

# Morphological and geographic variation of the cycad *Dioon edule* Lindl. (Zamiaceae): ecological and evolutionary implications

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Received October 2002; accepted for publication January 2003

The relationship in geographical distribution and morphological variation of leaflet width and length (diagnostic trait), between and within populations of *Dioon edule* Lindl., has been investigated throughout its known range in eastern Mexico (from the states of Nuevo León to Veracruz, north to south, respectively). A total of 1832 leaflets were measured for width and length from 154 plants distributed amongst five populations using four leaflet replicas from each of three leaves per plant. For leaflet width and length the variation among populations indicated significant statistical differences ( $F_{4,147} = 125.83$ ;  $P < 0.0001$ ;  $R^2 = 92.17\%$  and  $F_{4,147} = 9.04$ ;  $P < 0.001$ ;  $R^2 = 26.8\%$ ), respectively. With respect to leaflet width, the multiple range test showed three groups with a north to south distributional relationship along the range of the species. The correlation coefficient among paired populations, respect to geographical distance and the absolute value of the mean difference of leaflet width in each population, was positive, and different from zero ( $r = 0.82$ ;  $P = 0.013$ ). A great variation of important ecological and evolutionary parameters was shown. © 2003 The Linnean Society of London, *Botanical Journal of the Linnean Society*, 2003, 141, 465–470.

ADDITIONAL KEYWORDS: Cycadales – Mexico – phenotypic plasticity – populations – species complex – speciation.

## INTRODUCTION

Cycads are the most primitive living seed plants with a fossil history going back to the Permian and possibly the Carboniferous (Mamay, 1976; Norstog & Nicholls, 1997). They are regarded popularly as ‘living fossils’ and thought to be an evolutionary ‘cul de sac’ awaiting their final extinction. This we believe to be dogma, as recent research appears to confirm. Molecular studies appear to indicate that some extant genera originated more recently during Miocene and Pleistocene times (Schneider *et al.*, 2002). Schneider *et al.* (2002) also suggested that the cycad–beetle association related to entomophily is recent and not a result of an old Mesozoic coevolution. In the genus *Ceratozamia*, some species appear to be of more recent origin (Pleistocene), and undergoing adaptive radiation, whilst others are

found in Cenozoic floristic refuges of southern Mexico (González & Vovides, 2002). The genus appears to form species complexes showing great morphological variation (Vovides *et al.* in press).

The study of natural variation in ecologically important traits has a long history in evolutionary ecology (Mayr, 1963; Grant, 1971; Endler, 1977, 1986). The initial motivation for studies of this nature is to further our understanding of the roles of natural selection, gene flow, inbreeding and gene drift in shaping levels of adaptation, and in affecting the distribution and abundance of populations and species.

The phenotypic and genetic differentiation between populations of plant species occupying an array of environments was first experimentally demonstrated by Turesson (1922), and plant ecotypes along broad environmental gradients have been detected in many studies (e.g. Grant & Wilken, 1988; Macdonald &

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Chinnappa, 1988, 1989). Such ecotypes were assumed to be locally adaptive, and several rigorous tests of this assumption have been conducted (Jordan, 1992; Bennington & McGraw, 1996). In this context, the cycads are plants that have a geographical distribution determined by ancient historical processes (Chamberlain, 1919; Delevoryas, 1982) and by demographic dynamics occurring at the present time (Negrón-Ortíz & Breckon, 1989; Vovides, 1990; Negrón-Ortíz, Gorchov & Breckon, 1996; Watkinson & Powell, 1997; Pérez-Farrera *et al.*, 2000).

The cycads are phylogenetically basal to the seed plants making sequences from the cycad genome invaluable for evolutionary and other studies (Daly, Cameron & Stevenson, 2001). For example the neurotoxin BMAA ( $\beta$ -N-methylamino-L-alanine) of biomedical importance, is a synapomorphy, occurring only in the cycads (Daly *et al.*, 2001) and is thought to be implicated in the degenerative disease amyotrophic lateral sclerosis (Norstog & Nicholls, 1997).

