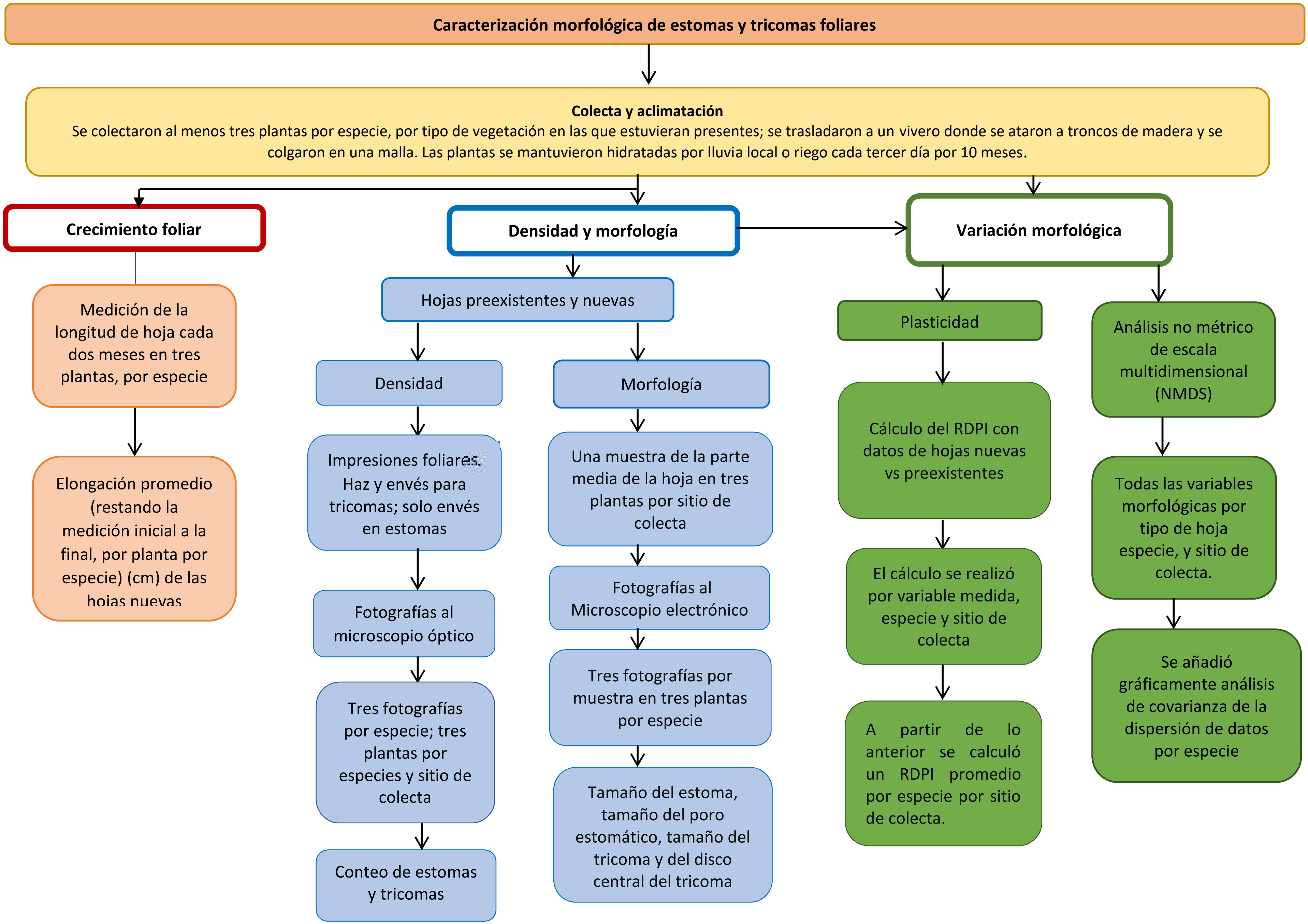
H4. Las especies con distribución amplia, particularmente las procedentes de los sitios más secos presentarán mayor plasticidad. En general se espera que los cambios en la densidad y morfología de estomas y tricomas converjan entre las distintas especies hacia un mayor aprovechamiento del agua disponible en el jardín común.

Estrategia experimental

Se colectaron cinco especies de bromeliáceas epífitas procedentes de cuatro tipos de vegetación caracterizando cada una de ellas (Tabla 1) y con la finalidad de cumplir con los objetivos planteados para este trabajo, se siguió una estrategia metodológica que permitiera la caracterización de la elongación de las hojas nuevas, determinación de la densidad y morfológica de estomas y tricomas, así como el cálculo de la plasticidad morfológica global y la determinación de las estrategias de respuesta de las plantas ante la variación ambiental mediante un análisis multivariado. En la figura 3 se presenta un esquema que resume la metodología seguida en esta tesis:

Tabla 1. Especies utilizadas en la presente tesis, así como los sitios de colecta de cada una. MDC.-matorral de duna costera; MC. -manglar chaparro; SBC. -selva baja caducifolia; SASP. - selva alta sub-perennifolia.

Especie	Tamaño promedio de una epífita en el jardín común	Sitios de colecta	Forma de vida	Ruta fotosintética
Tillandsia balbisiana Schult. f.	De entre 10-11.5 cm desde la base de las hojas, hasta la punta	SBC y SASP	Atmosférica	CAM
Tillandsia brachycaulos Schltdl.	Hasta unos 10-11 cm de largo	SBC y SASP	Atmosférica	CAM
Tillandsia dasyliriifolia Baker	De entre 10.5.-11.5 cm de largo	MDC Y SBC.	Tanque	CAM
Tillandsia recurvata (L.) L.	De entre 10-11 cm de largo	SBC y SASP	Atmosférica	CAM
Tillandsia yucatana Baker	Entre 8-12 cm de largo	MC, MDC y SBC	Atmosférica	CAM



Caracterización morfológica de estomas y tricomas foliares

Colecta y aclimatación
Se colectaron al menos tres plantas por especie, por tipo de vegetación en las que estuvieran presentes; se trasladaron a un vivero donde se ataron a troncos de madera y se colgaron en una malla. Las plantas se mantuvieron hidratadas por lluvia local o riego cada tercer día por 10 meses.

Crecimiento foliar

Medición de la longitud de hoja cada dos meses en tres plantas, por especie

Elongación promedio (restando la medición inicial a la final, por planta por especie) (cm) de las hojas nuevas

Densidad y morfología

Hojas preexistentes y nuevas

Densidad

Impresiones foliares. Haz y envés para tricomas; solo envés en estomas

Fotografías al microscopio óptico

Tres fotografías por especie; tres plantas por especies y sitio de colecta

Conteo de estomas y tricomas

Morfología

Una muestra de la parte media de la hoja en tres plantas por sitio de colecta

Fotografías al Microscopio electrónico

Tres fotografías por muestra en tres plantas por especie

Tamaño del estoma, tamaño del poro estomático, tamaño del tricoma y del disco central del tricoma

Variación morfológica

Plasticidad

Cálculo del RDPI con datos de hojas nuevas vs preexistentes

El cálculo se realizó por variable medida, especie y sitio de colecta

A partir de lo anterior se calculó un RDPI promedio por especie por sitio de colecta.

Análisis no métrico de escala multidimensional (NMDS)

Todas las variables morfológicas por tipo de hoja especie, y sitio de colecta.

Se añadió gráficamente análisis de covarianza de la dispersión de datos por especie

Figura 3. Esquema que ilustra la metodología seguida en este trabajo.

CAPÍTULO II

Morphological plasticity in epiphytic Bromeliaceae from four vegetation types transferred to common garden conditions

Running title: Plasticity in epiphytic Bromeliaceae

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Abstract

In epiphytic Bromeliaceae high physiological plasticity has been characterized but anatomic plasticity is not well studied. We set out to discern between observed anatomical divergence among different populations with marked rain seasonality and the within plant plasticity, after transference from natural to common garden conditions with continuous watering. Using five epiphytic *Tillandsia* species from four contrasting vegetation types of southern Mexico, we measured trichome and stomata density and morphology in new and preexisting leaves (formed prior to the transfer). Environmental conditions were monitored, and the relative distance plasticity index was calculated. We found very low plasticity values between the new and preexisting leaves in all the species (index <0.2), which may relate to the high light levels and high vapour pressure deficit of the common garden, that mimicked the original sites, despite the watering. However, large differences were found between our data and data from the same species at the driest vegetation site, measured almost a decade earlier, which may indicate environmental changes at the site. A correlated change in trichome traits was found, as an increase in size was related to a decrease in density, simultaneous in both leaf faces, despite the different microenvironmental conditions and processes involved.

Key words: Contrasting vegetation - Environmental variation - Stomata - Trichomes – Tillandsia

INTRODUCTION

The family Bromeliaceae has a wide geographical distribution within tropical America, covering a diverse range of environments, or habitats, from deserts to tropical forests (Zotz, 2016). About half of Bromeliaceae species occupy the epiphytic habitat, which has inherent variability, as the resources, such as water and nutrients are available only during diverse types of precipitation pulses (Kress, 1989; Benzing, 2000; Reyes-García, Mejia-Chang & Griffiths, 2012). Epiphytic species are expected to show high plasticity to cope with that variability.

Plastic responses have been observed in epiphytic bromeliads, with higher plasticity being described for physiological variables, compared to the more conservative anatomical variables (Cach-Pérez, Andrade & Reyes-García, 2018). These plasticity studies have been carried out under natural conditions with species that inhabit a range of habitats (Cach-Perez *et al*., 2016, 2018), and also among microenvironments within a site (e.g. shaded vs exposed canopy, Cavallero López & Barberis, 2009; North *et al.,* 2016). A transplant experiment documented fast physiological acclimation to contrasting environments in a *Tillandsia* species (Rosado-Calderon *et al.,* 2020), yet, to our knowledge, no studies have documented similar plastic responses in anatomical traits by species taken out of their natural habitat and placed into a new environment.

Stem and roots are highly reduced in epiphytic Bromeliaceae and leaves are responsible for both the absorption of water and nutrients and the production of energy through photosynthesis. Leaves are arranged in the form of a rosette and in some species form a water impounding “tank” at the base; while in others, termed “atmospheric species”, this structure is lacking or does not hold a significant amount of water (Pittendrigh, 1948; Benzing, 2000). Atmospheric species rely on succulent tissues and low transpiration rates to maintain water status (Adams & Martin, 1986). In both cases, water is absorbed mainly via the modified foliar trichomes characteristic of this family (Pierce *et al*., 2001). Among the epiphytic species of the Bromeliaceae, the genus *Tillandsia* shows the most diverse lifeforms, with the development absorptive trichomes (Benzing, 2000).

Key anatomical traits in epiphytic bromeliads that have received most attention are stomata and trichome traits, which may vary both between and within species, showing modified function, morphology and density (Benzing, 1990; Pierce, 2007; Zotz & Bader, 2009). These two structures are extremely important for plant-environment exchange of matter and energy.

Stomatal density in epiphytic Bromeliaceae has been shown to be generally higher in species with a tank lifeform, as compared to atmospheric species (Reyes-García *et al.,* 2008; Cach-Pérez *et al.,* 2016). Stomatal density was positively correlated with precipitation, except for sites such as mangrove swamps, with low precipitation but also very low vapor pressure deficit (VPD) year-round, wherein high stomatal density was observed (Cach-Pérez *et al.,* 2016). The same study found that stomatal size was negatively correlated to precipitation, which the authors interpreted as a positive response to higher light availability at the driest sites.

The trichomes of the Bromeliaceae are an important adaptation that has clearly contributed to the family´s success as epiphytes. Besides the role of absorbing water and nutrients, trichomes have been described as involved in photoprotection, as they increase leaf reflectance, which has been directly related to the level of photoprotection across species (Pierce *et al.,* 2001). The same study found that trichome density did not explain leaf reflectance values, instead, there was a high correlation with individual trichome size (Chávez-Sahagún, 2020). The function of trichomes positioned around stomata has been debated, as it has been suggested that these may increase the leaf boundary layer, diminishing water loss; however, the only study performed did not find a significant effect (Benz & Martin, 2006). Trichomes have also been found responsible for water transport through the leaf surface in *Tillandsia usneoides* (Herppich *et al.,* 2019).

