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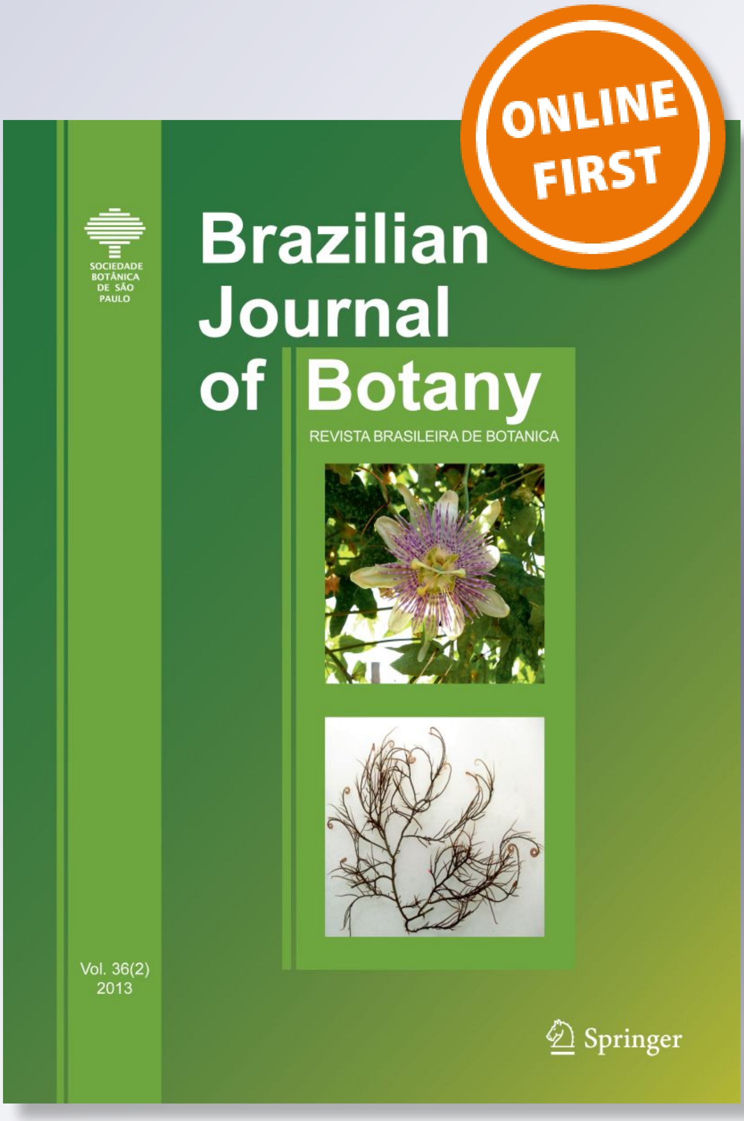

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ORIGINAL ARTICLE

Morphological plasticity of an endemic widespread columnar cactus and its congener

Gabriel Arroyo-Cosultchi[1] · **Salvador Arias**[2] · **Lauro López-Mata**[1] · **Teresa Terrazas**[3]

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Abstract The morphological variation among 11 populations belonging to two endemic congener species of *Neobuxbaumia*, columnar cacti, was studied. One of our hypotheses was that *N. mezcalaensis* (Bravo) Backeb. with widespread distribution would show a higher variation of morphological characters and geographic–environmental variables compared with *N. multiareolata* (E.Y. Dawson) Bravo, Scheinvar & Sánchez-Mej. displaying a narrow distribution range, thus *N. mezcalaensis* will have a higher plasticity in some of its morphological traits. For each population, 41 morphological variables, three geographic, 10 climatic, and six soil properties were generated and analyzed by the simplified relative distance plasticity index, principal component, and regression analyses. The plasticity index across all populations for *N. mezcalaensis* showed more levels of plasticity than *N. multiareolata* in 12 variables as we expected. Principal component analysis explained 74% of the total variation. The first principal component, 47.41%, resulted from environmental differences and contributes to species separation. Difference in fruits traits and plant height was correlated with longitude ($P < 0.0001$) and elevation ($P < 0.0001$), thereby indicating a steep clinal decrease in fruit size and plant height from east to west as well as with the increase of elevation ($P < 0.0001$). Moreover, this variation negatively correlated with the mean temperature of the driest quarter ($P < 0.0001$) and annual precipitation ($P < 0.0001$), thereby indicating also a steep clinal decrease from east to west in *N. mezcalaensis*, the species with widespread distribution. The vegetative characters show that plasticity could be influenced by geographic, climatic, and edaphic variables, while the reproductive characters are probably genetically fixed because no significant variation was detected.

Keywords Cactaceae · Environmental conditions · Geographic distribution · Phenotypic cline · Plasticity · Principal component analysis

1 Introduction

Phenotypic plasticity is the ability of an organism to change its morphology and physiology in response to environmental variation (Schlichting1986), and perennial plants respond to environmental changes primarily by phenotypic plasticity (Rubio de Casas et al. 2007). Plant morphology variation is frequently associated with a combination of local environmental conditions and intrinsic genetic variation (Ellison et al. 2004). Moreover, this variation is associated with plastic responses to environmental gradients and the biogeographic history of each species (Heslop-Harrison 1964; Briggs 1969; Bruschi et al. 2003; Warren et al. 2005). Such morphological variation seems to be a prerequisite for the formation of new species (Losos and Glor 2003). In several plant species, a significant correlation between morphological characteristics and geographic and climatic variables (Zobel and van Buijtenen 1989; Bruschi et al. 2003; Sugiyama 2003; Ellison et al. 2004; Li et al. 2006; Ruedas et al. 2006; Hernández