The neotropical cycad species *Dioon edule* Lindl., endemic to Mexico, has been investigated from various perspectives. These include natural history and morphology (Chamberlain, 1919, 1935), taxonomy and evolution (Stevenson, 1981; Sabato & De Luca, 1985), geographical distribution (Balduzzi, De Luca & Sabato, 1981; De Luca, Sabato & Vázquez Torres 1982; Vovides, Ress & Vázquez-Torres, 1983), population ecology (Vovides & Peters, 1987; Vovides, 1990), management and conservation (Vovides & Iglesias, 1994; Vovides *et al.* 2002), and phylogenetic aspects (Moretti *et al.*, 1993).

*D. edule* is arborescent with trunks often prostrate up to 5 m long. The plants produce cones the whole year, becoming mature from August to December, and are found in zones of ecotony or transition between tropical deciduous forest and groves of evergreen oaks, overhanging on steep sides or walls of canyons, in gullies or in gently sloping hillsides (Vovides *et al.*, 1983). This cycad is distributed along the Sierra Madre Oriental, from Veracruz to Nuevo León with disjunct populations in Tamaulipas (De Luca *et al.*, 1982). The conservation status of the populations of this species is threatened, and therefore it is characterized as a protected species and is incorporated, along with other Mexican cycads, in the Official Mexican Norm NOM-059-ECOL-1994–2000, covering wild species of threatened, rare and endangered flora and fauna that have special protection. Cycads are also considered priority for conservation and study (INE-SEMARNAP, 2000).

*D. edule* appears to form a complex of taxa and constitutes definite morpho-geographical varieties. In this study, we characterized important ecological and taxonomic traits across a latitudinal distribution of

populations of *D. edule*, as part of a study of the patterns and causes of adaptation limits of this cycad. De Luca *et al.* (1982) observed variation amongst different populations of *D. edule* throughout its range and report: 'The specimens from Tamaulipas and Nuevo León constantly showed leaflets narrower than those of specimens from further south.' They erected the varieties *D. edule* var. *angustifolium* and *D. edule* var. *edule* by using the bimodal distribution of leaflet width measurements they made on two population samples: those of Nuevo León and Tamaulipas (53 examined individuals); and the remaining from Hidalgo, Querétaro, San Luis Potosí and Veracruz (35 examined individuals). They thus obtained a bimodal distribution of leaflet width measurements and they correctly regarded leaflet width a more important diagnostic character. However, owing to their sampling method and analysis we felt that information on the *D. edule* populations intermediate within its range was overlooked.

## METHODS

Using leaflet measurement criteria we analysed this on five populations of *D. edule* (Table 1; Fig. 1) where a mean of 31 randomly selected plants per population from which three leaves were collected. From each of these leaves four leaflet replicas were taken from the middle portion of each leaf for length and width measurements by means of a vernier gauge. A correlation analysis was made between the measurements evaluated. Also a nested analysis of variance was performed, to show any differences among the characteristic means between the five populations and between plants within populations (Sokal & Rohlf, 1995). Tukey's multiple range test on unequal sample size per population (Zar, 1999), was done which permitted differentiation and grouping of the means between paired populations.

