

Work Placement at The New York Botanical Garden and Montgomery Botanical Center

June – August 2011

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Contents

1. Introduction	2
2. About the placement	3
3. Review of Cycads Collections	5
4. Phenology of the Genus <i>Zamia</i> L. in a Phylogenetic and Biogeographic Context	11
4.1 Purpose of project	11
4.2 Project background.....	11
4.3 Methodology.....	13
4.4 Strobili data gathered	13
4.5 Analysis of Data	13
5. Outcomes of research	14
Outcomes from Project	14
6. What was done and how this meets the aims and objectives	15
7. Expenditure and cost in NY and MBC	15
8. Bibliography	16

1. Introduction

Invitation to work with NYBG

2011 Kelly Research fellow

Cycads are botanical icons of the threats faced by plant populations and species throughout the world. Perhaps only orchids rival cycads in the level of interest from specialist collectors with wild populations of many cycad species badly depleted (IUCN and SSC 2003) due to illegal harvesting. The current diversity of cycads represented at about 316 extant taxon (Hill, Osborne and Stevenson 2007) with 10 genera split across two families Cycadaceae and Zamiaceae. The cycads date back to the pre- Mesozoic era some 350 million years ago (Norstog and Nicholls 1997).

Due to the increasing rarity of cycads still alive today it is imperative that we increase our awareness of these plants and improve their cultivation in order to relieve stress on wild populations and help their conservation (IUCN and SSC 2003). There were numerous institutions that specialise in cycad research around the world but these are now few and far between. The New York Botanical Garden and the Montgomery Botanical Center (Miami) are two of the world's most renowned institutions for cycad research. They have made some of the most significant advances of the last few decades in cycad research and have allowed us to improve our knowledge and understanding of the Cycadales.

During December 2010 I was offered the opportunity to work alongside Dennis Stevenson at both Montgomery Botanical Center and The New York Botanical Garden. This was an excellent opportunity to get some first-hand experience working with some of the most renowned cycad research centres. The placement was from 5 June to 14 August 2011 with the first two months spent in New York and the last three weeks at MBC, Miami Florida.

During my time at the Montgomery Botanical Center I became their 2011 Kelly Botanical Research Fellow. It was an honour to be presented with this award and will be acknowledged in any further publications.

2. About the placement

The original aim of the placement was to conduct work alongside and shadow Prof Dennis Stevenson at The New York Botanical Garden and the Montgomery Botanical Center, who is one of the world's leading researchers and authorities in the Cycadales. My initial aim was to assist Prof Stevenson in the daily workings of the collections and gain an understanding of the cultivation and the maintenance of plants within the living collection.

Both the Montgomery Botanical Center (MBC) and The New York Botanical Garden (NYBG), have significant large living collections with NYBG focusing on gymnosperms as group and the MBC focusing on palms, cycads and other tropical gymnosperms. The NYBG as an institution is known for one of the world's largest herbariums (over 6 million specimens) and its world renowned research facilities concentrating on molecular systematics, in particular the Cullman program, for plant molecular systematics.

Upon meeting Prof Stevenson I was greeted with a project proposal, looking at the phenology of the Cycadales with a particular focus on the genus *Zamia* L. Phenology of cycads is the study of the production of strobili (cones) (Jones 2002). Phenology is not only important to help ascertain determination of a taxon but is imperative for their cultivation. Understanding the phenological patterns of a particular taxon also important for horticulture because it can give an indication of when it is important to collect phenological characters from the field or in cultivation, it will aid with the understanding of when stock plants are ready to produce cones. With an understanding of phenology comes an understanding when the megasporangiate strobili (female cones) are receptive and with elongation and dehiscence of the microsporangia in the microsporangiate strobili (male cones) are ready for pollen collection.

Phenology is very important for horticulture because it highlights the phenological timings of plants so that in areas like Miami or South Africa where cycad pollinators also present they can be controlled by pesticides (if required). This helps prevents the crossing of microspores between separate taxon preventing hybridisation.



Figure 1 megasporangiate and microsporangiate strobili of *Zamia loddigesii* Miq, MBC Living collection (Clugston, 2011)



Figure 2 *Macrozamia communis* L.A.S. Johnson megasporangiate strobili crowing in the MBC living collection (Clugston, 2011)

3. Review of Cycads Collections

The New York Botanical Garden

The NYBG is known to be one of the world's leading botanical institutions, specialising in gymnosperm research. Much of the pioneering research done within the Cycadales has been conducted at NYBG by Kunut Norstog and Dennis Stevenson. The majority of work being conducted at the NYBG on Cycadales has been on the South American Zamiaceae, particularly *Zamia* L., *Ceratozamia* Brongn. and *Dioon* Lindl.

