

Phenotypic variation of *Zamia loddigesii* Miq. and *Z. prasina* W.Bull. (Zamiaceae, Cycadales): the effect of environmental heterogeneity

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Abstract The study of morphological variation in heterogeneous environments provides evidence for understanding processes that determine the differences between species and interspecific adaptive strategies. In 14 populations of two closely related cycads from the genus *Zamia* (*Zamia loddigesii* and *Z. prasina*), the phenotypic variation was analyzed based on 17 morphological traits, and this variability was correlated with environmental conditions across the populations. Despite the significant inter-population variation observed in the two species, greater inter-specific differences were observed based on generalized linear models. Individuals of all populations except for the Macuspana (Tabasco) population of *Z. prasina* were

separated into two discrete groups in a multivariate space (non-metric multidimensional scaling). Macuspana plants overlapped marginally with the multivariate space defined by plants in the four *Z. loddigesii* populations. Remarkably, Macuspana is geographically located at the distribution limits of both species that occur in close proximity expressing traits that resemble either of the two species. The heterogeneous environment seems to play a determining role in the phenotypic expression of both species. The variation found could be related to the local ecological adaptations that tend to maximize the populations adaptation.

Keywords Co-inertia and multivariate analysis · Mexico · Phenotype and environmental variation · Yucatan peninsula · *Zamia*

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Introduction

The study of the morphological variation in the geographical and environmental contexts is of paramount importance to understand proximal processes that determine species distribution, and the processes driving the origin of phenotypic variability (Stebbins 1950; Endler 1977). On an intraspecific level, the characterization of morphological variation in multiple environments provides evidence to understand the adaptive strategies of the species (Endler 1986), speciation mechanisms (Grant 1971; West-Eberhard 2005), and biogeographical processes (Mayr 1970; Cox and Moore 2005).

The phenotypic variation is determined by interactions among the genome (combination of different genetic variants), and epigenome (methylations in the amino acid sequences and stable chromatin modifications) with the physical environment (i.e., substrate type, soil use,

vegetation type, light availability, minimum and maximum temperatures, humidity, latitude, and altitude; Stearns 1989; Sultan and Bazzaz 1993) and biotic interactions (Feil and Fraga 2012) that include antagonists (i.e., herbivores, pathogens, seed predators, among others), and mutualists (i.e., seed and pollen dispersers, pollinators, root symbionts, among others).

Cycads are plants with an evolutionary history of approximately 250 my; however, molecular studies indicate that extant species date from a recent speciation in the Neogene (Nagalingum et al. 2011; Condamine et al. 2015). They occur in the tropics and subtropics of the world, in many cases in relict populations. The study of the phenotypic variation of cycads is mostly restricted to vegetative structures, and little is known on reproductive structures mainly because strobili are rare, ephemeral, and often asynchronous.

Some studies have evaluated the morphological variation of species and species complexes of cycads across their geographic range, e.g., *Dioon edule* Lindl. (De Luca et al. 1982; Gonzalez-Astorga et al. 2003), *Zamia pumila* L. (Newell 1989), *Ceratozamia norstogii* D.W. Stev. (Pérez-Farrera et al. 2014), *Zamia katzeriana* (Regel) E. Rettig (Nicolalde-Morejon et al. 2008), and *Ceratozamia miqueliana* H. Wendl. (Pérez-Farrera et al. 2009). Other studies have focused on the characteristics of the environments where different cycad populations occur (Pérez-Farrera et al. 2004; Vovides et al. 2008; Keppel 2009); however, the link between morphology and environment has not been explicitly explored yet.

The aim of this study was to analyze how the heterogeneity of the environment affects the phenotypic variation in 14 natural *Zamia prasina* and *Zamia loddigesii* populations in order to elucidate the processes of adaptation to local environments.

The genus *Zamia* L. is exclusive to the New World. It contains the greatest morphological, ecological, geographical, and karyological diversity of the entire order Cycadales (Caputo et al. 1996; Vovides and Olivares, 1996; Nicolalde-Morejon et al. 2009). *Zamia* includes some widely distributed species such as *Z. loddigesii* Miq. and *Z. prasina* W.Bull. These two species are likely to be closely related (Caputo et al. 2004), and at one time, they were considered as a single species (*Z. polymorpha* D.W.Stev, Moretti & Vázq. Torres, see Calonje and Meerman 2009) because of their adjacent geographic ranges (the Gulf of Mexico plains and the Yucatan Peninsula), and similar morphology of leaves and leaflets (Stevenson et al. 1996). However, later was shown that these two species occupy the vegetation types differently and have distinct karyologies (Napolitano et al. 2004).

Considering that *Z. loddigesii* and *Z. prasina* are closely related species (Caputo et al. 2004) of wide distribution

with adjacent distribution limits south of the Yucatan Peninsula, we expected to observe: (1) higher morphological variation in *Z. prasina* than in *Z. loddigesii* because the environment of the latter species is more heterogeneous than that of *Z. loddigesii*, and (2) correlation of characters with environment as a morphological adaptation to environmental conditions where the populations are distributed.