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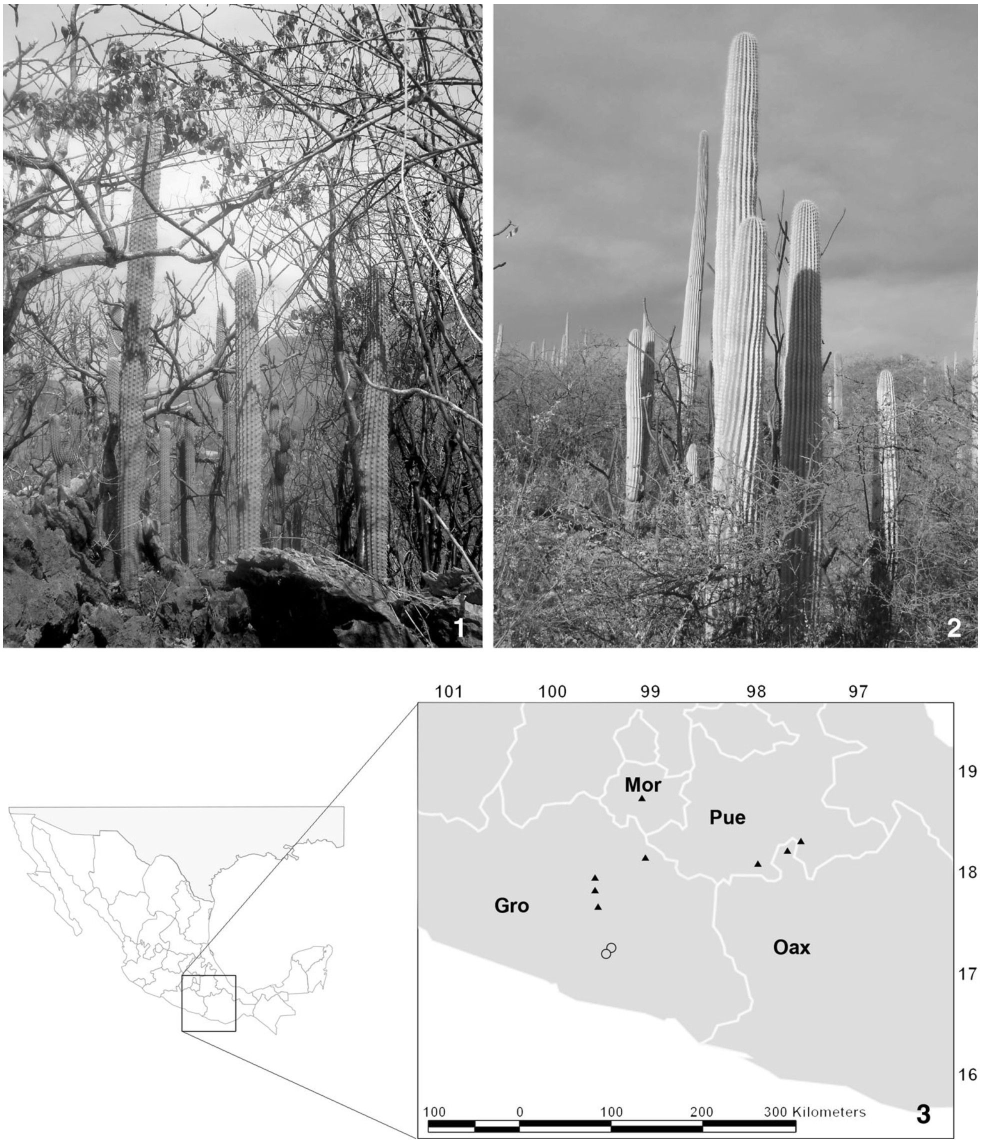
et al. 2007; Uribe-Salas et al. 2008; Pyakurel and Wang 2013) has been shown. Moreover, latitudinal or elevation gradients, with differences in climatic factors, may result in strong natural selection for local adaptation and ecological specialization for species with a broad distribution (Endler 1977) as compared with those with a narrow distribution (Karron 1987; Waller et al. 1987; Lavergne et al. 2004). However, modification of morphological characters depends on the extent of continuous or discrete variation along geographic range of species, which may result in clinal and ecotypic variation (Briggs 1969). Species that succeed in a rapidly changing climate are likely to have ample genetic variation for traits important in the new environment, broad ecological amplitudes, highly plastic phenotypes, short generation times or adaptations for long-distance seed dispersal (Vitt et al. 2010). The understanding of plasticity in diverse environments has been given importance in forecasting plant response to environmental change caused by global warming or anthropogenic disturbances (Valladares et al. 2007).