Furthermore, Pierce *et al.* (2001) observed under laboratory conditions that dew condensation, an alternative water source in epiphytic bromeliads, was favored on the bare leaf surface, and not on the trichomes. This suggests that a high trichome density does not favor dew condensation and subsequent absorption by the bromeliad, and these observations were supported by field data from Chávez-Sahagún *et al.* (2020). Thus, trichome density and size may influence, and be influenced by, a variety of environmental variables, such as precipitation, incident light, VPD and frequency of dew condensation conditions.

Our study set out to test for plasticity in stomata and trichome traits, using five epiphytic Bromeliaceae species from a range of environments and transplanting them into common garden conditions. There, plants were exposed to local environmental conditions, but water, presumably the most limiting factor for epiphyte growth (Laube & Zotz, 2003), was always available through natural precipitation or irrigation. We expected that the range of trait values related to the size and trichome and stomata density would be reduced in mature, but newly produced leaves (produced in the transplant site), compared to preexisting leaves (produced in the original habitat), as the species converged in the same environment. Though many environmental variables can influence trichome and stomata traits, as well as genetic constraints, if water is the most important factor, we expected changes to reflect lower water stress, through lower trichome density and higher stomata density in the mature, newly formed leaves, compared to the older leaves. Larger changes in trichome and stomatal traits were expected in plants from sites with more prolonged drought seasons, compared to plants from more mesic sites. We used a relative distances plasticity index (RDPI, Valladares, Sánchez-Gomez & Zavala, 2006) to quantify plasticity. A multivariate analysis was performed to observe the correlated changes in the different traits. We also monitored the rate of leaf formation and elongation at the transplant site (common garden). The observed leaf anatomic traits were discussed in relation to microenvironmental conditions in the original habitat and in the common garden.

MATERIAL AND METHODS

Collection sites

Plants were collected from four different vegetation sites in the Yucatán Peninsula, in Mexico. This region is characterized by presenting a precipitation gradient that goes from the north, with 500 mm of average annual precipitation, to the south, with 1600 mm of average annual precipitation, as well as marked seasonality, which, among other things, has led to the development of different types of vegetation (Bautista *et al.,* 2012). Within that scenario, four climatically and structurally contrasting vegetation types were chosen (Fig. 1): coastal sand dune scrub (CSD); scrub mangrove (SM); deciduous forest (DF) and semi-evergreen forest (SEF).

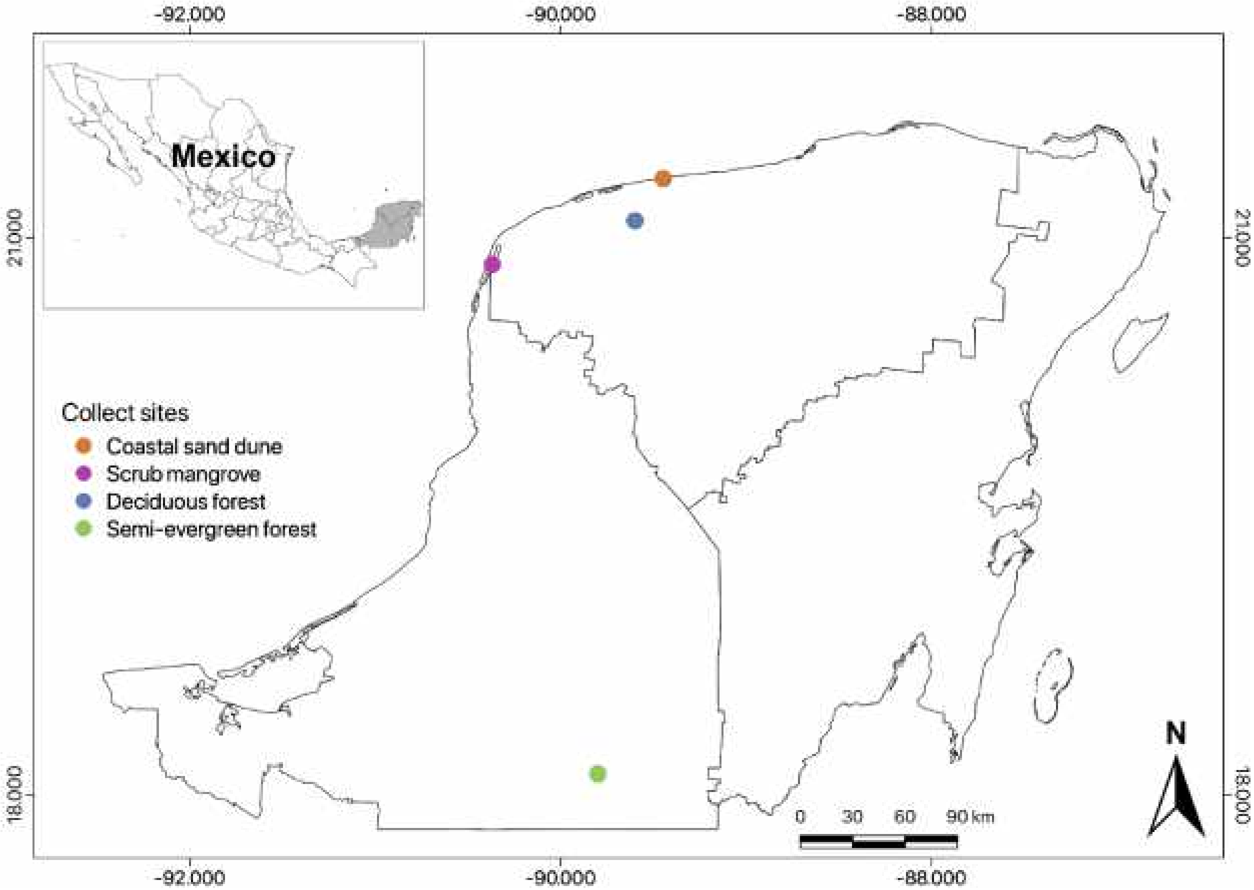

Figure 1. Map of the collection sites of the epiphytic bromeliads for this study.

Coastal sand dune (CSD). Characterized by small and succulent plants, this site is located between the coordinates 21° 19’11.28 "N and 89° 26’51.36" W, with an elevation of 2 m asl, an average annual rainfall of 500 mm and an average annual temperature of 30 ° C (Flores & Espejel, 1994; Bautista *et al.,* 2012).

Scrub mangrove (SM). With mangrove species that on average do not exceed 1.5 m in height, this site is located within the Ría Celestún Biosphere Reserve, between the coordinates 20° 51’23.30 "N and 90° 22’14.70" W, at 3 m asl, and represents a transition between semi-arid and sub-humid climates, with an average annual temperature of 28 ° C and an average annual precipitation of 675 mm (Flores & Espejel, 1994).

Deciduous forest (DF). Characterized by the low height of the deciduous tree components (8 - 10 m), organized in a single stratum, the site is located in an area of 538 ha undergoing regeneration, within the Dzibilchaltún National Park, with the coordinates 21° 5’33.72 "N and 89° 36’0.36" W, at 10 m asl (Bautista *et al.,* 2012). The site presents an average annual temperature of 26 ° C and a summer rainfall regime with precipitation varying from 872 to 1000 mm (Flores & Espejel, 1994).

Semi-evergreen forest (SEF). Between 25 and 50% of the trees are deciduous in this primary forest of approximately 713,185 ha, located within the Calakmul Biosphere Reserve, in the southern part of the Yucatan Peninsula. Situated between the coordinates 18° 6’48.96 " N and 89° 48’3.96 "W, at 260 - 380 m asl, with an average annual precipitation of 1500 mm (Flores & Espejel, 1994; Bautista *et al.,* 2012).

Collection of Bromeliaceae species

A detailed analysis of the epiphytic Bromeliaceae and tree composition of these four sites is described in Cach-Pérez *et al.* (2013). For the current study, five species of epiphytic bromeliads, each distributed in more than one of the vegetation types of the four mentioned above, were selected. The species were: *Tillandsia dasyliriifolia* Baker, *T. balbisiana* Schult.f., *T. brachycaulos* Schltdl., *T. recurvata* (L.) L., and *T. yucatana* Baker (Fig. 2). At least five individuals were collected per species, per vegetation type in which each one is present (Table 1) between January and May of the year 2018. The collected plants were transferred to the common garden site at El Colegio de la Frontera Sur, Villahermosa Campus (17º 54’39; 97” N; 93º 2’10.85” W) and were fixed onto wood plaques, and then hung from a wire mesh fence under 80% shade cloth (allowing 20% of total incident light to pass through it).

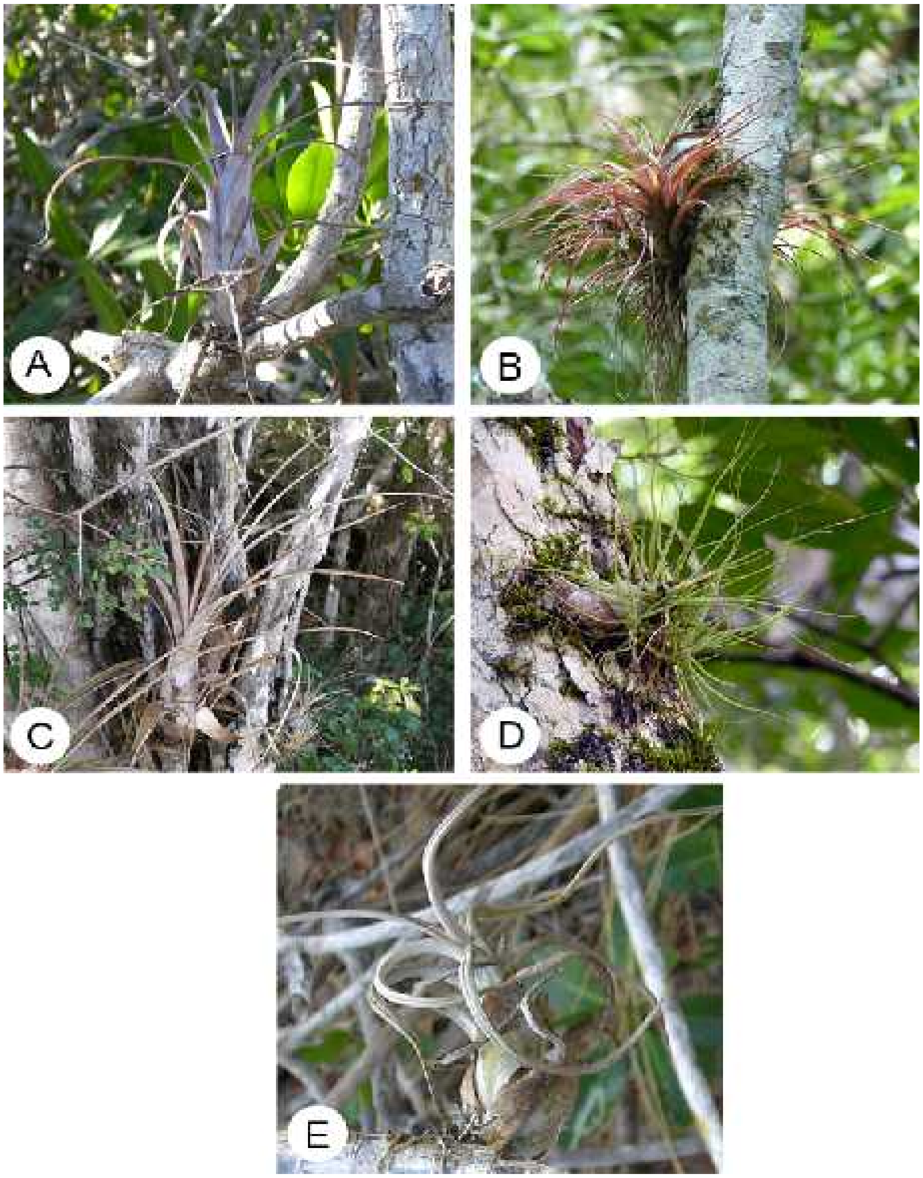

Figure 2. Illustration of the five epiphytic bromeliad species from four contrasting vegetation types in Yucatan, Mexico included in this study. A, *T. balbisiana*; B, *T. brachycaulos*; C, *T. dasyliriifolia*; D, *T. recurvata*; E, *T. yucatana*.

