Phytotaxa 270 (3): 161–181
http://www.mapress.com/j/pt/

Article

ISSN 1179-3155 (print edition)
PHYTOTAXA
ISSN 1179-3163 (online edition)

http://dx.doi.org/10.11646/phytotaxa.270.3.1

Anatomy and morphology suggest a hybrid origin of *Zamia katzeriana* (Zamiaceae)

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Abstract

The validity of *Zamia splendens* has been debated, mainly as a result of its synonymy under *Z. katzeriana*. Field explorations have uncovered previously unknown populations, and some do not appear to match the circumscription of *Z. katzeriana*. Some populations are morphologically more similar to, and often sympatric with, *Z. loddigesii.* This study aims at clarifying the morphological and anatomical distinction between the three taxa. To study the morphological variation, a total of 88 individuals from four populations were sampled, these spanning the geographical range of the three cycads in southern Mexico. Univariate, principal component and discriminant analyses of 12 vegetative morphological variables were carried out. To study variation in leaflet anatomy, three individuals per population were analysed for nine anatomical variables and their stomatal index. Standard plant histological techniques and bright field light microscopy were used for observations and measurements of leaflet anatomical variables. Both morphological and anatomical variables were analysed by multivariate statistical methods. Principal component and discriminant analyses on both morphological and anatomical variables and the stomatal index have shown a significant difference between the populations ($P = <0.0002$) with scatter diagram dispersion indicating three distinctive groups, with *Z. katzeriana* values consistently intermediate between those of *Z. splendens* and *Z. loddigesii*. Some individuals of *Z. katzeriana* have a leaflet morphology and anatomy similar to *Z. loddigesii*, yet others resemble *Z. splendens*. The three taxa comprise distinct species and we propose the acceptance of *Z. splendens* as independent from *Z. katzeriana*. The intermediate placement of *Z. katzeriana* in the analyses suggests a hybrid origin with *Z. splendens* and *Z. loddigesii* as putative ancestors.

Keywords: Cycads, hybrids, leaflet anatomy, morphological variation, *Zamia* speciation

Introduction

Zamia Linnaeus (1763: 1659; Zamiaceae, Christenhusz *et al.* 2011) is the second largest genus of living cycads with about 72 species (Osborne *et al.* 2012, with additions, e.g. Lindström *et al.* 2013) distributed in the new world from the southern USA (Georgia and Florida) and central Mexico to Bolivia. The genus occurs in a wide range of habitats, ranging from tropical wet forests, seasonally dry forests, coastal dune vegetation, and tropical montane forests to estuarine ecosystems. Elevation for the genus ranges from sea level to over 2000 meters in Colombia.

A series of taxonomic and nomenclatural problems persists in cycads in general, and *Zamia* is no exception, mainly arising from incomplete type specimens and poorly circumscribed localities. This is particularly true for species described during the 18th and 19th Centuries, or descriptions based on juvenile plants cultivated in European greenhouses (Dyer 1882–1886, Vovides *et al.* 1983). Some of these problems can be solved by more thorough field explorations, population genetic studies combined with *ex situ* and *in situ* morphological and anatomical surveys (González-Astorga *et al*. 2003, 2003a, 2005, Vovides *et al*. 2012, Pérez-Farrera *et al*. 2009, 2014).

Zamia katzeriana (Regel) Rettig (1896: 148–149) was originally described as *Ceratozamia katzeriana* by Regel

(1876: 261) and later transferred to *Zamia* by Rettig (1896:148–149). Stevenson and Sabato (1986:137) assigned Regel´s voucher of *Z. katzeriana* as the holotype: (*E.A. Regel s.n.* LE!). There is also a voucher of a male *Z. katzeriana* held at Kew (*E.A. Regel s.n.* K!) as well as an illustration of a living plant in Gartenflora (Rettig, 1896:149).

Hill & Stevenson (1998) and Hill *et al.* (2004) in The World List of Cycads placed both *Zamia katzeriana* and *Z. splendens* Schutzman (1984: 299) under synonymy of *Z. verschaffeltii* Miquel (1870: 31) as discussed by Schutzman (2004a). A population-based study by Nicolalde-Morejón *et al.* (2008) demonstrated that *Z. katzeriana* is a separate entity from *Z. verschaffeltii* but *Z. splendens* remained in synonymy.

This study is aimed at elucidating a suspected natural hybrid origin of *Zamia katzeriana* between the putative parents *Z. splendens* and *Z. loddigesii* Miquel (1843: 72–73) by means of anatomical and morphological techniques.

Material and Method

Explorations

Field explorations were carried out in the states of Veracruz (Las Choapas), Tabasco (Huimanguillo) and Chiapas (San Fernando) (Fig. 1). Herbarium vouchers were deposited at HEM and XAL and leaflets were collected for anatomical study. Living plants were also collected and cultivated under uniform conditions for five years or longer in order to eliminate phenotypic variation, after which time leaflets were again collected for comparative anatomical study. Additional herbarium vouchers were examined at CAS, FTG, HEM, LE, MEXU and XAL.

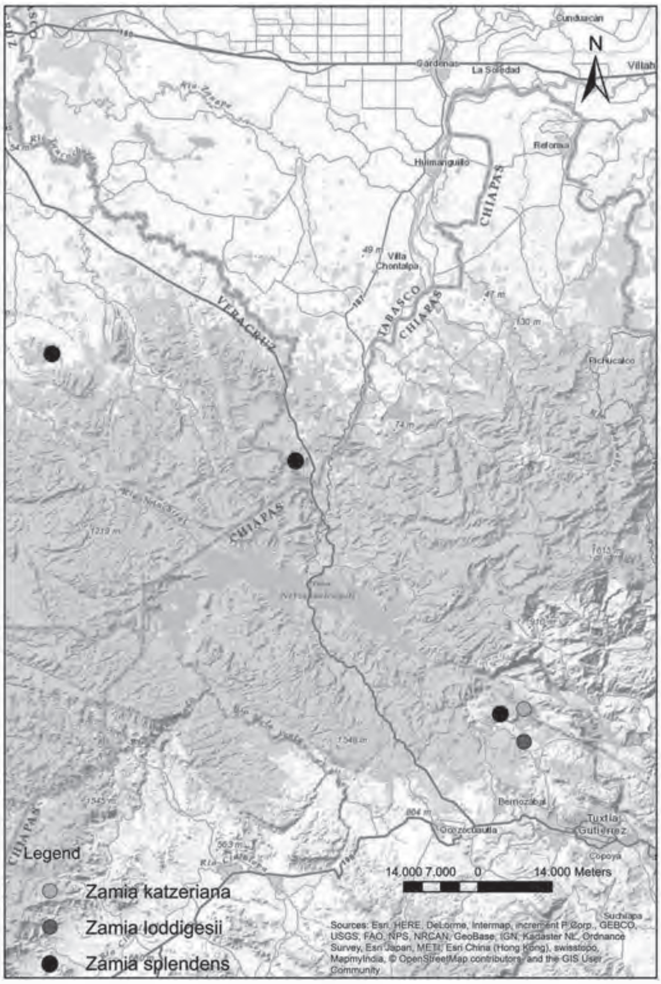

FIGURE 1. Distribution *Zamia splendens*, *Z. loddigesii* and *Z. katzeriana* in southern Mexico.

Taxon sampling

To study the morphological variation, one population of *Zamia katzeriana* (San Fernando, Chiapas; n = 17), two populations of *Z. splendens* (Las Choapas, Veracruz; n = 10 and Huimanguillo, Tabasco; n = 30) and one population of *Z. loddigesii* (San Fernando, Chiapas; n = 31) were sampled. Twelve morphological variables were analysed (Table

1) using (a) ANOVA (analysis of variance) and Kruskal-Wallis tests to determine the variation of parameters between populations of the three taxa, and (b) multivariate analysis PCA (Principal Component Analysis) and DA (discriminant analysis) to separate two or more groups based on measurements of the variables of each population. Data were transformed to Log_{10} and Mahalanobis distances were calculated. All data were analysed using Statistica v 8 and Statgraphics v 5.1 software for Windows. Missing data for some characters were handled by the mean substitution option of the programme. The analysis was focused to evaluate differences between populations only as we considered gender to be independent at this level.

