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CYCADS

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Cycad 2018
 South Africa
 First Announcement



I CONGRESO
 MEXICANO DE
 CÍCADAS

LOS CÍCADAS: NUESTROS COMPROMISOS EN CONSERVACIÓN, BIOPROSPERIDAD, 2017
 ESTRUCTURA, ETIMOLOGÍA Y GENÉTICA MOLECULAR. XALAPA, VERACRUZ

Feature Articles
 Research & Conservation News
 CSG Member News
 Upcoming Events

Magnificent female *Cycas pectinata* Buch.-Ham.
Assam, India. Photo: JS Khuraijam





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:
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Front Cover photo: Coastal population of *Zamia chigua* growing at waterfront in Malaga Bay, Valle del Cauca, Colombia.
Back Cover photo: *Ceratozamia decumbens* male cone. Photos: Michael Calonje

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IUCN/SSC CSG Meeting 2015 at Medellín, Colombia

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,

Behold the latest issue of *CYCADS*! Our tireless editor, JS Khuraijam, has worked to gather and compile the latest news in cycad conservation and science, presenting advancements in all aspects of these fascinating plants -- we are in his debt.

We include here two inspiring feature articles. Irene Terry and Claudia Calonje put forward an account of how *ex situ* collections improve the understanding of conservation challenges. On the *in situ* side, I am delighted to see an account by Nathalie Nagalingum of her recent expedition to Queensland, in search of four *Cycas* species -- including one named for Irene Terry! Nathalie's work will illuminate genetics of these imperiled plants, perils she confirms through firsthand experience. These direct observations are so valuable to the work of the Cycad Specialist Group (CSG), and we also include an update on pollinator taxonomy (Tang, page 10) based on recent fieldwork.

Please let me also use this space to share a plant I admire (photo). This *Encephalartos laurentianus*, growing at Montgomery Botanical Center, was obtained as seed from the National Botanic Garden of Belgium two decades ago. This individual has been used in numerous studies by CSG Members and others, much as the plants studied herein by Khuraijam *et al.* (page 19). The blue tape on this plant shows that we pollinated this individual (see Calonje *et al.*, page 16), to provide material which reduces pressure on wild populations. Here, this plant is featured in a tropical botany course, to teach conservation and diversity. Students from six different nations learned cycad conservation that morning -- each student can help spread the word about these living treasures, including through the use of CSG resources such as Clugston & Nagalingum's new video (page 22).

Please see also the series of recent updates on *Dioon*, including phylogeographic and anatomical findings, as well as a fascinating look at its microbiome, each led by CSG Members Jose Said Gutierrez-Ortega, Angelica Cibrian-Jaramillo, and Andrew Vovides. These advances in *Dioon* research are just a portion of the great concentration of cycad work underway in Mexico; see the announcement for the I CONGRESO MEXICANO DE CÍCADAS, this coming October.



And speaking of upcoming gatherings --CYCAD 2018 is announced here! The Cycad Society of South Africa is preparing a unique gathering, and our Specialist Group will be meeting on the days prior. I am working with the CSG leadership and our hosts in South Africa to organize our work for next summer. It will be great to get together again!

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Figure 1. Open-pollinated ovulate plant from 2016 coning year that has developing seeds.

Using cycads in *ex-situ* gardens for conservation and biological studies

Irene Terry & Claudia Calonje

Cycad *Aulacaspis* scale (CAS), *Aulacaspis yasumatsui*, has been attacking *Cycas* species throughout the world, but the greatest devastation has been to *Cycas micronesica* of Guam, Rota and other islands in the Western Pacific Ocean since the pest invaded these islands. In Guam, plant surveys that started soon after the CAS invasion in 2003 indicate that over 90% mortality of adult trees and 100% mortality of seedlings has occurred in less than ten years (Marler & Lawrence, 2012). Conservation efforts have focused on a number of management options including biological control, but none have been adequate to slow down tree mortality. In further attempts to save this cycad, seeds were collected in 2007 from multiple regions on Guam and Rota (Calonje, 2008) and then planted over several years at three *ex situ* locations: on the island of Tinian in the Marianas where this cycad and CAS are absent; and at two botanical gardens, Nong Nooch Tropical Botanical Garden in Thailand and Montgomery Botanical Center (MBC) in

Florida, where pests are managed. These plants serve as genetic and seed sources for future conservation work. At MBC we have been using these *ex situ* plants to further our investigations into the sexual reproduction of these trees. A thorough understanding of this cycad's pollination system will help determine management approaches for both long term plant and pollinator survival.

In Guam, we discovered that many insects visit both ovulate and pollen cones of this species, but only one species appears capable of being a primary pollinator, a microlepidopteran, *Anatrachyntis* sp. (Terry *et al.* 2009). We also determined that in certain habitats, specifically more open habitats where trade winds are strong, that wind is a potential vector for at least up to 8 m downwind from coning pollen trees and possibly farther (Hamada *et al.* 2015 a,b). To follow up on these studies, we have been investigating the pollination of this cycad at MBC. Seeds collected in 2007 from Guam and

Rota from individual mother plants were accessioned, sown in community pots, and tracked separately. Resulting seedlings were planted in eight different areas at MBC. The first seedlings were planted in 2009 in groups of up to 15 plants derived from the same individual populations. All plants were kept far from other *Cycas* species. Table 1 shows the year of the first appearance of cones for each surviving plant from each year of planting for both ovulate and pollen trees. Of interest is that pollen trees generally produce cones earlier than ovulate ones, and the second year after planting is the year that most plants produced their first cone. All cones emerged in the springtime with some as early as February, but most cones emerged in April. The peak timing of receptivity in ovulate cones and of pollen dehiscence in pollen cones was late April through May. We also found that some ovulate cones are open-pollinated and produce viable embryos (Figs. 1 and 2).

Table 1. The first coning events for ovulate and pollen plants from seeds collected in Rota and Guam in 2007, and planted during 2009-2011, and mortality as of July 2017.

Year assessed	Planting year					
	2009		2010		2011	
	Ovulate	Pollen	Ovulate	Pollen	Ovulate	Pollen
2011	1	5	0	0	0	0
2012	10	14	0	2	0	1
2013	5	5	10	16	4	6
2014	2	3	19	6	10	17
2015	2	0	2	3	5	0
2016	all coned	all coned	2	0	6	0
2017			all coned	all coned	all coned	all coned
Total coned	20	27	33	27	25	24
Died after coning	1	1	3	4	2	2
Died before coning		15		6		21



Figure 2. Embryo inside a developing seed from an open-pollinated plant that coned in 2014.

We are continuing studies to investigate whether insects or lizards act as pollen vectors. As this part of Florida is buffeted by northeastern trade winds we are also investigating wind as a possible vector.

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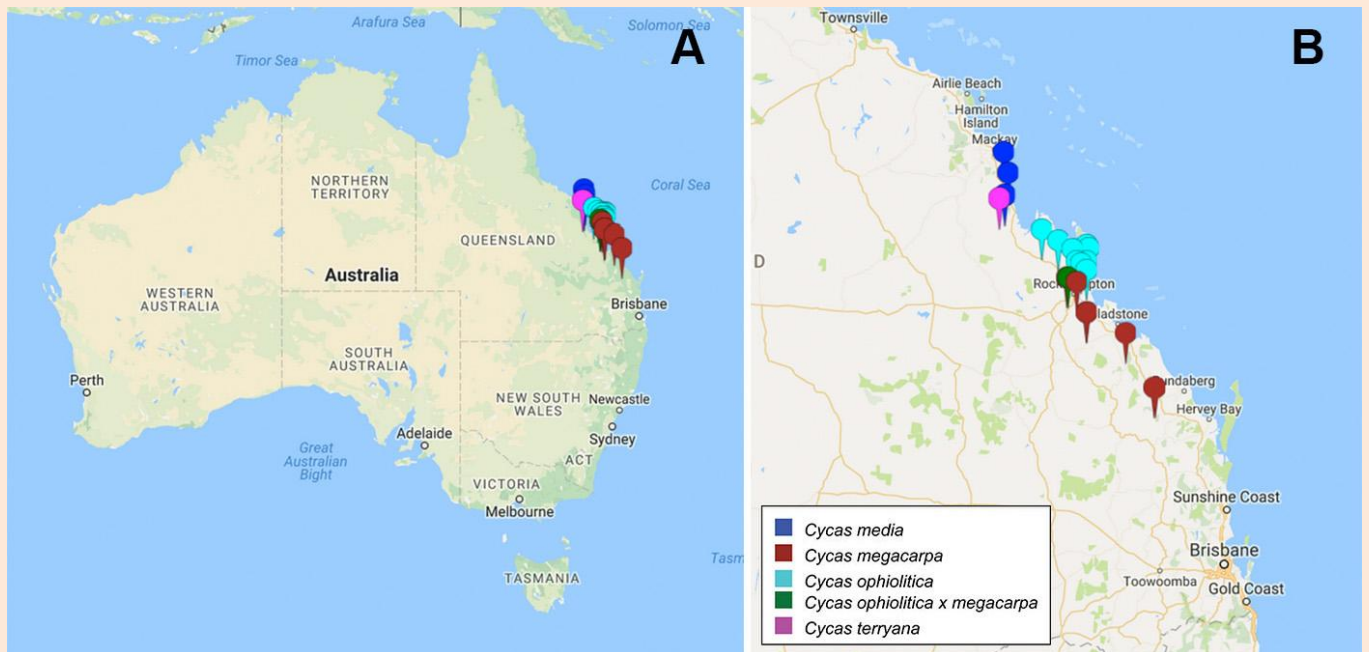


Figure 1. Collecting sites visited during field trip. A. Australia, B. Southeastern Queensland. Species are differentiated by different colored points, see legend.