Table 1. Original habitats of the five species of epiphytic bromeliads used in this study: CSD, coastal sand dune scrub; SM, scrub mangrove; DF, deciduous forest; SEF, semi-evergreen forest.

Species	**CSD**	**SM**	**DF**	**SEF**
Tillandsia balbisiana	X		X	X
Tillandsia brachycaulos			X	X
Tillandsia dasyliriifolia	X		X	
Tillandsia recurvata			X	X
Tillandsia yucatana	X	X		X

The plants were acclimatized during a preliminary period of ten months in the common garden site, before the start of the experiment, and produced new leaves under these new, prevailing conditions. During the acclimatization period, during the rainy season and the transition season (early dry) characterized by northerly winds and scattered showers, irrigation was fulfilled by precipitation; in the dry season, plants were watered every three days by rain water previously collected and stored. Vouchers of representative samples per species and collection site were deposited at the Universidad Juárez Autónoma de Tabasco (Juarez Autonomous University of Tabasco) herbarium.

Measurement of environmental conditions.

To compare the microclimatic conditions between the common garden and the collection sites, precipitation, vapor pressure deficit (VPD) and photosynthetically active radiation (PAR) were measured every 10 minutes in the common garden by installing a portable

HOBO U30 micrometeorological station, with connected humidity and air temperature sensors (S-THB-M002 and PAR S-LIA-M003, respectively). A second station was installed simultaneously in an open site (outside the common garden area) as a reference, to which a sensor was also connected to measure precipitation (S-RGB-M002) (equipment by Onset; Bourne, MA, USA). Microclimatic conditions in the common garden were compared to those reported by Cach-Pérez *et al.* (2013) and Cach-Pérez *et al.* (2018) at the collection sites, the data used for the comparison belonged to the lower layer of the canopy and were measured with a similar method and equipment. These measurements, made during the acclimatization period of the plants (in which new leaves were produced), covered the seasons mentioned (rainy, early dry and dry). Measurements were made every month throughout the experiment period.

The annual precipitation cycles were compared for each of the four original habitats, noting the number of months with zero or limited (>0 - 40 mm) precipitation. In the case of the common garden, we considered that the plants were not limited in water availability due to the supplementary irrigation (dry months = 0).

Elongation of new leaves.

The elongation of the new leaves produced in the common garden was determined as a way of comparing leaf growth rate between plants under these conditions. Characterization was made by subtracting the length (cm) at the first month of the acclimatization period (November 2018) from the length of these same new leaves at the end of the acclimatization period (May 2019); as before, average values of all new leaves were obtained for each plant per species and for each of the four original habitats.

Morphological characterization of stomata and trichomes

After the acclimatization period, stomata and trichome density were characterized in three plants per species, comparing healthy, mature leaves produced under the new environmental conditions of the common garden, and healthy, non-senescent preexisting leaves (present at the time of field collection). One new leaf and one preexisting leaf were cut per plant, and leaf impressions were made using transparent commercial nail polish, which was applied to the adaxial and abaxial surfaces of the base, middle and tip of each leaf. Once the nail polish had dried, it was removed using dissecting forceps. To observe the stomata, the leaf lamina was shaved with a scalpel to remove the wings of the trichomes before applying the nail varnish (Cach-Pérez *et al.,* 2016). Each section was mounted on a slide and viewed under a Primo Star light microscope (Carl Zeiss Microscopy GmbH, Gottingen, Germany) with a 10X objective. Photographs were taken with an Axiocam ERc 5s digital camera (Carl Zeiss, Gottingen, Germany). From the photographs obtained, the number of stomata and trichomes were counted; the densities (per mm2) of these structures obtained in each part of the leaf were averaged to obtain a value for the adaxial and abaxial surfaces of each leaf.

To characterize the morphology of the stomata and trichomes, leaf samples (3 cm2) were taken from the mid-section of the preexisting and new leaves of three plants for each species, which were fixed in FAA solution (formaldehyde, alcohol, acetic acid). The samples were taken to Centro de Investigación Científíca de Yucatan (CICY), where the morphology was determined from photographs obtained in a scanning electron microscope JEOL-JSM-6360LV (Jeol Ltd., Tokyo, Japan). The samples were previously dried to a critical point in a SAMDRI 795 dryer (Tousimis, Rockville, MD, USA), fixed to a copper base by double-sided adhesive tape, and gold plated in a Denton Vacuum Desk II Metallizer (Denton Vacuum LLC, Moorestown, NJ, USA). The trichomes were photographed on the abaxial and adaxial sides of the leaf, while the stomata were photographed only on the abaxial surface (no stomata were registered on the adaxial surface). Three photographs were obtained per sample from randomly selected fields. From the images obtained, measurements of stomatal size, stomatal pore area, total area of the trichome and area of the central disc (shield) of the trichome were made in one stoma and one trichome selected at random per photograph. In all cases, ImageJ version 3.0 software (Rasband, 2018) was used to make the measurements.

Plasticity index

The morphological data obtained were integrated to determine the response of each bromeliad species, transferred from one or more of four vegetation types to the same conditions within the common garden. Morphological plasticity was calculated using the plasticity index, based on phenotypic distances (RDPI), following the methodology proposed by Valladares *et al.* (2006):

$$\boldsymbol{R} \quad = \sum (d \quad \rightarrow i'j'/(X\ 'j' + X \quad))/n$$

Where dij → i´j´ / (Xi´j´ + Xij)) is the set of relative distances, or phenotypic distance between individuals from the four different vegetation types, and n is the number of distances. The index is obtained on a scale that goes from zero (low plasticity) to one (high plasticity). The variables used to calculate the index were: trichome density, trichome disc area, total trichome area, stomatal density, stomatal pore size, and total stomatal size in the five different species; similarly, from the previous data, an average value was calculated by species per vegetation type.

Statistical analyses

Variations in the density and morphology of the stomata and trichomes from different vegetation types was evaluated per species, using a simple ANOVA. A test including all the species was not performed since not all the species were found in all the sites. Collection sites were independent variables and the density and morphology of stomata and trichomes, both for the abaxial and adaxial faces, constituted response variables. A two-way ANOVA was performed with vegetation type and leaf type (new and preexisting) as factors per species. Tukey's post hoc test was used when necessary. Prior to the analyses, the normality and homogeneity of the variances of the data were verified. When the data did not show normality, the Kruskal-Wallis non-parametric test was used (stomatal pore size of the new leaves). To visualize the coordinated, integrated response of all the anatomical variables to both the original vegetation types and to the transplant, a non-metric multidimensional scale analysis (NMDS) was performed using all the data obtained from stomatal and trichome density and morphology, both from preexisting leaves and from new leaves; covariance for NMDS data was calculated per species for comparison of ranges of variation. This test does not assume a linear relationship between the variables. The Stress value was calculated to evaluate goodness of fit. All statistical analyses were performed with R software version 3.6.3.1 (R Core Team, 2019).

RESULTS

Microclimate

The PAR was higher within the common garden than in the different types of vegetation during the early dry and dry seasons, with the only exception being that of the semi-evergreen forest (SEF), which presented higher values (Fig. 3A); the VPD was similar between the common garden and SEF but higher than in the other types of vegetation during the rainy and dry seasons (Fig. 3B). The amount of precipitation recorded during the eight months of the acclimatization period in the common garden was less than that recorded in the natural environment (Fig. 3C). However, the number of months with limited precipitation (see Methods), that in the natural habitats can be up to seven months (Fig. 3C), was eliminated in the common garden as the plants were continuously supplied with additional water.

Elongation of new leaves.

Regarding the elongation of the new leaves (Fig. 4), the individuals of T. balbisiana and T. brachycaulos from the most humid collection site (SEF) presented greater elongation, compared to the individuals from their other collection sites ($P < 0.05$; $P < 0.05$, respectively). Similarly, individuals of T. yucatana from the CSD and SM presented the highest elongation compared to individuals from the deciduous forest (DF) ($P = 0.0024$). In contrast, individuals of T. dasyliriifolia and T. recurvata did not show significant differences in the elongation of new leaves, regardless of the site of origin (P = 0.646; P = 0.145, respectively).

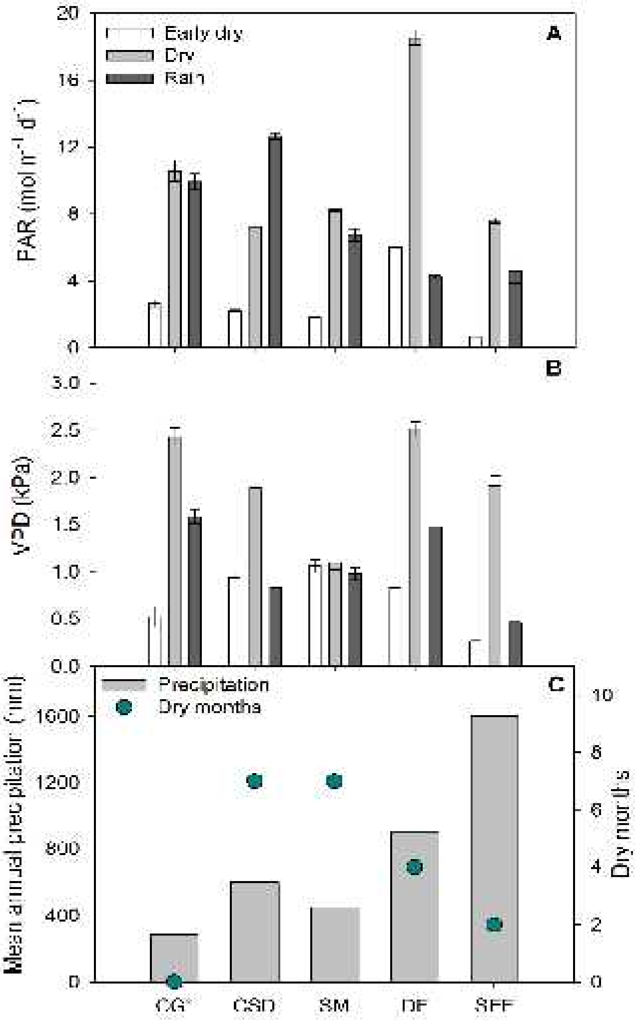

Figure 3. Microclimatic conditions in the common garden and original collection sites during the early dry, dry and rainy seasons**. CG, common garden; CSD, coastal sand dune; SM, scrub mangrove; DF, deciduous forest; SEF, semi-evergreen forest. Data are means ± SE for (a) and (b). * The data for CG correspond to that recorded by the microclimatic stations placed during the acclimatization period of the plants; ** data taken from Cach-Pérez et al., 2013 and Cach-Pérez et al., 2018.