TABLE 1. Twelve vegetative morphological variables used for morphological analysis of populations of *Zamia splendens*, *Z. katzeriana* and *Z. loddigesii*.

Variable	Character
1	Number of leaves
2	Number of leaflets
3	Petiole length
4	Rachis length
5	Leaflet length at leaf base
6	Leaflet length at leaf middle section
7	Leaflet length at leaf apex
8	Leaflet width at leaf base
9	Leaflet width at leaf middle section
10	Leaflet width at leaf apex
11	Inter-leaflet distance
12	Width of articulation

Leaflet anatomy

For study on variability in leaflet anatomy, leaflets were taken from the median part of mature leaves from three healthy *in situ* adult plants per population. The median part of each leaflet (ca.1 cm) was taken from three replicate individuals of each population for sectioning. The following transverse section (TS) measurements were taken on 20 randomly chosen replicates of each cell or tissue type (character) from each of the three leaflet replicates per population. A total of 208 samples were measured (*Zamia loddigesii* = 60, *Z. katzeriana* = 96, *Z. splendens* = 52). We recorded the thickness of the adaxial and abaxial cuticle (measurement taken from the top anticlinal limit of the epidermal cell where cuticle is thinnest), cuticle thickness at leaflet margin, TS dimensions of mega-lumen cells associated with the abaxial epidermal cells, depth of epi-stomatal chamber, number of layers of adaxial and abaxial girder sclerenchyma, and number of layers of sclerenchyma associated with the leaflet margin hypodermis (Table 2).

Sectioning, staining and slide preparation

Transverse sections were made with both hand microtome and sliding microtome. The sections were suspended in distilled water. The optimal sections were selected and subjected to histochemical staining with phloroglucinol-HCl for mapping lignified tissues and cells (Chamberlain 1932) and with a mixture of Sudan III and IV for cuticles. Permanent sections were obtained by double staining in safranin and fast green (Purvis *et al*. 1966) and mounted with Histoclad™ mounting medium.

Cuticular peel preparation

Approximately 1 cm^{-2} of the median part of a leaflet from each representative taxon was taken and macerated in a modified Jeffery´s solution (Stace 1965) until mesophyll and other lignified tissues were digested to expose the epidermis. Staining was done with 1% aqueous Bismarck brown, modified from Purvis *et al*. (1966), for 10–20 minutes, dehydrated through ethanol stages from 70% EtOH in increments of 10%, followed by two changes in absolute EtOH, then cleared for 2–3 minutes in methyl salicylate and mounted. Stomatal indices were calculated from microphotographs taken from five individuals from each population with five replicates per individual. Number of stomata and epidermal cells were scored off from the images with the aid of the ImageJ software and stomatal index calculated. Stomatal index (S.I.) is expressed by:

$$\text{S.I.} = \frac{\text{No. of stomata}}{\text{No. of stomata} + \text{No. of epidermal cells}} \times 100$$

Microphotography was done with a Canon a digital camera fitted to a Zeiss Fomi III photomicroscope.

TABLE 2. Nine leaflet anatomical variables used for anatomical analysis of two populations of *Zamia katzeriana* and one population of *Z. loddigesii*

Variable	Anatomical character
1	Adaxial cuticle thickness (μm)
2	Abaxial cuticle thickness (μm)
3	Margin cuticle thickness (μm)
4	Adaxial grider sclerenchyma (layers)
5	Abaxial grider sclerenchyma (layers)
6	Margin hypodermal sclerenchyma (layers)
7	Mega-lumen cell length (μm)
8	Mega-lumen cell width (μm)
9	Epi-stomatal chamber depth (μm)

Analysis

The Kruskal-Wallis test was carried out on the nine leaflet anatomical variables. Principal Component Analysis (PCA) and Discriminant Analysis (DA) of the anatomical data were made using Statistica v 8 and Statgraphics v 5.1 software for Windows. This procedure is designed to develop a set of discriminant functions that can be used to classify the operational taxonomic units (OTUs) based on quantitative values. A further *posteriori* analysis was done to test the robustness of the classification by dividing each of the four populations into two randomly generated subsets of equal size. One subset was chosen at random for a first discriminant analysis and the functions of this analysis used to classify the second subset. The Wilks Lambda test and Mahalanobis squared distances were calculated to determine significance levels. The two discriminant functions with $P \leq 0.05$ were considered statistically significant at the 95% confidence level for the anatomical variables.

Results

Field observations and habitat

The study of Nicolalde-Morejón *et al.* (2008) was based on the three then known populations of *Zamia katzeriana* in Veracruz and Chiapas. Their study included the type locality of *Z. splendens* and cultivated specimens totalling 32 individuals as well as the holotype of *Z. katzeriana*. However, our recent field explorations have uncovered new populations of *Z. katzeriana* (*sensu* Nicolalde-Morejón *et al.* 2008), extending its range in Chiapas and Tabasco. Our observations are based on this extended population range and on *ex situ* collections. *Zamia splendens* grows in the understory of primary evergreen tropical rain forests on karstic rock or clay soil between 100 and 800 m elevation. Sometimes the tropical rain forest habitat of *Z. splendens* borders or is in the vicinity of *Quercus* forest and seasonal tropical forest where *Z. loddigesii* occurs on varying soil substrates. *Zamia katzeriana* always grows in the understory of secondary, successional, tropical rain forest between 600 and 700 m elevation, also on karstic rock or clay soil.

Morphology

Zamia splendens (Las Choapas, Veracruz and Huimanguillo, Tabasco) has up to 16 pairs of leaflets per leaf, whereas *Z. katzeriana* (San Fernando, Chiapas) has rarely over six pairs (Fig. 2). All *Z. splendens* individuals have broad, long-elliptic through oblong to oblanceolate leaflets with a highly lustrous cuticle, while *Z. katzeriana* has fewer, but narrower, leaflets, also with a glossy cuticle (Figs 3, 4). *Zamia splendens* has megastrobili with aristate apices and long, decumbent peduncles, longer than the strobilus (Fig. 5), whereas the populations of *Z. katzeriana* have megastrobili with apiculate to rounded apices and short erect cone peduncles, shorter than the strobilus (Fig. 6). The megastrobilus of *Z. loddigesii* is erect with rounded or apiculate cone apex (Fig. 7) and we noticed the short erect cone peduncle in the illustration of *Z. katzeriana* by Rettig (1896) in Gartenflora (Fig. 8) and in the voucher by Regel (*E.A. Regel s.n.*, K! Fig. 9). All populations except *Z. loddigesii* correspond to the *Z. splendens* description by Schutzman (1984) in having glossy adaxial cuticles. However, the shiny cuticle is less so in *Z. katzeriana* that is also distinguished from *Z. splendens* in having narrower, oblong-lanceolate leaflets (Nicolalde-Morejón *et al*. 2008).

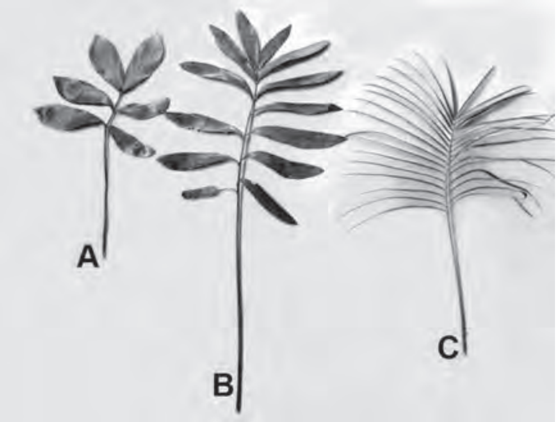

FIGURE 2. Comparison of leaves between species: A, *Zamia splendens*; B, *Z. katzeriana*; C, *Z. loddigesii.*

FIGURE 3. Comparison of leaflets between species: A, *Zamia splendens*; B, *Z. katzeriana*; C, *Z. loddigesii.* Scale Bar = 2 cm.

