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Morphological variation of *Cosmos bipinnatus* (Asteraceae) and its relation to abiotic variables in central Mexico

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Abstract

Background: Morphological variability can lead to serious taxonomic problems in species with wide distribution ranges. Although morphological variability is partly due to ontogenetic programming, abiotic variables can also exert a significant effect on micro- and macromorphological characters. In this paper, we studied the morphological variability (43 characters) of *Cosmos bipinnatus* associated to different vegetation types in central Mexico. We searched for significant correlations between the overall morphology of *C. bipinnatus* and abiotic variables such as altitude and soil parameters (pH, organic matter content, NH₄, NO₃, PO₄, total N and total P content). We also analyzed the Simplified Relative Distance Plasticity Index (RDPI_s).

Results: Locality had a significant effect in all but three morphological characters measured. Also, 71.43 % of the characters had a significant correlation with at least one abiotic variable. PO₄ content was significantly correlated with paleae characters, while pH had a significant effect in ligule coloration. Discriminant function analysis revealed that *C. bipinnatus* individuals collected at grasslands and *Pinus* forests form separate clusters, while individuals collected at scrubs and *Quercus* forests showed considerable overlap. The RDPI_s across all sites showed very low levels of plasticity in almost all characters.

Conclusions: Some abiotic variables (altitude, soil NH₄ and PO₄ content, and soil pH) largely contribute to the differential phenotypic expression of *C. bipinnatus* in central Mexico. However, we found that the number of external phyllaries, the trichome length, and the petiole area can be considered diagnostic traits of *C. bipinnatus* as they did not show differences within and between collected sites. We hypothesize that the low levels of plasticity found in *C. bipinnatus* across sites is due to the high tolerance of the species to different environmental conditions.

Keywords: Macromorphology; Micromorphology; Phenotype; Phenotypic plasticity reproductive character; Vegetative character

Background

Species with wide distribution ranges offer the opportunity to explore the evolutionary and ecological factors acting on their phenotypic expression. Usually, these species show local adaptations or an increased phenotypic plasticity, leading to different morphological and physiological characters in response to environmental conditions (Kollmann and Bañuelos 2004). If there is a great

variability in the abiotic conditions, species with wide distribution ranges may exhibit differential phenotypes that ultimately lead to taxonomic confusion (Ponsie et al. 2009; Semir et al. 2014). In extreme cases, morphological variability within a species may cause the split of one real taxon into two, or it may cause the merging of two real taxa into one species (Kieltyk and Mirek 2014).

A large quantity of studies employ a morphometric approach of natural populations or herbarium material to solve taxonomic problems due to high levels of intraspecific morphological variability (Conesa et al. 2012; Kieltyk and Mirek 2014). However, the main disadvantage in using

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solely a morphometric approach in natural populations is the difficulty to distinguish between factors that originate the observed variation. Genetic diversity and phenotypic plasticity may contribute importantly to the phenotypic variance observed in natural populations (Schlichting 1986; Nicotra et al. 2010). Unless plants are cultivated in uniform conditions (greenhouse experiments), the relative contribution of these factors is unknown. In order to avoid such constraints of morphometric analysis, several authors combine morphological as well as molecular data to disentangle the taxonomic status of a certain population (e.g., Reed and Frankham 2001; Kuta et al. 2013; Stenøien et al. 2014). However, even when molecular data reveals the genetic structure of populations, these studies are time consuming and they do not provide with sufficient data that permit the identification of a species under field conditions. Additionally, genetic diversity has been found to be a poor predictor of quantitative genetic variability (Reed and Frankham 2001). Moreover, the study of the morphology of a certain taxon in contrasting environmental conditions may permit the identification of morphological diagnostic markers and, thus, avoid potential taxonomic confusions.

Abiotic variables such as water stress, mineral nutrient deficiency, and geographical position may have an effect in the overall plant morphology. For example, latitude is negatively correlated with plant height (Moles et al. 2009), while altitude may have a significant effect in leaf morphology and plant height (Ran et al. 2013; Vitasse et al. 2013). Soil parameters also contribute to different macromorphological character expression in plants. Nitrogen (N) and phosphorous (P) are the two critical limiting elements in plant growth due to their poor availability in the soil. Shifts in availability of these nutrients may lead to changes in root architecture (López-Bucio et al. 2003) and in macromorphological characters (Trubat et al. 2006). Another abiotic factor that greatly limits plant growth

and development is water availability. Drought reduces plant growth by affecting several biochemical and physiological processes (Holmgren et al. 2011). As a result, plants under water stress show reduced height measured as stem length (Specht et al. 2001; Zhang et al. 2004; Manivannan et al. 2008) and reduced leaf growth and leaf area (Wullschleger et al. 2005; Manivannan et al. 2008; Farooq et al. 2009), and also, water stress reduces head diameter and yield of achenes in sunflowers (Mozaffari et al. 1996).

Cosmos bipinnatus Cav. (Asteraceae, Coreopsidae) is an herbaceous annual species with a wide distribution range in Mexico. It can be found mainly in the montane regions of the country (Vargas-Amado et al. 2013), where it occupies disturbed sites associated to several vegetation types with contrasting environmental conditions (grasslands, scrubs, *Quercus* forests and *Pinus* forests). The stems and leaves of *C. bipinnatus* are almost glabrous; with the leaves being bipinnate with threadlike segments. The inflorescences usually exhibit purple to pink ligules. However, atypical individuals with white ligules have been recorded. Recently, Paniagua-Ibáñez et al. (2014) employed a morphometric approach to propose the intraspecific taxon *C. bipinnatus* var. *albiflorus*, where white ligule individuals are included.

