Leaf morphospace in *Euphorbia tithymaloides* (Euphorbiaceae) was likely shaped by evolutionary contingencies rather than climate

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Abstract

Background and aims – Understanding whether variation in plant attributes is primarily driven by selection or historical contingencies is a main goal in evolutionary biology. We characterize leaf diversity in *Euphorbia tithymaloides* and identify patterns related to taxonomy, geography, biogeography, and climate that provide insights on the role of ecological and evolutionary forces in shaping its leaf diversity.

Material and methods – We constructed a leaf morphospace using linear morphometric measurements derived from images (leaf maximum length and width, area, and perimeter), and calculated indexes that reflect aspects of leaf shape (leaf aspect ratio, area-perimeter ratio, obovate index, and circularity). Climatic data were extracted from WorldClim layers based on occurrence data. We visualized leaf and climate spaces with principal components analyses and used Kruskal-Wallis tests, linear models, and Mantel tests to test predictors of leaf variation (taxonomy, geography, climate).

Key results – We document differences in the foliar morphospace occupied by subspecies of *Euphorbia tithymaloides*, and a substantial overlap in the climatic space they occupy, suggesting that foliar differences among subspecies are not likely driven by climate. Foliar morphology can be used as a proxy for subspecies in *E. tithymaloides*, as taxonomy explains a large proportion of variation in leaf morphology (10–60%). Geography and climate explain a small proportion of foliar variation not explained by subspecies (~3% and 5%, respectively). Temperature, precipitation, and seasonality are the climate variables with most explicative power.

Conclusion – Leaf diversity in *E. tithymaloides* is not driven by climate, instead, it is likely the result of evolutionary contingencies faced by this species throughout its historical range expansion across the Caribbean Basin. This study shows that historical contingencies in addition to selection acting on ecological processes can shape foliar diversity and expand a lineage's potential to explore morphological and climatic spaces.

INTRODUCTION

Understanding whether plant attributes and their variation are driven primarily by natural selection or evolutionary contingency (i.e. idiosyncratic events experienced by lineages) is one of the main goals of evolutionary ecology and, more broadly, of biology (Blount et al. 2018). Approaches to this question are often macroevolutionary and aim at identifying patterns across a broad phylogenetic spectrum. However, much of evolution and divergence happens at much smaller scales, in processes that are commensurable with the population-species interphase. Investigations at these scales tend to be narrower, usually focused on young lineages and one trait at a time, and often unable to predict the long-term

evolutionary consequences of outcomes at shallower time scales.

Systems that bridge these scales, where evolutionary, ecological, or morphological divergence is happening, offer great opportunities to explore questions related to potential drivers of divergence. Ring-species, dubbed by Ernst Mayr as "perfect demonstrations of speciation" are an example of systems in which evolutionary divergence is an active process (Mayr 1942; Pereira and Wake 2015). These systems consist of a circular chain of populations achieved by historical range expansion along two fronts that expand surrounding unsuitable habitat, such that when both fronts reunite, their ends have already evolved barriers to free gene flow as would be expected of incipient species (Irwin and Irwin 2002).

Leaves are the main photosynthetic organs of plants and exhibit a great deal of diversity in structure, morphology, and size. Leaf morphology plays an important role in a plant's ability to survive and compete in a given environment, and is therefore a key factor in plant performance and fitness (Givnish 1987; Ferris 2019). Thus, it is to be expected that changes that translate into any performance or fitness advantage would be passed on and eventually become fixed. Despite their paramount importance, both from a diversity and ecosystem function standpoint, our understanding of factors shaping leaf diversity is quite limited.

Many climatic and environmental factors have been associated with leaf shape in angiosperms, including water availability, amount of light, and temperature (Dilcher 1974; Givnish 1987; Schuepp 1993; Nicotra et al. 2011). No universal pattern has been identified linking leaf traits and environment, yet there is ample evidence that supports the functional significance of leaf shape and thus its ability to affect fitness and have adaptive value (Vogel 1968; Givnish 1979, 1987; Nicotra et al. 2011; Leigh et al. 2017). For example, it has been shown that in tropical environments, the morphology and structure of the leaf apex can play a fundamental role in draining water off the leaves, whose accumulation would otherwise interfere with gas exchange and photosynthetic activity, and even potentially cause structural damage (Wang et al. 2020). A tight relationship between foliar morphology and environment can also drive variation in leaf features among populations, individuals within a population, and even plasticity at the individual level, such that leaves of a single individual that are exposed to contrasting environments will differ in traits, including shape (Vogel 1968; Schuepp 1993; Royer et al. 2009).

The leaf boundary layer – the air that is relatively stationary resulting from friction due to being in direct contact with the leaf surface (Schuepp 1993) – has a direct influence on the exchange rates of gas, water, and heat between leaves and their environment, all of which influence photosynthetic activity with implications for plant performance (Vogel 1968; Givnish 1979; Schuepp 1993). The characteristics of the leaf boundary layer are, to a certain degree, determined by the shape of the leaf blade (Vogel 1968; Leigh et al. 2017). For example, leaves with lobed or narrow blades tend to have thinner boundary layers, which allow a more efficient thermal regulation that could be of critical importance in environments with high temperatures or to face sudden temperature changes (Vogel 1968; Schuepp 1993). It has also been shown that thinner boundary layers are associated with smaller leaf areas (Givnish 1987; Schuepp 1993; Sack and Frole 2006). On the other hand, thicker boundary layers can protect against freezing during drastic and sudden drops in temperature (Ferris 2019). Classic studies across taxa have put forward experimental evidence that is consistent with deeply lobed blades and high density of marginal teeth facilitating leaf temperature regulation (Vogel 1968; Schuepp 1993).

Correlations between leaf shape and environment have been documented in a diversity of angiosperm lineages, including *Viburnum* (Adoxaceae), *Pelargonium* (Geraniaceae), and *Musa* (Musaceae) (Vogel 1968; Schuepp 1993; Royer et al. 2009; Schmerler et al. 2012; Jones et al. 2013; Mitchell et al. 2015; Spriggs et al. 2018). In Poaceae, species with narrower leaves are associated with more open and exposed environments (Gallaher et al. 2019). Leaves of two varieties of red maple (*Acer rubrum* L. var. *rubrum* and *A. rubrum* L. var. *trilobum*) cultivated in colder and drier environments were more dissected and more profusely toothed than those cultivated in warmer and more humid environments, independent of variety and provenance of cultivars (Royer et al. 2009). More broadly, the relationship between leaf morphology and environment has been the basis for using fossil traits to infer the climate in which fossil floras thrived and to perform paleoclimatic reconstructions (Bailey and Sinnott 1916; Dilcher 1974; Calvillo-Canadell and Cevallos-Ferriz 2005; Sack et al. 2012; Ayala-Robledo 2015).

Here, we evaluate whether climate or evolutionary contingence are likely drivers of leaf shape in *Euphorbia tithymaloides* L., a system that exhibits a wide variation of leaf morphology across its native range in the Caribbean (Fig. 1) and that has been shown to be experiencing ecological, morphological, and genetic divergence (Dressler 1957; Cacho and Baum 2012; Cacho et al. 2019). In this system, which is considered to be the best example of a ring-species in plants, evolutionary processes are not limited to divergence but also include convergence (Cacho et al. 2019). First, we characterize the leaf morphospace in *E. tithymaloides* using a linear morphometric approach and assess whether quantitative leaf shape variation alone can be used as a proxy for taxonomy (subspecies). Then, we ask if leaf shape variation in *E. tithymaloides* is likely to have been shaped by ecological (climatic) factors or is rather the result of contingencies encountered along the historical range expansion of the species.

MATERIAL AND METHODS

The system: *Euphorbia tithymaloides* L.

Plants of *Euphorbia tithymaloides* (Euphorbiaceae) are evergreen woody succulents that inhabit dry environments, from tropical deciduous forests to inland and coastal xeric scrublands and are often associated with calcareous soils. This is the most variable and widely distributed species of the Pedilanthus clade of *Euphorbia*, a mainly Mexican clade that stands out due to its markedly bilateral inflorescences that suggest hummingbird pollination in an otherwise insect-pollinated genus (Dressler 1957; Cacho et al. 2010). In contrast to the rest of the species in the Pedilanthus clade that exhibit rather narrow geographic ranges (Olson et al. 2005), the range of *E. tithymaloides* spans the whole Caribbean Basin, including Florida, México, Central America, the northern portion of South America, and practically all Caribbean islands (Dressler 1957; Olson et al. 2005; Cacho and Baum 2012).

Euphorbia tithymaloides exhibits a remarkable morphological diversity for a single species, which includes variation in leaf shape as well as in other foliar attributes such as leaf base and apex, the presence and morphology of a keel, and features related to venation and indumentum (Fig. 1). In an attempt to make sense of morphological diversity in *E. tithymaloides*, Dressler (1957) proposed eight subspecies in this system, seven of which are located in the Caribbean. Subspecies assignation is complicated because of character intergradation among subspecies, to the point that geography plays a fundamental role in

Dressler's proposal (i.e. subspecies *E. tithymaloides* subsp. *bahamensis* and *E. tithymaloides* subsp. *jamaicensis*; Supplementary file 1). That is, the provenance of an individual is a key element for taxonomic determination.

Studies examining the historical biogeography of *E. tithymaloides* based on morphological (Dressler 1957), molecular phylogenetics (Cacho and Baum 2012), and landscape genetic approaches (Cacho et al. 2019) all point to *E. tithymaloides* originating in Mesoamerica (in particular, an area between Mexico and Guatemala) and expanding its range towards the Caribbean along two geographic fronts (Fig. 2A). One of these fronts colonized the Greater Antilles and continued east towards the Caribbean Basin, and the other travelled south through Central America, then east through northern South America, and north towards the Lesser Antilles. The two fronts appear to reunite near the Virgin Islands, where they remain morphologically, ecologically, and genetically distinct (Cacho and Baum 2012; Cacho et al. 2019; Fig. 2B).