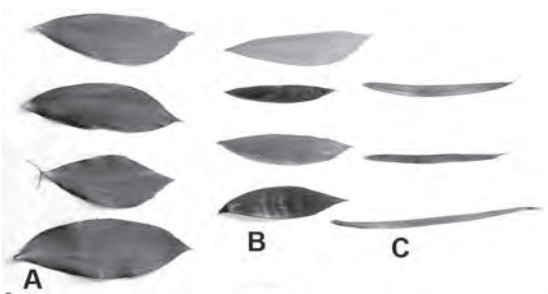

FIGURE 4. Variation of median leaflet of leaves: A, *Zamia splendens*; B, *Z. katzeriana*; C, *Z. loddigesii.*

Univariate analysis of morphological and leaflet anatomical variables

The univariate analysis shows significant statistical difference for all morphological variables except for number of leaves (Tables 3, 4), as all species usually have one or two leaves per trunk. Among the leaflet anatomical variables only the large lumen cell length and width were not statistically significant (Table 5).

FIGURE 5. Megastrobilus of *Zamia splendens*, showing aristate apex and long decumbent peduncle.

FIGURE 6. Megastrobili of *Zamia katzeriana*, showing apiculate to rounded apex and short erect peduncles.

FIGURE 7. Megastrobilus (immature) of *Zamia loddigesii.*

TABLE 3. Analysis of variance (ANOVA) for 6 morphological variables used in the analysis of *Zamia splendens*, *Z. loddigesii* and *Z. katzeriana* populations (F = F value, *P* = probability).

Variable	F	*P*
1	1.4	0.25
3	86.18	0.0001
4	85.6	0.0001
5	27.8	0.0001
6	27.63	0.0001
8	499.62	0.0001

TABLE 4. Kruskal-Wallis test for 6 morphological variables used in the analysis of *Zamia splendens*, *Z. loddigesii* and *Z. katzeriana* populations.

Variable	Kruskall-Wallis test	*P*
2	38.81	0.0001
7	44.62	0.0001
9	64.38	0.0001
10	62.05	0.0001
11	66.4	0.0001
12	68.52	0.0001

TABLE 5. Kruskal-Wallis test for 9 leaflet anatomical variables used in the analysis of *Zamia splendens*, *Z. loddigesii* and *Z. katzeriana*.

Variable	Kruskal-Wallis test	*P*
1	95.25	0.0001
2	79.25	0.0001
3	66.95	0.0001
4	89.38	0.0001
5	158.32	0.0001
6	37.35	0.0001
7	3.62	0.164
8	4.99	0.082
9	102.46	0.0001

PCA on morphological characters

The result of the PCA analysis shows three clusters corresponding to the three species defined *a priori* (Fig. 10). The first component explains 56.2% and the two components together explain 77.5% of the observed morphological variation of the three taxa (Table 6). The variables that best explain the variations are inter-leaflet distance and width of articulation represented by component 1 and number of leaflets and rachis length represented by component 2 (Table 7).

TABLE 6. Percent accumulated variance from PCA analysis of *Zamia splendens*, *Z. loddigesii* and *Z. katzeriana* for 12 morphological characters.

Character	Eigenvalue	% Total Variance	Cumulative Eigenvalue	Cumulative %
1	6.75	56.24	6.75	56.24
2	2.56	21.36	9.31	77.59
3	0.96	8.02	10.27	85.61
4	0.63	5.27	10.91	90.88
5	0.28	2.35	11.19	93.23
6	0.27	2.25	11.46	95.49
7	0.21	1.72	11.67	97.21
8	0.12	1.02	11.79	98.23
9	0.09	0.72	11.87	98.95
10	0.05	0.44	11.93	99.39
11	0.05	0.38	11.97	99.77
12	0.03	0.23	12	100

TABLE 7. Coordinate factors of morphological variables explaining maximum variation, based on correlations for *Zamia splendens*, *Z. loddigesii* and *Z. katzeriana*. The numbers in bold explain the variables that have greater variation for each factor.

Character	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
1	-0.0395	0.3641	-0.8927	0.2553	0.0523	-0.0266
2	-0.1190	**-0.8916**	-0.2905	-0.2343	-0.0621	0.1443
3	-0.8620	0.0582	-0.0402	-0.2637	0.3225	0.0267
4	-0.4852	**-0.7801**	-0.1789	-0.2259	0.0377	-0.0632
5	-0.7572	-0.4336	0.0699	0.3267	-0.0880	-0.2592
6	-0.7427	-0.5315	0.0811	0.2961	-0.0371	-0.0126
7	-0.8239	-0.0957	0.1253	0.3761	0.1151	0.3518
8	-0.8787	0.4066	-0.0028	-0.0713	-0.1654	-0.0229
9	-0.8672	0.4046	-0.0338	-0.1200	-0.1358	0.0159
10	-0.8707	0.4250	0.0253	-0.1188	-0.0881	0.1007
11	**-0.8892**	0.1832	0.0886	-0.0148	0.2471	-0.2054
12	**-0.9365**	-0.0205	-0.1006	-0.1487	-0.1830	0.0091

Discriminant Analysis

The Wilks lambda test was highly significant ($P < 0.0001$) for the two factors thus showing that all the species were classified correctly (Table 8). The F test on quadratic Mahalanobis distances showed significant differences between species (Table 9). The scatter diagram derived from discriminant function analysis groups the populations into three species that are separate and ordinately bidimensional in space (Fig. 11). Of the 12 variables included in the standardized discrete canonical function, the three variables with the highest values in factor 1 were rachis length, leaflet width at leaf base and leaflet width at leaf apex. In factor 2, leaflet length at leaf middle section, leaflet width at leaf base and inter-leaflet distance. The first canonical variable showed that about 86% of the variation is largely due to vegetative morphology and positive correlations of all the variables show differences between species (Table 10).

TABLE 8. Summary of the discriminant analysis results from the morphological analysis of populations of *Zamia splendens, Z. loddigesii* and *Z. katzeriana* populations (λ = lambda, χ^2 = chi-squared factor, DF = degrees of freedom, *P* = probability).

Derived Functions	Wilks λ	χ^2	DF	*P*
1	0.0121547	350.5979	24	0.0000
2	0.241231	113.0490	11	0.0000

TABLE 9. Mahalanobis distances (above asterix), F values (below asterix). All cases were significant (P< 0.0001).

	Zamia loddigesii	*Z. katzeriana*	*Z. splendens*
Zamia loddigesii	*	75.72074	87.24358
Z. katzeriana	60.3137	*	24.13817
Z. splendens	110.5423	20.89151	*

TABLE 10. Standardized discriminate function values for each of two factors used in the analysis of *Zamia splendens, Z. loddigesii* and *Z. katzeriana* populations. The numbers in bold explain the variables that have greater variation for each factor.

Variable	1	2
1	0.0058	0.0950
2	-0.2426	0.3916
3	-0.0498	0.1937
4	**0.3697**	0.5533
5	-0.2077	0.4167
6	0.2351	**-0.7175**
7	-0.2890	0.0988
8	**0.5672**	**-0.6048**
9	0.1597	-0.2074
10	**0.5779**	0.2077
11	-0.0967	**0.5748**
12	0.1508	0.3809
Eigenvalue	18.8467	3.1454
Canonical correlation	0.9745	0.8711
Among group variance	85.7 %	14.3 %

PCA on leaflet anatomical variables

The result of the PCA based on leaflet anatomy also shows three clusters corresponding to the three species defined *a priori* (Fig. 12). The first component of the PCA explains 35.1% and the two components together explain 50.9% of the variation (Table 11). The variables that best explain the variation for component 1 are adaxial cuticle thickness, abaxial girder sclerenchyma layers and epi-stomatal chamber depth, while for component 2 the variables are leaflet margin cuticle thickness, mega-lumen cell length and width (Table 12).