In this study, we explored the intraspecific micro- and macromorphological variability of *C. bipinnatus* in four different vegetation types in central Mexico (grasslands, scrubs, *Quercus* forests, and *Pinus* forests). We searched for significant relations of the measured morphological characters of *C. bipinnatus* with abiotic variables such as altitude and several soil parameters (pH, organic matter [OM], NH₄, NO₃, PO₄, total N and total P content). We hypothesized that the environmental pressure operating at each vegetation type will have a significant effect in the overall morphology of *C. bipinnatus*. More specifically, we expect that *C. bipinnatus* individuals growing at

Table 1 Sampled populations of *Cosmos bipinnatus* in central Mexico

Vegetation type/locality	State	Coordinates (UTM)		Altitude (m)	Soil type
Grassland					
Huehuetoca	Mexico State	0470736	2193770	2277	Vertisol
Santa Martha	Mexico State	0459458	2108525	2840	Andisol
Scrub					
Botanical Garden	Mexico City	0479893	2135838	2324	Phaeozem
Concordia	Mexico State	0471449	2158680	2406	Phaeozem
<i>Pinus</i> forest					
Tres Marías	Morelos	0474982	2108707	2882	Andisol
San Salvador	Mexico City	0489057	2122730	2667	Phaeozem
<i>Quercus</i> forest					
Cerro de la Estrella	Mexico City	0490294	2137729	2315	Phaeozem
Parque Ecológico	Mexico City	0479145	2129959	2573	Leptosol

Table 2 Micro- and macromorphological characters measured in *Cosmos bipinnatus*

Character abbreviation	Character description	Units
Macromorphological		
Vegetative		
TH	Total individual height	cm
H1P	Height to first peduncle	cm
Ple	Peduncle length	cm
PW	Peduncle width	mm
NEP	Number of external phyllary series	no.
NIP	Number of internal phyllary series	no.
MWL	Maximal width of lamina	cm
TL	Total laminal length	cm
PL	Petiole length	cm
TLL	Total leaf length	cm
MWPe	Maximal width of petiole	mm
MVD	Midvein diameter	mm
NS	Number of leaf segments	no.
FA	Foliar área	cm ²
PA	Petiole área	cm ²
TPL	Total palea length	mm
MWP	Maximal width of palea	mm
OPW	Outer phyllary series width	mm
LOP	Length of outer phyllary series	mm
IPW	Inner phyllary series width	mm
LIP	Length of inner phyllary series	mm
Reproductive		
FW	Fruit width	mm
FL	Fruit length	mm
TIW	Total inflorescence width	mm
DW	Disk width	mm
IH	Inflorescence height	mm
LN	Ligule number	no.
CL	Total corolla length of disk floret	mm
MWC	Maximal width of disk floret's corolla	mm
DFL	Total disk floret length	mm
LL	Length of ligule	mm
MWLi	Maximal width of ligule	mm
R	Red	no.
G	Green	no.
B	Blue	no.
C	Cian	no.
M	Magenta	no.
Y	Yellow	no.

Table 2 Micro- and macromorphological characters measured in *Cosmos bipinnatus* (Continued)

Micromorphological characters		
TrL	Trichome length	µm
SI	Stomatal index	no.
SL	Stoma length	µm
SW	Stoma width	µm

drier and warmer sites (i.e., scrubs and grasslands) will exhibit reduced height and shorter reproductive and vegetative structures compared to individuals growing at wetter and more temperate sites (i.e., *Pinus* forest and *Quercus* forest).

Methods

Study species

Cosmos bipinnatus Cav. is a composite annual herbaceous species belonging to the tribe Coreopsideae (Funk et al. 2009). This species is usually associated to disturbed sites such as roads and agricultural sites. It usually grows as an herb up to 2 m tall with erect, almost glabrous, and slightly branched stems. The leaves of *C. bipinnatus* may be sessile or with a winged petiole. The lamina is bipinnated with threadlike segments 5–20 mm long and 0.5–3 mm wide with an acuminate apex. The peduncles are up to 30 cm long and may bear one or two inflorescences. The inflorescences exhibit two series of phyllaries which may be lanceolate to ovate-lanceolated. The ligulated flowers are usually lilac, but pink and white may also occur (Rzedowski and Rzedowski 2001). Recently, Paniagua-Ibáñez et al. (2014) used a morphometric approach to distinguish between the white and lilac types of *C. bipinnatus*; the proposed name for the white morph is *C. bipinnatus* var. *albiflorus* (Paniagua-Ibáñez et al. 2014).

Cosmos bipinnatus is distributed in the montane regions of Mexico, particularly in the The Eje Neovolcánico

Table 3 Mean ± standard error and two-way ANOVA results for each soil parameter measured in eight localities in central Mexico

Soil parameter	F		Mean ± s.e.	Units
	Locality (d.f. = 7)	Transect (d.f. = 16)		
pH	10.60***	2.21**	6.65 ± 0.05	
OM	17.96***	1.65 ns	6.02 ± 0.37	%
NH ₄	5.68***	1.91**	15.32 ± 0.74	ppm
NO ₃	3.09***	2.13**	9.92 ± 0.28	ppm
PO ₄	26.77***	2.32***	2.89 ± 0.18	ppm
N _t	11.82***	1.65 ns	0.41 ± 0.02	%
P _t	14.13***	1.71 ns	0.07 ± 0.01	%

ns not significant
P < 0.01, *P < 0.001

(EN), an orographic system that traverses the central part of the Mexico in an east–west direction (Vargas-Amado et al. 2013). *Cosmos bipinnatus* may be found at altitudes between 2250 and 2750 m. Due to its complex topography, climates, and geological history, the EN is usually considered a center of diversification of a large number of taxa, including the Asteraceae family (Ramamoorthy et al. 1993, Turner and Nesom 1998, Vargas-Amado et al. 2013). Within EN, *C. bipinnatus* may be associated to grasslands, scrubs, and temperate forests dominated by *Pinus* and/or *Quercus*. Due to the wide distribution range of *C. bipinnatus*, this species exhibit a wide phenotypic variation across its range (Rzedowski and Rzedowski 2001).

