Morphometric and geographical variation in the *Ceratozamia mexicana* Brongn. (Zamiaceae) complex: evolutionary and taxonomic implications

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The Cycadales contain 11 nonproblematic genera and approximately 331 species where, in a number of cases, delimitation and circumscription is not so clear. *Ceratozamia*, the most diverse cycad genus in Mexico, clearly illustrates this situation because a large percentage (close to 70%) of the 28 currently valid species in the genus was recently described on the basis of inconspicuous differences. We evaluated population-level morphological variation (i.e. phenotype) in related plants of *Ceratozamia* that share geological and ecological units, seeking a refined taxonomic assessment of their populations in an explicitly evolutionary context. The populations/species of interest included the type species *Ceratozamia mexicana*, as well as the taxa *Ceratozamia brevifrons*, *Ceratozamia decumbens*, and *Ceratozamia morettii*. Data analysis was performed using uni- and multivariate techniques on morphological variability, which showed statistically significant differences. The characters 'leaflet width' and 'leaflet shape' supported diagnostic distinctions between groups of populations, and implied a classificatory rearrangement with nomenclatural consequences. This research is an initial step in the detailed, statistics-oriented study of phenotypic variation in the *Ceratozamia* populations of central Veracruz in Mexico that will allow the construction of evolutionary hypotheses in the context of phylogeography and historical demography. © 2016 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2016, **119**, 213–233.

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INTRODUCTION

'If you know a thing only qualitatively, you know it no more than vaguely. If you know it quantitatively – grasping some numerical measure that distinguishes it from an infinite number of other possibilities – you are beginning to know it deeply' (Sagan, 1997). The quantitative study of natural variation in ecologically important morphological traits has a long history in evolutionary ecology (Stebbins, 1950; Mayr, 1963; Grant, 1971). Studies in this particular biological area have traditionally attempted to understand the role of natural selection, gene flow, inbreeding, and genetic drift, and aimed to determine how these processes have influenced adaptation and organismal distribution and abundance. In plant species, phenotypic and genetic differentiation between populations that occupy environmental mosaics was demonstrated experimentally for the first time by Turesson (1922) and the existence of ecotypes across environmental gradients has been reported in several studies (Stebbins, 1950; Grant & Wilken, 1988; Stevenson, Sabato & Vázquez-Torres, 1986; González-Astorga, Vovides & Iglesias, 2003a; González-Astorga *et al.*, 2003b; Vovides *et al.*, 2004a).

The 'taxonomic species concept', which assumes the existence of morphologically similar populations within a defined geographical area that are morphologically distinct from other populations assigned to other species, is perhaps the most commonly used species concept in botany (Davis & Heywood, 1963); the order Cycadales does not appear to be an exception (Walters, Osborne & Decker, 2004). Such recognition and delimitation has frequently been carried out on the basis of subjective and arbitrary

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procedures. However, the introduction of numerical methods has allowed the quantification of these operations, making them reproducible and explicit practices (Sneath & Sokal, 1973). Within these methods, the main purpose of an analysis of geographical variation is to describe and synthesize patterns of variation in the characteristics of organisms distributed within a given area. Such analysis contemplates both continuous variables and discrete attributes, which can be categorized in taxonomic terms for the recognition of a population or series of populations, or for the interpretation of patterns such as adaptations to environmental factors (González-Astorga *et al.*, 2003a). Moreover, geographical analysis can lead to the probabilistic assignment of specimens without a clearly defined identity in terms of population or location of origin (Sneath & Sokal, 1973).

In Mexico, 54 cycad species are recognized in the three genera *Ceratozamia* Brongn., *Dioon* Lindl., and *Zamia* L. (Nicolalde-Morejón *et al.*, 2014). Morphological variation in these taxa, related to the geographical distribution of the type species *Dioon edule* Lindl. and *Zamia pumila* L., has been studied and defined by González-Astorga *et al.* (2003a) and Newell (1985), respectively. However, the type species of the genus *Ceratozamia* has not yet been studied from this perspective. This situation is notable given that, during the last 30 years, *Ceratozamia* has increased its taxonomic diversity and species richness by 70%, based on the analysis of strictly morphological differences.

The type species *Ceratozamia mexicana* was described by the French botanist Adolphe Brongniart in 1846 from living specimens that were taken from Mexico (with no information regarding the specific original location) and brought to the Muséum National d'Histoire Naturelle of Paris. In accordance with the historical usage, the protologue of *C. mexicana* characterized the species in strictly qualitative terms (Brongniart, 1846). From a contemporary perspective, Brongniart's qualitative description could fit several different species. The subsequent, complex taxonomic history of the genus involved fluctuations in its associated species richness. Although some studies recognized differences, others concentrated on the similarities (Regel, 1857; Miquel, 1861; Thiselton-Dyer, 1883; Schuster, 1932). Species complexes were proposed on the basis of morphological affinities and geographical distributions, although common agreement between the proposals was lacking (Moretti, Sabato & Vázquez-Torres, 1980; Stevenson *et al.*, 1986; Vovides *et al.*, 2004a, b). In the state of Veracruz, in Mexico, Vovides, Rees & Vázquez-Torres (1983) recognized only two species: *Ceratozamia miqueliana* H. Wendl. and *Ceratozamia mexicana* Brongn., and three varieties were described for the latter, acknowledging a mainly central distribution, with some northern and southern forms. It should be noted that, for these studies, the *mexicana* variety defines the species, which is therefore represented by the natural populations occurring in the municipalities of Jilotepec and Coacoatzintla, Veracruz. The importance of this observation relates to the specimen lectotypified by Stevenson & Sabato (1986) for the species differing morphologically from individuals found in these localities.

More recently, Vovides *et al.* (2004a) divided the genus *Ceratozamia* into seven species complexes. In the context of the present study, we highlight the proposal by Vovides *et al.* (2004a) of a complex that includes *C. mexicana*, restricted to the eastern slope of the Trans-Mexican Volcanic Belt. In the face of divergent taxonomic proposals regarding the identity and geographical distribution of the populations and species associated with *C. mexicana*, recognition of the biological boundaries of this species becomes particularly difficult (Moretti *et al.*, 1980; Stevenson *et al.*, 1986; Vovides *et al.*, 2004a, b; Chemnick *et al.*, 2008). In the present study, we conducted a meristic and morphometric evaluation of the relevant phenotypic variation aiming to answer three questions: (1) how can the extent of detectable and detected phenotypic variation be explained in an evolutionary ecology context; (2) is it possible to taxonomically diagnose the units (populations, species) evaluated through morphometric analyses in an evolutionary ecology context; and (3) what is the geographical identity of the type specimen of *C. mexicana*? To address these questions, we employed descriptive statistics, as well as uni- and multivariate morphometric analytical techniques (Sneath & Sokal, 1973; Marhold, 2011; Koutecký, 2015), with consideration given to the recommendations recently issued by the Cycad Classification Concepts Workshop (Osborne & Walters, 2004; Walters *et al.*, 2004).

MATERIAL AND METHODS

POPULATION SELECTION

Selection of the number and distribution of the populations was conducted on the basis of *exsiccatae* reviewed at the XAL, MEXU, FCME, CIB, P, HEM, and MO herbaria. The populations correspond to a geographical zone encompassing the eastern slope of the Trans-Mexican Volcanic Belt and the north of the South Range Mountain. This delimitation took into account the (ambiguous) proposals for distribution of the *C. mexicana* complex provided by Vovides *et al.* (2004a) and Chemnick *et al.* (2008) and the historical record of field collections by A. B.

Ghiesbreght, who collected the holotype (Hemsley, 1891; Rovirosa, 1891; Rzedowski, Calderón & Butanda, 2009). The plant species of this complex are included in 'vulnerable and endangered categories' by the IUCN Red List of Threatened Species (IUCN, 2015) and listed as 'under threat and endangered' by the Official Mexican Norm NOM-059-SEMARNAT-2010 [SEMARNAT (Secretaría de Medio Ambiente y Recursos Naturales), 2010]. Overall, the *Ceratozamia* populations distributed throughout the area of study consisted of the species *Ceratozamia brevifrons* Miq., *Ceratozamia decumbens* Vovides, Avendaño, Pérez-Farrera & González-Astorga, *Ceratozamia morettii* Vázquez-Torres & Vovides, and *C. mexicana* Brongn. (Vovides *et al.*, 1983). For each species, we sampled the type location and the furthest recorded population (i.e. two populations per species). Moreover, five geographically intermediate populations (but with no specific assigned epithet) were collected. The distributional range of the 13 populations comprised the montane arc in the centre of Veracruz, ranging from the Yecuatla municipality at the northern end to Tequila at the southern tip (Fig. 1, Table 1).