Molecular phylogenetic and landscape genetic approaches support *E. tithymaloides* subsp. *tithymaloides* as the most variable and geographically extended subspecies, with a continental geographic range that spans from Mexico through Venezuela. *Euphorbia tithymaloides* subsp. *jamaicensis*, *E. tithymaloides* subsp. *smallii*, *E. tithymaloides* subsp. *bahamensis*, *E. tithymaloides* subsp. *parasitica*, and *E. tithymaloides* subsp. *angustifolia* are supported as part of the Greater Antillean front, and *E. tithymaloides* subsp. *padifolia* as part of the Lesser Antillean one. Subspecies *angustifolia* and *padifolia* are the most recent subspecies, and the extremes of either
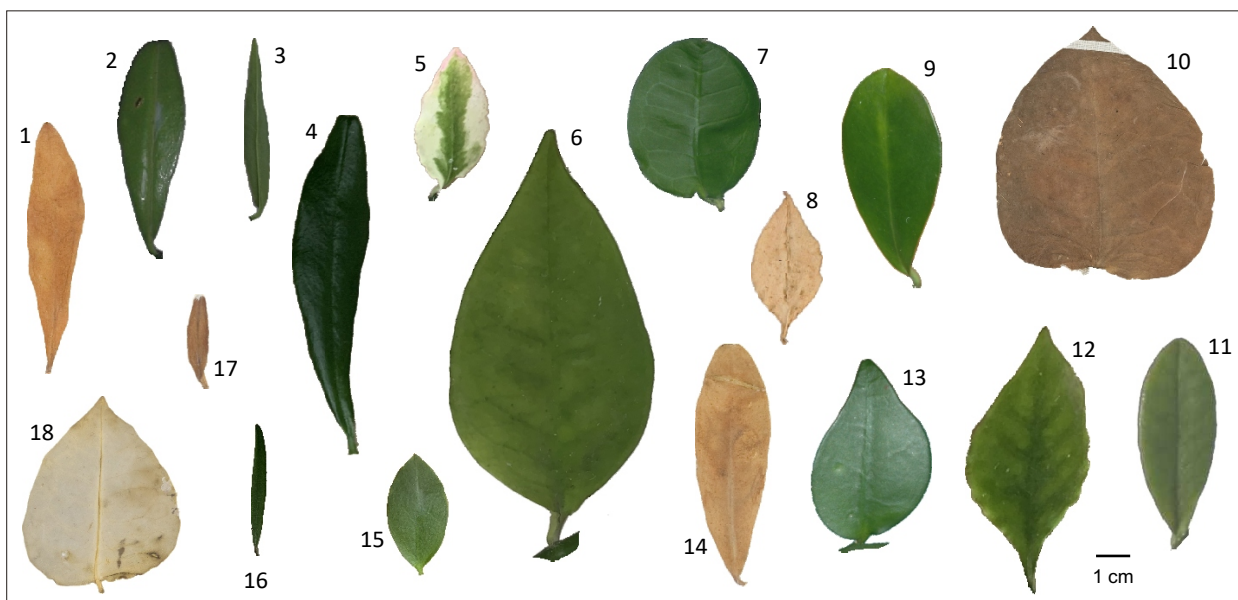

Figure 1. Leaf shape diversity in *Euphorbia tithymaloides*. Subspecies are as follows: *E. tithymaloides* subsp. *angustifolia* (2, 3, 4, 16), *E. tithymaloides* subsp. *bahamensis* (17), *E. tithymaloides* subsp. *jamaicensis* (1, 14), *E. tithymaloides* subsp. *padifolia* (9, 11), *E. tithymaloides* subsp. *parasitica* (10, 18), *E. tithymaloides* subsp. *smallii* (5, 8), *E. tithymaloides* subsp. *tithymaloides* (6, 7, 12, 13, 15). Image by Luis Emiliano Jacobo-Arteaga.
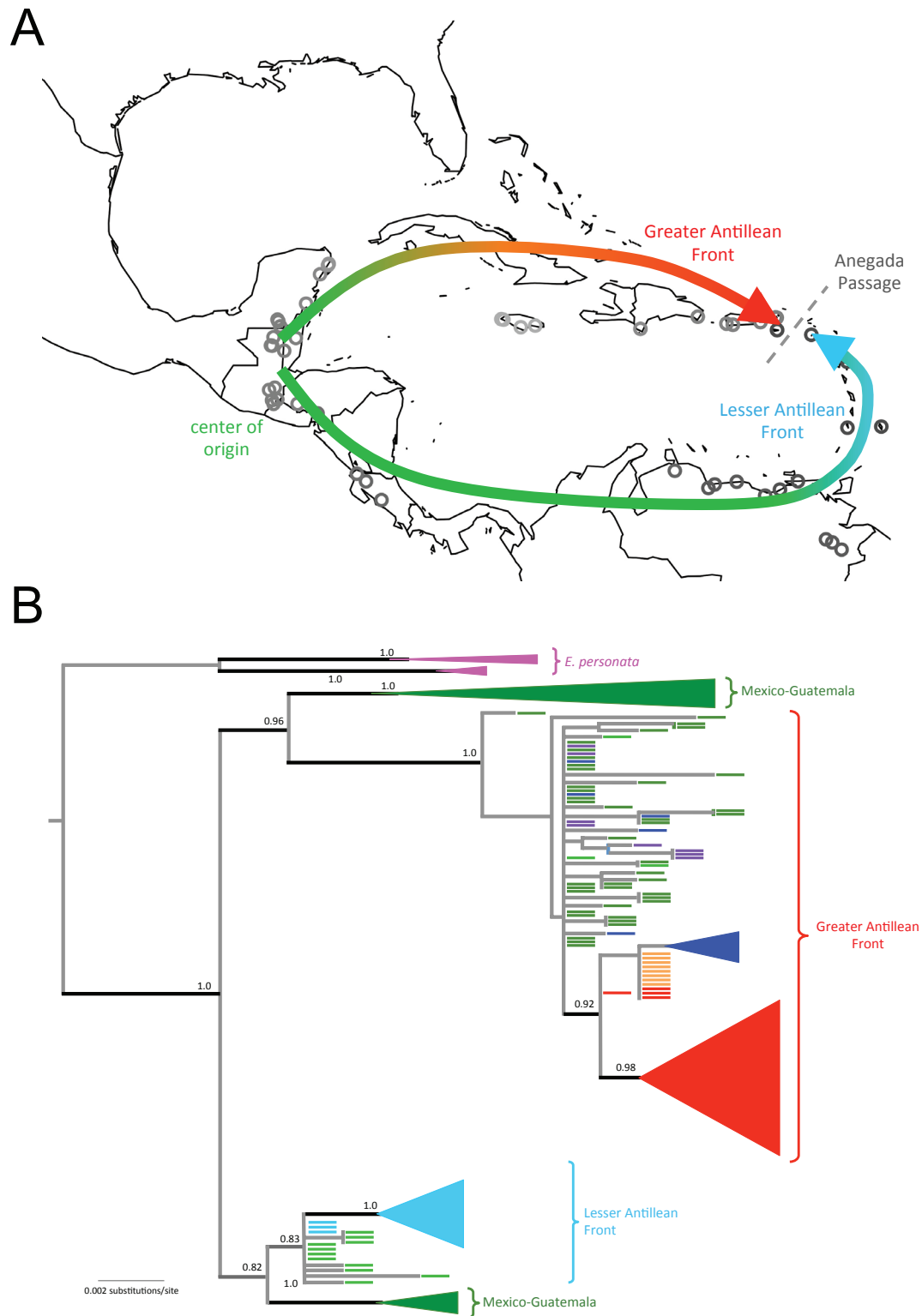
Figure 2. Historical biogeography of *Euphorbia tithymaloides*. **A**. This species expanded its geographic range from its area of origin in Mexico-Guatemala-Belize towards the Caribbean along two geographic fronts, one that extended through the Greater Antilles, and one that travelled south, then east, and then north through Central and South America, and the Lesser Antilles (drawn with information from Cacho et al. 2019). **B**. Representation of an allele phylogenetic tree of gene SGN-U342009 showing historic relationship between geographic fronts and subspecies. Colours: magenta, *Euphorbia personata* (sister species); green, *E. tithymaloides* subsp. *tithymaloides* (light green, Venezuelan accessions); purple, *E. tithymaloides* subsp. *parasitica*; navy, *E. tithymaloides* subsp. *jamaicensis*; yellow, *E. tithymaloides* subsp. *bahamensis*; red, *E. tithymaloides* subsp. *angustifolia*; cyan, *E. tithymaloides* subsp. *padifolia* (drawn with information from Cacho and Baum 2012).

biogeographic front, thus representing lineages with independent evolutionary trajectories (Fig. 2).

In *E. tithymaloides,* clear geographic patterns in floral morphology have been documented: the floral involucre has shortened from the centre of origin towards the Anegada Passage, and this has happened in parallel along both biogeographic fronts (Cacho et al. 2019). On the other hand, there is no evidence supporting geographic patterns in leaf morphology in relation to the Anegada Passage (Cacho et al. 2019). Dressler (1957) presents some data on leaf length/width ratio in two subspecies (i.e. *angustifolia* and *padifolia*), also in the context of discussing floral morphology, but he does not discuss the leaf data. Apart from these two instances, there is no study focusing on variation in leaf shape in *E. tithymaloides* throughout its range.

