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# CYCADS

Official Newsletter of IUCN SSC Cycad Specialist Group



December 2016



Dr. Dennis Stevenson  
awarded the Cycad D'or

Feature Articles  
Research & Conservation News  
CSG Member News  
New CSG Members (2015)



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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Cover photo: Male cone of *Cycas  
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Participants from 21 countries at Cycad 2015, Medellin, 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](mailto:jskhuraijam@yahoo.com)

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<http://www.cycadgroup.org/>

CSG Programme Office: Montgomery Botanical Center, 11901 Old Cutler Road, Coral Gables, FL 33156, USA

# MESSAGE

Welcome to the first issue of *Cycads*, the Newsletter of the Cycad Specialist Group! I am excited to introduce our new venue for communicating important new findings and conservation updates on this fascinating group of living treasures.

And, it is a wonderful issue to begin with, with a great diversity of papers on subjects that range from workshop reports, to updates on long term conservation efforts, previews of research, and improvements in taxonomy and nomenclature.

At its foundation, our Specialist Group derives its strength from its members, who bring their considerable expertise and experience to bear to help conserve these wonderful plants. I am pleased to report that our recent effort to broaden and expand the CSG has brought forward many accomplished scientists from around the world, as well as students working on cycad projects. I am especially proud of the CSG's effort to include these enthusiastic, early-career dynamos in our group – our long-term work absolutely depends on this next generation. Our newsletter thus also features these new members, of all levels of experience, who are helping to understand and conserve cycads around the world through their work.



Patrick Griffith at the IUCN World Conservation Congress 2016, Hawaii

You do not have to look far to see how our broadened membership moves us forward – this newsletter itself is the result of the vision, enthusiasm, and skill of one such expert, JS Khurajam, who joined the CSG in August 2015. He saw our need for enhanced communication, both within our group and with the broader public, and today you are reading the results of his tireless efforts to produce this new publication. The next time we are all together, JS will deserve a round of applause!

At the recent IUCN World Conservation Congress, the crucial role of our Specialist Groups was made very clear and very prominent. Bringing science-based, accurate information to inform conservation action is the way forward. I was pleased to see some of our members there, and left with even greater enthusiasm and energy to carry our work forward.

Again, please let me welcome you to our first issue. I look forward to reading your contributions in many more such issues over the years!

Sincerely,

Patrick Griffith  
Co-Chair, IUCN/SSC Cycad Specialist Group  
Executive Director, Montgomery Botanical Center, Florida  
✉ patrick@montgomerybotanical.org





Dennis Stevenson (right) receiving the award from Michael Calonje | Photo: Alan Meerow

## Dr. Dennis Wm. Stevenson awarded the Cycad D'or at Cycad 2015

The Cycad d'Or, or "Golden Cycad" was awarded to Dr. Dennis Wm. Stevenson at Cycad 2015 (10th International Conference on Cycad Biology, Medellin, Colombia) for his contributions to cycad science, his longtime support for international conferences on cycad biology and his commitment to training students in cycad research.

The specially designed plaque included the silhouette of a leaf of *Zamia stevensonii*, one of two species named in his honor. Dennis is the only person to have attended all 10 international conferences on cycad biology, beginning with the 1st conference held in France in 1987.

Dennis obtained his Ph.D. in 1975 from the University of California, Davis and is currently Cullman Curator & Vice President for Botanical Research at The New York Botanical Garden. He is editor of *The Botanical Review*, *Cladistics* and associate editor of *Blumea*. His primary areas of expertise include Cycads, Monocots, and Genomics. His major research interests in the past few years have focused upon the evolution and classification of the Cycadales (cycads) and their placement in seed plant phylogeny. Dennis has published 28 different cycad species names, including 20 names within the genus *Zamia*, the most for any author.



*Zamia stevensonii* Photo: Michael Calonje



*Dioon stevensonii* Photo: Michael Calonje



Patrick, Cristina, Dennis, Alan, and Angelica | Photo: James Clugston

For decades, Dennis W. Stevenson has been the catalyst for numerous studies of cycad morphology, anatomy and systematics, as well as the organizing force behind the tri-annual international meetings on cycad biology, and the IUCN/SSC Cycad Specialist Group. He has mentored numerous young botanists, many of whom are now leaders in the field. Dennis, in his 8th decade, still approaches the world of plants with the enthusiasm and wonder of someone a quarter his age. It is only fitting that he be the recipient of the first Cycad D'Or award.

- Alan Meerow



Dennis Stevenson with a female *Zamia lindleyi* in the Fortuna Dam area in Panama

Photo: Greg Holzman



## Nong Nooch Cycad Horticulture Workshop (19 -24 September, 2016): A Report

Anders Lindstrom

Cycad horticulture is often neglected despite huge money spent on acquiring plants and whole collections, more often than not cycads have a particular place in most botanical gardens or private gardens but their special requirements are often not of major concern. Nong Nooch Botanical garden has been growing cycads for almost 30 years now and has developed some insight on what each species requires and more importantly how to reproduce and propagate these often critically endangered plants. The workshop started in Bangkok and we travelled towards the West to see Nong Nooch Cycad nursery. This nursery have just been created and stocked with artificially propagated seedlings that have been produced at the main garden over the years. The area includes a large shade house for those species that do not tolerate full sun. The participants toured the nursery and looked at soil conditions and growing conditions.



Author with the participants

The next day we visited a population of *Cycas siamensis* in habitat and went on to visit two other cycad nurseries nearby. The third day was spent touring Nong Nooch Botanical Garden near Pattaya. Every night we had 2 speakers from different countries or climatic areas that talked about their experiences, trial and errors as well success in cultivating cycads.

The fourth day was spent looking at the Cycad collection and doing hands on work with repotting, hand pollination, both wet and dry as well as sucker removals. Suggestions and improvement of equipment and expertise was freely shared and discussed. The afternoon was spent conducting a brainstorm session with a moderator that guided the participants through 6 horticulture questions. Notes were taken and the whole session was recorded in order to provide more information about cycad horticulture. Prior to the Workshop, I was approached by William Tang, he has written an excellent but in some ways outdated cycad horticulture manual years ago. He volunteered to make the write up for an updated version if participants and speakers were willing to share their experiences. He made a questionnaire with an accompanied table to be filled.



We have so far got a very positive response to the questions and they will all be sent to Tang for reviewing. By request a small Editorial team was created consisting of Tang, Michael Calonje, Chip Jones and the author to edit the results and provide it to a wider audience.

**Anders Lindstrom**

✉ [ajlindstrom71@gmail.com](mailto:ajlindstrom71@gmail.com)

Nong Nooch Botanical Garden, Thailand

### FEEDBACK

This workshop was a unique occasion for growers from various countries, cultivating Cycads under very diverse climates, to share their experiences and discuss them. With such a tremendous amount of plants and the representation of most species, Nong Nooch Botanical Garden was an excellent place to set it up.

Simon Lavaud, France  
Participant of the Workshop



Simon (left) with *Cycas tansachana*

### FEEDBACK

The workshop allowed botanical garden employees, professional horticulturists, and enthusiasts from a broad geographic range to share their experiences in cultivating and propagating cycads. Nong Nooch's comprehensive ex-situ conservation cycad collection and massive cycad horticultural production facilities were a great inspiration for all attendees of this well-organized, highly informative workshop.

Michael Calonje, Cycad Biologist



### FEEDBACK

Cycad aficionados worldwide converged for a horticulture workshop hosted by Nong Nooch Tropical Botanical Garden. Everyone left amazed, improved, and a little jealous because the collection is truly more amazing than words can describe.

Chip Jones  
Jones Landscaping, Florida



Chip Jones (left) with *Kampon Tansacha*



*Cycas siamensis* in habitat at Kanchanaburi Province. Population visited during Cycad Horticulture Workshop field trip.

Photo: Michael Calonje



# Cycad Nomenclature Notes: Regarding Specimens and Epitypes

Michael Calonje



The rules currently governing the naming of plants are published in the International Code of Nomenclature for Algae, Fungi and Plants (ICN, McNeill et al., 2012) and revised at successive International Botanical Congresses. The ICN stipulates that newly published names of species or infraspecific taxa are only valid if the nomenclatural type is indicated at the time of publication (Art. 40.1). The nomenclatural type for the name of a species or infraspecific taxon is a botanical specimen (referred to as 'type specimen') or illustration (Art. 8.1) that is permanently attached to a plant name, thus serving as a reference point from which to interpret the name's correct application. As type designation was not required prior to 1958 (Art. 40.1), many older plant names have their types designated later by other authors seeking to clarify the application of these names. For names (of new taxa) published since 2007, the type must be a specimen (Art. 40.4).

Despite the fact that cycad researchers routinely collect and work with herbarium specimens, I have encountered many different opinions as to what exactly constitutes a specimen. Fortunately, the ICN provides a specific definition of the term as

well as guidance for the use of specimens for the purposes of typification.

Understanding the ICN's concept of a specimen is of particular importance to workers involved in taxonomic and nomenclatural research, as misunderstanding this term may have important consequences affecting the application and even the validity of published plant names.

In this short article I attempt to shed some light on the ICN's concept of what a 'specimen' is as well as provide some notes regarding the correct designation of epitypes.

## What is a specimen?

The ICN provides the following articles relevant to our understanding of specimens for the purposes of typification:

*"8.1. The type (holotype, lectotype, or neotype) of a name of a species or infraspecific taxon is either a single specimen conserved in one herbarium or other collection or institution, or an illustration.*

*8.2. For the purpose of typification a specimen is a gathering, or part of a gathering, of a single species or infraspecific taxon made at one time, disregarding admixtures. It may consist of a single*

*organism, parts of one or several organisms, or of multiple small organisms. A specimen is usually mounted on a single herbarium sheet or in an equivalent preparation, such as a box, packet, jar, or microscope slide.*

*8.3. A specimen may be mounted as more than one preparation, as long as the parts are clearly labelled as being part of that same specimen. Multiple preparations from a single gathering that are not clearly labelled as being part of a single specimen are duplicates, irrespective of whether the source was one organism or more than one."*

From the above it is clear that despite a popular misconception, a single specimen does not have to comprise of parts collected from a single individual plant, but can instead include diverse parts collected from multiple individuals within a gathering. Some researchers prefer to assign individual collection numbers to each individual plant sampled within a population, whereas others prefer to assign a single number to the entire population. Either approach is valid and just a matter of preference for the individual collector. Perhaps the most important thing to keep in mind is that a specimen must be collected from a single gathering (such as a wild population) of a taxon at a single time.



