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A reassessment of the *Ceratozamia miqueliana* species complex (Zamiaceae) of southeastern Mexico, with comments on species relationships

Abstract The *Ceratozamia miqueliana* species complex has been revised from a morphological, anatomical and biogeographical perspective. The complex consists of five closely related species, one of which is new, *C. santillanii* sp. nov., and is described and illustrated from the northern highlands of Chiapas. The five species, although not sympatric, occur in the 'Arc' Pleistocene floristic refuge that covers southern Mexico, including northern Oaxaca and Chiapas, as well as southern Tabasco and Veracruz. The five species are characterised by oblong, oblanceolate to widely oblanceolate leaflets with decumbent or erect megastrobili when ripe. The species complex consists of *C. becerrae* Pérez-Farr., Vovides & Schutzman *C. euryphyllidia* Vázq.Torres, Sabato & D.W. Stev., *C. miqueliana* H. Wendl., *C. zoquorum* Pérez-Farr., Vovides & Iglesias and the new species which is related to *C. zoquorum*, but differs from these in leaf, male and female cone growth habit, as well as inter-leaflet distance. Discriminant analysis using the five species with 14 variables showed significant differences (Wilks λ test, $P < 0.01$) for four functions among the five species. Mahalanobis distances were also highly significant among the five species ($P < 0.01$). *Ceratozamia euryphyllidia* and *C. becerrae* were the most distant, whereas *C. zoquorum* and *C. becerrae* were closest.

Key words Chiapas, Cycad species complex, endangered species, floristic refuges, Mesoamerica, Pleistocene

Introduction

The genus *Ceratozamia* is a highly diverse neotropical cycad group that can be considered endemic to Mexico with the exception of two species from Central America. It is largely distributed along the Sierra Madre Oriental and Occidental, Sierra Madre del Sur and the Sierra Madre de Chiapas with its centre of diversity in southern Mexico (Vovides *et al*., 2004) and the extreme limit of the genus reaching Belize, Guatemala and Honduras (Whitelock, 2004). These cycads are known from diverse, but always mesic habitats ranging from oak forest, tropical rain forest and cloud forest (Vovides *et al*., 2004) and there are 23 known species for Mexico, 50% of which are from the south and southeast of the country.

Moretti *et al*. (1980) were the first to recognise species complexes within the genus and Stevenson *et al*. (1986) proposed two main groups in *Ceratozamia* based on leaflet morphology. Using morphological criteria, various other related groups or complexes can also be found, and we recognise seven species (Vovides *et al*., 2004). Recently, during a botanical exploration to collect material for a revision of the genus *Ceratozamia* for Mesoamerica, we collected and studied a species from northwestern Chiapas in the physiographical region proposed by Müllerried (1957) as the northern highlands or 'montañas del norte' in an area adjacent to the 'Selva Zoque' that includes 'The Ocote Biosphere Reserve'. This species was cultivated under uniform greenhouse conditions at the Escuela de Biología, UNICACH, and the Clavijero Botanic Garden at Xalapa along with many of its congeners for 4 years to rule out plasticity. The differences observed in female cone morphology, growth habit, trunk, leaf and leaflet morphology under cultivated and habitat conditions over a 4-year period, lead us to the conclusion that this taxon is new to science. We therefore included this species along with its four congeners of the *C. miqueliana* complex in a discriminant analysis to elucidate relationships.

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No.	Abbreviation	Character and measurement units (in parentheses)
1	Largtr	Trunk length (cm)
2	Permtr	Trunk perimeter (cm)
3	Nhoja	Number of leaves per trunk
4	Largopec	Petiole length (cm)
5	Largoraq	Rachis length (cm)
6	Nfoliol	Number of leaflets per leaf
7	Largofol	Leaflet length (cm)
8	Anchofol	Leaflet width (cm)
9	Nvenas	Number of veins per leaflet
10	Largomicro	Microsporophyll length (mm)
11	Anchomicro	Microsporophyll width (mm)
12	Anchomegas	Megasporophyll distal face width (mm)
13	Largomegas	Megasporophyll distal face length (mm)

Table 1 List of morphometric variables used in the analysis of populations in the *Ceratozamia miqueliana* complex.

	Discontinuous variables used
1	Leaflet veins visible on abaxial surface
2	Translucent veins
3	Coriaceous leaflets
4	Membranaceous leaflets;
5	Pruinose leaflets
6	Branched trunk
7	Globose or subglobose trunks
8	Erect megastrobilus
9	Descendant megastrobilus
10	Armed petiole
11	Unarmed petiole
12	Erect leaves
13	Descendant leaves
14	Yellowish leaflet articulation
15	Green leaflet articulation
16	Circinate vernation
17	Erect vernation

Table 2 The discontinuous variables used for the *Ceratozamia miqueliana* complex on presence/absence characters.

Materials and methods

Field work

Five natural populations and 62 adult individuals of the four species and the new taxon were studied (15 plants of *C. miqueliana*, 10 of *C. euryphyllidia*, 10 of *C. zoquorum*, 15 of *C. becerrae* and 12 of the new taxon). All material collected was from evergreen tropical rain forest from the mountains of north and north-western Chiapas, southern Tabasco and the Uxpanapa region of southern Veracruz. For the morphological analysis nine vegetative and four reproductive characters were used (Table 1). These variables were those found to be most useful for delimiting species within *Ceratozamia* by Vovides & Rees (1983), Vovides *et al*. (1983), Vovides *et al*. (1993), Vázquez-Torres & Vovides (1998), Pérez-Farrera *et al*. (2004, 2007) and Pérez-Farrera (2005). Measurements were taken using a flexible measuring tape (Truper® 3 m) and a digital vernier with 0.01 mm resolution (Truper®). Data were captured on to an Excel for Windows® spreadsheet.

Analyses

Analysis of variance (ANOVA) was conducted on the parameters within and between populations of the five taxa. This was done to determine the amount of morphological variation that is present between the populations of the complex by using the JMP v. 3.2 for Windows® program. Discriminant analysis was applied to separate two or more groups of individuals based on the morphological measurements taken for each taxon. Data was transformed ($\log_{10}$) and Wilks λ (lambda) test were calculated and a graph for the discriminants was generated using Statigraphics® v. 2 for Windows®. The squared Mahalanobis distances and their significant level were calculated using Statistica v. 5.5 for Windows® program.