Leaf data and measurements

We assembled a collection of images of *E. tithymaloides* leaves (with scale) representing as wide a geographic range for the species as possible. We included our own images, taken during various field trips, as well as pictures we took from herbarium specimens (COL, HUA, MEXU) and images obtained from websites of individual herbaria (AAH, FLAS, FSU, GH, NY, P) or portals like the Global Biodiversity Information Facility (GBIF).

We excluded specimens without leaves, specimens with leaves that were damaged to the point of limiting our ability to measure them, specimens with locality data that would not allow georeferencing, and specimens whose native status was questionable. Subspecies assignation followed information on the specimen label, or when absent, we followed the subspecies key and descriptions by Dressler (1957).

We measured the following five leaf traits: blade maximum length (Lmax) and maximum width (Wmax), distance from the base to the point of maximum width (DbWmax), leaf area (A), and leaf perimeter (P), and calculated the following four metrics, which are described below: leaf aspect ratio, leaf area-perimeter ratio, obovate index, and circularity.

Leaf aspect ratio (Lmax / Wmax) – This is a robust metric that reflects the ratio between the two axes of an ellipse that is related to how round or elongated leaves are (Li et al. 2018). For leaves of similar values of Wmax, the leaf aspect ratio will be determined by Lmax, so that it will be larger for longer leaves.

Area-Perimeter ratio (A / P) – A metric that captures aspects related to leaf shape. A circular shape maximizes the area for a given perimeter. Given a constant perimeter, leaves with larger areas will have larger A/P values and be the most circular, and those with smaller areas will be less circular, either through elongation or the presence of lobing or dissection, and this metric will have smaller values.

Circularity (4π * (area / perimeter²)) – Another metric related to foliar shape, also based on area and perimeter.

This metric is more sensitive to lobing and captures aspects of the degree of how elongated leaves are (Li et al. 2018) and rather than being redundant with the metric AP, it can complement it in certain groups, as shown in *Vitis* (Chitwood et al. 2014).

Obovate index (DbWmax / Lmax) – This metric aims at capturing leaf shape along an ovate-obovate axis. Along this axis, for leaves that have the same values for Lmax, this index will be determined by DbWmax (the distance between the leaf base and the point of Wmax), so that ovate leaves will have smaller values and obovate leaves larger ones.

Whenever possible, we measured and calculated the metrics described above for as many as three leaves per individual. Averages per individual were calculated prior to subsequent analyses. Lmax, Wmax, and DbWmax were measured in Geogebra Classic v.5 (Supplementary file 2.1). Area and perimeter were measured in ImageJ v.2.0 (Schindelin et al. 2015), which required some image processing as illustrated in Supplementary file 2.2.

Geography and climate data

When we did not have geographic (latitude/longitude) information, we geo-referenced images based on information in the label of the specimens, using tools in Google Earth or Google Maps. Geo-referenced data were curated with QGIS v.3.16 (QGIS 2021) and geo-referencing errors (in cities, bodies of water, etc.) were corrected on a case by case basis.

We extracted climate data associated with curated occurrence points for 19 climatic variables and elevation from WorldClim v.2.0 (Fick and Hijmans 2017) at a scale of 30 s, which roughly corresponds to 1 km² at the equator.

Statistical analyses

To evaluate the extent to which leaf morphology alone predicts current taxonomy, as a first approximation we used a combination of Kruskal-Wallis and posthoc tests on leaf traits given that several variables did not conform to normality and homoscedasticity (per Shapiro and Levene tests, respectively). We then used linear models (of the type: morphology ~ subspecies) to estimate the proportion of variation in foliar morphology that is captured by subspecies assignation. To evaluate the correspondence between morphology and geography or climate, we used linear models and implemented Mantel tests using matrices of morphological, climatic, and geographic Euclidean distances. Variables were transformed as shown in Supplementary file 3.1 to improve the normality of the data, and we verified that the nature of leaves from where images were derived (fresh vs dry) would not introduce a systematic bias in our analyses (Supplementary file 4).

We used Principal Component Analyses (PCA) to account for collinearity among variables and reduce the dimensionality of our data. We implemented this

approach for both morphologic and climatic data (which is multivariate by definition). PCA was based on the correlation matrix (function princomp, cor = TRUE) to ensure data would be all at the same scale.

Because we found significant differences among subspecies and because subspecies are not randomly distributed across the landscape, to evaluate the effects of geography or climate as possible predictors of foliar morphology, we eliminated the subspecies effect by using the residuals of linear models by subspecies (syntax of model: variable ~ subspecies) as the response variable in all our analyses.

Patterns of geographic variation in foliar shape in *E. tithymaloides* across the Caribbean were evaluated using three approaches. First, we used linear models to assess variation in leaf shape (using morphological PCs) in relation to latitude and longitude. Then, based on the results and the loadings of the morphology PCs, we assessed latitude and longitude as potential predictors of specific variables reflecting leaf shape variation. For this, we focused on the variables with the highest loadings for every PC of morphology: A/P ratio and Wmax for PC 1, L/W ratio and Lmax for PC 2, and obovate index for PC 3. Finally, to evaluate if individuals that co-occur would share foliar morphology, driven by geographical proximity alone, we used Mantel tests (method = Pearson, permutations = 999), implementing the model $D_{morphologic} \sim D_{geographic}$.

Linear models to evaluate association between foliar morphology and climate focused on the first three PCs for both climate and leaf shape. As with geography, we used the results of these models to guide variable selection for subsequent models focusing on the variables with the highest loadings on relevant PCs (for both morphology and climate), which due to the nature of PCA have a lower probability of being collinear. We selected one variable per climatic PC, and two for every morphological PC (except for PC 3 which is mostly correlated with only one variable, see loadings below). The three climatic variables selected with this approach were: Mean annual temperature (BIO 1, PC 1), mean annual precipitation (BIO 2, PC 2), and temperature seasonality (BIO 4, PC 3), and the five morphological variables were the same as above (A/P ratio and Wmax for PC 1, L/W ratio and Lmax for PC 2, and obovate index for PC 3). To test the hypothesis that similarity in leaf morphology could be explained by the occupation of similar climatic envelopes (i.e. proximity in climatic space), we implemented Mantel tests as above. Climate data are known to be spatially autocorrelated (Portier et al. 2018), and this is true for our data (model: $D_{climatic} \sim D_{geographic}$; adj. R^2 = 0.494, p < 0.0001). To account for such autocorrelation, we implemented the following partial Mantel test: $D_{morphologic} \sim D_{climatic} + D_{geographic}$.

All analyses were implemented using base functions in R (R Core Team 2018) except for distance matrix calculations which were derived with functions of the R package vegan v.2.5-6 (Oksanen et al. 2018).
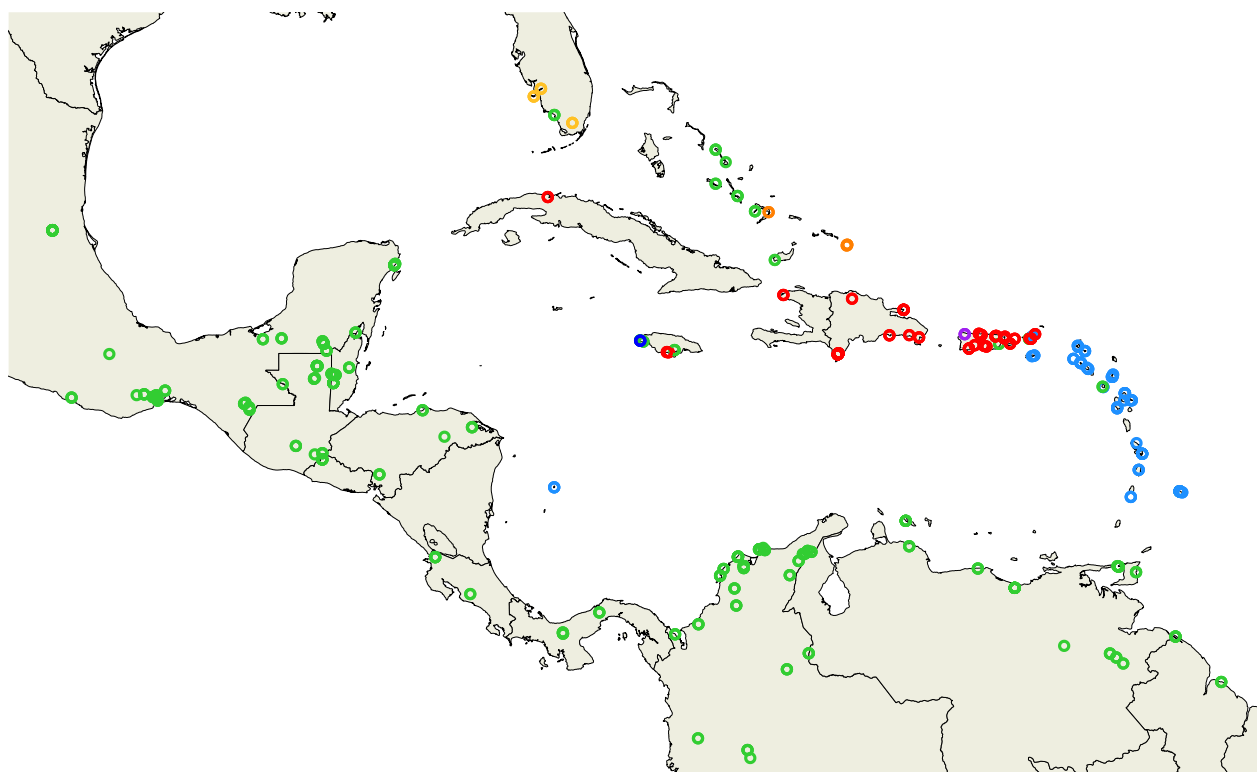

Figure 3. Map depicting the geographical placement of the 578 images used in this study. Colours correspond to subspecies assigned as follows: *E. tithymaloides* subsp. *angustifolia* (red), *E. tithymaloides* subsp. *bahamensis* (orange), *E. tithymaloides* subsp. *jamaicensis* (navy), *E. tithymaloides* subsp. *padifolia* (light blue), *E. tithymaloides* subsp. *parasitica* (purple), *E. tithymaloides* subsp. *smallii* (yellow), *E. tithymaloides* subsp. *tithymaloides* (green).