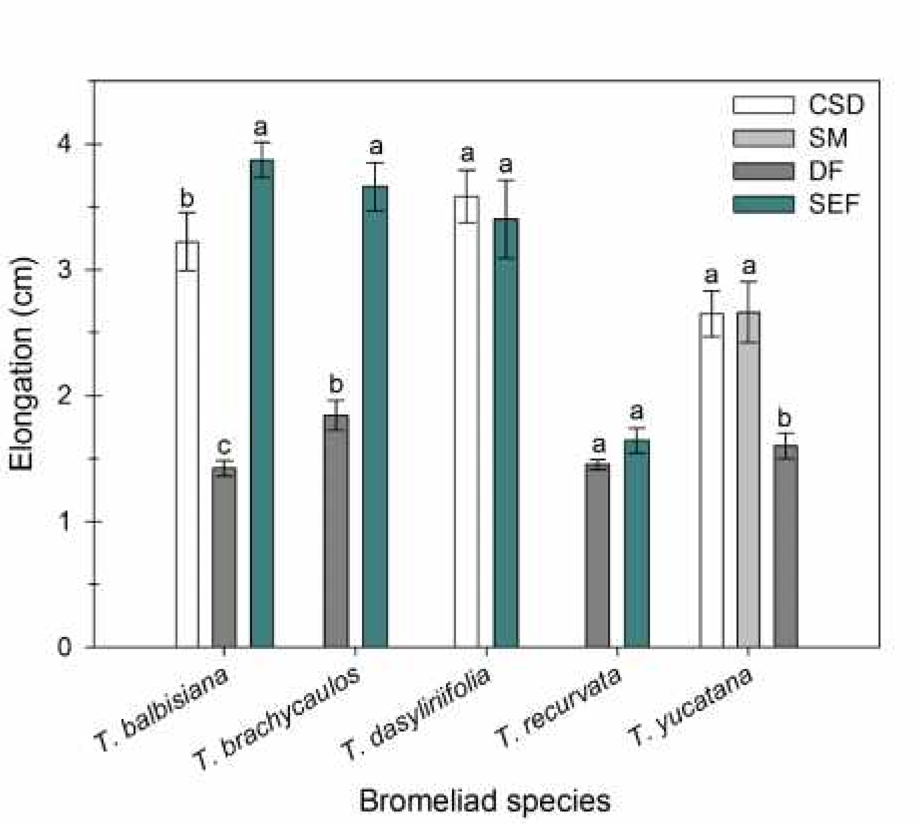

Figure 4. Elongation (growth in cm) of new leaves produced in the common garden, of five species of epiphytic bromeliads from four climate-contrasting vegetation types. Data are means ± SE. Different letters indicate significant differences between collection sites for the same species.

Trichome and stomatal traits

Total trichome density on the adaxial surface in preexisting leaves (Table 2) for individuals of *T. brachycaulos* was similar between plants regardless of their place of origin (P = 0.061). Individuals of *T. balbisiana*, *T. dasyliriifolia* and *T. yucatana* from CSD presented the lowest trichome density compared to individuals from other sites ($P = 0.004$; $P = 0.004$ and $P = 0.001$, respectively); On the other hand, individuals of *T. recurvata* from the SEF presented a higher total trichome density compared to individuals from the DF ($P = 0.003$).

For the new leaves (Table 2), we found the same pattern as for the preexisting leaves in *T. balbisiana*, where plants from CSD had the lowest trichome density, compared to the other two sites ($P < 0.05$). *Tillandsia brachycaulos* and *T. recurvata* had the lowest trichome density in plants from the driest site of their distribution, DF ($P = 0.022$ and $P < 0.05$, respectively), as well as *T. dasyliriifolia* from CSD in relation to plants from SEF ($P <0.05$). Finally, individuals of *T. yucatana* presented up to 30% fewer trichomes in the MS, compared to individuals from the DF ($P < 0.01$).

Regarding the abaxial surface, the density of trichomes in preexisting leaves (Table 2) of *T. balbisiana*, *T. dasyliriifolia* and *T. yucatana* was up to 20.5%, 69.5% and 27.5% lower, respectively, in individuals from the CSD, with respect to those from their other collection sites ($P < 0.01$; $P <0.05$ and $P <0.05$, respectively). *Tillandsia recurvata* individuals from the DF had a lower trichome density compared to those from the CEF ($P < 0.05$), while *T. brachycaulos* individuals from all collection sites had similar trichome density on the adaxial surface of the leaves.

Finally, with regard to the comparison of the same surface of the new leaves (Table 2), the results for individuals of *T. balbisiana*, *T. dasyliriifolia* and *T. yucatana* were similar, as for the preexisting leaves, where plants from the CSD presented the lowest trichome density, compared to its other collection sites ($P < 0.05$ in all cases). For *T. recurvata*, the lowest trichome density in both new and preexisting leaves was recorded in individuals from the DF ($P < 0.05$). Finally, to the contrary, the preexisting leaves of *T. brachycaulos* from the SEF presented 11% fewer trichomes in new leaves compared to individuals from the DF (P = 0.023).

When comparing trichome density on the adaxial surface of preexisting leaves and new leaves, by species, we found that individuals of *T. balbisiana* from the CSD and DF presented up to 13.2% and 9.6% higher trichome density on new leaves (P = 0.034 and P = 0.0113, respectively), while individuals from the SEF showed no such differences. There were no significant differences between leaf types in the rest of species and on the abaxial surface of the leaf.

Stomatal density on the abaxial surface of preexisting leaves (Table 3) of individuals of *T. balbisiana*, *T. brachycaulos*, *T. dasyliriifolia* and *T. recurvata* from the SEF were 15.38%, 25.29%, 19.85% and 36.90% less, respectively, than individuals from the other collection sites (P = 0.032; P = 0.024; P < 0.01; P = 0.002, respectively). For *T. yucatana*, the individuals from the MS and DF presented lower stomatal density when compared with individuals from the CSD (P < 0.05).

New leaves (Table 3) of individuals of *T. balbisiana* from the SEF presented up to 32.9% fewer stomata compared to individuals from the other two collection sites (P = 0.01), somewhat similar as observed for *T. yucatana* where individuals from the MS and DF presented lower stomata density compared to individuals from the CSD (P < 0.05). No significant differences were found in any case when comparing stomatal density between preexisting and new leaves.

Analysis of the stomatal density on the abaxial surface of preexisting leaves (Table 3), showed that individuals of *T. balbisiana*, *T. brachycaulos, T. dasyliriifolia* and *T. recurvata* from the SEF presented 15.38%, 25.29 %, 19.85% and 36.90% fewer stomata, respectively, compared to the individuals from the other collection sites (P = 0.032; P =

0.024; P = 0.005; P = 0.002, respectively). In the case of *T. yucatana*, the individuals from the MS and DF presented lower stomatal density compared to those from the CSD (P < 0.05). In the new leaves (Table 3), individuals of *T. balbisiana* from the SEF presented up to 32.9% fewer stomata compared to individuals from the other two collection sites (P = 0.01), similar to *T. yucatana*, where individuals from the MS and DF presented lower stomata density compared to individuals from the CSD (P < 0.05). No significant differences were found in any case when comparing stomatal density between preexisting leaves and new leaves

On the adaxial surface of preexisting leaves the size of the central disc of the trichome (Table 4), in individuals of *T. balbisiana* from the CSD were smaller than those from the DF and SEF (P = 0.034), while individuals of *T. yucatana* from the CSD had trichomes with larger central discs than those from SM and DF (P = 0.004). The rest of the species did not present significant differences. Regarding the same adaxial surface in new leaves, differences were only found in the size of the central disc for individuals of *T. balbisiana* from DF, which was up to 35.8% larger compared to the other two collection sites (P = 0.039) (Table 4).

The total area of the trichomes on the adaxial surface (Table 4) of preexisting leaves of *T. brachycaulos* individuals from the DF was up to 32.5% greater compared to the individuals from the SEF (P = 0.023). Similarly, individuals of *T. yucatana* from the CSD had trichomes up to 63.7% larger than those from SM and DF (P = 0.023). The rest of the species did not show differences; similarly, there were no differences in new leaves between collection sites for any species.

On the abaxial surface, differences were only found for the size of the central disc of trichomes of preexisting leaves of individuals of *T. balbisiana* from the CSD, which were up to 30.1% smaller than those of individuals from the other two collection sites ($P < 0.01$) (Table 5). There were no significant differences in the size of the central disc and total size of the trichome of the new leaves.

When comparing preexisting leaves and new leaves, we found that the central disc size and size of the trichomes on the adaxial surface of *T. yucatana* individuals from the CSD was up to 26.9% and 36.2%, respectively, greater in new leaves compared to preexisting leaves ($P = 0.004$). In the abaxial surface only the central disc size of the trichomes presented differences: individuals of *T. yucatana* from the DF were smaller in the new leaves when compared to the preexisting leaves ($P = 0.026$). There were no significant differences between preexisting and new leaves for the total area of the stomata and the size of the stomatal pore (Table 6) for any of the species, vegetation types.

Morphological plasticity and NMDS

The highest plasticity obtained in the morphological variables measured was registered in the stomatal pore size in all cases, followed by trichome size in *T. dasyliriifolia*, *T. recurvata* and *T. yucatana*, trichome central disc size and stomatal size in *T. balbisiana* and *T. brachycaulos*, respectively (Fig. 5 A-E). Average plasticity was similar between species (Fig. 5F); the RDPI index showed values between 0.09 and 0.22, indicating low average plasticity. *Tillandsia dasyliriifolia* had the highest plasticity in this study, within which individuals from the SEF had 40% higher values than those from the CSD. In contrast, individuals of *T. yucatana* from the CSD had the lowest plasticity (0.09), being 40% lower compared to individuals from the SM and SEF. Similarly, *T. balbisiana* showed a variation of up to 27% in its plasticity indices between individuals from the CSD (0.13) and SEF (0.18). *Tillandsia brachycaulos* and *T. recurvata* showed minimal variation in plasticity between vegetation types (0.01 points of difference).

Table 2. Number of trichomes per mm2 on the adaxial and abaxial surface of preexisting and new leaves in epiphytic bromeliads. CSD, coastal sand dune; SM, scrub mangrove; DF, deciduous forest; SEF, semi-evergreen forest. Data are means ± SE. Different letters denote significant differences (P < 0.05) between sites for the same species and type of leaf. Different symbols denote significant differences (P < 0.05) between preexisting and new leaves for the same leaf surface.

Species	Vegetation type	Adaxial trichomes per mm^{-2}		Abaxial trichomes per mm^{-2}	
		Preexisting	New	Preexisting	New
T. balbisiana	CSD	45.3 ± 2.2^{b††}	52.2 ± 2.1^{b†}	36.1 ± 2.6^{b}	36.0 ± 2.7^{c}
T. balbisiana	DF	59.3 ± 9.0^{a††}	65.6 ± 2.7^{a†}	53.7 ± 2.1^{a}	52.6 ± 2.7^{a}
T. balbisiana	SEF	55.1 ± 1.6^{a}	50.9 ± 2.8^{b}	48.2 ± 1.5^{a}	46.2 ± 2.6^{b}
T. brachycaulos	DF	48.4 ± 2.7^{a}	48.6 ± 3.8^{a}	38.7 ± 1.5^{a}	36.2 ± 0.9^{b}
T. brachycaulos	SEF	42.2 ± 2.5^{a}	39.6 ± 2.0^{b}	36.0 ± 2.9^{a}	33.4±1.2^{a}
T. dasyriliifolia	CSD	29.5 ± 1.1^{b}	28.2 ± 1.2^{b}	17.1 ± 1.7^{b}	18.1 ± 2.1^{b}
T. dasyriliifolia	SEF	61.0 ± 5.0^{a}	53.1 ± 3.9^{a}	48.7 ± 4.1^{a}	45.9 ± 2.7^{a}
T. recurvata	DF	19.8 ±1 .2^{b}	22.1 ± 1.2^{b}	20.7 ± 1.7^{b}	20.5 ± 1.0^{b}
T. recurvata	SEF	45.4 ± 2.6^{a}	44.3 ± 1.9^{a}	41.7 ± 2.0^{a}	41.9 ± 1.9^{a}
T. yucatana	CSD	35.5 ± 4.7^{c}	39.2 ± 3.4^{b}	25.3 ± 3.3^{c}	23.4 ± 1.9^{b}
T. yucatana	SM	45.8 ± 2.0^{b}	43.7 ± 2.9^{b}	32.2 ± 1.8^{b}	32.7 ± 1.3^{a}
T. yucatana	DF	56.4 ± 2.8^{a}	51.9 ± 2.1^{a}	38.6 ± 2.1^{b}	39.4 ± 2.2^{a}

Table 3. Number of stomata per mm2 on the abaxial surface of preexisting and new leaves in epiphytic bromeliads. CSD, coastal sand dune; SM, scrub mangrove; DF, deciduous forest; SEF, semi-evergreen forest. Data are means ± SE. Different letters denote significant differences ($P < 0.05$) between sites for the same species and type of leaf. There were no significant differences between leaf types.

