

A review of research on the cycad genus *Ceratozamia* Brongn. (Zamiaceae) in Mexico

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The genus *Ceratozamia* contains more than 20 species distributed in eastern and southern Mexico, with extensions into Central America. Morphology, anatomy, and molecular (ITS) data allow resolution of some aspects of species relationships, provide correlations with ecology, and suggest phylogeographic hypotheses. Some species complexes, such as those centered around *C. norstogii* and *C. miqueliana*, are restricted to southern-west Mexico, in what are regarded as Pleistocene floristic refugia. The low level of divergence of ITS sequences among taxa to the north of the Trans-Mexican Neovolcanic mountain range suggests a pattern of recent, and perhaps rapid, speciation.

KEYWORDS: Cycadales, Middle America, phylogeography, refugia, speciation.

INTRODUCTION

After a period of relative stagnation since the pioneering work of Charles Joseph Chamberlain in the early 20th century (Norstog & Nichols, 1997), renewed interest in cycads has occurred during the last 25 years. Cycads are distributed within subtropical and tropical regions of the world, with 303 species validly published (Osborne & al., 1999; Hill & al., 2004). This number may reach as high as 400 species when all potential cycad habitats have been investigated and taxonomic studies are completed (R. Osborne, pers. comm.). Countries particularly rich in cycads (Vovides, 2000) are Australia (ca. 78 spp.), Mexico (ca. 45 spp.), and South Africa (ca. 40 spp.). In the Neotropics, cycads occur in different habitats (Vovides & al., 1983; Jones, 1993; Stevenson, 2001) including evergreen tropical rainforests, seasonally dry tropical forests, cloud forests, oak and pine-oak forests, grasslands, savannahs, mangrove communities (e.g., the Colombian *Zamia roezlii* Linden), and even as epiphytes (the Panamanian *Zamia pseudoparasitica* Yates in Seem; Stevenson, 1993).

Renewed interest in cycads has resulted in work being done on toxicity, population ecology, phytochemistry, cytotaxonomy, and ecophysiology (De Luca & al., 1982; Newell, 1983; Vovides, 1983, 1985, 1990; Moretti & Sabato, 1984; Clark & Clark, 1988; Moretti, 1990; Charlton & al., 1992; Donaldson, 1995; Kokubugata & Kondo, 1996, 1998; Vovides & Olivares, 1996; Pérez-Farrera & al., 2000; Vovides & al., 2002). With regard to

secondary products, certain toxins are peculiar only to cycads, such as cycasin. Macrozamin and the neurotoxin 2-amino-3-(methylamino)-propanoic acid (BMAA) are considered synapomorphies of the cycad clade of the seed plants (Daly & al., 2001).

Despite these many previous investigations, phylogenetic studies using molecular techniques on the Neotropical cycads are scarce. Those so far published have examined Restriction Fragment Length Polymorphisms (RFLPs) and sequence data at the generic level (Caputo & al., 1991, 1993; De Luca & al., 1995; Bogler & Francisco-Ortega, in press). Moretti & al. (1993), using morphological and RFLP data in the genus *Dioon*, showed that the cladogram obtained with molecular data gave better resolution among taxa than that based on morphology. Correlation between these molecular data and phylogeography in *Dioon* shows a concentration of morphological and cpDNA diversity along the mountain system centred in Puebla, Oaxaca and Veracruz (Moretti & al., 1993). González & Vovides (2002), using DNA sequences of non-coding regions from chloroplast and nuclear genomes, found few molecular characters for the genus *Ceratozamia*.