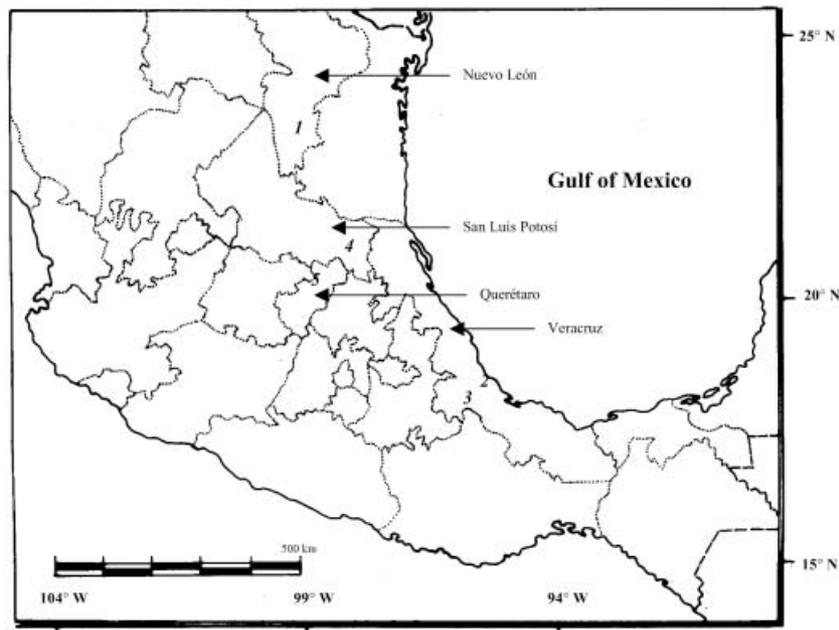
Comparison between geographical distance matrices and difference of absolute value of the means between populations was done to find any relation between geographical distance and variation of the evaluated characters by using the Mantel test (Mantel, 1967; Smouse, Long & Sokal 1986).

## RESULTS

From the five populations sampled 1832 leaflets were measured (width and length). The correlation coefficient between both attributes was 0.08. For leaflet width, the population of El Farallón, Veracruz, gave the highest mean ( $0.75 \pm 0.08$  cm) and the lowest was the Linares, Nuevo León population ( $0.48 \pm 0.06$  cm). For leaflet length the Farallón population showed the highest mean ( $9.16 \pm 1.73$  cm) and the Sierra

**Table 1.** Geographical location, sizes of sample and averages (standard diversion) of the width and length of leaflets of five populations of *D. edule*. *N* is the number of plants and *n* the number of leaflets evaluated

Population	Coordinates	Altitude (m a.s.l)	<i>N</i>	<i>n</i>	Width of leaflet (cm)	Length of leaflet (cm)
Linares, Nuevo León (1)	24°44'55"N 99°48'29"W	815	43	528	0.4788 (0.0586)	9.112 (3.951)
El Farallón, Veracruz (2)	19°36'49"N 96°22'40"W	10	30	360	0.7484 (0.0827)	9.163 (1.733)
Monte Oscuro, Veracruz (3)	19°21'52"N 96°46'58"W	640	30	360	0.7365 (0.0658)	8.405 (1.227)
Sierra del Abra, San Luis Potosí (4)	21°58'53"N 98°55'02"W	216	30	360	0.5477 (0.0684)	9.151 (4.559)
Sierra Gorda, Querétaro (5)	21°10'21"N 99°19'34"W	1100	21	224	0.5379 (0.0658)	6.607 (1.235)

**Figure 1.** Geographical distribution of *Dioon edule* in Mexico. The numbers show the geographical position of each population evaluated.

Gorda, Querétaro population showed the lowest ( $6.61 \pm 1.23$  cm), (Table 1; Fig. 1).

The nested analysis of variance results between populations for mean leaflet width and length indicated significant differences between populations (Table 2 A,B, respectively). Although analysis indicated significant differences between the populations, the intraclass correlation coefficient for leaflet width is 92.16%, and for leaflet length only 26.8%. This showed that the amount of variance in leaflet width is higher than that for leaflet length. The multiple range test

between paired populations for leaflet width clearly indicated three groups: (i) the Linares population to the north; (ii) the population of the Sierra del Abra in San Luis Potosí and the Sierra Gorda population in Querétaro at the centre of the distribution and, (iii) the populations of El Farallón and Monte Oscuro of Veracruz to the south-east (Table 3).

