The Mexican living cycad collection at the Jardín Botánico Francisco Javier Clavijero, Xalapa.

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Abstract

Over the last 30 years Mexico has increased its cycad flora by over 300%, from 12 species known in the late 1970's to over 50, due to botanical explorations into previously unexplored areas and has positioned Mexico as second worldwide for cycad diversity. Parallel to field explorations a living collection was built up at the Francisco Javier Clavijero Botanic Garden known as the National Cycad Collection now completing its 30th anniversary. It is the most comprehensive documented living cycad collection in Latin America and includes some species from old world genera. Research has been largely systematic with recent molecular phylogenies based on the collection in the genera *Ceratozamia* and *Dioon* as well as DNA bar-coding. Improvement in cultivation techniques such as use of native *arbuscular mycorrhizal* fungi and root pruning experiments aimed at sustainable management projects promoted and assessed by the Garden.

Key words

Botanic Gardens, Cycadales, DNA-Barcoding, Mexico, Meso-America, Systematics, Zamiaceae,

Introduction

The cycad collection began before the Jardín Botánico Francisco Javier Clavijero (JBC) was established with *Dioon edule* accession 1975-002 being the second plant accessioned at the Garden collected by the author. The decision to build a living cycad collection was mainly to complete the Flora of Veracruz fascicle on the cycad family Zamiaceae owing to the scarcity of reproductive structures on plants in the field (Vovides *et al*., 1983). The cycads were cultivated in a makeshift backyard greenhouse (Fig. 1.) and when coning occurred the descriptions could be completed. Plants were later transferred into better greenhouses at the JBC that were inaugurated in February of 1977. Since 1980 the collection grew into what is now the National Cycad Collection and is registered with the Mexican Botanic Gardens Association. The Collection holds representatives of the known species of Mexico and also at least one species of the remaining world genera (Table 1, Fig. 2.).

Aims and objectives

The principal aims of the Collection are to supply living material for research and *ex situ* conservation, for botanic garden extension and outreach to schools, the general public and conservation authorities. Also for assessment to rural communities engaged in propagation of cycads aimed at sustainable management. These activities cover Articles of the Convention on Biological Diversity (Given, 1997), *ex situ* conservation in country of origin (Article 9), education and awareness (Article 13) and technical and scientific collaboration (Article 18). Many plants previously held in greenhouses are now established in outside plantings of the JBC (Fig. 3.).

Conservation through propagation

Based on ecological studies (Vovides, 1990), as well as germination trials at the Garden, the first rural cycad nursery at Monte Oscuro, Veracruz, was established for the propagation of *Dioon edule* in 1990. The farmers who own cycad habitat were given talks and invited to take part in a sustainable management project where hands-on basic horticultural training was given. The nursery is the forerunner of similar nurseries established in other Mexican states under the auspices of the national conservation authorities that license them (Vovides *et al*., 2002). Though much has to be solved, especially marketing, we have noticed a growing interest in cycads among landscape architects and it is becoming fashionable to use native plants in residential estates, hotels and municipal landscaping (Fig. 4).

New cultivation techniques

Pruning of principal root

The pruning of primary roots of *D. angustifolium* seedlings encouraged development of multiple primary roots and more fibrous roots. This method, developed by Dehgan & Johnson (1987) who used growth regulators was found to be successful with *Zamia floridana.* We varied this technique by inoculating with native arbuscular mycorrhizal fungi on previously root-pruned *D. angustifolium* seedlings.

Inoculation with native arbuscular mycorrhizal fungi (AMF)

When *D. angustifolium* seedlings were inoculated with AMF two years after principal root pruning a faster growth rate was noted compared to the control plants ($P = 0.019$). This technique encourages faster growth and better container handling of plants. Though still experimental, it is being adopted in the Monte Oscuro nursery (Vovides *et al*., in press).

Identification of AMF in cycads

Knowing that AMF is beneficial to cycad growth (Vovides, 1991: Fisher & Vovides, 2004) we isolated AMF in cycad species covering the three genera, *Ceratozamia mirandae*, *C. matudae*, *Dioon merolae* and *Zamia soconuscensis* grown in native soil. Multiple AMF species were found on the cycads that ruled out AMF specificity in cycads, which is in agreement with the literature for other plant groups. The following six AMF species were identified; *Acaulospora denticulata*, *A. scrobiculata*, *A.* aff. *delicata*, *Glomus claroideum, G. fulvum* and *G. microaggregatum* (Hernández et al., in press).

Systematics

There has been an unprecedented increase in cycad species in Mexico since the 1970s when only about 12 species were known to 54 species described to date. This is an increase of over 300%. This is due to the renewed interest in cycad biology and taxonomy by national and international botanists coupled with extensive field explorations and collections to enrich the living cycad collection at the JBC. Nineteen of these new species descriptions were based on specimens in the National Cycad Collection (Table 2.). For species described up to 2002 see Whitelock (2002) thereafter see (Avendaño *et al*., 2003; Gregory *et al*., 2003; Pérez-Farrera *et al*., 2007; Vovides *et al*., 2008a,b; Nicolalde *et al*., 2009).