Collecting sites and micro- and macromorphometric procedure

For this study, we chose eight localities where *C. bipinnatus* was the dominant species (Table 1). We chose two sites where *C. bipinnatus* is associated with each of the following vegetation types: grassland (Huehuetoca and Santa Martha), scrub (Botanical Garden, Concordia), *Pinus* forest (Tres Marías, San Salvador), and *Quercus* forest (Cerro de la Estrella, Parque Ecológico). The study sites present four soil types: Vertisol, Leptosol, Andisol, and Phaeozem (Driessen and Deckers 2001) (Table 1). In each of these sites, three transects of 50 m were drawn; 10 squares of 1 m² were marked each 5 m. In each quadrant, we randomly chose one individual of *C. bipinnatus* with at least three inflorescences and no apparent mechanical damage (*n* = 60 individuals per vegetation type).

For the macromorphological measurements, we collected six leaves of each individual. Also, from each of the three inflorescences collected, we chose randomly six of the following structures: ligules, inner phyllaries, outer phyllaries, disk florets, pales, and achenes. Due to the fragility of these structures, these parts were attached by adhesive tape to paper, where the macromorphological characters were measured (Table 2). In addition, to these characters, we also used computer digitalized images of the ligules of each individual in order to quantify their color (parameters cyan, magenta, red, blue, yellow). Images were processed with Adobe Photoshop software (Adobe Systems). Macromorphological characters were classified as *reproductive* or *vegetative* in accordance to Table 2.

For micromorphological analysis, we selected two leaves per individual. Each leaf was conserved with ethanol 70 %. Next, three semipermanent slides of the leaf were done by the replicate technique. These slides were used to determine the types of stoma in *C. bipinnatus* and the stomatal index (Salisbury 1968). Also, permanent slides of the leaves' epidermis were done. These

Table 4 Mean ± standard error for each morphological variable measured in eight populations of *Cosmos bipinnatus* in central Mexico. Nested ANOVA results (*F*) are also shown

Morphological variables	<i>F</i>		Mean ± s.e.
	Locality (<i>d.f.</i> = 7)	Transect (<i>d.f.</i> = 16)	
Macromorphological characters			
Vegetative			
PLe	48.82***	3.43***	152.81 ± 2.33
PW	77.21***	9.03***	1.31 ± 0.01
NEP	1.17 ns	1.63 ns	8.01 ± 0.01
NIP	2.55*	2.95***	8.01 ± 0.01
MWL	119.42***	39.29***	66.19 ± 0.66
TL	162.35***	31.40***	83.86 ± 0.61
PL	32.76***	25.16***	6.43 ± 0.07
TLL	182.29***	39.56***	90.67 ± 0.64
MWPe	64.59***	35.75***	3.12 ± 0.03
MVD	71.99***	18.85***	1.41 ± 0.02
NS	132.98***	30.94***	9.41 ± 0.04
FA	177.83***	73.75***	9.77 ± 0.18
PA	1.84 ns	1.51 ns	0.14 ± 0.01
TPL	172.36***	10.88***	8.07 ± 0.03
MWP	204.28***	27.60***	1.48 ± 0.01
OPW	813.39***	35.56***	2.72 ± 0.1
LOP	2147.51***	65.10***	7.95 ± 0.03
IPW	1615.34***	66.48***	4.02 ± 0.02
LIP	5.94***	1.27 ns	10.31 ± 0.25
Reproductive			
FW	25.21***	17.28***	1.43 ± 0.02
FL	169.80***	17.88***	7.04 ± 0.03
TIW	230.20***	3.47***	51.68 ± 0.5
DW	1.01 ns	1.00 ns	19.56 ± 9.79
IH	71.78***	12.41***	10.84 ± 0.12
LN	10.99***	8.98***	8.07 ± 0.03
CL	114.91***	72.34***	5.34 ± 0.02
MWC	25.21***	17.28***	1.43 ± 0.02
DFL	169.80***	17.88***	7.04 ± 0.03
LL	1741.35***	18.78***	28.53 ± 0.11
MWLi	2857.95***	67.06***	14.85 ± 0.07
Micromorphological characters			
TrL	1.61 ns	2.27 ns	111.72 ± 2.01
SI	7.60***	0.07 ns	76.85 ± 1.17
SL	13.92***	3.71***	30.16 ± 0.25
SW	12.71***	6.27***	20.64 ± 0.22

ns not significant
 P* < 0.05, *P* < 0.01, ****P* < 0.001

slides were analyzed by high vacuum scanning electron microscopy (HVSEM) and by differential interference contrast microscopy (DICM). We used these slides to characterize the trichomes and papillae present in *C. bipinnatus*.

Soil analysis

In order to characterize the soil, we systematically collected four soil samples along a transect of 40 m (we

collected a sample each 10 m). For the soil analysis, we weighted 200 g of dry soil which was previously sieved with a 2-mm mesh. For each sample, we quantified the following variables: pH, content of organic matter (MO; measured with a Total Organic Carbon Analyzer, the CO₂ carbon released during the combustion was measured by Non-Dispersive Infrared Detection), ammonium content (NH₄; quantified after a extraction with KCl), nitrate content (NO₃; quantified through ion

Table 5 Regression analysis between morphological variables measured in *Cosmos bipinnatus* and abiotic variables in central Mexico. Only variables with significant correlations ($P < 0.05$) are shown