Materials and methods

Study area and geographic distribution

During March 2013, a total of 14 populations were sampled throughout the distribution of the species in Mexico. Four (1–4) corresponded to *Zamia loddigesii* and ten (5–14) to *Z. prasina* (Table 1; Fig. 1). All samples corresponded to field collections taken *ex profeso* for this study, except for three cases: Aldama, Tamaulipas; Tecolutla, and El Guanal, Veracruz. These three populations were evaluated from living individuals sheltered at the National Mexican Cycad Collection, in the Instituto de Ecología A.C. (Francisco Javier Clavijero Botanical Garden, Online Resource 1).

Locally, all individuals of each population share similar environmental characteristics, owing to an aggregate spatial distribution (not beyond 100 m of distance between individuals in the same population). In all the populations examined, their elevation and geographic position were registered with a GPS. To visualize the spatial distribution of the populations, their geographic coordinates were projected onto a digital map of Mexico. In order to determine current soil use and vegetation type in each population, a soil use and vegetation map was consulted (INEGI 2012) that follows the Rzedowski (1981) classification for vegetation types associated with *Zamia loddigesii* and *Z. prasina* populations. The information was processed with the ArcMap 10.2 software (ESRI 2013).

Species studied

Zamia loddigesii is endemic to Mexico and distributed in the coastal plain of the Gulf of Mexico along approximately 400 km from Tamaulipas, in the north, to Tabasco, in the south (Fig. 1; Table 1). It occupies elevations ranging from sea level up to 1000 m high. The average annual temperature in the localities where this species is found ranges between 22.8 and 26 °C. *Z. loddigesii* is associated with secondary vegetation of deciduous tropical forest, disturbed habitats due to seasonal, annual and permanent agriculture as well as cultivated pasturelands. The plants bare two to three leaves, 12–23 leaflet pairs of narrow leaflets between 0.4 and 0.7 cm wide (Nicolalde-Morejon et al. 2009).

Table 1 Population, sample size (*N*), geographical coordinates, elevation, mean annual temperature, annual rainfall, and vegetation-type-associated populations of *Zamia loddigesii* (1–4) and *Z. prasina* (5–14)

Population	<i>N</i>	Latitude (N)	Longitude (W)	Elevation (m)	Mean annual temperature (°C)	Annual rainfall (mm)	Vegetation type
1. Aldama, Tamaulipas	18	23°00'	98°03'	245	22.8	921	SVD
2. Tecolutla, Veracruz	15	20° 26'	97°00'	22	24.3	1473	APA
3. El Guanal, Tabasco	24	17°48'	93°51'	11	25.8	2539	CP
4. Huimanguillo, Tabasco	15	17°48'	93°29'	37	26	2381	SA
5. Macuspana, Tabasco	20	17°47'	92°34'	18	26.2	2314	CP
6. Balancán, Tabasco	6	17°39'	91°32'	40	26.6	2096	CP
7. Tenosique, Tabasco	21	17°16'	91°24'	373	25.5	2280	SER
8. El Tormento, Campeche	20	18°36'	90°48'	90	26	1382	DTR
9. Xbacab, Campeche	20	18°55'	90°38'	45	26.1	1354	STR
10. Calakmul, Campeche	20	18°31'	89°43'	256	24.9	1140	STR
11. Alfonso Caso, Yucatán	19	20°05'	89°08'	41	25.9	1061	SSR
12. Xcalacoop, Yucatán	19	20°39'	88°31'	24	25.8	1214	SSR
13. Puerto Morelos, Q. Roo	21	20°51'	87°00'	9	25.6	1148	DTR
14. Carrillo Puerto, Q. Roo	21	19°28'	88°01'	12	25.9	1284	STR

SVD secondary vegetation of deciduous tropical forest, APA annual and permanent agriculture, CP cultivated pastureland, SA seasonal agriculture, SER secondary succession of evergreen tropical rainforest, DTR sub-deciduous tropical rain forest, STR secondary succession of sub-deciduous tropical rain forest, SSR secondary succession of seasonally dry rainforest. According to INEGI (2012). Temperature and precipitation derived from Hijmans et al. (2005)

In Mexico, *Zamia prasina* is distributed in the Yucatan Peninsula, encompassing the states of Quintana Roo, Campeche, Yucatan, Tabasco and Chiapas. The elevation range is from sea level to 400 m, and the average temperature oscillates between 24.9 and 26.6 °C. This species is associated with evergreen tropical rainforest, sub-deciduous tropical forest and secondary vegetation in disturbed areas (Fig. 1; Table 1). The plants produce wide as well as narrow leaflets between 15 and 17 pairs (Calonje and Meerman 2009).