Species	Vegetation type	Leaf	
		Preexisting	New
T. balbisiana	CSD	17.2 ± 0.6^{a}	18.6 ± 1.7^{a}
T. balbisiana	DF	16.2 ± 1.4^{a}	15.9 ± 2.0^{a}
T. balbisiana	SEF	14.0 ± 0.6^{b}	12.3 ± 1.3^{b}
T. brachycaulos	DF	10.0 ± 1.2^{a}	10.6 ± 1.0^{a}
T. brachycaulos	SEF	6.9 ± 0.8^{b}	8.7 ± 0.9^{a}
T. dasyriliifolia	CSD	11.8 ± 1.4^{a}	15.1 ± 1.6^{a}
T. dasyriliifolia	SEF	13.8 ± 1.8^{a}	15.9 ± 2.3^{a}
T. recurvata	DF	10.1 ± 0.8^{a}	9.8 ± 0.9^{a}
T. recurvata	SEF	6.5 ± 0.8^{b}	7.3 ± 0.6^{a}
T. yucatana	CSD	12.1 ± 1.1^{b}	11.6 ± 1.9^{a}
T. yucatana	SM	7.9 ± 0.6^{a}	7.9 ± 0.3^{b}
T. yucatana	DF	8.3 ± 1.9^{a}	7.2 ± 0.7^{b}

Table 4. Morphology of trichomes in preexisting and new leaves on the leaf adaxial surface of five species of epiphytic bromeliads from four types of vegetation (VT). CSD, coastal sand dune; SM, scrub mangrove; DF, deciduous forest; SEF, semi-evergreen

forest. Data are means ± SE. Different letters denote significant differences ($P < 0.05$) between collection sites per specie. Different symbols denote significant differences ($P < 0.05$) between preexisting and new leaves for the same character, species and site.

		Central disc size (μm^{-2})		Total area (μm^{-2})	
Species	VT	Preexisting	New	Preexisting	New
T. balbisiana	CSD	3396 ± 801^{b}	5077 ± 891^{b}	17603 ± 2718^{a}	14376 ± 2748^{a}
T. balbisiana	DF	8207 ± 1618^{a}	7914 ± 1250^{a}	26924 ± 5339^{a}	31045 ± 4407^{a}
T. balbisiana	SEF	7832 ± 404^{a}	5988 ± 1175^{ab}	25605 ± 4401^{a}	23642 ± 4489^{a}
T. brachycaulos	DF	8335 ± 964^{a}	8052 ± 1366^{a}	42788 ± 476^{a}	41049 ± 3857^{a}
T. brachycaulos	SEF	6931 ± 2110^{a}	7024 ± 733^{a}	28858 ± 3869^{b}	29067 ± 3634^{a}
T. dasyriliifolia	CSD	6961 ± 1172^{a}	6331 ± 835^{a}	40705 ± 1839^{a}	41318 ± 5345^{a}
T. dasyriliifolia	SEF	4361 ± 1326^{a}	5990 ± 668^{a}	25350 ± 7625^{a}	23994 ± 4990^{a}
T. recurvata	DF	10325 ± 1079^{a}	8394 ± 1122^{a}	73292 ± 4344^{a}	80829 ± 7958^{a}
T. recurvata	SEF	5634 ± 1912^{a}	6399±1836^{a}	16781 ± 6932^{a}	28227 ± 8252^{a}
T. yucatana	CSD	12119 ± 881^{a*}	8855 ± 361^{a**}	86049 ± 3844^{a†}	54858 ± 2113^{a††}
T. yucatana	SM	5878 ± 1406^{b}	6133 ± 488^{a}	32125 ± 2765^{b}	37365 ± 5866^{a}
T. yucatana	DF	5032 ± 324^{b}	6492 ± 1354^{a}	31214 ± 1158^{b}	31459 ± 8185^{a}

Table 5. Morphology of trichomes in preexisting and new leaves on the leaf abaxial surface of five species of epiphytic bromeliads from four types of vegetation (VT). CSD, coastal sand dune; SM, scrub mangrove; DF, deciduous forest; SEF, semi-evergreen forest. Data are means ± SE. Different letters denote significant differences ($P < 0.05$) between collection sites per specie. Different symbols denote significant differences ($P < 0.05$) between preexisting and new leaves for the same character, species and site.

		Central disc size (μm^{-2})		Total area (μm^{-2})	
Species	VT	Preexisting	New	Preexisting	New

T. balbisiana	CSD	4889 ± 108^{b}	5626 ± 1243^{a}	18162 ± 2532^{a}	18716 ± 1040^{a}
T. balbisiana	DF	6289 ± 186^{a}	7091 ± 1523^{a}	18822 ± 1967^{a}	24649 ± 2521^{a}
T. balbisiana	SEF	6997 ± 274^{a}	5851 ± 2127^{a}	15232 ± 2410^{a}	19368 ± 5138^{a}
T. brachycaulos	DF	8299 ± 335^{a}	5882 ± 4360^{a}	42122 ± 3653^{a}	31949 ± 5832^{a}
T. brachycaulos	SEF	6273 ± 777^{a}	6933 ± 870^{a}	35419 ± 1221^{a}	38381 ± 4859^{a}
T. dasyriliifolia	CSD	4443 ± 246^{a}	5119 ± 1817^{a}	42965 ± 912^{a}	39509 ± 1747^{a}
T. dasyriliifolia	SEF	4384 ± 953^{a}	6329 ± 3618	22569 ± 6731^{a}	29566 ± 8073^{a}
T. recurvata	DF	7776 ± 867^{a}	9261 ± 616^{a}	53737 ± 8097^{a}	66050 ± 7185^{a}
T. recurvata	SEF	6382 ± 1480^{a}	6037 ± 1706^{a}	32411 ± 6235^{a}	26730 ± 8137^{a}
T. yucatana	CSD	7321 ± 635^{a}	6804 ± 717^{a}	50688 ± 3055^{a}	47941 ± 1158^{a}
T. yucatana	SM	9396 ± 1259^{a}	6877 ± 3676^{a}	72877 ± 7952^{a}	56265 ± 11736^{a}
T. yucatana	DF	7171 ± 179^{a*}	5197 ± 697^{a**}	45552 ± 8337^{a}	35177 ± 3989^{a}

Table 6. Morphology of stomata in mature and new leaves in five species of epiphytic bromeliads from four types of vegetation. Data are means ± SE. Different letters denote significant differences ($P < 0.05$) between sites for the same species, character and leaf type. There were no significant differences between leaf types in any character.

Species	Vegetation types	Total area (µm^{-2})		Stomatal pore (µm^{-2})	
		Preexisting leaves	New leaves	Preexisting leaves	New leaves
T. balbisiana	CSD	891 ± 73^{a}	1190 ± 176^{a}	27 ± 1^{a}	32 ± 10^{a}
T. balbisiana	DF	920 ± 177^{a}	992 ± 50^{a}	44 ± 19^{a}	32 ± 7^{a}
T. balbisiana	SEF	992 ± 112^{a}	995 ± 114^{a}	30 ± 7^{a}	12 ± 1^{a}
T. brachycaulos	DF	812 ± 128^{a}	777 ± 237^{a}	36 ± 4^{a}	28 ± 8^{a}
T. brachycaulos	SEF	691 ± 22^{a}	634 ± 11^{a}	24 ± 8^{a}	16 ± 8^{a}
T. dasyriliifolia	CSD	638 ± 130^{a}	644 ± 67^{a}	13 ± 4^{a}	18 ± 5^{a}
T. dasyriliifolia	SEF	793 ± 80^{a}	686 ± 51^{a}	20 ± 2^{a}	22 ± 15^{a}

T. recurvata	DF	604 ± 79^a	790 ± 131^a	18 ± 3^a	21 ± 9^a
T. recurvata	SEF	941 ± 99^a	754 ± 10^a	18 ± 3^a	14 ± 3^a
T. yucatana	CSD	917 ± 4^a	924 ± 27^a	28 ± 3^a	27 ± 3^a
T. yucatana	SM	1037 ± 139^a	980 ± 60^a	17 ± 14^a	34 ± 13^a
T. yucatana	DF	964 ±79^a	932 ± 56^a	26 ± 5^a	15 ± 7^a

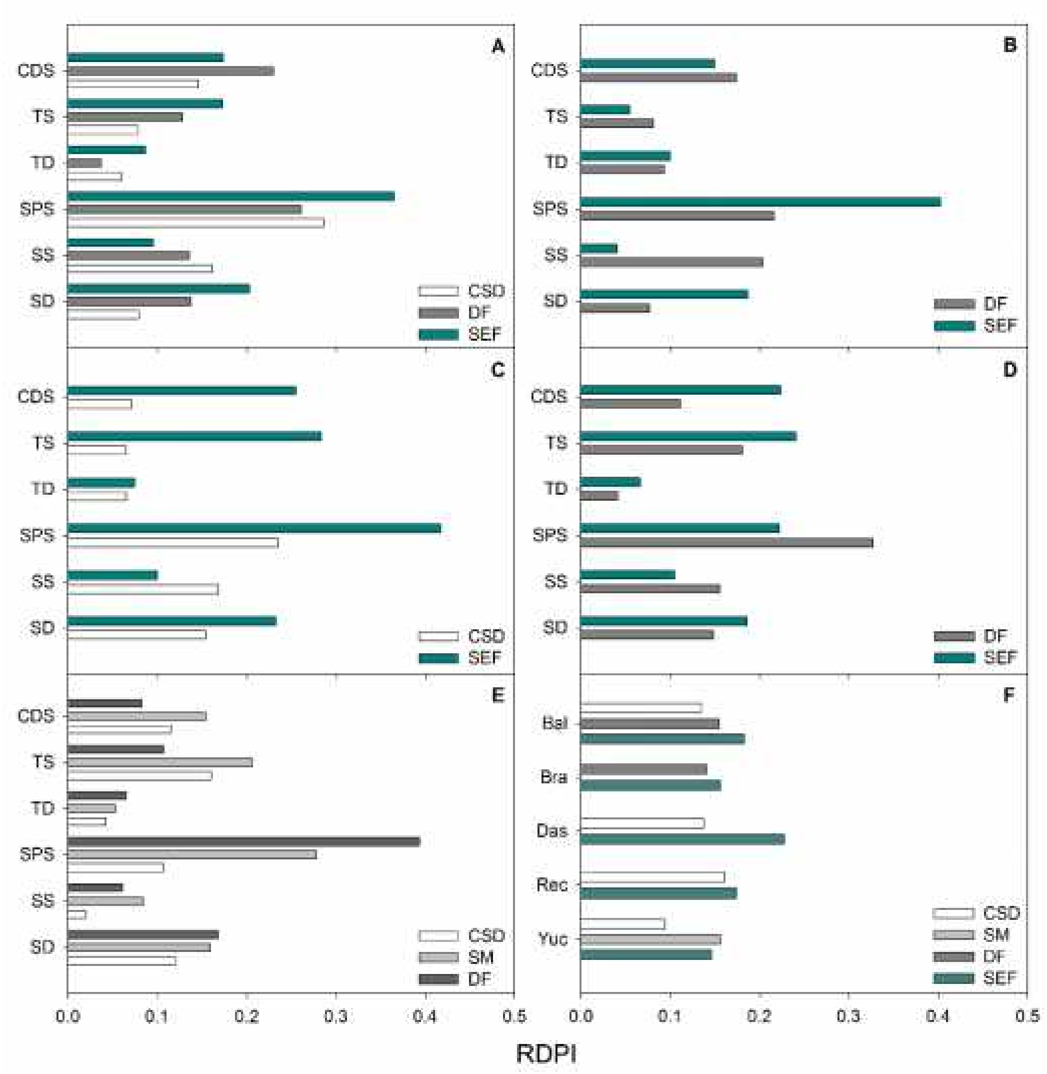

Figure 5. Plasticity index (RDPI) by morphological variable (A-E) and average (F) for five species of epiphytic bromeliads from four vegetation types calculated from the comparison

between preexisting and new leaves by species per collection site. A, *T. balbisiana*; B, *T. brachycaulos*; C, *T. dasyliriifolia*; D, *T. recurvata*; E, *T. yucatana*. CSD, coastal sand dune; SM, scrub mangrove; DF, deciduous forest; SEF, semi-evergreen forest. CDS, central disc size; TS, trichome size; TD, trichome density; SPS, stomatal pore size; SS, stomatal size; SD, stomatal size; Bal, *T. balbisiana*, Bra, *T. brachycaulos*; Das, *T. dasyliriifolia*; Rec, *T. recurvata*; Yuc, *T. yucatana*.

