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CYCADS

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Cycad Specialist Group

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Botanic Garden In Focus: MBC, Florida
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The Cycad Specialist Group (CSG) is a component of the IUCN Species Survival Commission (IUCN/SSC). It consists of a group of volunteer experts addressing conservation issues related to cycads, a highly threatened group of land plants. The CSG exists to bring together the world's cycad conservation expertise, and to disseminate this expertise to organizations and agencies which can use this guidance to advance cycad conservation.

Official website of CSG:
http://www.cycadgroup.org/

Cover photos
Front: *Encephalartos latifrons* at San Diego Zoo, California. Photo by Christy Powell
Back: *Encephalartos middelburgensis*, South Africa. Photo by Simon Lavaud

Design & layout: JS Khuraijam
Copy Editors: M Calonje & JS Khuraijam

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All contributions published in *Cycads* are reviewed and edited by IUCN/SSC CSG Newsletter Committee and members. IUCN/SSC CSG members can send contributions to **jskhuraijam@yahoo.com**

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MESSAGE

Dear Friends,

A very informative issue awaits you -- I am excited to see the important content assembled here. These pages show both the severity of threats our beloved cycads face, as well as the wide diversity of approaches and techniques needed to stop the loss of these living treasures.

I am especially encouraged by the engagement and expertise brought forward to address these threats. These pages show how many kinds of methods are needed help preserve these plants -- genetics, horticulture, seedbanking, and even *armed deterrence*. I believe all of these methods are essential to preventing cycads extinctions, so I am glad to see our group exploring all possibilities. Integrating these ideas and practices into the broader framework of our Specialist Group strategy will lead to many positive outcomes.

I am also glad to see the detailed attention being given to cycad collections. Our strategy recognizes the vital role that assurance colonies play to prevent catastrophic loss. My own studies in this area have convinced me that the best path forward is to share these vital resources, and steward them collaboratively – the genetic evidence clearly points that way. Adapting the mindset of zoos can help us help cycads in this regard.

I am honored to have the opportunity to discuss in these pages, with my colleague Michael Calonje, our own collection at Montgomery. As I have said before to this esteemed Group – *our collection is your collection*. Montgomery's work depends on your input, collaboration and expertise, so we grow these plants for you.

This is our decade to prevent cycad extinctions – keep up the great work and let no cycad vanish!

Thank you,

Patrick Griffith
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Dioon merolae in natural habitat Photo by Takuro Ito

Botanic Garden: In Focus

Montgomery Botanical Center
Florida, USA

Montgomery Botanical Center's Cycad Collection – Focus on research and conservation

Michael Calonje and Patrick Griffith

Montgomery Botanical Center (MBC) is a 120-acre botanical garden in Coral Gables, Florida (USA) which focuses on advancing science, education, conservation, and horticultural knowledge of tropical plants, with an emphasis on palms and cycads. The cycad collection on MBC's grounds began in 1932 when the property was first developed as Colonel Robert Montgomery's winter home. Among the early introductions by Montgomery is a plant of the Cuban endemic species *Microcycas calocoma* (Photo at right). The plant is still alive today and is likely the largest individual of the species in cultivation outside of Cuba (photo, page 6). Col. Montgomery continued to develop his cycad collection until his death in 1953, and in 1959, his wife Nell Montgomery created The Montgomery Foundation (now Montgomery Botanical Center) to support research on tropical plants and to honor her husband's names. Nell Montgomery passed away in 1990, leaving the property and plant collections to the oversight of its Board of Directors.

Since that time, MBC has quickly developed one of the most comprehensive and well-documented cycad collections in the world. It includes 7,213 individual plants, 1,920 accessions, and 259 taxa representing approximately two thirds of all known species. Plants from the ten cycad genera are hosted at MBC, with the most comprehensive collections being those of the Neotropical genera *Ceratozamia*, *Dioon*, *Microcycas*, and *Zamia*. MBC develops its collections during research expeditions in close collaboration with partner institutions worldwide, and the collections are world-renowned for their scientific and conservation value, as they focus on first-generation, wild-collected material from well-documented sources sampled in a way that maximizes genetic diversity. In fact, MBC has conducted research on genetic capture of cycad diversity in ex-situ collections to better understand the role botanic garden ex-situ collections can play in cycad conservation, and how to plan

Magnificent plant of *Cycas conferta* at MBC. Photo: Michael Calonje

Cycas cairnsiana Photo: Michael Calonje

for and build genetically appropriate cycad collections (Griffith et al., 2015; Griffith et al., 2017).

Microcycas planting at MBC

MBC cooperates with cycad researchers around the world by making its plant collections, knowledge, and expertise available for study. Scientists, educators, and students regularly visit our collections to conduct their studies or are able to receive materials and data useful for their research projects. In the last two years, over 20 published scientific articles made use of MBC's cycad collection.

MBC maintains one of the most active seedbank programs for cycads. Cycad pollen is routinely collected from plants in our collection and either used immediately for hand-pollination, or stored in an ultra-low freezer for later use or to distribute to other botanical institutions wanting to pollinate their cycads. Cycad seeds are recalcitrant and therefore cannot be reliably stored long-term. Therefore, seeds produced at MBC are immediately distributed. The widespread distribution of artificially propagated cycads helps reduces pressure on wild cycad populations by making them abundantly available in the horticultural trade, and this is one of the primary reasons for MBC's commitment to sharing its living collections. Seeds produced from Montgomery's living collections are routinely distributed to botanic gardens, research institutions, universities, schools, and plant societies worldwide. After conservation, research, and educational seed distributions are made, the remaining seeds are auctioned off in perhaps the first-ever online seed auction, in partnership with the Miami-Dade Chapter of the Florida Nursery, Growers and Landscape Association (FNGLA). Proceeds from this auction support the Montgomery Seedbank.

Since June of 2015, MBC hosts the Programme Office for the IUCN Species Survival Commission Cycad Specialist Group (CSG). In this capacity, MBC supports some of its staff members as officers of the CSG, helps provide administrative and technical capacity, manages funding, collaborates with other partner organizations, and helps develop the next generation of cycad conservation expertise.