Morphological characters evaluated

We analyzed a single leaf per plant, considering the biology of this group. Newell (1985, 1986, 1989) showed no significant difference in the morphological variation between leaf attributes of the same individual in *Zamia* species; in the biology of *Z. prasina* and *Z. loddigesii*, it is common to observe individuals with few leaves (1–3, up to 7 in *Z. prasina*) and because negligible intra-individual variation occurs in fully developed leaves (see Calonje and Meerman 2009), the probability of detecting extra variability useful within the scope of this manuscript, if more leaves were assessed, are minimal and not worth the effort. Therefore, in our study, morphometric evaluation was conducted only in adult specimens. In each individual, a fully developed leaf was randomly selected to evaluate 17 morphological characters (Table 2). All units were recorded in centimeters, except for the count values for LP and NL.

These characters have been referred in a recent taxonomy of the genus *Zamia* and in some cases, used as diagnostic characters on the species level (Stevenson et al. 1996; Nicolalde-Morejon et al. 2008, 2009).

Statistical analysis

The basic statistics—mean and standard error—was calculated for each population. Given the possible correlation between the evaluated characters, we used a multidimensional nonparametric scaling analysis in all 17 morphometric variables of all recorded individuals that were evaluated. The distance matrix was constructed based on the modification of the Bray Curtis similarity index proposed by Aguirre et al. (2010) to condense the variability between and within the populations of each species. To explicitly test for differences in traits among populations of the two species, we used generalized linear models (GLM) with estimates based on the gamma distribution and the inverse link function (McCullagh and Nelder 1989). In each model, the proportion of deviance accounted for was calculated as follows: 1—residual deviance/null model deviance (analogous to R^2 in ANOVA). We used this ratio as an indicator to select traits with major differences across populations (Zar 1999). To identify homogeneous groups (populations), we used contrast based on *t* test comparison between pairs of populations with standard errors derived from the linear predictor of the model.

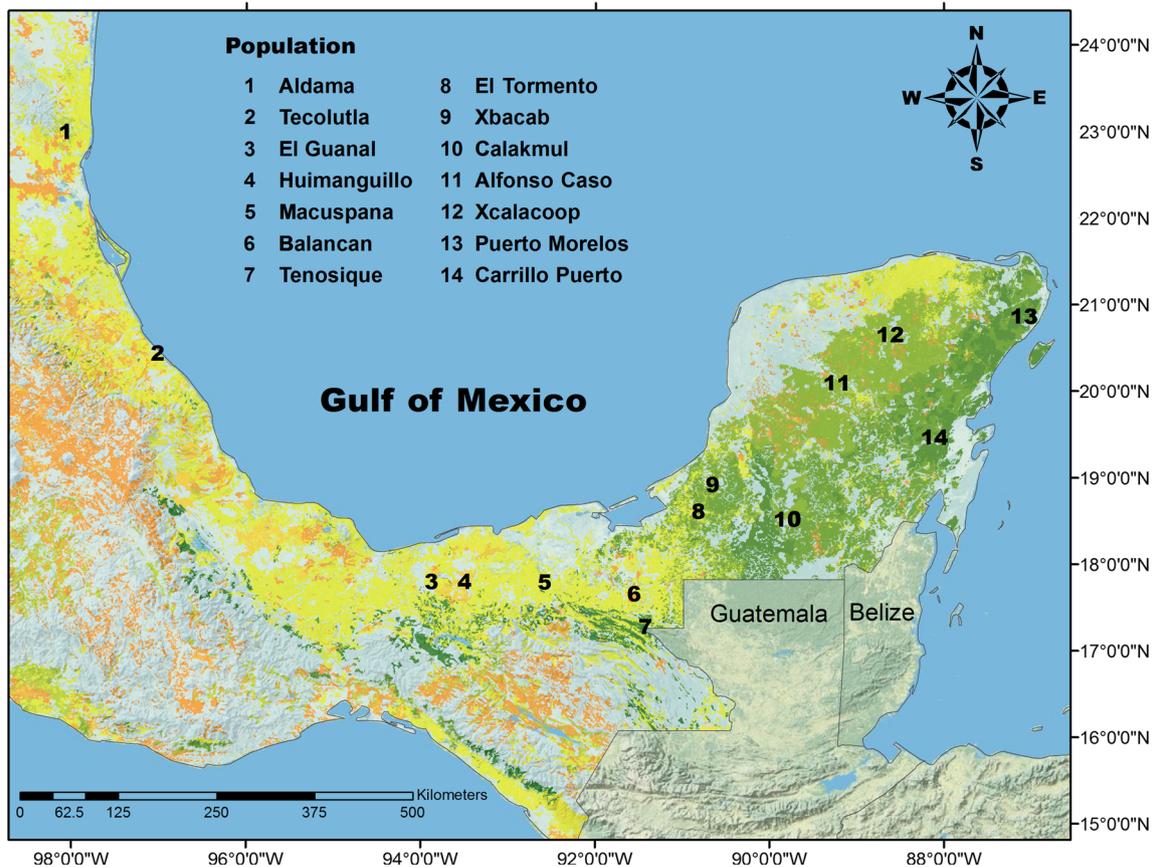


Fig. 1 Geographical distribution of *Zamia loddigesii* (population 1–4) and *Z. prasina* (population 5–14). Associated vegetation types (according to INEGI 2012) are highlighted