VEGETATIVE VARIATION

We analyzed 13 populations comprising the distributional range of the four species studied (15 ± 4 individuals per population) for a total of 196 adult individuals. From each foliar sample, we measured 17 quantitative morphological variables (Fig. 2, Table 2) selected from among those previously evaluated for morphological variation in Mexican cycads (González-Astorga *et al.*, 2003a; Pérez-Farrera *et al.*, 2004, 2007a; Vovides *et al.*, 2004a, b; Nicolalde-Morejón *et al.*, 2008), some of them deemed diagnostic in taxonomic treatments within the genus *Ceratozamia* (Brongniart, 1846; Miquel, 1861; Regel, 1876; Thiselton-Dyer, 1883; Schuster, 1932; Vovides *et al.*, 1983). Each specimen was herborized and deposited in the XAL herbarium.
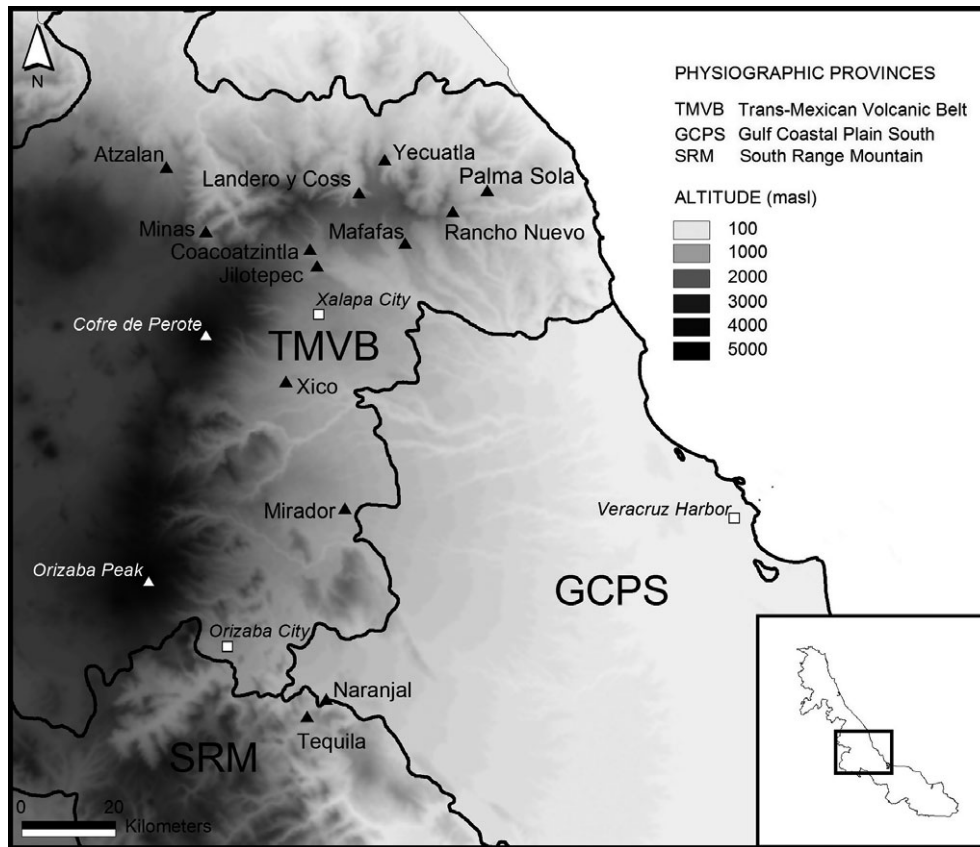

Figure 1. Geographical distribution of *Ceratozamia* complex populations. The black triangles represent the sampled populations distributed on the east slopes of the Trans-Mexican Volcanic Belt and the South Range Mountain in Mexico. The white triangles represent the mountain peaks as natural terrain references; squares correspond to the main cities of the state of Veracruz.

Table 1. Geographical location and sample size of the *Ceratozamia mexicana* complex populations

Taxon	Population (code)	Municipality	Co-ordinates	Altitude*	N	Herbarium voucher
Ceratozamia brevifrons[†]	Palma Sola (PAL)	Alto Lucero	19°46′03.38″N 96°36′47.32″O	864	20	A. Medina 327 (XAL)
Ceratozamia aff. *brevifrons*	Rancho Nuevo (RCHO)	Alto Lucero	19°43′38.66″N 96°40′43.64″O	1052	17	F. Nicolalde 1716 (CIB)
Ceratozamia decumbens[†]	Naranjal (NAR)	Naranjal	18°47.3′4.53″N 96°55.1′7.66″O	630	10	A. Medina 418 (XAL)
Ceratozamia aff. *decumbens*	Tequila (TEQ)	Tequila	18°45′32.65″N 96°57′27.83″O	840	6	A. Medina 430 (XAL)
Ceratozamia morettii[†]	Landero y Coss (LAN)	Landero y Coss	19°45′46.34″N 96°51′30.22″O	1312	14	A. Medina 247 (XAL)
Ceratozamia aff. *morettii*	Yecuatla (YEC)	Yecuatla	19°49′34.34″N 96°48′34.22″O	1325	18	A. Medina 353 (XAL)
Ceratozamia aff. *mexicana*	Mafafas (MAF)	Tepetlán	19°40′00.40″N 96°46′15.04″O	1420	15	F. Nicolalde 1703 (CIB)
Ceratozamia aff. *mexicana*[‡]	Coacoatzintla (COA)	Coacoatzintla	19°39′18.08″N 96°57′08.89″O	1574	20	A. Medina 288 (XAL)
Ceratozamia aff. *mexicana*[‡]	Jilotepec (JIL)	Jilotepec	19°37′23.70″N 96°56′19.30″O	1316	16	A. Medina 353 (XAL)
Ceratozamia aff. *mexicana*	El Mirador (MIR)	Comapa	19°09′28.99″N 96°53′07.36″O	1003	12	A. Medina 198 (XAL)
Ceratozamia aff. *mexicana*	Las Minas (MIN)	Las Minas	19°41′20.79″N 97°09′05.97″O	1586	19	A. Medina 217 (XAL)
Ceratozamia aff. *mexicana*	Atzalan (ATZ)	Atzalan	19°48′44.50″N 97°13′39.41″O	1400	19	A. Medina 227 (XAL)
Ceratozamia aff. *mexicana*	Xico (XICO)	Xico	19°24′05.42″N 96°59′54.72″O	1162	10	A. Medina 270 (XAL)

*m a.s.l.
[†]Locality of type specimens.
[‡]Representative locality associated with the taxon.

REPRODUCTIVE VARIATION

Reproductive variables were evaluated from strobili obtained in the field, as well as from samples from the 'Colección Nacional de Cícadas' at the Jardín Botánico Francisco Javier Clavijero (JBC) and the XAL herbarium (Instituto de Ecología, A.C.) (Fig. 2; Table 2, see also Supporting information, Table S1).

QUALITATIVE VARIATION

In addition to the above estimates, we coded 19 qualitative characters that included vegetative, reproductive, and overall habit traits (see Supporting information, Table S2). Each character state was recorded in the field and/or before herborization.

STATISTICAL ANALYSIS

Each variable per population was subjected to tests of normality (Shapiro–Wilk's test) and homoscedasticity (Bartlett's test) in advance. However, the analyses were ultimately carried out with the raw data set because the behaviour was the same as with the transformed data set.

We performed an analysis of correlation for the measured variables to determine the corresponding iso- and allometry. To compare the variation in magnitude of the variables in populations with different means in species of other genera within the Cycadales, we calculated coefficients of variation (CV) and the shape index for leaflets. We also performed analyses of variance (ANOVA) to estimate the intra- and interpopulation variation for each variable and to test whether all means were the same. To detect groups of means between population pairs, we conducted Tukey's multiple range analysis, which are also valuable for establishing statistically significant and phenotypically recognizable quantitative limits.

Finally, we performed a discriminant analysis to estimate the multivariate differentiation between groups and to determine which vegetative variables have greater influence on the dispersion of variation. From this analysis, we obtained centroids for each population, and then calculated paired Euclidean
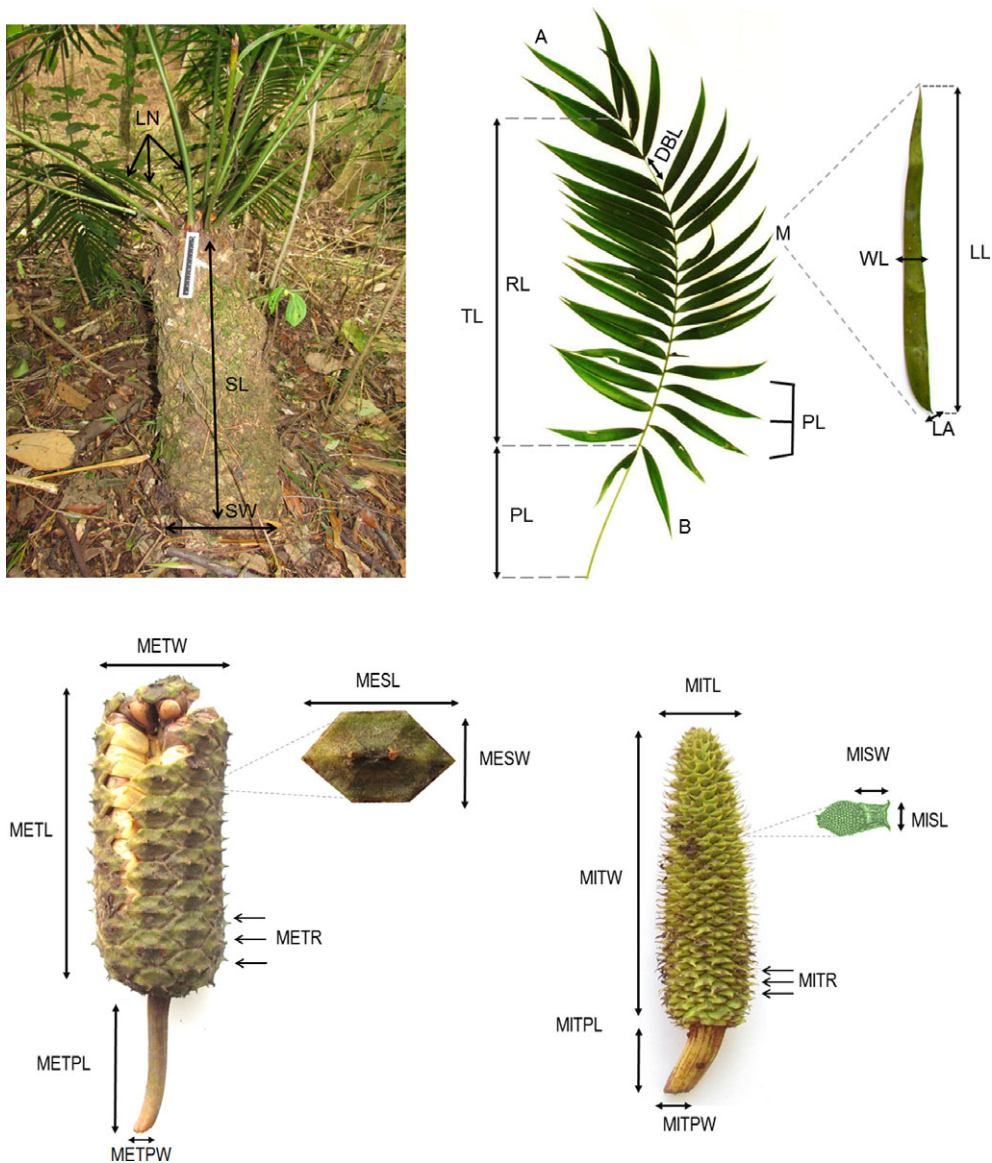
Figure 2. Morphometric variables evaluated in the present study. Upper left: LN, leaf number; SL, stem length; SW, stem width. Upper right: TL, total length; PL, petiole length; RL, rachis length; LP, leaflet pairs; B, basal level; M, middle level; A, apical level; DBL, distance between leaflets; LL, leaflet length; LW, leaflet width, LA, leaflet articulation. Lower left: METL, megastrobilus length; METW, megastrobilus width; METPL, megastrobilus peduncle length; METPW, megastrobilus peduncle width; METR, megastrobilus rows; MESL, megasporophyll length; MESW, megasporophyll width. Lower right: MITL, microstrobilus length; MITW, microstrobilus width; MITPL, microstrobilus peduncle length; MITPW, microstrobilus peduncle width; MITR, microstrobilus rows; MISL, microsporophyll length; MISW, microsporophyll width.