RESULTS

Leaf images and data

Our image database consisted of a total of 578 leaf images of *E. tithymaloides* specimens spanning across this species' range in the Caribbean (Fig. 3), including specimens from the following countries or territories:

- Greater Antilles region (161/578, ~28%): Bahamas, Cuba, Dominican Republic, Haiti, British Virgin Islands (Guana), US Virgin Islands (St Croix, St John, St Maarten), Jamaica, Puerto Rico, Turks and Caicos.
- Lesser Antilles region (108/578, ~19%): Antigua, Barbados, Becquia, Curaçao, Guadeloupe, Martinique, Montserrat, Saba, St Barthelemy, St Eustatius, St Kitts, St Lucia, Tortola, Trinidad.
- Mainland (309/578, ~53%): Belize, Colombia, Costa Rica, El Salvador, Guatemala, Guyana, Honduras, Mexico, Panama, United States, Venezuela.

From a taxonomic standpoint, all seven Caribbean subspecies described by Dressler (1957) were represented in our database. The three subspecies with the largest ranges and evolutionary significance, i.e. *angustifolia*, *padifolia*, and *tithymaloides*, also had a noticeable larger

representation in our dataset (with 107, 98, and 351 leaves measured from 41, 42, and 146 specimens respectively) than the other four subspecies whose ranges are more limited (number of images: subsp. *bahamensis*, 7; subsp. *jamaicensis*, 3; subsp. *parasitica*, 3; subsp. *smallii*, 9). Thus, these three subspecies are the focus of most of our analyses, especially those related to geography and climate. On average, the number of leaves per specimen measured for the three main subspecies was 2.55 for subsp. *angustifolia*, 2.39 for subsp. *padifolia*, and 2.4 for subsp. *tithymaloides*.

Euphorbia tithymaloides subsp. *tithymaloides* was the most variable of all subspecies, which would be expected given its significantly larger geographic range. However, for certain metrics (e.g. circularity and obovate index), subsp. *angustifolia* was the most variable (Supplementary file 3.2). In contrast, there is not one variable for which subsp. *padifolia* was noticeably the most variable, and it is rather common that it is the least variable subspecies in the system.

Leaf morphospace of Caribbean *Euphorbia tithymaloides*

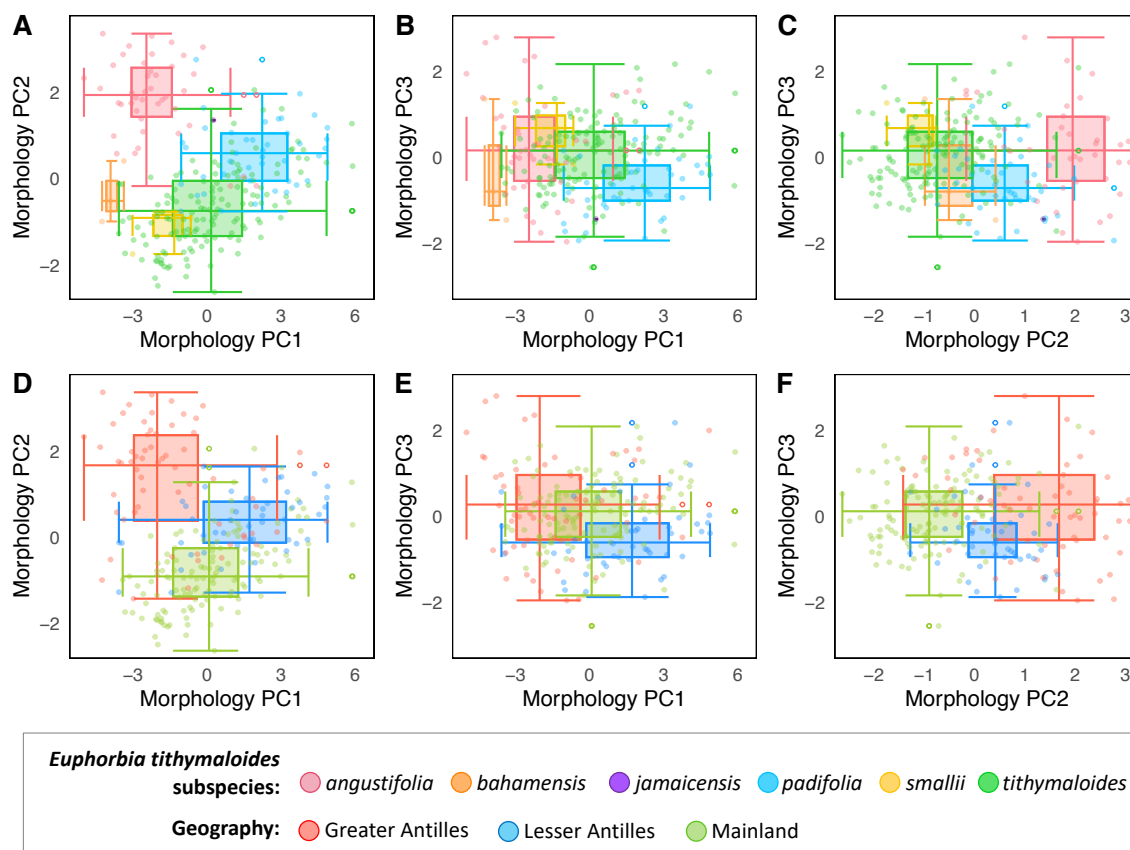Our PCAs on leaf form reveal that foliar variation in *E. tithymaloides* is well represented by three principal

Figure 4. Leaf morphospace of Caribbean *Euphorbia tithymaloides*. **A**–**C.** Highlighting the taxonomy by Dressler (1957). **D**–**F.** Highlighting the main geographical areas in this system (Greater Antilles, Lesser Antilles, and mainland).

components (PCs) that are related to how elongated and how obovate a leaf is, as well as leaf dimensions (Lmax and Wmax). The three first PCs capture 96.7% of variation in leaf morphology in *E. tithymaloides* (Supplementary file 5). Based on the loadings of the variables on the PCs (Supplementary file 5), PC 1 (which captures 64% of total variance) is mostly positively correlated with leaf shape as summarized by A/P ratio (also with Wmax, area, and perimeter). PC 2 (22% of total variance) represents how elongated a leaf is due to its positive correlation with leaf aspect ratio (L/W) and Lmax, and negatively with circularity. PC 3 (10.9% of total variance) represents how ovate is a leaf (inverse relationship with obovate index).

When visualizing the foliar morphospace of Caribbean *E. tithymaloides*, either with a taxonomic perspective (subspecies) or a geographic one (focused on the three main regions: Greater Antilles, Lesser Antilles, and Mainland), it is possible to appreciate a substantial correspondence between three main subspecies and the main geographic areas (Fig. 4). Individuals of subsp. *angustifolia* and those that occur in the Greater Antilles occupy areas of morphospace that are highly coincident, but not identical. Similarly, individuals of subspecies *padifolia* occupy a portion of the foliar morphospace of *E. tithymaloides*

that overlaps with the one occupied by individuals of the Lesser Antilles, and the morphospace occupied by individuals of subsp. *tithymaloides* corresponds broadly to that occupied by individuals form the mainland. This might be expected, in part because geography is a quite important component of current taxonomy.

Foliar variation of Caribbean *Euphorbia tithymaloides* in the context of taxonomy