Collecting cycads in Queensland, Australia

Nathalie Nagalingum

In Australia, *Cycas* is found in the north of the continent principally in the Northern Territory and the state of Queensland, plus a smaller number of species in Western Australia. After a field trip in 2015 to the Northern Territory, in late 2016 I set out to collect samples in Queensland (Fig. 1). My goal was to collect four species: *Cycas media*, *Cycas terryana*, *Cycas ophiolitica*, and *Cycas megacarpa*, plus the hybrid *Cycas ophiolitica x megacarpa*.

I began the field trip in the beachside city of Mackay, known for its large sugar cane industry. I visited many locations with the goal of collecting *Cycas media* in its most southerly occurrences. However, in this

region much of the land is used for agriculture, and so I found very few populations of *C. media* (Fig. 1, dark blue points).

I then travelled south and collected *Cycas terryana* (Fig. 1, pink points). It is the most recently described species of *Cycas* from Australia and was formerly considered a hybrid between *Cycas media* and *Cycas ophiolitica* (Forster, 2011; Hill, 1992). The species was described by Dr Paul Forster (Queensland Herbarium, Brisbane) and named for the cycad biologist Dr Irene Terry (University of Utah). There were few plants in the population, and they showed signs of

significant leaf damage with no recent leaf growth.

The main part of the field trip was centered around the city of Rockhampton, called Rocky by locals, and is famous for beef production. Here I collected *Cycas ophiolitica* (Fig. 1, teal points; Fig. 2), so called because it occurs on serpentine-derived soils (ophios, Greek for serpent or snake). The species faces pressures from development, and there was evidence that some plants had been relocated (Fig. 3).

A hybrid of *Cycas ophiolitica* and *Cycas megacarpa* occurs south of Rockhampton (Fig. 1, green point, Fig. 4).

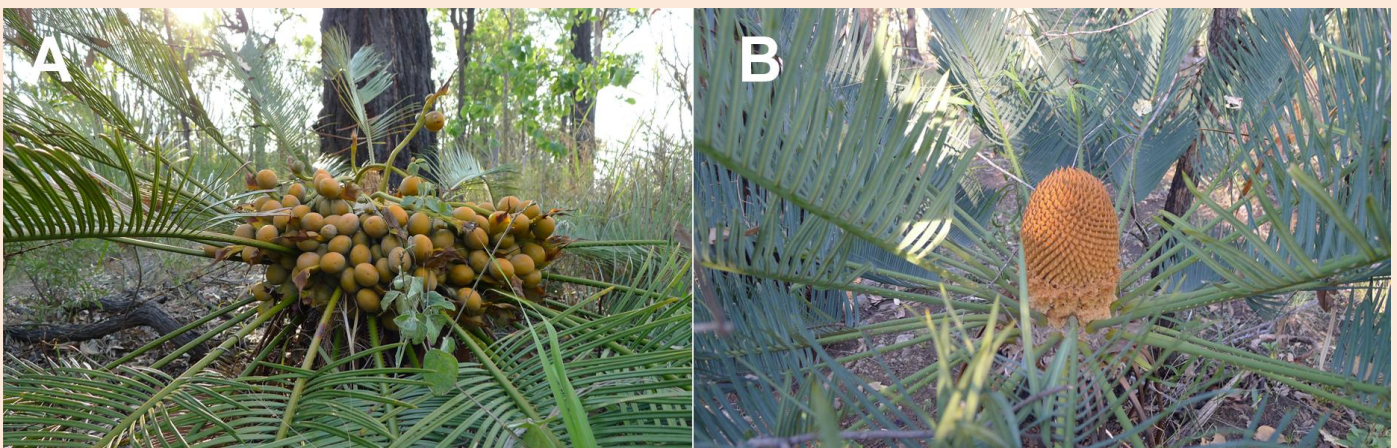


Figure 2. Reproductive structures of *Cycas ophiolitica*. A. Mature seeds, B. Young pollen cone.



Figure 3. Relocated plants of *Cycas ophiolitica*



Figure 4. Habitat of the hybrid *Cycas ophiolitica* x *Cycas megacarpa* in a *Eucalyptus* woodland. Black arrows indicate position of plants.

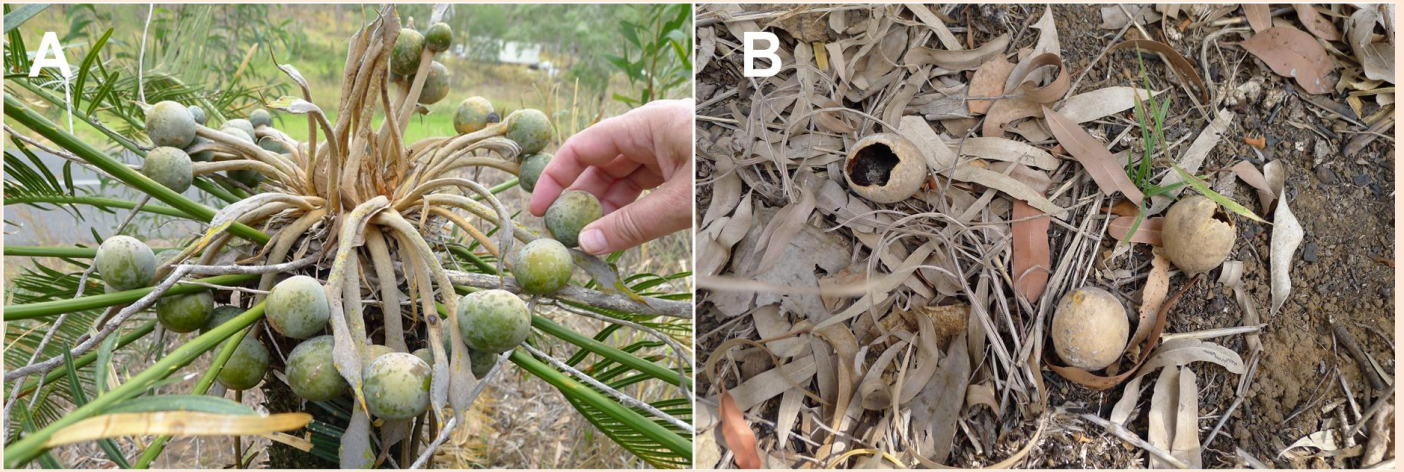


Figure 5. Seed of *Cycas megacarpa*. A. Maturing seeds, B. Dispersed, ungerminated seeds located at the base of the mother plant.

The hybrid occurs in close proximity with *Cycas megacarpa* (Fig. 1, burgundy points; Fig. 5). *Cycas megacarpa* is the most southerly species of *Cycas* in Australia. Again I noticed that some plants have been relocated, due to developments. Other plants were growing along roadsides, in private properties, and in national parks.

In 10 days, I travelled almost 3,000 km, and I collected leaflet samples from 153 plants in 17 populations. These samples are being used to improve our understanding of cycad

conservation, population genetics, and taxonomy. There were fewer sites than I expected—land has been cleared for agriculture and plants likely stolen from the wild. Unfortunately, cycads are the world's most endangered plants and searching unsuccessfully for these plants reinforces this sad reality.

This field work was supported by a grant from the Palm and Cycad Society of Australia.

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I CONGRESO MEXICANO DE CÍCADAS

LAS CÍCADAS: NUESTROS CONOCIMIENTOS EN CONSERVACIÓN, DEMOGRAFÍA,
ESTRUCTURA, ETNOBOTÁNICA Y GENÓMICA/SISTEMÁTICA.

2017
XALAPA, VERACRUZ

See announcement on page 23

News from the Entomology Group

Willie Tang

The international effort to identify and taxonomically describe insect pollinators of cycads in the northern hemisphere achieved a major breakthrough in May 2017. Based on recent field surveys in Thailand, India, Vietnam and other Asian countries, as well as study of museum specimens the erotylid beetle genus *Cycadophila* was reviewed (Skelley *et al.* 2017). Erotylid beetles have been found on cycad cones in all major cycad regions, including Africa, Australia, Asia and the Americas. Xu *et al.* (2015) erected the Asian genus *Cycadophila*, based on both morphology and molecular characters, as a sister genus of *Pharaxonotha*, which they recognized as being confined to the New World. In this latest publication Skelley *et al.* (2017) recognized a total of 21 species of *Cycadophila*, 13 of which are new to science. While many species of *Cycadophila* appear to be obligate specialists on the genus *Cycas*, feeding and reproducing only on *Cycas* cones, other members of *Cycadophila* may be facultative visitors to cones or may have no association with cycads. This contrasts with the situation in the Americas

where all known species of *Pharaxonotha* are specialists on cycads, with the exception of just one species. With this latest publication and two previous publications (O'Brien & Tang 2015, Xu *et al.* 2015) approximately half of the cycad cone beetles in the northern hemisphere have been described. Two other beetle groups, the cycad weevils of Asia and the erotylid beetles of the New World, remain to be reviewed before this initiative on northern hemisphere cycad beetles is complete. Once a fairly thorough taxonomic framework has been erected for northern hemisphere cycad pollinators, it will facilitate studies on cycad pollination ecology by local students and researchers and conservation policies for



Figure 1. Above: adult of *Cycadophila* (subgenus *Strobilophila*) *hiepi*, a probable pollinator of *Cycas elongata* and *C. pachypoda*; below: larva of the genus *Cycadophila* (subgenus *Strobilophila*); reproduced with permission from Zootaxa.

both cycads and cycad pollinators can be more confidently devised.