distances and resampled randomly to build a null model (with 10 000 replicas) as a test of significance. To corroborate the identity of the taxonomic identities defined a priori, we cross-validated the population assignation of each individual, from which we obtained a confidence value (i.e. the probability of correctly classifying an individual with respect to its population of origin). With these values and the 'predict' function obtained from the discriminant analysis, we probabilistically estimated the population of origin for the type specimens of each species. The analyses described in this subsection were executed in the statistical environment R (R Development Core Team, 2012).

Table 2. Vegetative and reproductive variables measured

Variable	Code
Vegetative	
Leaf number	LN
Leaflet pairs	LP
Petiole length	PL
Rachis length	RL
Total length	TL
Basal leaflet length	BLL
Basal leaflet width	BLW
Basal leaflet articulation	BLA
Distance between basal leaflets	DBBL
Middle leaflet length	MLL
Middle leaflet width	MLW
Middle leaflet articulation	MLA
Distance between middle leaflets	DBML
Apical leaflet length	ALL
Apical leaflet width	ALW
Apical leaflet articulation	ALA
Distance between apical leaflets	DBAL
Reproductive	
Megastrobilus rows	METR
Megastrobilus length	METL
Megastrobilus width	METW
Megastrobilus volume	METV
Megastrobilus peduncle length	METPL
Megastrobilus peduncle width	METPW
Megasporophyll length	MESL
Megasporophyll width	MESW
Megastrobilus rows	METR
Female stem length	FSL
Female stem width	FSW
Female stem volume	FSV
Microstrobilus rows	MITR
Microstrobilus length	MITL
Microstrobilus width	MITW
Microstrobilus volume	MITV
Microstrobilus peduncle length	MITPL
Microstrobilus peduncle width	MITPW
Microsporophyll length	MISL
Microsporophyll width	MISW
Microstrobilus rows	MITR
Male stem length	MSL
Male stem width	MSW
Male stem volume	MSV

RESULTS

CORRELATIONS: VEGETATIVE VARIABLES

From the matrix of correlations (Table 3), the variables that were highly positively and significantly correlated were: (1) total length vs. petiole length (0.73) and total length vs. rachis length (0.91), which appears to indicate that, although the rachis and petiole contribute to the increased total length, they do not grow isometrically; (2) the widths of the paired leaflets (0.89, 0.83, and 0.89, respectively), which also indicate asymmetry in their growth; and (3) leaflet widths vs. distances between leaflets at the three levels (0.42, 0.77, and 0.69, respectively), which indicate that the wider the leaflet, the greater the distance between the insertion points on the rachis.

Among the highly negatively and significantly correlated variables are the leaflet pairs vs. leaflet widths at the three levels (−0.66, −0.70, and −0.73, respectively), which indicate that the higher the number of leaflet pairs, the lower their width. With the values of each population's mean of leaflet pairs vs. middle leaflet width a highly significant negative correlation is obtained ($r = -0.86$); similarly, this pattern is also found in a parallel population study of two *Zamia* species morphological and taxonomically related (Fig. 3, left). Furthermore, using only the *Ceratozamia* species population data and the two groups of statistically differentiated populations, we found significant negative correlation ($r = -0.71$) (Fig. 3, right).

CORRELATIONS: REPRODUCTIVE VARIABLES

From the matrix of correlations of the male reproductive variables (Table 4), the highly positively and significantly ones were: (1) microstrobilus rows vs. microstrobilus length and microstrobilus rows vs. microstrobilus volume (0.91 and 0.87, respectively), which indicates that the longer the strobilus and more robust the stem, the more rows contained in the strobilus, and (2) the correlations of the microstrobilus volume vs. male stem volume, microstrobilus length and microstrobilus width (0.85, 0.84, and 0.72, respectively), which indicates that the more robust the stem, the larger are the strobili and their sporophylls.

From the matrix of correlations of the female reproductive variables (Table 5), the highly positively and significantly ones were: (1) megastrobilus rows vs. megastrobilus length and female stem volume (0.82 and 0.78, respectively), which indicates that the longer the megastrobilus and larger the stem, the higher the number of rows in the strobilus, and (2) megastrobilus volume vs. female stem volume ($r = 0.95$), which indicates that the more robust the stems, the larger the strobili produced.

COEFFICIENT OF VARIATION AND SHAPE INDEX

From the exhaustive search of studies of populations within the order Cycadales, the coefficients of variation [CV = (SD/mean) × 100] of the evaluated

Table 3. Pearson's coefficient matrix of vegetative variables

	LP	PL	RL	TL	BLL	BLW	BLA	DBBL	MLL	MLW	MLA	DBML	ALL	ALW	ALA	DBAL
LN	0.667*	0.235*	0.539*	0.500*	0.268*	−0.368*	0.007	0.165*	0.372*	−0.389*	0.147*	−0.344*	0.086	−0.451*	−0.206*	−0.368*
LP		0.093	0.794*	0.625*	0.167*	−0.666*	0.005	−0.065	0.413*	−0.700*	0.194	−0.703*	0.109	−0.733*	−0.260*	−0.636*
PL			0.402*	0.739*	0.593*	0.191*	0.035	0.409*	0.501*	0.167*	0.076	0.248*	0.247*	0.078	−0.057	0.186*
RL				0.914*	0.360*	−0.346*	0.067	0.253*	0.543*	−0.349*	0.267*	−0.355*	0.221*	−0.445*	−0.108	−0.325*
TL					0.528*	−0.170*	0.065	0.368*	0.621*	−0.183*	0.230*	−0.151*	0.272*	−0.293*	−0.104	−0.157*
BLL						0.111	0.204*	0.185*	0.760*	0.001	0.229	0.141*	0.416*	−0.022	0.069	0.114
BLW							0.221*	0.421*	−0.126	0.899*	0.052	0.725*	0.056	0.839*	0.356*	0.592*
BLA								0.020	0.183*	0.137	0.810*	−0.045	0.112	0.079	0.607*	−0.051
DBBL									0.158*	0.382*	0.011	0.372*	0.070	0.250*	0.086	0.188*
MLL										−0.181*	0.292*	−0.044	0.583*	−0.180*	0.071	−0.037
MLW											0.074	0.770*	0.045	0.892*	0.377*	0.662*
MLA												−0.147*	0.162*	−0.005	0.574*	−0.192*
DBML													0.119	0.743*	0.235*	0.805*
ALL														0.156*	0.221*	0.169*
ALW															0.423*	0.693*
ALA																0.238*

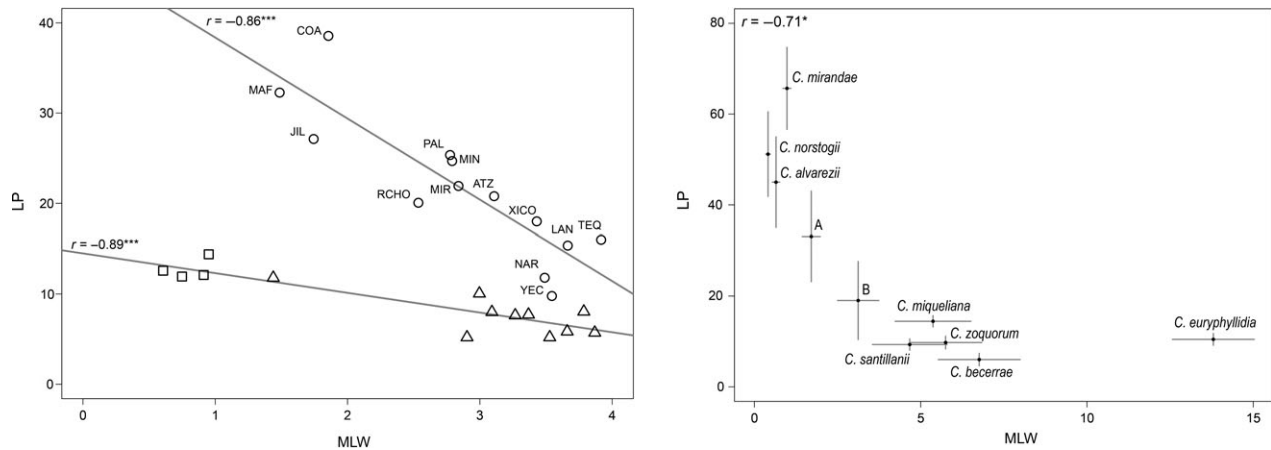$N = 196$. For codes, see Table 2.
*$P < 0.01$.