FIGURE 8. Habit of *Zamia katzeriana* with megastrobilus. Illustration from Rettig (1896).

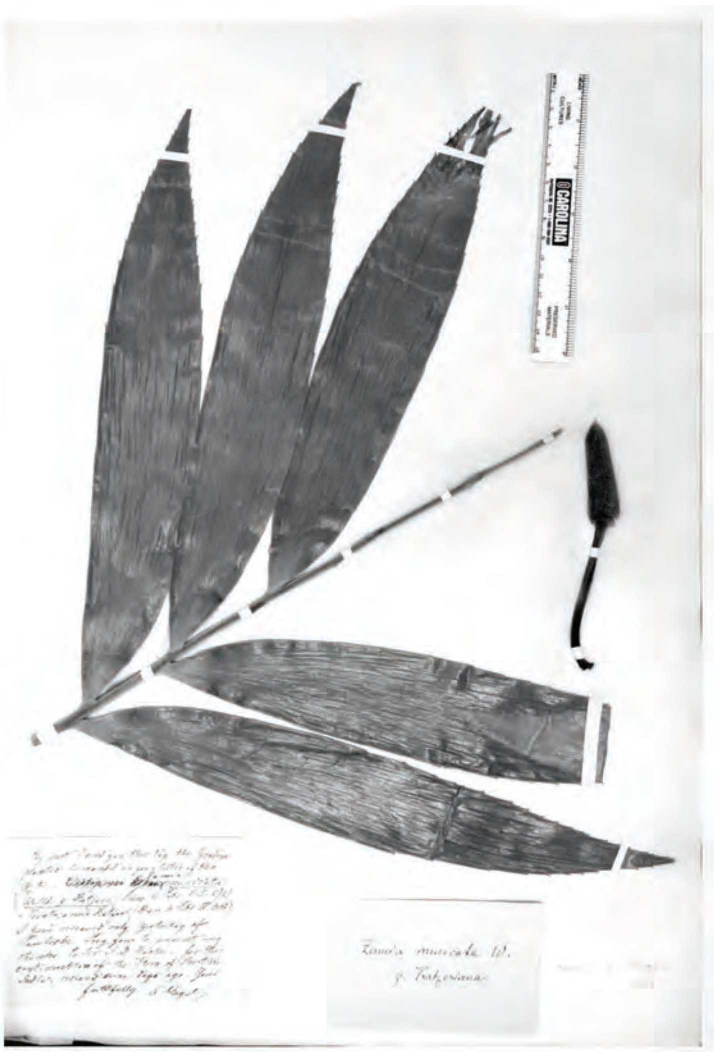

FIGURE 9. *Zamia katzeriana* (male) *E.A. Regel sn* (K).

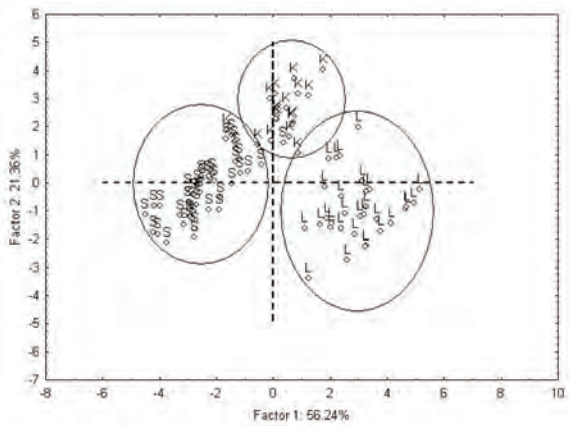

FIGURE 10. Principal components analysis on morphological data between populations of *Zamia splendens, Z. loddigesii* and *Z. katzeriana:* S = *Z. splendens*, K = *Z. katzeriana*, L = *Z. loddigesii.*

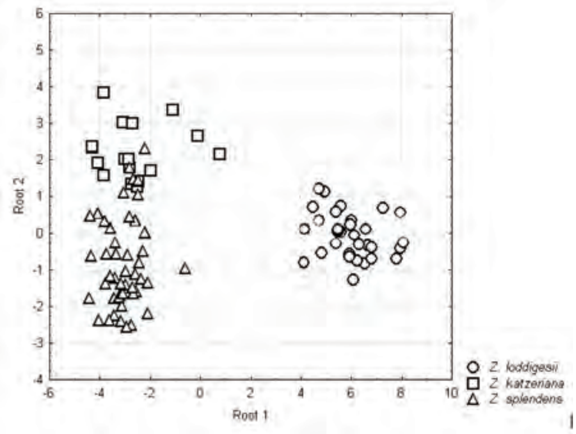

FIGURE 11. Scatter plot of scores derived from the functions produced by stepwise discriminant analysis of 12 morphometric ratios from populations of *Zamia splendens*, *Z. loddigesii* and *Z. katzeriana*

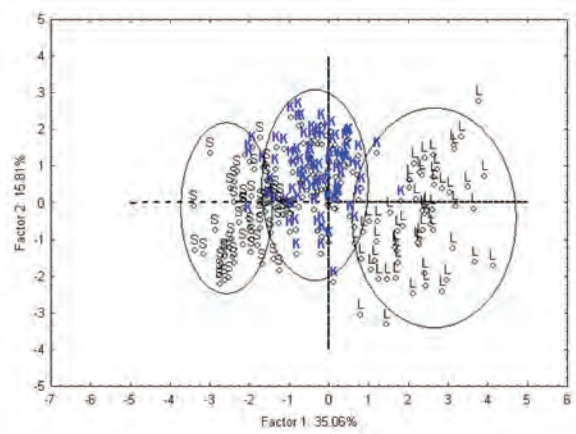

FIGURE 12. Principal components analysis on 9 anatomical leaflet variables: S = *Zamia splendens*, K = *Z. katzeriana*, L = *Z. loddigesii.*

Discriminant analysis on leaflet anatomical variables

The Wilks lambda test was highly significant ($P < 0.0001$) for the two factors thus showing that all the species are classified correctly for leaflet anatomical variables (Table 13). The F test for quadratic Mahalanobis distances showed significant differences between species (Table 14). The discriminant function analysis scatter diagram grouped the populations into three species (Fig. 13). Of the nine variables included in the standardized discrete canonical function, the three variables with the highest values in factor 1 are abaxial girder sclerenchyma, adaxial girder sclerenchyma and adaxial cuticle thickness. In factor 2, abaxial cuticle thickness, leaflet margin cuticle thickness and epi-stomatal chamber depth being variables with highest values. The first canonical variable shows that about 96.2% of the variation is largely due to leaflet anatomy. The positive correlations of all the variables show differences between species (Table 15).

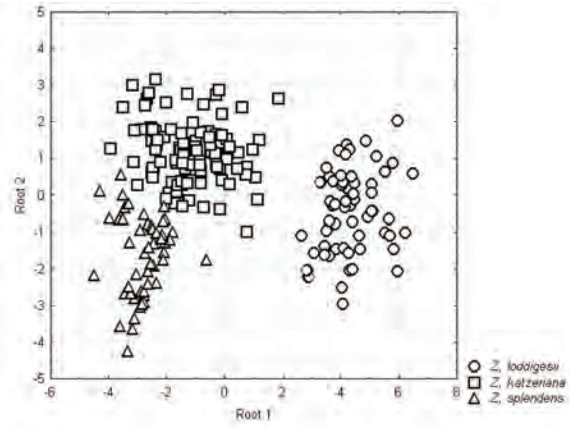

FIGURE 13. Discriminant analysis on 9 leaflet anatomical variables of *Zamia splendens, Z. loddigesii* and *Z. katzeriana*.