Morphological character	Altitude		NH ₄		PO ₄		P _t		pH		OM		N _t		NO ₃		
	R	R ²	R	R ²	R	R ²	R	R ²	R	R ²	R	R ²	R	R ²	R	R ²	
Macromorphological characters																	
Vegetative																	
TH	-0.45	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
H1P	-0.67	0.44	0.44	0.19	-	-	-	-	-	-	-	-	-	-	-	-	-
Ple	-	-	-	-	-	-	-	-	-	-	0.67	0.45	0.44	0.19	-	-	-
PW	0.74	0.55	-0.51	0.26	-	-	0.69	0.47	-	-	-	-	-	-	-	-	-
MWL			0.45	0.20	0.43	0.18	-	-	-	-	-	-	-	-	-	-	-
TL	-0.46	0.21	0.43	0.18	-	-	-	-	-	-	-	-	-	-	-	-	-
TLL	-0.46	0.21	0.42	0.18	-	-	-	-	-	-	-	-	-	-	-	-	-
MWPe	0.45	0.20	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
MVD	-	-	-	-	-	-	-0.52	0.26	-	-	-0.54	0.28	-0.42	0.17	-	-	-
PA	-	-	-0.41	0.16	-	-	-	-	-	-	-	-	-	-	-	-	-
TPL	-0.56	0.31	-	-	0.68	0.46	-	-	0.42	0.18	-	-	-0.55	0.30	-	-	-
MWP	-0.57	0.32	0.59	0.34	0.52	0.27	-	-	-	-	-	-	-	-	-	-	-
OPW	-	-			0.41	0.17	0.49	0.24	-	-	0.49	0.24	-	-	-	-	-
LOP	-	-	0.53	0.28	0.49	0.24	0.59	0.34	-	-	0.56	0.31	-	-	-	-	-
IPW	-	-	0.46	0.21	0.47	0.22	0.41	0.16	-	-	-	-	-	-	-	-	-
LIP	-	-	-	-	0.73	0.53	-	-	-	-	-	-	-	-	-	-	-
Reproductive																	
TIW	-	-	-	-	0.52	0.28	0.41	0.16	-	-	-	-	-	-	-	-	-
DW	-	-	-0.41	0.16	-	-	-	-	-	-	-	-	-	-	-	-	-
IH	-	-	0.42	0.18	-0.45	0.21	-	-	-	-	-	-	-	-	-	-0.56	-0.31
DFL	-	-	-	-	0.62	0.39	-	-	-	-	-	-	-	-	-	-	-
LL	-	-	0.41	0.17	0.53	0.28	-	-	-	-	-	-	-	-	-	-	-
MWLi	-	-	0.46	0.21	0.50	0.25	0.43	0.19	-	-	0.45	0.16	-	-	-	-	-
R	-0.52	0.27	-	-	0.48	0.23	-	-	0.56	0.32	-	-	-	-	-	-	-
G	-0.49	0.23	-	-	-	-	-	-	0.56	0.31	-	-	-	-	-	-	-
B	-	-	-	-	-	-	-	-	0.58	0.34	-	-	-	-	-	-	-
C	0.49	0.24	-	-	-0.48	0.23	-	-	-0.51	0.26	-	-	-	-	-	-	-
M	0.42	0.17	-	-	-	-	-	-	-0.46	0.21	-	-	-	-	-	-	-
Micromorphological characters																	
SI	-	-	-0.42	0.17	-	-	-0.47	0.21	-	-	-0.46	0.21	-	-	-	-	-
SL	0.55	0.30	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

DF discriminant function

capillary electrophoresis), phosphate content (PO_4), total nitrogen content (N_t ; following the method of Bremner (1965)) and total phosphorous (P_t ; measured through the method developed by Bray and Kurtz (1945); Table 3).

Data analyses

In order to determine the effect of collecting site (locality), transect and individual on the micro- and macromorphological characters measured in *C. bipinnatus*, we conducted nested Analysis of Variance (ANOVA). Individuals were nested within transect, and the latter within collecting site. All discontinuous data were transformed as $[(x) \frac{1}{2} + 0.5]$ (Zar 2010). We conducted two-way ANOVA to determine the effect of collecting site and transect on the soil parameters measured. Also, regression analyses were performed between altitude and the soil parameters measured.

Then, to determine the relation between the abiotic variables (altitude, soil parameters) and the morphology of *C. bipinnatus*, we conducted regression analysis between these data sets. Discriminant function analysis (DFA) was used to determine how macromorphological characters separate individuals into groups. We establish vegetation type as the predictor variable of the groups. For DFA, we excluded the variables that showed correlation coefficients >0.6 in order to improve the performance of the analysis. Also, we performed linear regression analyses with vegetation type as independent variable and the morphological characters as dependent variables. For this analysis, we rank vegetation types along an environmental gradient according to increasing water availability and decreasing temperature (Scrub—Grassland—*Quercus* forest—*Pinus* forest).

For each character measured, we calculated the Simplified Relative Distance Plasticity Index (RDPI_s; Valladares et al. 2006). The RDPI_s measures the relative distances between the mean values of the chosen characters measured for all pairwise comparisons of vegetation types. The values of RDPI_s range from 0 (no plasticity) to 1 (maximal plasticity). For more details of calculation of RDPI_s revise Valladares et al. (2006).

Finally, to determine the intercorrelation within vegetative and reproductive characters, the Pearson product moment correlation was computed between both data sets. JMP software version 11 (SAS Institute, Cary, NC) was used for all statistical analyses.

Results

Nested ANOVA revealed a significant effect of collecting site (locality) in all macromorphological vegetative (except for number of external phyllary series and petiole area) and reproductive characters (except disk width), as well as micromorphological characters (except trichome length; Table 4). Transect had a significant effect in all

macromorphological vegetative (except number of external phyllary series, petiole area and length of inner phyllary series) and reproductive characters (except disk width). Also, transect had an effect in all but two (trichome length and stomatal index) micromorphological characters. In a similar fashion, locality had a significant effect in all soil parameters measured, while locality had a significant effect in all but three (OM, N_t , and P_t) soil parameters (Table 3). However, altitude did not have a significant effect on any soil parameter, except for NH_4 content, which exhibited a negative correlation with altitude ($R^2 = 0.29$, $R = -0.54$, $P < 0.05$).

Regression analyses showed that 71.43 % of the morphological characters measured in *C. bipinnatus* had an effect with at least one of the abiotic variables (altitude and soil parameters) considered in this study (Table 5). Five vegetative characters, five reproductive characters, and two micromorphological characters did not have a significant effect with any of the abiotic variables. The remainder characters were significantly related with at least one abiotic variable (Table 5). No obvious patterns

Table 6 Standardized canonical discriminant function coefficients of morphological variables measured in *Cosmos bipinnatus* in eight populations in central Mexico

Morphological variable	DF1	DF2
TH	-0.49	0.28
H1P	0.14	0.01
Ple	0.15	0.32
PW	0.26	-0.69
NEP	0.06	0.02
NIP	0.07	-0.03
MWL	-0.14	0.39
PL	0.04	-0.08
MWPe	0.36	-0.23
NS	-0.40	0.23
PA	0.03	0.01
TPL	-0.02	0.34
MWP	0.14	-0.15
LIP	0.16	0.17
FW	-0.10	-0.33
FL	-0.57	-0.39
DW	0.58	0.18
IH	0.29	0.09
LN	0.05	-0.10
CL	0.09	0.06
MWC	0.17	0.13
Y	-0.06	-0.24
Eigenvalue	2.67	1.93
Percent variation	52.98	38.25

arise from these analyses. However, it is interesting to outline the relation between PO₄ content and palea and phyllary-related characters. Soil pH had a significant effect in all characters related with ligule color. Altitude and PO₄ also had an effect in ligule color (Table 5). Finally, NH₄ content was significantly related with the length and width of leaves, paleae, and ligules. It is noteworthy that the vast majority (92.30 %) of the values of R² < 0.35 (Table 5).