**Figure 1.** Holotype of *Zamia tolimensis*, Esquivel et al. 3601 (TOL), consisting of material collected from one individual plant, mounted on three cross-labelled herbarium sheets.

Another common misconception is the assumption that each herbarium sheet is a single specimen. As exemplified in Art. 8.3, a specimen does not necessarily have to be mounted on a single herbarium sheet (preparation) but can be comprised of numerous preparations such as multiple herbarium sheets (Fig. 1), boxes, and jars. A fact that is often overlooked by collectors is that for the purposes of typification, the number of specimens in a particular collection is defined by the way the collected material is later mounted and labeled at the herbarium repository. Collections consisting of multiple preparations must be cross-labeled if they are to be considered a single specimen, so it is critical for the collector to communicate with herbarium staff regarding the number of specimens included in each collection so that they can be mounted and cross-labeled appropriately. The cross-labelling of type specimens consisting of multiple preparations can be made at any time prior to type designation at the request of the typifying author(s) (Sennikov & Calonje 2016), and the number of preparations included in the specimen should be stated in the protologue (Rec. 8A.4) or other typifying publication.

### Designation of epitypes

The 'Tokyo Code' of Botanical nomenclature (Greuter et al., 1994) introduced the concept of epitypes, which are specimens or illustrations that can be designated to support a type that is ambiguous and difficult to interpret. Epitypes can indeed be very useful in clarifying the application of ambiguous types, and their usage has recently been

adopted by cycad taxonomists (e.g. Lindstrom, 2009; Singh et al., 2015; Vovides et al., 2016).

The ICN provides the following definition of an epitype:

*"9.8. An epitype is a specimen or illustration selected to serve as an interpretative type when the holotype, lectotype, or previously designated neotype, or all original material associated with a validly published name, is demonstrably ambiguous and cannot be critically identified for purposes of the precise application of the name to a taxon. Designation of an epitype is not effected unless the holotype, lectotype, or neotype that the epitype supports is explicitly cited."*

Two key points to remember from the above ICN article are that 1) an epitype is a single specimen, so duplicate collections deposited at other herbaria should not be included in the epitype designation and 2) a single type supported by the epitype must be explicitly cited, so if the name is supported by multiple types (syntypes), a lectotype must be designated from one the syntypes before an epitype can be designated to support it.

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**Michael Calonje**

✉ michaelc@montgomerybotanical.org  
Montgomery Botanical Center,  
11901 Old Cutler Road, Miami, FL 33156, USA



## Visiting the equatorial cycads of Uganda

### A collaborative and knowledge sharing with Ugandan researchers

Phakamani Xaba & De Wet Bösenberg

During April 2016 we visited Uganda for two weeks, where we were able to visit populations of both *Encephalartos equatorialis* (CR) in the vicinity of Jinja and *E. whitelockii* (CR) in the vicinity of Kamwenge near to the Rwenzori Mountains. Uganda has another two species further to the north viz. *E. septentrionalis* (NT) and *E. macrostrobilus* (EN).

Our journey started in Kampala where we met Mr. Dennis Kamoga and Mr. Simon Luwemba of the Joint Ethnobotanical Research Advocacy (JERA), Uganda. An international collaboration between JERA, KEW Royal Botanic Garden (Prof. Hugh Pritchard – project leader - Kew, RBG), South African National Botanical Institute (SANBI) and Nong Nooch Tropical Botanical Garden, Thailand, was established in 2014. The aim of the collaboration was to facilitate knowledge sharing and skills transfer on the biology and ecology of Ugandan cycads and general threats so as to advise on practical conservation of these plants. Professor John Donaldson oversees the general project,

whilst Mr Phakamani Xaba is the project coordinator/technical advisor and Mr De Wet Bösenberg is the technical advisor from the South African side.



Figure 1. A field of cassava (*Manihot esculenta*) with *Lantana camara* on the fringes partially hiding a group of *E. equatorialis* in habitat.

The first part of our journey was to visit *E. equatorialis* to the west of Jinja. Once permission from a local forestry company was acquired and sitting out a thunderstorm, we set off with local guides to a population of profusely coning plants. It was evident from the approach to the population that agriculture and alien invasives (e.g. *Lantana camara*) were two visible threats to the survival of this species.

This visit was an ideal opportunity to do some preliminary temperature measurements of male and female cones to establish whether they exhibit similar heating patterns found in other *Encephalartos* spp. Loggers were inserted in both male and female cones. At the same time headspace samples were taken on both male and female cones to determine which the main chemical odours are that are produced during pollen shed and female receptivity. Other populations visited showed signs of recent fires, a tool used to promote the quality of pastures for grazing. This would be detrimental to the establishment of seedlings.

There are ~80 plants remaining, with a 1:3 male dominated ratio, with no evidence of recruitment. A thorough search in a few areas revealed the complete absence of seedlings or juveniles. The possible collapse of the pollinator system in *E. equatorialis* was confirmed by a hunt for any possible pollinator beetles on fresh and older cones that turned up no evidence of pollinator presence. Cone thermogenesis was detected in male cones in the early evening which is a similar diurnal pattern detected in cycads from Southern Africa.

Further work is needed during a whole coning season to make any conclusions but the initial results paint a bleak picture for *E. equatorialis*.

After an overnight stop in Kampala, we made our way to the east going via Fort Portal to the Kamwenge area. This was to be our base for the next few days while visiting the beautiful Mpanga river gorge, habitat of *E. whitelockii*.



Figure 3. Head space sampling on male cone of *E. equatorialis*.



Figure 2. De Wet training Ugandan scientists, Dennis and Simon as well as Isaac (local farmer) on temperature logger programming.

A similar protocol was followed here in collecting both temperature data and doing headspace sampling. We found *E. whitelockii* in a far better state of conservation than *E. equatorialis*. Population data shows that there is recruitment with the presence of seedlings and juveniles in all areas visited. The relative abundance of adult plants with the majority of them coning bodes well for this species. Dissection of fresh and older male cones led to at least two beetle species being collected.

The first species found was an *Antliarhis* spp., a likely seed predator and later unidentified potential pollinators were collected. Similar to the habitat of *E. equatorialis*, this area is also affected by alien invasives, agriculture, regular burns and development. Most of the plants are fortunately found on very steep slopes in areas where agriculture would be almost impossible.

These two equatorial cycads are unfortunately also prone to consistent poaching activities.



Figure 4. Dennis explaining the importance of cycads during a community meeting.

Conservation interventions that are currently being applied include: collection of more data to further understand reproduction; marking of plants using covert microdots; artificial hand pollination (in the case of *E. equatorialis*) to facilitate viable seed set and training on propagation of seedlings for replanting in the wild and sales to the general public. More importantly, public awareness in local schools and inclusive community and stakeholder participation in decision making is necessary.



Figure 5. Remarkable success in seedling propagation.

The current collaboration and assistance with the conservation efforts of the Ugandan NGO

is a positive step in ensuring the survival of these species.



Figure 6. At the end of a successful day in the field with Phakamani Xaba (4<sup>th</sup> from left) and Simon Luwemba (extreme right).

#### Acknowledgements

This work is related to the activities of a Defra-funded Darwin Initiative project on 'Protecting Ugandan endemic cycads from biodiversity loss and trafficking' (21-003), with Uganda, RSA, Thailand, China, the Philippines and the UK (Royal Botanic Gardens, Kew).

All photographs are by the authors.

**Phakamani Xaba**

✉ P.Xaba@sanbi.org.za

**De Wet Bösenberg**

✉ D.Bosenberg@sanbi.org.za

South African National Biodiversity Institute (SANBI)

## News from the Entomology & Sustainable Nurseries Subgroups

Willie Tang

The *Cycas debaoensis* conservation project in China began in 1999 as a sustainable nursery project managed by Liu Nian of Zhongkai University, Anders Lindström of Nong Nooch Tropical Garden and myself (see Tang et al. 2004). As local villagers and government authorities became more involved and committed to this project it evolved over the years. In 2011 the Forestry Dept. of Guangxi Province declared the land where this population of cycad is situated as a reserve, funding two foresters to help manage it (Tang 2012). In response the conservation effort by project managers has evolved as well. As an initiative of the Entomology Subgroup to support this new reserve, the insect pollinators of *Cycas debaoensis* have been studied and, employing both morphological and molecular techniques, four probable pollinating beetles have been identified (Xu et al. 2015, figure 1). Two of these beetles are new species, whose names, *Cycadophila debaonica* and *C. fupingensis*, honor the locality and people of the region. The tabulation of biodiversity and the discovery of new species helps to justify the existence of reserves and facilitate their management.

Future work by the Entomology Subgroup include expeditions to the site to tabulate the hemipteran and molluscan fauna of the reserve.

In the Americas the Entomology Subgroup is involved with identifying and describing cycad pollinators. With the field assistance of many members of the Cycad Specialist Group, Charles W. O'Brien, one of the world's leading weevil specialists, and I published a review of the New World cycad weevils (O'Brien & Tang 2015). In recognition of their invaluable support, 9 of the 25 species recognized in this review are named after members and former members of the Cycad Specialist Group and one species is named for the Montgomery Botanical Center. It is hoped that the description of pollinators will facilitate studies of cycad pollination by students and future workers.



**Figure 1.** Adult and larva of *Cycadophila fupingensis*, one of the probable pollinators of *Cycas debaoensis*.

Another initiative of the Entomology Subgroup over the years, has been controlling the spread of the destructive cycad aulacaspis scale (CAS), an effort that has been largely championed by Jody Haynes. Recently a review of biocontrol efforts of CAS was published (Tang & Cave 2016) and will be posted on the Cycad Specialist Group website.

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**Willie Tang**

✉ william.tang@aphis.usda.gov  
 USDA APHIS PPQ South Florida,  
 P.O. Box 660520,  
 Miami, FL 33266, USA



Complete dominance of *Zamia erosa* on the understory, Vega State Forest, northern Puerto Rico.

## Reassessment of the Conservation status of *Zamia* in Puerto Rico

Julio C. Lazcano Lara

*Zamia* is one of the four cycad genera present in the New World, and is also the only cycad genus widely distributed in the West Indies. In Puerto Rico there are three species: *Zamia erosa*, *Z. portoricensis*, the only endemic, and *Z. pumila*. Recent molecular studies support the existence of the three taxa.