TABLE 11. Percent accumulated variance from PCA analysis of *Zamia splendens, Z. loddigesii* and *Z. katzeriana* for 9 leaflet anatomical characters.

Variable	Eigenvalue	% Total Variance	Cumulative Eigenvalue	Cumulative %
1	3.155677	35.06308	3.155677	35.0631
2	1.422454	15.80505	4.578131	50.8681
3	1.009531	11.21701	5.587662	62.0851
4	0.867727	9.64141	6.455389	71.7265
5	0.72303	8.03367	7.178419	79.7602
6	0.579284	6.43649	7.757704	86.1967
7	0.556443	6.1827	8.314147	92.3794
8	0.427694	4.75216	8.741841	97.1316
9	0.258159	2.86844	9	100

TABLE 12. Coordinate factors of leaflet anatomical variables explaining maximum variation based on correlations for *Zamia splendens, Z. loddigesii* and *Z. katzeriana.* The numbers in bold explain the variables that have greater variation for each factor.

Variable	Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6	Factor 7	Factor 8	Factor 9
1	**0.6827**	0.1195	-0.3636	0.1538	0.0650	-0.1893	0.5385	-0.1521	0.1030
2	0.6805	-0.1588	-0.0449	-0.0280	-0.4011	-0.5040	-0.3040	-0.0185	0.0349
3	0.5555	**0.4942**	-0.0396	0.4718	-0.1685	0.1903	-0.0600	0.3917	0.0378
4	0.6495	-0.3342	0.2544	-0.4644	-0.1374	0.2648	0.1042	0.1288	0.2639
5	**0.8346**	-0.2332	-0.0691	-0.2288	0.0823	0.0792	0.0785	0.1260	-0.3960
6	0.4784	-0.2378	0.5226	0.2879	0.5578	-0.1922	-0.0708	0.0424	0.0610
7	-0.2452	**-0.6026**	-0.6514	0.0469	0.2396	-0.0273	-0.1196	0.2611	0.0977
8	-0.0333	**-0.7287**	0.1368	0.5149	-0.3245	0.2090	0.0911	-0.1559	-0.0495
9	**0.7138**	0.1478	-0.2969	0.0496	0.1734	0.3091	-0.3546	-0.3522	0.0521

TABLE 13. Summary of the discriminant analysis results from the analysis of leaflet anatomical variables of *Zamia splendens*, *Z. loddigesii* and *Z. katzeriana* (λ = lambda, χ^2 = chi-squared factor, DF = degrees of freedom, *P* = probability).

Derived Functions	Wilks λ	χ^2	DF	*P*
0	0.045849	619.5634	18	<0.001
1	0.423332	172.7793	8	<0.001

TABLE 14. Quadratic Mahalanobis distances (above asterix) between species for leaflet anatomical variables of *Zamia splendens, Z. loddigesii* and *Z. katzeriana* F values (below asterix). All cases were significant (P< 0.0001).

	Zamia loddigesii	*Z. katzeriana*	*Z. splendens*
Zamia loddigesii	*	33.30	52.92
Z. katzeriana	131.29	*	10.46
Z. splendens	157.42	37.67	*

TABLE 15. Standardized discriminate function values for each of two factors used in the leaflet anatomical analysis of *Zamia splendens, Z. loddigesii* and *Z. katzeriana.* The numbers in bold explain the variables that have greater variation for each factor.

Variable	1	2
1	0.14756	**0.502918**
2	**0.35204**	-0.249791
3	0.146813	**0.511135**
4	**0.502147**	-0.423959
5	**0.769335**	-0.194033
6	0.127718	-0.009258
7	0.023077	-0.034727
8	0.013693	-0.006406
9	0.269073	**0.580759**
Eigenvalue	8.233209	1.362212
Canonical correlation	0.944296	0.759387
Among group variance	96.17 %	3.83 %

Stomatal index

The mean stomatal indices for *Zamia splendens* (S.I.= 6.7), *Z. katzeriana* (S.I.= 6.9) and *Z. loddigesii* (S.I.= 9.4) are significantly different in the Kruskal-Wallis test = 23.09, *P* = < 0.0001 with the multiple range test, but *Z. splendens* and *Z. katzeriana* are closest for these values.

Anatomical features observed

Although certain variation is observed within and between species, there is a consistent difference in girder sclerenchyma associated with the vascular bundles. In one population that we identified as *Zamia splendens*, there consistently is a single layer of girder sclerenchyma between the abaxial epidermis and vascular bundle. In *Z. loddigesii* there is abundant girder sclerenchyma of several layers between the abaxial epidermis and vascular bundle, also abundant G-fibres of several layers between the adaxial epidermis and vascular bundle. In *Z. katzeriana* the amount of girder sclerenchyma is variable from one to two layers between the abaxial epidermis and vascular bundle, and sporadic presence of fibres between the adaxial epidermis and vascular bundle, varying from none to a few isolated cells (Fig. 14).

Leaflet anatomy description in TS

Zamia katzeriana (Fig. 14 A–C; Fig. 15 A, B)

The adaxial epidermis is lignified, 15.0–22.5 µm thick. The lignified hypodermis is very discontinuous at times, single cells or two together, variable and continuous only at the revolute margin, up to two layers extending to the first vascular bundle on the adaxial side. Abaxial epidermis is lignified and 10–25 µm thick, hypodermis is absent. Palisade mesophyll is 30–70 µm long with some cells containing tannins. Girder sclerenchyma is incipient from one to two layers and not reaching the vascular bundle, but isolated sclerenchyma fibres are sometimes present on adaxial side of the bundle. Few interfascicular G-fibres are sometimes present, but a discontinuous ring of perivascular G-fibres surrounds the vascular bundle.

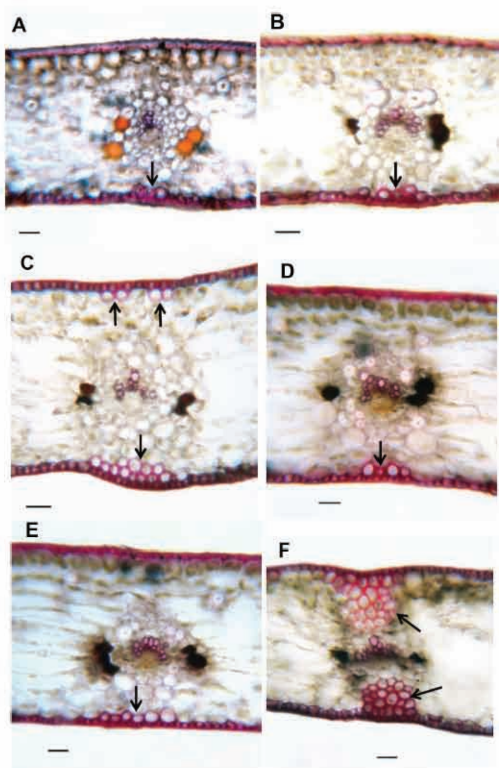

FIGURE 14. A–C, variation in girder sclerenchyma in T.S. of leaflet of *Zamia katzeriana* (arrows); D and E, variation in girder sclerenchyma in T.S. of leaflet of *Z. splendens* (arrows); F, multiple layers of Girder sclerenchyma (lower arrow) and G-fibres (upper arrow) in T.S. of leaflet of *Z. loddigesii*. Stain, Phloroglucinol HCl reaction for lignin. All scale bars = 50 µm.