The genus *Ceratozamia* is largely distributed along the Sierra Madre Oriental in the warm temperate and tropical regions of eastern and southern Mexico, including mid-elevation pine-oak forests from the state of Tamaulipas at its extreme northern limit in Mexico, to Belize and Guatemala in Central America (Balduzzi & al., 1981–82). Recently a species has been reported for

Honduras (Whitelock, 2004) that appears to belong to the *C. miqueliana* species complex. Much of our knowledge of the distribution of *Ceratozamia* is due to the early exploratory work of Chamberlain (1919). Some *Ceratozamia* species are basically arborescent with stems rarely more than one meter tall. They are often leaning or curved but rarely branching (Norstog & Nicholls, 1997). Others are semi-hypogeous and often branching (*Ceratozamia hildae* Landry & Wilson; *C. zaragozae* Medellín-Leal; *C. microstrobila* Vovides & Rees and *C. alvarezii* Pérez-Farrera, Vovides & Iglesias). In recent years information on the genus has been greatly expanded by workers from Mexico and Italy (see also Balduzzi & al., 1982; Moretti & Sabato, 1988; Norstog & Nicholls, 1997; Pérez-Farrera & al., 1999, 2001; Vovides & al., 2001; Avendaño & al., 2003). By 1980, only six species were known, and by 2003 over 20 species had been described for Mexico. With future studies, we anticipate that this figure may increase to about 26.

The purpose of this paper is to review the morphological, anatomical and molecular data available for *Ceratozamia* and to use these to interpret phylogeographic trends within the genus.

MORPHOLOGY

Botanical explorations for *Ceratozamia* throughout Mexico over several years and herbarium consultations (CHIP, F, MEXU, MO, XAL, XALUV, ENCB, IEB) have enabled us to complete regional floras (Vovides & al., 1983; Vovides, 1999), as well as to make morphological measurements, such as leaflet shape and width and correlate these with vegetation types (Pérez-Farrera & al., 2004) using quantitative ecological multivariate techniques (McCune & Mefford, 1997).

Throughout the distribution of *Ceratozamia*, various related groups or species complexes can be found (Moretti & al., 1980; González & Vovides, 2002; Vovides & al., 2004). On the basis of gross morphology, and especially that of leaf, leaflets, and reproductive structures, we now believe that there are at least seven species complexes in the genus (Vovides & al., 2004) with groups in southern and southeastern Mexico (which appear to coincide with floristic refuges according to Toledo, 1982 and Wendt, 1987), and three outlying groups in northeastern Mexico (Vovides & al., 2004).

Plants from northeastern Mexico are generally characterised as having small and often branching trunks with few leaves per crown, and leaflets are usually narrow (i.e., less than 2 cm wide), with the exception of *Ceratozamia microstrobila* Vovides & Rees, *C. huastecorum* Avendaño, Vovides & Castillo-Campos,

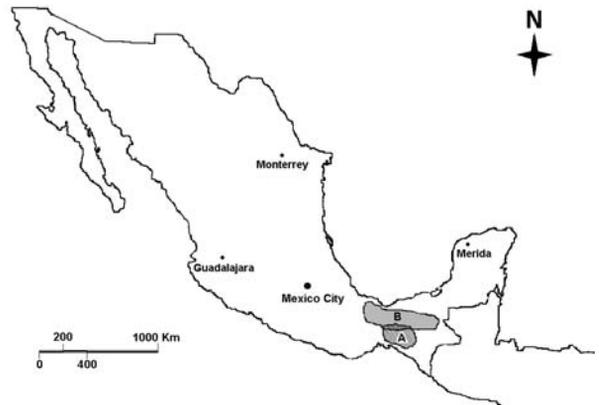


Fig. 1. Distribution of *Ceratozamia norstogii* species complex (A) and *C. miqueliana* species complex in southern Mexico (B).

and *C. latifolia* Miq. These plants also bear smaller cones than those from southern and southeastern Mexico, which are generally larger and with solitary trunks (although branching has been observed in some taxa, e.g., *Ceratozamia alvarezii* Pérez-Farrera, Vovides & Iglesias from Chiapas).