*Zamia* species, as many other species, have been severely affected by habitat destruction. The landscape of southern Puerto Rico reflects the negative effects of many years of extensive habitat transformation to establish pastures, quarries, crop fields, roads, urbanizations, and landfills. In addition to habitat destruction, in the 1960s all species of *Zamia* were subjected to an eradication campaign promoted by the Department of Agriculture in response to an increase in cases of cattle poisoning after the ingestion of cycad leaves. The most recent conservation assessment of the Puerto Rican zamias, accessible at The IUCN Red List of Threatened Species, was submitted by Dennis W. Stevenson in 2010, and proposed that the endemic *Z. portoricensis* is Endangered (EN), while *Z. erosa* is Vulnerable (VU), and *Z. pumila* is Near Threatened (NT). In all cases, the assessment process was mainly based on rough estimates of population size and population decline. Thus far, there is no

official recognition of the threatened status of *Zamia* in Puerto Rico; none of its species are included in the USFWS (2015) regional list of endangered and threatened species, and consequently none are protected under the Endangered Species Act.



**Figure 1.** Female cone of *Z. portoricensis* with ripe seeds, an extremely rare event at Guánica State Forest, southern Puerto Rico.

From 2009 to 2015 I conducted field studies to produce the first conservation assessment of the genus in Puerto Rico that is based on quantitative information on plant density, population structure, and habitat range. I located the populations using herbarium records accessed online, available literature, data from interviews with persons with field experience on the island, and Google Earth to identify suitable but unexplored habitats. For each species I estimated the extent of occurrence, the area of occupancy, and the number of mature individuals using polygons

in Google Earth, and randomly set 25 × 20 m plots in which I recorded vegetative and reproductive data of every individual. I carried out the Red List assessment following the instructions established in the IUCN Red List Categories and Criteria Version 3.1, second edition.

New IUCN Red List categories are proposed for the three species assessed. *Zamia erosa* should be considered Least Concern (LC), *Z. portoricensis* should be considered Vulnerable (VU), and *Z. pumila* should be considered Critically Endangered (CR) in Puerto Rico. Habitat destruction and a past eradication campaign are the major factors that drove *Z. portoricensis* and *Z. pumila* to a threatened status since extant populations, at undisturbed sites, are healthy, well-structured, and self-sustainable.

Currently, baseline information that can be used to monitor the conservation status of the group is available. Additionally, six critical actions that should be taken to provide this extraordinary group of plants with an opportunity for long-term persistence have been recommended.

**Julio Lazcano Lara**

✉ jlazcano1@yahoo.com

Departamento de Biología Universidad de Puerto Rico, Río Piedras San Juan, Puerto Rico



*Zamia manicata* - natural population

## An international program of long-term ecological research for Cycad conservation

Cristina Lopez-Gallego

One of the main goals of our CSG is to promote Cycad conservation. Biodiversity conservation should be based on the best available knowledge about species and ecosystems, because only with an adequate understanding of their functioning we can make good decisions to implement strategies with real impact. For Cycad species, research to understand the natural dynamics of populations and their response to threats is fundamental for management and conservation. This means studying how populations grow in their natural habitats and react to environmental changes, and how threats like habitat destruction and degradation or over-exploitation affect them. For example, if we have enough information on habitat requirements for recruitment and we understand the survival and fecundity trends of a species, we can predict (with some level of certainty) how many seeds can be extracted without harming a population, or we can determine actions to protect and restore threatened populations to ensure their long-term persistence.

Within the last two decades many ecological studies about the population biology of

Cycads have been published. We have a good amount of information on population abundance and recruitment patterns, as well as studies on ecological interactions like pollination, herbivory and symbiosis with microorganisms. These studies represent only a small diversity of the Cycads, but provide important knowledge and suggest some guidelines for conservation. We have learned for example that many Cycad populations can vary greatly in their fecundity from year to year, and that survival of well-established adults is crucial for population persistence. Furthermore, interacting species of pollinators and the soil microbiota are fundamental to ensure healthy populations. Nevertheless, the population biology of Cycads is complex and diverse, therefore general guidelines might not be universal. Population dynamics can differ greatly in time (for example between 'El Niño' years and wetter years) or in space (for example across soil types) between species and even across populations of the same species, therefore monitoring multiple populations for long periods of time is necessary to create models for describing and predicting the ecology of a species.

In addition to understanding the natural dynamics of populations, we need to generate information about how threats like habitat changes and exploitation affect Cycads, but these type of studies are scarcer. A recent study with *Cycas circinalis* in India used population models to explore the effects of traditional seed, leaf and pith harvesting in several populations. This study suggested that the current rates of harvesting are damaging to most populations, and highlighted the need for recommendations on how to achieve sustainable levels of harvesting (Krishnamurthy et al. 2013). In the other hand, studies with *Ceratozamia matudai* in Mexico suggested that populations in degraded habitats have lower growth rates than populations in their natural habitats, and these populations differ in leaf production, plant size and seedling mortality, among others (Perez-Farrera et al. 2006, Perez-Farrera et al. 2000). We need more studies like these, so that solid knowledge can help us design and implement conservation strategies in the face of multitude of threats for Cycads.

Implementing population biology research for Cycads is not a simple task, as many of these studies are labor intensive (but not necessarily expensive), especially in species with long and complex life cycles (for an introduction on how to implement these studies, see Lopez-Gallego 2007). Fortunately, we have several Cycad researchers with ample experience in this type of research in countries like South Africa, Mexico and Colombia, and most of them are members of our CSG. I believe that we can coordinate efforts to try to acquire resources (economical, technical, etc.) and to exchange experiences to improve our research, so that we can continue studying the ecology of Cycad populations. We need to create a forum for discussing and defining a short list of relevant research topics (to understand population responses to natural habitat variation and to threats), and also potential species and sites to establish long-term

monitoring projects for Cycads. Perhaps we can discuss about such a proposal using the CSG communication opportunities we have currently available (like this newsletter and our webpage), and eventually set up a large international program of long-term ecological research for Cycad conservation. For updates on this proposal and potential contributions to it, please keep checking our CSG webpage.

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**Cristina Lopez-Gallego**

✉ clopezgallego@gmail.com

Biology Institute, Universidad de Antioquia, AA 1226, Medellin, Colombia



*Zamia encephalartoides* - measuring individuals in the field





*Dioon edule* used in municipal landscaping in Xalapa

## Cycad propagation by rural nurseries in Mexico as an alternative conservation strategy: 25 years on

A.P. Vovides, M.A. Pérez-Farrera & C. Iglesias

The trafficking of decapitated leaf-crowns of *Dioon edule* inspired a demographic study of a population of the cycad, that in turn, resulted in the establishment of a rural nursery in Monte Oscuro, Veracruz, Mexico, since 1990. It has been under the supervision of staff at Francisco Javier Botanic Garden, with the principal objective of addressing illegal trafficking and habitat destruction. This model and objective was taken up by similar nurseries in Chiapas. The nurseries work through dependency on a healthy habitat with good recruitment, seed harvesting, cultivation, financial benefit through plant sales, and in return, conservation of the habitat with reintroduction of plants. Plant sales have been limited. Nevertheless, along with the sale of other managed forest products, have given the cycad producers and other villagers enough incentive to conserve 80 hectares of cycad habitat at Monte Oscuro, and to discourage illegal collecting. The Chiapas nurseries are in two biosphere reserves, where four additional cycad species and two endangered *Chamaedorea* palms are being propagated. A further biosphere reserve in Puebla hosts a similar nursery for the critically endangered *D. caputoi*. Here, the producers are paid through the Reserve authority for cultivation and reintroduction of the cycad. All species were studied at the population level prior to and during nursery establishment. Cultivation knowledge has been passed on to the farmers, as well as

limited help in marketing. Seedling reintroduction experiments have been carried out, but further demographic studies of *D. edule* and *Ceratozamia mirandae* have given reason to re-think reintroduction strategies. The marketing problem is still an issue. It has been approached by the involvement of conservation authorities in Chiapas to assist the producers with permit paperwork and to seek markets, but this is not so for the remaining nurseries. This experience is an important example of botanic garden extension to rural communities in Mexico that covers several articles of the Convention on Biological Diversity.



Nursery grown cycads with official label for sale at Clavijero Botanic Garden shop

Though these nurseries have not been a total success, they have not at all totally failed. Monte Oscuro is still operating albeit with one person over 25 years on. The main problems are marketing issues, transport and

paperwork that most rural producers cannot deal with efficiently. There is a need for a horticultural marketing specialist assessor, and help with transport and paperwork logistics. In short, an intermediary linking the nurseries to markets and aiding with bureaucracy.

TOTAL PLANT SALES OVER A PERIOD OF APPROX. 25 YEARS	
SPECIES	\$ US
<i>Dioon edule</i>	10,075
<i>Dioon merolae</i>	1,000
<i>Ceratozamia matudae</i>	800
<i>Ceratozamia mirandae</i>	820
<i>Zamia soconuscensis</i>	820
<b>TOTAL</b>	<b>13,515</b>

Source: Vovides, A.P. et al. 2010. *Kew Bulletin*. 65: 603-611

**A.P. Vovides**

✉ andrew.vovides@inecol.mx

*Biología Evolutiva Curador Jardín Botánico Fco. J. Clavijero Instituto de Ecología, A.C. Apdo Postal 63 Carretera Antigua a Coatepec 351 El Haya Xalapa Veracruz, Mexico, 91070*

**M.A. Pérez-Farrera**

✉ perezfarreram@yahoo.com.mx

*Instituto de Ciencias Biológicas Universidad de Ciencias y Artes de Chiapas Tuxtla Gutierrez, Chiapas, Mexico*

**C. Iglesias**

*Jardín Botánico Fco. J. Clavijero, Instituto de Ecología, A.C., Xalapa, Veracruz, Mexico*

*Cycas armstrongii*

Photo by Nathalie Nagalingum

## Towards a comprehensive phylogeny and classification of *Cycas*

Nathalie S. Nagalingum

With 114 species, *Cycas* is the most diverse and widespread of all the cycad genera, occurring in Asia, Australia, Madagascar, and the southwestern Pacific Ocean islands (Calonje et al., 2013-2016). It is the first diverging genus in the cycads, and is the sole member in the family Cycadaceae (Nagalingum et al., 2011; Salas-Leiva et al., 2013; Stevenson 1992). The genus is strongly and consistently resolved as monophyletic, and is characterised by a prominent midvein in the leaflets, and the arrangement of the megasporophylls into a loose crown (Griffith et al., 2014; Hill, 1996; 2004; Hill & Osborne, 2001; Lindström, 2004; Nagalingum et al., 2011).