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Dioon: the cycads from forests and deserts

José Said Gutiérrez-Ortega, Karen Jiménez-Cedillo, Takuro Ito, Miguel Angel Pérez-Farrera & Andrew P. Vovides

There are few plant groups exhibiting greater habitat versatility than cycads. Despite their conservative morphologies and ecological functionalities, modern cycads were able to evolve into a great variety of habitats in almost all continents. It is interesting that some large cycad genera, such as *Zamia* and *Cycas*, have achieved a great habitat variation by reaching broad continental and island distributions. But now our attention is focused on a relatively small cycad genus, *Dioon*, due to its surprising combination of a restricted distribution, and wide habitat variation ranging from humid forests through mesic environments to deserts.

Fourteen species of the genus are restricted to tropical ecosystems occurring along the main Mexican mountain chains. One disjunct species is endemic to Honduras. The congruency between Mexican topography and the geographic distribution of *Dioon* (Fig. 1) suggests that orogeny has given rise to tropical habitats where the genus *Dioon* inhabits. At the same time, evolution of tropical ecosystems might have driven the diversification of *Dioon*. To test this hypothesis, we are researching into the evolutionary history of *Dioon* in the light of associations between habitat variation and interspecific variation.

Some of the most interesting climate variables explaining habitat variation within *Dioon* are those related to aridification. Aridification has been an important climatic factor in the diversification of tropical ecosystems in Mexico, and it might have also influenced habitat divergence among *Dioon* populations/species (Fig. 1). We have surveyed the populations within two different clades to document their habitat and population differentiations (Fig. 2). We plan to use a multidisciplinary approach to obtain evidence on ecological, genetic and structural factors that allowed the genus *Dioon* to attain such remarkable habitat differentiation in relation to aridification (Fig.3). We think that the evolution of *Dioon* towards arid zones might represent an important step in understanding cycad adaptation to changing environments. Such knowledge will contribute to make effective

conservation strategies for *Dioon* in the future.

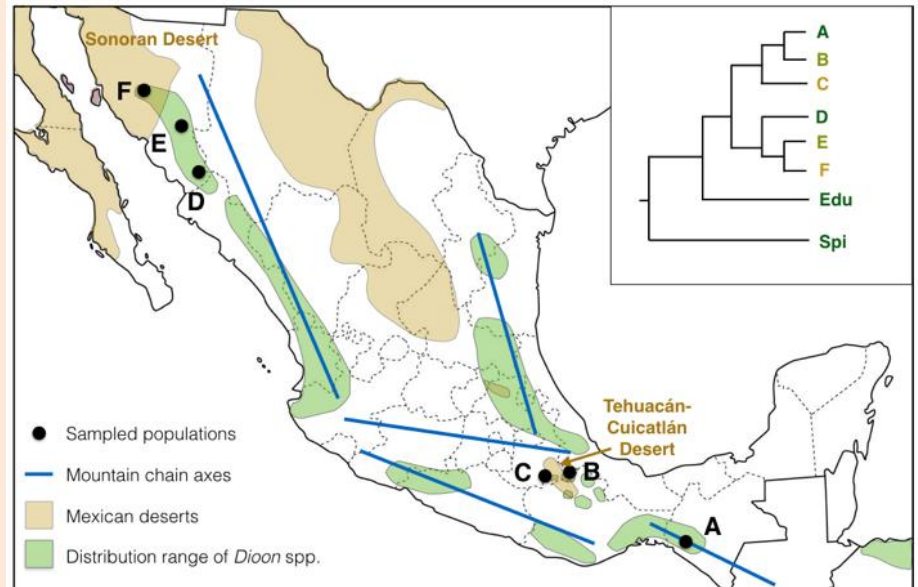


Figure 1. The distribution of the genus *Dioon* depicts the occurrence of the tropical forests along the main mountain chains of Mexico. However, at least two deserts have expanded towards the distribution ranges of *Dioon*. The expansion of the Tehuacán-Cuicatlán Desert reached the distribution range of one clade containing the populations A (*D. merolae*), B (*D. califanoi*) and C (*D. caputoi*). Parallel to this, the expansion of the Sonoran Desert promoted habitat differentiation among different populations of *D. sonorensis* (D-F). Inset shows the phylogenetic relationships of the populations we are studying, revealed by chloroplast DNA sequences. Edu = Edule clade; Spi = Spinulosum clade. Populations A-F correspond to those shown in Fig. 2.

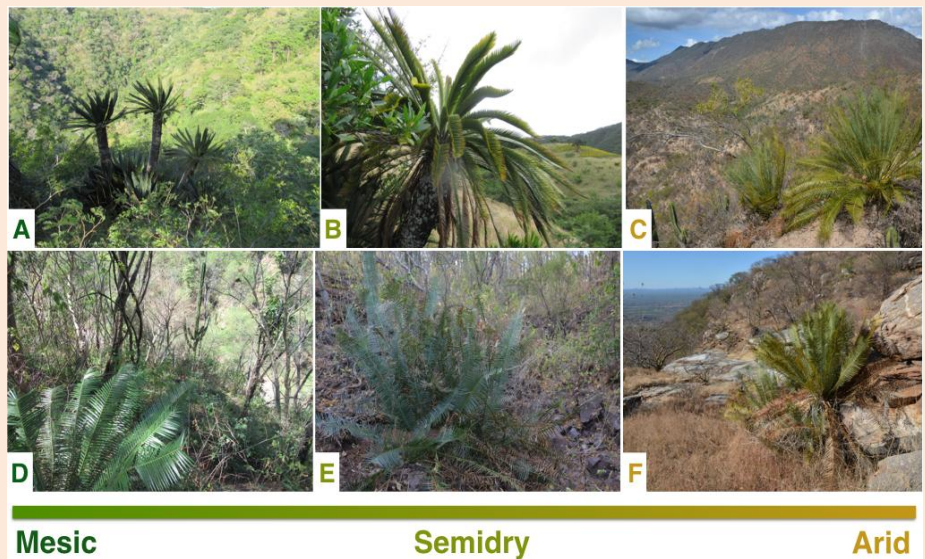


Figure 2. At least two *Dioon* clades show habitat differentiation throughout aridity gradients. Whereas *D. merolae* (A) inhabit mesic habitats in the State of Chiapas, *D. califanoi* (B) and *D. caputoi* (C) occur in drier conditions in the State of Puebla. Moreover, *D. sonorensis* occurs from the northernmost tropical forests of northwestern Mexico (D), towards the populations colliding (E) and embedding by the expansion of the Sonora Desert (F).



Figure 3. *Dioon caputoi* from the Tehuacán-Cuicatlán Desert in Puebla, Mexico, might be the best example of a cycad adapted to aridity.

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**The authors own all photographs in this article.

The biodiverse microbiome of cycad coralloid roots

Pablo Suárez-Moo & Angelica Cibrian-Jaramillo*

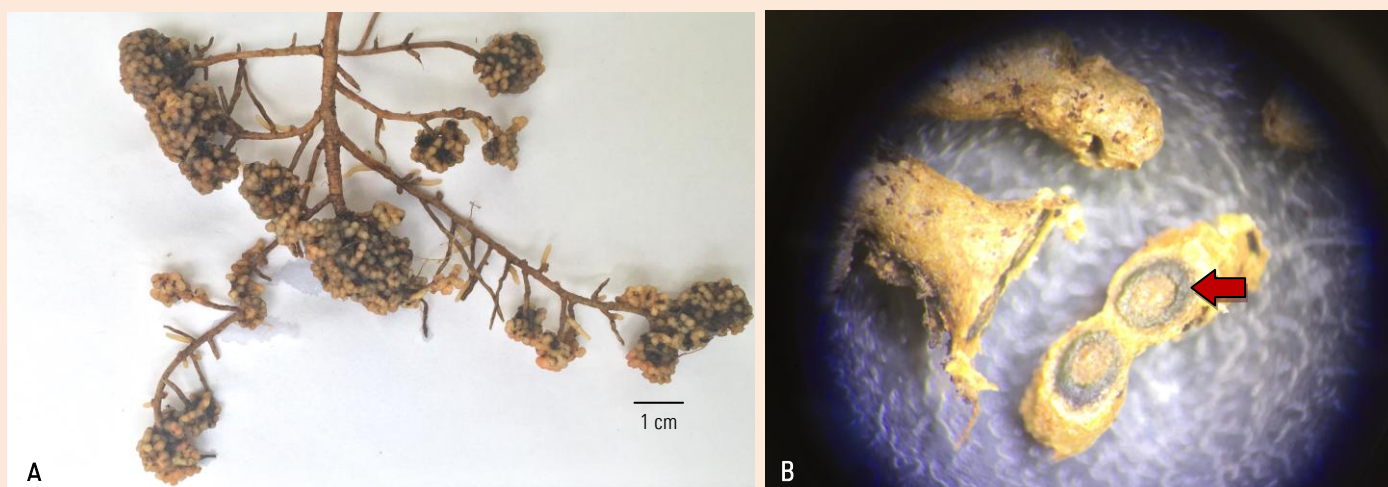


Figure 1. A. Photo showing an example of coralloid roots from *Dioon merolae* from Mexico. The coral-like structures can be observed in small clusters, B. Transversal cut of the coralloid root tip, the cyanobacterial zone can be seen inside the opened root (arrow).

Cycads are the only gymnosperms and the oldest lineage of plants that have specialized roots in small coral-like shapes, termed coralloid roots, which have the function to harbor symbiotic bacteria. Previous studies of the coralloid roots that trace back as far as the 19th century, mostly report a simple bacterial community composed of a few or many strains of cyanobacteria, *Nostoc*, and less frequently, *Calothrix*. We were inspired to look in more depth at the coralloid root microbiome, understood as bacteria communities and their genomes, based on the observation that in the process of infection of the coralloid roots bacteria can enter the plant through natural breaks in the roots epidermis layer and thus the infection could be open to many bacterial groups. But, why is it important to know if the bacterial community in the coralloid roots is taxonomically simple or diverse? We think that the association cycad-bacteria has conferred important evolutionary advantages to the plant so it can colonize and survive in environments that other plants cannot tolerate, in particular soils with low or difficult to access nutrients. Knowing the taxonomic and functional diversity of the coralloid root would therefore allow us to understand how microbiomes contribute to cycads fitness and their survival over evolutionary time, but also provides other practical knowledge like how to improve their success in *in vitro* or *in situ* propagation using its own bacteria to help it survive.