Figure 3. Middle leaflet width (MLW) vs. leaflet pairs (LP) correlation. Left: ○, populations of the present study; □, *Zamia loddigesii* Miq.; Δ, *Zamia prasina* Bull (*P < 0.05, **P < 0.01, ***P < 0.001). The *Zamia* data are from Francisco Limón-Salvador (unpubl. data). Right: each point corresponds to the mean values of middle leaflet width (MLW) and leaflet pairs (LP) from population studies of *Ceratozamia* species; the lines represent the variance (i.e. ± SD). The uppercase letters corresponds to statistical differentiated groups of populations at MLW and shape index (SI) variables: A, COA-MAF-JIL; B, PAL-RCHO-MIN-MIR-ATZ-XICO-NAR-TEQ-YEC-LAN. The *Ceratozamia* species data were obtained from Pérez-Farrera *et al.* (2004, 2009). For codes, see Tables 1, 2.

Table 4. Pearson's coefficient matrix of male reproductive variables

	MITL	MITW	MITV	MITPL	MITPW	MISL	MISW	MSL	MSW	MSV
MITR	0.919***	0.9***	0.906***	0.285	0.157	0.755*	0.621	0.889**	0.866**	0.87***
MITL		0.792*	0.88**	0.173	0.264	0.821**	0.745*	0.844	0.804**	0.78*
MITW			0.971***	0.334	0.138	0.788*	0.614	0.745*	0.856**	0.857**
MITV				0.237	0.206	0.844**	0.727*	0.79*	0.857**	0.855**
MITPL					−0.282	0.249	−0.012	0.072	0.159	0.165
MITPW						−0.2	−0.218	0.525	0.165	0.214
MISL							0.931***	0.473	0.733*	0.662
MISW								0.402	0.584	0.506
MSL									0.781*	0.819**
MSW										0.982***

N = 9. For codes, see Table 2.
*P < 0.05; **P < 0.01; ***P < 0.001.

attributes were calculated with the values of the statistics reported. The variables reported differed from one study to the other; however, leaflet length and width were invariably recorded (see Supporting information, Table S3). Figure 4 shows that the CV values of the studied populations occur with the greatest frequency in the interval 10–20 (mean ± SD: 17.7 ± 6.9). The minimum value was presented by *Ceratozamia euryphyllidia* Vázquez-Torres, Sabato & D.W.Stev. (7.89) and the maximum by *Zamia loddigesii* Miq. (38.43). *Zamia* and *Cycas* L. were the genera with the widest range of CV values, whereas *Dioon*, *Encephalartos* Lehm., and *Ceratozamia* were found around the mean, within the

SD, and within the interval with the highest frequency.

Regarding the shape index, *Ceratozamia* was the genus with the most extreme values and, consequently, presented the most divergent leaflet shapes (Fig. 5). The minimum value was 1.86 in *C. euryphyllidia* and ranged up to 86.7 in *Ceratozamia norstogii* D.W.Stev. In the genus *Zamia*, the species *Zamia prasina* W.Bull presented the widest leaflet, whereas *Z. loddigesii* had the narrowest. The populations of *Encephalartos*, *Cycas*, and *Dioon* presented intermediate values, from 8.58 to 22.5. The populations of the complex evaluated here also presented intermediate values, from 8.29 of the Yecuatla

Table 5. Pearson's coefficient matrix of female reproductive variables

	METL	METW	METV	METPL	METPW	MESL	MESW	FSL	FSW	FSV
METR	0.824*	0.408	0.652	0.093	0.646	−0.305	−0.03	0.693	0.651	0.789*
METL		0.704	0.932**	0.518	0.647	0.111	0.399	0.802*	0.924**	0.97***
METW			0.899**	0.653	0.358	0.62	0.748	0.455	0.75	0.745
METV				0.627	0.595	0.398	0.659	0.693	0.926*	0.954***
METPL					−0.202***	0.805*	0.748	0.055	0.753	0.512
METPW						−0.324	0.119	0.848*	0.435	0.677
MESL							0.889**	−0.295	0.421	0.208
FSW								0.051	0.655	0.52
FSL									0.562	0.717
FSW										0.931**

N = 7. For codes, see Table 2.
*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.
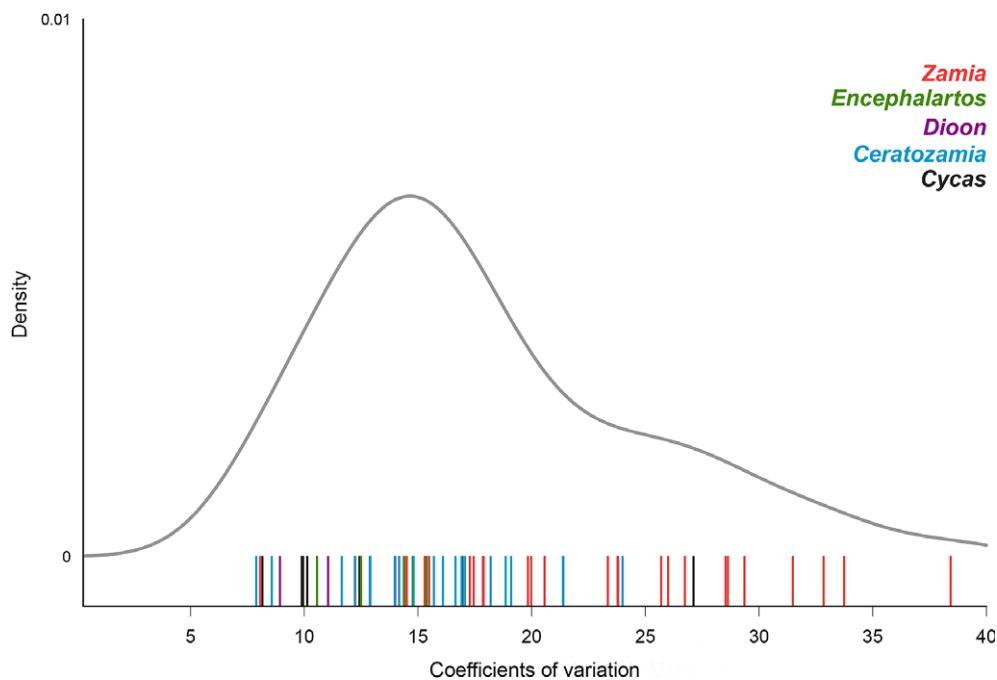
Figure 4. Distribution of coefficient of variation of middle leaflet width (MLW) in different populations of Cycadales.

population to the maximum value of 23.5 in the Mafafas population.

LINEAR DISCRIMINANT ANALYSIS

In the discriminant analysis, the first and second linear coefficients contain 66% of the entire variation of the vegetative variables (Table 6). The Wilk's λ was low (0.0019) and significant ($F_{12,183}$ = 7.3901, $P < 0.001$), which indicates that there is a global effect of the grouping by populations, despite overlap among individuals in the diagram of dispersion

(Fig. 6). The variables with most influence in axis one correspond to leaflet width at the middle level towards its positive extreme, and the variable leaflet pairs towards its negative extreme. On axis two, the variables were rachis length towards the positive extreme and total length towards the negative extreme.

Significant differences were detected among the centroids of the populations (Table 7; see also Supporting information, Fig. S1). Almost all of the population pairs were significant, except for Minas-Atzalan, Landero-Xico, Landero-Yecuatla,
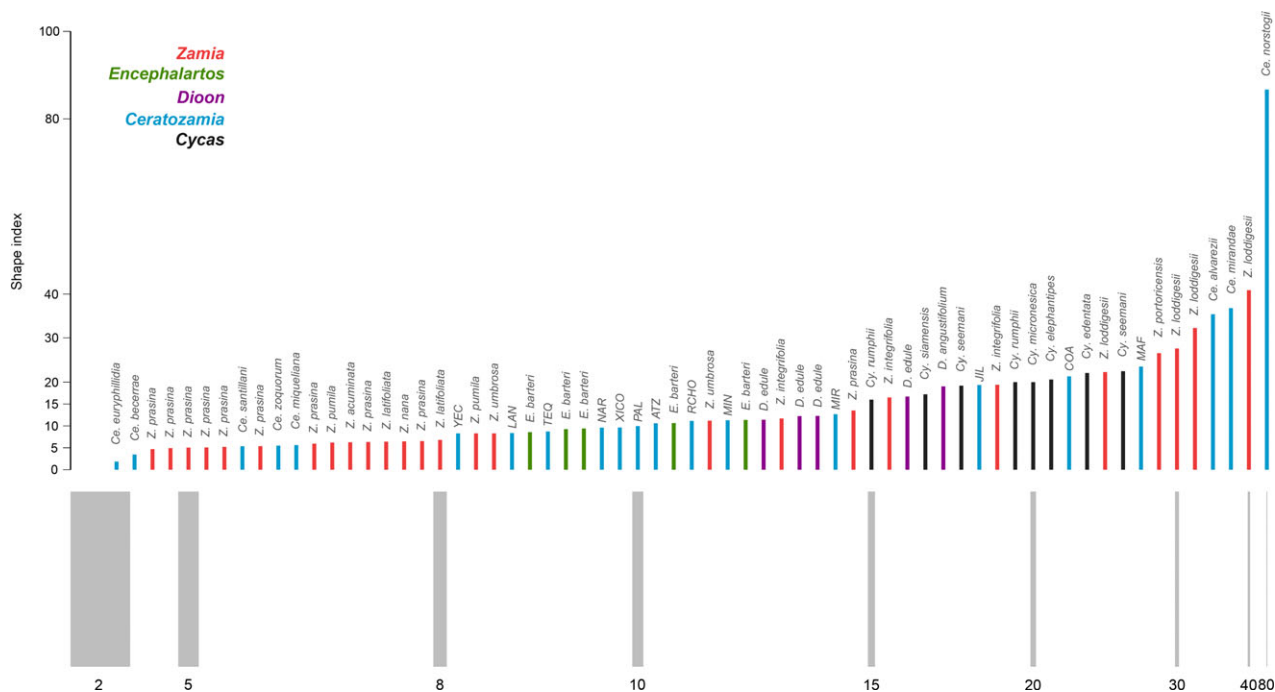
Figure 5. Shape index in different populations of Cycadales. The populations of the present study are indicated by its code (see Table 1), and the grey bars represent the shape of the leaflets based on the length/wide ratio.