Dioon spinulosum cone at MBC - largest cycad cone ever recorded.

As part of its CSG duties, MBC's Cycad Biologist Michael Calonje serves as coordinator of The World List of Cycads, a taxonomic and nomenclatural reference for cycad research that serves as the taxonomic backbone for the IUCN Red List, CITES, and the Catalogue of Life.

To meet its mission and goals, Montgomery cherishes the participation and input of cycad experts worldwide. We are always happy to host cycad researchers, provide material for study and conservation, and to collaborate in support of cycad conservation. Supporting and working with the CSG is an essential part of this collaborative endeavor, and we look forward to working with you in the years to come.

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Cristina Lopez-Gallego (CSG Vice Chair) and her students with *Microcycas calocoma* at Montgomery Botanic Center

Figure 1. View of impressive *Encephalartos* collection at the Kirstenbosch National Botanic Garden, South Africa.

Towards an approach for the conservation and illegal trade prevention of South Africa's endangered *Encephalartos* spp.

James A. R. Clugston, Michelle Van Der Bank and Ronny M. Kobongo

Cycads worldwide are under threat from habitat destruction caused by human activity such as over-collection of plants from wild populations, causing a reduction in the size of these populations and increasing the threat of extinction (Donaldson 2003). Species in the genus *Encephalartos* Lehm. (Fig. 1) are some of the most sought after of all cycads, which includes species such as *E. hirsutus* and *E. eugene-maraisii* (Fig. 2). These species can cost hundreds of US dollars per seedling due to their rarity and value in private collections. This rarity has spurred demand for many rare species in both horticultural and private collections (Dehgan 1997), and ultimately has led to their over collection from the wild and an increase in illegal trade (Williamson *et al.* 2016). Although many of these species are regularly cultivated, this has been insufficient to satisfy the demand for these species and prevent illegal collection from wild populations (Whiting *et al.* 1966).

In recent years illegal trade of cycads for both horticultural and medicinal purposes has caused a significant increase in unidentified plants being confiscated by authorities (Cousins *et al.* 2012). Many of these plants require identification to species level, but the morphological characteristics that are often used to identify them are not always present (Cousins *et al.* 2013). The advent of DNA barcoding technologies was considered to help identify plants that are confiscated due to illegal trade (Sass *et al.* 2007). However, due to recent radiation (in the last 10-15 million years) of the extant cycads and their close genetic relationship among many species, traditional DNA barcoding markers are often ineffective at resolving close genetic relationships between species (Condamine *et al.* 2015, Nagalingum *et al.* 2011).

Due to the ineffectiveness of the traditional methods, new approaches are needed to help conserve *Encephalartos* in Africa. This is because we need to be able to identify plants in illegal trade to species level and find out which populations they were collected from so they can be returned to that same population without affecting genetic diversity of the species (Neale 2012). Recent advances in genomic sequencing and DNA fingerprinting has so far proved valuable in understanding close species relationships, yet significant costs associated with sequencing large and complex genomes (e.g. *Encephalartos* c. 58 Gbp) are prohibitive (Roodt *et al.* 2017, Zonneveld 2012). However, new next generation sequencing approaches such as targeted capture sequencing (Brewer *et al.* 2019), and restriction site associated DNA sequencing (RADseq), has been shown to be very successful for both population genetics and phylogenetics (Andrews et al. 2016). Additionally, RADseq has already been shown to be successful in its application to cycads and other orgasms with large and complex genomes (Clugston *et al.* 2019).

With these recent advances in mind, this opens new doors for testing RADseq and its application to tackle conservation and track illegal trade in *Encephalartos*. To do this, a team of experts was formed to help formulate, establish and implement new protocols for the conservation of *Encephalartos*, led by African Centre for DNA barcoding at the University of Johannesburg (Prof. Michelle Van Der Bank and Ronny "Beads" Kobongo), Royal Botanic Garden Edinburgh (Prof. Peter Hollingsworth), The University of Sydney (Dr. James Clugston), California Academy of

Figure 2. James Clugston next to a large mature specimen of *Encephalaertos eugene-maraisii* in the wild at South Africa.

Sciences (Dr. Nathalie Nagalingum) and in South Africa, the Endangered Wildlife Trust and the Department of Environmental Affairs. We are actively working to develop new methods to genotype species and populations of *Encephalartos*. Using RADseq methodology proposed by Clugston *et al.* (2019), we will not only be able to provide an accurate species level identification, but also determine the origin of wild *Encephalartos* specimens that have been confiscated by customs or recovered from illegal trade, and facilitate their return to their original populations.

We have already obtained data from a successful pilot study which was completed in the molecular laboratories of the Royal Botanic Garden Edinburgh (Fig 3). The samples from this project represented multiple accessions for 23 species of *Encephalartos*, which will be used to develop a set of custom markers to further genotype populations and species. Ultimately, our findings will be used to build better conservation management plans for wild populations and aid in the conservation of *Encephalartos* for future generations.

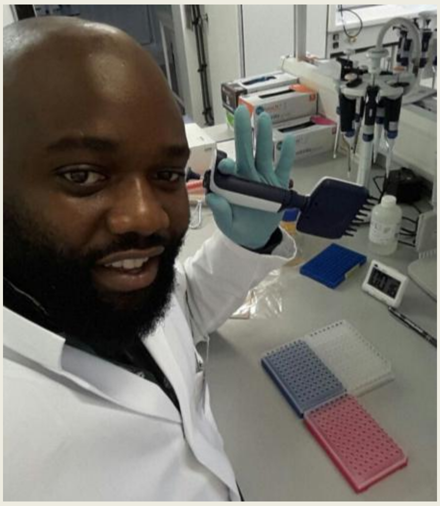

Figure 3. Ronny "Beads" Kobongo undertaking library preparation in the molecular laboratories of the Royal Botanic Garden Edinburgh.