Our research group searched for different bacterial species and their functions in the microbiome of coralloid roots of species of the Mexican genus *Dioon* that we sampled from several natural habitats. We sequenced bacteria isolates and shotgun metagenomes of co-cultures of many bacteria simultaneously, and also massively amplified the 16s rRNA gene directly from coralloid roots, ending up with millions of DNA sequences that belong to different bacteria groups. The usual suspects of *Nostoc* cyanobacteria were found, as well as newly reported *Calothrix* and *Tolypothrix* cyanobacteria. Incredibly, we also found at least 200 different genera living inside the coralloid root, including *Burkholderia*, *Stenotrophomonas*, *Achromobacter* and *Rhizobium*, which are known nitrogen fixers in other plants. There were also many taxa we still do not know to which family or genus they belong to, and could be new species or genera (Cruz *et al*, 2017 <http://biorxiv.org/content/early/2017/03/27/121160>). We also looked outside of the root in the soil, and in the close region surrounding roots (rhizosphere) and we found that 22% of taxa were shared with the coralloid root, so we are investigating how and why only some bacterial groups get into the coralloid root and manage to survive (Suarez-Moo *et al*, unpublished). When we analyzed the function of the root microbiome, we found genes that can produce specialized metabolites implicated in the cyanobacteria-cycad interaction such as chemotaxis proteins and

peptide protease inhibitors and others that we are figuring out what they do inside the cycad. Interestingly, some of these genes were not found in genomes of free-living cyanobacteria compared to the group that only includes cycad symbionts, which from its phylogeny appears to be a very tight group indeed (Cruz *et al*, <http://biorxiv.org/content/early/2017/03/27/121160>). These results suggest some degree of co-evolution between cyanobacteria and their plant host, which we are in the process of confirming. Finally, we thought that if the coralloid root microbiome is really so important, the mother cycad has to ensure its inheritance via the seed. We looked in the embryo and the megagametophyte of *Dioon* and we found four genera that are the same as in its coralloid root, and that cannot be found in the male cone. Our next step is make sure they are indeed the same species as the root via the deep comparison of their genomes. So thus far our work shows that the coralloid root microbiome is an essential part of cycad life, and likely key to their lineage survival over millions of years. And excitingly, we are only at the tip of root of the coralloid in our discoveries.

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Unnoticed micromorphological characters in *Dioon* leaflets

Andrew P. Vovides, Sonia Galicia & M. Ydelia Sánchez-Tinoco

Cuticle information and epidermal characters have great potential in systematic studies. However, micromorphology and anatomical studies on cycads are relatively scarce in comparison to similar studies on other gymnosperms. In this study leaflet anatomy and cuticles in the genus *Dioon* have been investigated with bright field microscopy, epifluorescence, and scanning electron microscopy. Structures hitherto not completely studied for the genus are reported, especially G-fibers, and Florin ring-like structures and wax plugs associated with the stomatal apparatus.

Throughout the leaflets of *Dioon* spp in cross section there can be seen a varying number of thick-walled hyaline fibers both in the spongy mesophyll and surrounding the vascular bundles. These are non-lignified but have a lignified outer wall (S1 and S2 layers) that reacts positive when stained with phloroglucinol/HCl (a specific histochemical stain for lignin) and a very thick cellulose secondary wall (S3 layer) that does not react with the lignin test (Vovides & Galicia, 2016). Furthermore these fibers tend to tear apart when sectioning owing to the differing toughness of these layers. These are G-fibers and they provide certain flexibility to the leaflet and toughness that characterizes these cycad leaves (Fig. 1).

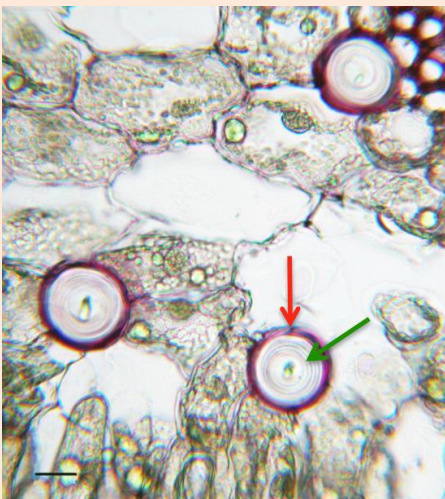


Figure 1. Section through *Dioon* leaflet showing G-fibers; lignified outer cell wall (red arrow) S1 and S2 layers (red arrow) and an unstained thick cellulose secondary wall (green arrow). Stain phloroglucinol HCl. Bar = 20µm.

Florin rings *sensu stricto* are cuticular thickenings of the periclinal walls of the stomatal subsidiary cells (Deckert *et al.*, 2001). In *Dioon* there are an additional three layers of encircling cells above the subsidiary cells, and here we have a cuticular thickening of the periclinal walls of the upper encircling cells forming the ring (Fig. 2) and is also evident under epi-fluorescence (Fig. 3). We recognize two types of Florin ring; that which is presented by the Pacific seaboard *Dioon* spp that we named the Purpusii type and that of the *Dioon* spp that occur along the Gulf of Mexico seaboard and Caribbean that we regard as the Edule type. These anatomical features agree with recent phylogenies (Moretti *et al.*, 1993; González *et al.*, 2008) and Gutiérrez-Ortega *et al.* (in review). In the Pacific seaboard species the epistomatal chambers are deep with varying numbers of papillae projecting into and partially occluding the chambers, the epistomatal pores being irregular in shape to elongate giving rise to an irregular Florin ring of the Purpusii type (Vovides & Galicia, 2016) though *Dioon merolae* and *D. holmgrenii* representing the southernmost range of the genus along the Pacific seaboard appear to be exceptional to this where epistomatal chambers do not present papillae or vary rarely so. These, along with the Gulf of Mexico seaboard species the epistomatal chambers are less deep than those of the Pacific seaboard clade. The depth of the epistomatal chamber and presence of papillae appear to be correlated with drier climatic conditions (Gutiérrez-Ortega *et al.*, in review; Vovides *et al.*, in review). In all species the epistomatal chamber is often filled with an epicuticular wax plug (Figs 4, 5) and along with the projecting papillae the occlusion by wax could mitigate temperature fluctuations, high light levels and also restrict transpirational water loss (Deckert *et al.*, 2001; Evert, 2006). Wax plugs are also found in the Pinaceae and are of guard cell and subsidiary cell origin (Johnson & Riding, 1981). Stomatal plugs occur also in other conifers (Podocarpaceae, Araucariaceae, and Cupressaceae (Evert, 2006).

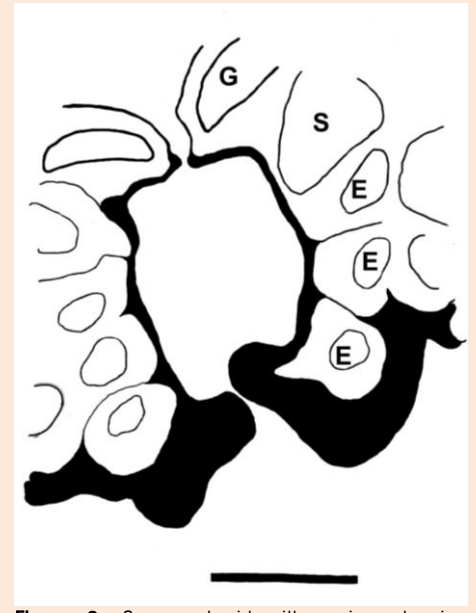


Figure 2. Camera Lucida illustration showing section through stomatal apparatus of *Dioon purpusii* showing thickened cuticle (black), G = guard cell, S = subsidiary cell, E = encircling cells. Cuticular thickening (black). Bar = 20 µm. From *Botanical Sciences* 94: 263–268 (2016)

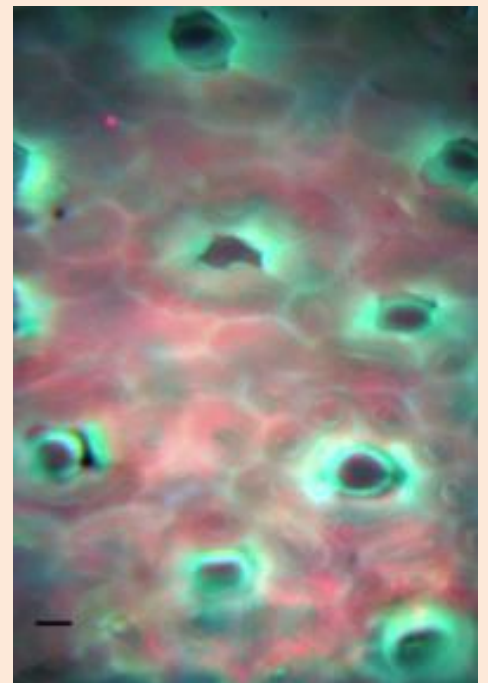


Figure 3. Epi-fluorescent image of stomatal pores and Florin rings (green). Bar = 20µm. From *Botanical Sciences* 94: 263–268 (2016)