Additionally the complex was analysed by means of conglomerates using the UPGMA grouping method on discontinuous variables to generate a dendogram with Jaccard's coefficient. The discontinuous variables used for the complex on a presence/absence analysed with MVSP® v. 3.1 for Windows® are presented in Table 2.

Results

Univariate analysis

The mean and standard deviations values for five species are shown in Table 3. *Ceratozamia euryphyllidia* had the largest trunk, leaves and leaflets within the *C. miqueliana* complex, while *Ceratozamia becerrae* had the smallest of these characters within the complex. The ANOVA results show that species characters are not overlapping (Table 4). All the variables analysed were highly significant ($P < 0.0001$).

Discriminate analysis

A scatter diagram was generated from data derived from discriminate function analysis (Fig. 1). The five species separated by ordination in bi-dimensional space and did not present any overlapping between groups. Of the 13 variables included in the standardised discrete canonical function, the variables with the highest values in factors were trunk perimeter, microsporophyll width, megasporophyll width and leaflet width and the first canonical variable showed that 69% of the variation was between species. In addition to these variables, other variables such as leaflet length and number of veins per leaflet emerged as discriminant variables between species. The positive correlations of all the variables showed differences between species (Table 5), and the variables with the highest values of variation were trunk perimeter, leaflet length, number of veins per leaflet, microsporophyll width and megasporophyll distal face

	C. euryphyllidia		*C. santillanii*		*C. becerrae*		*C. miqueliana*		*C. zoquorum*	
Character	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
Largtr	1.28	0.08	**1.52**	0.2	1.14	0.13	1.31	0.15	1.32	0.17
Permtr	**1.95**	0.07	1.48	0.07	0.88	0.07	1.06	0.11	1.01	0.15
Nhoja	0.54	0.12	0.72	0.09	0.43	0.14	0.92	0.22	0.5	0.15
Largopec	**1.97**	0.11	**1.7**	0.19	**1.67**	0.17	**1.9**	0.13	**1.94**	0.12
Largoraq	**2.13**	0.16	**1.7**	0.15	**1.59**	0.22	**1.97**	0.09	**1.9**	0.11
Nfoliol	1.02	0.12	0.97	0.1	0.78	0.14	1.16	0.1	0.99	0.15
Largofol	1.41	0.06	1.4	0.07	1.37	0.08	1.48	0.05	**1.5**	0.07
Anchofol	1.14	0.09	0.67	0.05	0.83	0.09	0.73	0.06	0.76	0.04
Nvenas	**1.8**	0.11	1.55	0.02	**1.58**	0.06	1.49	0.07	**1.56**	0.05
Largomicro	0.837	0.03	**2.55**	1.16	1.05	0.04	1.12	0.06	1.02	0.02
Anchomicro	0.76	0.03	−0.11	0.02	0.69	0.05	0.98	0.05	0.77	0.04
Anchomegas	**1.6**	0.03	**1.6**	0.02	**1.41**	0.06	**1.74**	0.02	**1.43**	0.04
Largomegas	1.28	0.11	1.17	0.05	1	0.09	1.39	0.02	1	0.06

Table 3 Mean value and standard deviations (SD) for the ratios used in the analysis of populations in the *Ceratozamia miqueliana* complex. Highest values in bold.

Character	F	*P*
Largtr	10.33	<0.0001
Permtr	224.29	<0.0001
Nhoja	21.78	<0.0001
Largopec	10.59	<0.0001
Largoraq	23.85	<0.0001
Nfoliol	17.94	<0.0001
Largofol	9.77	<0.0001
Anchofol	73.7	<0.0001
Nvenas	32.2	<0.0001
Largomicro	21.66	<0.0001
Anchomicro	1230.3	<0.0001
Anchomegas	180.03	<0.0001
Largomegas	74.73	<0.0001

Table 4 Summary of analysis of variance of the 13 morphometric characters used in the analysis of populations in the *Ceratozamia miqueliana* complex (F = F value, *P* = probability).

length (Table 6). The discriminant analysis showed that there were distinct morphological characters between species. The Wilks–Lambda test was highly significant ($P < 0.0001$) for the four factors; thus showing that all the species were classified correctly (Table 7). The values of squared Mahalanobis distances are shown in Table 8. In all cases, the values were highly significant ($P < 0.001$). The highest values about Mahalanobis distances were between *C. santillanii* and *C. miqueliana*, while the lower values were between *C. becerrae* and *C. zoquorum.*

Description

Ceratozamia santillanii Pérez-Farr. & Vovides, sp. nov. (Fig. 3)

Diagnosis: Plantae rupestrae, truncus cylindricus, parvus, semi-hypogaeus vel hypogaeus, 20–67 cm altus; cataphylla lanata, triangularia, stipulata, folia pinnata; petiolus 25–115 cm longus; rachis 22–72.5 cm longa, foliolis pendentibus et foliolis aculeatissimis; foliola opposita vel subopposita, 6–13 juga, linearia; strobilus masculinus lineari-cylindricus, 20–22 cm longus, pedunculum tomentosum 3–4 cm longum; strobilus femineus, erectis 13–20 cm longus, pedunculum tomentosum 2–3 cm longum.

Type: Mexico, Chiapas, northern mountain range. 15 Oct. 2004 *M. A. Pérez-Farrera* 3030 (female) (Holotype: HEM. Isotypes: XAL, MEXU). Paratypes: mexico, chiapas, northern mountain range. 15 Oct. 2004 *M. A. Pérez-Farrera* 2944 (male) (HEM).