DFA with vegetation type as the grouping variable generated two significant discriminant functions (DF) that explained 91.23 % of the variation in the original data set. Length variables of disk, fruit, and total individual height had the highest standardized canonical discriminant function coefficients in DF1, while the width of lamina, paleae, and peduncle length and the maximal width of lamina showed the highest discriminant function coefficients in DF2 (Table 6). A plot of canonical scores of DF2 vs DF1 was generated (Fig. 1). In general, *C. bipinnatus* individuals collected at grasslands and *Pinus* forests show almost no overlap with individuals collected at another vegetation type. However, individuals collected at scrubs and *Quercus* forests show extensive overlap in the ordination space (Fig. 1).

Regression analyses with vegetation type as independent variable and all morphological characters as dependent variables revealed that 44 % of the characters were affected by the environmental gradient (scrub—grassland—*Quercus* forest—*Pinus* forest) total

individual height and leaf size-related characters decreased along the environmental gradient. In contrast, reproductive characters tend to increase along the gradient. In particular, ligule characters and floral characters were affected by the environmental gradient. Also, the length and width of stomata increased along the environmental gradient (Table 7).

The RDPI_s showed that the morphological characters measured in *C. bipinnatus* showed low plasticity values across sites. Only 6.97 % of all characters showed plasticity values above 0.2, while 44.18 % of the characters showed plasticity values close to zero (<0.9; Table 8).

A matrix of pairwise correlation between macromorphological characters is shown in Additional file 1. All characters showed correlation with at least one character. We found that ligule, bract, foliar, and phyllary size-related characters showed massive correlations within themselves. Particularly, ligule width and ligule length were correlated with 76.31 and 68.42 % of the characters, respectively. On the other hand, ligule number was correlated with only 8 % of the characters. Fruit width showed correlation with only 18.42 % of the characters. Finally, characters related with ligule color showed an overall low percentage of correlations with other characters (Additional file 1).

Finally, permanent slides analyzed through HVSM and DICM revealed the presence of simple uniseriated trichomes in the leaf epidermis of *C. bipinnatus*. Also, papillae

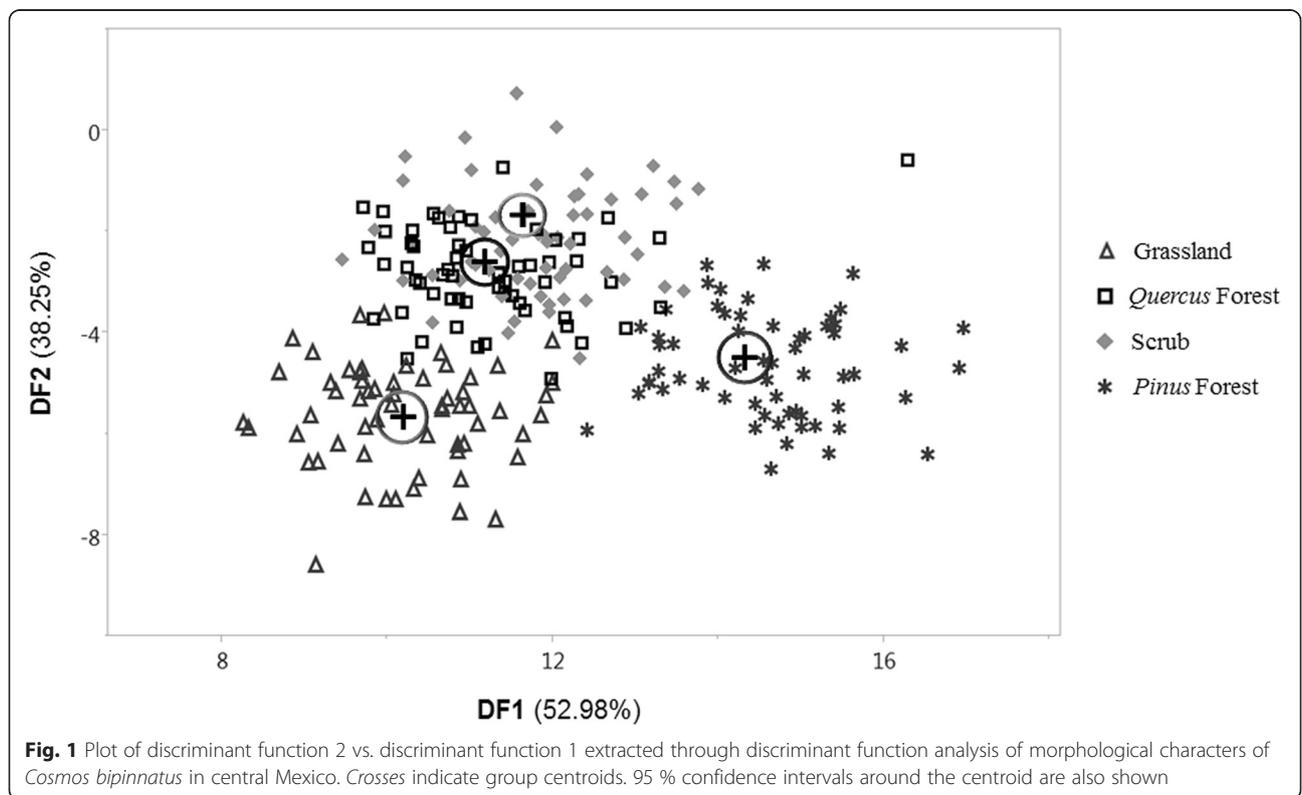


Table 7 Effect of the environmental gradient (scrub—grassland—*Quercus* forest—*Pinus* forest) on morphological characters revealed by regression analysis. Only morphological variables with significant relations with the environmental gradients are shown ($P < 0.05$). For character description refer to Table 2