Historic collection at National Botanic Garden, Glasnevin, Ireland

David Moore, curator of the National Botanic Gardens 1838-1879 acquired a *Ceratozamia* from Cuba that he tentatively named *C. fusca-viridis* in 1878 a year before his death. The origin of this species has since remained a mystery (no ceratozamias are native to Cuba) however Dr. Dennis Stevenson found a voucher at Kew that connected this name to a plant at Glasnevin. This plant is still in cultivation at the Garden and has been so for over 130 years, which speaks very highly of generations of horticulturists at the National Botanic Garden for keeping alive a specimen for so long. This plant was compared to living specimens collected recently in Mexico and has cleared up a vexing taxonomic problem on the identity of *C. fuscoviridis* (Osborne *et al*., 2006).

Cytotaxonomy

The living collection at the JBC has been used for research into chromosome numbers and karyotypes in cycads. Many of the Mexican species have been karyotyped based on the collection and the entire genus *Ceratozamia* is currently undergoing karyotipification. Somatic chromosome number and overall karyotype morphology in *Ceratozamia* is very stable typically 2n = 16, but in *Zamia* chromosome number and morphology is very variable mainly due to fusions and or fissions known as Robertsonian changes see (Vovides, 1983; Moretti & Sabato, 1984; Vovides & Olivares, 1996; Jones, 1977, 1998).

Anatomy

Old techniques and traditional tools that can still provide new information are useful to complement modern techniques in systematics. The Collection is used to explore potential anatomical characters that can help resolve problems of species relationships in cycads. Techniques such as histochemical staining, bright field microscopy, epiflourescence microscopy, Nomarsky differential interference microscopy and scanning electron microscopy are being used.

An example of this is the separation of *Ceratozamia brevifrons* from synonymy with *C. mexicana.* Only the description by Miquel exists of *C. brevifrons* and no type has so far been found. A small population of ceratozamias that fit the Miquel description have been found in central Veracruz. These were compared with *C. mexicana* and several morphological differences have been noted as well as a distinct leaflet anatomy that differs from that of *C. mexicana*. A neotype for *C. brevifrons* is being assigned.

Phylogenies

A molecular phylogeny hypothesis on the genus *Ceratozamia* using nuclear ribosomal DNA ITS and chloroplast *trnL-F* DNA non-coding region have generated a tree with a topology that agrees with the Pleistocene floristic refugia hypothesis as well as with species complexes based on gross morphology. Two main branches, one above or north and another below or south of Mexican Transition Zone divided taxa. Though resolution for all species was not obtained a deeper understanding of the biogeography of *Ceratozamia* was obtained (González & Vovides, 2002).

A molecular phylogeny of *Dioon* also reflected biogeography and morphology where the tree consists of three clades, two of which separated species from the Atlantic seaboard, the *Dioon edule/angustifolium* group from eastern and northeastern Mexico and the larger *Dioon spinulosum/rzedowski/mejia* group from southeastern Mexico and

Honduras. A third clade contained species from the Pacific seaboard and central Mexico. Species from both seaboards in their northerly distributions mirror each other by presenting narrow leaflets and small seeds (González *et al*., 2008).

DNA Barcoding

A promising and novel technique for species identification that has potential for use in law enforcement to control traffic of endangered and threatened species. However identification at 100% certainty has not yet been achieved for plants including cycads. The National Cycad Collection at the JBC has been barcoded using the recommended loci for plants. No unique loci exists for species identification, however using a combination of two to four loci up to 79% certainty has been achieved for *Dioon*, 78% for *Ceratozamia* and 75% for *Zamia* (Table 3). Much work is still needed to improve on this largely searching for new loci. Not withstanding, for CITES purposes that only require genus identification for most cycads, this technique appears to be very promising (Nicolalde *et al*., 2010).

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Table 1. The Mexican National Cycad Collection at the Jardín Botánico Clavijero

Families	Genera	Species	Plants
3	**10**	**96**	**1805**

Table 2. New Species Descriptions Based on Material in the National Cycad Collection since 1980

GENUS	No. Species
Ceratozamia	**12**
Dioon	**2**
Zamia	**5**

Over 300% increase in cycad flora since late 1970s

Table 3. Comparative performance of number of species uniquely identified of the various combinations of loci used, after character-based DNA barcoding analyses with the CAOS software (Sarkar et al., 2008).

Combination of loci	***Ceratozamia***	***Dioon***	***Zamia***
psbK-psbI	4/23 (17%)	8/14 (57%)	12/24 (50%)
psbK-psbI + atpF-atpH	9/23 (39%)	11/14 (79%)	15/24 (63%)
psbK-psbI + atpF-atpH + rpoC1	9/23 (39%)	11/14 (79%)	16/24 (67%)
psbK-psbI + atpF-atpH + rpoC1+ rpoB		1/14 (79%)	
psbK-psbI + atpF-atpH + rpoC1+ matK	12/23 (52%)		
psbK-psbI + atpF-atpH + rpoC1+ matK+ITS	18/23 (78%)		
psbK-psbI + atpF-atpH + rpoC1+ ITS2			18/24 (75%)

From Nicolalde et al. (2010) *Cladistics* **26** (on-line)

FIGURES

Fig 1. Makeshift greenhouse housing the first cycad collections prior establishment of the Botanic Garden.

Fig. 2. Present day greenhouse for the national cycad collection at the Botanic Garden.

Fig. 3. Outside plantings of the cycad collection at the Botanic Garden.

Fig. 4. Roadside municipal landscaping in Xalapa with *Dioon edule* produced at the Monte Oscuro nursery.