Description: TRUNKS medium sized, rupiculous, cylindrical, erect or arched, subterranean or partially so, 20–67 cm long, 7.6–12.4 cm in diameter, armed with light brown persistent leaf bases in the upper part of the trunk. LEAF CATAPHYLLS triangular, stipulate, silvery sericeous in the middle and apical part, 2–4.5 cm long, and 1.7–3 cm wide. LEAVES 4–7, descending to decumbent, pinnate, olive-green with circinate vernation upon emergence, forming an open crown, 62–211 cm long, 36–59 cm wide, tomentose, light green, pruinose with light brown-reddish waxy bloom when young, dark green and glabrous at maturity; seedling eophylls 2. PETIOLES erect, terete, 25–115 cm long, 0.6–1.3 cm diameter, armed to unarmed, when armed, with few stout slightly erect prickles being more numerous towards the base; rachis erect, semi terete, 22–72.5 cm long, with few or without prickles. LEAFLETS coriaceous, flat, pruinose, oblong to oblanceolate, 6–13 pairs, opposite to sub opposite, apical portion asymmetrical, apex acuminate, base truncate, margins entire, dark green on adaxial surface, light green on abaxial surface, 18.5–31.5 cm long, 3.3–5.5 cm wide, venation not visible on abaxial surface, 32–39 veins, inter-vein distance 0.07–(0.09)–0.12 cm ($n = 9$); leaflet articulations yellow to orange-green 0.68–(0.88)–1.1 cm wide ($n = 9$) in mature plants. MICROSTROBILI conical, erect, light to olive green at emergence, creamy to light yellow when mature, 20–22 cm long,

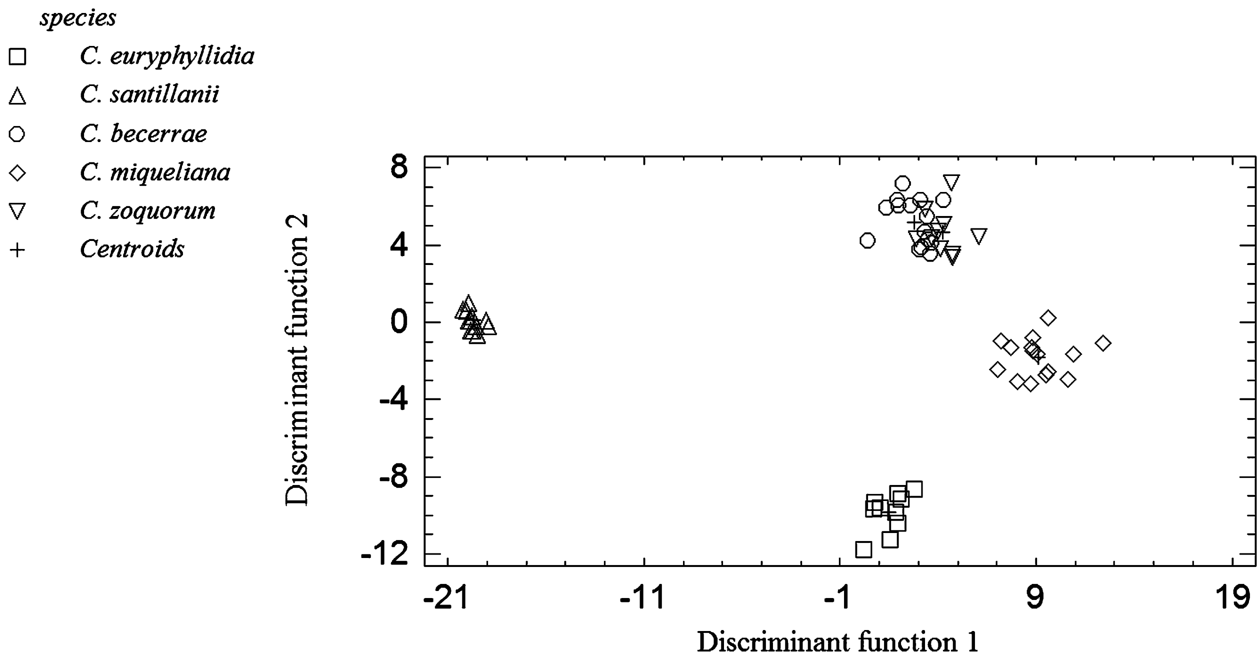

Figure 1 Scatter plot of scores derived from functions produced by stepwise discriminant analysis of 14 morphometric ratios from populations in the *Ceratozamia miqueliana* complex.

	1	2	3	4
Largtr	−0.13	−0.16	−0.18	−0.37
Permtr	−0.20	**−0.91**	−0.43	−0.47
Nhoja	−0.10	−0.11	0.11	0.39
Largopec	0.18	−0.17	−0.09	−0.71
Largoraq	−0.45	0.24	0.33	0.42
Nfoliol	0.21	0.04	−0.18	−0.14
Largofol	0.18	0.34	0.32	−0.57
Anchofol	0.04	−0.49	−0.64	**1.19**
Nvenas	−0.07	0.14	−0.20	−0.68
Largomicro	−0.26	0.06	0.23	0.15
Anchomicro	**1.05**	−0.13	−0.07	−0.15
Anchomegas	−0.08	−0.47	**0.78**	0.09
Largomegas	0.11	−0.26	0.30	0.27
Eigenvalue	109.201	28.7677	18.1735	1.72357
Among-group variance	69.17	18.22	11.51	1.09
Canonical correlation	0.99545	0.98306	0.97357	0.79551

Table 5 Standardised discriminate function values for each of four factors used in the analysis of populations in the *Ceratozamia miqueliana* complex. Highest values in bold.

3.2–5 cm diameter; PEDUNCLES tomentose, light brown at emergence turning brown at maturity, 3–4 cm long, 1.1–1.5 cm diameter; MICROSPOROPHYLLS number indeterminate, cuneiform, bicornate at distal end, inserted spirally on cone axis forming apparent vertical rows, red tomentulose, fertile portion covering 1/2–2/3 of the abaxial surface 0.8–(0.87)–1 cm from cone axis excluding the horns, 0.53–(0.59)–0.63 cm wide, distance between horns 0.52–(0.61)–0.65 cm; MICROSPORANGIA number indeterminate in sori of 3–4, dehiscence by a longitudinal slit. MEGASTROBILI cylindrical or barrel shaped, erect and light green when emergent, olive green when mature, 13–20 cm long, 8–9.2 cm diameter at median portion; PEDUNCLES slightly white-tomentose, 2–3 cm long, 1.3–1.4 cm diameter; MEGASPOROPHYLLS number indeterminate, peltate, spirally inserted on cone axis forming apparent vertical rows, distal face hexagonal, bicornate, with reddish epidermal colouring on the margins of the lobes partly covered with greyish-light brown tomentulum extending to about a third of the distance towards the horns, pedicel length 2.6–(2.8)–3 cm from cone axis, distal face long axis 3.7–(3.9)–4.1 cm, short axis 1.3–(1.6)–1.9 cm, distance between horns 1.7–(1.9)–2.1 cm, horn length 0.4–(0.6)–0.8 cm (all $n = 6$). SEEDS angular-ovate 2.2–(2.4)–2.4 cm long, 1.8–(1.9)–2.1 cm in diameter, sarcotesta white when immature, light yellow