Character	R^2	R	$F_{(3238)}$
Macromorphological vegetative			
TH	0.18	−0.42	54.62
PW	0.13	0.36	36.77
TL	0.10	−0.32	26.93
TLL	0.09	−0.30	24.17
OPW	0.10	0.32	28.78
LOP	0.06	0.24	16.28
IPW	0.15	0.39	43.82
Macromorphological reproductive			
FW	0.02	0.14	6.89
TIW	0.19	0.44	58.91
DW	0.17	0.41	51.88
IH	0.05	0.22	14.96
CL	0.10	0.32	27.08
MWC	0.07	0.26	18.05
DFL	0.13	0.36	36.54
LL	0.14	0.37	39.5
MWLi	0.11	0.33	30.21
C	0.11	0.33	31.49
Micromorphological			
SL	0.11	0.33	5.99
SW	0.10	0.32	5.23

were found in the leaf margins as well as in the secondary veins, while stomata were also present (Fig. 2). Two types of stomata were found: anisocytic and anomocytic.

Discussion

Species with wide distribution ranges usually exhibit broad morphological variability. Abiotic variables may have a significant effect on the phenotype of a species. As a consequence, assessing morphological variability in widespread species often brings taxonomic confusion, due to the wide array of phenotypes that taxa may exhibit (Geng et al. 2006; Conesa et al. 2012; Kiełtyk and Mirek 2014; Paniagua-Ibáñez et al. 2014). In this study, we assessed the morphological variability of *C. bipinnatus* under field conditions and its relation to abiotic conditions (altitude and soil parameters).

The analyses performed in this study revealed that 40 out of 43 characters had a significant effect on vegetation type and transect as revealed by nested ANOVA results (Table 4). This finding reveals that *C. bipinnatus* shows a

broad morphological variation. This result is in accordance with previous taxonomic work (e.g., Rzedowski and Rzedowski 2001). Despite the great morphological variability shown by *C. bipinnatus*, we were able to identify some characters that may be considered as diagnostic traits. The number of external phyllaries, trichome length, and petiole area did not show a significant effect of locality and transect as revealed by ANOVA. These characters show low dispersion values, and thus, we propose that these can be employed as species diagnostic morphological characters (Table 4).

Although we found high morphological variability of *C. bipinnatus*, the RDPI_s across all sites showed that almost all characters showed plasticity values close to zero (Table 8). We explain these findings with the following argument. *Cosmos bipinnatus* is a species associated to human-disturbed sites (e.g., roadsides, agricultural sites) and early succession stages (Rzedowski and Rzedowski 2001; Vargas-Amado et al. 2013). As such, these species often exhibit a high tolerance to adverse conditions such as high solar exposure and drought (Hobbs and Huenneke 1992; Andraski and Bundy 2003), exhibiting physiological adaptations to these particular conditions (Caplan and Yeakley 2013). So, we hypothesize that the populations of *C. bipinnatus* sampled in this study are not in severe physiological stress, resulting in low values of phenotypic plasticity across all sites. Previous studies have demonstrated that agricultural habitats and human-disturbed habitats are heterogeneous habitats regarding nutrient availability, leading to population differentiation and ultimately leading to contrasting morphologies within sites (Leiss and Müller-Schärer 2001). Thus, the heterogeneity of the environmental conditions on a small spatial scale (within sites and transects) could be the main source of phenotypic variation for *C. bipinnatus*. In order to test this hypothesis, future studies should obtain pure lines of *C. bipinnatus* and perform reciprocal transplants in contrasting environments in order to evaluate precisely the contribution of phenotypic plasticity in this species.

Differential morphological expression of *C. bipinnatus* under different vegetation types

We expected that individuals growing at scrubs and grasslands would exhibit a smaller height with small reproductive and vegetative structures compared with individuals growing at *Quercus* forests and *Pinus* forests. Interestingly, we found the opposite pattern in some vegetative characters. In particular, individuals which tend to be smaller with shorter leaves were present at *Quercus* and *Pinus* forest compared with *C. bipinnatus* individuals growing at scrubs and grasslands. However, all reproductive characters (flower-size-related characters) showed an increase in size along the environmental

Table 8 Simplified Relative Distance Plasticity Index (RDPI_s) across all sites for all macro- and micromorphological characters measured of *C. bipinnatus* in Central Mexico

Character abbreviation	RDPI _s
Macromorphological	
Vegetative	
TH	0.43
H1P	0.23
PLe	0.16
PW	0.15
NEP	0.00
NIP	0.00
MWL	0.15
TL	0.12
PL	0.06
TLL	0.12
MWP	0.08
MVD	0.05
NS	0.03
FA	0.16
PA	0.08
TPL	0.05
MWP	0.03
OPW	0.12
LOP	0.15
IPW	0.14
LIP	0.12
Reproductive	
FW	0.06
FL	0.11
TIW	0.19
DW	0.13
IH	0.10
LN	0.00
CL	0.08
MWC	0.15
DFL	0.10
LL	0.19
MWLi	0.24
R	0.02
G	0.02
B	0.00
C	0.11
M	0.03
Y	0.05

Table 8 Simplified Relative Distance Plasticity Index (RDPI_s) across all sites for all macro- and micromorphological characters measured of *C. bipinnatus* in Central Mexico (*Continued*)

Micromorphological characters	
TrL	0.04
SI	0.05
SL	0.05
SW	0.06

gradient. Also, size-related stomata characters also increased following the environmental gradient (Table 7).