Currently, *Cycas* is segregated into five sections (*Asiorientales*, *Wadeanae*, *Indosinensis*, *Stangerioides*, and *Cycas*), and there are an additional four subsections in section *Cycas* (Hill, 2004; Singh et al., 2015). The sections are distinguished principally by seed structure as well as by a few mega- and microsporophyll characters and a stomatal feature. This classification into the six

sections was based on a phylogeny of 40 species that was inferred from morphology and ITS sequence data (Hill, 2004). At the time of publication, Hill (2004) noted that “placement of a number of species... is uncertain”, and that the classification was an “informal interim arrangement”.

A phylogeny for *Cycas* is vital—in 2007 Forster remarked “a major impediment to any recovery plan for Australian species of *Cycas* is the lack of a species phylogeny”. The most sampled phylogeny of *Cycas* sampled 64 species, and was presented as part of a larger study of cycads (Nagalingum et al., 2011). There is agreement between this phylogeny and the current infrageneric classification for section *Cycas*, however, three sections are highly polyphyletic while section *Wadeanae* cannot be tested as its two species were not sampled. On evaluating some of the leaf anatomical characters for the genus *Cycas*, Griffith et al. (2014) noted that many are homoplasious or under phylogenetic stasis. Nonetheless, it is promising that there were several reliable

characters that could be used to define clades in the Nagalingum et al. phylogeny (Griffith et al., 2014).

To assist in the conservation and understanding of this iconic but threatened genus, a modern phylogeny and classification are needed. The “*Cycas* classification group” aims to address these needs.

For this project, new DNA sequence data for all species are being generated to infer a new phylogeny. In addition, morphological characters will be reviewed. The molecular phylogeny and morphological data will then be used to produce the first comprehensive infrageneric classification for the genus.

All members of the cycad community are welcome to join the *Cycas* classification group. Currently we have collaborators from five countries who have sent leaf tissue for DNA extraction. The DNA is being used to generate a Next Generation Sequencing (NGS) phylogeny. New members are welcome to assist in the coding of morphological

characters and providing input on the circumscription of sections.

For more information and to join the *Cycas* classification group, contact Dr. Nathalie Nagalingum: evolutionofplants@gmail.com

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**Nathalie Nagalingum**

Royal Botanic Garden Sydney, and  
California Academy of Sciences  
✉ evolutionofplants@gmail.com



*Cycas micronesica*

Photo by Nathalie Nagalingum



Female cones on sale in local market, Manipur (India).

Photo. BS Konjengba

## Illegal trade of *Cycas* cones in India, Bangladesh and Myanmar

JS Khuraijam & Rita Singh

Non-timber forest products are biological resources other than timber that have been extracted by indigenous people and different aboriginal groups throughout the Tropics. Use of plants in traditional medicine and cultural rituals has been an age-old practice in Asia. Ayurveda and Traditional Chinese medicine are two of the oldest medical practices in the world that includes herbal medicine, exercise (yoga, qigong) and dietary therapy. Besides these, there are many folk medicine practices in South Asia, Southeast Asia and Far East. Out of about 16000 species of angiosperms in India, 6000 are used for Ayurvedic or other traditional medicine, and over 3000 are officially recognized by the government for their medicinal uses. Persistent exploitation of these plant species over the years have resulted in the depletion of many species but the level of mortality in the exploited population depends on the method of extraction and whether vital parts are removed.

As such, the exploitation of non-timber forest produce is assumed to be sustainable and often viewed as a promising arrangement between the conservation of biodiversity and source of income to tribal or non tribal rural population. However, during our study of *Cycas* species of India and adjoining countries we have found a widespread systematic

illegal trade of reproductive units (male and female cones) of *Cycas pectinata* which is against the standard assumptions about sustainability of extraction of non-timber forest produce. We believe that prolonged extraction of vital reproductive cones of *Cycas pectinata* in particular the female cones has severely damaged the natural pattern of seedling recruitment and is one of the major causes of rapidly dwindling populations of this species in the Northeast Indian states of Assam, Manipur, Meghalaya.

Illegal trade originating from Assam was recorded in Shillong (Meghalaya), Agartala, Kalashahar and Pecharthal (Tripura), Guwahati, Goalpara, Dibrugarh and Silchar (Assam) and bordering areas of Bangladesh. It was observed that the illegally collected and smuggled male and female cones were collected from Nagaon district of Assam. The range of trade is broader in comparison to Manipur and is executed by well established local medicine healers supported by a network of illegal collectors.

Cross border illegal trade of *Cycas* cones between India and Myanmar was also identified after the sale of female cones collected from either side of the borders were found to be sold in local markets of Moreh

and Kakching in Manipur and Tamu in Myanmar.

The demand of both female cones for folk traditional medicine practice in Imphal, state capital of border state Manipur have triggered the illegal trade across the border threatening the cycad populations on both sides of the border. Both immature ovules and mature seeds are sold at INR 10 per ovule/seed. Plant parts for traditional medicine, cultural and religious ritual practices. Traditional use of medicine with religious and cultural belief system in these countries is very strong and is major reason for pushing several species into illegal trade including *Cycas*.

*Cycas* are slow growing and to attain reproductive stage, it takes around 15 years. They are obligatory dioecious i.e. male and female reproductive organs are always borne on different plants. Over-harvesting of either of the cones will result in the depletion of population dynamics and unsustainable harvesting will lead to imbalance sex-ratio thereby threatening their existence.

We believe that cycad experts of South Asia and Southeast Asian countries have to collaborate to draw the attention of their respective governments declare *Cycas* as one of the National Natural/Biological Heritages

to protect them from the prevailing threats that include the illegal trades of plants and plant parts. Inter-state and cross-border trade of non-timber products should be tighten. Government of India should include all the species of *Cycas* under Schedule VI (Section 2) of the Wildlife Protection Act 1972 and should

encourage their propagation through seeds in government agencies.

**JS Khuraijam**

CSIR-National Botanical Research Institute,  
Rana Pratap Marg, Lucknow-226001,  
Uttar Pradesh, India  
✉ jskhuraijam@yahoo.com

**Rita Singh**

GGs Indraprastha University, Dwarka  
Sector 16C, New Delhi-110078, India  
✉ rsinghipu@yahoo.co.in

RESEARCH & CONSERVATION NEWS



*Ceratozamia mexicana*

**A reassessment of *Ceratozamia mexicana***

Andrew P. Vovides, Dennis Wm. Stevenson, Miguel Angel Pérez-Farrera, Sergio López-Mendoza & Sergio Avendaño

Various populations of *Ceratozamia* Brongn. exist in central Veracruz on the Mexican Transvolcanic Belt whose taxonomic status is not yet completely clear. Especially two populations of *Ceratozamia mexicana* with distinct morphology; one population at El Esquilón and Coacoatzintla considered as *C. mexicana* Brongn. by Chamberlain and others, and another population at El Mirador. Dennis Stevenson located a voucher collected by Brongniart at the herbarium of the Museum of Natural History in Paris and identified it as the holotype of *C. mexicana*. The plants we found at El Mirador are comparable to the holotype of *C. mexicana*, but those at El Esquilón do not compare. Stevenson later also found another voucher at Kew by labeled by Thiselton-Dyer as *C. mexicana* var. *tenuis*

Dyer from Mexico that is similar to the plants at the El Esquilón population. An analysis of 11 leaflet anatomical characters of both *Ceratozamia* populations gave a significant discriminant pattern (Wilk's lambda 0.00015  $P < 0.01$ ). The squared Mahalanobis distances were also significant ( $P < 0.05$ ) and the first canonic variable showed that 93.4 % of the variation was due to the anatomical characters. Thiselton-Dyer's voucher was designated as the lectotype of *C. mexicana* var. *tenuis*, and the new nomenclatural combination *Ceratozamia tenuis* (Dyer) D.W.Stev. & Vovides was proposed for this taxon.

Both the holotype of *C. mexicana* as well as the lectotype for *C. mexicana* var *tenuis* were

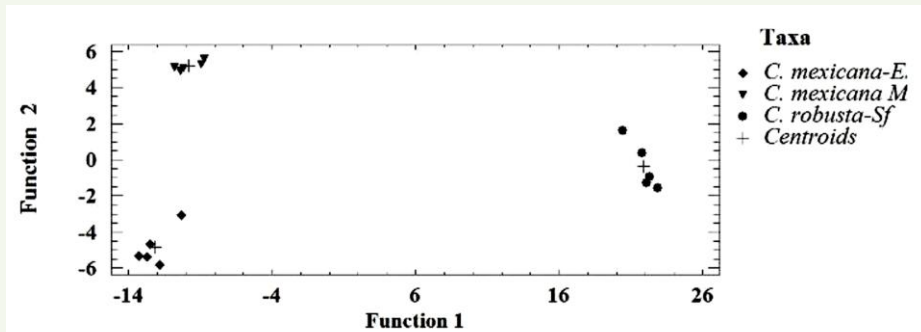


Epitype: *Ceratozamia tenuis* (Dyer) D. W. Stev.

based on sterile cultivated material of unknown provenance and were therefore demonstrably ambiguous. Consequently, specimens collected at El Mirador and El Esquilón were designated as epitypes for the two names therefore fixing these names to plants from known localities whose distinctiveness is supported by our anatomical analyses. Revised descriptions for both species were also provided.



Epitype: *Ceratozamia mexicana* Brongn. et Vovides



Scatter plot score derived from the functions produced by stepwise discriminant analysis of 11 anatomical characters occurring in *Ceratozamia tenuis* from El Esquilón, *C. mexicana* from El Mirador, and *C. robusta* from San Fernando, Chiapas (included to satisfy analysis).



*Ceratozamia tenuis*

Source : Vovides, A. P., D.Wm. Stevenson, M.A. Pérez-Farrera, S. López, & S. Avendaño. 2016. What is *Ceratozamia mexicana* (Zamiaceae)? *Botanical Sciences* 94(2): 419-429.