Character	*Ceratozamia euryphyllidia*	*Ceratozamia santillanii*	*Ceratozamia becerrae*	*Ceratozamia miqueliana*	*Ceratozamia zoquorum*
Largtr	28.26	26.99	5.21	−0.22	12.86
Permtr	**374.46**	**292.93**	**215.41**	**234.37**	**232.14**
Nhoja	−59.93	−48.73	−66.49	−62.65	−76.36
Largopec	97.62	56.72	73.27	89.56	94.14
Largoraq	−274.12	−182.53	−246.00	−259.54	−259.69
Nfoliol	55.86	13.02	58.24	54.69	63.38
Largofol	**485.54**	**513.11**	**563.63**	**601.28**	**606.03**
Anchofol	−467.37	−617.64	−563.85	−616.72	−637.64
Nvenas	**599.03**	**620.09**	**607.09**	**571.84**	**641.70**
Largomicro	−1.88	13.11	0.45	0.29	−1.06
Anchomicro	**533.43**	**−39.46**	**510.79**	**681.22**	**561.82**
Anchomegas	**972.08**	**1040.70**	**826.62**	**1082.37**	**842.97**
Largomegas	233.23	193.13	193.61	262.71	187.29
Constant	−1959.46	−1721.01	−1460.44	−2062.00	−1608.22

Table 6 Classification of coefficient function by species. Highest values in bold.

Derived functions	Wilks Lambda (λ)	χ^2	df	*P*
1	0.000005	626.6627	52	0.0000
2	0.000643	382.1426	36	0.0000
3	0.019149	205.6845	22	0.0000
4	0.367165	52.1010	10	0.0000

Table 7 Summary of discriminate analysis of the populations in the *Ceratozamia miqueliana* complex (χ^2 = chi-squared factor, df = degrees of freedom, P = probability).

to light-cream or beige upon maturity, sclerotesta light-brown, smooth with 7–10 visible coronulae radiating from the micropyle with a dark brown areole 0.7 cm in diameter circumscribing the micropylar end, chalazal end presenting 6–8 vascular trace perforations on a raised portion of the sclerotesta 0.8–1.2 cm in diameter. Chromosome number 2n = 16.

Etymology: The specific epithet was chosen to honour Professor Trinidad Alemán Santillán in recognition for his academic accomplishments as Professor of Botany and Ecology at the University of Science and Arts of Chiapas (UNICACH) and in his tireless efforts in the training of young biologists in Chiapas, Mexico.

Key to the *Ceratozamia miqueliana* species complex

The following key permits the separation of species in the *Ceratozamia miqueliana* species complex of southern Mexico: *Ceratozamia santillanii*, *C. becerrae*, *C. euryphyllidia*, *C. miqueliana* and *C. zoquorum*.

1a. Leaflets papyraceous to moderately membranaceous, translucent .... *C. euryphyllidia*
1b. Leaflets coriaceous, not translucent.
  2a. Petioles armed, leaflets with green articulations .... *C. miqueliana*
  2b. Petioles unarmed or very scarcely so, leaflets with yellow articulations.
    3a. Leaflets subfalcate, oblong to widely oblanceolate, ... *C. becerrae*
    3b. Leaflets not subfalcate, oblanceolate.
      4a. Leaflets with visible veins, distance between leaflets 1.7–2.4 cm, female cones decumbent at maturity ....*C. zoquorum*
      4b. Leaflets without visible veins, distance between leaflets 3.4–7.5, female cones erect at maturity. ....*C. santillanii-*

Habitat description

Ceratozamia santillanii grows on karstic rocks on walls of ‘*cimas*’ (holes in the rock, about 10 m diameter) within an

	C. becerrae	*C. zoquorum*	*C. miqueliana*	*C. euryphyllidia*	*C. santillanii*
C. becerrae		7.17	80.10	84.70	229.03
C. zoquorum	21.55		49.54	69.30	202.37
C. miqueliana	188.42	148.91		88.44	345.14
C. euryphyllidia	254.60	253.59	265.84		196.38
C. santillanii	612.23	673.19	922.62	653.28	

Table 8 F-values (df = 13.45) and the squared Mahalanobis distances. $P < 0.001$ in all cases.

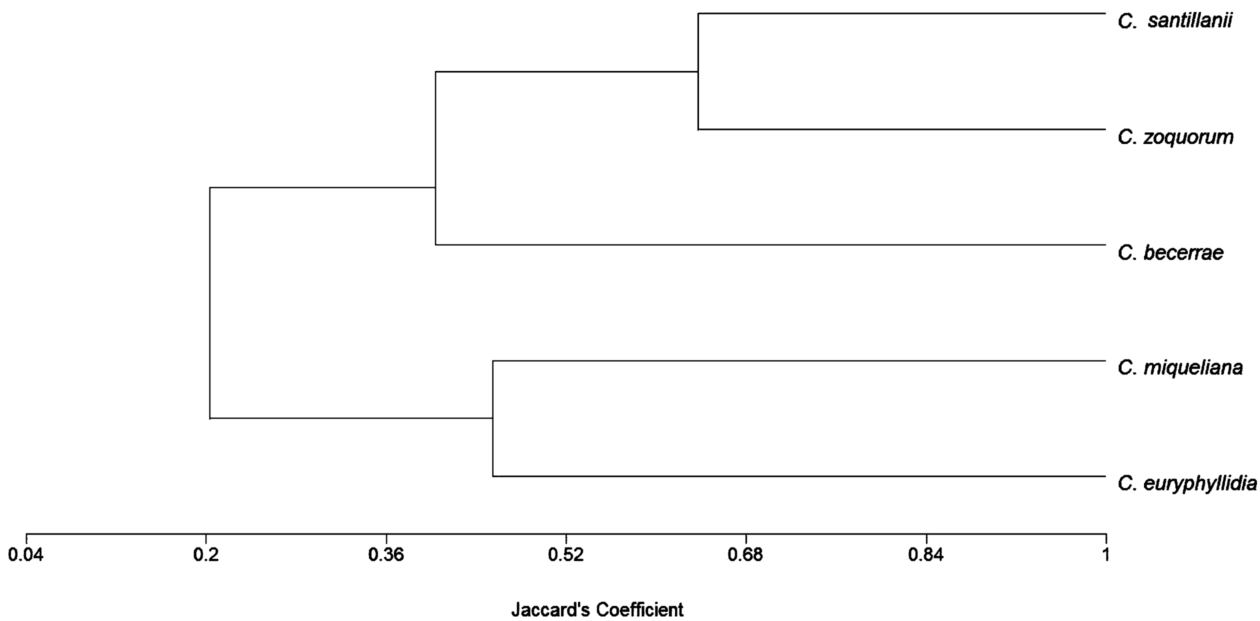

Figure 2 UPGMA and Jaccard's coefficient cluster analysis of the *Ceratozamia miqueliana* complex.