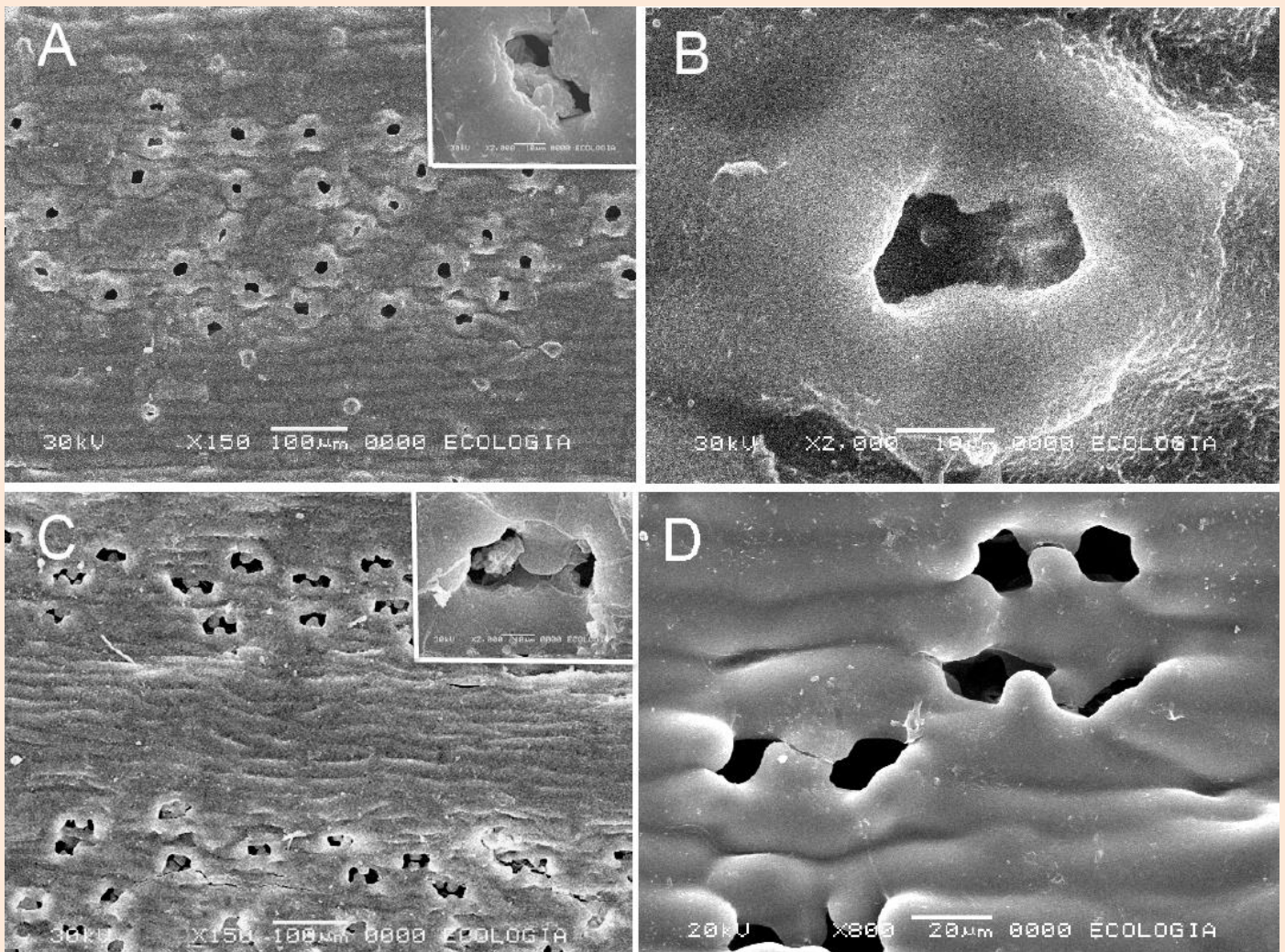


Figure 4. A-B: SEM of *Dioon edule* leaflet abaxial surface showing regular epistomatal pores, insert close-up of stomatal epistomatal pore with wax plug. B, Edule type Florin ring. C-D: SEM of *Dioon purpusii* leaflet abaxial surface showing irregular epistomatal pores with projecting papillae, insert close-up of epistomatal pore with wax plug. D, Detail of Purpusii type Florin ring. Bars: 100 µm in A and C, 10 µm in B, 20 µm in D. From *Botanical Sciences* 94: 263–268 (2016)

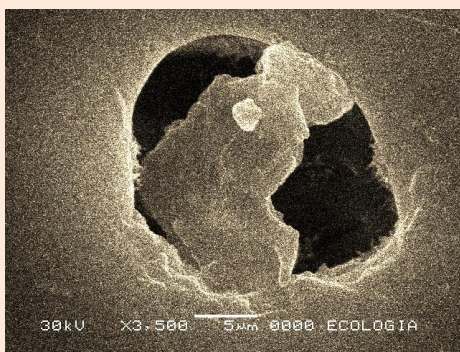


Figure 5. SEM image of wax plug in epistomatal pore of *Dionopanax spinulosum*. Bar = 5 µm.

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Optimizing the long-term storage and viability testing of cycad pollen

Michael Calonje, Claudia Calonje, Gregory Barber, Phakamani Xaba, Anders Lindstrom & Esperanza M. Agoo

Ex-situ conservation of plant genetic resources can be achieved by seedbanking, field genebanks, in vitro storage methods, pollen banks, and DNA banks (Laliberté 1997). Cycad seeds are recalcitrant and unfortunately cannot be stored for the long-term in seedbanks. In addition, in-vitro tissue culture propagation of cycads has not been implemented successfully at a large scale. Therefore, the conservation of cycad germplasm is limited to field genebanks, also known as living collections, and pollen banks. These activities are usually undertaken by botanical gardens housing well-documented, scientifically valuable, and actively managed cycad collections.

The long-term storage of cycad pollen, also known as pollen banking, is of critical importance for maintaining living collections, as pollen is necessary to produce seeds, and fresh pollen may not always be available when needed due to the lack of reproductive synchronicity between ovulate and pollen-bearing plants. In addition, pollen banking allows institutions housing living collections to exchange pollen and be able to propagate plants that they would otherwise be unable to. Unlike recalcitrant cycad seeds, cycad

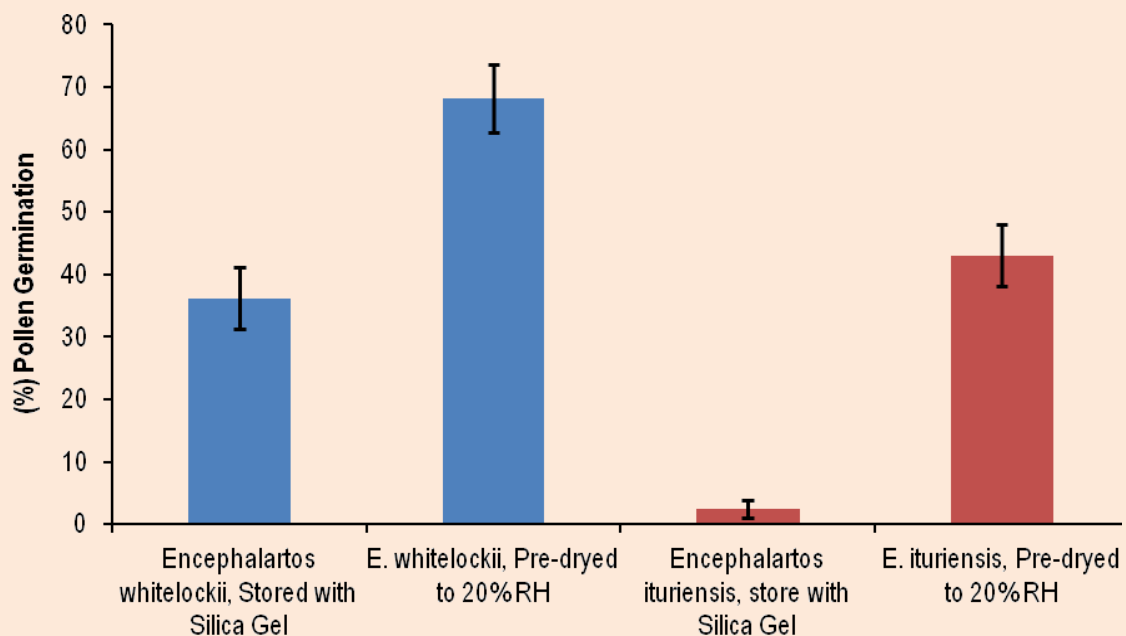
pollen is desiccation tolerant and can be stored long-term in freezing subzero degrees and dry conditions (Osborne *et al.*, 1992), serving as a gene repository of sorts, although with the caveat that some maternally-inherited genes such as chloroplast and mitochondrial genes (Zhong *et al.*, 2011) are not preserved in pollen.

Longevity of cycad pollen

While several articles have been written that include techniques for cycad pollen collection, storage, and hand pollination (e.g. Tang 1986a; Broome 2004, Calonje *et al.* 2011), a very limited amount of work has been published evaluating the longevity of fresh or cold-stored cycad pollen (e.g. Tang 1986b, Osborne 1989, Osborne *et al.* 1992).

Cycad pollen can be stored for up to two years at 0°C and three years at -15°C while retaining adequate (>50%) germinability (Osborne *et al.*, 1992), and it has been shown to survive after three months of cryopreservation at 196°C in liquid nitrogen, so it could potentially be stored indefinitely (Osborne, 1989). Osborne *et al.* (1992) showed that storing cycad pollen over desiccant has a slightly detrimental effect on the pollen's

viability compared to pre-drying previous to storage. Indeed, some recent trials conducted by Phakamani Xaba, Anders Lindstrom, and Esperanza Maribel Agoo suggest that predrying *Encephalartos* pollen in a moisture cabinet with 20% relative humidity results in significantly higher viability than drying and storing the pollen along with silica gel (fig. 1). Even so, many institutions store cycad pollen along with the desiccant primarily because it serves as a protection against moisture intrusion during cold storage, but also because the optimum moisture content for pollen has not been determined and no published protocols exist detailing methods for pre-drying pollen prior to cold storage. Moisture content significantly affects its longevity (Marks *et al.*, 2014; Towill, 1985) yet the optimum moisture levels required to retain cycad pollen viability in cold storage remain unknown and consequently no pre-drying protocols exist. Future research on cycad pollen storage should include the determination of optimum moisture content for cycad pollen storage and the creation of simple desiccation protocols to quickly reach this optimum without significant loss of pollen viability in the process.