The southern and southeastern *Ceratozamia* spp. have obovate leaflets usually more than 2 cm wide, the widest being found in *C. euryphyllidia* Vázq. Torres, Sabato & Stevenson with exceptions to this seen in *C. norstogii* Stevenson, *C. mirandae* Vovides, Pérez-Farrera & Iglesias and *C. alvarezii* (all three of which have linear or linear-lanceolate leaflets less than 1.5 cm wide).

Species complexes include the *C. norstogii*, *C. miqueliana*, and *C. robusta* groups (Pérez-Farrera & al., 1999, 2001; Vovides & al., 2001, 2004; Fig. 1). *Ceratozamia miqueliana* H. Wendland and related species occur in evergreen tropical rain forest and are not generally found at elevations greater than 1,000 m. In contrast to this, species of the *C. norstogii* group are largely found in cooler, drier oak and pine-oak forests between 800–1,400 m. The *C. robusta* species complex



Fig. 2. Distribution of *Ceratozamia robusta* species complex in southern Mexico, Belize and Guatemala.

(Fig. 2) appears to have a wider geographic range and habitat type.

Using multivariate analysis on taxa of *Ceratozamia* and their habitat types, it has been observed that species with wide-oblongate to oblong leaflets are confined to evergreen tropical rain forest and a number of species with narrow, linear or falcate leaflets are frequently associated with cloud forests, oak and pine/oak forests (Fig. 3).

Spatial distribution patterns of both *C. mirandae* and *C. matudae* were found to be grouped or aggregated on shallow soils and the population structure a reverse ‘J’ or Deevey type-III curve, reflecting a higher frequency of seedlings and juvenile plants than adults (cf. Vovides, 1990; Pérez-Farrera & al., 2000). This population structure is typical of perennial species, including certain short-lived herbaceous plants where high investment in reproduction is an important phase of the life cycle. The long-lived cycads studied (Vovides, 1990; Pérez-Farrera & al., 2000) also present this type of curve, due to arborescent branches that enable production of several cones per plant. Gravity appears to be the major distribution agent, since the majority of seedlings and juveniles are found near the mother plants, in shade under trees, or at the bottom of steep slopes. However, the peccary (*Tayassu tajacu*) has been observed to disperse seeds of *C. matudae* and *C. mirandae* (Pérez-Farrera & al., 2000; 2004). This pattern analysis, population structure, and life cycle is similar to that found in *Dioon edule* Lindl. (Vovides, 1990).

Ceratozamia species so far studied appear to have

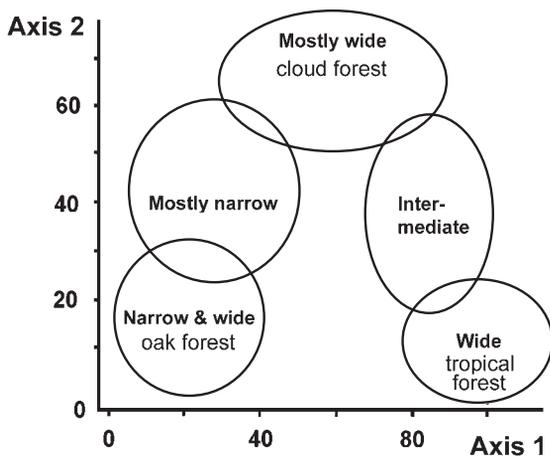


Fig. 3. Graph showing results of multivariate analysis in species of *Ceratozamia* on leaflet width of taxa and their habitat types: species with wide-oblongate to oblong leaflets are confined to evergreen tropical rain forest, and species with narrow, linear or falcate leaflets are frequently associated with cloud forests, oak or pine/oak forests. Modified from Vovides & al. (2004).

seeds with physiological dormancy owing to immature embryos, because seeds do not germinate immediately after cone dehiscence (Pérez-Farrera & Vovides, 1997; Sánchez-Tinoco & al., 2000). The embryo requires a further six to eight months or more of maturation before germinating.