Quercus and *Pinus* forests usually exhibit a greater water and nutrient availability compared with grasslands and scrubs in central Mexico. Thus, we expected an overall increase in size along the environmental gradient. The finding of the inverse pattern in some vegetative characters suggests that resource allocation towards vegetative characters is greater at grasslands and scrubs. Meanwhile, resource allocation in *Pinus* forests and *Quercus* forests favors an increase in size of reproductive structures (measured as flower-size-related characters). As *C. bipinnatus* is an annual herb, this strategy seems advantageous. In favorable sites, more nutrients will be available and the production of pollen and seeds would be enhanced, while at adverse conditions the production of higher plants with larger leaves would favor the photosynthetic activity of *C. bipinnatus*.

On the other hand, discriminant function analysis (DFA) revealed that individuals of *C. bipinnatus* collected at grasslands and *Pinus* forests were grouped into two distinct clusters, while individuals growing at scrubs and *Quercus* forests showed significant overlap (Fig. 1). The differences between the environmental conditions between grasslands and *Pinus* forests may act as an environmental pressure that leads to contrasting phenotypic expression of *C. bipinnatus*.

DFA also showed that individuals collected at scrubs and *Quercus* forest exhibited significant overlap (Fig. 1). Following the above argument, this pattern may arise because local conditions are very similar between sites. We hypothesize that the similarity in the environmental conditions between the sampled *Quercus* forests and scrubs may explain the observed pattern. In central Mexico, some *Quercus* forests (dominated by *Quercus deserticola* and *Quercus frutex* among others) are often associated with water-stress conditions similar to those found in scrubs (Medina Lemus and Tejero-Díez 2006). Also, severe deforestation in the last century in central Mexico has led to the establishment of shrub- or scrub-type vegetation in sites previously occupied by *Quercus* or other tree species (Rzedowski 1978; Challenger 1998; Galicia and García-Romero 2007). In particular, in one scrub locality sampled in this study (Concordia), we found several

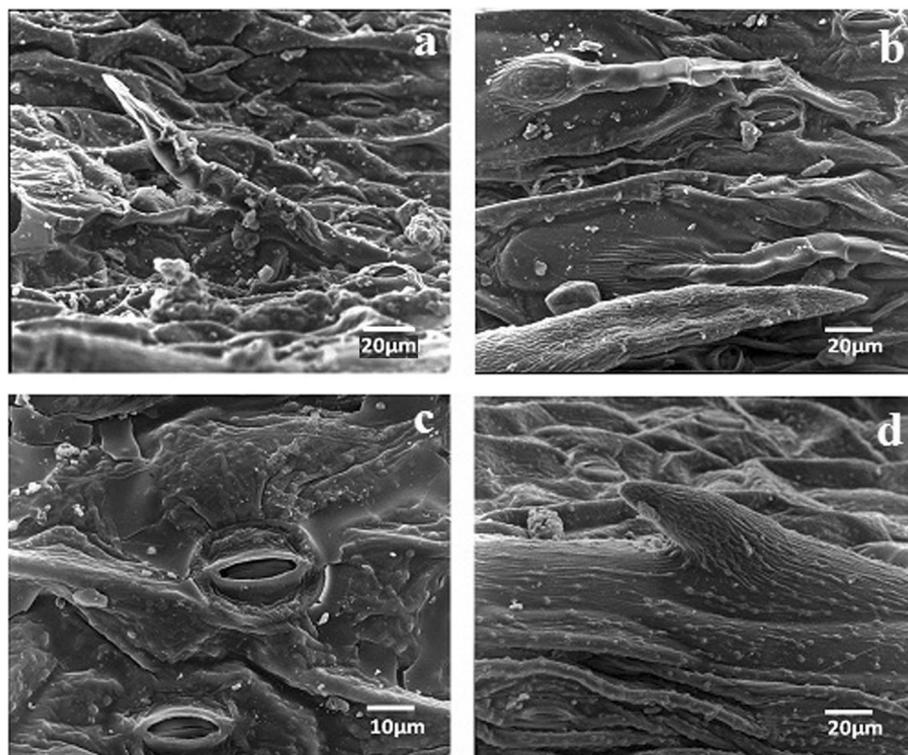


Fig. 2 Trichomes (a, b), stomata (c), and papillae (d) in leaf epidermis of *C. bipinnatus* revealed by high vacuum scanning electron microscopy. See text for details

sparsely *Quercus* sp. individuals in poor condition, suggesting that this site was formerly a *Quercus* forest (Medina Lemus and Tejero-Díez 2006). Thus, the similarity in the conditions between the Scrub and *Quercus* forest sampled in this study and the recent deforestation of *Quercus* forests may account for the overlap of *C. bipinnatus* individuals revealed by DFA.

Abiotic variables and character expression of *C. bipinnatus*

We found a significant effect of locality in all soil parameters measured (Table 3). Also, locality showed a significant effect in all but three micro- and macromorphological characters of *C. bipinnatus* (Table 4). Regression analysis between morphological and abiotic variables revealed some interesting patterns. We are aware that relatively low determination coefficients were obtained through the regression analyses. That is, variables not considered in this study (i.e., genetic) may also have a significant effect in morphological expression in *C. bipinnatus*. However, we briefly discuss the general patterns deduced in this study.

First, a negative correlation was found between total individual height and altitude ($r = -0.45$). This pattern may arise because altitude has a significant effect in precipitation and temperature (Dillion et al. 2006). Reduced

temperatures and shifts in precipitation may account for the observed pattern in height of *C. bipinnatus*. Smaller plants at high altitudes may also occur in response to NH_4 content. In this study altitude was negatively correlated with soil NH_4 content ($r = -0.54$). As nitrogen is a macronutrient found in proteins and nucleic acids, the increase in altitude will reduce the nitrogen available to the plant, leading to smaller plants. This pattern is coincident with other studies reporting smaller plants at increasing altitudes (Lavorel and Grigulis 2012). Besides the effect of soil NH_4 content in plant height, we found that this soil parameter was also significantly correlated with the overall size characters related with leaves, ligules, and paleae (Table 5). The role of NH_4 in plant nutrition may also account for this pattern.