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Cycas tansachana, Saraburi Province, Thailand Photo by Simon Lavaud

Adult *D. merolae* burning on the fire, April 2019 at the hill Nambiyigua

Fire is the most important threat for conservation of *Dioon merolae* (espadaña) in the hill Nambiyigua, municipality of Villaflores, Chiapas, Mexico

Miguel Angel Pérez-Farrera and Mauricio Martínez Martínez

Dioon merolae is a cycad species endemic to Chiapas and Oaxaca states (Chemnick *et al.*, 1997). This species grows in the physiographic regions of the sierra madre and in the central depression of Chiapas; in Oaxaca it grows in the Sierra Madre Sur in the deciduous tropical forest and in the pine-oak forest between 500 and 1200 mls, reaching up to 5 m in height. This species has been studied from the ethnobotanical (Pérez-Farrera & Vovides 2006), ecological (Lazaro-Zemeño *et al.*, 2011), eco-physiological (Pérez-Farrera *et al.*, 1999), morphogenetic (Cabrera-Hilerio *et al.*, 2008) and genetic points of view (Cabrera-Toledo *et al.*, 2010; 2012) and recently its morphological variation has been studied through its geographical distribution range (Pérez-Farrera *et al.*, 2018).

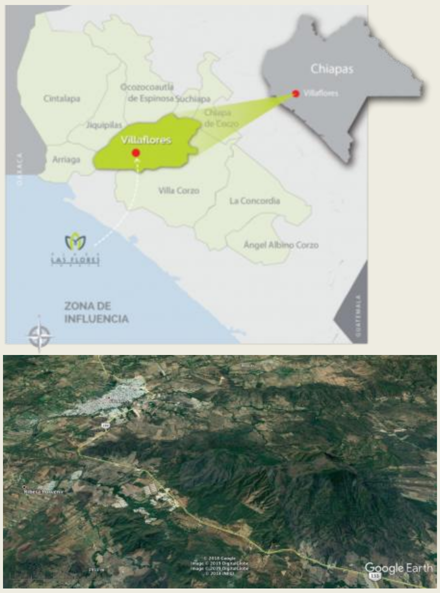

Figure 1. Geographic localization of Nambiyigua hills, Villaflores, Chiapas.

One of the localities registered for *Dioon merolae* in the original publication by De Luca and Sabato *et al.* (1981) "Cerro Grande, district of Villaflores", is located in the Central Depression of Chiapas (Fig. 1) and is known locally as "cerro Nambiyigua" (Nambiyigua in the Chiapanec language means hill of the witch monkey (Mangue and Montesinos, 1991). This site is suffering a great tragedy at this moment in that the cycad population is being impacted by fire (Fig. 2) caused by the drought, winds and agricultural activities. The individuals of *Dioon merolae* in this locality grow on steep slopes of up to 45° and begin to grow from 900 to 1200m in pine-oak forest.

Due to the cultural and biological importance of this species, the municipal government of Villaflores, Chiapas established an in-situ nursery in the municipality of Villaflores for propagation and reintroduction purposes. In this sense, the municipal government reintroduced more than 2000 seedlings to Nambiyigua. However, this locality has suffered constant burning throughout different years in 1998, 2000, 2018, 2019. Last year (2018) more than 50 ha were affected. In this year since the mountain began to burn, more than 100 hectares of forest have been destroyed.

Figure 2. *Dioon merolae* locality on fire at Nambiyigua Hill.

This population is important for the following reasons:

a) it is one of the localities where the espadañeros arrive to collect leaves for the ritual of the festivity of "Santa Cruz" in Suchiapa, Chiapas (Pérez-Farrera & Vovides, 2006),

b) it is one of the original localities referenced in the publication by De Luca and Sabato *et al.,* (1981) describing *D. merolae* and its morphological variation,

c) the hill Nambiyigua is an area that has been proposed as a natural reserve with approximately 4127 has for the national commission of protected natural areas (CONANP), although this has not yet been done officially, and it is an area within the La Sepultura biosphere reserve,

d) It was an important place for the Chiapanec culture. In this place, several archaeological remains of this culture have been found (Navarrete, 1966),

e) on this site individual of *Dioon merolae* up to 5 m high have been registered (Martínez com. pers.),

f) it represents one of the populations with the greatest abundance of individuals within their range of geographic distribution (Conanp, 2012)

In 2011, 447 individuals were registered in three sites within the Nambiyigua hill (Las Anonas, El Pashtal and San Angel) (Conanp, 2012). In 2018 a group of students from The Eizi Matuda Herbarium, Universidad de Ciencias y Artes de Chiapas (UNICACH) evaluated two populations, one with disturbance (through fires) and the other without disturbance, and evaluated the abundance and structure of the population by reproductive condition and they found significant differences in relation to their abundance; the population conserved denominated "The Mangal" had a total of 295, whereas the population of "San Angel" which is considered with disturbance had 158 individuals (Fig. 3). Unfortunately, the study cannot be continued due to the current great fire, where the conserved populations were affected. However, this same problem has prompted us, to begin in August 2019 a research study to assess the situation and the state of conservation of newly discovered populations of *D. merolae* in the area of La Frailesca Chiapas (Villaflores, Villacorzo and La Concordia municipalities). These *Dioon* populations will be evaluated through various population parameters (abundance, class structure, spatial distribution, sex ratio), dendrometry and allometry (height, diameter of trunk, number of leaves, leaf width and long) and this data will be correlated with the history of use of the cycads, also a study about effect fire on population dynamic of *Dioon merolae* will be evaluated and the land with the purpose of providing information that generates a strategy for conservation.