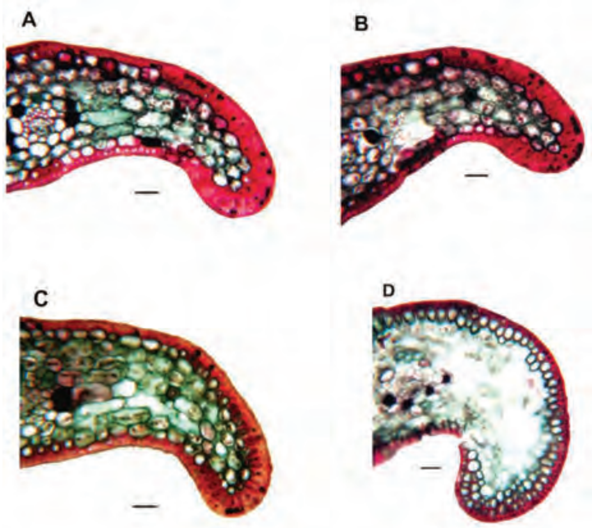

FIGURE 15. A–B, *Zamia katzeriana* TS leaflet at margin showing variation in hypodermal fibre layers; C, *Z. splendens* TS leaflet at margin showing single layer of hypodermal fibres; D, *Z. loddigesii* TS leaflet at margin showing up to three layers of hypodermal fibres and a continuous layer extending along adaxial portion. Stain Safranin O and Fast Green FCF. All scale bars = 50 µm.

Zamia loddigesii (Fig. 14 F; Fig. 15 D)
The adaxial epidermal cells are lignified with one continuous to discontinuous layer of sclerenchyma forming a lignified hypodermis of 40–65 µm thick, including the epidermis.

The abaxial epidermis is 20–30 µm thick, lignified and hypodermis absent. The subrevolute leaflet margin is reinforced with a lignified hypodermis from two to three layers of sclerenchyma. There are three to four layers of sclerenchymatous fibres forming a girder sclerenchyma associated with the abaxial region of the vascular bundle, whereas on the adaxial region there is a corresponding girder of several G-fibres, as only the outer wall (S1 and S2 layers) reacted for lignin with phloroglucinol-HCl. There are no intervascular fibres, but sometimes there is a discontinuous ring of perivascular G-fibres surrounding the vascular bundle.

Zamia splendens (Fig. 14 D, E; Fig. 15 C)
The adaxial epidermis is lignified, 15–20 µm thick, lacks hypodermis, the latter being restricted to the subrevolute leaflet margin consisting of one layer of sclerenchymatous fibres and stretching discontinuously under the adaxial epidermis up to the first vascular bundle. Abaxial epidermis is lignified, 17.5–22.5 µm thick, hypodermis is absent and an incipient girder sclerenchyma of one layer is associated with the vascular bundle, but does not reach it. No fibres are present on the adaxial side of the bundle. Interfascicular G-fibres are present, and a discontinuous ring of perivascular G-fibres is associated with the vascular bundle.

Taxonomy

Diagnostic key separating *Zamia splendens*, *Z. katzeriana* and *Z. loddigesii*

1. Megastrobili decumbent with aristate apex. Leaflets wide long-elliptic through oblong to oblanceolate .... *Z. splendens*
1. Megastrobili erect with rounded to apiculate apex .... 2.
2. Leaflets lanceolate to linear-lanceolate .... *Z. loddigesii*
2. Leaflets narrow oblong-lanceolate .... *Z. katzeriana*

Zamia katzeriana (Regel) Retting (1896: 148). Basionym: *Ceratozamia katzeriana* Regel (1876: 298). Type: CULTIVATED. ex hort. Katzer, *E. A. Regel s.n.* (lectotype, designated by Stevenson & Sabato (1986: 579) LE!).

= *Ceratozamia katzeriana* Regel. (1876: 298). Lectotype*: Regel s.n.* ex hort. Katzer (LE) designated by Stevenson & Sabato, (1986: 579).

Description:—*Stem* subterranean, rarely branched, up to 26 cm long, 6–8 cm in diameter. *Cataphylls* triangular basally, aristate apically, chartaceous, up to 9 cm long, 1.5–2.5 cm at base, tomentulose. *Leaves* 1–2 per crown pinnate, ascending to spreading, 40–102 x 30–42 cm; petioles 27–52 cm long, terete to subterete, armed with many prickles up to 3 mm long, bases swollen and glabrous; rachises subterete, up to 36 cm long, with varying amounts of prickles on half to lower third. *Leaflets* 4–10 pairs, opposite to subopposite, coriaceous, narrow oblong-lanceolate, apices acute, bases attenuate, 16–28 x 2.5–4.6 cm, margins dentate along distal third, subrevolute, green to dark-green when mature, cuticle lightly lustrous, articulations 0.6–0.9 cm wide. *Pollen strobili* 1–2 per crown, light brown tomentulose erect, cylindrical to conical, 10.5–11.5 cm long, 1.7–3.3 cm in diameter, apex apiculate; peduncle light grey tomentose, 5.8–6.3 x 2–2.3 cm in diameter; microsporophylls cuneiform, distal face hexagonal truncate, 0.7 cm long, fertile abaxial surface with 4–5 bisporangiate synangia per lobe. *Ovulate strobili* up to two per crown, erect, beige-tomentulose, cylindrical, 9 cm long, 6 cm in diameter, apex rounded to apiculate; peduncle brown-tomentose, to 5.5–7 cm long, 0.8–1.4 cm in diameter; megasporophylls peltate, distal face hexagonal-truncate, 1.3–1.5 cm high, 1.7–2 cm wide. *Seeds* angular-ovoid, sarcotesta pink when immature, turning red at maturity, 1.3–1.5 cm long, 0.8–0.9 cm in diameter, sclerotesta smooth.

Distribution and habitat:—Endemic to Mexico in secondary succession forest nearby *Quercus* and deciduous tropical forests between 600 and 700 m elevation in the San Fernando area, Chiapas.

Reproductive phenology:—Both ovulate and pollen strobili have been observed from March through to September on plants under cultivation.

Etymology:—The specific epithet is in honour of Herr Katzer, a previous inspector of the Paullowsk gardens in Russia (Regel 1876).

Conservation status:—The IUCN (2015) Red List considers *Zamia katzeriana* as Endangered (EN) sub category A4ac; B1ab(i,v) taking into account five known populations that include *Z. splendens* (as synonymy), with population trend decreasing. When *Z. splendens* is been removed from synonymy to *Z. katzeriana*, it leaves the latter with only one known population in the San Fernando area of Chiapas and possibly another in the Ocote Biosphere Reserve. We suggest a re-assessment of the IUCN category to Critically Endangered (CR) for *Zamia katzeriana*, with population trend decreasing.

Additional specimens examined:—MEXICO. Chiapas**:** San Fernando. 710 m, 18 May 1988, *Dennis E. Breedlove; Mona Bourell 68259* (CAS); 850 m, 29 April 1995, *Pérez-Farrera M.A. 294* (HEM); 680 m, 29 April 1995, *Vovides A. 1266* (XAL); 685 m, *Nicolalde F. & Pérez Farrera M.A. 1420* (XAL); 650 m, *Pérez Farrera M.A. sn* (XAL).

Zamia loddigesii Miquel (1843: 72–73). Type: CULTIVATED. *Van Houtte 3374* (U) (Lectotype designated by: Nicolalde-Morejón *et al*. 2009: 318).

= *Zamia cycadifolia* Dyer (1884: 195). Type: *Bourgeau s.n.* (C). *nomen illegit*.

= *Zamia galeotti* De Vriese (1845: 24). Isoneotype: *D.W. Stevenson 538* (XAL).

= *Zamia lawsoniana* Dyer (1884: 195). Holotype: *Fielding 209* (OX; isotype K).

= *Zamia leiboldii* Miquel, Linnaea (1847: 425). Holotype: *Miquel s.n.* (U).

= *Zamia leiboldii* var. *angustifolia* Regel (1876: 307). Neotype: *D.W. Stevenson 559* (NY; isoneotype XAL).

= *Zamia leiboldii* var. *angustifolia* (Regel) J. Schuster (1932: 148). Type: *Karwinsky 1028b* (lectotype, designated by Stevenson & Sabato, (1986: 137) LE; isolectotype, LE).

= *Zamia leiboldii* var. *latifolia* Regel (1876: 307). Type: ex hort. Petropolitano, 1875, *Regel s.n.* (holotype: LE).