In the cactus family, around 1475 (73%) species display a narrow distribution, few populations, small populations size, low population density, habitat specificity or a combination of those factors (Rabinowitz et al. 1986) resulting in a high proportion of local endemism (Hernández and Godínez-Álvarez 1994; Ortega-Baes and Godíınez-Álvarez 2006). Few cacti species have a wide distribution (26%) and are frequently found in many habitats (Ortega-Baes and Godínez-Álvarez 2006). Groups of parental species located in different and distant geographic sites are rare. Although they have a common origin, the divergence among them may have occurred as a consequence of recent evolution of each group separately (Bevill and Louda 1999). Mexico concentrates nearly 45% of all known cactus species, of which around 80% are endemic (Ortega-Baes and Godínez-Álvarez 2006). Thus, Cactaceae is an ideal system to study the morphological variation either in species with wide or narrow distribution as well as for endemic species with high or low population densities. For example, most studies on cactus concern the morphological variation of wild species using univariate and regression analyses (Felger and Lowe 1967; Racine and Downhower 1974; Rundel 1977; Felker et al. 2002) as well as multivariate analyses (Chamberland 1997; Hicks and Mauchamp 2000; Muñoz Urias et al. 2008) have focus on species complexes. Multivariate analyses have primarily been applied to answer taxonomical questions at different hierarchical levels (Chamberland 1997; Baker and Johnson 2000; Schmalzel et al. 2004; Muñoz Urias et al. 2008; Arroyo-Cosultchi et al. 2010; Baker and Butterworth 2013; Sánchez et al. 2013; Vázquez-Benítez et al. 2016). In these studies, the multivariate analyses favor the recognition of few diagnostic morphological characters that allow differentiating species, especially when many variables are analyzed. In addition, PCA is also an appropriate statistical technique to examine the relationships among multiple intercorrelated environmental variables (McCune and Grace 2002). For example, other studies (Parker 1991; Ruedas et al. 2006; Bárcenas-Argüello et al. 2010; Ribeiro-Silva et al. 2016) applying univariate and multivariate analyses and mineralogy assessments were conducted to identify which of the edaphic properties (e.g., soil development, depth, texture, as well as calcium carbonate and phosphorous content) contribute the most to the establishment, early developmental stages, distribution, and abundance of different cactus species. In contrast, the presence/absence of many cactus species is associated with elevation or latitude constraints (Gurvich et al. 2014; Bauk et al. 2015), above which freezing temperatures reach or appear to reach the threshold (Jordan and Nobel 1981).

Neobuxbaumia Backeb. is an endemic genus to Mexico (Guzmán-Cruz et al. 2003; Ortega-Baes and Godínez-Álvarez 2006), which ranges from Tamaulipas in northeastern Mexico to Oaxaca in southwestern Mexico, associated with thorn scrubs and deciduous forests. The genus comprises branched or unbranched trees with stems that are stout, gray–green, and cylindrical. The genus is also characterized by numerous ribs and usually stiff or flexible spines; flowers that are commonly funnel-shaped, covered by small fleshy scales and bristles and mostly open at night; fruits that are globose or ovoid dehiscing by vertical slits; and seeds that are large to extremely large and black–brown with a glossy surface and periphery keeled (Bravo-Hollis 1978; Arroyo-Cosultchi et al. 2007). This genus has nine species with a distribution from broad to narrow (Bravo-Hollis 1978; Guzmán-Cruz et al. 2003). However, species limits have been controversial for some ones as for *Neobuxbaumia multiareolata* (E.Y. Dawson) Bravo, Scheinvar & Sánchez-Mej (Fig. 1) which was described originally as a variety of *N. mezcalaensis* (Bravo) Backeb (Fig. 2). Although, Bravo-Hollis et al. (1972) first recognized as a new distinct species, Hunt (2006) does not recognize this independent taxon, so treat it as a subspecies of *N. mezcalaensis*. However, Arroyo-Cosultchi et al. (2010) and Tapia et al. (2016) proposed that they represent independent species based on morphological evidences such as areole size and form, central spine length and distribution pattern of the radial spines, plus four reproductive characters (size of the flower, pericarpel, fruit, and seed) mentioned previously by other authors. These reproductive characters do not overlap between species and are diagnostic for each one (Arroyo-Cosultchi et al. 2010). One of the questions that arise was what makes that one of these two species to have a wide distribution and the other to be restricted to a few nearby hills at Tierra Colorada and Acahuizotlá at the Costa Chica-Rio Verde hydrological



Figs. 1–3 –Species studied and their location. **1** *Neobuxbaumia mezcalaensis*, **2** *Neobuxbaumia multiareolata*, **3** distribution of studied sites

region? The most common species of *Neobuxbaumia* (*N. mezcalaensis*, *N. polylopha* (DC.) Backeb., and *N. squamulosa* Scheinvar & Sánchez-Mej.) are found in a wide range of habitats, while the endemic species (*N. laui* (P.V. Heath) D.R. Hunt, *N. macrocephala* (F.A.C. Weber ex K.Schum.) E.Y. Dawson, and *N. multiareolata*) have a limited narrow distribution (Guzmán-Cruz et al. 2003; Ruedas et al. 2006; Arroyo-Cosultchi et al. 2010). *N. mezcalaensis* is a keystone and critical for survival of many other plant and animal species in some communities in states of Guerrero and Puebla (Esparza-Olguín et al. 2005). It is found in a wide range of habitats and exhibits high levels of genetic and phenotypic diversity in most distribution areas (Esparza-Olguín 2005). In contrast, distribution of *N. multiareolata* is narrow and restricted to specific habitats resulting in a suitable and limited number of sites for species presence and low density.

N. mezcalaensis encounters widespread range of habitats in south of Mexico and different ecological conditions. Due to the heterogeneous nature of *N. mezcalaensis* habitats, it can therefore be assumed that the species has developed adaptive phenotypic plasticity to enable it to occupy all the natural range opposite to a species with narrow distribution like *N. multiareolata*. The aim of this study was to elucidate the influence of geographic, climatic, and edaphic gradients on morphological character variation among populations of *N. mezcalaensis* and *N. multiareolata* using the simplified relative distance plasticity index, multivariate, and regression analyses. We hypothesized that species with a widespread distribution range would show a higher level of morphological character variation compared with species displaying a narrow distribution range. Moreover, we predicted that vegetative characters would display more variation than reproductive ones that are less plastic.