The relation of the variation of the morphological traits across the 14 populations with the environmental conditions was explored with a co-inertia analysis based on the principal component analysis (Doledec and Chessel 1994). For this analysis, each matrix of quantitative data (environment and morphology) is ordered in a multivariate space, and after rescaling them, one space is rotated on the top of the second space both pinned through their origins. By matching the identity of each site across ordinations, the rotation that minimized the overall distance between pairs is selected. Then, the co-inertia axes represent the co-structure between variation of morphological traits and environmental factors (Franquet et al. 1995).

In the first matrix (morphological) from each population, we used the mean values of the 17 morphological traits, transformed into Napierian logarithms, to minimize the effect of size for the largest structures. The second matrix (environmental) was constructed by associating the geographical coordinates of each population and obtaining the associated values of the environmental layers Annual Mean Temperature (BIO1) and Annual

Precipitation (BIO12) both obtained from the WorldClim database (Hijmans et al. 2005). These data layers were generated through interpolation of a high number of climatic observations around the world from monthly average climate data (from the period 1950 to 2000) and topographical data from the Shuttle Radar Topographic Mission (SRTM) (Hijmans et al. 2005). The other layer was potential evapo-transpiration (Global-PET), which is the maximum quantity of water that can evaporate from a soil completely covered with vegetation, as an indicator of humidity/aridity. This was obtained from the ‘Consortium for Spatial Information’ (CGIAR-CSI) (Trabucco and Zomer 2009). The Global-PET is modeled using the available data from the WorldClim as input parameters and the methodology presented in Allen et al. (1998). All layers were with a resolution of ca.1 km². Finally, the elevation of each population was obtained directly from the field and from the database of the Francisco Javier Clavijero Botanic Garden. All analyses were conducted with the R statistics software (R Core Team Group 2013).

Table 2 Morphometric variables evaluated and abbreviations

Character	Code
Apex	
Length of leaflets at leaf apex	LLA
Width of leaflets at leaf apex	WLA
Distance between leaflets at leaf apex	DLA
Leaflet articulation width at leaf apex	AWA
Median	
Length of leaflets at leaf median	LLM
Width of leaflets at leaf median	WLM
Distance between leaflets at leaf median	DLM
Leaflet articulation width at leaf median	AWM
Base	
Length of leaflets at leaf base	LLB
Width of leaflets at leaf base	WLB
Distance between leaflets at leaf base	DLB
Leaflet articulation width at leaf base	AWB
Total length of the leaf	TL
Petiole length	PL
Rachis length	RL
Leaflets pairs	LP
Number of leaves per plant	NL

Results

Interspecific morphometric variation

A total of 259 individuals in 14 populations were evaluated (72 *Z. loddigesii* individuals and 187 *Z. prasina* individuals), and the average of the 17 attributes differed between the two species in a 300–15 % range (Fig. 2a–q). Consequently, in the multidimensional space of non-metric scaling (Fig. 2r), a clear separation was observed between the two species, except for the Macuspana (Tabasco) population, which 95 % confidence ellipse marginally overlapped with those of the four *Z. loddigesii* populations and 66 % of the remaining *Z. prasina* populations. Overall, *Zamia loddigesii* is characterized by having a greater number of leaves with narrow leaflets, whereas in *Z. prasina*, the leaflets are much wider.

Intraspecific morphometric variation

The GLM models indicate that the morphometric differences in the two species are greater between populations than between individuals of the same population (Table 3). In *Z. prasina*, the differences between populations in all the characters evaluated were statistically significant. In *Z. loddigesii*, the characters: distance between middle leaflets, number of leaflet pairs, width of leaflets at leaf apex, and

the distance between basal leaflets show no significant differences among the populations.

For *Z. loddigesii*, petiole length is the factor with the highest ratio of deviance accounted for (67 %) by the population factor, followed by total leaf length (59 %), leaflet width at the leaf apex (41.4 %), and average leaflet length (36.6 %). The contrast tests (Table 4) show that the population from Huimanguillo (Tabasco) is different from the other three populations in terms of these four attributes, and that these three populations form a second homogeneous group with regard to length of the petiole and total leaf length. The Aldama (Tamaulipas) and El Guanal (Tabasco) populations differ in terms of leaflet width at the leaf apex and average leaflet length, but the Tecolutla (Veracruz) population is indistinguishable from the other two populations.