= *Zamia loddigesii* var. *angustifolia* Regel, (1857: 190). Type: *ex Horto Petropolitano*, 1856, *Regel s.n.* (Holotype, LE).

= *Zamia loddigesii* var. *leiboldii* (Miquel) A. DC. (1868: 541). Basionym: *Zamia leiboldii*.

= *Zamia loddigesii* var. *longifolia* J. Schuster (1932: 147) Type: Mexico. Veracruz: Colipa, *Karwinski 1029* (LE; isolectotype, LE). Lectotype, designated by Stevenson & Sabato (1986: 138).

= *Zamia loddigesii* var. *obtusifolia* Regel, (1857: 190). Type: t. 186, figs 27–28 in Gartenflora vol. 6, 1857. (Lectotype, designated by Stevenson & Sabato (1986: 138) LE).

= *Zamia mexicana* Miquel, (1861: 13–14, 25–26). Type: *Eriozamia mexicana* H. Belg., 1847, *Miquel s.n.* (Holotype, U).

= *Zamia sylvatica* Chamberlain (1926: 223). Type: Mexico, Oaxaca, Tuxtepec, Sep 1910, *C.J. Chamberlain s.n.* (NY; isolectotype, F). Lectotype, designated by Stevenson & Sabato, (1986: 142).

Description:—*Stem* subterranean, contractile, branching with age or after damage, 46 x 8–15 cm in diameter. *Cataphylls* triangular basally, aristate apically, chartaceous, 8.4 cm long, 3.6–3.8 cm at base, tomentose. *Leaves* 1–7 pinnate, ascending to spreading, 45–96 x 30–41 cm; petioles 13–26 cm long, green in young leaves, subterete, armed with varying amounts of prickles up to 4 mm long, bases swollen and glabrous; rachises subterete, up to 59 cm long, with sparse prickles on lower third. *Leaflets* 12–80 pairs, opposite to subopposite, coriaceous, linear-lanceolate, apices acute to rounded, bases attenuate, 10–26 x 1.0–2.9 cm margins serrulate along distal third, subrevolute, green to dark-green adaxially, light green abaxially; articulations 0.4–0.7 cm wide. *Pollen strobili* 1–2 per crown, light brown tomentulose erect, cylindrical, 7–14 cm long, 1.7–3.3 cm in diameter, apex apiculate; peduncle light grey tomentose, 5.8–6.3 x 1.0–1.2 cm; microsporophylls cuneiform, distal face hexagonal truncate, 0.3 cm long, fertile abaxial surface with 6–8 bisporangiate synangia per lobe. *Ovulate strobili* usually 1–2 per crown, erect, beige-tomentulose, ellipsoid to conical, 11–16 cm long, up to 3.5–6 cm in diameter, apex rounded to apiculate; peduncle brown-tomentose, to 5.9–6 cm long, 1.5–1.6 cm in diameter; megasporophylls peltate, distal face hexagonal-truncate, 0.7–1.1 cm high, 1.9–2.6 cm wide. Seeds ovoid, sarcotesta pink when immature, turning red at maturity, 1.5–1.9 cm long, 0.8–1.1 cm in diameter, sclerotesta smooth. Chromosome number 2n = 18 (Vovides 1983; Moretti 1990).

Distribution and habitat:—Endemic to Mexico and distributed widely along the Gulf of Mexico seaboard and parts of Oaxaca with few known localities in Chiapas. The species occurs between 0 and 1000 m elevation in evergreen tropical forest, tropical deciduous and sub-deciduous forests as well as secondary succession and disturbed habitats such as pastures, cornfields and roadside vegetation.

Reproductive phenology:—Both pollen and ovulate strobili have been observed from May through December.

Etymology:—The specific epithet honours George Loddiges (1786–1846), a past British horticulturist and nurseryman.

Conservation status:—The IUCN Red List of Threatened Species version 2015-4 lists *Zamia loddigesii* as near threatened (NT). However, owing to its wide distribution coupled with the continuing expansion of agriculture and pastureland has made a more precise assessment difficult; in some areas it is becoming virtually extirpated whereas in others not so. *Zamia loddigesii* occurs in some national parks, especially the Ocote Biosphere Reserve and Villa de Allende of Chiapas and archaeological sites.

Additional specimens examined:—MEXICO. **Chiapas:** Ocozocoautla, 800m, 28 April 1995, *Pérez-Farrera M. 290* (HEM); San Fernando, 870 m, 6 April 2015, *Pérez-Farrera M. 3031*, 22 November 2015, *3053* (HEM).

Zamia splendens Schutzman (1984: 299) Type: CULTIVATED. Type locality. MEXICO, Chiapas, *J. Watson 1870* (holotype NY, isotypes FLAS, FTG!, MEXU!).

Description:—*Stem* subterranean, occasionally branched in old specimens up to 30 cm long, 4–7 cm in diameter. *Cataphylls* triangular basally, aristate apically, chartaceous, fragile, irregularly twisted, up to 10 cm long, 1.5–3.6 cm at base. *Leaves* 1–4 per crown pinnate, ascending to arching, up to 70 cm long, 15–42 cm wide, brilliant red at emergence turning salmon pink to cream as leaf matures before turning green; petioles up to 40 cm long, 0.4–0.7 cm in diameter, dark brown, terete to subterete, armed with many prickles up to 4 mm long, bases swollen, puberulent to glabrous; rachises subterete, 5–24 cm long with varying amounts of prickles on half to lower third. *Leaflets* 3–16 pairs, opposite to subopposite, coriaceous, wide long-elliptic through oblong to oblanceolate, apices acuminate, bases attenuate, 9–35 x 3–7.5 cm margins serrulate to denticulate along distal 2/3 to 4/5, subrevolute, green to dark-green when mature, cuticle highly lustrous, articulations 0.5–1 cm wide. *Pollen strobili* 1–3 per crown, light brown tomentulose, conical, decumbent 3–7.8 cm long, 1.1–1.5 cm in diameter, apex rounded to apiculate; peduncle light grey tomentose, 8–14 x 0.5–0.7 cm in diameter, decumbent; microsporophylls cuneiform, in regular orthostichies, distal face hexagonal with slightly truncate tomentulose dome-shaped apices, 0.3–0.4 cm long, fertile abaxial surface with 6–7 microsporangia per lobe. *Ovulate strobili* up to two per crown, beige-tomentulose, subglobose to ellipsoid, 7 cm long, 6.4 cm in diameter, apex aristate, light brown tomentulose turning dark green and glabrescent at maturity; peduncle light brown brown-tomentose, decumbent, up to 10 cm long, 1.1–1.4 cm in diameter; megasporophylls peltate, in regular orthostichies, distal face hexagonal-truncate, 1.3–1.8 cm high, 2.5–3.3 cm wide. *Seeds* angular-ovoid, sarcotesta pink when immature, turning red at maturity, 1.5–1.7 cm long, 1–1.1 cm in diameter, sclerotesta smooth. Chromosome number 2n=16 (Schutzman 1984).

Distribution and habitat:—Endemic to Mexico and distributed in evergreen and seasonal tropical rain forest on karstic topography in north western and north eastern Chiapas, also in southern Tabasco and south eastern Veracruz at elevations 100 to 800 m. **Reproductive phenology**:—Both pollen and ovulate strobili have been observed over most part of the year between March and October on cultivated specimens. **Etymology**:—The specific epithet alludes to the handsome leaves that have a highly lustrous cuticle giving the leaves a brilliant shining appearance (Schutzman 1984).

Conservation status:—The IUCN Red List of Threatened Species version 2015-4 lists the species (included within the concept of *Zamia katzeriana*) as Endangered (EN) subcategory A4ac; B1ab (i,v) and declining. In view of the declining and endangered status of evergreen tropical rain forests we recommend a reassessment for *Z. splendens* as CR. It is protected in the Ocote Biosphere Reserve of Chiapas.