Table 1 Species, locality names, states, code letters, geographic coordinates, and elevations for the studied populations of *Neobuxbaumia mezcalaensis* and *Neobuxbaumia multiareolata*

Specie-localities	State	Code	Lat *N*	Long *W*	Elevation (m)
N. mezcalaensis					
Las Estacas	Morelos	a▲	18° 42′ 18″	99° 06′ 19″	945
Colonia San Martín	Puebla	b▼	18° 17′ 00″	97° 32′ 13″	1896
Santiago Chazumba	Oaxaca	c■	18° 12′ 06″	97° 40′ 26″	1700
Atenango del Río	Guerrero	d◆	18° 07′ 01″	99° 05′ 31″	638
Petlalcingo	Puebla	e✱	18° 06′ 07″	97° 57′ 05″	1380
Mezcala	Guerrero	f✚	17° 55′ 14″	99° 34′ 44″	486
Casa Verde	Guerrero	g●	17° 47′ 57″	99° 34′ 03″	742
Xochipala	Guerrero	h▶	17° 47′ 38″	99° 34′ 47″	884
Zumpango del Río	Guerrero	I◀	17° 38′ 06	99° 32′ 38″	1092
N. multiareolata					
Tierra Colorada	Guerrero	j○	17° 08′ 25″	99° 32′ 11″	450
La Venta	Guerrero	k□	17° 11′ 36″	99° 30′ 38″	192

Additionally, we postulated that morphological variations in *N. mezcalaensis* may be associated with geographic location, soil properties, or climatic variables and that a null overlap exists for the morphological characters between both species.

2 Materials and methods

Species studied – *Neobuxbaumia mezcalaensis* is a non-branched columnar cactus than may reach between two and 14 m height. Its flowers emerge along the stem between April and June and are white (occasionally green–red) with nocturnal anthesis; they are pollinated by bats and seeds dispersed by bats and birds between May and June (Valiente-Banuet et al. 1997). Populations are dense, with 1000–17,000 ind. ha^{-1} (Ruedas et al. 2006). This cactus species inhabits in thorny forest and tropical dry forest, on calcareous soils. It is commonly found between 486 and 2000 m elevation, with a broad geographic distribution range that partially covers the Tehuacán-Cuicatlán valley (Mexican states of Puebla and Oaxaca), as well as the Balsas River Basin (Guerrero and Puebla, southwest of Mexico). *Neobuxbaumia multiareolata* is an unbranched, columnar cactus that reaches between 2 and 7 m in height in the adult stage. Its red–purple flowers, which bloom between April and May, are borne both along the stem and in crowns near the stem tips. *Neobuxbaumia multiareolata* inhabits tropical dry forest, growing on cliff faces (Bravo-Hollis 1978; Arroyo-Cosultchi et al. 2010; Ojedzi-Aley and Rodríguez-López 2011), with a narrow elevation distribution between 192 and 450 m. This species is endemic to a small region in the Costa Chica-Rio Verde hydrological region in the state of Guerrero, Mexico.

Study area and fieldwork – Eleven populations of *N. mezcalaensis* and *N. multiareolata* were sampled along their geographic distribution (Table 1; Fig. 3). A total of 20 mature individuals per population were randomly sampled ($N = 220$ individuals). For each set of 20 plants, we measured the length of 13 vegetative characters. Five areoles were selected, and in each one, the length of the central and lateral spines was counted and measured. Five mature flowers and fruits were collected and fixed (50% ethanol) per individual, and later in the laboratory, 28 reproductive variables were measured and counted (Table 2). The geographic location and elevation of each population site was georeferenced using a handheld GPS (global positioning system) unit accurate to ±50 m (Table 3). Soil properties were characterized from ca. 1 kg of soil collected at each population.

Laboratory procedures – Climatic variables for the 11 population sites were obtained applying the bioclimatic modeling approach implemented in the BIOCLIM program (Houlder et al. 2000), which uses interpolated climatic surfaces estimated from a standard network of meteorological stations. The climatic surfaces or digital files obtained were generated using the thin-plate smoothing spline methods in the ANUSPLIN package (Hutchinson and Gessler 1994). Derivation of the bioclimatic profiles was based on selected-simple-matching thresholds and the limits throughout a grid of data points for each of the 10 bioclimatic parameters selected (Table 3).

The soil analyses were carried out in the Soil Fertility Laboratory at Colegio de Postgraduados. For each sample, pH and mean electric conductivity (E.C) (1:5 mmhos $\mathrm{cmdS\text{-}m}^{-1}$) were measured. Organic matter was assessed by determining the organic carbon decay of animal and vegetal tissues from the humic fraction on the

Table 2 Relative distance plasticity index (RDPIs) across all populations for the all morphological characters measured of *Neobuxbaumia mezcalaensis* and *Neobuxbaumia multiareolata*

Morphometric variable	*N. mezcalaensis*	*N. multiareolata*
RDPI		
Vegetative		
Height (m)	0.109	0.005
Perimeter (cm)	0.099	0.001
Number of ribs	0.054	0.004
Height ribs (cm)	0.226	0.040
Distance ribs (cm)	0.044	0.013
Thickness ribs (cm)	0.053	0.010
Distance areole (cm)	0.037	0.082
Areole length (cm)	0.250	0.064
Areole width (cm)	0.262	0.056
Number of central spines	0.246	0.000
Central spine length (cm)	0.192	0.111
Number of radial spines	0.137	0.063
Radial spine length (cm)	0.076	0.049
Reproductive (flower)		
Flower length (cm}	0.031	0.002
Flower width (cm)	0.035	0.014
Floral tube (cm)	0.042	0.034
Pericarpel length (cm)	0.043	0.020
Podary of the tube length (cm)	0.042	0.034
Podary of the tube width (cm)	0.043	0.068
Podary length (cm)	0.030	0.002
Filament length (cm)	0.043	0.034
Outer filament length (cm)	0.043	0.071
Style length (cm)	0.035	0.022
Number of stigma lobes	0.048	0.000
Stigma lobes length (cm)	0.096	0.012
Stigma lobes width (cm)	0.018	0.044
Nectar chamber length (cm)	0.056	0.002
Nectar chamber width (cm)	0.079	0.001
Ovary length (cm)	0.081	0.011
Ovary width (cm)	0.083	0.024
Flower scales length (cm)	0.100	NA
Reproductive (fruit and seed)		
Fruit length (cm)	0.060	0.065
Fruit width (cm)	0.136	0.018
Fruit volume (ml)	0.155	0.109
Fruit spine length (cm)	0.144	0.057
Podary length (cm)	0.068	0.021
Podary width (cm)	0.170	0.078
Number of fruit areoles	0.057	0.146
Seed length (mm)	0.042	0.020
Seed width (mm)	0.025	0.017
Seed area (mm^2)	0.057	0.027