The non-metric multidimensional scale analysis (NMDS; Fig. 6) found correlation among the different anatomical variables (Stress=0.1079). The first axis (NMDS1) showed species with a positive correlation in trichome size among both leaf faces (abaxial and adaxial) and a negative effect of trichome density (also on both leaf faces, Table 7). The second axis (NMDS2), showed less coordination between the leaf faces, as the variables most relevant were adaxial trichome size, and abaxial trichome density and central disc size. Stomata variables, which could be important for the plasticity index, were secondary in the NMDS analysis.

Using both NMDS variables, there was an overall separation of the different species, except for *T. recurvata*, which also showed very high separation between its original populations (SEF and DF; Fig. 6). *Tillandsia balbisiana* tended towards lower comparative trichome size and higher density, followed by *T. dasylirifolia*, while at the other extreme, DF *T. recurvata* had larger trichomes and lower density. Coastal sand dune and DF populations tended to have lower values in NMDS2, irrespective of the species. Particularly for *T. dasyliriifolia* and *T. yucatana*, there was a clear pattern where the values of the newly produced leaves from different populations showed large shifts in both axes and tended to converge towards the center of the plot. This was not seen in the other species, either because the shifts were small in the newly produced leaves, compared to preexisting (*T. recurvata* and *T. brachycaulos*), or because the changes were in different directions for the different populations (*T. balbisiana).*

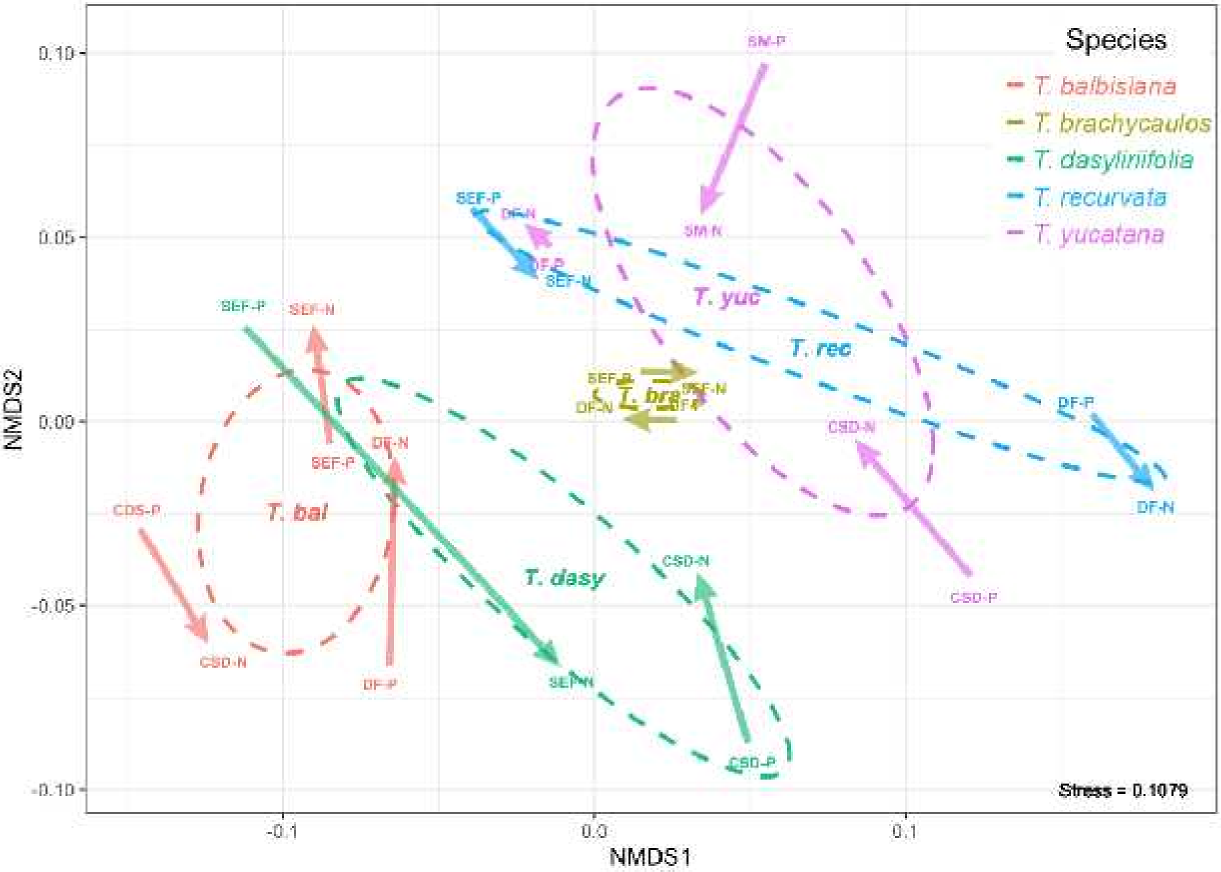

Figure 6. Non-metric multidimensional scale analysis (NMDS) that includes density and morphology data for the stomata and leaf trichomes of five species of epiphytic bromeliads. The dotted lines indicate the degree of dispersion of the data for each species, constructed from a covariance matrix of the data. Arrows were drawn to give an estimate of the magnitude and trajectory of the changes between preexisting and new leaves. CSD, coastal sand dune; SM, scrub mangrove; DF, deciduous forest; SEF, semi-evergreen forest; N, new leaves; P, preexisting leaves.

DISCUSSION

Our results found consistently low overall anatomic plasticity per species (RDPI less than 0.22; Fig. 5), even where there was high variation in the plasticity of each anatomic component, with stomata pore size being the most variable in all species. In contrast, trichome density, a highly studied anatomic trait (Benzing et al., 1978; Benzing, 2000; Benz & Martin, 2006, Reyes-García & Griffiths, 2009; Males, 2016; Cach-Pérez *et al.,* 2016; 2018; Chávez-Sahagun, 2020) was among the most conserved traits according to the RDPI values. Correlated increases in trichome size in both leaf faces, and diminished trichome density were observed through the NDMS, while stomata size and density were not as highly correlated (Fig. 6).

The low plasticity observed could partly be due to similarity of the environmental conditions prevailing in the common garden with those found within the vegetation types where the plants were collected. Even though we effectively eliminated the drought, the high light and VPD values may have contributed to stress the plants, making the overall environmental differences insufficient to stimulate the expected morphological responses. Figure 2 shows that the microclimate in the common garden was very similar to that found in the CSD (Coastal Sand Dune) and DF (Deciduous Forest) in terms of PAR and VPD, and individuals of *T. yucatana* from the CSD registered the lowest plasticity in this study (0.09; Fig. 5). There was also similarity with the VPD values obtained for the common garden and for the SEF (Semi-Evergreen Forest), particularly during the dry season.

The limited variation in environmental conditions and the unexpected low plasticity shown by the plants growing in the common garden environment set up in this study contrasts with data reported by Cach-Pérez *et al.* (2018) for the same species. However, in that case, the calculations were based on the morphological variations observed between individuals of the same species growing in different types of vegetation with microclimatically contrasting environments, without moving them to a new environment. In the aforementioned study, four of the five species that we analyzed here are included, for which greater plasticity was reported for *T. balbisiana* (mean RDPI 0.19, greater in all cases), *T. yucatana* (mean RDPI 0.15, greater than in individuals from the CSD and similar to that of the other collection sites), while for *T. dasyliriifolia* the reported value (mean RDPI 0.20) is similar to that found in individuals from the SEF, and higher than those from the CSD.

Despite the limited variation observed between the preexisting and new leaves, we found differences in the sensitivity to environmental variation between stomata and trichomes. For example, like Cach-Pérez *et al.* (2016), we found that, with the only exception of *T. brachycaulos* and *T. dasyliriifolia*, comparing the natural vegetation types, higher stomatal density coincided with the driest sites (preexisting leaves, Table 3). However, even though there was an apparently more variable response in terms of trichome density on the adaxial surface of leaves between species and collection sites, stomatal density had higher adaptation capacity (RDPI, Fig. 5) than trichome density to the change in environmental conditions when the plants were transferred to the common garden. However, there was no change for any of the species in the morphology of these structures between the preexisting leaves and the new leaves formed after transferal to the common garden (Tables 2 and 4).

We observed greater variation between the preexisting leaves, formed in the original habitat prior to collection, and previous reports for the same species from the same collection sites (Cach-Pérez *et al.,* 2016). While species like *T. balbisiana* and *T. yucatana* presented a reduction in trichome density on the adaxial leaf surface of up to

41.8% and 39.9%, respectively, in plants from the two driest places of their distribution, individuals of both species from SEF were similar to our results. In contrast, plants of *T. recurvata* and *T. dasyliriifolia* from SEF presented up to 50% and 56% more trichomes, respectively, with respect to the aforementioned report. Although stomatal density was less variable than that of the trichomes, their morphology was more affected, since in all cases the stomatal pore size was reduced compared to that characterized by the aforementioned authors between 2011 and 2012. This suggests that the climate in the different collection sites, particularly in the driest, has varied significantly in the last eight to 10 years, causing variation in density and morphology of stomata and trichomes of epiphytic bromeliads. At the same time, we can observe the response of these plants to environmental variation, particularly those from SEF which was the most climatic contrasting collection site, with respect to the common garden. However, in most cases we can assume that the degree of climatic variation for these morphological responses to be expressed must be greater than that to which we subjected to the plants in the present study.

Non-metric multidimensional scale analysis allowed us to identify correlated patterns of variation in the different anatomical traits between species by original vegetation type (Fig. 6). This ordination method searches for axes that explain the most variation, thus finding the correlation between the different anatomical traits. Given the array of environmental variables that can influence trichome and stomata traits, plastic responses are expected to be similarly complex. For example, if trichome size can influence light reflectance (Chávez-Sahagún, 2020), while density may increase water absorption (Stefano, Papini & Brighigna, 2008), these traits may vary independently according to the environment and differently among the adaxial or abaxial leaf faces responding to microenvironmental differences. The first axis, NMDS1 showed that actually, the most common combination is a similar response in both leaf faces, with increasing trichome size related to decreasing trichome density. The results from this analysis cannot be equated to the RDPI results, since variations in stomatal traits are diminished, presumably because these do not have high covariance with other traits. From the NDMS analysis, *T. dasyliriifolia* and *T. yucatana* are the species displaying the greatest variation between the preexisting and new leaves (Fig. 6).

However, parallel to that, we observed convergence in the response of plants from the different vegetation types to the common garden environment, as shown in Figure 6, where the points representing the new leaves are closer together, when compared to the points representing the preexisting leaves. *Tillandsia brachycaulos* showed no variation, while *T. balbisiana* presented an intermediate response, with an apparent divergence of response by the new leaves, as compared to the preexisting ones. *Tillandsia recurvata* presents a clear differentiation between plants from different vegetation types, but with little response to the new environment as shown by the similarity between preexisting and new leaves.

The second NDMS axis showed a lower correlation between the abaxial and adaxial trichome traits. These differential responses among leaf faces correlated more to site differences, i.e. CSD individuals had lower values than SEF individuals, irrespective of the species. This opens new ways to investigate the effects of different environmental factors in trichome size and arrangement and further, controlled studies may be relevant to pick out the differential effects of abiotic conditions such as water source, light and temperature.

We refer to Laube & Zotz (2003), who suggested that water is the limiting factor for the development of vascular epiphytes, from which we may interpret the response of the plants as related to the different strategies that are followed in the field for the acquisition of water versus what happened under the conditions of the common garden. For example, the convergence in characters in the new leaves of the different populations of *T. dasyliriifolia* may suggest that this species modified its water acquisition strategy. The clear separation between the preexisting leaves suggests that the plants in the CSD have one strategy, probably using condensation, mist or dew as their source of water, while plants in the SEF will depend upon precipitation, i.e. rainfall. In the common garden, constant irrigation eliminated seasonal variability in sources and availability of water, unifying the strategy for acquiring water for all five species and regardless of original vegetation type. In this sense, Chávez-Sahagún *et al.* (2020) showed that dew is not a relevant source of water for species such as T. yucatana, particularly in the DF during the dry season, which explains the convergence of the morphological response observed in the new leaves of plants of this species towards maximizing the use of water from the irrigation to which they were subjected in our experiment.