In *Z. prasina*, leaflet width at the leaf apex (60.1 %), mid leaf (58.9 %) and basal (49.4 %), and the distance between the middle leaflets (34.6 %) are the traits with most deviance accounted for by the population factor. The Macuspana (Tabasco) population presented the narrowest leaflets and shortest inter-leaflet distance between leaflets at mid leaf separating it from the rest of the populations. Also, El Tormento and Xbacab populations (both in Campeche) are separated from the remaining populations by having narrow leaflets (only at the leaf apex leaflets in the case of Xbacab) and with little separation between the mid leaf leaflets. The leaflets in these populations were almost twice as wide and the inter-leaflet distance at mid portion of the leaf was twice as that of the Macuspana (Tabasco) population. Additionally, Alfonso Caso (Yucatan), Tenosique (Tabasco), and Carrillo Puerto (Quintana Roo) populations presented wider leaf apex leaflets separating them from the rest of the populations of this species (Table 4).

Influence of the environment on the morphology

The co-inertia analysis (Fig. 3) showed a significant relationship between the average morphology in the 14 populations and the environmental variables that define their geographic areas ($P < 0.025$). Overall, the width of the leaflets increased as the evapo-transpiration potential increased, and to a lesser degree the leaflets were also wider in higher temperatures but thinner in sites with more precipitation. In contrast, the number of leaves decreased as evapotranspiration and temperature increased. It is important to highlight that the populations with the greatest environmental similarity, the one *Z. loddigesii* and the other *Z. prasina*, present morphological differences between them, even though they were also different from the rest of the populations of the corresponding species.

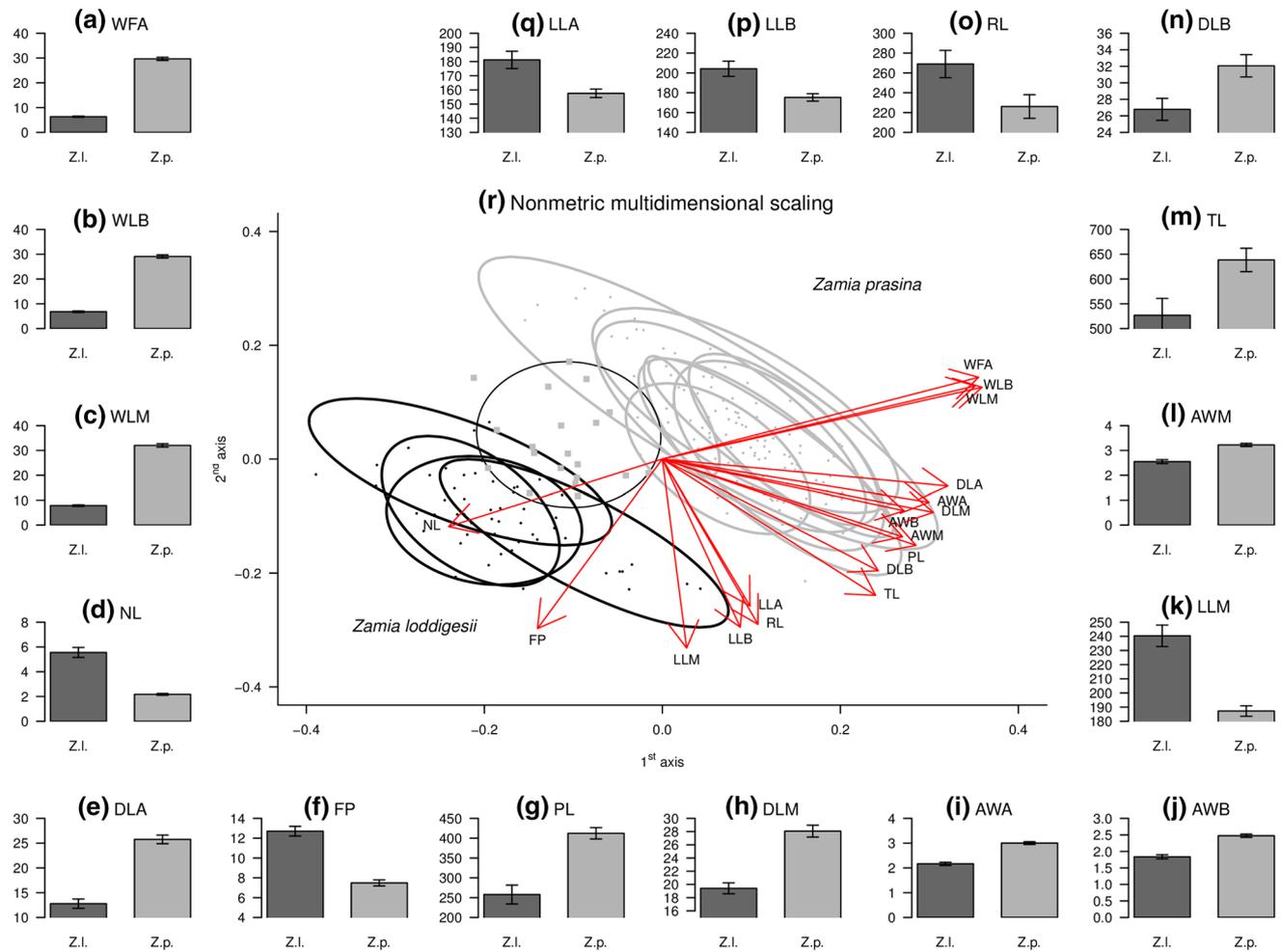


Fig. 2 a–q Average variation in each character evaluated in *Zamia loddigesii* and *Z. prasina*, r non-metric multidimensional scaling of *Zamia loddigesii* and *Z. prasina* with ellipses covering 95 % of the individuals in each population