Morphometric variables with $\geq$0.10 are indicated in bold

Table 3 Principal component analysis results for three components based on 12 morphometric and 16 environmental variables of *Neobuxbaumia mezcalaensis* and *Neobuxbaumia multiareolata*

	PC-1	PC-2	PC-3
Eigenvalue	15.17	5.30	3.35
Percentage	47.41	16.56	10.45
Cumulative percentage	47.41	63.97	74.42
Morphometric variables			
Height (cm)	−0.2136	**−0.5807**	0.0028
Height ribs (cm)	−0.4162	0.4169	0.2105
Areole length (cm)	−0.0113	−0.1999	**−0.6517**
Areole width (cm)	−0.0169	−0.2120	**−0.6780**
Number of central spines	−0.7218	0.4779	−0.1240
Central spine length (cm)	−0.5895	0.4312	−0.0686
Number of radial spines	**−0.8003**	0.1195	0.0267
Fruit width (cm)	−0.2682	**0.8204**	0.2286
Fruit volume (ml)	−0.4182	0.5042	0.3439
Fruit spine length (cm)	−0.4580	**0.6299**	−0.1252
Podary width (cm)	−0.1429	**0.8275**	0.1805
Number of fruit areoles	−0.7041	−0.3213	0.3899
Flower scales length (cm)	−0.3303	0.3949	0.3437
G*eographic variables*			
Longitude (*W*)	**0.8108**	−0.5141	0.0225
Latitude (*N*)	**−0.8087**	−0.1575	0.3373
Elevation (masl)	**−0.9136**	−0.046	−0.0762
Climatic variables			
Annual mean temperature (°C)	**0.8738**	−0.2062	0.3633
Mean diurnal range (°C)	−0.5941	−0.1061	**0.7370**
Temperature seasonality (%)	**0.7754**	0.2053	−0.5127
Mean temperature of wettest quarter (°C)	**0.8585**	−0.1526	0.3975
Mean temperature of driest quarter (°C)	**0.9132**	−0.2393	0.1977
Mean temperature of warmest quarter (°C)	**0.7873**	−0.2624	0.4702
Mean temperature of coldest quarter (°C)	**0.9268**	−0.1710	0.2560
Annual precipitation (mm)	**0.9347**	0.1050	0.0603
Precipitation of wettest period (mm)	**0.8734**	0.3363	0.0096
Precipitation seasonality (%)	**0.8778**	0.2521	0.0646
Edaphic variables			
pH	**−0.8221**	−0.4954	0.1759
Electric conductivity	**0.8583**	0.1584	0.1878
Organic matter	0.7269	0.4526	−0.0134
Phosphorus	0.6401	**0.6350**	−0.3054
Sand (%)	0.4567	0.3146	0.4397
Clay (%)	−0.6933	−0.5263	0.0545

Variables with high loadings on each of the principal components are indicated in bold

mineralization process and from inert elementary carbon, via K2Cr2O7 oxidation using the Walkley–Black method. The phosphorus (P) (NH4O Ac1 N pH 7 Meq 100 g^{-1}) was assessed using the Olsen method. Soil texture was determined via granulometric analysis based on the dispersion and sedimentation of soil particles, which is known as Bouyoucos' density method (Etchevers 1988) (Table 3).

Statistical analyses – A simplified relative distance plasticity index (RDPI) was carried on (Valladares et al. 2007). The RDPI measures the relative distances between mean values of the variables for all pairwise comparisons between populations of each species. The values of RDPIs were estimated after Valladares et al. (2007) and range from 0 (no plasticity) to 1 (maximal plasticity).

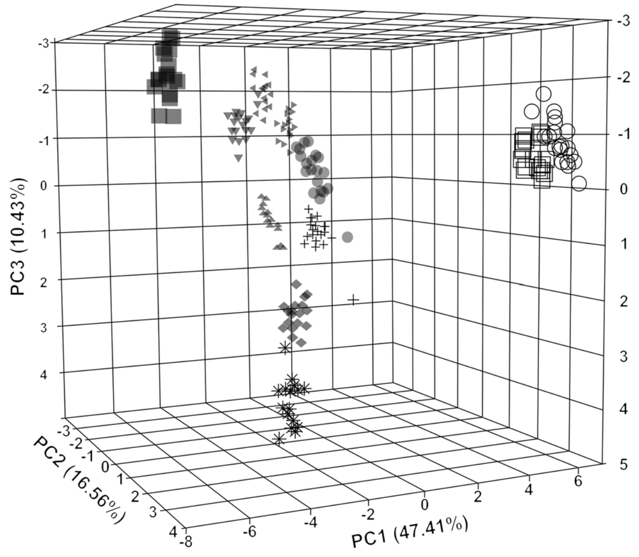


Fig. 4 Tri-plot resulting from principal component analysis (1–3 PC) of *Neobuxbaumia mezcalaensis* (*solid symbols*, 9 populations) and *Neobuxbaumia multiaereolata* (*open symbols*, 2 populations). Las Estacas (*filled black up triangle*), Colonia San Martín, (*filled black down triangle*), Santiago Chazumba (*filled black square*), Atenango del Río (*filled black diamond*), Petlalcingo (*filled black star*), Mezcalas (*filled black plus*), Casa Verde (*filled black circle*), Xochipala (*filled black right pointer*) and Zumpango del Río (*filled black left pointer*), Tierra Colorada (*open white circle*), and La Venta (*open white square*)