Our analyses reveal differences in foliar variation among subspecies of Caribbean *E. tithymaloides*. Differences are subtle where our sampling is shallow (subspp. *bahamensis, jamaicensis, parasitica,* and *smallii*) possibly due to limited statistical power. However, for better-represented subspp. *angustifolia, padifolia,* and *tithymaloides*, our analyses reveal clear differences among subspecies (Fig. 5; Supplementary file 6.1). While subspecies differ for all three morphology PCs, posthoc tests reveal that not all three subspecies differ in all three PCs: there are significant three-way differences for PC 1 and 2, but not for PC 3, in which subsp. *padifolia* differs from subspp. *angustifolia* and *tithymaloides*, which are not statistically different for this axis (Fig. 5).
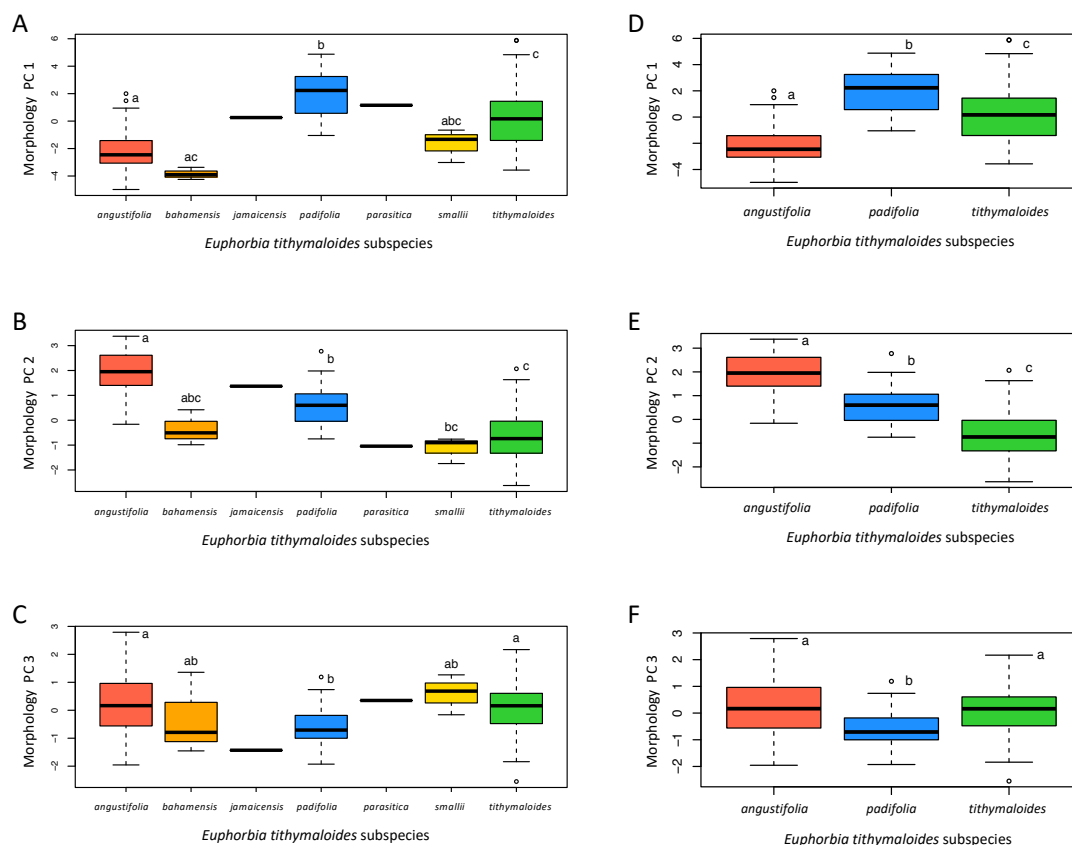

Figure 5. Kruskal-Wallis tests on the first three morphology PC axes (that capture 96.7% of variation in leaf morphology) reveal significant differences in foliar morphology among subspecies of *Euphorbia tithymaloides*. **A–C.** Results from tests including all seven subspecies described for Caribbean *E. tithymaloides*. **D–F.** Results from tests that focus on the main three subspecies of this system (*E. tithymaloides* subspp. *angustifolia, padifolia,* and *tithymaloides*). Letters depict statistically different groups as identified by posthoc tests.
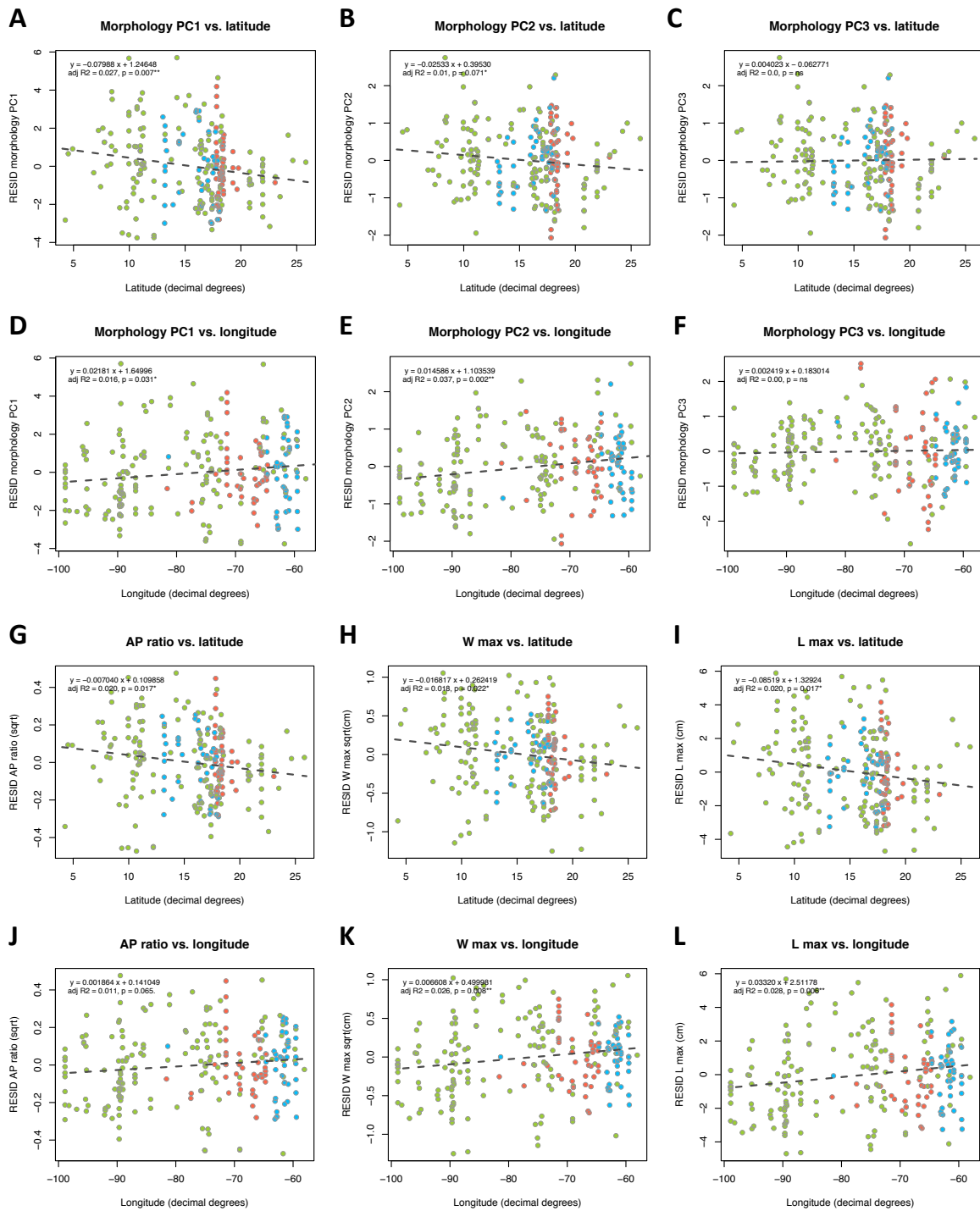
Figure 6. Linear models examining variation foliar morphology in relation to geography in *Euphorbia tithymaloides*, after removing subspecies effect. Colours represent subspecies (red, *E. tithymaloides* subsp. *angustifolia*; blue, *E. tithymaloides* subsp. *padifolia*; green, *E. tithymaloides* subsp. *tithymaloides*). No significant relationships were identified for Leaf Aspect Ratio (not shown).

Statistically significant differences in PC 1 (correlated with Wmax, Lmax, A/P ratio, as well as A and P) among three main subspecies suggest that the leaves of subsp. *angustifolia* leaves are smaller in size (smaller values of Wmax, Lmax, A, and P) and more elongated (smaller A/P ratios) than those of subspp. *padifolia* and *tithymaloides*. On the other hand, leaves of subsp. *padifolia* are characterized by values of PC 1 in the opposite direction (larger values of Lmax, Wmax, A, P, and A/P ratio), suggesting that leaves are larger and rounder. Leaves of subsp. *tithymaloides* are intermediate for PC 1.

Values along leaf PC 2 (associated with leaf aspect ratio L/W, Lmax, obovate index, and P) reveal that leaves of subsp. *angustifolia* are more elongated (higher values of A/P ratio) than those of the other two subspecies. For PC 2, subsp. *padifolia* has intermediate values to those of subspp. *angustifolia* and *tithymaloides*.

Morphology PC 3 (how ovate a leaf is – negatively correlated with obovate index) has a lower power to discriminate among subspecies. Results reveal that on average, leaves of subsp. *padifolia* are statistically more obovate (less ovate) than those of subspp. *angustifolia* and *tithymaloides*.

In summary, our analyses provide statistical support for leaves of subsp. *angustifolia* being smaller and more elongate in shape than those of subspp. *padifolia* and *tithymaloides*, and less obovate than those of subsp. *padifolia* but not statistically different along an ovate-obovate axis from those of subsp. *tithymaloides*. On the other hand, the leaves of subsp. *padifolia* are statistically larger, rather round, and markedly obovate, and those of subsp. *tithymaloides* are quite variable and mostly elliptical in shape.

Results from a linear model approach are consistent with Kruskal-Wallis tests and provide evidence that a significant proportion of the variation in leaf morphology – ranging from 11% (PC 3) to 57% (PC 2) (Supplementary file 6.2) – is captured by taxonomy.

Geographic patterns in leaf shape variation in Caribbean *Euphorbia tithymaloides*

Mantel tests ($D_{morphologic} \sim D_{geographic}$) suggest a correlation between morphology and geography (adj. R^2 = 0.056, p = 0.0025). However, this relationship disappears when eliminating variation in morphology due to subspecies (p = 0.58; Supplementary file 7). Thus, there is no evidence supporting that similarity in leaf morphology could be explained by geographical proximity alone but rather that variation in leaf morphology is structured by processes that are captured by taxonomy (i.e. subspecies).