Discussion

This study indicated that a multivariate morphological differentiation exists between *Zamia loddigesii* in the coastal plain of the Gulf of Mexico and *Z. prasina* in the Yucatan Peninsula, with the exception of the Macuspana (Tabasco) population. Also, this study showed that within each of the two species, one population is markedly different from the rest; in *Z. loddigesii*, the Huimanguillo (Tabasco) population in the southeast border of the distribution is distinct from the other three populations. Similarly, the Macuspana (Tabasco) population in the southwest limit of the *Z. prasina* distribution is different from all the other populations of this species. Consistently with these findings, the co-inertia analysis detected a positive relationship between the leaflets width and the potential evapotranspiration. The results suggest that the morphological differences found between the two species involve an environmental component, as it has been postulated for

Zamia acuminata and *Z. pseudomonticola* (Acuña-Castillo and Marín-Méndez 2013) and between species in the genus *Dioon* (Barone Lumaga et al. 2015) and *Ceratozamia* (Pérez-Farrera et al. 2014) in which the form of the leaflets (particularly length and width), present differences owing to adaptations to different environments (xeric to mesic). Also, López-Gallego (2008) evaluated habitat quality of *Z. melanorrhachis* and found that plant size (i.e., petiole length, leaflet length and width) was lower in disturbed habitats, which is congruent with the correlation between morphological traits and environmental variables observed in this study.

Environmentally, *Zamia prasina* grows principally under a dense canopy, in tropical rain forests and semi-deciduous tropical forests (Fig. 1), and therefore, wide leaflets could represent an adaptation to facilitate evapotranspiration and to capture more sunlight under shade conditions. In contrast, *Z. loddigesii* grows on sites with lower potential evapotranspiration, associated with

Table 3 Proportion of deviance accounted in both species for each variable. *Zamia loddigesii* (four populations), (DF: 3.68); *Zamia prasina* (ten populations) (DF: 9177)

Character	<i>Zamia loddigesii</i>			Variable	<i>Zamia prasina</i>		
	F	P value	PDA		F	P value	PDA
PL	48.641	***	0.670	WLA	31.566	***	0.601
TL	35.392	***	0.590	WLM	29.226	***	0.589
WLA	15.915	***	0.414	WLB	19.492	***	0.494
LLM	13.763	***	0.366	DLM	12.124	***	0.346
WLM	11.666	***	0.361	DLA	9.144	***	0.309
NL	11.188	***	0.354	PL	6.938	***	0.28
LLB	12.767	***	0.337	FP	6.925	***	0.276
LLA	11.894	***	0.335	DLB	5.785	***	0.218
WLB	7.257	***	0.254	AWA	4.598	***	0.187
DLA	7.87	***	0.237	TL	4.224	***	0.185
RL	7.094	***	0.211	LLM	4.152	***	0.168
AWM	4.86	**	0.179	LLA	4.02	***	0.165
AWB	3.847	*	0.149	NL	3.44	***	0.146
DLM	1.533	NS	0.061	RL	3.283	**	0.126
FP	1.106	NS	0.046	AWM	2.594	**	0.116
AWA	0.604	NS	0.027	LLB	2.606	**	0.115
DLB	0.291	NS	0.011	AWB	2.075	*	0.099

PDA proportion of deviance accounted, NS not significant, analogous to R^2 in ANOVA; *** $P < 0.0001$; ** $P < 0.001$; and * $P < 0.05$

Table 4 Contrast tests on the four characters with higher values of PDA in *Zamia loddigesii* and *Zamia prasina*

Population	PL	TL	WLA	LLM
(a) <i>Zamia loddigesii</i>				
1. Aldama	12.17b	35.45b	0.674b	20.35c
2. Tecolutla	17.51b	39.19b	0.605bc	20.65bc
3. El Guanal	21.75b	48.20b	0.490c	24.78b
4. Huimanguillo	56.84a	94.01a	0.841a	30.67a
Population	WLA	WLM	WLB	DLM
(b) <i>Zamia prasina</i>				
5. Macuspana	1.30c	1.44d	1.41d	1.23c
8. El Tormento	2.71b	2.90c	2.56c	2.45b
9. Xbacab	2.74b	3.00bc	2.80bc	2.50b
6. Balancán	2.80ab	3.09abc	2.80abc	2.25bc
10. Calakmul	3.09ab	3.53abc	3.11abc	3.36ab
12. Xcalacoop	3.19ab	3.27abc	3.04abc	3.05ab
13. Puerto Morelos	3.22ab	3.37abc	3.23abc	3.00ab
11. Alfonso Caso	3.38a	3.66ab	3.12abc	3.17ab
7. Tenosique	3.47a	3.79a	3.50a	3.07ab
14. Carrillo Puerto	3.63a	3.87a	3.39ab	3.57a

secondary tropical deciduous forest vegetation and disturbed sites (Nicolalde-Morejon et al. 2009) with greater sunlight incidence; therefore, the narrow leaflets could probably represent a strategy to minimize water loss.