A data matrix was generated for the 12 morphometric characters ($N = 220$ individuals) which showed RDPI ≥ 0.1 (Table 2), 13 climatic and geographic variables, and six edaphic variables; all of them were analyzed using principal component analysis (PCA). Variables were transformed to logarithms (continuous characters), square root (counts), and arcsine (percentage or proportion characters) prior to performing the PCA. From variable loading of the PCA, we identified the most important morphometric and environmental variables given by their highest loading factors, which explained the global variation in the populations of both species. For those morphological variables highlighted by the first three principal components (PC1–PC3) as a result of the PCA, linear regressions of geographic–climatic variables against morphological variables of the PCA scores were made exclusively for the *N. mezcalaensis* populations. All analyses were performed with SAS (2008).

3 Results

Simplified Relative Distance Plasticity Index – The RDPIs showed that the 41 morphological variables measured for both species have low plasticity values. RDPI values were lower for *N. multiareolata* than *N. mezcalaensis* (Table 2). For *N. mezcalaensis* seven vegetative variables (height, ribs height, areole length and width, number of central spines, central spine length, number of radial spines), four of fruit (fruit width, fruit volume, fruit spine length and podary width), and one floral variable (flower scales length) reached above 0.1, whereas only three variables (central spine length, fruit volume, and number of fruit areoles) had a value higher than 0.1 for *N. multiareolata* (Table 2).

Principal component analysis – The PCA including the 12 morphometric variables with RDPI values ≥ 0.1 (Table 2) plus environmental variables showed that three components accounted for 74.42% of the total variance of *N. mezcalaensis* and *N. multiareolata*. PC-1 explained 47.41% of the total variance with 15 variables of high loadings. One of these variables was morphometric (Nr. of radial spines); 12 variables were geographic and climatic (i.e., latitude, longitude, elevation, annual mean temperature, temperature seasonality, mean temperature of wettest quarter, mean temperature of driest quarter, mean temperature of warmest quarter, mean temperature of the coldest quarter, annual precipitation, precipitation of the wettest period, and precipitation seasonality), and two were edaphic (i.e., pH and electric conductivity). PC-2 explained 16.56% of the residual variance with five variables as follows: four morphometric (i.e., plant height, fruit width, spine fruit length, and podary fruit width) plus one edaphic (i.e., phosphorus of the soil). PC-3 explained 10.45% of the residual variance with three variables: areole length and width and mean diurnal range (Table 3). Figure 4 shows that PC-1 contributed mostly to separate the two species mainly due to their geographic distribution and their associated climatic variables, while PC-2 and PC-3 contributed to the separation among populations into *N. mezcalaensis* due to morphometric variables.

Regression analyses – For *N. mezcalaensis*, a multiple regression analysis between PC-2 (fourth morphological characters) against geographic and climatic variables with higher loading values for PC1 were performed. The morphological variables (associated with fruit size and plant height) corresponding to the second principal component (PC-2) displayed a significant correlation between the population average score and the longitude and elevation of the populations ($r = -0.80$; $P < 0.0001$, Fig. 5a; $r = 0.58$; $P < 0.0001$, Fig. 5b). This finding suggests that fruit characters and plant height decrease clinally from east–west and positively with elevation. According to these results, the populations from the Tehuacán-Cuicatlán valley would have larger fruits than the western populations (i.e., Balsas River Basin). The PC-2 was also significantly correlated with mean temperature of the driest quarter ($r = -0.51$; $P < 0.0001$; Fig. 5c), mean temperature of the coldest quarter ($r = -0.46$; $P < 0.0001$; Fig. 5d), and annual precipitation ($r = -0.47$; $P < 0.0001$; Fig. 5e).