Finally the relationship between geographical distance and the absolute value of the means of leaflet width between paired populations analysed by the Mantel test indicated a positive and significant rela-

**Table 2.** Analysis of variance nested for the leaflet width (A) and leaflet length (B) of five populations of *D. edule* in Mexico

Source of variation	Degrees of freedom (d.f)	Sum of squares(SS)	Mean square (MS)	F	P
<b>A<sup>a</sup></b>					
Populations	4	22.7014	5.6753	125.83	0.0001
Plants in populations	147	6.6274	0.0451	32.51	0.00001
Error	1680	2.2869	0.0014		
Total	1831	31.6157			
<b>B<sup>b</sup></b>					
Populations	4	1222.59	305.65	9.04	0.001
Plants in populations	147	4971.25	33.81	4.48	0.001
Error	1680	12679.10	7.54		
Total	1831	18872.94			

<sup>a</sup>R<sup>2</sup> = 92.16%. <sup>b</sup>R<sup>2</sup> = 26.8%

**Table 3.** Analysis of multiple ranges with Tukey's test for the five populations of *D. edule* in Mexico

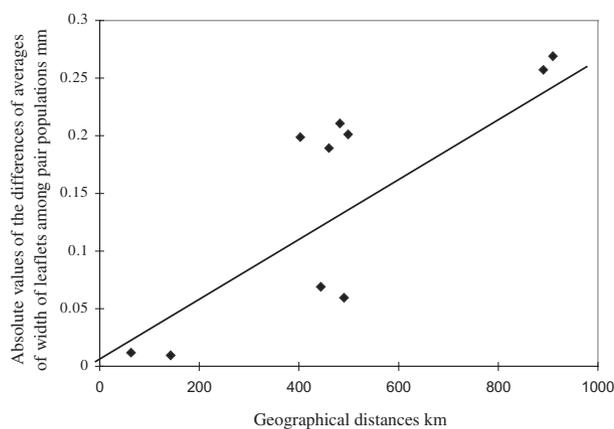
Population	Mean	Range
El Farallón, Veracruz (2)	0.7484	a
Monte Oscuro, Veracruz (3)	0.7365	a
Sierra Gorda, Querétaro. (5)	0.5379	b
Sierra el Abra, San Luis Potosí (4)	0.5477	b
Linares, Nuevo León (1)	0.4788	c

tionship (Fig. 2) ( $r = 0.82$ ;  $P = 0.013$ ); for the leaflet length, the relationship not was significantly different from zero ( $r = -0.24$ ;  $P = 0.276$ ).

## DISCUSSION

Cycads show heterobathmic characters where morphology has changed little from their predecessors for some characters whereas others appear to be of recent evolution. Classical examples of this are seen in the genus *Cycas*, which is phylogenetically basal to the cycads (Stevenson, 1985) with leaflets having mid-ribs, and platyspermic seeds, considered derived, coupled with the primitive ancestor-like megasporophylls resembling the Permian genus *Crossozamia* (Zhifeng & Thomas, 1989; Moretti, 1990).

Karyological studies have been so far unsuccessful in correlating karyotypic with phenotypic characters, at least in the American genera (Moretti, 1990). However, some biogeographical or ecological trends may be seen from these studies. There is a high diversity of karyotypes in *Zamia*, which also reflects the wide variety of morphological traits, habitats and ecological niches that this genus occupies, the Panamanian *Z. pseudoparasitica* for example, is entirely epiphytic



**Figure 2.** Relationship among geographic distances and absolute values of the differences of averages of width of leaflets among pair populations of *Dioon edule* in Mexico. The Mantel test indicates a positive and significant relationship ( $r = 0.82$ ;  $P = 0.013$ ).

on rain forest trees. Cytotypes of *Zamia loddigesii* Miq. (or *Z. polymorpha* D.W. Stev.) on the Yucatan Peninsula of Mexico as well as *Z. paucijuga* from the western coastal regions of Mexico appear to reflect the drier climates of these regions (Moretti & Sabato, 1984; Vovides & Olivares, 1996). This is in contrast to the relative stability of karyotypes in *Ceratozamia* and *Dioon* (Vovides, 1983; Moretti, 1990).

In this study we detected great variation in both width and length of leaflets in *D. edule*. The results largely agree with those reported by De Luca *et al.* (1982); though it is important to emphasize that in this study, the data were obtained in such a way that could be analysed with a design that allowed detection of variation between and within populations. The fractions of variance that explained the evaluated

attributes, with regard to the total variance among populations, were also likewise obtained.