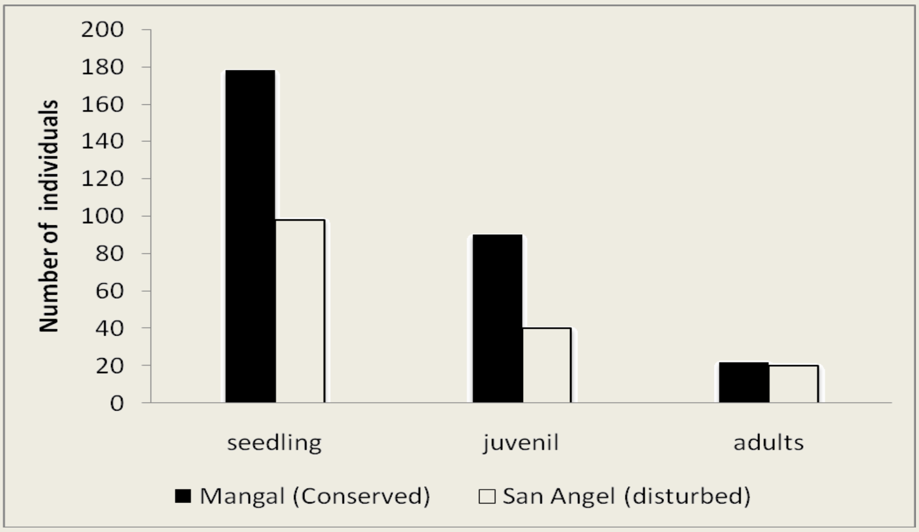

Figure 3. Population structure by reproductive condition in *D. merolae*.

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Cycas panzhihuaensis Photo: Patrick Griffith

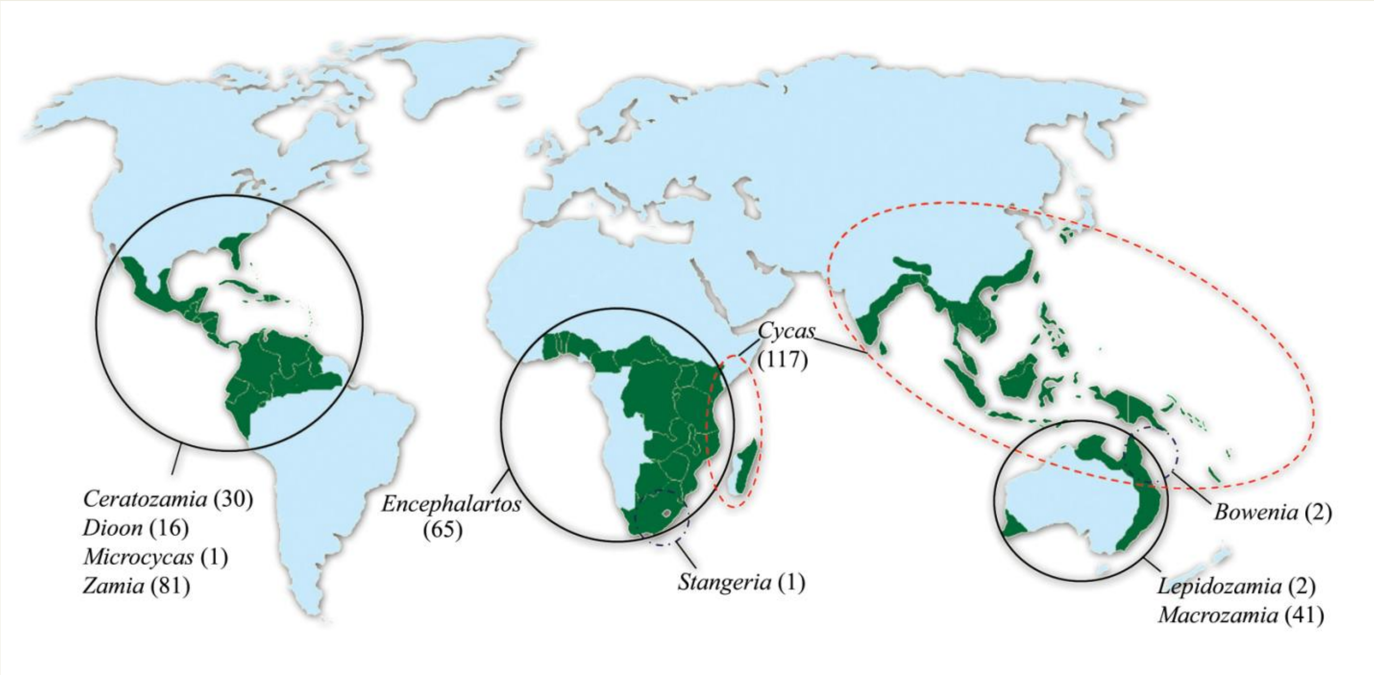

Figure 1. World distribution of cycads (356 species, Calonje *et al.*, 2019).

Ex-situ Cycad Conservation [1]: Public and Private Collections

Chip Jones & JS Khuraijam

When you hear the word "collection" many things might come to mind in the context of cycads. Botanists, horticulturalists, conservationists, public, and private plantsmen have connotations of collection. The definition of collection from Merriam Webster is an accumulation of objects gathered for study, comparison, or exhibition or as a hobby. A collection is typically thought to be a group of objects or people together but can refer to a single specimen or a verb as the act of collecting. Often a plant collection is thought to be a living group of plants brought together for a purpose. Collections can be privately owned by individuals. Often larger cycads collections are kept by institutions, museums and botanical gardens collectively called public gardens. Public gardens offer varying degrees of access to plant collections often keeping rare or sensitive species in more secure areas. A collection can be for professional reasons such as a specimen collection for a botanist who would see the value of a herbarium collection to preserve details of a plant for a theoretically indefinite time. An individual might see a herbarium as a destructive sample for few to see and prefer to keep living collections of whole plants. Too often there is a dichotomy between individuals with collections and professionals who collect specimens for botanical gardens or herbaria. Occasionally the two sides of collecting join. This article is introduction to the public gardens and private collections, and we plan to have a follow-up on detail collection information in next issue.