Another pattern found between abiotic variables and the morphology of *C. bipinnatus* is the relation between ligule colors and pH. Interestingly, all color characters were related with soil acidity (Table 5). At greater soil acidity there is a trend for ligules to exhibit predominantly cyan and magenta colors (i.e., white), while at alkaline soils red, blue, and green will be the predominant colors (i.e., purple). This pattern may be partially explained by the differential colors that anthocyanins may exhibit when subjected to different pH levels (Bueno et al. 2012). These pigments are water soluble and exhibit a red coloration

when subjected acid solutions, purple in near neutral pH and blue in alkaline solutions (Bueno et al. 2012). Thus, soil pH may be partially involved in ligule pigmentation. However, genetic factors may greatly contribute to ligule color. As mentioned earlier, Paniagua-Ibáñez et al. (2014) proposed the intraspecific taxa *C. bipinnatus* var. *albiflorus* where white ligule individuals are included. Individuals of *C. bipinnatus* var. *albiflorus* were found in close proximity to *C. bipinnatus* with purple ligules. The soil pH of the locality where both taxa were found was nearly neutral (pH = 7.07). So, we hypothesize that ligule color is under genetic control, while soil pH may be playing a minor role.

In addition to soil pH and NH_4 content, PO_4 content in soil also had a significant effect in a number of characters. In particular, PO_4 showed a positive significant effect in several reproductive characters related with the overall size of ligules and inflorescences. Also, vegetative characters such as the size of phyllaries were positively and significantly correlated to soil's PO_4 content (Table 5). Phosphorous is considered one of the most important nutrients in terrestrial ecosystems (Donahue et al. 1997) and is considered a macronutrient in plants. As such, the lowered levels of phosphorous have been related with a diminished production of flowers and leaves as well as poor performance of plants (Marschner 2002; Agusti 2003; Lavorel and Grigulis 2012).

Character correlation in *C. bipinnatus*

Morphological character correlation is a well-known feature of plants (Price and Weitz 2012). However, the study of character correlation in a particular taxon is important, as this will facilitate the selection of characters in a morphological study: the characters that exhibit more independence would be the ideal ones to be included in morphological analysis.

For *C. bipinnatus*, we found massive character correlation; all macromorphological characters were correlated with at least one character (Additional file 1). This raises an interesting question regarding morphometric studies: which characters should we employ in the data analysis? In this particular case, we employed a DFA analysis to discriminate between individuals growing at different vegetation types. DF1 clearly separated individuals into groups (Fig. 1). Variables total individual height (TH), number of leaf segments (NS), fruit length (FL), and disk width (DW) had the highest standardized canonical discriminant function coefficients in DF1 were. These variables should be assessed when comparing the overall morphology of *C. bipinnatus* growing at different vegetation types.

However, when assessing the morphological variability of *C. bipinnatus* within a population, other characters should be employed. As suggested by some authors (e.g.,

Tetsana et al. 2014), we recommend that characters exhibiting massive intercorrelation should be omitted of morphological analyses. In the case of *C. bipinnatus*, we found that all characters exhibited correlation with at least one character. However, leaf, ligule, and bract size-related characters showed high and significant correlations within themselves. Thus, a limited number of size-related characters of these structures should be employed in this kind of studies (i.e., only one length measurement). Also, the length and width of ligules should be avoided, as these showed correlations with 68–76 % of the characters. On the other hand, some characters clearly exhibited low correlations. In particular, the number of ligules and the fruit width showed low percentage of correlations with other characters. Also, characters regarding ligule colors showed almost no correlations with other vegetative characters. So, we suggest that these characters may be helpful in morphological studies of *C. bipinnatus* and in members of the Asteraceae family in general.

Conclusions

In this study we explored the intraspecific morphological variability of *Cosmos bipinnatus*. The results obtained in these study revealed that this species shows a broad range of morphological variation partly due to the environmental pressure acting at each site (e.g. altitude and NH_4 content). Despite its great morphological variability, we were able to identify diagnostic morphological characters for *Cosmos bipinnatus*: number of external phyllaries, trichome length and petiole area. We propose that these characters should be explored in detail when taxonomic confusion arises. Also, massive character intercorrelation was found in the morphological characters of this species. Although this was an expected result, we found that flower-related characters (number of ligules, fruit width and ligule color) showed low intercorrelation and thus, they should be employed in morphometric studies of *Cosmos bipinnatus* and other members of the Asteraceae family.

We hypothesize that individuals growing at wetter and more temperate habitats (*Pinus* and *Quercus* forest) will display greater height and longer structures compared to drier and warmer habitats (Scrub and Grasslands). Regarding reproductive structures, we found bigger flowers and associated structures in temperate habitats. However, the opposite pattern was found when we explored vegetative characters. The differential resource allocation between sites may partly explain this pattern.

Finally we found low levels of phenotypic plasticity in all characters analyzed. As a weedy species, *C. bipinnatus* shows high tolerance to environmental stress. So, we hypothesize that the plants collected at contrasting vegetation types were not in severe physiological stress. Also, we propose that the heterogeneity of the environmental

conditions on small spatial scales is the main source of phenotypic variation of *C. bipinnatus*. However, future studies should explore the phenotypic plasticity in more detail by performing reciprocal transplants of pure lines growing at contrasting environments in order to evaluate precisely the contribution of the phenotypic plasticity to the phenotypic variation of *C. bipinnatus*.

Additional file

Additional file 1: Character intercorrelation within and between vegetative and reproductive macromorphological characters of *Cosmos bipinnatus*. Only significant correlation coefficients ($P < 0.05$) are shown. (XLS 52.0 KB)

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

MG contributed with the micromorphological analyses and revision of the manuscript. OH and SS contributed with the edaphological analyses and revision of the manuscript. PI contributed with the field sampling and experimental work. LC contributed with the statistical analyses and manuscript writing. RR contributed with the taxonomical determination and field work. TS contributed with the original idea, experimental design, statistical analyses and manuscript writing. All authors participated in the review topic design, in the data analyses, and in the manuscript writing. Also, all authors read and approved the final version of the manuscript.

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