Negrón Ortiz and Gorchov (2000) showed that in *Z. pumila*, the production of leaves significantly increases when there are controlled fires in the previous year as part of the agricultural management. Controlled fires are common in the Aldama (Tamaulipas) populations and central Veracruz in *Zamia loddigesii* (Nicolalde-Morejón and González-Astorga, personal observations). Therefore, the morphological characters, such as leaf architecture, could be adaptations that permit them to grow under different environmental conditions (Picotte et al. 2009) and could provide a connection between various environmental factors and the functions of the leaf.

According to the observations of Stevenson et al. (1996), *Zamia prasina* from intermediate environmental zones can present intermediate morphological forms, which could be related to hybridization or speciation processes. Moretti and Sabato (1984) point out that the habitat differences can cause chromosomal fission, allowing the species to invade and survive in a much greater range of habitats than their relatives with lower chromosomal numbers. While *Z. loddigesii* has a stable chromosomal number of $2n = 18$ (Norstog 1980), in *Z. prasina*, there is a large karyological variation $2n = 17, 22, 23, 24, 25, 26, 27, \text{ and } 28$ (Stevenson et al. 1996; Vovides and Olivares 1996; Napolitano et al. 2004), and therefore, it has been proposed that this phenomenon may generate adaptive radiations on a small scale in this species (cf. Olson and Gorelick 2011). Our findings are consistent with the