ANATOMY

Results obtained so far from leaflet anatomy (for delimiting species) appear promising, even though homogeneity of states has been observed in only about 50% of the characters studied. From a suite of 19 characters analysed using morphometric and phenetic techniques only nine were useful, which enabled separation of only nine groups from a total of 18 OTUs. Figs. 4 and 5 show examples of a useful anatomical character: differences in lower leaflet cuticle thickness associated with the stomatal pore between *C. miqueliana* and *C. zaragozae*. There are also differences in the number of fibres associated with the vascular bundles of the leaflets of *C. robusta* and *C. kuesteriana* (Vovides & al., 2004). The very thick leaflet cuticle in *Ceratozamia miqueliana* and *C. euryphyllidia* appears to be inconsistent with the present day moist tropical rain forest habitat. This character may have developed during cooler and more arid climates of the Pleistocene (Toledo, 1982; Leyden, 1984).

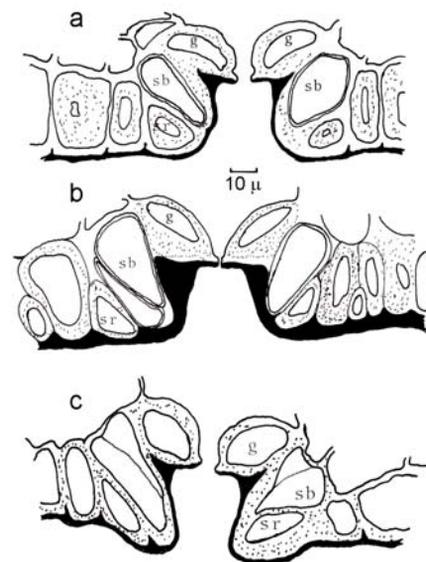


Fig. 4. Transverse sections through stomatal apparatus of *Ceratozamia* leaflets showing differences in cuticle thickness (black); a, *C. mexicana*; b, *C. miqueliana*; c, *C. zaragozae*.

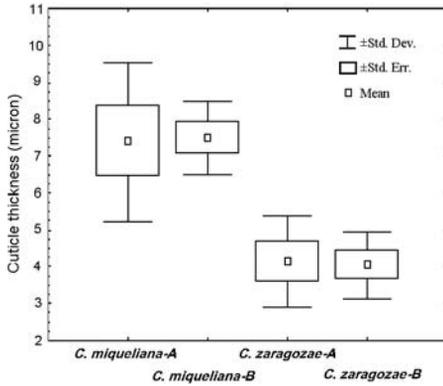


Fig. 5. Box diagram showing differences in cuticle thickness between two species of *Ceratozamia* (*C. miqueliana* and *C. zaragozae*) but no differences between individuals within the same species. From Vovides & al. (2004).

MOLECULAR DATA

Recent studies using sequences of non-coding regions from the chloroplast and nuclear genomes (ITS and *trnL-F*) have given insights into phylogenetic rela-

tionships within *Ceratozamia* (González & Vovides, 2002). The two regions have been used in phylogenetic studies at the intrageneric level for a variety of plants (Schilling & al., 1998; Potter & al., 2000), but very little variation was detected among species of *Ceratozamia*. Only 29 characters out of 1,083 of the ITS region were informative among 24 exemplars, and two out of 998 were informative from the *trnL-F* non-coding region. Despite low levels of variation, cladistic analyses showed that *Ceratozamia* is monophyletic and identified *Zamia* as sister group (González & Vovides, 2002).