We can, therefore, assume that water availability had a greater effect than light on the morphological response of the five epiphytic bromeliad species included in this study. Chávez-Sahagun *et al.* (2020) proposed that the size of the central disc of the trichome is what determines the reflectance and, therefore, the role of trichomes in photoprotection, while the trichome density is directly related to the acquisition of water. Although in our case we found few statistical differences between preexisting and new leaves (Table 2), there is a clear trend towards an increase in the trichome density in the new leaves, particularly on the adaxial side, while there is no such trend in modifying the size of the trichome shield, supporting the idea that the elimination of seasonality in water availability was the key factor driving the plasticity values of our plants. In fact, trichome size and density were the main variables that guided the dispersion between preexisting and new leaves in the NMDS, more than trichome central disc size (Table 7). A higher trichome density on the adaxial surface in humid sites would allow plants to absorb greater amounts of water and nutrients from the environment, compared to the driest places where trichomes could play a greater role in photoprotection (Benzing, 2000; Pierce, 2007; Cach-Pérez *et al.,* 2016).

Finally, the above-mentioned observations are reflected in the elongation of the new leaves (Fig. 4). In most cases, plants from the SEF (the most humid of the four vegetation types) are the ones with the greatest growth in three of the four species collected there, compared to their other collection sites (Fig. 4); these individuals were used to high water availability. Of the four species mentioned above, only *T. balbisiana* and *T. brachycaulos* showed statistical differences in growth (elongation), while *T. dasyliriifolia* and *T. recurvata showed* no change in growth in response upon transferal to the common garden. The first two show a divergence, or practically no morphological response to the change in the environment (Fig. 6), which suggests that they may need more time to acclimatize to the new conditions, compared to the other two that present a convergence and / or faster response in the modification of stomatal and trichome density and morphology, which allows them to maximize the use of resources (in this case water), and, therefore, continue to grow in a similar way, regardless of their place of origin.

Although previous studies report that mist and dew could be the main source of water for arid zone bromeliads (González *et al.,* 2011; Pinto, Barría & Marquet, 2006), as could be considered the SM, CSD and, to a lesser extent, DF, our study shows that these plants can modify their morphology to maximize the use of water from other sources, in this case the rain or irrigation to which they were subjected. Likewise, we found differences between the preexisting leaves of the plants collected in our experiment, with respect to previous reports in the same study sites, which supports the hypothesis that certain characteristics of epiphytic bromeliads can be an excellent indicators of climate change (Cach-Pérez, Andrade & Reyes-García, 2014; Cach-Pérez *et al.,* 2018).

CONCLUSIONS

Although plasticity indices, as expressed in terms of the density and morphology of stomata and trichomes, were low, the present study shows how five species of epiphytic bromeliads from four structurally and climatically contrasting vegetation types respond morphologically to a change in the environment in which they grow. Variation in trichome characteristics could indicate a response associated with a change in the strategy of water acquisition in plants from dry sites to take advantage of the availability of water in the common garden. However, a more drastic degree of modification in environmental conditions seems to be necessary to trigger a clearer response for these variables, in these plants. We observed that our plants were already undergoing changes, as shown by differences in the characteristics of the preexisting leaves in this study, with previous reports for the same species and collection sites; those changes suggest climate change occurring in the Yucatán Peninsula, which may have diluted the response to the changes imposed in our study. Likewise, it is necessary to carry out an evaluation of the physiological response of plants transferred from dissimilar environments to converge in the same environment, to have a more complete information of other compensatory mechanisms which may occur.

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REFERENCES

Adams WW, Martin CE. 1986. Morphological changes accompanying the transition from juvenile (atmospheric) to adult (tank) forms in the Mexican epiphyte *Tillandsia deppeana* (Bromeliaceae). *American Journal of Botany* 73: 1207–1214.

Bautista F, Palacio G, Páez-Bistraín R, Carmona-Jiménez E, Delgado-Carranza C, Cantarell W, Tello H. 2012. Regionalización edáfica del territorio de México. In: Krasilnikov P, Jiménez FJ, Reyna T, García, NE, eds. *Geografía de suelos de México*. Mexico City: Universidad Nacional Autónoma de México, 17–56.

Benz BW, Martin CE. 2006. Foliar trichomes, boundary layers, and gas exchange in 12 species of epiphytic *Tillandsia* (Bromeliaceae). *Journal of Plant Physiology* 163: 648–656.

Benzing DH, Seemann J, Renfrow A. 1978. The foliar epidermis in *Tillandsioideae* (Bromeliaceae) and its role in habitat selection. *American Journal of Botany* 65: 359–365.

Benzing DH. 1990. Vascular epiphytes: general biology and related biota. New York: Cambridge University Press.

Benzing DH. 2000. Bromeliaceae: profile of an adaptative radiation. New York: Cambridge University Press.

Cach-Pérez MJ, Andrade JL, Chilpa-Galván N, Tamayo-Chim M, Orellana R, Reyes-García C. 2013. Climatic and structural factors influencing epiphytic bromeliad community assemblage along a gradient of water-limited environments in the Yucatan Peninsula, Mexico. *Tropical Conservation Science* 6: 283–302.

Cach-Pérez MJ, Andrade JL, Reyes-García C. 2014. Susceptibility of epiphytic bromeliads to climate change. *Botanical Sciences* 92: 157–168.

Cach-Pérez MJ, Andrade JL, Cetzal-Ix W, Reyes-García C. 2016. Environmental influence on the inter- and intraspecific variation in the density and morphology of stomata and trichomes of epiphytic bromeliads of the Yucatan Peninsula. *Botanical Journal of the Linnean Society* 181: 441–458.

Cach-Pérez MJ, Andrade JL, Reyes-García C. 2018. Morphophysiological plasticity in epiphytic bromeliads across a precipitation gradient in the Yucatan Peninsula, Mexico. *Tropical Conservation Science* 11: 1–10.

Cavallero L, López D, Barberis IM. 2009. Morphological variation of Aechmea *distichantha* (Bromeliaceae) in a Chaco Forest: Habitat and size-related effects. *Plant Biology* 11: 379–91.

Chávez-Sahagún E, Andrade JL, Zotz G, Reyes-García C. 2019. Dew can prolong photosynthesis and water status during drought in some epiphytic bromeliads from a seasonally dry tropical forest. *Tropical Conservation Science* 12: 1–11.

Chávez Sahagún E. 2020. *Balance de Energía y Relaciones Hídricas en bromeliáceas epífitas de la Península de Yucatán*. Un published D. Phil. Thesis, Centro de Investigación Científica de Yucatán. México.

Flores Guido JS, Espejel Carvajal I. 1994. Tipos de vegetación de la Península de Yucatán. Mérida, Yucatán, México: Universidad Autónoma de Yucatán.

González AL, Fariña JM, Pinto R, Pérez C, Weathers KC, Armesto JJ, Marquet PA. 2011. Bromeliad growth and stoichiometry: Responses to atmospheric nutrient supply in fog-dependent ecosystems of the hyper-arid Atacama Desert, Chile. *Oecologia* 167: 835–845.

Herppich WB, Martin CE, Tötzke C, Manke I, Kardjilov N. 2019. External water transport is more important than vascular transport in the extreme atmospheric epiphyte *Tillandsia usneoides* (Spanish moss). *Plant, Cell & Environment* 42: 1645–1656.

Kress WJ. 1989. The systematic distribution of vascular epiphytes. In: Lüttge U. ed. *Vascular Plants as Epiphytes*. Berlin: Springer, 234–261.

Laube S, Zotz G. 2003. Which abiotic factors limit vegetative growth in a vascular epiphyte?. *Functional Ecology* 17: 598–604.

Males, J. 2016. Think tank: water relations of the Bromeliaceae in their evolutionary context. *Botanical Journal of the Linnean Society* 181: 415–440.

North GB, Marvin GB, Kyle F, Maharaj FDR, Phillips, CA, Woodside, WT. 2016. A tale of two plasticities: Leaf hydraulic conductances and related traits diverge for two tropical epiphytes from contrasting light environments. *Plant, Cell & Environment* 39: 1408–19.

Pierce S, Maxwell K, Griffiths H, Winter K. 2001. Hydrophobic trichome layers and epicuticular wax powders in Bromeliaceae. *American Journal of Botany* 88: 1371–89.

Pierce S. 2007. The jeweled armor of *Tillandsia* multifaceted or elongated trichomes provide photoprotection. *Aliso* 23: 44–52.

Pittendrigh CS. 1948. The bromeliad-*Anopheles*-malaria complex in Trinidad. I The Bromeliad Flora. *Evolution* 2: 58–89.

Pinto R, Barría I, Marquet PA. 2006. Geographical distribution of *Tillandsia* in the Atacama Desert, northern Chile. *Journal of Arid Environments* 65: 543–552.

Rasband WS. 2018. ImageJ. National Institutes for Health, U.S.A. Bethesda, Maryland, U.S.A. https://imagej.nih.gov/ij/

Reyes-García C, Mejia-Chang, Griffiths H. 2012. High but not dry: diverse epiphytic bromeliad adaptations to exposure within a seasonally dry tropical forest community. *New Phytologist* 193: 745–54.

Reyes-García C, Griffiths H. 2009. Ecophysiological studies of perennials of the bromeliaceae family on a dry forest: strategies for survival. In: De la Barrera E, Smith WK, eds. *Perspectives in biophysical plant ecophysiology: a tribute to Park S. Nobel*. Mexico City: Universidad Nacional Autónoma de México, 121–151.

Reyes-García CA, Griffiths H, Rincón E, Huante P. 2008. Niche differentiation in tank and atmospheric epiphytic bromeliads of a seasonally dry forest. *Biotropica* 40: 168–175.

Rosado-Calderón AT, Tamayo-Chim M, de la Barrera E, Ramírez-Morillo IM, Andrade JL, Briones O, Reyes-García C. 2020. High resilience to extreme climatic changes in the CAM epiphyte *Tillandsia utriculata* L. (Bromeliaceae). *Physiologia Plantarum* 168: 547–562.

R Core Team. 2019. R: A Language and Environment for Statistical Computing. https://doi.org/10.1038/sj.hdy.6800737

Stefano M, Papini A, Brighigna L. 2008. A new quantitative classification of ecological types in the bromeliad genus *Tillandsia* (Bromeliaceae) based on trichomes. *Revista de Biología Tropical* 56: 191–203.

Valladares F, Sanchez-Gomez D, Zavala MA. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. *Journal of Ecology* 94: 1103–1116.

Zotz G, Bader MY. 2009. Epiphytic plants in a changing world-global: Change effects on vascular and non-vascular epiphytes. In: Lüttge U, Beyschlag W, Büdel B, Francis D, eds. *Progress in Botany*. Berlin: Springer, 147–170.

Zotz G. 2016. *Plants on plants - The biology of vascular epiphytes*. Switzerland: Springer.

CAPITULO III

Conclusiones generales

El ambiente en el que las plantas se aclimataron tuvo poco efecto en la densidad y morfología de estomas y tricomas foliares; se pudo observar que, si bien hubo tendencias entre especies hacia una misma respuesta en los caracteres medidos, ésta depende de la especie. A pesar de que las diferencias microclimáticas entre el jardín común y los sitios de colecta no fue de la magnitud que se esperaba, el cambio fue suficiente para que especies como *T. yucatana* presentaran baja densidad estomática en la superficie abaxial de sus hojas nuevas respecto a las hojas preexistentes, particularmente en las plantas procedentes de los sitios más secos; también fue el caso de *T. dasyliriifolia* procedente de sitios húmedos como la selva alta sub-perennifolia que registró un aumento de la densidad de tricomas en la superficie adaxial de las hojas nuevas en comparación con las hojas preexistentes.