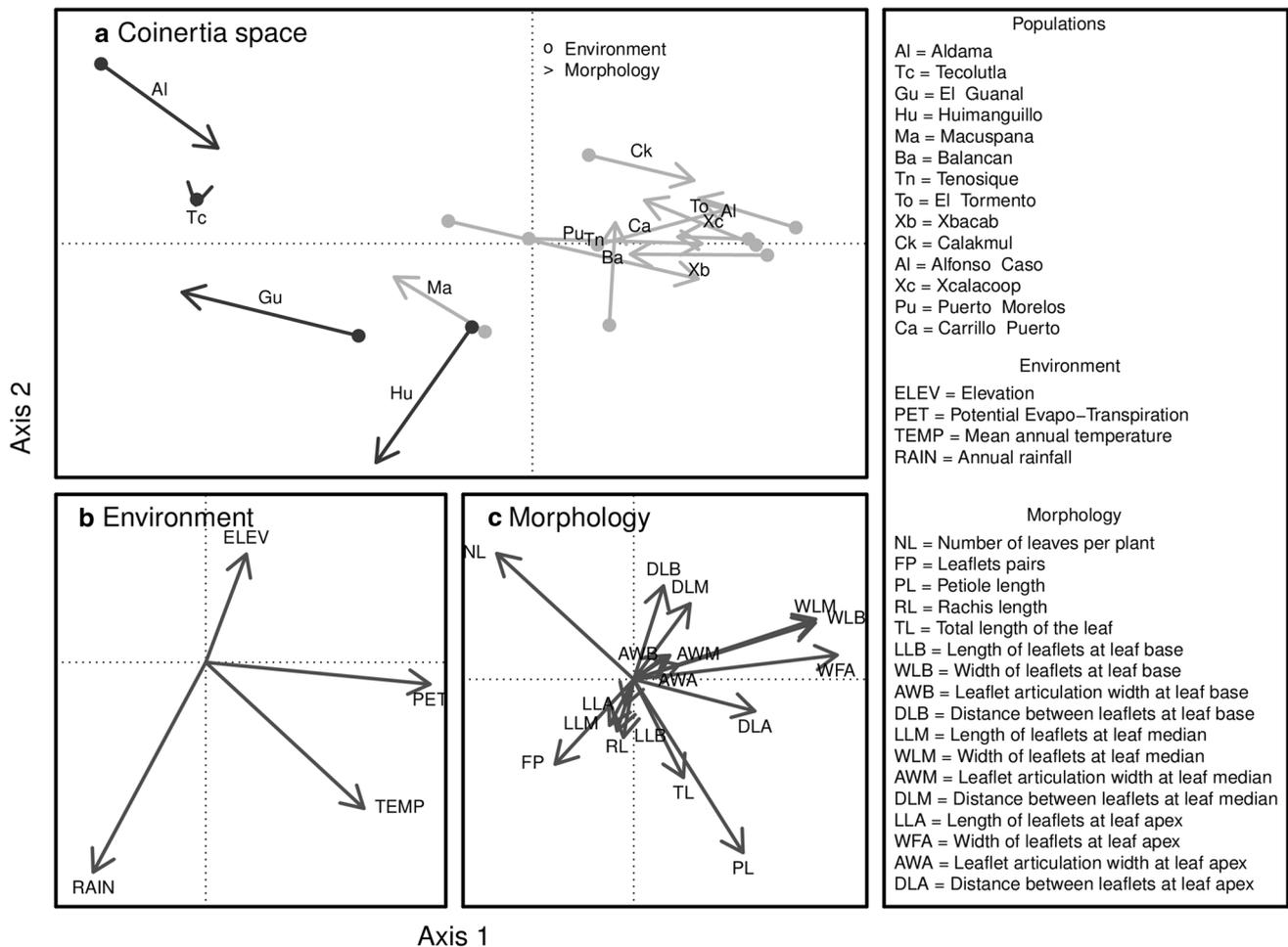


Fig. 3 Co-inertia analysis of four populations of *Zamia loddigesii* and ten from *Z. prasina*. Co-inertia space (a), the stem ends of the arrows (circles) represent populations based on morphological attributes, while arrowheads represent the environments in which

the populations studied occur. Morphological attributes panels (b) and environmental (c) the arrows indicate in which direction the panel co-inertia stocks with the highest values are located

observations of Stevenson et al. (1996), since our data indicate a relationship of morphological variation with the environment. Nevertheless, while all these patterns (i.e., chromosomal fission, karyological variation, or hybridization) appear to be related in some way to environmental heterogeneity, it is important to note that these relationships have never been tested empirically. There is therefore insufficient information to enable us to directly associate the morphological and environmental variation in our study with some of these processes.

Cycad taxonomists have frequently overlooked the implications that geographic and morphological (polymorphisms) variation ranges have on the delimitation of taxa (Newell 1985, 1986) leading them in many cases to establish controversial taxonomic decisions. For instance, *Z. loddigesii* with a total of 11 synonyms identified so far (Osborne et al. 2012) is a typical example of taxonomic ambiguity mainly caused by the high levels of

morphological polymorphisms observed across their distribution range (Nicolalde-Morejon et al. 2009).

Originally, *Z. loddigesii* was considered the species of *Zamia* with the greatest morphological variation and widest geographical distribution, from the state of Tamaulipas to the Yucatan Peninsula in Mexico, south into the Petén region in Belize and Guatemala; however, Stevenson et al. (1996) described the populations in Guatemala, Belize, and the Yucatan Peninsula as *Z. polymorpha* (= *Z. prasina*, Calonje and Meerman 2009), based on the great morphological variation observed in the populations in this region, and especially, megastrobilus indument used to separate it from *Z. loddigesii*.

Jointly, the evidence shows the existence of polymorphisms associated with different environmental conditions within each one of these species, and this seems to be an important characteristic in tropical species of wide distribution (e.g., *Quercus rugosa*, Uribe-Salas et al. 2008;

Ficus petiolaris, Piedra-Malagón et al. 2011). The mechanisms that permit the existence of these polymorphisms are a topic that must be investigated. With the evidence presented here, it is not possible to distinguish between phenotypic plasticity, polymorphisms with a genetic basis or with methylations and structural changes in the chromatin.

In general, cycads are plants that have had a geographic distribution determined as much by ancient historical processes, as by recent dynamics (Nagalingum et al. 2011). Therefore, their morphological variation is shaped by some deep geological process as well as recent ecological events. For instance, the Aldama (Tamaulipas) population grows on formations that date from 65 my ago, whereas the populations from the Yucatan Peninsula are on a substrate that is approximately 5.3 my old, while the contact zone between the two species (Tabasco) is from the most recent origin (ca. 2.5 my old, INEGI 2002). This scenario is important to understand the processes that unite geological dynamics, evolutionary processes of colonization, and distribution of these two species. For that reason, future investigations require the integration of this information with molecular data and lineage dating (e.g., Nolasco-Soto et al. 2015), this to be able to understand the evolutionary processes that have molded these species, aspects that can be addressed from the phylogeography, and the historical demography of the species.

Conclusion

The morphological and geographical variation in two phylogenetically related species of cycads throughout their ranges allowed inferences regarding the differential effect of environmental variation (vegetation types, temperature, altitude, and precipitation) on the phenotype, which generates local adaptations in varying environments. Also, under this perspective, multivariate analysis can distinguish between two species with geographically close populations and in similar ecological conditions. Finally, to further corroborate these findings, phylogeographic and population genetics studies at various levels are required.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Information on Electronic Supplementary Material

Online Resource 1. Plants evaluated in *Zamia loddigesii* by access number from the National Collection of Mexican Cycads, Francisco Javier Clavijero (JBC) Instituto de Ecología A. C.

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