Effects of Pollen Desiccation Prior and During Storage

Figure 1. Comparison of germination of pre-dried and silica gel stored *Encephalartos* pollen. Cold-storage temperature was -18C, (\pm SE) (n =20).

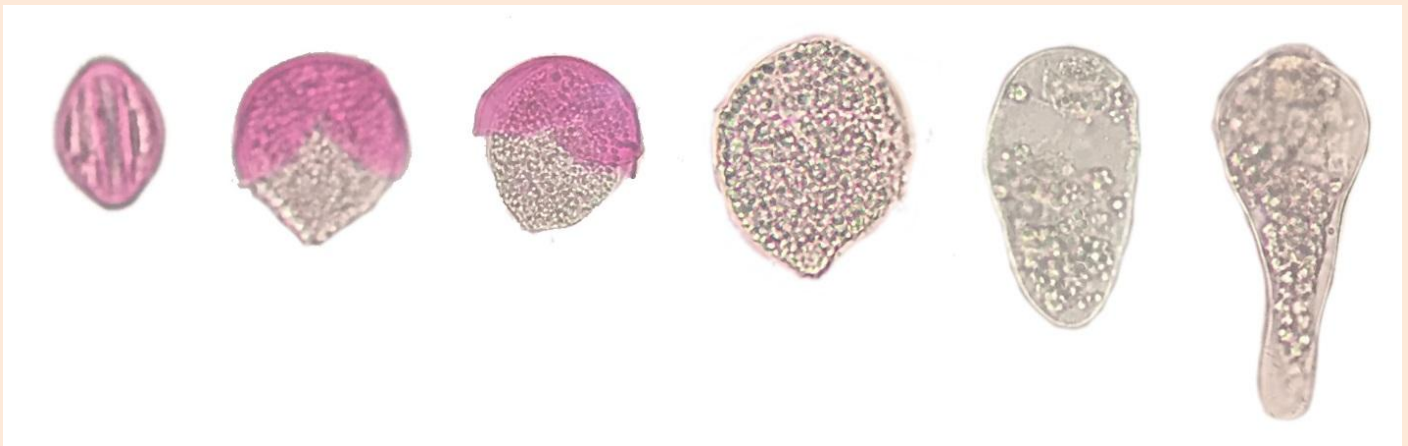


Figure 2. Stages in the germination of pollen of *Lepidozamia peroffskyana*.

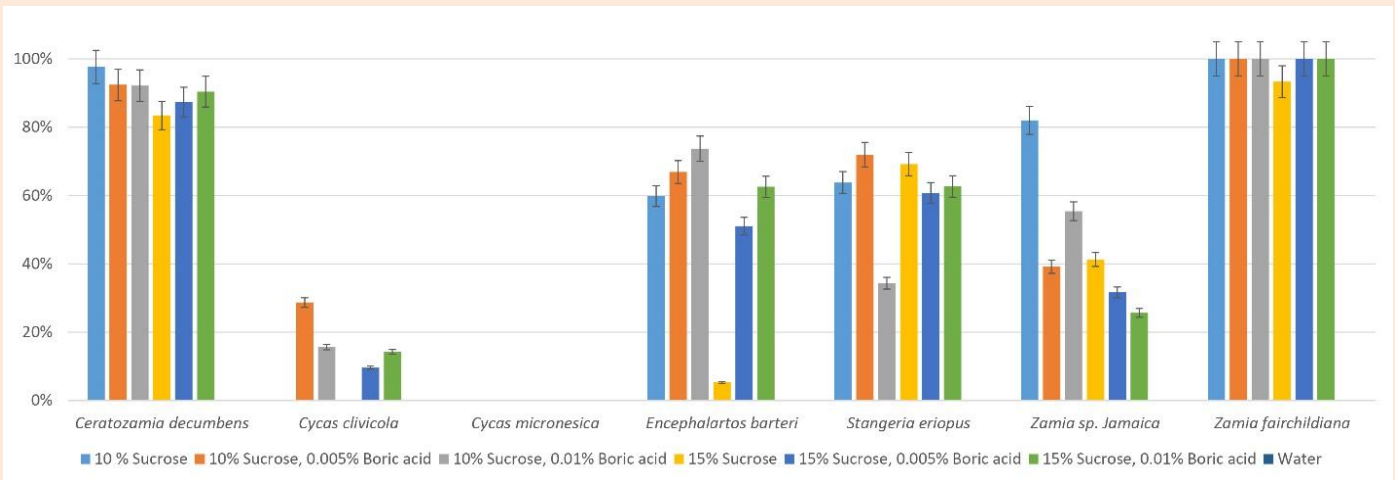


Figure 3. Comparison of germinability of fresh pollen of seven different species germinated under seven different treatments

Pollen viability testing

Evaluating the effect of different drying and storage regimes on pollen viability requires reliable methods for evaluating pollen viability. A few chemical stains have been tried to estimate cycad pollen viability (Tang 1986b, Calonje *et al.* 2011) but very few of them have been tested on cycads (Calonje *et al.*, 2011), some of them may not work for all genera (e.g. Nitroblue Tetrazolium with *Dioon*, Tang 1986b). Furthermore, some of these chemical stains can be extremely hazardous and require great care and access to specialized laboratory equipment (such as fume hoods) for safe handling.

The preferred method for cycad pollen viability testing is to actually germinate the pollen (Fig. 2). Germinability is considered a reliable estimate of viability as this is what pollen does naturally when coming into contact with the micropylar droplets on ovules. Pollen germination is typically done in a nutrient media including sucrose and one or several additional chemical compounds. One of the challenges with germinating cycad pollen is that the growth of pollen tubes is very slow compared to that of flowering plants, and at least three days are required to observe measurable germination (Osborne *et*

al. 1992). The long duration required for cycad pollen germination also means there is a higher chance that fungal contamination detrimentally impacting germination can occur.

Preliminary pollen germination trials using germination solutions with different concentrations of sucrose and boric acid have been conducted at Montgomery Botanical Center, with successful germination observed in most cycad genera, but very weak or no germination of species of the genus *Cycas* (Fig. 3). Preliminary data obtained during these trials suggests that there is no single optimum germination solution, but rather the best solution may vary between taxa. However, trials including additional taxa and germination solutions are required to determine which may be the best performing solutions for pollen germination. Additional chemicals may be required to optimize germination of *Cycas* pollen, but so far, trials including the addition of Calcium Nitrate by Phakamani Xaba have been unsuccessful, and separate trials by Esperanza Maribel Agoos have had limited success in germinating pollen for only a very small percentage of species and treatment combinations attempted.

Conclusion

As several researchers and institutions associated with the Cycad Specialist Group are currently collaborating on evaluating different cycad pollen storage and viability testing protocols, we expect these collaborations to result in improved protocols to improve the quality of stored pollen and therefore increase the number of viable seeds that can be produced by hand pollination.

Acknowledgements

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Former seedbank coordinator Claudia Calonje and volunteer Debb DuMond pollinate *Encephalartos sclavoi* at Montgomery Botanical Center, Florida, USA

Abnormal forking of pinnae in some Asian cycads

JS Khurajam*, Rita Singh, SC Sharma, RK Roy, Simon Lavaud & Sathitvong Chayangsu

Cycas are ancient group of plants that resemble palms and tree ferns in having columnar trunks and crowns of pinnately-compound leaves at the apex. *Cycas* produces two types of leaves - small scale leaves (cataphylls) and large green foliage leaves. Both these leaves are arranged compactly around the apex of the trunk in a continuous alternation. Scale leaves are small, dry, brown, triangular in shape, and covered with hairs and are non-photosynthetic. Young foliage leaves have circinate vernation like those of ferns and are always covered with brown hairs which are shed on maturation. Each foliage leaf consists of a central vascularised rachis which bears numerous laterally-flattened photosynthetic pinnae having decurrent bases. The lower pinnae of rachis are often modified into spines which may be arranged opposite or alternate. The pinnae are mostly narrow and linear with a single midrib running their entire length. The margins of pinnae may be revolute (as in *Cycas beddomei* Dyer) or they may be flat (as in *C. annaikalensis* Singh & Radha, *C. circinalis* L. and many others). Pinnae are usually simple in most of the species but are once or twice forked as in some Southeast Asian species e.g. *Cycas bifida* (Dyer) Hill, *C.*

debaensis Zhong & Chen, *C. micholitzii* Dyer and *C. multipinnata* Chen & Yang. *Cycas* species in India all have the more typical foliage type, i.e. unipinnately compound leaves with numerous thick and leathery, sessile, ovate-lanceolate, spine-tipped pinnae which may be alternately or oppositely arranged on the rachis. However, abnormal formations of forked pinnae have been observed in some leaves of *C. pectinata* Buch-Ham. and *C. revoluta* Thunb. (Pant, 1953; Chavan *et al.*, 1962; Satake, 1981). Likewise, a number of abnormalities in *C. circinalis* and *C. revoluta* have been recorded from plants growing in the gardens at Baroda (Chavan *et al.*, 1962). The abnormalities consisted of fusion and branching of pinnae and formation of additional pinnae. This phenomenon is not known from other Indian species. In the present article, we report on abnormal forking in pinnae of six Asian species, namely *C. beddomei*, *C. elongata*, *C. nayagarhensis*, *C. pectinata*, *C. siamensis* and *C. sphaerica*.