The vast majority of researchers in cycads at the NYBG, tend to specialise in the South American Zamiaceae (*Ceratozamia*, *Dioon*, *Microcycas* and *Zamia*). with the living collections at the NYBG consisting of many *Ceratozamia*, *Dioon* and many specimens of *Zamia*. One particular specimen of interest NYBG had within the collection was the only described epiphytic cycad species *Zamia pseudoparasitica* Yates and Seem found growing epiphytically on trees in Panama (South America) Figure 1.



Figure 3 *Zamia pseudoparasitica* Yates in Seem, NYBG Living collections (Clugston, 2011)

Although NYBG have an excellent display collection consisting mostly of South American species, they have many rare African *Encephalartos* Lehm. species that are held within the backup collection. The backup collection also contains large quantities of *Cycas multipinata* group which in the recent years had become a particular interest for collectors and researchers due to their uniqueness of having bi-pinnate or tri-pinnate leaflets.

Overall the NYBG has an impressive cycad collection although the specimens which have the most impact are not on public display due to their rarity and chance of theft. Because of the growing conditions cycads often require, the NYBG is more involved in cycad research rather than having an extensive cycad collection represented within its living collection.



Figure 4 *Cycas rumphii* Miq. at the central point of the main drive within the MBC Collections (Clugston, 2011)

The living collection at the Montgomery Botanical Center is perhaps one of the largest and most impressive collections of cycads in cultivation around today, stated as “*The world’s largest legal cycad collection*” (Stevenson 2010). The collection holds 235 (Montgomery Botanical Center 2011) of the 315 described taxa (Hill, Osborne and Stevenson 2007).

The collection has all extant genera of Cycadales represented, with over 80% of material being known wild origin and being of significant scientific value.



Figure 5 *Cycas maconochiei* ssp. *lanata* megasporophylls at the pre maturation stage (Clugston, 2011)

Within its living collection there are some rare species of cycads that have a significant importance for conservation. The collection has no particular emphasis on country, region or specialisation which is both a good and bad thing. The MBC living collection tends to expand depending on the research being undertaken, for example there is extensive work being carried out on the South American *Zamia* L. and because of this, the collection has one of the most extensive collections of this genus in the world.

Some of the rarest species of the cycads in cultivation are the Australian members of the genus *Cycas* L. Shown in figure 3 & 4,. They are rare in cultivation due to the heavy restrictions applied by the Australian government, and are a good example of cycads not in the hands of collectors. Australia is known to have the greatest diversity of cycads in the world and the only country to have both families represented.

Although the collection is impressive, it is distinctly lacking in many of the South African *Encephalartos* Lehm. which is mainly due to the lack of research that is undergone within the United States on this particular genus. Yet the living collection has an extensive collection of African *Encephalartos* containing some very rare species such as *Encephalartos brevifolius*.



Figure 6 *Cycas maconochiei* ssp. *lanata* an exceptional species from Australia, Montgomery botanical centre living collection (Clugston, 2011)



Figure 7 *Encephalartos concinnus* R.A. Dyer & I. Verd if megasporangiate strobili growing within the MBC living collection (Clugston, 2011)

4. Phenology of the Genus *Zamia* L. in a Phylogenetic and Biogeographic Context

4.1 Purpose of project

To provide an understanding of the strobili production times across a range of taxa in the genus *Zamia* L. (where possible) of both cultivated and wild collected plants. With the gathered data, circular statistics will be applied to create statistical diagrams and charts to help provide an understanding into the yearly and monthly coning cycles of a taxon.

The collected phenology data will be analysed and combined with molecular data (nrITS regions ITS1,5.8S & ITS2 and regions from chloroplast DNA matK & trnk) to create concise phylogenetic tree to aid a better understand of the cyclical production of strobili in a phylogenetic perspective of the premeditated taxa.

This data could be exceedingly valuable for the botanist and horticulturalist by providing information about the production of strobili within wild populations and for plants in a cultivated environment. A range of taxa have been recorded to switch the cyclical production strobili when situated in a different hemisphere. The collected data will also provide important information which could provide aid for future plant collections, due to improved knowledge of the seasonal production of strobili.

4.2 Project background

The extant Cycadales are split between two families the monotypic Cycadaceae containing only *Cycas* L.(105) and Zamiaceae; *Bowenia* (2), Hook. ex Hook. f. *Ceratozamia* Brongn.(27), *Dioon* Lindl. (14), *Encephalartos* Lehm. (65), *Lepidozamia* Regel (2), *Macrozamia* Miq. (41), *Microcycas* (Miq.) A. DC. (1), *Stangeria* T. Moore (1) and *Zamia* L. (71) (Hill, Osborne and Stevenson 2007) updated using 2010-2011 world list (Griffith, et al. 2011)

With current classification systems cycads are manly separated using clear diagnostic morphological characters, but many of these characters can be highly variable. The strobili are known to be a very stable character among the majority of taxa, because stable characters are imperative to ascertain species level determination (Norstog and Nicholls 1997).