Parsimony analysis of the ITS sequence dataset yielded 112 trees of 51 steps (CI = 0.82; RI = 0.92; RC = 0.75). The strict consensus of these trees is shown in Fig. 6 in relation to the biogeographic boundary of the Trans-Mexican Neovolcanic mountain range. The low level of variation detected among *Ceratozamia* spp. limited the conclusions on phylogenetic relationships because few clades were resolved. However, the cladograms obtained enabled us to infer a tentative phylogenetic hypothesis for the genus (González & Vovides, 2002). The molecular phylogeny of *Ceratozamia* revealed three main clades (Fig. 6). Molecular data suggest that isolation and speci-

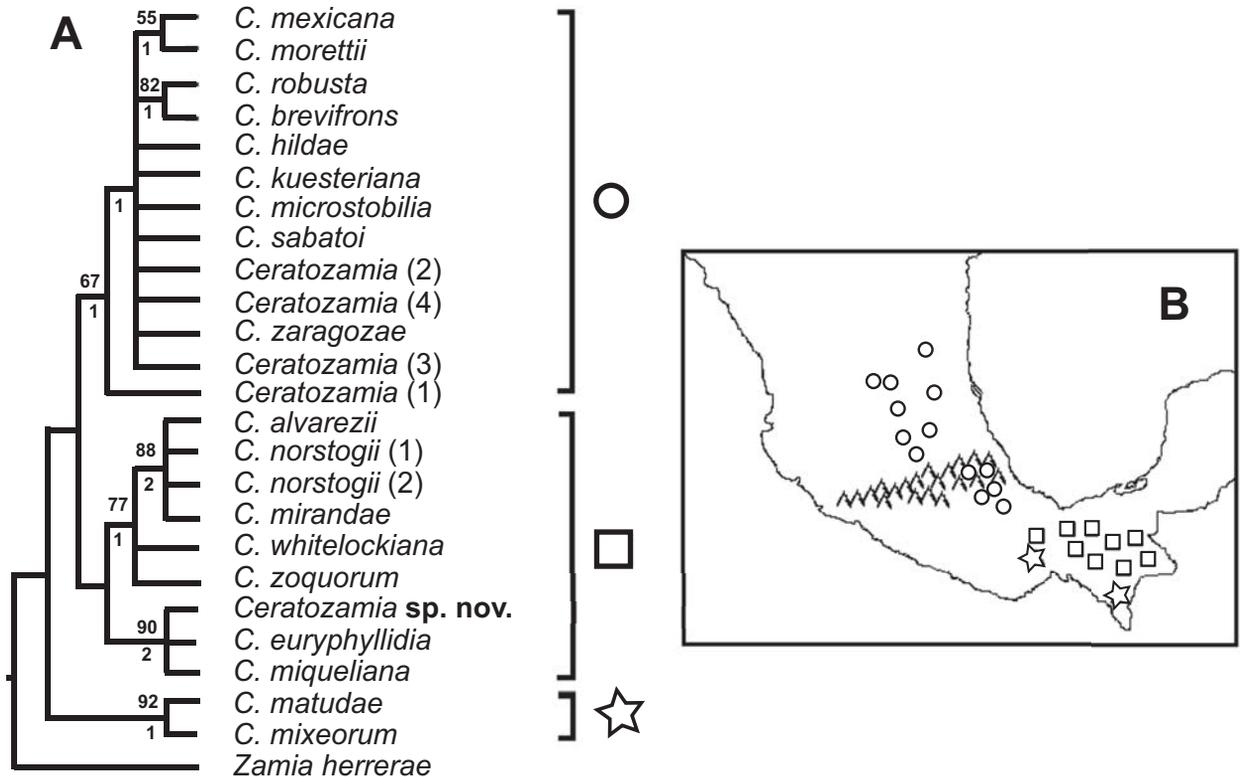


Fig. 6. Main clades (A; ITS strict consensus tree) within *Ceratozamia* in relation to the biogeographic boundary of the Trans-Mexican Neovolcanic mountain range (B). From González & Vovides (2002). Numbers above branches are bootstrap values; those below are decay values. Numbers above are bootstrap estimates for 500 replicate analyses, numbers below are decay values. Modified from González & Vovides (2002) according to Walters & Osborne (2004) where infraspecific status in cycads has been dropped.

ation in the genus appear to be relatively recent because DNA sequences show low levels of divergence among species (Gonzales & Vovides, 2002).