Table 6. Linear discriminant coefficients of the 17 vegetative quantitative variables (from highest to lowest). For codes, see Table 2

Variable	LD1	LD2	Variable
MLW	0.7590	3.2969	RL
ALW	0.4892	2.1918	PL
BLW	0.4599	1.1621	MLA
RL	0.4543	0.5163	BLA
ALA	0.3795	0.4323	ALA
MLA	0.2946	0.3510	ALL
DBML	0.2108	0.1554	LN
TL	0.1127	−0.0069	DBML
DBBL	0.0330	−0.0887	BLL
LN	0.0220	−0.2700	DBBL
BLA	−0.0258	−0.2962	DBAL
DBAL	−0.0364	−0.4255	MLW
PL	−0.1518	−0.4451	ALW
MLL	−0.1895	−0.6845	BLW
ALL	−0.2067	−0.7360	MLL
BLL	−0.3994	−1.0012	LP
LP	−0.5443	−4.9579	TL
Percentage of variation	38%	28%	

Xico-Yecuatla, and Naranjal-Tequila. The value of P is interpreted as the probability of obtaining by chance an equal or greater distance than that observed (i.e. that the nonsignificant population pairs do not differ among themselves within the multivariate space).

The cross-validation provided high 'confidence values', which is considered as the probability of correctly classifying the individuals to their original population (Table 8). The geographical distribution of correctly classified individuals, as well as those that were identified with other populations, is shown in the Supporting information (Fig. S2). Figure 7 summarizes the individual posterior probabilities from the cross-validation, which is interpreted as the individual 'phenotypic proportion' (i.e. a degree of morphological similarity).

Regarding the classification of 'type specimens' (Table 9), the analysis assigns the maximum probability of 1.0 that the type specimen of *C. brevifrons* comes from the population of Rancho Nuevo. The type *C. morettii* is assigned to the population of Yecuatla with a maximum probability of 0.62. The type *C. decumbens* is assigned to the population Mirador with a maximum probability of 1.0. The type *C. mexicana* (Collection of A. B. Ghiesbreght w.n.) is assigned to the population Rancho Nuevo with a maximum probability of 0.98. It is possible to graphically visualize the positions occupied by the 'type specimens' in the multivariate space of the discriminant analysis (see Supporting Information, Fig. S3), which coincide probabilistically with the determined populations.
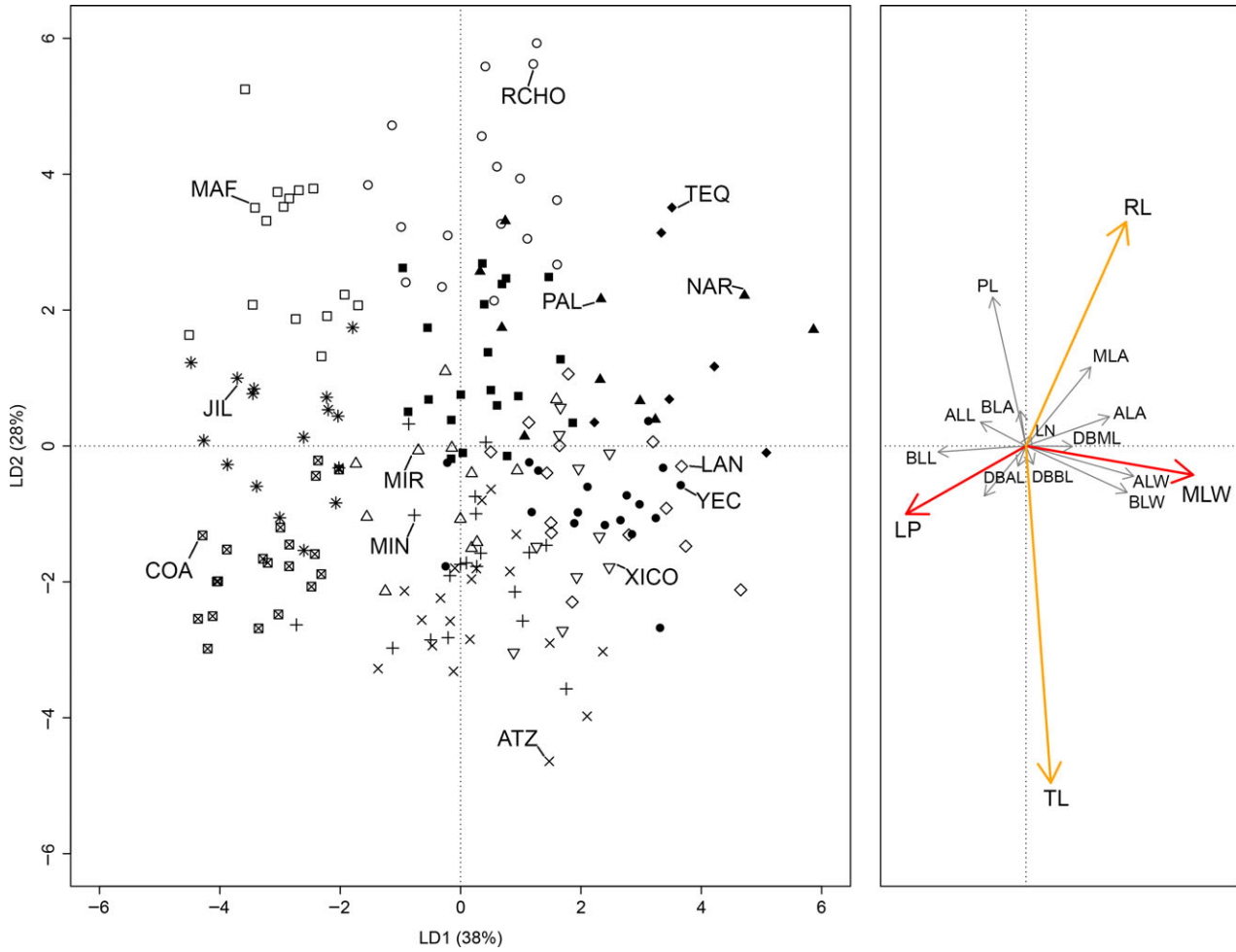
Figure 6. Scatterplot of discriminant analysis. This plot contains the 196 adult individuals that were evaluated. The arrows represent the most influential variables of axes one (red) and two (yellow). For codes, see Tables 1 and 2.

Table 7. Significance of Euclidean distances between population centroid pairs

	MAF	RCHO	MIR	MIN	ATZ	LAN	XICO	COA	JIL	PAL	YEC	NAR
RCHO	***											
MIR	***	***										
MIN	***	***	*									
ATZ	***	***	***	0.14								
LAN	***	***	***	***	***							
XICO	***	***	***	***	***	0.28						
COA	***	***	***	***	***	***	***					
JIL	***	***	***	***	***	***	***	***				
PAL	***	***	***	***	***	***	***	***	***			
YEC	***	***	***	***	***	0.84	0.51	***	***	***		
NAR	***	***	***	***	***	***	***	***	***	***	***	
TEQ	***	***	***	***	***	***	***	***	***	***	***	0.09

For codes, see Table 1.

*P < 0.05; **P < 0.01; ***P < 0.001.

Table 8. Confidence value corresponding to the proportion of individuals correctly identified with respect to the total number of individuals in each population (N)

Population	Correctly identified/N	Confidence
PAL	20/20	1.00
RCHO	16/17	0.94
JIL	15/16	0.94
XICO	9/10	0.90
MAF	13/15	0.87
LAN	12/14	0.86
COA	17/20	0.85
MIR	10/12	0.83
TEQ	5/6	0.83
NAR	8/10	0.80
MIN	15/19	0.79
ATZ	15/19	0.79
YEC	13/18	0.72

For codes, see Table 1.

UNIVARIATE ANALYSIS

All of the analyses of variance of the vegetative variables were significant, indicating that at least some of the population means are different (Table 10). The variable leaflet width at the middle level is notable for presenting the highest r^2 value, with 0.75 ($F_{12,183} = 46.21$, $P < 0.001$), which thus coincides with the discriminant analysis because it was the variable that obtained the highest influence value (Table 6). Among the vegetative variables with $r^2 > 0.50$ (i.e. where the majority of the variation is contained between populations), these were the values of leaflet width, distances between leaflets, shape indexes, leaflet pairs, and petiole length.