altitudinal range of 800–900 m.a.s.l., in lower montane rain forest according to classification of Breedlove (1981) or '*bosque tropical perennifolio*' according to Rzedowski (1978) predominated by three strata. The dominant emergent tree species between 20 and 30 m tall are: *Zanthoxylum culantrillo* Kunth, *Psychotria* aff. *horizontalis* Sw., *Persea steyermarkii* C.K. Allen., *Ocotea effusa* Hemsl., *Mastichodendron foetidissimum* (Jacq.) H.J. Lam., *Ardisia verapazensis* Donn.Sm., *Picramnia hirsuta* W.W. Thomas and *Pseudolmedia oxyphyllaria* Donn.Sm. The mid stratum consists of trees between 10 and 20 m tall among which are commonly found: *Viburnum guatemalense* Gand., *Malmea depressa* R.E.Fr., *Sapranthus campechianus* Standl., *Oreopanax salvinii* Hemsl., *Eugenia uliginosa* Lundell, *Inga oerstediana* Benth., *Saurauia oreophila* Hemsley, *Chiococca sessilifolia* Miranda, *Hamelia calycosa* Donn.Sm. and *Corchorus siliquosus* L. The lower stratum consists of trees, shrubs and herbaceous plants between 1 to 10 m tall and represented by: *Kohleria elegans* Loes., *Lobelia xalapensis* Kunth, *Astrocaryum mexicanum* Liebm. ex Mart., *Malvaviscus arboreus var mexicanus Schltdl.*, *Witheringia nelsonii (Fernald) Hunz.*, *Chamaedorea arenbergiana* H. Wendl., *C. ernesti-augusti* H.Wendl., *C. elegans* Mart., *C. tenella* H. Wendl., *Anthurium clarinervium* Matuda, *A. huixtlense* Matuda, *Lasiacis* aff. *Rhizophora* Hitchc. and *Heliconia librata* Griggs. Some epiphytes and hemi-epiphytes are also found: *Stelis bidentata* Schltr., *S. chiapensis* R.Solano Gómez, *Gongora galeata* Rchb.f., *Pecluma* sp., *Polypodium chiapense* A.M.Evans & A.R.Sm., *Anthurium pentaphyllum* G.Don var. *bombacifolium* (Schott) Madison, *A. scandens* Engl., *Disocactus macranthus* (Alexander) Kimnach & Hutchison and *Philodendron seguine* Schott. The habitat presents an irregular topography with slopes of up to 80% and cliff faces. The geology of this region corresponds to early tertiary continental marine strata from the Cainozoic, middle and upper Cretaceous. The rock type is limestone forming a karstic topography and the soil a shallow tropical rendzina (Ferrusquía-Villafranca, 1998).

The geographic region of this habitat has an annual precipitation of 818 mm and average annual temperature of 23.9 °C, minimum temperature of 21.2–21.7 °C during December and January and maximum temperature of 25.2–26 °C during April to June (García, 2004). The habitat lies within an area of southern Mexico noted for its floristic hot spots and refugia.

Discussion

The results of discriminant analysis showed that five species can be separated morphologically. The reproductive structures (megasporophylls and microsporophylls) have emerged as important discriminant variables, but unfortunately these are often absent in herbarium vouchers due to the unpredictable phenology in the complex. This complex has shown low levels of genetic variation in spite of its high morphological variation (Pérez-Farrera *et al.*, 2007). González and Vovides (2002) have suggested a recent speciation within the genus as shown by its low intralineage divergence presented by nuclear ribosomal DNA ITS and chloroplast DNA *trn*L-F non-coding region and Pérez-Farrera (2005) has suggested a recent ecological speciation probably due to specialisation. The species of this complex have colonised very specific microhabitats. For example, *C. miqueliana* grows on basaltic rock and clay soil while *C. zoquourum*, *C. becerrae* and *C. santillanii* grow only on karstic rock and occupy different geographic areas.

Ceratozamia santillanii is related to *C. zoquorum* (Fig. 2), both having descending to decumbent leaves (Figs 4, 5), ptyxis circinate, tomentose, pruinose, flat, coriaceous, oblong to oblanceolate leaflets with unarmed petioles or scarcely so with few stout prickles and yellow-orange articulations. However, *C. santillanii* has erect female cones, non-visible leaflet veins and greatest inter-leaflet distance. In contrast, *C. zoquorum* has descending female cones (Fig. 6), visible leaflet veins and short inter-leaflet distance. Also *C. santillanii* is similar to