Analyses from linear models based on PCs and where variation from subspecies has also been accounted for (using as a response variable the residuals of models focused on subspecies: morphology PCs ~ subspecies) reveal that PC 1 and 2 are negatively related to latitude (p = 0.007 and p = 0.071, respectively) and positively to longitude (p = 0.031 and p = 0.002) (Fig. 6). PC 3 does not show a significant relationship to either latitude or longitude (Fig. 6C, F). These results suggest patterns by which leaves of *E. tithymaloides* tend to be larger and of somewhat roundish shape in the south and west portions of its range, of smaller sizes and more elongate with increasing latitude (towards the north), and of larger sizes and more rounded shapes with increasing longitude (towards the east) (Fig. 6). These patterns were confirmed by analyses of individual focal variables that showed the largest loadings on the PCs: Lmax, Wmax, and A/P ratio tend to decrease with increasing latitude and increase with decreasing longitude (Fig. 6G–L). Smaller and less round leaves (those that are like those of subsp. *angustifolia*) are found in larger latitudes and smaller longitudes (NW of the distribution of *E. tithymaloides* in the Caribbean) and leaves towards the SE of the species range tend to be of larger sizes and rounder shapes, as are those of *E. tithymaloides* subsp. *padifolia*.

Leaf morphospace of Caribbean *Euphorbia tithymaloides* in relation to climate

A first visualization of the climatic space occupied by Caribbean *E. tithymaloides* suggests no marked differences with respect to portions of climate space occupied by different subspecies, nor that the main geographic areas in the system imply different climatic spaces (Fig. 7). Rather, subspecies of *E. tithymaloides* tend to occupy subsets of the climatic space already occupied by subspecies *tithymaloides* (Fig. 7A–C). From a geographic perspective, the Antillean individuals occupy only a portion of a climatic space that is otherwise already occupied by *E. tithymaloides* individuals from the mainland (Fig. 7D–F).

Because there are significant differences in leaf morphology across subspecies, to examine the proportion of variation in leaf shape that could be attributable to climatic factors alone, we implemented linear models in which we used as response variable the residuals of models where subspecies was used as a factor (X ~ subspecies). For these analyses, only data for the main three subspecies were included (subspp. *angustifolia*, *padifolia*, and *tithymaloides*).

Results reveal that there is an association between foliar morphology and climate. However, only a very small fraction of the variation in leaf morphology (between 1.4 and 2.8%) not accounted for by subspecies is explained by climatic variation (Fig. 8; Supplementary file 8). Larger and rounder leaves (Lmax, Wmax, A, P, A/P, all metrics related to morphology PC 1) are associated with sites that experience higher temperatures (mean annual, coldest month, driest quarter, and coldest quarter, BIO 1, BIO 6, BIO 9, and BIO 11, respectively, all variables related to climate PC 1; Fig. 8; Supplementary file 9) and less annual variation in temperature (BIO 7; p = 0.007; Fig. 8A). Also, PC 1 of leaf morphology is negatively related to climatic PC 3 (p = 0.039; Fig. 8C) suggesting that larger leaves are associated with larger isothermality (BIO 3)

and precipitation seasonality (BIO 15) but with lower temperature seasonality (BIO 4).

Leaf shape in relation to aspect ratio (L/W) and degree of obovate shape as well as some leaf Lmax and perimeter (morphology PC 2) are related to higher annual temperatures (climatic PC 1; p = 0.033; Fig. 8D). Morphology PC 2 is also negatively related to climate PC 2 (p = 0.016; Fig. 8E), which is negatively correlated with precipitation (mean annual, of the driest month and quarter, and of the coldest quarter, BIO 12, BIO 14, BIO 17, BIO 19) and positively to maximum temperature of the warmest month (BIO 5).

The metric with the highest loading on morphology PC 3 is the obovate index (Supplementary file 5.1, negative relationship). And morphology PC 3 only is significantly related to climatic PC 2 (Fig. 8H, negative relationship), suggesting that leaves that are more markedly obovate are associated to sites that experience more precipitation (BIO 12, BIO 14, BIO 17, BIO 19) and lower temperatures in the warmest month (BIO 5).

Results of models focusing on the variables with the highest loadings on morphological PCs (Supplementary file 5.1) and one variable per climatic PCs (Supplementary file 9.1) reveal that while there are significant relationships between morphology and climate, the proportion of variation in morphology that is explained by the climatic component is quite low (~1–4.5%). Most significant correlations were those related to temperature seasonality (A/P ratio and Wmax, p = 0.0006 and p = 0.0005, respectively). Four of the seven models that were evaluated were marginally significant: A/P ratio, Lmax, and Wmax in relation to mean annual temperature (p = 0.038, p = 0.052, and p = 0.064; Fig. 9D–F), and Lmax in relation to annual mean precipitation (p = 0.072; Fig. 9G), and three were non-significant (Fig. 9). These models suggest that in *E. tithymaloides*, once the effect of subspecies has been accounted for, larger and rounder leaves (as per A/P ratio and Wmax) are associated to warmer and less seasonal climates, while leaves with rather elongate and narrow shapes are related to climates that are less warm but more seasonal (Fig. 9). Also, longer leaves (Lmax) are related to sites that receive more mean annual precipitation (Fig. 9). Analyses do not find a significant relationship between the degree to which leaves are obovate and climate.

Climatic distance is not a good predictor of leaf morphologic distance in this system (Supplementary file 10), and this does not change when considering the spatial autocorrelation in climatic data: the partial Mantel test ResidD$_{morphologic}$ ~ D$_{climatic}$ + D$_{geographic}$ was not significant (p = 0.14).
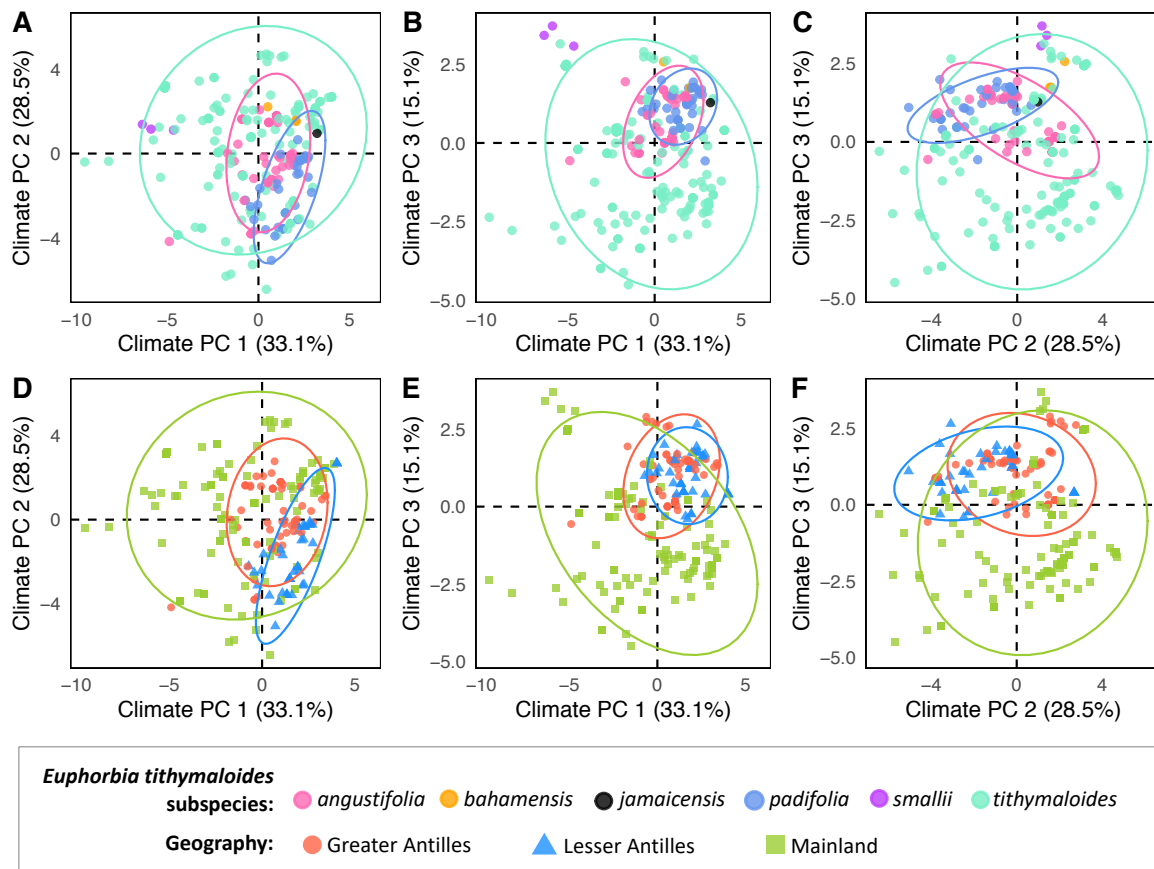

Figure 7. Climatic space occupied by *Euphorbia tithymaloides*, based on a PCA of 19 bioclimatic variables and elevation obtained from WorldClim v.2.0 (Fick and Hijmans 2017). **A–C.** Visualization with respect to subspecies as proposed by Dressler (1957). **D–F.** Visualization with respect to geographic occurrence (Greater Antilles, Lesser Antilles, and mainland).