TUKEY'S MULTIPLE RANGE ANALYSIS

Although the ANOVA of each variable indicates significant differences among the populations, only six of the 20 vegetative variables evaluated identified two different groups of means that are congruent among them. In the other 17 variables, the means of the 13 populations overlap, impeding the determination of homogeneous groups (Table 11). The variables related to the width and shape index of the leaflet in the three levels (base, middle, and apex) differentiate the group with narrow linear leaflets vs. the group of wide oblong leaflets. The populations that integrate the first group are Mafafas, Coacoatzintla, and Jilotepec, whereas the second group comprises the populations of Palma Sola, Rancho Nuevo, Yecuatla, Las Minas, Landero y Coss, Atzalan, El Mirador, Xico, Naranjal, and Tequila (Table 12). Considering the leaflet width at the middle level as the structure least susceptible to alteration during its growth (by herbivory or mechanical damage), and by being one of the diagnostic attributes of the species in most of the literature, this attribute presents a clear gap among the means of the population groups (Fig. 8).

FREQUENCY OF QUALITATIVE CHARACTERS

Seven of the 19 characters evaluated were variable (Table 13), whereas the other 13 uniformly presented one single invariable state. The character state
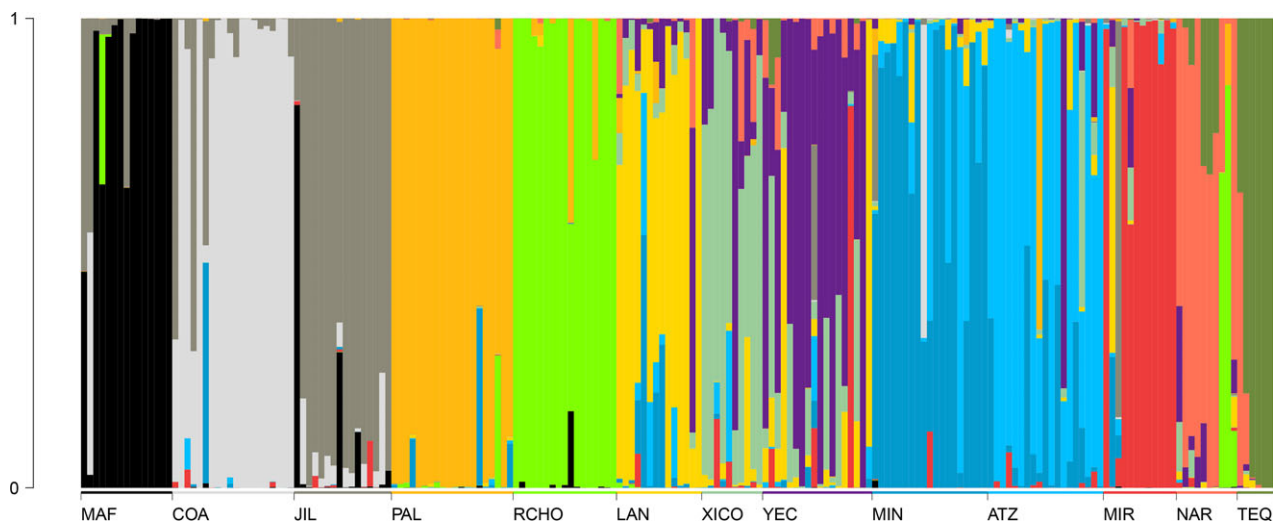

Figure 7. Probabilistic assigment plot. Each vertical bar corresponds to an individual that contains the population's allocation posterior probabilities used in the cross-validation. For codes, see Table 1.

Table 9. Probabilistic assignment of type specimens based on linear discriminant analysis

Type specimens	MAF	RCHO	MIR	MIN	ATZ	LAN	XICO	COA	JIL	PAL	YEC	NAR	TEQ
Ceratozamia brevifrons	0.0000	**1.0000**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000*	0.0000	0.0000	0.0000
Ceratozamia morettii	0.0000	0.0000	0.0018	0.0190	0.1202	0.0770*	0.1561	0.0000	0.0000	0.0000	**0.6258**	0.0000	0.0000
Ceratozamia decumbens	0.0000	0.0000	**1.0000**	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000*	0.0000
Ceratozamia mexicana	0.0155	**0.9832**	0.0001	0.0000	0.0000	0.0000	0.0000	0.0000†	0.0006†	0.0000	0.0000	0.0005	0.0000

For codes, see Table 1. Values shown in bold correspond to the highest probability value assigned by the model.
*Type localities.
†Representative locality associated with the taxon.

'aculeate' of the petiole was presented in all of the populations, except for some individuals in the population of Xico. The 'aculeate' rachis occurred in all of the populations; however, eight populations, including Yecuatla, Rancho Nuevo, Mafafas, Coacoatzintla, Xico, Mirador, Tequila, and Naranjal, presented 'inermous' individuals. Naranjal presented the 'inermous' character state in 80% of its individuals. The 'flat' insertion of the leaflets on the rachis is the predominant character state in all of the populations, whereas the 'keeled' insertion was presented in four populations: Yecuatla, Landero y Coss, Palma Sola, and Rancho Nuevo. The colour 'green' of the articulation is the character state that prevails in almost all of the populations, except for those of Palma Sola, Yecuatla, Rancho Nuevo, and Landero y Coss; in these two latter populations, the colour 'yellow' dominates over 'green' in 53% and 71% of individuals, respectively. In the population Naranjal, the colour 'reddish–ochre' was recorded in 30% of the individuals. The direction 'straight' (or perpendicular) of the leaflets relative to the rachis was the predominant character state in almost all of the populations, apart from Mafafas, Palma Sola, and Jilotepec, where the status 'falcate' was found in 20%, 25% and 50%, respectively. The 'asymmetric' character state of the leaflet apices dominated in almost all of the populations, apart from those of Mafafas, Jilotepec, and Coacoatzintla, where 'symmetric' was the only character state observed.

DISCUSSION

The molecular phylogenetic analysis performed by Nagalingum *et al.* (2011), which was calibrated with palaeobotanical dates, questioned the idea of considering cycads as classical examples of living fossils because the results indicated synchronic diversification over a time period not greater than 12 Myr into the past. However, Nagalingum *et al.* (2011) indicate that the short terminal branches obtained in their phylogenetic reconstructions could raise doubts regarding the validity of some currently accepted cycad species, despite the supporting taxonomic evidence. This evidence involves the genera *Encephalartos* (van der Bank *et al.*, 2001) and *Zamia* (Nicolalde-Morejón, Vovides & Stevenson, 2009). From a character-based DNA barcoding perspective, Nicolalde-Morejón *et al.* (2011) found that, using up to six genic regions, it is only possible to achieve 78% of correct species identifications in *Ceratozamia*, a finding that suggests that < 20% of the currently accepted diversity in this genus might be a taxonomic overestimation. In our view, taxonomically ambiguous entities could hinder the construction of

Table 10. Analysis of variance of the variables with $r^2 > 0.50$

Variable	DFM	DFE	SSM	SSE	SST	MSM	MSE	F	P	r^2
MLW	12	183	100.84	33.28	134.12	8.404	0.182	46.210	< 0.001	0.75
MLL/MLW	12	183	5192	1820	7012	432.7	9.900	43.510	< 0.001	0.74
MLA	12	183	93.13	43.03	136.16	7.761	0.235	33.010	< 0.001	0.68
MLB	12	183	100.05	48.25	148.3	8.338	0.264	31.620	< 0.001	0.67
DBML	12	183	240.2	124.7	364.9	20.015	0.681	29.380	< 0.001	0.66
BLL/BLW	12	183	3870	2194	6064	322.5	12.000	26.900	< 0.001	0.64
DBAL	12	183	316.6	255.4	572	26.38	1.396	18.900	< 0.001	0.55
LP	12	183	12 560	10 489	23 049	1046.7	57.3	18.26	< 0.001	0.54
ALL/ALW	12	183	5489	4641	10 130	457.4	25.4	18.04	< 0.001	0.54
PL	12	183	34 739	33 572	68 311	2894.9	183.5	15.78	< 0.001	0.51

DFM, degrees of freedom model; DFE, degrees of freedom error; SSM, sum of squares model; SSE, sum of squares error; SST, sum of squares total; MSM, mean square model; MSE, mean square error. For codes, see Table 2.

adequate evolutionary explanations of the mechanisms that triggered speciation in the genus.

EVOLUTIONARY IMPLICATIONS

Most evolutionary trends in the morphology of vascular land plants are related to greater specialization. In turn, specialization can be obtained from three types of structural change: reduction, fusion, and symmetry. Reduction, which is understood as a reduced growth period between the successive differentiation of appendages or internodes (Stebbins, 1950), is the commonest of these trends. In the context of the present study, the character 'leaflet width', which negatively correlates with the quantity of leaflet pairs in a leaf, deserves special attention. The relationship between appendage reduction and multiplication suggests that phylogenetic tendencies of this type are the result of changes in growth synchronization and phases of differentiation of structures with a determinate growth; a phenomenon known as allometry (Stebbins, 1950). The correlation between leaflet pairs and the width of the middle leaflet (Fig. 3) suggests a fixed allometric behaviour in species or groups of populations, as supported by statistically significant negative correlations, and this is probably associated with phylogenetic restrictions. In both genera, a 'trade-off' between leaflet width and quantity is apparent (i.e. an increase in leaflet pair number corresponds to narrower leaflets, and vice versa). This compensation perhaps belongs to an equilibrium maintained by the foliar area to optimize the performance of physiological functions, as determined previously in other vascular plants (Parkhurst & Loucks, 1972; Givnish & Vermeij, 1976; Givnish, 1987). The stability of this correlation in plants from different populations cultivated under uniform 'common garden' conditions suggests genetic control of the corresponding variables (González-Astorga et al., 2003a).