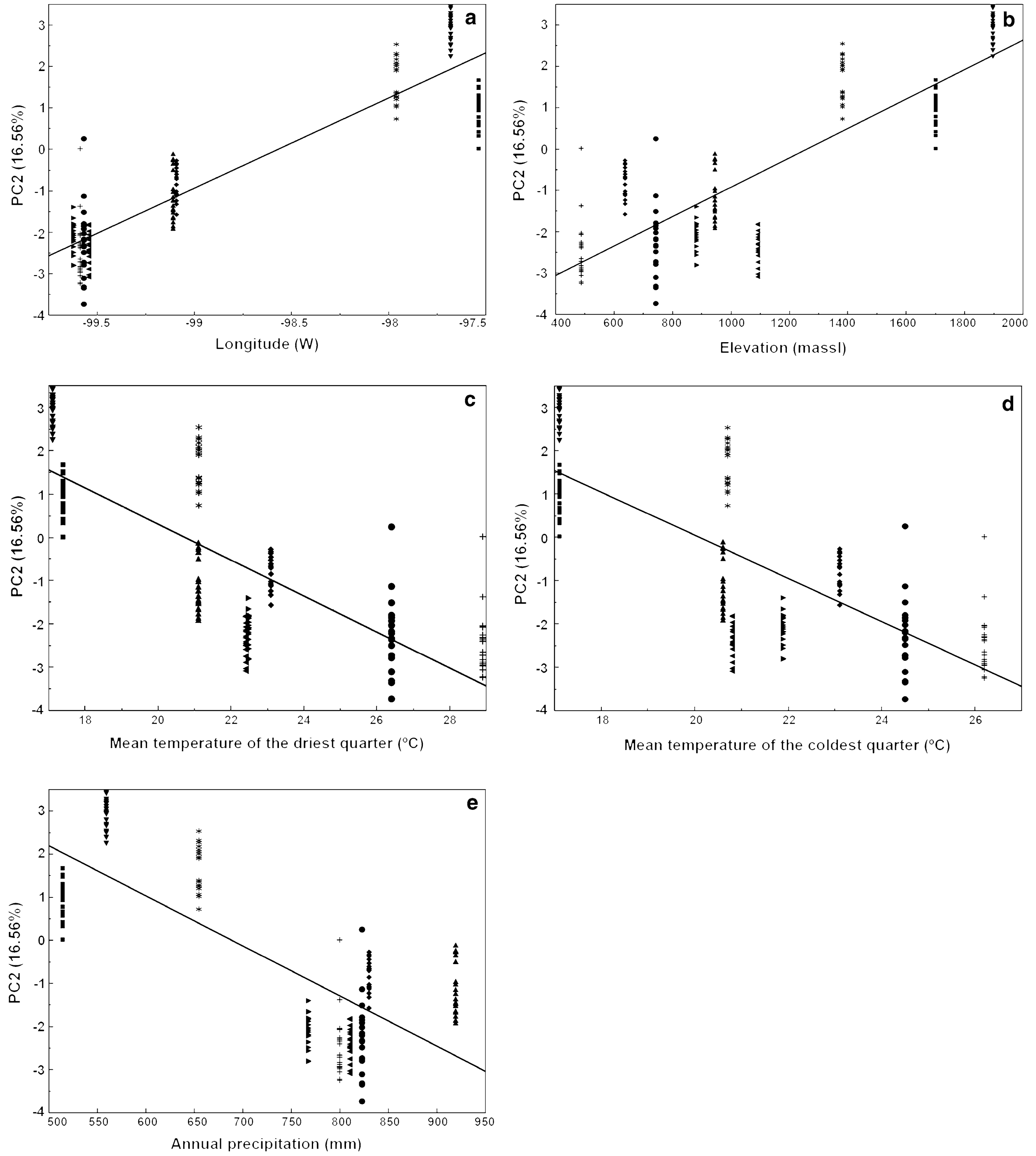


Fig. 5 Linear relationships between geographic locations, climatic variables, and PC2 morphological characters of *Neobuxbaumia mezcalaensis*. **a** Longitude, **b** elevation, **c** mean temperature of the driest quarter, **d** mean temperature of the coldest quarter, **e** annual precipitation. *Neobuxbaumia mezcalaensis* populations: Las Estacas (*filled black up triangle*), Colonia San Martín (*filled black down triangle*), Santiago Chazumba (*filled black square*), Atenango del Río (*filled black diamond*), Petlalcingo (*filled black star*), Mezcala (*filled black plus*), Casa Verde (*filled black circle*), Xochipala (*filled black right pointer*), and Zumpango del Río (*filled black left pointer*). In all cases, the response variable is the second principal axis (fruit characteristics and plant height) (see Table 3 for all loading)

4 Discussion

Species with wide distribution ranges usually show larger morphological variability compared with species with narrow distribution (Rapson and Maze 1994; Lavergne et al. 2004). This assertion was supported with the RDPI values obtained for *N. mezcalaensis*. Moreover, most reproductive characters showed less plasticity than vegetative ones and those variables used to distinguish species by Arroyo-Cosultchi et al. (2010) as for flower length, tube length, seed area, and podaria fruit length showed very low RDPI. Abiotic variables may have a significant effect on the phenotype of a species. This work is the first of its kind to study the relationship between the morphological variables and the geographic, edaphic, and climatic variables. The results showed that the environmental variables did contribute to the expressed plasticity of some characters of *N. mezcalaensis* and *N. multiareolata*, although they are different between species (see below) and the floral ones showed less variation in both taxa.

This study indicates that individuals of *N. mezcalaensis* modify their fruit characters and plant height along a longitudinal gradient. The pronounced east–west longitudinal clinal variation in morphological fruit characters indicates a strong phenotypic variation among populations of *N. mezcalaensis* in response to a climatic gradient of temperature and precipitation (Figs. 2, 3). Three relevant climatic variables: mean temperature of the driest quarter, mean temperature of the coldest quarter, and annual precipitation, showed a significant variability along the longitudinal gradient. The pattern of morphological variation suggests that the fruit characters are larger in the east, where sites are drier and cooler (i.e., Tehuacán-Cuicatlán), whereas the size of fruits and plant height are gradually smaller toward the west, where population sites are wetter and warmer (i.e., Balsas River Basin; Zopilote Canyon). These morphological attributes, which gradually change along with the longitudinal gradient, elevation, and precipitation, also appear to mean the response to other environmental factors such as local rainfall, soil nutrient deficiency, and extreme temperatures. Fruit size is among the reproductive characters that have been found to covary more often with longitudinal, elevation, and environmental variables in several cactus species (*Echinocactus polycephalus* (Engelm. & Bigelow) complex, Chamberland 1997; *Opuntia ficus-indica* ((L.) Mill.), Felker et al. 2002; *Ferocactus cylindraceus* subsp. *cylindraceus* (Engelm.) Orcutt, *Carnegiea gigantea* (Engelm.) Britton & Rose, *Lophocereus schottii* (Engelm.) Britton & Rose, and *Stenocereus thurberi* (Engelm.) Buxb., Gibson and Nobel 1986; *Coryphantha robustispina* (Ant.Schott ex Englem.) Britton & Rose, Schmalzel et al. 2004). Therefore, the current results suggest that the local environmental variations play an important role in shaping local plant morphology.