DISCUSSION

This study is the first to characterize geographic patterns in leaf morphology in *Euphorbia tithymaloides*. It expands previous efforts both in kind and number of measurements as in approach and sampling scope (Dressler 1957; Cacho et al. 2019). We contribute a quantitative perspective on the foliar morphospace of *E. tithymaloides* in the Caribbean and show that in this species leaves tend to be smaller and narrower as latitude increases, of larger size and elliptical in shape towards the southwestern portion of the geographic range, and increase in size and become rounder as longitude increases (towards the eastern portion of the species range). These patterns are consistent with the geographical distribution of subspp. *angustifolia*, *padifolia*, and *tithymaloides*, with previous reports on leaf aspect ratio (Dressler 1957), and with field

observations in this system: some of the largest leaves in this species belong to subsp. *padifolia,* which tends to have more markedly obovate leaves (we did not detect a significant geographical pattern in this metric, possibly because in some cases leaves of subsp. *angustifolia* are obovate as well). Despite these patterns, Mantel tests show that geographic proximity alone is not enough to explain the degree of foliar variation observed in *E. tithymaloides*, and that incorporating variation explained by subspecies increases the explanatory power of geography with respect to foliar morphology. This could be explained in part by the marked geographic structure of subspecies in the landscape, which is the result of historical (evolutionary) processes in this species, and also a factor in subspecies delimitation (Dressler 1957). That is, in this system, taxonomy and geography are deeply intertwined.
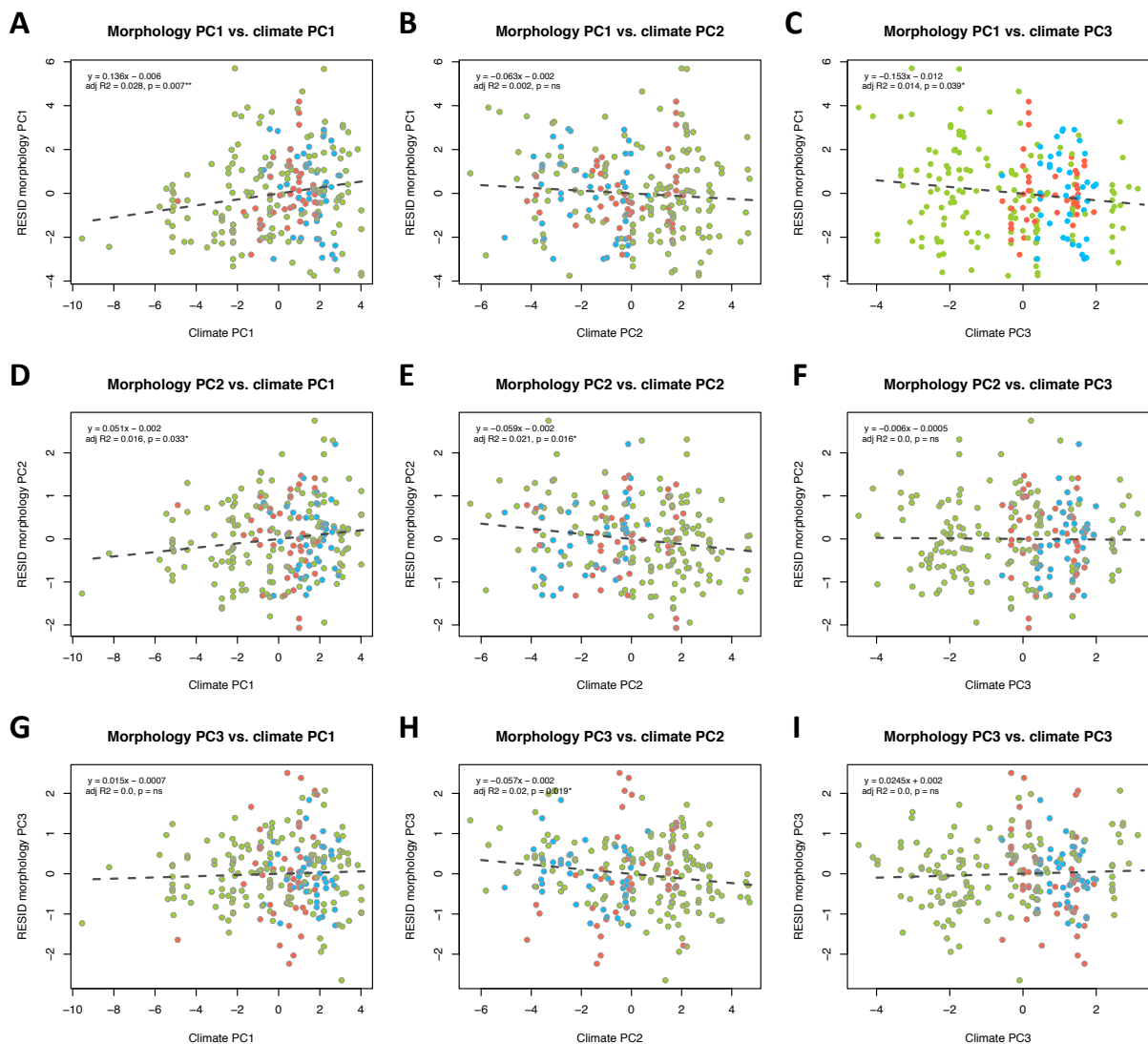

Figure 8. Linear models examining foliar morphology in *Euphorbia tithymaloides* (focused on the three main PC axes that capture 96.7% of variation in morphology) in relation to climate eliminating variation due to subspecies (response variable are residuals from morphology ~ subspecies models). Colours represent subspecies (red, *E. tithymaloides* subsp. *angustifolia*; blue, *E. tithymaloides* subsp. *padifolia*; green, *E. tithymaloides* subsp. *tithymaloides*).

Our study shows, like have others (Valcárcel and Vargas 2010; Huang and Knowles 2016), that the use of continuous morphological characters can be valuable when assessing diversity at shallow phylogenetic scales where overlaps in variation among recognized entities are expected (such as the population-subspecies-species continuum). Here, mainland subsp. *tithymaloides* occupies a larger portion of the foliar morphospace, with more variable leaves in both size and shape (larger variance in almost all metrics analysed). The levels of variation in leaves we observe across subspecies contrasts with subsp. *angustifolia* being a more recent and cohesive entity (Dressler 1957; Cacho and Baum 2012). While such cohesiveness might be true from a phylogenetic standpoint, it is not reflected in foliar morphology: subsp. *angustifolia* is rather variable, sometimes even

more so than subsp. *tithymaloides*. Subspecies of *E. tithymaloides* overlap in their leaf morphospaces, but as would be expected in a system ongoing active divergence (Cacho and Baum 2012; Cacho et al. 2019) they also show differences. Leaf shape is one of the attributes Dressler (1957) recognized as markedly discontinuous between subspp. *padifolia* and *angustifolia,* which he otherwise considered "closely allied", mainly given their shared relatively short inflorescences. In the light of our current understanding of the historical biogeography of *E. tithymaloides*, these two subspecies are recognized as the extremes of the two geographic expansion fronts, and thus are the most phylogenetically distant entities of the system. In this context, shared morphological traits that are not present in subsp. *tithymaloides* can only be the result of evolutionary convergence, as it has been shown
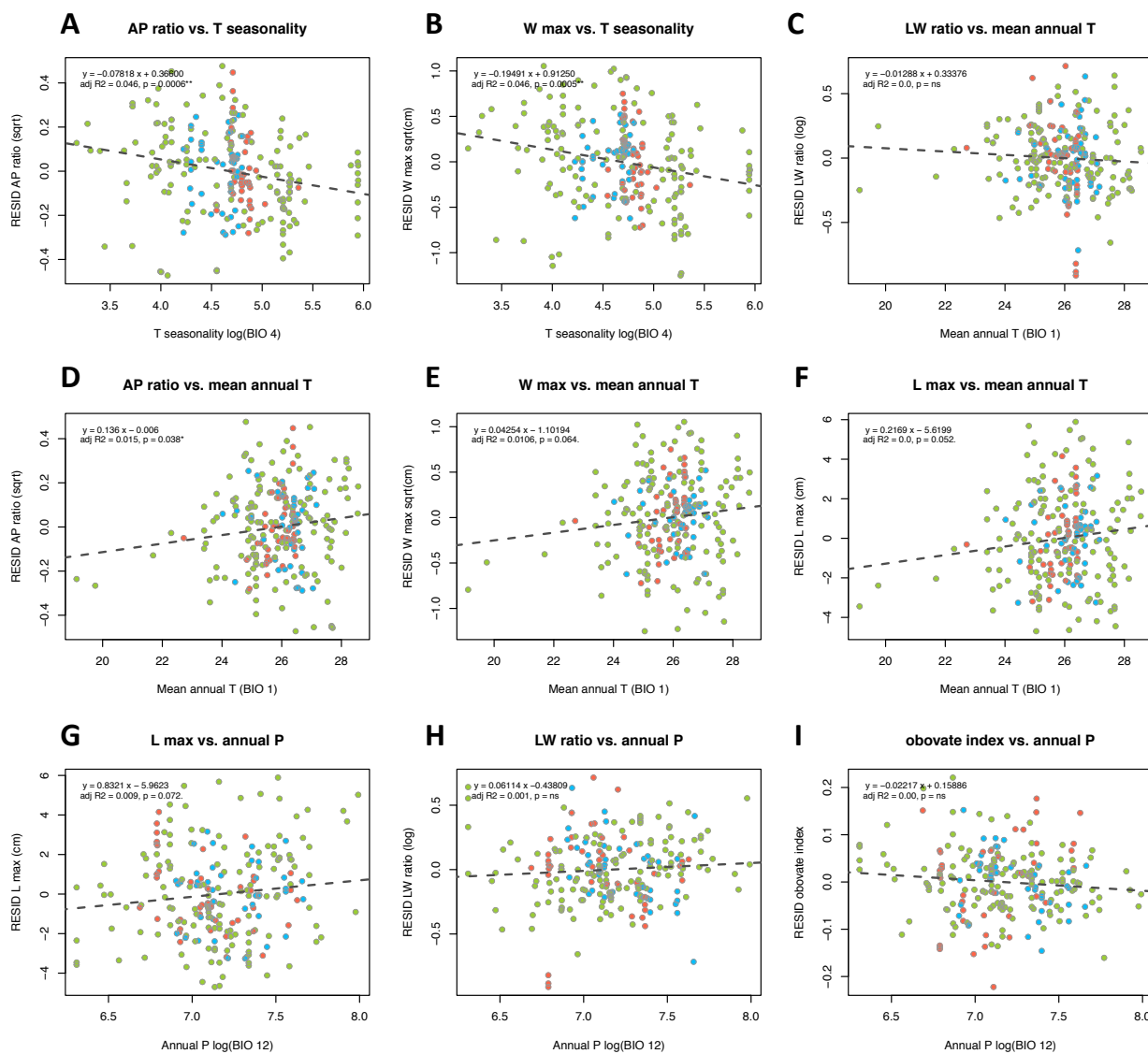

Figure 9. Linear models examining foliar morphology in *Euphorbia tithymaloides* (focused on variables with highest loadings on three PCs) in relation to climate after variation due to subspecies was eliminated (response variable are the residuals from morphology ~ subspecies models). Colours represent subspecies (red, *E. tithymaloides* subsp. *angustifolia*; blue, *E. tithymaloides* subsp. *padifolia*; green, *E. tithymaloides* subsp. *tithymaloides*).

with floral morphology (Cacho et al. 2019). This could be the case of obovate leaves evolving in parallel in subspp. *angustifolia* and *padifolia*, but this matter requires deeper investigation.