**Andrew P. Vovides**

✉ andrew.vovides@inecol.mx  
 Biología Evolutiva Curador Jardín Botánico  
 Fco. J. Clavijero Instituto de Ecología, A.C.  
 Apdo Postal 63 Carretera Antigua a Coatepec  
 351 El Haya Xalapa Veracruz, Mexico, 91070

**Dennis Wm. Stevenson**

✉ dws@nybg.org  
 New York Botanical Garden, New York City,  
 USA

**M.A. Pérez-Farrera**

✉ perezfarreram@yahoo.com.mx  
 Herbario Eizi Matuda (HEM) Instituto de  
 Ciencias Biológicas Universidad de Ciencias y  
 Artes de Chiapas Tuxtla Gutierrez, Chiapas,  
 Mexico

**Sergio López-Mendoza**

Instituto de Ciencias Biológicas Universidad  
 de Ciencias y Artes de Chiapas Tuxtla  
 Gutierrez, Chiapas, Mexico

**Sergio Avendaño**

Instituto de Ecología A.C., Xalapa, Veracruz,  
 Mexico

## The application of RADseq for conservation genomics of Australian *Cycas*

James A.R. Clugston and Nathalie Nagalingum

Understanding genetic variation of wild cycad populations is vital for conservation, highlighting populations with the highest levels of genetic diversity and identifying populations of conservation priority. Genetic data also allows the safeguarding of wild populations through identifying if ex-situ conservation collections represent the genetic diversity of natural wild populations. Knowledge of the genetic diversity in botanic garden collections is critical because they are a safe haven for conserving natural populations (Griffith et al. 2015).

Next generation sequencing represents the future for molecular research and offers major advantages to traditional approaches by using genomic data to understand genetic relationships and ancestry. However, the large genomes of cycads (~25-60 Gigabase pairs (Gbp)) (Zonneveld 2012), make full genome sequencing intractable. Restriction-site associated DNA sequencing (RADseq) is an approach for reducing the genome with restriction enzymes. RADseq captures

hundreds to thousands genetic markers throughout the genome, and allows a large number of samples to be sequenced at relatively low cost (Puritz et al. 2014)

### How will we use RADseq to understand genetic diversity in cycads?

Using genetic data, we are assessing several *Cycas* species in order to fine-tune conservation management plans, and to gain a better understanding to the genetic diversity of natural populations. In addition, we are comparing the genetic diversity from ex-situ conservation collections directly with wild populations. We will then determine how the genetic diversity of the natural populations compares to *ex-situ* collections. Ultimately, this will allow us to improve the representation of wild genetic diversity in botanic garden collections.

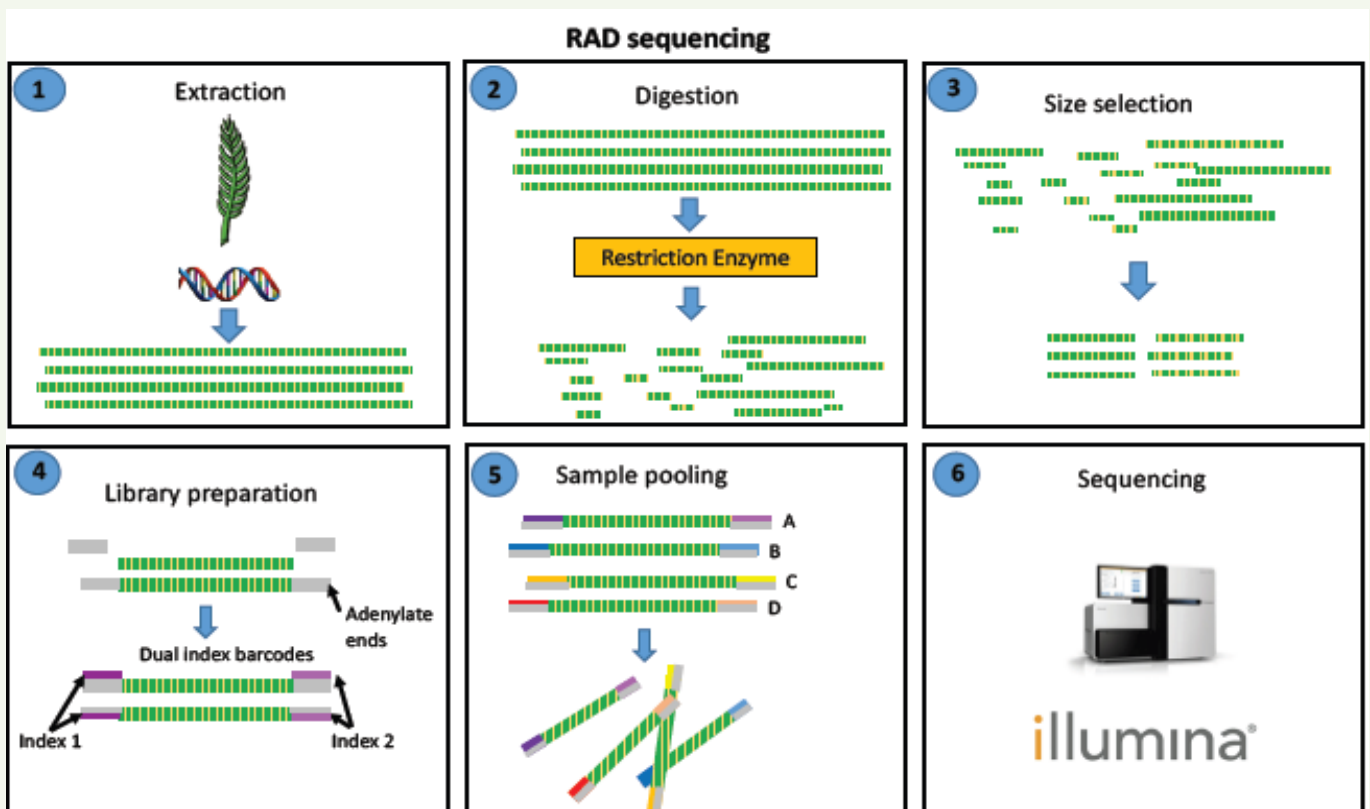
### What RADseq protocol have we tested for cycads?

We are using the ezRAD protocol (Toonen et al. 2013) to generate population genomic data

for three species of *Cycas* from the Northern Territory, Australia, *C. armstrongii* Miq., *C. calcicola* Maconochie and *C. maconochiei* Chirgwin & K.D.Hill, and for a hybrid population of *C. armstrongii* x *C. maconochiei*. Leaf samples were collected from 247 wild plants and 47 botanic garden plants. Genomic DNA was extracted from the dried leaf samples, digested using a combination of two restriction enzymes, and library preparation was carried out using an Illumina TruSeq Nano DNA Library Preparation Kit (Figure 1). Furthermore, we tested these protocols across the nine other cycad genera in Zamiaceae, as a proof of concept.

### How did we work with the data?

For each sample, there was approximately 1 GB of data, in the form of roughly three million sequence reads. As a test, we mapped reads from a sample of *C. armstrongii* to reference chloroplast- and mitochondrial genomes of *C. taitungensis* C.F. Shen et al. (Wang et al. 2007).



**Figure 1.** Our RADseq protocol involves six major steps in preparing samples from extraction to sequencing: (1) DNA is extracted from a leaf sample, (2) DNA is digested into smaller fragments using restriction enzymes, (3) DNA undergoes a size selection process, selecting fragments between 200-600 base pairs in length, (4) the ends of the fragments are blunted and prevented from sticking together (adenylated) and dual index barcodes are attached (ligated) to fragments. (5) PCR is carried out to amplify the fragments and samples are pooled (=multiplexed) and (6) Pooled fragments are then sequenced using the illumina NextSeq platform.

From over three million reads, less than 5% were mapped to the reference chloroplast and only 2.5% to the mitochondrial genome. This indicates that the remaining 92.5% of reads were derived from the nuclear genome, demonstrating that RADseq is an effective technique for obtaining large numbers of reads from unknown parts of a genome. Then using the software ipyrad (Eaton et al. 2016), we carried out de-novo assembly of reads. The reads were filtered, trimmed, and assembled into clusters to generate consensus sequences to find single nucleotide polymorphism (SNPs). Because *Cycas* has a large genome (25-30 Gbp), this could be problematic for downstream analysis (due to a lower number of clusters). However, ipyrad identified between 2000-11,000 potentially informative linked SNPs, which will be used to genotype individuals. For the nine cycad genera in Zamiaceae, large numbers of linked SNPs were also detected using ipyrad, demonstrating the effectiveness of RADseq across the cycads.

Our results have tested the utility of RADseq for obtaining genomic data from *Cycas* and the Cycadales. RADseq has yielded a high number of informative, markers, which are potentially useful for both population genomics and phylogenetics. Furthermore, when these methods are results are published, we hope that it will enable others to use these approaches for population genetics across all members of cycads.

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**James Clugston**

Royal Botanic Garden Edinburgh and  
University of Edinburgh  
✉ [jclugston@rbge.ac.uk](mailto:jclugston@rbge.ac.uk)

**Nathalie Nagalingum**

Royal Botanic Garden Sydney, and  
California Academy of Sciences  
✉ [evolutionofplants@gmail.com](mailto:evolutionofplants@gmail.com)



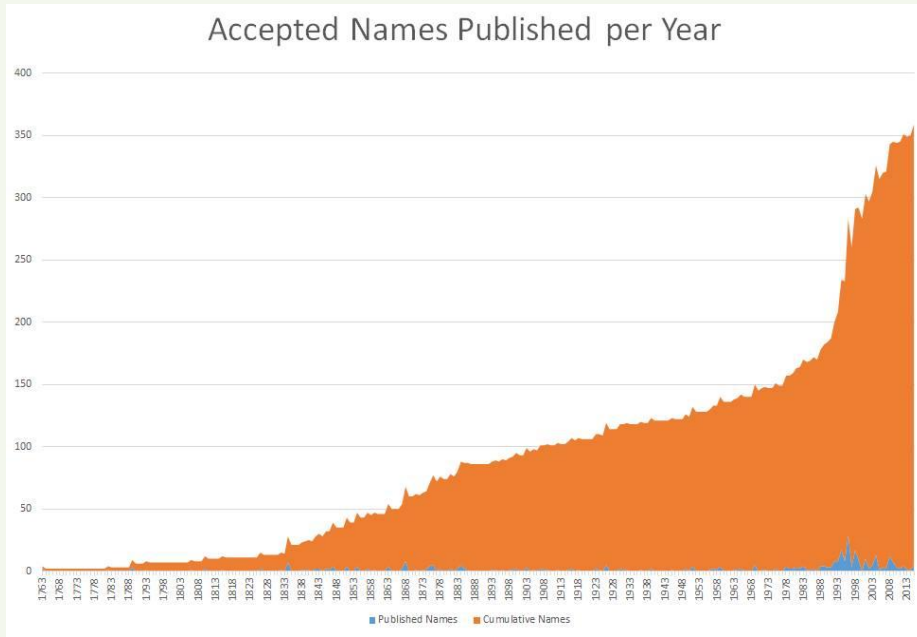
*Cycas maconochiei* growing in habitat around the Darwin Cox Peninsula, Northern Territory, Australia | Photo: James Clugston



## Call for image contributions to The World List of Cycads website

Michael Calonje

The World List of Cycads (TWLoC), published under the auspices of the Cycad Specialist Group (CSG), is a list of validly published cycad names including accepted names, synonyms, and illegitimate names. It currently serves as the nomenclatural reference for the IUCN Red List, CITES, and The Catalogue of Life. The World List of Cycads was first published by Osborne & Hendricks in 1985 and has since been published eleven times in print. Last year, TWLoC celebrated its 30th anniversary. It is currently edited by CSG members Dennis Stevenson, Leonie Stanberg, and Michael Calonje. During this time, the World List has expanded from 195 taxa to the 363 taxa accepted today. During the Cycad Biology Conference held in Shenzhen in 2011, CSG members agreed that an easily searchable, easily updateable online version of TWLoC would be helpful in order to stay current with changes in cycad nomenclature and taxonomy. Consequently, the online edition of TWLoC (<http://cycadlist.org>) was launched in June of 2013.