Cycad collections in Botanic Gardens

Name of Botanic Garden	No. of Taxa
1. Nong Nooch Tropical Garden, Pattaya*:	347
2. Montgomery Botanical Center, Florida :	261
3. The Huntington, California :	178
4. Lotusland, California :	142
5. Flamingo Gardens, Florida :	120
6. University of California Botanical Garden :	114
7. Fairchild Tropical Garden, Florida :	105
8. Royal Botanic Gardens, Kew :	81
9. CSIR-NBRI Botanic Garden, Lucknow :	72
10. Kirstenbosch NBG, Capetown:	52

*Private Garden

Figure 2. A magnificent *Cycas debaoensis* at Nong Nooch Tropical Garden, Thailand
[Right to left: A Vogel, A Lindstrom, W Tang & JS Khuraijam]

Botanical gardens are collections of living plants often used by botanists and other researchers. The botanical garden can reduce the need for extensive travel to analyze multiple specimens or species. In

Figure 3. Cycad Collection at Kirstenbosch National Botanical Garden, South Africa.

addition to the value to science gardens can educate individuals and give an introduction to the living world to people of all ages and especially to children. Public gardens often follow a mission statement describing purposes of horticulture and education and conservation. Cycads are among the most threatened plants with extinction and well suited to fit the missions of most botanical gardens. Many individuals cannot obtain specimens of rare plants due to scarcity, price, and legal protections placed on cycads. Botanical gardens are an important way for individuals to appreciate and better understand cycads. In the way zoos have breeding programs and assurance colonies for research and conservation so too botanical gardens play important roles in cycad research and conservation.

Public gardens can easily obtain important representations of cycad taxa and often serve as sites for *ex-situ* conservation for living collections of rare cycads. CITES is virtually impossible for individuals but relatively easy via CITES institutional exchange (Conf. 11.15 Rev. CoP18) www.cites.org. Most botanical gardens have ample space for plants to achieve mature size and usually space for multiple specimens. Also, most public gardens have security, an infrastructure for maintenance and record keeping.

Figure 4. Chip Jones at a private collection in South Africa.

Public gardens have staff that can watch for coning events and document phenology. Pollen viability research and access to -80 freezers and stand-by generators assure pollen banks function for sexual propagation. Botanical gardens often produce seeds and distribute plants. Plant distribution furthers conservation efforts and removes pressure for illegal collecting. If an individual can obtain a plant from a local botanical garden there is little chance that individual would want to collect from habitat.

In collecting data from public collections some observations are made. With only 10 genera and 356 species of extant cycads (www.cycadlist.org, see Fig. 1), many botanical gardens have complete or near complete collections of cycad genera (Table 1). Most collections focus on endemic species to the region of the garden. For example Kirstenbosch National Botanical Garden is known to have an impressive collection of cycads of both tax and specimen quality and yet has few non-endemic taxa other than African *Encephalartos* and *Stangeria*. A public garden in Brazil, Inhotim, in 2011, joined the Brazilian government's official botanical

Figure 5. Cycad Collection, Montgomery Botanical Center, USA Photo by Michael Calonje

garden association, and the staff has begun an inventory of its 5,000 plant species, including 1,300 types of palm alone. Inhotim has only 2 Dioon, 2 *Cycas*, 2 *Encephalartos*, and a few *Zamia* accessions (pers. comm.). In 2018, Inhotim received its 3 millionth visitor while the same year Kirstenbosch National Botanical Garden saw over 2 million visitors (SANBI annual report 2017/18). Conversely where cycads are not native we see high diversity in collections such as Europe (zero extant native cycads) and the United States (one endemic species). This is explained by climate and the interests of the public in exotic or charismatic flora. Horticulturalists know what plants will survive and perform well in the soils and climates where they maintain collections. Exotic plants from faraway places make good stories for curators that write interpretation for botanical displays. The living fossil moniker is as popular as dinosaur food in describing the history of cycads in evolutionary terms.

Public garden collections have a unique opportunity to introduce millions of people to cycads. Because of habitat degradation, public garden collections might be the only opportunity for some people to see a cycad. The introduction to cycads could lead to public awareness and further support for conservation.

The authors would like to compile a database of living collections worldwide. Any information to further this goal is appreciated and can be sent to us by email.

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Figure 1. Armed guards at Lillie Flora Nature Reserve, protecting *E. dyerianus* around the clock.

The Cycad Extinction Crisis in South Africa

Wynand van Eeden & Tim Gregory

The IUCN Red List lists only four species of cycad as extinct in the wild (EW) and all four were endemic to South Africa. Only one, *Encephalartos woodii*, was known from one single clone. All the others were multiple plants or even populations, although they occurred in a confined area.All four species became extinct due to human intervention and specifically for the horticultural trade. If one considers that fourteen more species of *Encephalartos* are now listed as critically endangered (CR) or endangered (E) and that a few of these are functionally extinct in the wild already, the South African situation is dire indeed! Nowhere else in the world are cycads so sought after and do they fetch such high prices, as in South Africa.

What can be done about this?

Currently only a few species occur in official reserves where some kind of protection is offered. However, most reserves focus on animals and not specifically the plants and it is usually a coincidence if cycads do occur in reserves. The only exception to this is Lillie Flora Nature Reserve in which *E. dyerianus* is growing and the cycads have survived only because armed guards are on duty 24/7.

To remedy the lack of focus, a new conservation oriented, non-profit company, *Wild Cycad Conservancy NPC*, or *WCC*, was registered in South Africa this year, with the aim to acquire land on which endangered cycad species occur, to have this land declared as reserves and to make the reserves sustainable. The reserves will be managed by the WCC and as far as possible in co-operation with the original landowner. As a non-profit organisation, the WCC is run by volunteers and entirely dependent on donations.WCC will be registered as a public benefit organisation (PBO) in South Africa and a 501c3 in the US. The management structure currently is made up of a board of directors and an advisory board with members from across the world.