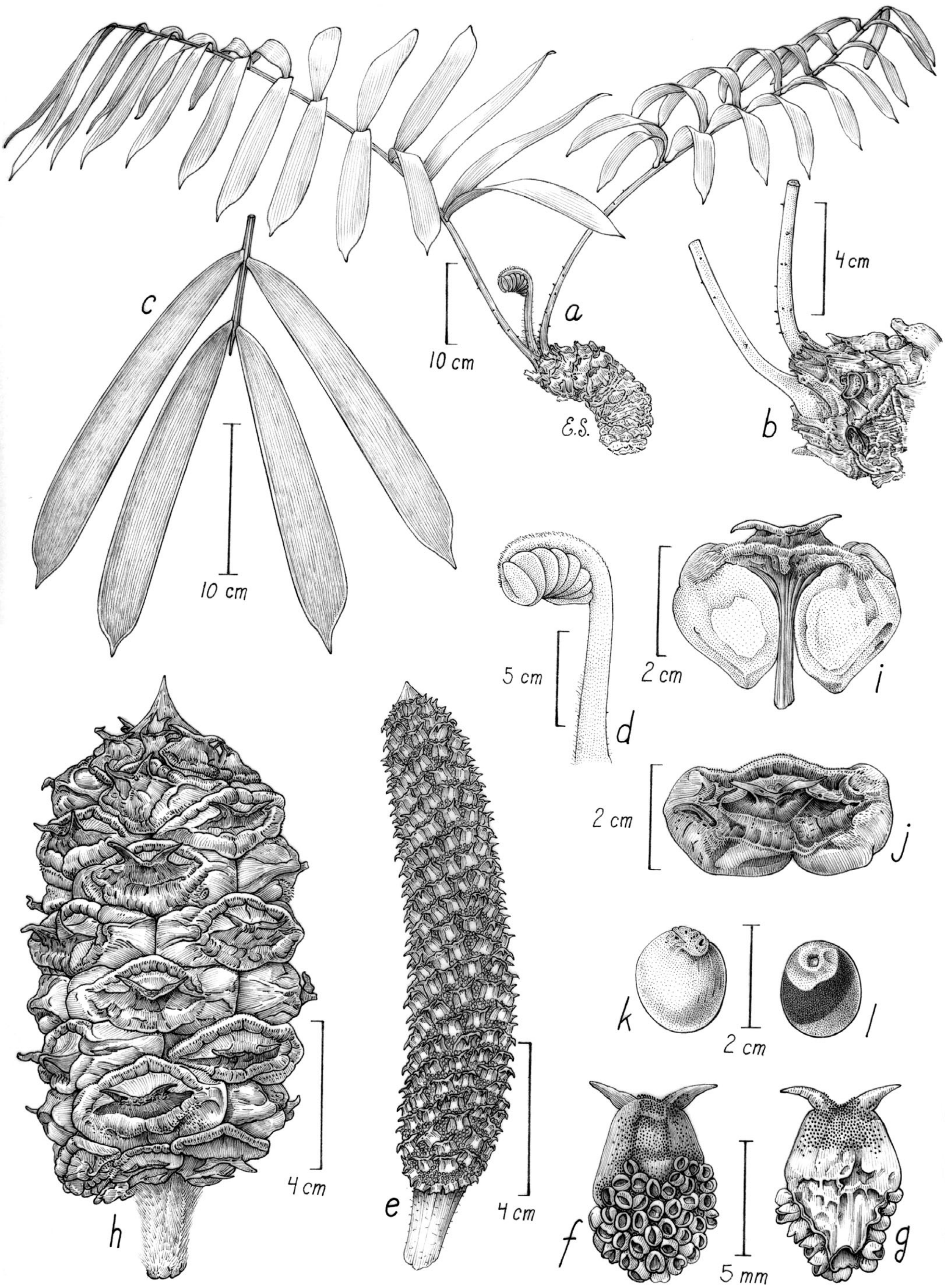

Figure 3 *Ceratozamia santillanii*; a, habit of plant; b, detail of trunk and petiole bases; c, detail of distal portion of leaf; d, detail of emerging leaf with circinate ptyxis; e, male strobilus; f, abaxial view of microsporophyll; g, adaxial view of microsporophyll; h, female strobilus; i, megasporophyll with ovules; j, view of distal face of megasporophyll; k–l, detail of seed.

Figure 4 Leaf morphology of *C. zoquorum* (left) and *C. santillanii* (right).

Figure 5 Leaf morphology of *Ceratozamia becerrae.*

Figure 6 Female cone morphology and habit of *C. becerrae* (erect).

C. becerrae in leaves and female cone habit (Figs 7, 8), both species have descending to decumbent leaves, pruinose young leaflets and erect female cones, but differ in leaflet and trunk morphology, as well as female cone peduncle tomentulum and megasporophyll colour. *Ceratozamia becerrae* has oblong leaflets, globose trunk, reddish female cone peduncle tomentulum and olive-green megasporophyll tomentulum, while *C. santillanii* has oblanceolate leaflets, cylindrical trunk, female cone peduncle with white tomentulum and greyish-light brown megasporophyll tomentulum.

The morphological characteristics of *C. santillanii* place it within the first group of the genus *Ceratozamia* according to Stevenson *et al.* (1986). This includes *C. zoquorum*, *C. becerrae*, *C. miqueliana*, *C. huastecorum* Avendaño, Vovides & Cast.-Campos, *C. euryphyllidia* Vazq.Torres, Sabato & Stevenson, *C. latifolia* Miq., *C. moretti* Vazq. Torres & Vovides, *C. microstrobila* Vovides & Rees. We consider that *C. santillanii* forms part of a complex of *Ceratozamia* populations in the northwestern mountains of Chiapas that includes *C. zoquorum*, *C. becerrae*, *C. miqueliana* and *C. euryphyllidia*, the latter species from lowland southern Veracruz and Oaxaca. All species showed a separate separation in the cluster analysis (Fig. 1), except *C. becerrae* and *C. zoquorum* that tend to overlap for the characters analysed. This closeness may be explained by a probable historic vicariance and/or dispersal events. *Ceratozamia becerrae* occurs on isolated 'mogotes' of southern Tabasco as well as in the neighbouring 'Montañas del Norte' of northern Chiapas. Though *C. zoquorum* occurs in the latter mountain range (both ranges are relatively close) the two species form separate populations and are never sympatric, being separated by steep canyons. There are qualitative differences in strobilar morphology between the two species (Vovides *et al*., 2004) and also *C. miqueliana*, *C. becerrae* and *C. euryphyllidia* group out on a separate clade from that of *C. zoquorum* in a molecular phylogeny (González & Vovides, 2002). The geology where *C. becerrae* grows is Tertiary sedimentary rock on a calcareous base, while the geology of the *C. zoquorum* habitat is sedimentary rock of late/upper Cretaceous on calcareous substrate. For both species the topography is abrupt with steep slopes.

This mountainous region forms part of Cenozoic and Pleistocene floristic refugia, according to Toledo (1982) and the 'Arc' refugium of Wendt (1987), that extends from northern Oaxaca, southern Veracruz and northern Chiapas and forms a biological corridor between the 'Ocote' Biosphere Reserve,

Figure 7 Female cone morphology and habit of *C. santillanii* (erect).

Figure 8 Female cone morphology and habit of *C. zoquorum* (descending).

'Chimalapas' and the 'Uxpanapa' rain forest, which is noted for its floristic affinities of great age.