Una de estas diferencias microclimáticas entre el jardín común y los sitios de colecta, fue la disponibilidad de agua, la cual fue mayor en el primer caso debido al riego y la lluvia local captada por la hojas a través de los tricomas, lo que en su caso favoreció el crecimiento de hojas nuevas; como fue el caso de las plantas procedentes de los sitios más secos como el matorral de duna costera y el más húmedo como la selva alta sub-perennifolia, ya individuos de *T. balbisiana* y *T. brachycaulos* procedentes de estos tipos de vegetación, mostraron una mayor elongación de las hojas nuevas en comparación con las preexistentes. Esta respuesta sugiere una estrategia para el aprovechamiento de agua en el nuevo sitio de aclimatación para su supervivencia.

Esta variación microclimática también tuvo efectos sobre el tamaño de los tricomas en las hojas de *T. balbisiana* y *T. yucatana* procedentes de los sitios más secos, los cuales mostraron tricomas más grandes en la superficie adaxial de las hojas nuevas en comparación con las hojas preexistentes, mientras que en la superficie abaxial el disco central de los tricomas es más pequeño.

Dicha respuesta, para la superficie abaxial podrían deberse a una interacción con los estomas, es decir, un mayor tamaño del tricoma y una disminución del área de los estomas y poros estomáticos, puede resultar en una disminución de la transpiración. En el caso superficie adaxial, la respuesta registrada en las plantas sugiere una estrategia orientada a reflejar luz, o en su caso conducir el agua hacia la base de las hojas, ya que el agua siempre estuvo disponible para las plantas a través del riego.

A pesar de no encontrarse diferencias significativas en la densidad y morfología de estomas y tricomas entre algunas especies, *T. dasyliriifolia*, *T. recurvata* y *T. yucatana* registraron mayores cambios entre las hojas nuevas y las preexistentes, demostrando que son especies con mayor capacidad de respuesta a los cambios en el ambiente, particularmente en lo relacionado con las estrategias de fotoprotección y aprovechamiento de agua. Esto sugiere que pequeños cambios en el ambiente, pueden tener un efecto sobre la morfología de estas plantas, mientras que individuos de especies como *T. brachycaulos*, podría tener mayor grado de tolerancia a las fluctuaciones ambientales antes de manifestar una modificación morfológica en sus estructuras foliares.

En cuanto a la plasticidad, contrario a lo que se esperaba, fue baja en las cinco especies de bromeliáceas epífitas, procedentes del matorral de duna costera, manglar chaparro, selva baja caducifolia y selva alta sub-perennifolia, probablemente asociado a la poca diferencia ambiental entre el jardín común y la mayoría de los sitios de colecta, lo que se reflejó únicamente en caracteres asociados a la captación de agua como ya se ha mencionado.

Probablemente antes de que ocurra una modificación morfológica, las plantas ajustan en primera instancia sus procesos fisiológicos en respuesta a variaciones ambientales poco severas. Por lo tanto, se sugiere llevar a cabo mayor trabajo experimental bajo condiciones de jardín común en donde se evalúen la respuesta fisiológica de las plantas, con estos datos se podría complementar los datos obtenidos en esta tesis y permitirá tener una perspectiva más amplia sobre la respuesta de las bromeliáceas epífitas ante la variación climática, así como de la posible influencia de componentes genéticos asociados a dicha respuesta (o la falta de esta) en estas plantas.

Literatura citada

Andrade JL, De la Barrera E, Reyes-García C, Ricalde MF, Vargas-Soto G, Cervera YJC. 2007. EL Metabolismo Ácido de las Crasuláceas: Diversidad, fisiología ambiental y productividad. Bol Soc Bot Méx. 81:37-50.

Benzing DH, Renfrow A. 1971. The significance of photosynthetic efficiency to habitat preference and phylogeny among tillandsioid bromeliads. Bot. gaz. 132:19-30.

Benzing DH. 1976. Bromeliad trichomes: structure, function and ecological significance. Selbyana. 1:330-348.

Benzing DH. 1990. Vascular epiphytes: general biology and related biota. New York Port Chester: Cambridge University Press.

Benzing DH. 2000. Bromeliaceae: profile of an adaptive radiation. New York: Cambridge University Press.

Benz BW, Martin CE. 2006. Erratum to “Foliar trichomes, boundary layers, and gas exchange in 12 species of epiphytic *Tillandsia* (Bromeliaceae). J Plant Physiol. 163:648-656.

Brighigna L, Ravanelli M, Minelli A, Ercoli L. 1997. The use of an epiphyte (*Tillandsia caput-medusae morren*) as bioindicator of air pollution in Costa Rica. Sci. Total Environ. 198:175-180.

Cach-Pérez MJ. 2008. Influencia ambiental sobre la fisiología y anatomía foliar de *Tillandsia heterophylla,* bromelia endémica de México. Tesis de Maestría en Ciencias. División de Posgrado, Instituto de Ecología, A. C. Xalapa, Veracruz, México.

Cach-Pérez MJ, Andrade JL, Cetzal-Ix W, Reyes-García C. 2016. Environmental influence on the inter- and intraspecific variation in the density and morphology of stomata and trichomes of epiphytic bromeliads of the Yucatan Peninsula. Bot J Linn Soc. 181:441-458.

Cach-Pérez MJ, Andrade JL, Reyes-García C. 2018. Morphophysiological plasticity in epiphytic bromeliads across a precipitation gradient in the Yucatan Peninsula, Mexico. Trop Conserv Sci. 11:1-10.

Chávez-Sahagún E, Andrade JL, Zotz G, Reyes-García C. 2019 Dew can prolong photosynthesis and water status during drought in some epiphytic bromeliads from a seasonally dry tropical forest. Trop Conserv Sci. 12:1-11.

Chávez Sahagún E. 2020. Balance de energía y relaciones hídricas en bromeliáceas epífitas de la Península de Yucatán. Tesis Doctoral, Centro De Investigación Científica de Yucatán. México.

Ciha AJ, Brun WA. 1975. Stomatal size and frequency in soybeans. Crop Science. 15:309-313.

Crayn DM, Winter K, Smith JA. 2004. Multiple origins of crassulacean acid metabolism and the epiphytic habit in the neotropical family Bromeliaceae. Proc Natl Acad Sci EE. UU. 101:3703-3708.

De la Rosa-Manzano E, Andrade JL, Zotz G, Reyes-García C. 2014. Epiphytic orchids in tropical dry forests of Yucatan, Mexico-species occurrence, abundance and correlations with host tree characteristics and environmental conditions. Flora. 209:100-109.

Freschi L, Rodrigues MA, Tiné MAS, Mercier H. 2010. Correlation between citric acid and nitrate metabolisms during CAM cycle in the atmospheric bromeliad *Tillandsia pohliana*. J Plant Physiol. 167:1577-1583.

Gentry AH, Dodson CH. 1987. Diversity and Biogeography of neotropical vascular epiphytes. Ann. Missouri Bot. Gard. 74:205-233.

Gotsch SG, Nadkarni N, Amici A. 2016. The functional roles of epiphytes and arboreal soils in tropical montane cloud forests. J Trop Ecol. 32:455-468.

Granados-Sánchez D, López-Ríos GF, Hernández-García MÁ, Sánchez-González A. 2004. Ecology of epiphyte plants. Rev. Chapingo, Ser. Cienc for Ambient. 9:101-111.

Griffiths H. 1992. Carbon isotope discrimination and the integration of carbon assimilation pathways in terrestrial CAM plants. Plant, Cell and Environment 15:1051-1062.

Hetherington AM, Woodward FI. 2003. The role of stomata in sensing and driving environmental change. Nature. 424:901-908.

Kathleen CS, Craig EM. 1996. Effects of drought stress on CO2 exchange and water relations in the CAM epiphyte *Tillandsia utriculata* (Bromeliaceae). J Plant Physiol. 149:721-728.

Kress WJ. 1986. The systematic distribution of vascular epiphytes: an update. Selbyana. 9:2-22.

Luther HE. 2014. An Alphabetical list of bromeliad binomials. Saratosta, FL: Marie Selby Botanical Garden and Bromeliad Society International.

Murren C, Auld J, Callahan H. 2015. Constraints on the evolution of phenotypic plasticity: limits and costs of phenotype and plasticity. Heredity. 115:293-301.

Nadkarni NM. 1983. Epiphyte biomass and nutrient capital of a neotropical elfin forest. Biotropica. 16:249-256

Ohrui T, Nobira H, Sakata Y, Taji T, Yamamoto C, Nishida K, Tanaka S. 2007. Foliar trichome-and aquaporin-aided water uptake in a drought-resistant epiphyte *Tillandsia ionantha* Planchon. Planta. 227:47-56.

Pierce S, Maxwell K, Griffiths H, Winter K. 2001. Hydrophobic trichome layers and epicuticular wax powders in Bromeliaceae. Am J Bot. 88:1371-1389.

Pierce S. 2007. The jeweled armor of *Tillandsia*-multifaceted or elongated trichomes provide photoprotection. Aliso. 23:44-52.

Pittendrigh CS. 1948. The bromeliad-*Anopheles*-malaria complex in Trinidad, the bromeliad flora. Evolution. 2:58-89.

Reyes-García AC, Griffiths H, Rincón E, Huante P. 2008. Niche differentiation in tank and atmospheric epiphytic bromeliads of a seasonally dry Forest. Biotropica. 40:168-175.

Reyes-García C, Mejia-Chang M, Griffiths H. 2012. High but not dry: diverse epiphytic bromeliad adaptations to exposure within a seasonally dry tropical forest community. New Phytol. 193:745-754.

Rosado-Calderón AT, Tamayo-Chim M, de la Barrera E, Ramírez-Morillo IM, Andrade JL, Briones O, Reyes-García C. 2020. High resilience to extreme climatic changes in the CAM epiphyte *Tillandsia utriculata* L. (Bromeliaceae). Physiol. Plant. 168: 547-562.

Salazar-Tortosa D, Castro J, Villar-Salvador P, Viñegla B, Matías L, Michelsen A, de Casas R, Querejeta JI. 2018. The “isohydric trap”: A proposed feedback between water shortage, stomatal regulation, and nutrient acquisition drives differential growth and survival of European pines under climatic dryness. Glob Chang Biol. 24:4069-4083.

Smith JAC. 1989. Epiphytic bromeliads. In Vascular Plants as Epiphytes: Evolution and Ecophysiology ed. U. Lüttge pp. 109-138. Springer, Berlín.

Sperry JS, Wang Y, Wolfe BT, Mackay DS, Anderegg WRL, McDowell NG, Pockman WT. 2016. Pragmatic hydraulic theory predicts stomatal responses to climatic water deficits. New Phytologist. 212:577-589.

Stancato GC, Mazzafera P, Buckeridge MS. 2001. Effect of drought period on the mobilisation of non-structural carbohydrates, photosynthetic efficiency and water status in an epiphytic orchid. Plant Physiology and Biochemistry. 39:1009-1016.

Turcotte MM, Levine JM. 2016. Phenotypic Plasticity and Species Coexistence. Trends Ecol Evol. 31:803-813.

Valladares F, Matesanz S, Guilhaumon F, Araújo MB, Balaguer L, Benito-Garzón M, Cornwell W, Gianoli E, van Kleunen M, Naya DE. 2014. The effects of phenotypic plasticity and local adaptation on forecasts of species range shifts under climate change. Ecol Lett. 17:1351-1364.

Valladares F, Sanchez-Gomez D, Zavala MA. 2006. Quantitative estimation of phenotypic plasticity: bridging the gap between the evolutionary concept and its ecological applications. J Ecol. 94:1103-1116.

Winkler U, Zotz G. 2009. Highly efficient uptake of phosphorus in epiphytic bromeliads. Ann Bot. 103:477-484.

Winkler U, Zotz G. 2010. “And then there were three”: Highly efficient uptake of potassium by foliar trichomes of epiphytic bromeliads. Ann Bot. 106:421-427.

Willmer CM, M Fricket. 1996. Stomata. 2nd. ed. London, UK. Chapman & Hall. 375 p.

Zotz, G. 2013. The systematic distribution of vascular epiphytes-a critical update. Bot J Linn Soc. 171:453-481.