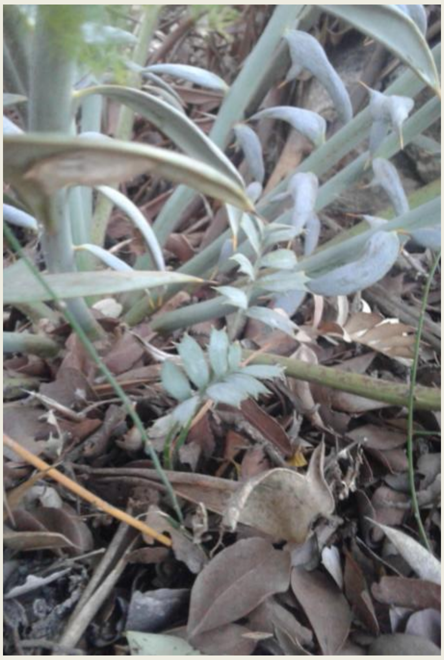

Figure 2. Healthy seedlings of *E. horridus* in what will soon be the first reserve to protect the species.

Encephalartos horridus has come under severe pressure from poachers lately. A property was identified with a healthy population that will be the pilot project for WCC. A preliminary population survey was done by Alicia Wain, a botanist and expert on habitat restoration, and it has shown healthy recruitment with seedlings, as well as sub-adults, in the population. The sex ratio appears to be close to 1:1 and pollinators are present. Once this reserve has been established, it will be an excellent starting point to get other landowners to collaborate and work with WCC to stop *E. horridus* from moving from being listed as EN to CR in the future.

Securing land for reserves is the main, but not only, activity of WCC. Future projects will expand their scope to include the establishment of assurance populations of threatened species, as well as re-introduction programmes when possible.

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Encephalartos latifrons in natural habitat at Eastern Cape Province, South Africa Photo by Simon Lavaud

What is *Ceratozamia becerrae* ?

Andrew P. Vovides, Miguel Angel Pérez-Farrera and José Said Gutiérrez-Ortega

Cycad descriptions are traditionally based on vegetative morphology, especially, leaf, leaflet and strobilar traits. Anatomical characters, as aid to species delimitation in cycads, have been particularly useful when compared with molecular data, at times ratifying or contradicting previously reported phylogenetic relationships. Anatomical traits have enabled us to bring *Ceratozamia brevifrons* out of synonymy with *C. mexicana* (Vovides et al., 2012). Likewise, a morphology and leaflet anatomy study has helped the definition of the species within the *Ceratozamia norstogii* complex (Pérez-Farrera *et al.,* 2014).

Ceratozamia becerrae in the *C. miqueliana* species complex has recently come under scrutiny questioning its validity by Martínez-Domínguez et al. (2017) based on the absence of overall variation among some morphological traits, and the lack of variation in the internal transcribed spacer (ITS) of the ribosomal DNA. The close geographic distribution of *C. becerrae* to that of *C. zoquorum* has also added to the confusion between the two species. However, this study ignored previous morphological and molecular evidence reported in the studies by González and Vovides (2002) and Vovides *et al.* (2004).Also, the recent molecular study by Medina-Villarreal et al., (2019) places the two species on different clades; *C. zoquorum* on the *C. mexicana* clade and *C. becerrae* on the *C. miqueliana clade*. Morphologically, the two species are distinguishable mainly by number of leaflets and spacing between them, leaflet shape and articulation width. Megastrobili are erect in *C. becerrae* but decumbent in *C. zoquorum*, see Pérez-Farrera *et al.* (2001) and Vovides *et al.* (2004). In our recent analyses, we have found that the leaflet anatomy of the two species also differs. Transverse sections of leaflets taken midway along the leaf show that *C. zoquorum* presents a continuous lignified hypodermis and very pronounced girder sclerenchyma associated with the vascular tissue whereas these two traits are absent in *C. becerrae* (Fig. 1). Furthermore, the leaflets of *C. zoquorum* are much thicker than those of *C. becerrae*. These results suggest a clear species distinction of *C. becerrae*.

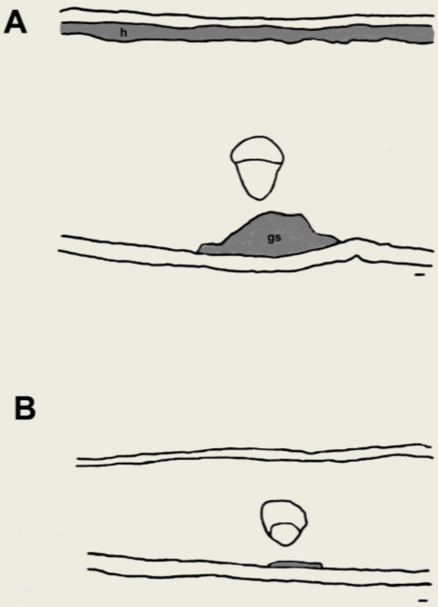

Figure 1. Camera Lucida illustrations. A; transverse section *Ceratozamia zoquorum* leaflet, shaded areas h = lignified hypodermis; gs = girder sclerenchyma. B; transverse section of *C. becerrae* leaflet, note lack of lignified hypodermis and very scarce girder sclerenchyma. Bars = 20µm.

For meaningful taxonomic changes in cycads, such as the invalidation or raising of lumped of taxa, it is necessary to identify the potential mistakes that previous studies have committed, re-evaluate the total biological evidence that is known, and propose the new taxon status based in explicit predictions leading to testable hypotheses. Furthermore, it is well known that cycad species show low genetic differentiation (*e. g.* González and Vovides, 2002; Salas-Leiva *et al.,* 2013), and we discourage the delimitation of cycad species based in the variation in only one molecular marker. Poor criteria might be problematic for the taxonomy of cycads, as in textual words of Kadereit *et al.* (2012) "*Lumping similar species into single taxa would therefore produce polyphyletic taxa, which is unacceptable to any taxonomist who believes that species should reflect evolutionary history*". With the rationale mentioned above, and additional evidence that we are preparing, we propose the recognition of *C. becerrae* as a valid taxon.