Additional specimens examined:—MEXICO. Chiapas: Chicoasen, 174 m, 25 May 2009, *H. Gómez-Domínguez 2079* (HEM); Malpaso, *Gómez-Pompa 705* (MEXU), 250 m, *Nicolalde 1455*, 150 m, 8 March 2005, *457, 1458, 1459, 1460* (XAL); Ocozocuautla, 900 m, 5 November 1971, *D.E. Breedlove & A.R. Smith 21832*[a], 400 m, *32579* (CAS); Tecpatán, 500 m, 11 April 1984, *J.B. Watson & J.B. Watson 1870* (MEXU). Tabasco: Teapa, 340 m, 16 April 1996, *Pérez-Farrera 899* (HEM); Huimanguillo, 480 m, 8 August 2015, *Pérez-Farrera 3032* (HEM). Veracruz: Las Choapas, 195 m, 11 January 2004, *R. Martínez-Camilo & R. Martínez-Meléndez 825* (HEM).

Zamia verschaffeltii Miquel (1870: 31) Type: *Miquel s.n.* (holotype U), *species dubium* (Nicolalde-Morejón *et al.* 2009: 333).

Neither extensive field, herbarium and living collections explorations no living or preserved material of this species other than the holotype has been found (Stevenson & Sabato 1986) and the voucher comprises only one leaf fragment. According to Nicolalde-Morejón *et al.* (2009) *Zamia verschaffeltii* is a *species dubium* and we consider this species probably extinct. Therefore a formal description cannot be made.

Discussion

Habitat

As *Zamia splendens* is found only in evergreen tropical rain forest, with some areas bordering *Quercus* forest or seasonal tropical forest where *Z. loddigesii* grows. However, sometimes after clear cutting of the evergreen tropical rain forest, *Z. splendens*, having a subterranean trunk, will appear in the secondary succession stages. Both species are occasionally found together in these zones. It is possible that these (as putative parent species) may have crossed due to changes in forest dynamics over time. Thus giving rise to a hybridization process producing *Z. katzeriana* that colonized nearby secondary succession forests. Introgression or back crossing to *Z. splendens* probably explains the variation found in populations of *Z. katzeriana*.

Morphology, PCA and discriminate analyses

Morphological variables such as leaflet width at leaf base, mid-section and apex, rachis length, and inter-leaflet distance emerged as discriminant variables between species. These same variables were reported by Nicolalde-Morejón *et al*. (2008), but their argument for placing *Zamia splendens* under *Z. katzeriana* in their discriminant analysis was based on the criterion that the variables of the type specimen (*Regel s.n.*) of *Z. katzeriana* grouped with the *Z. splendens* cluster (see Fig. 4. in Nicolalde-Morejón *et al*. 2008). However, we note that in their Fig. 4 there are two groups labelled "*Z. splendens*" and the *Regel* specimen coincided only with the "*Z. splendens*" plants from San Fernando, Chiapas, which we consider a hybridization zone. Our PCA and DA account for over 77% and 86% of the variation respectively due to morphological variables separating three clusters with almost no overlap, placing *Z. katzeriana* between *Z. splendens* and *Z. loddigesii*. We therefore suspect that the San Fernando population may be of ancient hybrid origin and coincides with the concept of *Z. katzeriana* reported by Retting (1896). Interestingly, other hybrid plant species were reported in this area such as *Anthurium clarinervium* × *A. pedatoradiatum* (Araceae; Croat 1983) and hybrids in the palm genus *Chamaedorea* (Hodel 1992).

Leaflet anatomy and stomatal index

Both the PCA and DA have shown that 86% and over 90% of the variables respectively, are attributed to leaflet anatomy. This places *Zamia katzeriana* between *Z. splendens* and *Z. loddigesii*. The same pattern is repeated with the mean stomatal indices where that of *Z. katzeriana* is also between those of *Z. splendens* and *Z. loddigesii*. Visible anatomical features can be observed in the scarce, but consistent, amount of girder sclerenchyma present in *Z. splendens*, scarce but varying amounts in *Z. katzeriana* and larger amounts of both girder sclerenchyma and G-fibres in *Z. loddigesii* (Fig. 13). In *Z. katzeriana* the hypodermal fibres at the subrevolute leaflet margin vary from one layer and a discontinuous to continuous second layer stretching into an adaxial hypodermis. In *Z. splendens* this is consistently one layer and in *Z. loddigesii* over two layers, giving rise to a thicker margin (Fig. 14). Morphological and leaflet anatomical univariate results as well as stomatal indices have shown statistical significance between the variables of the taxa. The multivariate analysis supports these results by grouping the populations into three discontinuous entities with *Z. katzeriana* always intermediate between *Z. loddigesii* and *Z. splendens* with almost no overlap (Figs 10–14). Some anatomical and morphological similarities exist between *Z. splendens* and *Z. katzeriana* and this may probably be due to backcrossing or introgression where *Z. katzeriana* and *Z. splendens* occur sympatrically, particularly in the San Fernando area.

Hybridization in Zamiaceae has been well documented. Johnson (1963) indicated hybrid swarms in the Australian genus *Macrozamia* and Norstog (1987) carried out artificial crosses between *Zamia furfuracea* Linnaeus (1789: 477) and *Z. spartea* De Candolle (1868: 539). Norstog obtained an F1 generation showing leaflet variation similar to the variation found in a wide range of *Z. loddigesii* populations in eastern and southern Mexico, indicating that *Z. loddigesii* may itself probably have arisen through historic hybridization, which was also discussed in Schutzman *et al*. (1988) and Schutzman (2004b). Diploid chromosome counts are 2n = 18 for *Z. furfuracea*, *Z. spartea* and *Z. loddigesii*, and the karyotype morphology of these species are similar (Moretti *et al*. 1991), indicating probable high gamete compatibility. Additionally, *Z. loddigesii* occupies a broad geographical range and habitats varying from primary and secondary vegetation types as well as cultivated plots. The related *Z. paucijuga* Weiland (1916: 212) of the Mexican Pacific seaboard and *Z. prasina* Bull (1881: 20) formerly *Z. loddigesii* of the Yucatan Peninsula show karyotype variation by Robertsonian changes allowing the colonization of secondary succession vegetation and water stressed habitats (Moretti & Sabato 1984, Vovides & Olivares 1996).

Further argument supporting our hypothesis lies in the female strobili. The megastrobilus habit (pendent) and aristate apex in *Z. splendens* differs from that of populations of *Z. katzeriana*, in which it is erect with apex acuminate or sometimes rounded, being also typical of *Z. loddigesii* megastrobili. The intermediary characteristic of *Z. katzeriana* is also supported by preliminary genetic variation data using microsatellite markers (Ruiz-Castillejos *et al*. in preparation).

Conclusion

Field studies expanded the range and showed occasional sympatric occurrence of *Z. splendens* and *Z. loddigesii*, setting the scene for possible hybridization events resulting in the development of *Z. katzeriana* in adjacent secondary succession forest. This coupled with morphological and anatomical data, consistently showing an intermediary position of *Z. katzeriana* with almost no overlap, made us conclude that *Z. katzeriana* is an independent species from *Z. splendens*, probably arising from ancient hybrid origin between *Z. splendens* and *Z. loddigesii* as putative parents. Possible backcrosses or introgression may have caused confusion in past classifications of this lineage. We therefore propose the removal of *Z. splendens* from the synonymy of *Z. katzeriana*. We recognize *Z. katzeriana* as a species derived through processes of hybridization through time.

Acknowledgments

The authors were financially supported by SEP-CONACYT project No. CB-2011-01-169468 awarded to APV and the Project “Floristic diversity and evolutionary ecology of endangered species in protected natural Areas in Chiapas, México” awarded to MAPF by Christopher Davidson and Sharon Christoph and we are grateful for their support. We also thank Maarten Christenhusz for his useful comments and suggestions that greatly improved this manuscript and Roy Osborne who suggested improvements to style.

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