Plants evaluated from five populations were significantly different with respect to variation of leaflet width and length. Only leaflet width presented relationship with the geographical distribution of the populations at the species level. In addition, there was a high (92.16%) interregional component for phenotypic variation, which is probably related with adaptation to environmental conditions of differential annual mean luminosity at each location.

It is likely that the greatest proportion of total variation is due to environmental factors, by induced modification of the phenotype over time (since the Pleistocene). Therefore patterns of variation observed in *D. edule* in the field are likely due primarily to phenotypic plasticity. The effects of ecotype differentiation were also detectable. The correlation of morphological and habitat type suggest phenotypic plasticity and/or ecotype differentiation between populations.

In this sense Snow & Body, (1984) detected different ecotypes of *Hordeum spontaneum* in one altitudinal gradient, which was explained as local adaptation to environmental gradient, as in the case of the populations of *D. edule* in Mexico. Here the probable effect of intensity of light is higher in the northern part of the distribution than in the southern region (García, 1981). Leaflet width variation could be an adaptation to environmental factors, where plants with narrow leaflets are exposed to higher levels and periods of sunlight and therefore water stress, but much less so with plants with wider leaflets in the south. The fact that leaflet width does not change in newly produced leaves when plants from different populations are grown under uniform conditions in the botanic garden indicates a genetically controlled character (A. P. Vovides & D. González-Astorga, pers. observ.). However, it can be regarded as a highly plastic attribute early at the species level, that later became genetically fixed over time.

In taking into account geographical variation for species delimitation, studies on population genetic structure and variation (J. González-Astorga, A.P. Vovides, M.M. Ferrer & C. Iglesias, unpubl. data) must also be considered, and appear to support this hypothesis. We believe that only then can we confidently propose three species within the *D. edule* complex. However, we based this study taking into consideration the recommendations put forward during the Cycad Classification Concepts Workshop (Walters & Osborne, in press) in using population criteria, morpho-geographical data and to avoid infra specific ranking such as variety, subspecies, etc. Our findings appear to be in agreement with Moretti *et al.* (1993) where their two exemplars of *D. edule* var. *angustifolium* and *D. edule* var. *edule sensu* De Luca

*et al.* (1982) used in their cpDNA RLFP analysis appear as sister taxa on their consensus tree. It appears that speciation processes in these taxa have been influenced by the Pleistocene glaciations and climate warming during the post Pleistocene giving rise to increasingly arid environments (Toledo, 1982; Moretti *et al.*, 1993).

## CONCLUSION

If we take into account a recent speciation scenario as suggested by molecular studies and this study, which suggests that phenotypic plasticity has become fixed in post Pleistocene times, we predict that future population genetic information will also reflect this trend.

## ACKNOWLEDGEMENTS

This study was supported by a FMCN, A.C. No. B-2.00/013 and CONACyT No. 34077-N to G.-A. J grants and CONACyT no. 29379-N to A. V. We are grateful to Carlos Montaña and Miriam Ferrer for their comments on a previous draft of the manuscript. We thank Pablo O. Aguilar who helped us with the editing of the manuscript, and also the anonymous reviewers. Thanks to Edmundo Saavedra for drawing Figure 1.