The distribution of variation coefficients (Newell, 1985, 1986, 1989; González-Astorga et al., 2003a; Lindström, 2004; Pérez-Farrera et al., 2004; Ekué et al., 2008; Pérez-Farrera et al., 2009; Lindström et al., 2013; F. Limón-Salvador, unpubl. data) suggests that there is a tendency for the maintenance of low variation levels with respect to the leaflet width variable. However, there are differences between genera that could be related to their alpha diversity. According to Osborne et al. (2012), the most diverse genus in Cycadales is Cycas (110 spp.), followed by Zamia (76 spp.). Both genera coincide in terms of presenting the highest variation levels in population analyses of their species. In turn, populations of Ceratozamia (27 spp.) and Dioon (14 spp.) show more conservative variation values, which are possibly reflected in their relatively low species diversity within cycads. Encephalartos (65 spp.) appears to be an exception; this genus presents low variation and high species diversity, although this could be the result of insufficient sampling at the population level not accurately reflecting intrageneric variation.

Despite the homogeneity that Ceratozamia shows in variation coefficient values for leaflet width, this genus has (along with Zamia) the widest spectrum of leaflet shape (shape index). The 13 Ceratozamia populations evaluated in the present study are placed in the median zone of this range, although certain populations have a tendency towards elongation and narrowness (e.g. Jilotepec, Coacoatzintla, and Mafafas). These localities coincide with the group A of populations where a relatively high number of leaflet pairs co-occur with narrow leaflets (Fig. 3, right).

TAXONOMIC IMPLICATIONS

Our discriminant analysis, which produced high confidence values, supported the prediction of the origin of the C. mexicana holotype, and its assignment to

Table 11. Tukey's multiple range analysis from all vegetative variables ($P < 0.05$)

Population	LN	LP	PL	RL	TL	BLL	BLW	BLA	DBBL	B	MLL	MLW	MLA	DBML	M	ALL	ALW	ALA	DBAL	A
YEC	d	e	bcd	d	de	bc	A	cdef	bcd	b	cd	ab	de	a	d	ab	a	bcd	a	b
XICO	bcd	cde	abc	abc	abc	abc	Ab	bcd	abcd	b	abcd	abc	bcde	abc	cd	ab	abc	bcdef	ab	b
NAR	d	de	bcd	cd	cde	abc	Ab	ab	bcd	b	bcd	ab	abc	ab	cd	ab	ab	ab	abc	b
LAN	cd	de	cd	bcd	cde	c	A	bcde	bcd	b	bcd	a	cde	bc	d	a	a	abc	bc	b
MIR	bcd	cd	a	ab	ab	a	abc	abc	abc	b	ab	cde	bcd	cde	c	ab	d	bcdef	bc	b
TEQ	bcd	cde	a	abcd	abc	abc	a	ab	abcd	b	abcd	a	a	abc	cd	ab	a	a	bcd	b
MIN	bcd	bc	de	abc	cde	c	bc	f	bcd	b	bcd	de	de	def	cd	ab	bcd	defg	cd	b
ATZ	bcd	cd	ab	abc	abc	bc	abc	ef	a	b	bcd	bcd	e	cd	cd	ab	cd	efg	cd	b
RCHO	bcd	cd	e	cd	e	c	a	a	de	b	d	e	ab	fg	cd	b	d	abc	de	b
PAL	ab	bc	cd	bcd	cd	c	c	bcd	ab	b	d	de	abc	fg	cd	ab	d	cdef	de	b
JIL	bc	bc	bcd	bcd	bcd	abc	d	ef	cde	a	abcd	f	de	efg	b	a	e	fg	de	a
COA	a	a	ab	a	a	ab	d	def	cd	a	a	f	de	fg	ab	a	e	g	de	a
MAF	bcd	ab	cde	abc	cd	bc	d	bcd	e	a	abc	f	abc	g	a	a	e	bcde	e	a

For codes, see Tables 1, 2.
B, basal leaflet shape index; M, middle leaflet shape index; A, apical leaflet shape index.

Table 12. Tukey's multiple range analysis ($P < 0.01$) of middle leaflet width (MLW) and shape index (SI)

Population	MLW	Range	SI	Range
TEQ	3.92 (0.63)	a	8.73 (2.31)	a
LAN	3.66 (0.57)	ab	8.37 (1.45)	a
YEC	3.54 (0.45)	ab	8.30 (1.63)	a
NAR	3.49 (0.74)	abc	9.60 (2.34)	a
XICO	3.43 (0.30)	abcd	9.64 (1.26)	a
ATZ	3.11 (0.46)	bcde	10.61 (2.03)	a
MIR	2.84 (0.39)	cdef	12.68 (1.96)	a
MIN	2.79 (0.39)	def	11.30 (3.12)	a
PAL	2.78 (0.39)	ef	9.97 (1.86)	a
RCHO	2.54 (0.47)	f	11.14 (3.11)	a
COA	1.86 (0.23)	g	21.32 (3.85)	b
JIL	1.74 (0.20)	g	19.34 (4.07)	b
MAF	1.49 (0.24)	g	23.56 (6.38)	b

For codes, see Table 1. Mean values are shown (cm), with the SD in parentheses.

the Rancho Nuevo population. This result is in contrast to the traditional identification of this taxon with the populations of Coacoatzintla and Jilotepec, as designated by Chamberlain (1912) and Vovides *et al.* (1983). It should be noted that the taxonomic identification in these previous studies comes from a direct comparison with the original description, and not with the type specimen (much less from statistic morphometric sampling) as in the present study. The incorporation of a spectrum of population-level morphological variation in the multivariate and probabilistic interpretation of the type specimens of the species evaluated in the present study increases the probability of correct identification, even though these do not correspond to the most 'typical' or representative of the corresponding taxa (the latter observation is congruent with Article 7.2 of the International Code of Botanical Nomenclature; Mcneill *et al.*, 2012).

In our discriminant analysis, the leaflet width variable has the highest influence. This explains the dispersion of variation towards the positive extreme on axis one, whereas the number of leaflet pairs has the highest influence towards its negative side. This is highly relevant from an evolutionary point of view because the correlation between these variables appears to indicate clear phylogenetic trends in vascular plants (Givnish, 1987). The influence of the middle leaflet width variable in the analyses is congruent with its corresponding analysis of variance, given that it was statistically significant and had the highest value of the variation explained by the model (i.e. $r^2 = 0.75$). The discriminant analysis was also congruent with Tukey's multiple range
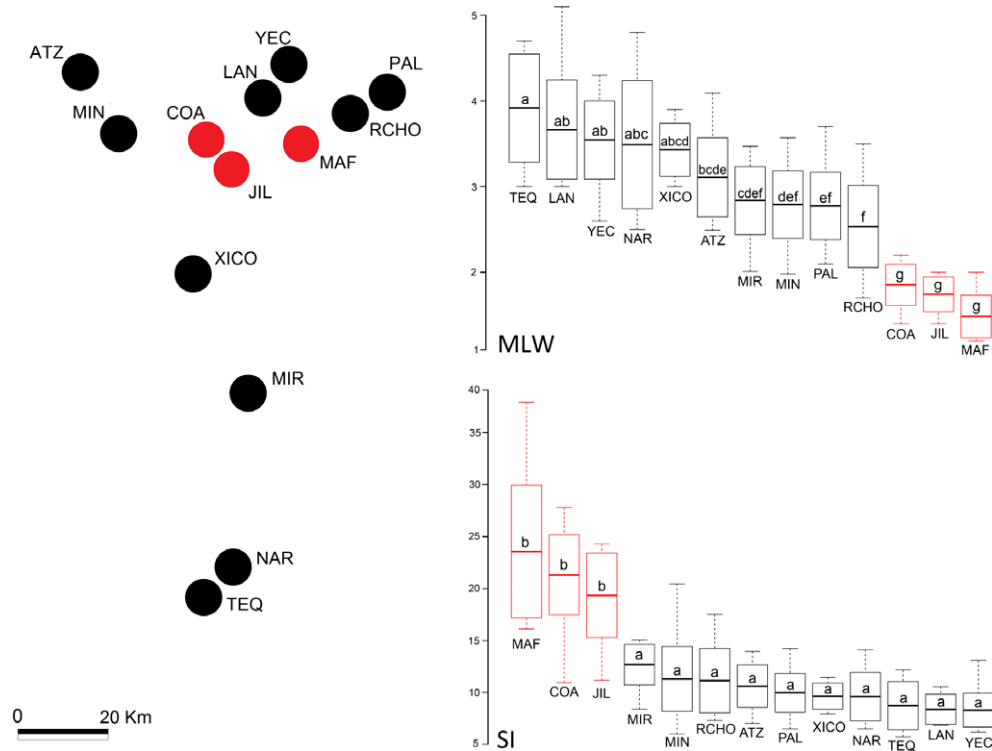
Figure 8. Geographical distribution of the morphotypes by width and shape leaflet. The linear leaflet morphotype is shown in red, and the oblong leaflet morphotype is shown in black. In the boxplot, the black line corresponds to the population mean, the box is ± 1 SD, and the whiskers are the maximum and minimum values (units in cm). The letters inside the boxes correspond to the range detected by Tukey's multiple range analysis ($P < 0.01$). For codes, see Table 1. MLW, middle leaflet width; SI, shape index.

analysis, which detected significant differences between population groups.

The leaflet width and shape index variables allowed the detection of two groups. The first is characterized by short, wide leaflets (oblong, in botanical terminology) and includes the populations of Rancho Nuevo, El Mirador, Palma Sola, Yecuatla, Minas, Atzalan, Xico, Tequila, Naranjal, and Landero y Coss. The second group corresponds to long, narrow leaflets (linear, in botanical terminology) and includes the Mafafas, Coacoatzintla, and Jilotepec populations. These variables behave discretely as a true taxonomic character, given that their oblong and linear states present defined ranges of ± 1 SD around the mean, without overlap. This produces a gap of 0.52 cm in the width and 3.71 in the shape index values between the linear and oblong shapes of the middle leaflet, respectively. The highly statistically significant difference between these two means demonstrates the negligible probability of a common origin for our data, in terms of statistical populations (Fig. 8).