Our models and the substantial overlap in climatic space among subspecies of *E. tithymaloides* suggest that foliar variation in this system is not likely to be driven by climate. When foliar variation explained by subspecies is accounted for, the proportion of variation in leaf morphology explained by climate is quite small (< 5%). Taken together, our results do not support ecology (climate) as being an important force shaping leaf diversity in this system. Thus, it is likely that variation in leaf traits in *E. tithymaloides* has been shaped by historical contingencies encountered along the evolutionary trajectory of this species as it colonized the Caribbean from its area of origin in Mexico-Guatemala.

While it is impossible to completely rule out a possible role of climate in shaping foliar variation in *E. tithymaloides* in the past, our analyses suggest that at least in the present, the role of climate in shaping foliar variation in this species is limited. Even so, we show that there are correlations between leaf morphology and climate. We show that temperature and precipitation, as well as seasonality, are factors with a significant (yet limited) predictive power of leaf size and shape in *E. tithymaloides*. Once variation due to subspecies effect is considered, trends in foliar morphology in *E. tithymaloides* due to climate alone consist of larger and rounder leaves associated with warmer, more humid, and less seasonal sites. These trends are consistent with what is known about larger leaf areas favoured in sunny and humid environments (Ferris 2019). On the other hand, it is also amply documented that in xeric environments – like those occupied by *E. tithymaloides* – a reduction in leaf size can also be an adaptation to avoid high foliar temperatures due to extreme solar exposure (exposed leaves can achieve temperatures 10–20ºC higher than ambient temperature, sometimes reaching 55–60ºC; Vogel 1968). In these environments, a more efficient temperature regulation mediated by smaller boundary layers associated with smaller leaves could be adaptive (Givnish 1987; Schuepp 1993; Nicotra et al. 2011; Sack et al. 2012; Ferris 2019). However, it is important to highlight that there are other attributes, both from leaves and environmental that could modulate such expectations. In particular, individuals can occupy microsites with higher water availability, or shaded environments, where individuals could avoid leaves overheating (Vogel 1968), and our climate data are unlikely to capture environmental conditions at these scales. Also, leaf traits other than the ones considered here (such as trichomes, cuticle, and anatomical modifications) can offset the effects of high temperature, high solar radiation, and low water availability (Peguero-Pina et al. 2020).

A shortcoming of this study is that our working definition of climatic space – which is based on climatic variables available at a 30 s resolution – is likely a quite limited approximation to the true environments experienced by plants. As mentioned before, factors and processes that operate at smaller micro- and local environmental scales (and that are important in defining species' niches) have not been considered in this study. Factors such as vegetation (canopy) structure, soil type, and microsite preferences related to elevation, vegetation cover, and topography (among others) can delineate environmental differences that are undetectable when focusing on climatic variables alone (as we did). These elements can make sites that are climatically distant more ecologically similar, and vice versa.

From a morphological standpoint, our approach could be extended in various ways. First, there are aspects of foliar variation with taxonomic, ecological, and functional significance that have not been considered and that could offer a more nuanced understanding of the leaf morphospace. Among these, trichome type and density, cuticle thickness, and anatomical and phenological aspects may be important. Both trichomes and cuticle can limit water loss due to evapotranspiration, as well as offer protection against radiation (Peguero-Pina et al. 2020). Anatomical features and differences in phenology can offset what is perceived as different ecologies, and the ability to drop leaves can have adaptive significance in xeric environments like those occupied by *E. tithymaloides* (Peguero-Pina et al. 2020). The leaves of subsp. *angustifolia* are rather thin, with thin cuticles and trichomes that can vary in density (Dressler 1957; N. Ivalú Cacho pers. obs.). In subsp. *padifolia* leaves are thicker and with thicker cuticles too, markedly glabrous, and aphylly is common in individuals of this subspecies when growing in quite exposed environments (N. Ivalú Cacho pers. obs.). Studies incorporating broader perspectives, including morpho-anatomic and phenological attributes could unveil significant differences where this study has not. It is also possible that studies implementing more sophisticated approaches (e.g. geometric morphometrics (Chitwood et al. 2014) or topological analyses of computerized X-ray tomography data (Li et al. 2018)), might be able to identify dimensions of variation that escape this study.

In summary, our analyses support a scenario in which variation in foliar morphology across the geographic range of Caribbean *E. tithymaloides* is not explained by climate and it is likely the result of historical contingencies, thus reflecting the independent evolutionary trajectories among lineages in this system. Subspecies *angustifolia* and *padifolia*, despite occupying largely overlapping climatic spaces, differ quite substantially in foliar attributes related to both, leaf size and shape, and in the portion of the foliar morphospace they occupy. This could be interpreted as two different solutions to similar climatic scenarios, solutions that were achieved independently as these lineages diverged from their common mainland ancestor. Historical contingencies can be of evolutionary significance by means of traits that could in turn amplify a species ability to explore both, an expanded foliar

morphospace, and a broader climatic envelope, facilitating its ecological expansion and its evolutionary potential.

CONCLUSION

Variation in foliar morphology in *Euphorbia tithymaloides* is mainly explained by significant differences among subspecies and not by climate or geography. Because taxonomy captures important aspects of foliar morphospace in this species, leaf morphology can be used as an appropriate proxy for subspecies assignation.

In general, leaves of *E. tithymaloides* subsp. *angustifolia* (Greater Antilles) are smaller and narrower than those of subspp. *padifolia* and *tithymaloides*; those of subsp. *padifolia* (Lesser Antilles) are relatively large and distinctly obovate, and; those of subsp. *tithymaloides* (mainland) are mostly elliptical, but also quite variable, both in size and shape.

Differences in foliar morphology in this system are likely to be the result of historical contingencies encountered by this species sub-lineages during their geographic expansion and colonization of the Caribbean Basin from its centre of origin in Mesoamerica. Yet some climatic variables have some predictive power of foliar attributes in *E. tithymaloides*, but the proportion of variation they explain is quite small (< 5%). Variables with most predictive power are temperature seasonality (negatively related to roundness and foliar size), mean annual temperature, and mean annual precipitation (both positively related to roundness and leaf size).

In *Euphorbia tithymaloides*, once variation in subspecies is accounted for, neither climatic distance nor geographic distance alone suffice to explain distance in foliar morphospace. A small proportion of variation in foliar morphology (~3%) can be explained by geography, so that leaves tend to decrease in size and be less round as latitude increases, and as longitude decreases.

This study shows how, at a rather shallow time scale, historical contingencies rather than ecological processes can shape variation in foliar morphology, and expand a lineage's potential to explore both morphological and climatic spaces.

ACKNOWLEDGEMENTS

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SUPPLEMENTARY FILES

Supplementary file 1
Characteristics of the Caribbean subspecies of *E. tithymaloides*.
Link: https://doi.org/10.5091/plecevo.91487.suppl1

Supplementary file 2
Procedures for the leaf measurements.
Link: https://doi.org/10.5091/plecevo.91487.suppl2

Supplementary file 3
Variables included in this study.
Link: https://doi.org/10.5091/plecevo.91487.suppl3

Supplementary file 4
Data from images from dry vs fresh leaves do not differ systematically.
Link: https://doi.org/10.5091/plecevo.91487.suppl4

Supplementary file 5
Principal Component Analysis (PCA) of foliar variation in *E. tithymaloides*.
Link: https://doi.org/10.5091/plecevo.91487.suppl5

Supplementary file 6
Kruskal-Wallis tests and linear models testing differences in leaf morphospace among *E. tithymaloides* subspecies.
Link: https://doi.org/10.5091/plecevo.91487.suppl6

Supplementary file 7
Mantel tests examining geographic distance as predictor of distance along the leaf morphospace in *E. tithymaloides*.
Link: https://doi.org/10.5091/plecevo.91487.suppl7

Supplementary file 8
Linear models examining climatic variation as predictor of variation in leaf morphospace in *E. tithymaloides*.
Link: https://doi.org/10.5091/plecevo.91487.suppl8

Supplementary file 9
Principal Component Analysis (PCA) of climate associated to sites occupied by *E. tithymaloides*.
Link: https://doi.org/10.5091/plecevo.91487.suppl9

Supplementary file 10
Mantel tests examining climatic distance as predictor of distance along the leaf morphospace in *E. tithymaloides*.
Link: https://doi.org/10.5091/plecevo.91487.suppl10