## REFERENCES

- Balduzzi A, De Luca P, Sabato S. 1981–1982.** A phytogeographical approach to the New World Cycads. *Delpinoa, N.S.* **23–24:** 185–202.
- Bennington CC, McGraw JB. 1996.** Environmental dependence of quantitative genetic parameters in *Impatiens pallida*. *Evolution* **50:** 1083–1097.
- Chamberlain CJ. 1919.** *The living cycads*. New York: Hafner.
- Chamberlain CJ. 1935.** *Gymnosperms. Structure and evolution*. Chicago: University of Chicago Press.
- Daly DC, Cameron KM, Stevenson DW. 2001.** Plant systematics in the age of genomics. *Plant Physiology* **127:** 1328–1333.
- De Luca P, Sabato S, Vázquez Torres M. 1982.** Distribution and variation of *Dioon edule* (Zamiaceae). *Brittonia* **34:** 355–362.
- Delevoryas T. 1982.** Perspectives on the origin of cycads. *Review of Palaeobotany and Palynology* **37:** 115–132.
- Endler JA. 1977.** *Geographic variation, speciation, and clines*. Princeton New Jersey: Princeton University Press.
- Endler JA. 1986.** *Natural selection in the wild*. Princeton New Jersey: Princeton University Press.
- García E. 1981.** Modificaciones al sistema de clasificación climática de Köppen. *México D.F. Instituto de Geografía*. UNAM.
- González D, Vovides AP. 2002.** Low intralinesage divergence in the genus *Ceratozamia* Brongn. (Zamiaceae) detected with nuclear ribosomal DNA ITS and chloroplast DNA *trnL-F* non-coding region. *Systematic Botany* **27** (4).