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Cycas pectinata at MBC, Florida — Photo by Patrick Griffith

Preliminary Finding: Seed longevity of *Encephalartos* in controlled storage

Ngawethu Ngaka and Phakamani Xaba

Introduction

Cycads have the oldest currently known seed lineage and seed is reported to be recalcitrant (Forsyth & Van Staden, 1983; Woodenberg et al., 2007). Recalcitrant seeds are defined as seeds that are sensitive to desiccation and cannot be dried below a certain critical moisture level (Farrant *et al.,* 1989). Since cycads seeds are recalcitrant, this means traditional seed banking methods cannot be employed to conserve seeds. Moreover, cycads are one of the most threatened living plant species to date (IUCN, 2010). Knowledge and understanding in cycad seed longevity and storage is scant, therefore limiting conservation efforts. This preliminary study is aimed at broadening the understanding of cycad seeds longevity in storage.

Materials and Methods

The research was conducted in 2018 at Kirstenbosch National Botanical Garden (KNBG) to determine seed longevity of different *Encephalartos* cycad species, by analysing seed moisture content and viability in seed stored at different periods. *Encephalartos* were hand pollinated and harvested upon naturally disintegrating. Seed was cleaned and stored at 15RH and 15°C at KNBG Seed Department. *Encephalartos* seed of the six species, that were available at the time at KNBG, were used for the experiment, included *E. altensteinii*, *E. aemulans, E. natalensis*, *E. trispinosus, E. lebomboensis,* and *E. horridus*. Rescued seed embryos of all the above seed were treated with tetrazolium chloride to determine the viability of the seeds (de Carvalho *et al.,* 2014). Viable seeds were confirmed after treated embryos turned pink; non-viable seed did not change colour (Fig 1). To determine seed moisture content, fresh and dry weight were measured. After fresh weight was determined, seed was then dried in an oven for 17 hours at 103°C (Tang and Sokhansanj, 1991) then both weights were subtracted to determine seed moisture content. The number of experimental samples in each species is (n =10).

Results and Discussion

Seed moisture is considered to be one of the important factors determining seed longevity (Baskin & Baskin, 2007). Results show that, *Encephalartos* seed species stored for one year had variation of <5%amongst the two species *E. altensteinii* and *E. horridus* (Fig. 2). However, seeds stored between two and seven years showed consistent drop amongst the four species tested with an average mean of 21%. The results also show that there is significant difference in seed moisture content of different *Encephalartos* seed stored over different periods (in years) (ANOVA, $P < 0.05$), however, seed moisture content in *Encephalartos* species drops with an increase in years of storage. Results indicate that cycads loose moisture during storage from > 30% at the first year to < 20% by the seventh year (Fig 1). Moisture content consistently drops with years of storage in all species, although seed viability is not consistent in all the species (Fig 2).

The tetrazolium test conducted showed highly significant difference in seed viability that was stored for different years(χ^2, $p < 0.05$.) (Fig 2). Viability in seeds stored for one year ranged between 70–90%.The results confirm that *Encephalartos* cycad seed are desiccation sensitive, as stated by Berjak and Pammenter (2008) & Chin *et al.* (1989), and seed viability drops with an increase in years of storage.

Seed viability drops in the second year and none are viable after seven years, a result consistent with desiccation sensitive seed behaviour. However, *E. aemulans* seed stored for three years was inconsistent with seed desiccation storage period compared to one and two years storage. Perhaps the inconsistency is a result of original environmental conditions (Osborne, 1995) and genetic variation of different species. Seeds stored for seven (7) years tend to be non-viable.

Figure 1. Results of seed viability test in *Encephalartos aemulans*, (left) example of viable seed with pink stain embryos, (right) example of non-viable seed with no colour stain.

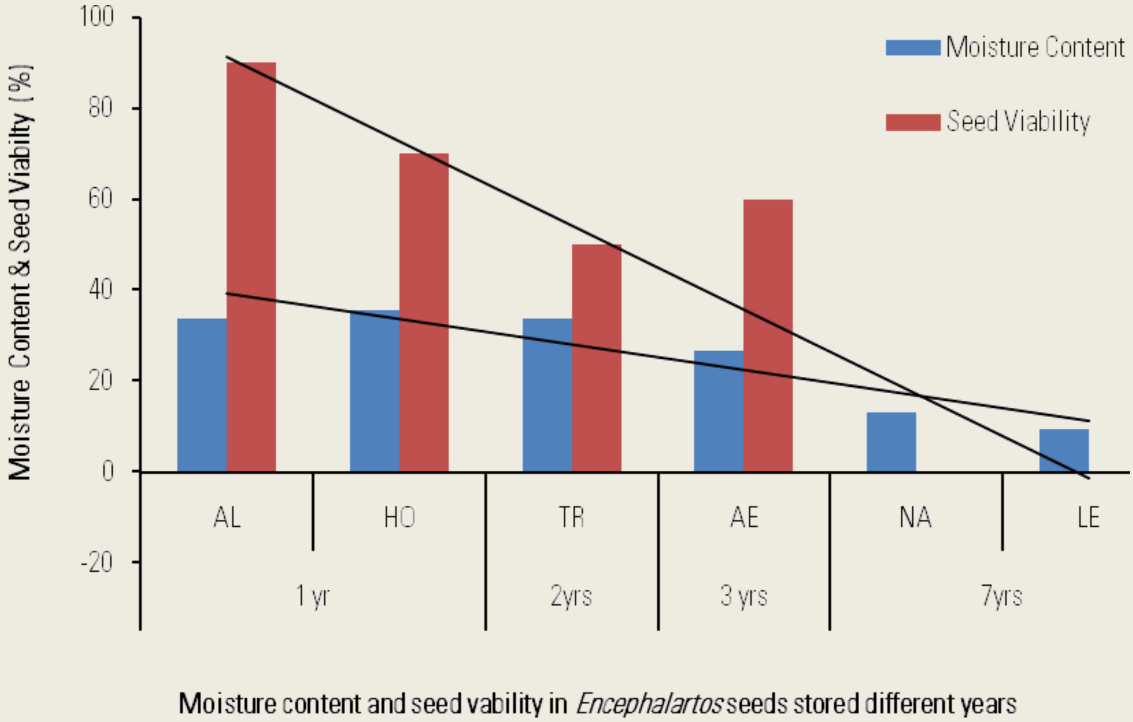

Figure 2. Seed moisture content and seed viability in *Encephalartos* seed stored at different years. Al = *Encephalartos altensteinii,* Ho = *Encephalartos horridus*, TR = *Encephalartos trispinosus, AE = Encephalartos aemulans,* LE = *Encephalartos lebomboensis,* NA= *Encephalartos natalensis*. Trend lines indicating drop in seed moisture content and seed viability. Seed stored at 15%RH and 15°C, each species (n =10).