Looking at the characteristics that comparatively describe the species (see Supporting information,

Table S4), 12 out of 43 show character states that are recognizable in at least one species; in principle, these are diagnostic characters (in the remaining 31 characters, there is either overlap or uniformity in the four species evaluated). According to the protologues, of the 12 diagnostic characters, only leaflet width coincides with our results in the sense of being a truly discriminant character among species. On the other hand, the frequency of the qualitative characters is evenly distributed among populations, without being exclusive to any population or group of populations. The 'ochre colour of leaflet articulation', a diagnostic character for *C. decumbens* (Vovides *et al.*, 2008) was found in some individuals in the Naranjal population, although the character state in the remaining individuals was green. The 'translucid (or conspicuous) veins' diagnostic character in *C. morettii* (Vázquez-Torres & Vovides, 1998) was recorded in varying proportions in additional populations, even in all individuals in populations such as Palma Sola and Rancho Nuevo. 'Circinate ptyxis', which is also considered diagnostic in *C. morettii*, was also found in the Mafafas, Jilotepec, Xico, and Naranjal populations, as well as in representative

Table 13. Frequency of qualitative vegetative characters in terms of proportion

Population	Petiole prickles*	Rachis prickles[†]	Leaflet insertion[‡]	Leaflet veins[§]	Leaflet articulation colour[¶]	Leaflet direction**	Leaflet symmetry[††]
MAF	1.00	0.87	1.00	0.00	1.00	0.8	1.00
RCHO	1.00	0.94	0.35	0.00	0.47	1.00	0.41
MIR	1.00	0.83	1.00	0.17	1.00	1.00	0.83
MIN	1.00	1.00	1.00	0.63	1.00	1.00	0.74
ATZ	1.00	1.00	1.00	0.37	1.00	1.00	0.79
LAN	1.00	1.00	0.93	1.00	0.29	1.00	0.21
XICO	0.90	0.60	1.00	0.30	1.00	1.00	0.70
COA	1.00	0.95	1.00	0.20	1.00	0.5	1.00
JIL	1.00	1.00	1.00	0.63	1.00	1.00	1.00
PAL	1.00	1.00	0.55	1.00	0.65	0.75	0.75
YEC	1.00	0.44	0.89	1.00	0.83	1.00	0.22
NAR	1.00	0.20	1.00	0.00	0.70[‡‡]	1.00	0.20
TEQ	1.00	0.50	1.00	0.00	1.00	1.00	0.00

For codes, see Table 1.
*Aculeate/inermous.
[†]Aculeate/inermous.
[‡]Flat/keeled.
[§]Conspicuous/inconspicuous.
[¶]Green/yellow.
**Straight/falcate.
[††]Symmetric/asymmetric.
[‡‡]Green/ochre–reddish.

individuals from *Ceratozamia hildae* and *Ceratozamia zoquorum* at JBC (see Supporting information, Fig. S4).

Microsporophyll length was recently employed by Vovides *et al.* (2012) as a diagnostic character to distinguish between *C. mexicana* Brongn. (Vovides *et al.*, 1983) and *C. brevifrons*. However, these differences might be correlated to the size of the plants from which the strobili were collected, rather than representing defined patterns (fixed, differential character states) between species. As a result of the high correlation between strobilus volume and stem volume, both in male and female plants, we can suggest that more robust plants produce larger strobili.

Throughout the taxonomic history of the genus *Ceratozamia*, continuous variables (e.g. PL, RL, TL, LL, LW, DBL, METL, MITL, METPL-W, and MITPL-W), qualitative character (e.g. leaflet shape, articulation colour, phyllotaxis, ptyxis, stem habit, presence/absence of prickles, sclerenchymatous shafts in vascular bundles, and geographical distribution patterns in terms of physiographical provinces and vegetation types), and meristic variables (e.g. leaflet pairs and leaf number) have been used for species delimitation (Miquel, 1847; Miquel, 1849; Wendland, 1854; Regel, 1857, 1876; Moore, 1878; Schuster, 1932; Lundell, 1939; Medellín-Leal, 1963;

Landry & Wilson, 1979; Moretti *et al.*, 1980; Moretti, Sabato & Vázquez-Torres, 1982; Stevenson, 1982; Vovides *et al.*, 1983, 1993, 2004b, 2007, 2008, 2012, 1983; Stevenson *et al.*, 1986; Chemnick & Gregory, 1995; Vázquez-Torres & Vovides, 1998; Pérez-Farrera, Vovides & Iglesias, 1999, 2001a, b, c; Pérez-Farrera *et al.*, 2004, 2007a, b, 2009; Avendaño, Vovides & Castillo-Campos, 2003; Haynes *et al.*, 2008).

We demonstrate that, for the *Ceratozamia* species complex evaluated in the present study, most characters traditionally considered as diagnostic are in fact spurious. This statement is based on the absence of detectable differences between populations belonging to species with previously accepted taxonomic descriptions and circumscriptions. The only variables that do not support this generalization are leaflet width and shape because these resisted statistical scrutiny.

The aggregation pattern resulting from the analysis of leaflet width and shape (Fig. 8) supports two cohesive groups of populations, which in turn match a defined geographical distribution. On the basis of the statistically significant morphological evidence, we postulate the existence of two phenotypically distinct species; in this context, we further propose the recognition of the Rancho Nuevo population as the

location of origin of the 'type specimen' of the genus *Ceratozamia*. Moreover, in the absence of morphological evidence supporting the taxonomic validity of the species *C. brevifrons*, *C. decumbens*, and *C. morettii*, we suggest that these three *Ceratozamia* species should enter in synonymy with *C. mexicana*, a name that bears nomenclatural priority on the basis of Brongniart's taxonomic description in 1846. We also highlight the need to update morphological descriptions for *Ceratozamia mexicana*, on the basis of the parameters described for the group of populations including Palma Sola, Rancho Nuevo, Yecuatla, Landero y Coss, Minas, Atzalan, Xico, Mirador, Tequila, and Naranjal, which are all distributed on the Montane Arc in the centre of Veracruz. Finally, in relation to the Mafafas, Coacoatzintla, and Jilotepec populations, we consider that these may merit description (in a separate publication) as a new species given the consistent presence of linear leaflets in the corresponding samples analyzed in the present study. In line with the previous observations, this population aggregate cannot be placed under the name *C. mexicana*, which implies a different circumscription. A description of the morphological and geographical limits of both species is currently in preparation.

CONCLUSIONS

In accordance with some classical philosophical perspectives on science (e.g. the post-positivist scientific realism of Popper, 1959), the interpretation of observed patterns should be considered fallible as a matter of principle. As long as there are data and data tests that can falsify hypotheses (as a Popperian would say), we might refine our understanding of reality as we discard untenable models of it. The use of strictly numerical methods in the study of morphometric and phenotypic variation in cycads, and *Ceratozamia* in particular, ensures reproducibility and clarifies explanations. In the present study, we have collected population-level data through careful fieldwork, and deposited the corresponding herborized botanical material in herbaria. This methodological prescription warrants the direct access of other researchers to the evidence employed for analysis, implicitly promoting reanalysis and further corroboration through repeated testing, or even the falsification of any conclusions.

From an evolutionary taxonomic viewpoint that is sympathetic to the unified species concept (De Queiroz, 2007), we consider that characters such as leaflet width and shape constitute legitimate evidence for the recognition of metapopulation lineages evolving independently. On this basis, we propose the recognition of two groups of populations as 'phenetically distinct species'. The adjective 'phenetically distinct' makes reference to analytical method employed in the present study, which implies phenotypic evaluation of morphological variables.

Determination of the dynamics of evolutionary processes such as inbreeding, gene flow, selection, and genetic drift, as well as their role in the determination of the distribution of morphological patterns in the populations investigated in the present study, requires a population-genetic perspective. Either through the lens of 'classical' population genetics (González-Astorga *et al.*, 2003b, 2005, 2006, 2008) or through phylogeography (Huang *et al.*, 2001; Chiang *et al.*, 2009; Kyoda & Setoguchi, 2010; Zhan *et al.*, 2011; Gutiérrez-Ortega, Kajita & Molinar-Freaner, 2014; Nolasco-Soto *et al.*, 2015), the results of further work in this direction will allow the construction of ancestor-descendant hypotheses involving known populations, as well as the assessment of demographic history and genetic differentiation between these populations. In accordance with our population-genetic research programme, evolutionary inferences derived from these phylogeographical interventions will constitute the starting point in species recognition and circumscription. In this context, we predict that population-genetic information will be consistent with the 'recent speciation for cycads' scenario (Nagalingum *et al.*, 2011) and that fixed phenotypes in the morphotype/population aggregates described in the present study will be shown to be connected to phenotypic plasticity.

paper is dedicated to Patricia Guerrero and Sagan Medina.

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SUPPORTING INFORMATION

Additional Supporting Information may be found online in the supporting information tab for this article:

Figure S1. Centroid scatterplot of discriminant analysis.
Figure S2. Spatial distribution of confidence values.
Figure S3. Multivariate spatial distribution of type specimens.
Figure S4. Circinate ptyxis of some of the individuals from different populations/species.
Table S1. Source of strobili measured in the present study.
Table S2. Characters and character states evaluated in the present study.
Table S3. Coefficient of variation of middle leaflet width (CV) and shape index (SI) for population studies in Cycadales.
Table S4. Comparison of characteristics of each *Ceratozamia* species investigated in the present study as obtained from their protologues.