The online version of TWLoC contains individual pages for each cycad name providing additional information about types, nomenclatural and taxonomic notes, and photographs of cycad taxa in habitat. The cycad image gallery for TWLoC is by far the most comprehensive gallery of cycad images available on the internet. It provides a vast searchable database of imagery which is readily available for research and education purposes, and to raise awareness about this imperiled plant group. It currently contains over 5,000 images encompassing approx-

imately 45% of all taxa. The images have been provided by over 50 different contributors, and all have been assigned Creative Commons Licenses (<https://creativecommons.org>) which allow anyone to use the images under the same license with the proper attribution, typically restricted to non-commercial purposes depending on the specific license selected by the contributor.

The World List of Cycads image database is almost half-way there in terms of cycad species representation and will only become a

more useful resource as more images are added. We are seeking additional habitat photographs to continue to enrich this invaluable resource, so if you are able to contribute images of cycads taken in habitat, please contact me at [michaelc@montgomerybotanical.org](mailto:michaelc@montgomerybotanical.org)

**Michael Calonje**  
Coordinator, The World List of Cycads

*Zamia pyrophylla* Calonje, D.W.Stev. & A.Lindstr. ([Visit species page](#))



Images of *Zamia pyrophylla* in The World List of Cycads image database



Cycad Garden in front of the Conservatory | Photo: JS Khuraijam

## ***Ex-situ* conservation of Cycads at CSIR-National Botanical Research Institute, Lucknow**

JS Khuraijam & RK Roy

The National Botanical Research Institute at Lucknow is one of the constituent laboratories of CSIR-India and a premier institute in the field of plant sciences. The institute started as the National Botanic Gardens which was originally laid out as a royal garden in 18<sup>th</sup> century. The botanic garden of the institute is one of the largest and oldest botanic gardens in South Asia.

### **Cycad conservation at CSIR-NBRI Botanic Garden**

Cycads are priority group of plants for research and conservation at CSIR-NBRI Botanic Garden. Cycad Conservation Center at the Garden is the only such center in South Asia for *ex-situ* conservation for this threatened group of plants. The center comprises of four plant houses namely Cycad House, Jurassic Gallery, Conservatory and RET Propagation House. The conservation center houses the largest collection of cycads in the Indian sub-continent with 60 species including 9 species of Indian *Cycas*. The germplasm collection at the Center is regularly enriched through field collection and exchange with institutes and botanic gardens of India and abroad. The major activities of the conservation center are collection, taxonomical study, multiplication and reproductive biology study. Priority has been given to exploration and conservation of Indian *Cycas*. In the last two years, exploration and collection of four species (*Cycas beddomei*, *C. nayagarhensis*, *C. pectinata*, *C. orixensis*) were undertaken



Interior view of the Cycad House

Photo: JS Khuraijam

resulting in the discovery and rediscovery of several populations.

### **Germplasm multiplication**

In natural habitats, cycads are pollinated primarily by beetles. Due to lack of natural pollinators in botanic gardens and nurseries, they are usually pollinated by artificial means. Hand pollination of 8 species viz., *Zamia loddigesii*, *Z. pumila*, *Z. fischeri*, *Z. furfuracea*, *Cycas zeylanica*, *Dioon spinulosum* and *Microcycas calocoma* were successfully carried out in this Center. Several seedlings of *Zamia* have been raised through germination of viable seeds. Besides this, 10 species are propagated at the center through either vegetative

propagation through bulbils and suckers or seed germination. Indian species (*Cycas beddomei*, *C. nayagarhensis*, *C. orixensis*, *C. pectinata*, *C. sphaerica*, *C. zeylanica*) are propagated through germination of seeds collected from natural populations.

In the year 2016, Montgomery Botanical Center, USA and Nong Nooch Botanical Garden, Thailand extended help in the enrichment of germplasm at the center by donating seeds of rare cycads and also in the propagation of *Microcycas calocoma* and *Dioon spinulosum*. by providing pollen for artificial pollination.



JS Khuraijam pollinating *Microcycas calocoma*

Photo: Hans Raj

#### Future ahead

CSIR-NBRI Botanic Garden aims to carry out extensive field surveys in Northeast India, Eastern Ghats, Western Ghats and Andaman & Nicobar Islands for germplasm collection, taxonomic studies, population estimation and threat assessment of Indian *Cycas* to formulate long term conservation strategy and restoration of cycad populations. Exploration, population study and propagation of poorly known Indian species, *Cycas indica*, *C. nathorstii* and *C. andamanica* will be given priority.

In order to popularize Indian *Cycas* and reduce the pressure on natural populations for horticultural use, regular workshop on cycad horticulture and training program will be organized to educate basics of cycad propagation and cultivation.

#### Acknowledgments

We are thankful to forest departments of Andhra Pradesh, Bihar and Manipur for their support and co-operation during surveys and plant collection. We are grateful to Montgomery Botanical Center, United States, Nong Nooch Botanical Garden, Thailand and GGS Indraprastha University, New Delhi for

donating plants, seeds and pollen grains. We are thankful to Director, CSIR-NBRI for his encouragement and support in the cycad research and conservation.

**JS Khuraijam  
RK Roy**

Botanic Garden, CSIR-National Botanical  
Research Institute, Rana Pratap Marg,  
Lucknow, Uttar Pradesh, India  
✉ jskhuraijam@yahoo.com  
✉ roynbri@rediffmail.com

## South Asian Cycad Research Collaboration: A new beginning

JS Khuraijam

Cycads in South Asia are least studied and need urgent exploration. Except for India, cycad localities in remaining other countries in Indian Subcontinent viz. Sri Lanka, Nepal, Bangladesh, Bhutan, Myanmar and Mauritius are yet to be investigated properly. Researchers in South Asia and adjoining countries who are interested in joint research, exploration and information/experience sharing in cycad conservation and taxonomical studies can contact me at jskhuraijam@yahoo.com



Project team members and collaborators posing next to *Zamia* donated to the botanic garden at Quinta de Los Molinos National Monument in Havana, Cuba

## Preliminary results of Cuban *Zamia* research and conservation Project

Ramona Oviedo-Prieto, Gabriel Brull-Puebla, Lisbet González-Oliva, Michael Calonje & Javier Francisco-Ortega

González-Geigel (2003) recognized seven species of *Zamia* in Cuba of which six are currently recognized (Osborne et al., 2012). Without a doubt, Cuba represents the center of diversity for the *Zamia pumila* species complex, a monophyletic assemblage of taxa comprised of relatively small plants with subterranean-stems and unarmed petioles occurring in Florida as well as several islands of the Greater Antilles and the Bahama Archipelago. We are currently conducting research in Cuba on a four-year collaborative Project between Cuban and U.S. based institutions titled: "Conservation status of populations of Zamiaceae (Cycadales) in Cuba 2013-2017". The project seeks to understand the diversity, distribution, and conservation status of Cuban *Zamia*, as well as to disseminate and make use of this knowledge in a way that benefits Cuban *Zamia* conservation. *Zamia* in Cuba occurs from sea level to approximately 1,000 m elevation in a large diversity of natural and seminatural ecosystems including coastal sand dune vegetation complex, coastal and subcoastal xeromorphic shrubland, xeromorphic shrubland over serpentine, pine

forest, evergreen forest, gallery forest, semideciduous forest, savannahs, and mogote vegetation complex, with a marked preference for sandy, karstic, and to a lesser degree, serpentine substrates.



Michael & Ramona preparing herbarium specimens of *Zamia angustifolia*.

So far, we have surveyed over seventy *Zamia* populations and identified the largest and most important ones maintaining a certain degree of ecosystem function and biological interactions. As a result of the project we have determined that approximately 60-70% of the populations surveyed occur within protected areas. This provides an important

safeguard for their conservation and management and enables the long term monitoring of populations by protected area staff who work on managing, monitoring, and conserving the native *Zamia* populations located by the project as well as their associated ecosystems.

Detailed data has been collected regarding the biology, biogeography, ecology, taxonomy, and conservation status of native *Zamia* populations and their associated ecosystems. This information will be used to implement a management and monitoring plan to ensure the conservation of Cuban *Zamia* in the short, middle and long term, and DNA samples collected during the project will support the first large-scale population genetics study of the *Zamia pumila* complex throughout its entire Caribbean range. The Project also includes an outreach component which involves capacity building and environmental education and the participation of environmental authorities, botanical garden technicians, protected area staff, and Cuban cycad enthusiasts. Seeds collected during this project have been

distributed to botanical gardens, protected areas, and local cycad enthusiasts who are propagating them for reintroduction purposes or to develop ex situ conservation for future seed production so that future demand can be obtained through horticultural instead of impacting wild populations.

### Acknowledgments

We are deeply thankful to colleagues and institutions supporting this project, including: Empresa Nacional para la Protección de Flora y Fauna and the Instituto de Ecología y Sistemática for collaboration, permission, and logistics. The fieldwork in Cuba, initially facilitated by Javier Francisco-Ortega of FIU and FTBG, was funded by The National Geographic Society and a grant from Charles and Dorothy Sacher. Raul Verdecia, Ramiro Chaves, and the Flora y



Gabriel Brull, Michael Calonje and Lisbet Gonzalez-Oliva collecting field data from *Z. angustifolia*.



*Zamia angustifolia* at Turquino National Park, with Michael Calonje and park staff - Aramis Espinosa Barrero and Rogelio Suarez Areia

Fauna protected areas staff provided invaluable field support. Labwork for the Caribbean *Zamia* project, coordinated by Alan Meerow of USDA-ARS, was funded by The National Science Foundation (DEB 1050340), USDA, and the Chris Tyson Research Fellowship.