Conclusion

From the results presented above, it can be concluded that seeds of *Encephalartos* species loose viability as years of storage increases, therefore consistent with reports of desiccation sensitive seeds. Moisture content of the seeds consistently drops with years of storage, as seeds stored at greater years (seven years) had the least moisture content among the species. *Encephalartos* cycad seeds remain viable for a short period of time, optimum one year and no viability at seven years. However, *Encephalartos* cycad seed viability in controlled storage varies with species over time of storage. This trend needs to be further investigated; particularly is highly threatened species, so as to optimise conservation.

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2nd Nong Nooch Cycad Horticulture Workshop

Anders Lindstrom

This year, the workshop was held from the 13-19 October at Kanchanaburi, Western Thailand and also at Nong Nooch main garden in Pattaya, Thailand. The workshop was attended by 39 participants from 11 countries. The participants enjoyed a guided tour of the extensive and world class cycad propagation and collections of cycads. Hands-on sessions with hand pollination, pollen collecting and storage as well as seed storage and even grafting of cycads were demonstrated.

A fantastic selection of speakers presented and shared their knowledge about cycad horticulture, Paul Mills from Ganna Walska Lotusland, U.S.A. was talking about their cultivation and propagation of *Encephalartos heenanii*, Gary Roberson from Huntington Botanical Garden, U.S.A. showed the latest landscape developments including the Cycad walk dedicated to the late Loren Whitelock.

Anders with participants of the workshop

Growth rate of cycads and its application to climate change was presented by UPhakamani Xaba from Kirstenbosch Botanical Garden, South Africa and ground breaking pollen germination studies were presented by Anna Nebot, Spain.

Presentations from private gardens and collectors by Chip Jones, U.S.A. and we learned how to transplant giant cycads from Hendrik Wentzel, South Africa.

At the 1st Cycad Horticulture workshop in 2016, all participants contributed with their knowledge to cycad horticulture and all this data was summarized by Willie Tang into an updated version of his cycad horticulture booklet and was released during the workshop.

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Photo by Jennifer Schwenk

Plant Conservation Genetics Workshop

Caroline Iacuaniello, Stephanie Steele & Christy Powell

The application of genomic tools to plant conservation is advancing our ability to make informed decisions about management and reintroduction of rare and endangered species. To advance San Diego Zoo Global's (SDZG) mission of saving species worldwide, plant conservationists at SDZG are harnessing genomic information to guide management of threatened species both within the zoo's extensive horticultural collection and within natural plant populations of conservation concern. While genomic data can greatly benefit management decisions, plant species provide unique challenges, from difficulties obtaining genetic material to the analysis of large and complex genomes. In recognition of the difficulties inherent to plant genomic research and the importance of conserving plant species, Christy Powell (SDZG Horticulture) and Caroline (Ruby) Iacuaniello (SDZG Plant Conservation) led a Plant Conservation Genetics Workshop with funding support from the SDZG Priority Species Project. Forty-eight attendees from across the U.S. including representatives from botanical gardens, research institutes, universities, plant nurseries, and government agencies convened to discuss their latest research programs, how genomic research can be applied to conservation of endangered plant species, and how to overcome common research, analytical, and bioinformatic challenges.

Small groups formed to brainstorm about solutions to common problems and share resources. Discussions involved what sequencing and bioinformatic pipeline methods worked for plants, as well as those that did not. For example, many of the bioinformatic pipelines that are currently being used were developed for diploid organisms thus polyploidy presents unique complications. Another topic that was addressed was genetic rescue, with consideration being given to when and under what conditions genetic rescue of a species should be attempted, how long an intervention should continue, and what levels of survivability should be expected. Participants further deliberated over how to best represent genetic diversity across species within live collections and seed banks, how to bridge lab-work with conservation action, and plant-specific climate related concerns.

Much of the presented research was focused on cycads, IUCN red-listed priority species at the zoo. Cycad-specific talks covered conservation genetics, population distribution, and other conservation concerns. A core group of cycad focused individuals, including Jeff Chemnick, Manuel Luján, Paul Mills, Stephanie Shigematsu, Christy Powell, Mike Diaz, Michael Letzring, Ruby Iacuaniello and Vanessa Handley gathered to discuss cycad-specific issues. One large challenge to cycad management is that natural seed production is not possible for some species due to distance between individuals. In order to assist reproduction, compatible pollen must be accessible when threatened cycads set cones. A database of individual plants, pollen, and seed availability would offer a possible solution to this problem. How to best create such a database and the specific difficulties surrounding individual plants in private collections was deliberated over. Other stated concerns included the anthropogenic loss of cycad habitat, changing climate conditions, and the likelihood of increasing fire frequency. Separately, a conversation was started about how to best transfer a collection of cycads including *Encephalartos kisambo, Encephalartos turneri,* and *Encephalartos arenarius* from the U.C. Botanical Garden to the San Diego Zoo and the Huntington Botanical Gardens.

Encephalartos latifrons at the San Diego Zoo. Photo by Christy Powell

Overall, plant conservation researchers built many new connections, shared a multitude of resources, and generated many new ideas about how to better apply genomic tools to the conservation of endangered plants. Generous support from an Institute Museum and Library Services grant to the Center for Plant Conservation allowed many of the presentations to be filmed; these are available for viewing at https://academy.saveplants.org/tag/plant-conservation-genetics-workshop-2019.

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