- Grant V.** 1971. *Plant speciation*. New York: Columbia University Press.
- Grant V, Wilken DH.** 1988. Racial variation in *Ipomopsis tenuituba* (Poemoniaceae). *Botanical Gazette* **149**: 443–449.
- INE-SEMARNAP.** 2000. Prep 6: Protección, conservación y recuperación de la familia Zamiaceae (Cycadales) de México. México: D.F. Instituto Nacional de Ecología.
- Jordan N.** 1992. Path analysis of local adaptations in two ecotypes of the annual plant *Diodia teres* Walt. (Rubiaceae). *American Naturalist* **140**: 149–165.
- Macdonald SE, Chinnappa CC.** 1988. Patterns of variation in *Stellaria longipes* complex: the effect of polyploidy and natural selection. *American Journal of Botany* **75**: 1191–2000.
- Macdonald SE, Chinnappa CC.** 1989. Population differentiation for phenotypic plasticity in the *Stellaria longipes* complex. *American Journal of Botany* **76**: 1627–1637.
- Mamay SH.** 1976. Paleozoic origin of the cycads. Professional Paper 934. Washington, DC: US Geological Survey.
- Mantel N.** 1967. The detection of disease clustering and generalized regression approach. *Cancer Research* **27**: 209–220.
- Mayr E.** 1963. *Populations, species, and evolution*. Cambridge, Massachusetts: The Belknap Press of Harvard University Press.
- Moretti A.** 1990. Karyotypic data on North and Central American Zamiaceae (Cycadales) and their phylogenetic implications. *American Journal of Botany* **77**: 1016–1029.
- Moretti A, Caputo P, Cozzolino S, De Luca P, Gaudio L, Gigliano GS, Stevenson DW.** 1993. A phylogenetic analysis for *Dioon* (Zamiaceae). *American Journal of Botany* **80**: 204–214.
- Moretti A, Sabato S.** 1984. Karyotype evolution by centromeric fission in *Zamia* (Cycadales). *Plant Systematics and Evolution* **146**: 215–223.
- Negrón-Ortíz V, Breckon GJ.** 1989. Population structure in *Zamia debilis* (Zamiaceae) I. Size classes, leaf phenology and leaf turnover. *American Journal of Botany* **76**: 891–900.
- Negrón-Ortíz V, Gorchov DL, Breckon GJ.** 1996. Population structure in *Zamia* (Zamiaceae) in northern Puerto Rico. II. Seed germination and stage-structured population projection. *Journal of Plant Science* **157**: 605–614.
- Norstog KJ, Nicholls TJ.** 1997. *The biology of the cycads*. Ithaca: Cornell University Press.
- Pérez-Farrera MA, Quintana-Ascencio PF, Salvatierra B, Vovides AP.** 2000. Population *Ceratozamia matudai* Lundell (Zamiaceae) in the Triunfo Biosphere Reserve, Chiapas, Mexico. *Journal of the Torrey Botanical Society* **127**: 291–299.
- Sabato S, De Luca P.** 1985. Evolutionary trends in *Dioon* (Zamiaceae). *American Journal of Botany* **72**: 1353–1363.
- Schneider D, Wink M, Sporer F, Lounibos P.** 2002. Cycads: their evolution, toxins, herbivores and insect pollinators. *Naturwissenschaften* **89**: 281–294.
- Smouse PE, Long JC, Sokal RR.** 1986. Multiple regression and correlation extensions of the Mantel test of matrix correspondence. *Systematic Zoology* **35**: 627–632.
- Snow L, Body T.** 1984. Genetic variation of *Hordeum spontaneum*. Israel: eco-geographical races, detected by trait measurements. *Plant Systematics and Evolution* **145**: 15–28.
- Sokal RR, Rohlf FJ.** 1995. *Biometry*, 3rd edn. New York: Freeman.
- Stevenson DW.** 1981. Observations on ptaxis, phenology, and trichomes in the Cycadales and their systematic implications. *American Journal of Botany* **68**: 1104–1114.
- Stevenson DW.** 1985. A proposed classification of the Cycadales. *American Journal of Botany* **72**: 971–972.
- Toledo VM.** 1982. Pleistocene changes of vegetation in tropical Mexico. In: Prance GT, ed. *Biological diversification in the Tropics: Proceedings of the 5th International Symposium of the Association for Tropical Biology, Caracas*. New York: Columbia University Press, 93–111.
- Turesson G.** 1922. The genotypical response of the plant species to the habitat. *Hereditas* **3**: 211–350.
- Vovides AP.** 1983. Systematic studies on the Mexican Zamiaceae. I. Chromosome numbers and karyotypes. *American Journal of Botany* **70**: 1002–1006.
- Vovides AP.** 1990. Spatial distribution, survival and fecundity of *Dioon edule* (Zamiaceae) in a tropical deciduous forest in Veracruz, Mexico, with notes on its habitat. *American Journal of Botany* **77**: 1532–1543.
- Vovides AP, Iglesias CG.** 1994. An integrated conservation strategy for the cycad *Dioon edule* Lindl. *Biodiversity and Conservation* **3**: 137–141.
- Vovides AP, Olivares M.** 1996. Karyotype polymorphism in the cycad *Zamia loddigesii* (Zamiaceae) of the Yucatan Peninsula, Mexico. *Botanical Journal of the Linnean Society* **120**: 77–83.
- Vovides AP, Pérez-Ferrera MA, González D, Avendaño S.** 2004. The genus *Ceratozamia*: relationships and phylogeography. In: Walters T, Osborne R, eds. *Cycad classification: concepts and recommendations*. Miami, Florida: Montgomery Botanical Center, in press.
- Vovides AP, Peters C.** 1987. *Dioon edule*: la planta más antigua de México. *Ciencia Y Desarrollo* **13**: 19–24.
- Vovides AP, Rees JD, Vázquez-Torres M.** 1983. *Flora de Veracruz. Zamiaceae*. México: Fascículo 26. INIREB, Xalapa, Veracruz.
- Vovides AP, Iglesias C, Pérez-Ferrera MA, Vázquez-Torres M, Schippmann U.** 2002. Peasant nurseries: a concept for an integrated conservation strategy for cycads in Mexico. In: Maunder M, Clubbe C, Hankamer C, Groves M, eds. *Plant conservation in the tropics: perspectives and practice*. London: Royal Botanic Gardens, Kew 421–444.
- Walters T, Osborne R.** 2004. *Cycad classification: concepts and recommendations*. Wallingford, UK: CABI Publishing, in press.
- Watkinson AR, Powell JC.** 1997. The life history and population structure of *Cycas armstrongii* in monsoonal Australia. *Oecologia* **111**: 341–349.
- Zar JH.** 1999. *Biostatistical analysis*, 4th edn. New Jersey: Prentice Hall.
- Zhifeng G, Thomas BA.** 1989. A review of fossil cycad megasporophylls, with new evidence of *Crossozamia* Pomel and its associated leaves from the lower Permian of Taiyuan, China. *Review of Palaeobotany and Palynology* **60**: 205–223.