There appears to be a strong correlation with the presence of cycads in these refugia (Schutzman *et al*., 1988). A tentative molecular phylogeny of the genus *Ceratozamia* by González and Vovides (2002), in spite of low levels of variation found, nevertheless allowed an interesting scenario for the genus where the three main clades revealed are consistent with the distribution ranges of the species. The two basal clades contained the species distributed in southern Mexico, south of the trans Mexican neovolcanic range, in or near floristic refugia, including the *C. miqueliana* complex of this study, and is consistent to the findings of Palacios and Rzedowski (1993), who reported on the fossil pollen of *Ceratozamia* (Cycadopodites) in the lower and mid Miocene of Pichucalco, Chiapas, and also Rzedowski and Palacios (1977) from the Miocene of the Chinantla region of Oaxaca along with fossil *Engelhardtia* pollen. *Engelhardtia* used to be widely distributed over the northern hemisphere during the Tertiary (Rzedowski & Palacios, 1977) that parallels the Tertiary presence of *Ceratozamia* fossils in Alaska and Europe (Hollick, 1932; Kvacek, 2002). The bringing together of knowledge of fossil and extant cycad taxa is important to understanding divergence and timing events within the group (Hermsen *et al*., 2006) and is complementary to the molecular results. The third clade of unresolved topology (González & Vovides, 2002) contains species north of the trans Mexican neovolcanic range and they appear to be the result of relatively recent speciation events probably due to a fast series of vicariance events during the Cenozoic orogeny and the spread of floristic communities during post Pleistocene climatic warming. All this appears to be in agreement with Marshall and Liebherr (2000), who identified two biogeographic biotic assemblages; one north of the Mexican transition zone (trans Mexican neovolcanic range) and another to the south. It is interesting to note that no floristic refugia of great age appear to have yet been reported north of this transition zone. Recent historical events effecting the present day distribution of certain species in the genera *Dioon* and *Zamia* have also been discussed from a population genetics viewpoint by González-Astorga *et al*. (2003, 2005, 2006) and appear to give support to the floristic refugium hypothesis for present-day cycad distribution.

Many endemic species of restricted distribution have been found in this karstic area such as *Chamaedorea stolonifera* H. Wendl. ex Hook. f., *C. tenella* H.Wendl. (Hodel, 1992), *Anthurium berriozabalense* Matuda, *A. clarinervium* Matuda (Croat, 1983), *Lepanthes acuminata* Schltr. *ssp. ernesti* Salazar & Soto Arenas, *Stelis chiapensis* R.Solano Gómez, *Epidendrum alvarezdeltoroi* Hágsater, *Pleurothallopsis ujarensis* (Rchb.f.) Pridgeon & M.W.Chase, *Stelis leucopogon* Rchb.f., S. *martinezii* R.Solano Gómez (Solano, pers. comm.), *Picramnia hirsuta* W.W.Thomas (Thomas, 1988), *Phanerophlebia gastonyi* Yatsk., *Polypodium chiapense* A.M.Evans & A.R.Sm. and *Selaginella chiapensis* A.R.Sm. (Yastkievych, 1992, 1996; Mickel & Smith, 2004), *Dioscorea sumiderensis* B.G.Schub. & O.Téllez (Tellez-Valdes & Schubert, 1991) and *Alfaroa mexicana* D.E.Stone. This area is better known for its diversity and high endemism in vertebrates (Johnson & Savage, 1995).

We have found only one population of *Ceratozamia santillanii* in the 'Selva Zoque' region of the northern highlands of Chiapas and we consider it to be endangered owing to the rapid transformation of its habitat into pastureland and coffee plantations. Precise locality information has been purposely omitted in order to discourage illegal collection of this rare and endangered species. Because of its small population size and highly restricted distribution, we propose the IUCN Red List Category of CR B.1 (IUCN, 2005).

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References

BREEDLOVE, D. E. 1981. *Introduction to the Flora of Chiapas*. Part 1. California Academy of Science, California.

CROAT, T. 1983. A revision of the genus *Anthurium* (Araceae) of Mexico and Central America, Part I. Mexico and Middle America. *Annals of the Missouri Botanical Garden* **70**, 211–420.

FERRUSQUIA-VILLAFRANCA, I. 1998. Geología de México: Una sinopsis. In: RAMAMOORTHY, T. P., BYE, R., LOT, A. & FA, J., Eds., *Diversidad Biológica de México: orígenes y distribución*. Universidad Autónoma de México, pp. 3–108.

GARCÍA, E. 2004. *Modificaciones al sistema de clasificación climática de Koppen 5a ed*. México, D.F., Instituto de Geografía, UNAM.

GONZÁLEZ, D. & VOVIDES, A. P. 2002. Low intralineage divergence in the genus *Ceratozamia* Brongn. (Zamiaceae) detected with nuclear ribosomal DNA ITS and chloroplast DNA *trn*L-F non-coding region. *Systematic Botany* **27**, 654–661.

GONZÁLEZ-ASTORGA, J., VOVIDES, A. P., FERRER, M. M. & IGLESIAS, C. 2003. Population genetics of *Dioon edule* Lindl. (Zamiaceae, Cycadales): biogeographical and evolutionary implications. *Biological Journal of the Linnean Society* **80**, 457–467.

GONZÁLEZ-ASTORGA, J., VOVIDES, A. P., CRUZ-ANGÓN, A., OCTAVIO-AGUILAR, P. & IGLESIAS, C. 2005. Allozyme variation in three extant populations of the narrowly endemic cycad *Dioon angustifolium* Miq. (Zamiaceae) from northeastern Mexico. *Annals of Botany* **95**, 999–1007.

GONZÁLEZ-ASTORGA, J., VOVIDES, A. P., OCATVIO-AGUILAR, P., AGUIRRE-FAY, D., NICOLALDE-MOREJÓN, F. & IGLESIAS, C. 2006. Genetic diversity and structure of the cycad *Zamia loddigesii* Miq. (Zamiaceae): implications for evolution and conservation. *Botanical Journal of the Linnean Society* **152**, 533–544.

HERMSEN, E. J., TAYLOR, T. N., TAYLOR, E. L. & STEVENSON, D. W. M. 2006. Cataphylls of the Middle Triassic cycad *Antarcticycas schopfii* and new insights into cycad evolution. *American Journal of Botany* **93**, 724–738.

HODEL, R. H. 1992. *Chamaedorea Palms. The Species and their Cultivation*. Allen Press, Lawrence, Kansas.

HOLLICK, A. 1932. Descriptions of new species of Tertiary cycads, with a review of those previously recorded. *Bulletin of the Torrey Botanical Club* **59**, 169–189.