In comparison with previous studies conducted on other cactus species (Felger and Lowe 1967; Rundel 1977; Chamberland 1997), our results reveal that the strong variation in the vegetative characters is a plastic response to the diverse environments where populations of *N. mezcalaensis* occurred along their natural distribution. Clinal variation may not only result from selection along environmental gradients but may also be influenced by the patterns of gene flow among populations (Endler 1977). For example, high rates of gene flow among populations located in geographic proximity would contribute to a stronger phenotypic similarity than would environmental variables. In contrast, clines may also be the product of historical isolation and the divergence of populations with subsequent expansion and contact (Endler 1977). The results of ongoing population genetic studies of *N. mezcalaensis* may help to discern between these alternatives. A study of isozyme variation in *N. mezcalaensis* showed a high and low degree of genetic variation between populations and within populations, respectively (Esparza-Olguín 2005), and no information exists for *N. multiareolata*, but lower genetic variation is expected.

The higher vegetative and fruit morphological variability were found of *N. mezcalaensis* than *N. multiareolata*, the RDPI of both species revealed that some morphological characters showed plasticity values close to zero. Clinal variation appears to be common for columnar cactus, considering that their cross-pollination and seed dispersion occur primarily via bats and birds, agents which are able to move great distances (15 km per night and 200 km per week) (Rojas-Martínez et al. 1999; Valiente-Banuet et al. 2004; Arias-Cóyotl et al. 2006).

As a possible consequence of their distribution range, the results clearly show that the types of habitats in which *N. mezcalaensis* may be found are more heterogeneous than the habitats in which *N. multiareolata* are distributed. The PCA allowed us to identify the geographic, climatic, and edaphic variables that are partially responsible for these patterns. In particular, *N. mezcalaensis* is found in habitats with alkaline water potential (7.8–8.3), lower electric conductivity percentage (15–31%), lower organic matter (4.6–16.6), lower phosphorus content (2–9), and soils with various textures. In contrast, *N. multiareolata* occupies habitats with acidic water potential (6.6–6.8), high electric conductivity percentage (32–35%), high organic matter (31.1–53.1), high phosphorus content (32–42), and loam soil texture as compared to *N. mezcalaensis*. Distribution of different cactus species has been associated with the diversity of soil properties, as they are affected by water and nutrient uptake (Parker 1991; Ruedas et al. 2006; Ribeiro-Silva et al. 2016). The populations of *N. mezcalaensis* in the Tehuacán-Cuicatlán valley inhabit higher elevation sites, with lower precipitation and soil with a higher content of organic matter and phosphorus compared with the populations in the Zopilote Canyon, which are located at lower elevations, higher temperature, and precipitation levels and poorer soils (i.e., low organic matter and phosphorus levels) as other studies have indicated (Ruedas et al. 2006) for the same species. Concerning the soil, most of the sites occupied by *N. mezcalaensis* are of sandy texture, although no other difference was observed with other columnar cacti (Hernández et al. 2007).

N. multiareolata grows in tropical dry forests, inhabiting cliff faces (Bravo-Hollis 1978; Arroyo-Cosultchi et al. 2010) between elevations of 192 and 450 m, with higher annual precipitation (1218–1400 mm), lower temperature seasonality (29–30), and distinctive soils features (see above) than *N. mezcalaensis*. The combination of these soil properties and climatic factors contributes to the narrow distribution and endemicity of *N. multiareolata* to a small region (Tierra Colorada and Acahuizotlá at the Costa Chica-Rio Verde hydrological region) in the state of Guerrero, Mexico. The geographic, climatic, and soil variables are clearly strong determinants of the presence and isolated distribution of both cactus species, thereby partially explaining their lack of sympatric distribution. Recent speciation or multiple origins for *N. multiareolata* may easily explain the morphological similarity to *N. mezcalaensis*. It is possible that a recent speciation event and hybridization have occurred (Smith and Pham 1996).

In conclusion, this study suggests that morphological differences between *N. mezcalaensis* and *N. multiareolata* across geographic and environmental gradients are the result of a combination of climatic and edaphic variables. It is worth noting that *N. multiareolata*, the species with the narrow distribution range, shows lower level of morphological character variation than to *N. mezcalaensis*, the species with the broader distribution range, which is similar with a study in two congenetic bunch grasses *Achnatherum* (=*Oryzopsis*) with different distribution (Rapson and Maze 1994). Therefore, we suggest that *N. multiareolata* could have originated from a recent speciation of another species of wider distribution, in this case *N. mezcalaensis*.

Both species show geographic–climatic ranges totally contrasting. *N. mezcalaensis* has greater amplitude for them and does not overlap with those of *N. multiareolata*. This explains that the first has been adapted to grow in a greater number of environments and shows evidence that both species have well-defined environmental niches.

Environmental factors tend to have greater influence on vegetative characters than on reproductive ones (Jones 1988). For instance, the flowers were consistent with a bat pollination syndrome (i.e., night flowering, nectar production, and large quantities of pollen) (Valiente-Banuet et al. 1997) and can show a slow phenotypic plasticity in response to spatiotemporal variation in both biotic (i.e., visitors assemblage) and abiotic conditions (i.e., resources availability; Herrera 1993; Rojas-Sandoval and Meléndez-Ackerman 2009); floral variation for some characters showed a conservative pattern of evolution between sister branches of the phylogeny (Martínez-Peralta et al. 2014). There is a clinal morphological variation in *N. mezcalaensis* from east to west associated primarily with longitude and elevation gradient, which mainly impacts fruit characters and plant size.

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