PHYTOGEOGRAPHY

Molecular data and geographic distribution patterns allow phylogeographic interpretations to be made (Fig. 6). The two basal clades contain the species that are distributed in southern and southeastern Mexico, both at and south of the Trans-Mexican Neovolcanic mountain range of Pliocene-Quaternary (Pleistocene) age (Marshall & Liebherr, 2000). Here the *C. norstogii* clade integrates well with what we consider (from morphological data) the *C. norstogii* complex. This conforms with the general findings of Marshall & Liebherr (2000), who identified two biogeographic assemblages, one north of the Neovolcanic range and another to the south. The large unresolved clade in *Ceratozamia* contains a group of morphologically distinct species that are to the north and northeast of the Neovolcanic range that appear to be of more recent origin. The few mutations detected in the genus could be due to the relatively long generation times for *Ceratozamia*.

The Pleistocene refugial hypothesis indicates that tropical species were restricted to a few areas within the geographical tropics where climatic changes were not as extreme. This hypothesis has been applauded by many (Rzedowski & Palacios 1977; Haffer, 1982; Prance, 1982; Toledo, 1982; Wendt 1987; Lewis & Crawford, 1995; Aide & Rivera, 1999; Wallace & Case, 2000; Renner & Meyer, 2001) and criticised by others (Endler, 1982; Bush, 1994; Colinvaux & al., 2000). Lowland forest was replaced by savanna/scrub vegetation in several sites during the last glacial maximum (Leyden, 1984). Sosa & al. (2003) using molecular data conclude that the genus *Chiangiodendron* is a relict of a wet-tropical Laurasian flora that has been preserved in Neotropical refugia. Evidence from leaflet morphometry on *Dioon edule* throughout its distribution range (Gonzalez-Astorga & al., 2003b) and allozyme electrophoresis to detect genetic diversity (González-Astorga & al., 2003a) also gives support to this theory as well as research by others on other taxa in other regions (Lewis & Crawford, 1995; Wallace & Case, 2000).

There is a consensus on the existence of floristic and faunistic refuges of great age in southern Mexico (Brown 1976; Toledo, 1982, 1988). During the past 40,000 years, tropical forests in Mexico have been disrupted and displaced to lower latitudes due to the oncoming of Pleistocene climatic changes, with cycles of cold-dry, cold-wet and warm-dry climates. The tropical forest was replaced by savannah/scrub vegetation in several sites

during the last glacial maximum (Leyden, 1984). This resulted in relict pockets or refuges that protected the biota from lowering of temperature, rainfall and precipitation (Toledo, 1982). Graham (1976, 1999), using palynological analysis, maintains that the modern tropical rainforest of Mexico is recent, the result being considerable alteration in range and composition since at least upper Miocene times. It appears that *Ceratozamia* (*Cycadopodites*) was present in the Miocene flora of the southern Mexican region of Pichucalco, Chiapas (Palacios & Rzedowski, 1993), and in Cenozoic *Engelhardtia* forests in Oaxaca where the fossil pollen spectrum is remarkably similar to that of modern forests. *Engelhardtia* used to be widely distributed over the Northern Hemisphere during the Tertiary (Rzedowski & Palacios, 1977). Owing to climatic changes during the Pleistocene, some members of *Ceratozamia* survived in the south of Mexico, where the greatest diversity and basal clades of the genus appear to be concentrated. Very probably an ancestral form became isolated in Honduras, a territory with a history of geographic isolation (Coney, 1982). These refugia, however, are apparently absent in the areas north of the Neovolcanic mountain range. This leads us to the hypothesis that *Ceratozamia* probably originated in southern/southeastern Mexico, the taxa at and north of the Neovolcanic range being the result of recent speciation (González & Vovides, 2002).

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