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### Ramona Oviedo

Instituto de Ecología y Sistemática-Herbario Nacional, CITMA. Cuba  
✉ roviedo@ceniai.inf.cu / hac@ecologia.cu

### Gabriel Brull Puebla

Empresa Nacional para la Protección de la Flora y la Fauna (ENPFF), MINAG, Cuba  
✉ dir.cons@ua.ffauna.co.cu

### Lisbet Gonzalez-Oliva

Instituto de Ecología y Sistemática-Herbario Nacional, CITMA. Cuba  
✉ lgonzalez-oliva@ecologia.cu

### Michael Calonje

Montgomery Botanical Center, Florida. U.S.A.  
✉ michaelc@montgomerybotanical.org

### Javier Francisco-Ortega

Fairchild Tropical Botanical Garden, Florida  
✉ ortegaj@fiu.edu



## CSG Member News

CSG member Angelica Cibrian at LANGEBIO-CINVESTAV, the National Laboratory for Genomics of Biodiversity (Mexico), has received a two-year, USD\$115,000 'Frontiers of Science' research grant from CONACYT, the Mexican National Science Council, for "Cycads and the Domestication of Maize in the Mesoamerican Landscape: Elucidating a Millennia-Old Relationship via Genomics, Archaeology, and Cultural Geography". The multidisciplinary research teams involved are led by Dr. Cibrian as well as by CSG member Mark Bonta (Pennsylvania State University), Andres Moreno (LANGEBIO), Joshua Englehardt (Colegio de Michoacan), and Michael Carrasco (Florida State Univ.).



*Dioon edule* in natural habitat. Photo: P. Suarez-Moo

The project is the first of its kind in the annals of cycad research, integrating diverse research streams in the natural and social sciences - in molecular biology, ethnography, ethnohistory, archaeobotany, iconology, and allied fields - to probe into the role of cycads in the origins of maize domestication among hunter-gatherers as well as maize diversification throughout the Mesoamerican region during the entire Holocene. The main focus is the geographic corridor stretching from Tamaulipas south to Oaxaca, and data will be gathered on species in the three local cycad genera (*Ceratozamia*, *Dioon*, and *Zamia*) and the people who have used them in the past and in the present-day. Along the way, substantial basic data on the

ethnobotany of cycads will be generated, and partnerships will be formed with several indigenous communities to aid in cycad conservation.

The project is expected to be expanded as it is renewed up to six years. Cycad researchers seeking more information should contact Dr. Cibrian.



*Ceratozamia fuscoviridis* leaves with maize. Photo: Teresa Vargas & M. Bonta

### Angelica Cibrian

✉ angelica.cibrian@cinvestav.mx  
 LANGEBIO-CINVESTAV  
 National Laboratory for Genomics of  
 Biodiversity, Mexico



**Tracy Monique Magellan**  
Montgomery Botanical Center  
✉ [tracym@montgomerybotanical.org](mailto:tracym@montgomerybotanical.org)

Tracy Magellan is the Outreach Manager at Montgomery Botanical Center (MBC). She earned a Bachelor of Science in Botany from the University of Florida (2002) and a Master of Environmental Science with a focus in Tropical Ecology from Yale University's School of Forestry and Environmental Studies (2007). Her background involves work on ecological restoration and conservation projects. The first time that she worked with cycads she published on the effect of coffee grounds on *Cycad aulacaspis* scale [CAS (*Aulacaspis yasumatsui*)] and worked on an Institute of Museum and Library Services grant that asked, "Can a botanic garden cycad collection capture the genetic diversity in a wild population?" She continues to work primarily on the educational outreach components of palm and cycad conservation projects at MBC, but she also works on cycad anatomy projects looking at fiber types in different locations within the cycads with Dr. P. Barry Tomlinson and Dr. M. Patrick Griffith.



**James Clugston**  
Royal Botanic Garden Edinburgh and  
University of Edinburgh  
✉ [jclugston@rbge.ac.uk](mailto:jclugston@rbge.ac.uk)

My research interests are currently focused around: conservation genomics, systematics and phylogenetics of members of the gymnosperms with a focus on the extant Cycadales. My research aims are to use and develop new techniques to gain further insights into the genomic and systematic

relationship of cycads and gymnosperms. My current research utilises next-generation sequencing technologies to better understand genetic diversity of wild Australian *Cycas* populations. Using restriction site associated DNA sequencing (RADseq), we are able to obtain a large numbers of genomic markers, which will be used to understand genetic diversity of wild populations and emphasise populations of conservation priority. This will enable us to compare representative species from wild populations to their counter parts in botanic garden collections and determine if the genetic diversity of wild populations is represented in botanic gardens. Ultimately the research aims help inform conservation management plans and conserve natural populations of *Cycas* in Australia. We can also build a conservation strategy to help ex-situ botanic garden collections better represent genetic diversity of the natural populations in Australia, thus acting as genetic reserve for natural populations.



**Jibankumar Singh Khuraijam**  
Botanic Garden Division  
CSIR-National Botanical Research Institute  
Lucknow, India  
✉ [jskhuraijam@yahoo.com](mailto:jskhuraijam@yahoo.com)

JS Khuraijam is Scientist Fellow at Botanic Garden Division of CSIR-National Botanical Research Institute, India. He did his PhD (2014) on taxonomy of *Cycas* of Eastern Ghats and Northeast India under the supervision of Prof. Rita Singh at GGS Indraprastha University, New Delhi. His PhD work was a major breakthrough in solving taxonomic puzzle of *Cycas* of northern Eastern Ghats resulting in the discovery of a new species besides a new combination and new subsection of *Cycas*. His work also helped in understanding the range of distribution, population structure and taxonomic complexity of *Cycas pectinata* in Northeast India. He is involved in *in-situ* conservation of *Cycas* in the states of Manipur, Assam and Odisha by involving

youths, local communities, NGOs and state forest departments.

At CSIR-NBRI Botanic Garden, he is in-charge of the *ex-situ* conservation of cycads besides other responsibilities of introduction, conservation and multiplication of different plant groups in the Conservatory, Orchidarium,, Palm House and other plant houses. He has introduced more than 100 plant species including Cycads which were collected from different parts of India and also through exchange program with other Botanic Gardens. He multiplied 8 species of cycads through artificial pollination and the seedlings were made available for exchange program with other institutes and botanic gardens. His ongoing and future research aims to a) solve taxonomic complexity of *Cycas pectinata* in South Asia and Southeast Asia through morpho-anatomical studies and molecular biology, b) Conservation status assessment of South Asian *Cycas* and c) adopt new techniques for *in-situ* and *ex-situ* conservation of Cycads.

JS Khuraijam is secretary of the recently formed Cycad Society of India. He edits and maintains the online portal Cycads of India (<http://www.cycadsofindia.in/>)



**M. Ydelia Sánchez-Tinoco**  
Plant Anatomy Laboratory, Instituto de  
Investigaciones Biológicas, Universidad  
Veracruzana (UV), México  
✉ [ytinoco@uv.mx](mailto:ytinoco@uv.mx)

I studied biology at the Universidad Veracruzana and I am actually employed at the Instituto de Investigaciones Biológicas of the UV as full time researcher. My post graduate research has been aimed at the study of seed anatomy, female reproductive cycles and phylogenetic relationships in *Ceratozamia mexicana*, *Zamia furfuracea* and recently *Dioon edule*. Currently I am working on the reproductive portion of the gametophyte in these species, particularly the archegonial ontogeny. Some of my

contributions have been the anatomical description of the ontogenetic stages from ovule to seed as well as the delimitation of their occurrence. The structure and ergastic substances contained in the seed coat and the vegetative gametophyte have been described. I have proposed that some ovule and seed characters could have taxonomic or discriminant value. Evidence has been put forward for discussion on the seed concept as applied to this plant group and new terminology has been introduced for the first time describing seed structures not reported before. By serial sections of the seed coat I have reconstructed and illustrated the manner in which the seed is supplied by a double system of vascular bundles. The way in which ergastic substances contained in the vegetative gametophyte pass to the interior of the archegonium through cytoplasmic connections has been described. This information has been published in articles and presented in specialized academic meetings. Some contributions have led awards. I am currently collaborating with colleagues of the Instituto de Ecología and the Colegio de Postgraduados.



**José Said Gutiérrez-Ortega**

Graduate School of Sciences, Department of Biology, Faculty of Science, Chiba University, Japan.

✉ [josesgo@chiba-u.jp](mailto:josesgo@chiba-u.jp)

My main interests are in understanding the natural processes that maintain, eliminate, or produce the diversity of cycads at the ecosystem, species and genetic levels, in terms of ecology and evolution. I am currently researching on the evolution of the genus *Dioon* from both macro- and micro-evolutionary perspectives. One of my objectives is to identify the spatial and temporal biogeographic patterns that facilitated speciation. For this, I use genetic and genomic data at genus level to complement methods concerning to other fields in biology. A second objective is to get evidences about the relative importance of the ongoing evolutionary mechanisms at population level. I use population genetic approaches to estimate the distribution and the levels of diversity within species to make

discussion on the underlying ecological factors in wild populations. The implications of my research are diverse. First, I want to clarify the speciation ways in *Dioon*, which can be extrapolated across cycad genera and other seed plant taxa. Also, I find to contribute in the taxonomy of cycads, by providing evidences that can clarify the species delimitation and the recognition of new taxa. I especially emphasise on the importance of considering the evolutionary significance of the groups to be classified or conserved. Thus, my research also contributes to the identification of units that should be considered in conservation management.



**Dra. Ramona Oviedo Prieto**

Herbario de la Academia de Ciencias (HAC) Instituto de Ecología y Sistemática (IES) Ministerio de Ciencia, Tecnología y Medio Ambiente (CITMA).

✉ [roviedo@ceniai.inf.cu](mailto:roviedo@ceniai.inf.cu) / [hac@ecologia.cu](mailto:hac@ecologia.cu)

I am currently involved in a research project focusing on Cuban *Zamia*. The Project, titled “Conservation status of Zamiaceae (Cycadales) populations in Cuba”, is a rare scientific collaboration between Cuban and U.S. based research institutions. My role includes planning and management aspects of the Project as well as extensive fieldwork throughout Cuba. The Project seeks to locate populations of Cuban Zamiaceae and gain a better understanding of the geographic distribution, ecological preferences, traditional uses, conservation status, and taxonomic status of these populations. Information obtained in the process is shared with diverse stakeholders such as researchers, environmental agencies, botanical gardens, and protected areas staff in order to promote additional research on Cuban Zamiaceae as well as provide support for additional activities such as species management plans, population monitoring, and environmental education programs. We are working together with local cycad enthusiasts and horticulturists to propagate

native species and promote their responsible use in Cuban horticulture and landscaping.