The morphological study of pinnae of six Asian *Cycas* was carried on mature plants and seedlings under ex-situ conservation at CSIR-NBRI Botanic Garden in India and under cultivation in private gardens at France and

Thailand. Seedlings were germinated from seeds collected from natural populations in India (*C. beddomei*, *C. nayagarhensis*, *C. pectinata*, *C. sphaerica*) and Myanmar (*C. pectinata*). Seeds of *Cycas elongata* and Thailand *C. pectinata* were collected from cultivated plants at Thailand. Seeds of *C. beddomei*, *C. nayagarhensis*, *C. pectinata*, *C. sphaerica* were germinated under controlled environment while those of *C. elongata* were germinated under natural conditions.

Seedlings of *Cycas beddomei* developed different forms of forking resembling the natural forking of pinnae in *Cycas bifida* and *C. micholitzii* (Fig.2, A-C). This unique feature is reported here for the first time for this endangered species. Pinnae of three months old seedlings developed these unusual characters. The forking usually starts at median portion of the pinnae but some started forking at the base. It was also observed that the pinnae dichotomously bifurcated from the base as in *Cycas bifida*. Likewise, six months old seedlings of *Cycas sphaerica* developed two types of forking – bifurcation at tip and at the median portion of the pinnae (Fig. 2, D-E; Table 1).

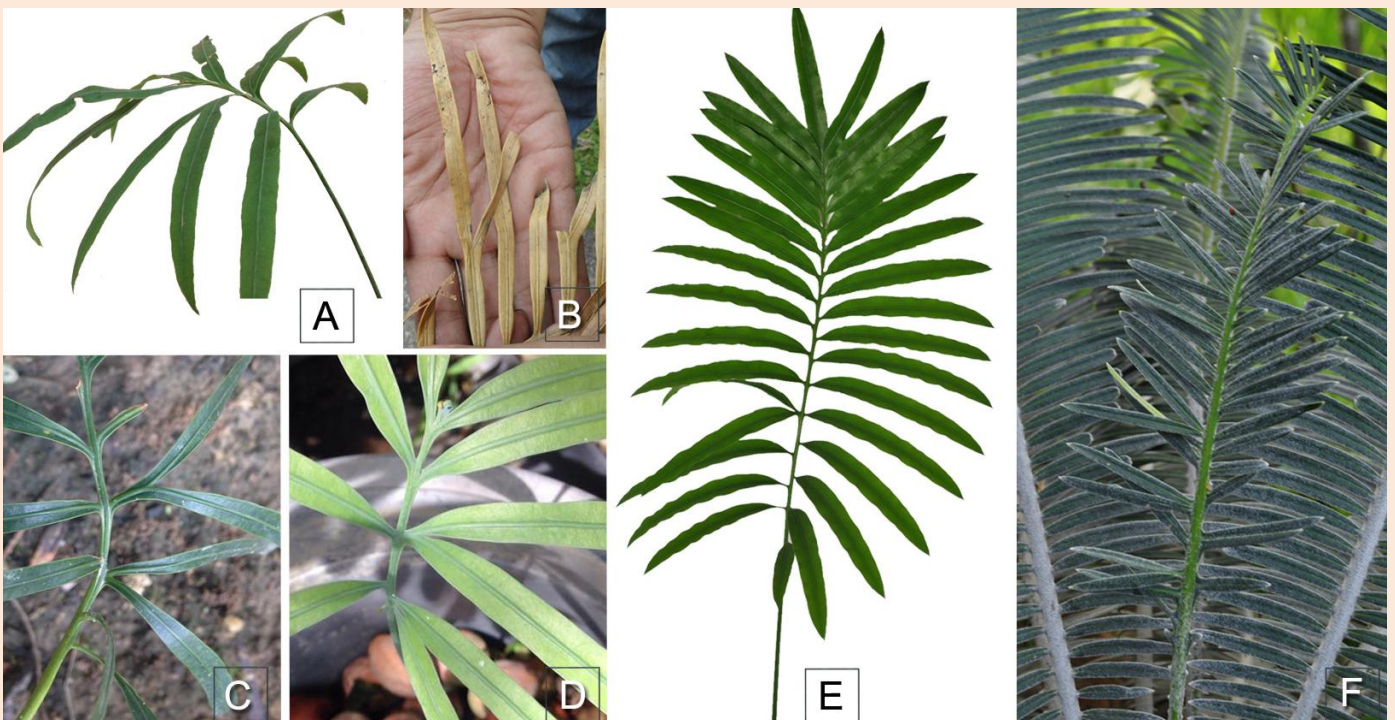


Figure 1. Abnormal pinnae of *Cycas pectinata* (A-D), *Cycas elongata* (E) and *Cycas siamensis* (F).

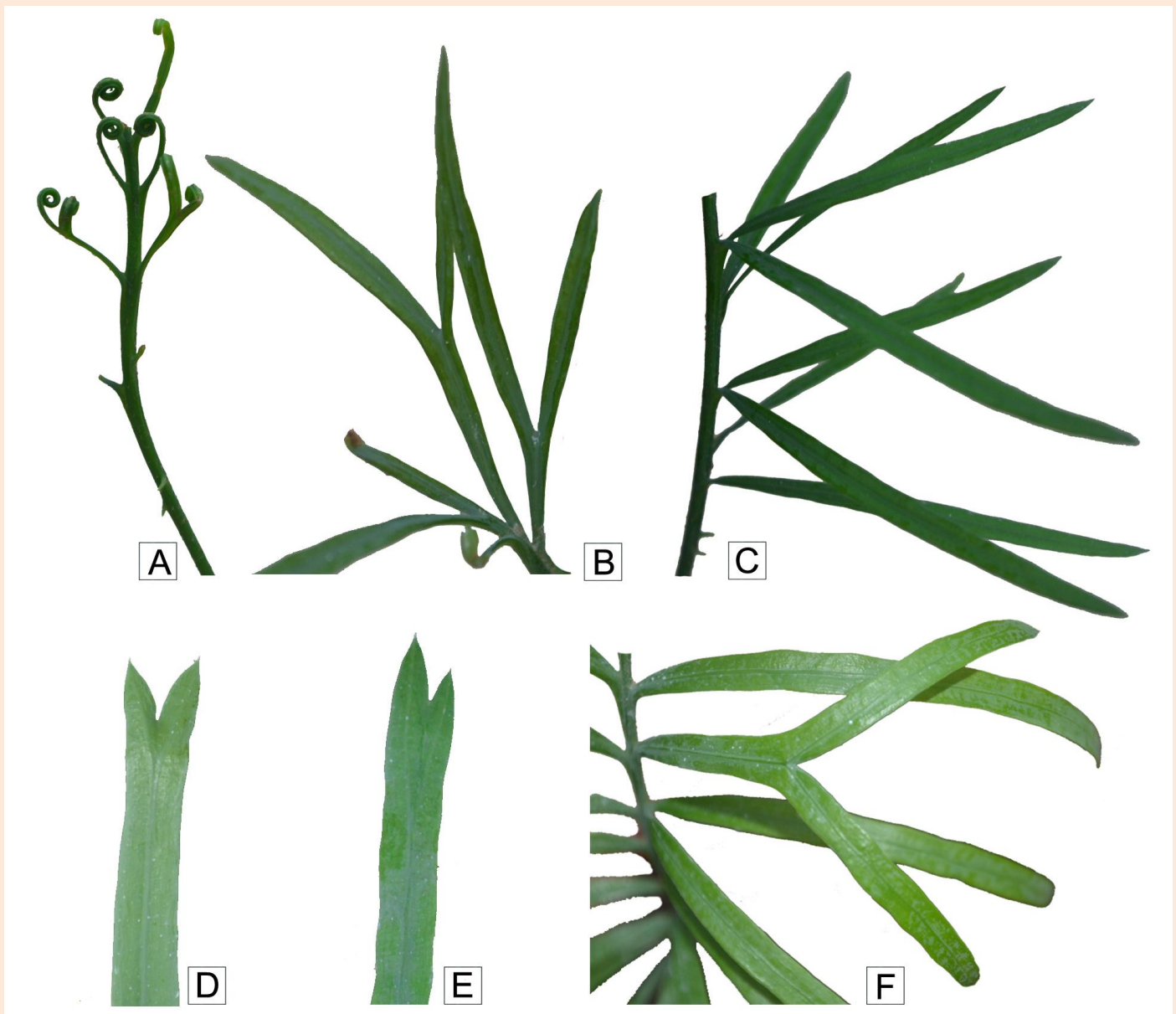


Figure 2. Pinnae of *Cycas beddomei*(A-C) and *Cycas sphaerica* (D-F) showing different forms of forkings.

Table 1. Pinnae abnormalities in seedlings of five *Cycas* species.

Species	Total number of seedlings	Number of seedlings with forked pinnae	Number of leaves with forked pinnae
<i>Cycas beddomei</i> Dyer	40	6	12
<i>Cycas sphaerica</i> Roxb.	40	4	9
<i>Cycas elongata</i> (Leandri) D. Yue Wang	8	1	1
<i>Cycas pectinata</i> Buch.-Ham (Myanmar)	20	5	10
<i>Cycas pectinata</i> Buch.-Ham (India)	15	4	12
<i>Cycas pectinata</i> Buch.-Ham (Thailand)	50	4	7
<i>Cycas nayagarhensis</i> Singh, Radha & Khuraijam	50	1	1

In all the cases, the midrib of the pinnae split into two but the divided midribs remained attached to the main midrib. After shedding leaves with forked pinnae, the seedlings developed normal pinnae without any forking. Since the unusual forkings in *Cycas beddomei*, *Cycas nayagarhensis* and *Cycas sphaerica* were observed in seedlings developed from seeds collected from natural habitats Eastern Ghats, the seedlings in natural habitats need to be examined to determine whether the

forking is an abnormality or a prominent character in young plants. The forking character was not observed in mature plants in natural populations or in cultivated plants.