IUCN 2005. *Guidelines for Using the IUCN Red List: Categories and Criteria* (April 2005). IUCN Species Survival Commission. IUCN, Gland, Switzerland and Cambridge, UK.

JOHNSON, J. D. & SAVAGE, J. M. 1995. A new species of the *Eleutherodactylus rugulosus* group (Leptodactylidae) from Chiapas, Mexico. *Journal of Herpetology* **29**, 501–506.

KVACEK, Z. 2002. A new Tertiary *Ceratozamia* (Zamiaceae, Cycadopsida) from the European Oligocene. *Flora* **197**, 303–316.

MARSHALL, C. J. & LIEBHERR, J. K. 2000. Cladistic biogeography of the Mexican transition zone. *Journal of Biogeography* **27**, 203–216.

MICKEL, J. & SMITH, A. R. 2004. The Pteridophytes of Mexico. *Memoirs of the New York Botanical Garden* **88**, 1–1054.

MORETTI, A., SABATO, S. & VAZQUEZ, T. M. 1980. *The distribution of Ceratozamia*. (Zamiaceae). *Delpinoa* **20**, 13–21.

PALACIOS, C. R. & RZEDOWSKI, J. 1993. Estudio palinológico de las floras fósiles del Mioceno inferior y principios del Mioceno Medio de la región de Pichucalco, Chiapas, México. *Acta Botánica Mexicana* **24**, 1–96.

PÉREZ-FARRERA, M. A., VOVIDES, A. P., HERNÁNDEZ-SANDOVAL, L., GONZÁLEZ, D. & MARTÍNEZ, M. 2004. A morphometric analysis of the *Ceratozamia norstogii* Stevenson complex (Zamiaceae) In: WALTERS T., & OSBORNE R. *Cycad classification: concepts and recommendations*. CABI Publishing, Wallingford, pp. 127–136.

PÉREZ-FARRERA, M. A. 2005. *Estudio sistemático de los complejos Ceratozamia norstogii D.W. Stev. y Ceratozamia miqueliana H. Wendl. (Cycadales, Zamiaceae).* Ph.D. Thesis. Universidad Autónoma de Queretaro, Santiago de Queretaro, Queretaro, México.

PÉREZ-FARRERA, M. A., VOVIDES, A. P., GONZÁLEZ, D., HERNÁNDEZ-SANDOVAL, L. & MARTÍNEZ, M. 2007. Variación morfológica y genética del complejo *Ceratozamia miqueliana* H.A.Wendl. (Cycadales, Zamiaceae). *Memoirs of the New York Botanical Garden* **97**, 519–541.

MÜLLERRIED, F. K. G. 1957. *La geología de Chiapas*. Gobierno Constitucional del Estado de Chiapas, Tuxtla Gutiérrez, México.

RZEDOWSKI, J. 1978. *Vegetación de México*. Editorial Limusa, México, D.F.

RZEDOWSKI, J. & PALACIOS, C. R. 1977. El bosque de *Engelhardtia* (*Oreomunnea*) *mexicana* en la región de la Chinantla (Oaxaca, México). Una reliquia del Cenozoico. *Boletín de la Sociedad de Botánica de México* **36**, 93–123.

SCHUTZMAN, B., VOVIDES, A. P. & DEHGAN, B. 1988. Two new species of *Zamia* (Zamiaceae, Cycadales) from southern Mexico. *Botanical Gazette* **149**, 347–360.

STEVENSON, D., SABATO, S. & VÁZQUEZ-TORRES, M. 1986. A new species of *Ceratozamia* (Zamiaceae) from Veracruz, Mexico with comments on species relationships, habitats, and vegetative morphology in *Ceratozamia*. *Brittonia* **38**, 17–26.

TELLEZ-VALDES, O. & SCHUBERT, B. G. 1991. Especies nuevas y colecciones notables de *Dioscorea* (Dioscoreaceae) en Mesoamerica. *Annals of the Missouri Botanical Garden* **78**, 245–253.

THOMAS, W. M. W. 1988. A conspectus of Mexican and Central American *Picramnia* (Simaroubaceae). *Brittonia* **40**, 89–105.

TOLEDO, V. M. 1982. Pleistocene changes of vegetation in tropical Mexico. In: PRANCE G. T., Ed., *Biological Diversification in the Tropics*. Columbia University Press, New York, pp. 93–111.

VÁZQUEZ-TORRES, M. & VOVIDES, A. P. 1998. A new species of *Ceratozamia* (Zamiaceae) from Veracruz, Mexico. *Novon* **8**, 87–90.

VOVIDES, A. P., PÉREZ-FARRERA, M. A., GONZALEZ, D. & AVENDAÑO, S. 2004. Relationships and phytogeography in *Ceratozamia* (Zamiaceae). In: WALTERS T. & OSBORNE R., Eds., *Cycad Classification: Concepts and Recommendations*. CABI Publishing, Wallingford, pp. 109–125.

VOVIDES, A. P. & REES, J. D. 1983. *Ceratozamia microstrobila* (Zamiaceae): a new species from San Luis Potosí, Mexico.*Madroño* **30**, 39–42.

VOVIDES, A. P., REES, J. D. & Vázquez TORRES, M. 1983. Zamiaceae. In: GÓMEZ-POMPA A. & SOSA V., Eds., *Flora de Veracruz*. Xalapa, INIREB Fasc. 26, pp. 1–31.

VOVIDES, A. P., VÁZQUEZ-TORRES, M., SCHUTZMAN, B. & IGLESIAS, C. G. 1993. A new species of *Ceratozamia* (Zamiaceae) from Querétaro and Hidalgo, Mexico. *Novon* **3**, 502–506.

WENDT, T. 1987. Las selvas de Uxpanapa, Veracruz-Oaxaca, México: evidencia de refugios florísticos Cenozoicos. *Anales del Instituto de Biología UNAM (Ser. Bot.)* **58**, 29–54.

WHITELOCK, L. 2004. Range and variation in the genus *Ceratozamia* (Zamiaceae). *The Botanical Review* **70**, 235–239.

YATSKIEVYCH, G. 1992. Innovations on the fern genus *Phanerophlebia*. *Novon* **2**, 445–446.

YATSKIEVYCH, G. 1996. A revision of the fern genus *Phanerophlebia* (Dryopteridaceae). *Annals of the Missouri Botanical Garden* **83**, 168–199.