**Angelica Cibrian**

LANGEBIO-CINVESTAV

National Laboratory for Genomics of Biodiversity, Mexico

✉ [angelica.cibrian@cinvestav.mx](mailto:angelica.cibrian@cinvestav.mx)

Angelica Cibrian-Jaramillo graduated with honors in Biology (UNAM, Mexico), and obtained a PhD in Ecology, Evolution and Environmental Biology at Columbia University (Fulbright-CONACYT) working in palm population genetics. She carried out her work in a multidisciplinary graduate program that included experience in environmental policy with a focus in plant genetic resources, and the humanities. Angelica obtained postdoctoral experience at the American Museum of Natural History/The New York Botanical Garden as a Cullman Fellow, at OEB/MHG Harvard University, and at New York University, in plant population genetics, plant genetics, and phylogenomics, with cycads as an important part of her research. She is a fellow of the Montgomery Botanical Center for her interest in cycad and palm conservation research, in particular in applying genetic tools to conservation decisions and management. As a Principal Investigator of the Ecological and Evolutionary Genomics Lab in Langebio, CINVESTAV, Mexico (<http://www.langebio.cinvestav.mx/?pag=426>), her group is interested the (i) population genetics and genomics of cycads; ii) genomics of domestication in Mesoamerica plants; (iii) plant phylogenomics; and iv) the adaptive role of bacterial microbiomes associated with plants. In cycads, she is currently leading projects on the landscape genetics of all of *Dioon* species in Mexico; transcriptomics and genomics of the coralloid root microbiome; and more recently, in the cultural importance of cycads and their role in maize domestication. Angelica is deeply interested in discovering and providing genetic and genomic information that is useful for cycad conservation and



management, and useful to understand their fascinating evolutionary history.



**Gabriel Brull Puebla**

Empresa Nacional para la Protección de la Flora y la Fauna (ENPFF), MINAG, Cuba

✉ [cons.director@ua.ffauna.cu](mailto:cons.director@ua.ffauna.cu)

Gabriel Brull Puebla was born in 1954 in Yara, Granma province, Cuba. He received a degree in Biology with an emphasis in pedagogy and worked for eight years as a Botany professor at the Superior Pedagogical Institute of Manzanillo, in Granma province. He has been a technical supervisor for 30 years with Empresa Nacional para la Protección de la Flora y la Fauna (ENPFF, National Enterprise for Protection of Flora and Fauna), first as Regional Technical Director of the five provinces of the Oriente Region, and since 2002 as National Technical Director of Conservation. ENPFF is in charge of the administration of 80 official protected areas, and 7 to be declared. Approximately 40 of these areas host native populations of Zamiaceae. Since 2013, ENPFF has been the executive institution on the Cuban side for

the international project “State of Conservation of Zamiaceae in Cuba”. As Technical Director of ENPFF, he has coordinated this project in Cuba with the other participants and has been in charge of the actions and local projects for conservation of *Microcycas calocoma* and *Zamia* species of the protected areas under his supervision. He has been part of the 5 expeditions of the team to study and sample populations of *Zamia* in Cuba. Presently he has participated in the preparation of the technical documents for the management of Zamiaceae population to be included in the protected areas’ Management Plans. He attended the Cycad 2015 meeting in Medellin, Colombia.

## IUCN/SSC Cycad Specialist Group Members



### **Alan Meerow**

Alan.Meerow@ars.usda.gov  
USDA-ARS-SHRS, National Germplasm  
Repository, Miami, Florida, U.S.A.

### **Alberto Taylor**

asidneyb@gmail.com  
Facultad de Ciencias Naturales, Exactas y  
Tecnología, Universidad de Panamá  
Panamá, Panamá

### **Anders Lindstrom**

ajlindstrom71@gmail.com  
Nong Nooch Tropical Botanical Garden  
Sattahip, Chonburi, Thailand

### **Andrew Vovides**

andrew.vovides@inecol.mx  
Laboratorio de Biología Evolutiva  
Instituto de Ecología, A.C. Xalapa, Veracruz,  
Mexico

### **Angélica Cibrian Jaramillo**

acibrian@langebio.cinvestav.mx  
Langebio Cinvestav, Guanajuato, México

### **Anita Varghese**

anita@keystone-foundation.org  
Keystone Foundation, Nilgiris District,  
Tamil Nadu, India

### **Cristina Lopez-Gallego**

clopezgallego@gmail.com  
Instituto de Biología, Universidad de Antioquia  
Medellín, Antioquia, Colombia

### **De Wet Bösenberg**

D.Bosenberg@sanbi.org.za  
South African National Biodiversity Institute  
Kirstenbosch National Botanical Garden  
Cape Town, South Africa

### **Dennis Stevenson**

dws@nybg.org  
The New York Botanical Garden, Bronx,  
New York, U.S.A.

### **Doris Francis**

dfrancis7@earthlink.net

### **Esperanza M. Agoo**

esperanza.agoo@dlsu.edu.ph  
Biology Department, De La Salle University  
Manila, Philippines

### **Fernando Nicolalde-Morejón**

enicolalde@uv.mx  
Instituto de Investigaciones Biológicas  
Universidad Veracruzana  
Xalapa, Veracruz, Mexico

### **Gabriel Brull Puebla**

cons.director@ua.ffauna.cu  
Empresa Nacional para la Protección de la  
Flora y la Fauna (ENPFF), MINAG, Cuba

### **Irene Terry**

irene.terry@utah.edu  
Department of Biology, University of Utah  
Salt Lake City, Utah, U.S.A.

### **J.S. Khurajiam**

jskhurajiam@yahoo.com  
Botanic Garden, CSIR-National Botanical  
Research Institute, Lucknow, India

### **James Clugston**

J.Clugston@rbge.ac.uk  
Royal Botanic Garden Edinburgh  
Edinburgh, United Kingdom

### **Javier Francisco-Ortega**

ortegaj@fiu.edu  
Department of Biological Sciences, Florida  
International University, Miami, Florida, U.S.A.  
Fairchild Tropical Botanical Garden, Miami,  
Florida, U.S.A.

### **Jeff Chemnick**

jeffchemnick@cox.net  
Santa Barbara, California, U.S.A.

### **Jody Haynes**

jody@cycadconservation.org  
Miami, FL, U.S.A.

### **John Donaldson**

J.Donaldson@sanbi.org.za  
South African National Biodiversity Institute  
Kirstenbosch National Botanical Garden  
Cape Town, South Africa

### **Jorge González Astorga**

jorge.gonzalez@inecol.mx  
Laboratorio de Genética de Poblaciones  
Instituto de Ecología, A. C. Xalapa, Veracruz,  
Mexico

**José Said Gutiérrez-Ortega**  
josesgo@chiba-u.jp  
Department of Biology, Chiba University  
Chiba, Japan

**Julio Lazcano Lara**  
jlazcano1@yahoo.com  
Departamento de Biología  
Universidad de Puerto Rico, Río Piedras  
San Juan, Puerto Rico

**Leonie Stanberg**  
Leonie.Stanberg@rbgsyd.nsw.gov.au  
Royal Botanic Gardens & Domain Trust  
Sydney, Australia

**Li Nan**  
andreali1997@126.com  
Shenzhen Key Lab on Biodiversity of Southern  
Subtropical Plant Diversity  
Fairylake Botanical Garden, Shenzhen & CAS  
Shenzhen, China

**Maria Ydelia Sánchez Tinoco**  
ytinoco@uv.mx  
Laboratorio de Anatomía Vegetal  
Instituto de Investigaciones Biológicas  
Universidad Veracruzana, Veracruz, México

**Mark Bonta**  
mab86@psu.edu  
Division of Mathematics and Natural Sciences  
Penn State Altoona, Altoona, Pennsylvania,  
U.S.A.

**Michael Calonje**  
michaelC@montgomerybotanical.org  
Montgomery Botanical Center, Coral Gables,  
Florida, U.S.A.

**Miguel Pérez-Farrera**  
perezfarreram@yahoo.com.mx  
Herbario Eizi Matuda (HEM)  
Instituto de Ciencias Biológicas  
Universidad de Ciencias y Artes de Chiapas  
Tuxtla Gutierrez, Chiapas, Mexico

**Nathalie S. Nagalingum**  
nathalie.nagalingum@rbgsyd.nsw.gov.au  
National Herbarium of New South Wales  
Royal Botanic Gardens & Domain Trust  
Sydney, New South Wales, Australia

**Patrick Griffith**  
Patrick@montgomerybotanical.org  
Montgomery Botanical Center, Coral Gables,  
Florida, U.S.A.

**Paul Forster**  
paul.forster@dsiti.qld.gov.au  
Brisbane Botanic Gardens, Toowong,  
Queensland, Australia

**Phakamani Xaba**  
p.xaba@sanbi.org.za  
South African National Biodiversity Institute  
Kirstenbosch National Botanical Garden  
Cape Town, South Africa

**Piet Vorster**  
pjvors@gmail.com  
Department of Botany and Zoology  
University of Stellenbosch, Stellenbosch,  
South Africa

**Quentin Luke**  
quentinluke1@gmail.com  
East African Herbarium, National Museums of  
Kenya, Nairobi, Kenya

**Ramona Oviedo Prieto**  
roviedo@ceniai.inf.cu  
Instituto de Ecología y Sistemática (CITMA)  
Cuba

**Rita Singh**  
rsinghipu@yahoo.co.in  
University School of Environment  
Management, GGS Indraprastha University  
New Delhi, India

**Santiago Mario Vázquez Torres**  
savazquez@uv.mx  
Instituto de Investigaciones Biológicas  
Universidad Veracruzana  
Xalapa, Veracruz, Mexico

**Tien Hiep Nguyen**  
hiep.nguyen@cpcvn.org  
Vietnam Center for Plant Conservation  
Ha Noi, Vietnam

**Tim Gregory**  
cycadtim@gmail.com  
UC Berkeley Botanical Garden  
Berkeley, California, U.S.A.

**Tracy Magellan**  
tracym@montgomerybotanical.org  
Montgomery Botanical Center  
Coral Gables, Florida, U.S.A.

**Virginia Hayes**  
vhayes@lotusland.org  
Ganna Walska Lotusland  
Santa Barbara, California, U.S.A.

**Willie Tang**  
william.tang@aphis.usda.gov  
USDA APHIS PPO South Florida  
Miami, Florida, U.S.A.



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