Cycas pectinata has the most widespread distribution among all the species studied. The species is known to produce abnormal pinnae under cultivation in India (Pant, 1953). In the present study, the seedlings of *Cycas pectinata* germinated from seeds collected

from India, Myanmar and Thailand, developed abnormal leaves with forked pinnae. Seeds of *Cycas pectinata* of Myanmar took around 6 months to germinate and out of the 20 seedlings, 5 of them showed abnormal pinnae. Out of the 15 seedlings of *Cycas pectinata* of India, four seedlings have abnormal pinnae with different levels of forking. Four seedlings of the species from Thailand have forked pinnae which in some cases being forking at the base attached to the rachis. Abnormal

pinnae were also observed in a single seedling of *Cycas elongata* out of eight under cultivation in France (Fig. 1,E).

Abnormal pinnae were also observed in mature plants of *Cycas pectinata* in natural populations in Assam, India (Fig. 1,B) and *Cycas siamensis* under cultivation (Fig. 1,F).

Conclusion

Cycas plants are cultivated in ornamental gardens due to their decorative crowns of evergreen leaves. Leaf characters in most of the species are not considered taxonomically conservative as is the case with female and male cones. However, in some species, such as *C. bifida*, *C. debaoensis*, *C. micholitzii* and *C. multipinnata*, their forked and dichotomous pinnae are diagnostic characters which clearly separate them from other species without necessarily observing their reproductive characters (Chen *et al.*, 2004). Abnormal forked pinnae developed in seedlings are inconsistent characters that are not taxonomically important. Even though these characters are not prominent, they can be useful for elucidating leaf development

studies and mutational aberration in cultivated plants and natural populations. These characters could also help in deciphering information on their adaptation, habitat and change in environment.

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Cycas orixensis in natural habitat, Odisha, India

Get to know the world's most endangered plants free online educational video

James A. R. Clugston & Nathalie S. Nagalingum



Introductory image of the video: cycads growing in South African beds in the living collection of the Royal Botanic Garden Edinburgh.

Cycads are a model group for both education and science communication because of their incredible evolutionary history, ancient origin, and conservation threats. Cycad are at risk due to illegal poaching, over-collection, and habitat loss, and communicating these threats is very important to cycad survival. In a highly connected world, science communication is vital and online media can quickly get the message across. To promote both cycads and the Cycad Specialist Group (CSG), we created a short video entitled "Get to know the world's most endangered plants". The video introduces the CSG, and explains cycad history, morphology, diversity, and reproduction. It is freely available on the CSG website, VIMEO, and YouTube. We hope the video will become a resource for all those who have an interest in getting to know and conserving the cycads.



Nathalie Nagalingum discussing cone morphology of *Stangeria eriopus* in the video.

QR code



To view the video follow
<https://vimeo.com/228256410>
<http://www.cycadgroup.org/?p=562>

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CONSERVACIÓN/DEMOGRAFÍA
Preside: **DR. PABLO OCTAVIO AGUILAR**
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ESTRUCTURA/FISIOLOGÍA
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FECHAS:

Límite para el envío de resúmenes es el viernes 22 de septiembre de 2017.

El resultado de los trabajos aceptados será dado a conocer el viernes 6 de octubre de 2017.

INSTRUCCIONES PARA LA ELABORACIÓN DEL RESUMEN:

El resumen deberá incluir: i) Al inicio de la página, arriba del texto, en la parte superior izquierda anotar la temática y presentación: oral o cartel, ii) Título, iii) Autores, iv) Institución de adscripción, v) Correo electrónico del autor que presentará la ponencia (señalado con un asterisco), de lado derecho, vi) El cuerpo del resumen: máximo 300 palabras, texto justificado, letra Arial 12, espacio entre regiones de 1, y márgenes superior e inferior 2 cm, derecho e izquierdo 2 cm, ver ejemplo en archivo adjunto, y vii), Palabras clave (un máximo de cinco).

El resumen deberá enviarse como documento Word 6.0 o posterior. Los autores serán los responsables del contenido de su resumen. Las contribuciones podrían ser rechazadas si el tema queda fuera del ámbito del congreso o que se trate de una investigación con resultados en un grado de avance incipiente.

TEMAS:
SISTEMÁTICA
ETNOBOTÁNICA
GENÓMICA
CONSERVACIÓN
DEMOGRAFÍA
ESTRUCTURA
FISIOLOGÍA

PREPARACION DE CONTRIBUCIONES ORALES Y CARTELES:

Las contribuciones orales tendrán una duración total de 20 min (15 min para la exposición y 5 min para preguntas). El tamaño de los carteles será de 90x120 cm; para su diseño debe considerarse que su contenido sea claramente visible a una distancia de 1 m.

La modalidad de exposición (oral o cartel) será seleccionada por los autores en el momento de enviar el resumen; sin embargo, la forma de presentación definitiva será dada a conocer a los ponentes a más tardar el 6 de octubre de 2017.

ATENTAMENTE,

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FOTOGRAFÍA: JOSÉ LUIS LLAGUNO ROQUE

CYCAD 2018

FIRST ANNOUNCEMENT



CYCAD 2018, the 11th Conference on Cycad Biology, is to be held next year in South Africa.

It will be hosted by the **Cycad Society of South Africa**. We are not exactly novices, having hosted the 3rd Conference in 1993. It will be organised under the auspices of the **Cycad Specialist Group** of the **International Union for the Conservation of Nature (IUCN)**.

In order to have your name entered on our mailing list for future announcements, send an E-mail message to Wynand van Eeden at wynand@ananzi.co.za or Dr Piet Vorster at pjvors@gmail.com.

When? The Conference will take place from 19 to 24 August 2018. The program will be announced as soon as it has been finalised. August is at the end of our winter, when the days are balmy rather than hot, the rains are still some way off, and the exquisite African plants start budding and flowering.

Where? The Conference will take place at a lodge near **Nelspruit**. This is at the foot of the escarpment, close to the Kruger National Park. The Lowveld National Botanical Garden in Nelspruit has a very good collection of *Encephalartos*.

What are we offering? The Conference will be similar in structure to previous Conferences in the series. It will be a weeklong event with four or five days of research presentations arranged as symposia, and a surprise mid-week excursion.

Because non-professional aficionados are not always interested in the procedures and results of cutting-edge research, we hope to

organise parallel symposia on subjects close to the hearts of collectors. We acknowledge the important contribution of non-professional cycadophiles to the expansion of our knowledge of cycads, and encourage as many collectors as possible to attend the Conference.

Proceedings of the Conference: as with previous Conferences, the papers presented will be published in a volume of Proceedings. These papers will be peer reviewed.

We see the Proceedings in a very serious light. We consider all the papers to further our knowledge of cycads, rather than contributing to disciplines like anatomy or physiology, and accordingly we would like to publish them in the Proceedings rather than have them scattered in some other journals, which may not be readily accessible to the general cycadophile. For this reason we would like to include all the presented papers in the Proceedings.

We aim to publish the Proceedings as rapidly as possible and therefore papers, with completed manuscripts handed in at registration, will get preference.

Field trips: there will be pre- as well as post-Conference field trips to see cycads in their natural habitats. Watch this space for details.

Accommodation: we are not going to put you in a 5-star hotel. Instead our accommodation will be convenient, clean, and safe; but breathing the atmosphere of

the African bush. We guarantee bird song by day, the voices of the bush at night, and who knows, the far-off call of a jackal or the roar of a lion before you enter dreamland.

Our food will be of the kind you only get in Africa. You definitely don't have to be adventurous to enjoy our dishes. However, if you have special dietary preferences, we can accommodate you.

Our beer is amongst the best in the world, not to mention the famous Cape wines, and we even manufacture Coca-Cola.

Acquisition of plants: in South Africa, cycads are strictly protected by local laws as well as international agreements. Nevertheless we will try to make available, especially to our foreign guests, a range of seedling plants at affordable prices. We will try to arrange for someone with experience of exporting plants to organise transport and export permits on your behalf, and ship your plants to your homes.

We fervently hope to see you here next year for the Conference of your life!



Visit CSSA for further information
<http://www.cycadsociety.org/>



CSG member Nathalie Nagalingum has recently been appointed as an Associate Curator of Botany and the Decker and Martha McAllister Chair of Botany at the California Academy of Sciences in San Francisco, USA.

The Cal Academy was established in 1853, and today is a combined natural history museum with public exhibits, and scientific institution housing millions of collections. With its mission to “Explore, Explain, and Sustain life on Earth”, there is a strong focus on education, outreach, science, and conservation.

The exhibits at Cal Academy attract 1.5 million visitors per year. One of the popular exhibits engages visitors to learn about Earthquakes by showing how shifting continents have shaped landscapes and life on earth.



The Earthquake exhibit includes a display on cycads with living specimens and models, as well as signs explaining the age of cycads

and the impact that the break-up of Gondwana had on cycads.

At the Cal Academy, Nathalie continues her research on cycad phylogenetics, population genetics, and conservation. A principal component of her work is on the genus *Cycas*, with a special focus on using NGS (genomics) to understand the genetic diversity of Australian species. There is a genomics laboratory (Center for Comparative Genomics), which is fully equipped for state-of-the-art molecular work. The botany department houses a herbarium (acronym CAS) with approximately 2.5 million collections, dating to the late 1800s onwards and with 11,000 types.

If you would like to join Nathalie’s research group, collaborate on cycad projects, or visit the Cal Academy, please email her at nnagalingum@calacademy.org



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