Many type and other herbarium specimens are lacking important key characters required for identification due to the collection when the plants are sterile and not producing strobili.



Figure 8 *Encephalartos hildebrandtii*, megasporangiate strobilus, MBC Living collection (Clugston, 2011)

Recently a study has been done, on the phenology of cultivated cycads (Griffith, et al.), but there is a lack of published data on the phenology of both wild origin and cultivated plants. Combined. Cycadales are protected under C.I.T.I.E.S (Convention on International Trade in Endangered Species of Wild Fauna and Flora) (C.I.T.I.E.S 2011) and are IUCN red list recognised (IUCN 2010). Understanding the phenology of taxa may provide new and vital conservation strategies required for conserving and ensuring the survival of the cycads.

4.3 Methodology

- Data from wild populations is gathered via herbarium material contained within The New York Botanical Garden (NY) and Fairchild Tropical Botanic Garden (FTG) and other collections on loan or held within the NY herbarium (US, MO, COL, K, FTG, L, XAL etc.)
- Cultivated data and plants records at Montgomery Botanical Centre used for comparison against wild collected specimens and vouchers. The MBC records will be used to expand species with missing or insufficient data (where available)
- Photographs of wild collected herbarium specimens of *Zamia*, provided by Dennis W. Stevenson (NY) recoded from herbariums around the world
- The study looks at an assortment of data obtained from specimen labels; barcode, genus, specific epithet, cultivated, locality, country, latitude, longitude, collector, collection, herbarium, collection date and onset of flush of leaves

4.4 Strobili data gathered

- Early megasporangiate strobili production (pre megagametophyte maturation)
- Late megasporangiate strobili production (post maturation of megagametophyte)
- Microsporangiate strobili: early microsporangiate year (pre elongation of central axis and pre dehiscence of microsporangia)
- late microsporangiate year (post elongation of central axis and post dehiscence of microsporangia and the release of microspores)

4.5 Analysis of Data

- Data analysed and check using MySQL (Oracle 2011) and R statistical software combined with a circular statistic package (Lund and Agostinelli 2007)
- DNA sequences provided from Damon P. Little (NY) for phylogenetic analysis
- FASTA files aligned with Crustal W and phylogenetic tree created using TNT (Goloboff, Farris and Nixon 2004), creating a combined tree using both molecular and phenological data

5. Outcomes of research

The data of collected from herbarium specimens; early megasporangiate, late megasporangiate, early microsporangiate & late microsporangiate, taking into account the; year, month and day were phenological data was available. The initial data was gathered from wild collected herbariums specimens. Specimens were sampled from numerous herbaria: AAH,AAU,AHUC,B,BM,COAH,BUS,COL,E,F,FTG,GH,HUA,JBSD,K,LA,LE,MEXU,MGR,MO,NY,P,PENN,PMA,SEL,SLPM,U,US,VEN & XAL.

A total of 1,265 wild origin material sampled from over 6,000 herbarium specimens including a total of 163 taxa from both Zamiaceae and Cycadaceae. From the gathered data 54 taxa from the genus *Zamia* L. were represented comprising of 76% of the recognised 71 taxa within the genus. Due to the large quantity of data from a single genus 22 taxa were selected from the 54 sampled taxa.

Montgomery Botanical Center, have 14 of the 22 chosen taxa represented within its living collection with a total 10,588 phenological events recorded. These records include; early megasporangiate, late megasporangiate, early microsporangiate, late microsporangiate and leaf new production.

Outcomes from Project

- Publication of peer reviewed, botanical/ scientific publication
- 2011 Kelly Botanical Research Fellow
- Data used for final year honours dissertation, Royal Botanic Garden Edinburgh
- Improve understanding of statistical analysis, data analysis and data collection procedures
- Gaining experience to further botanical career leading to potential PhD in systematic botany expanding work with Cycadales
- Continue working alongside MBC supporting and helping to further their research with Cycadales

6. What was done and how this meets the aims and objectives

What has been achieved

- Improve identification skills by increasing my understanding of morphological characters of the many species I have not yet seen in this group
- Gain an understanding of the current horticultural and botanical research that is being done in the Cycadales
- Understand how the collections in these two different botanical and horticultural institutions can play an active role in the conservation and protecting of these rare plants
- Increase my ability to teach others about the group - I will do this by taking a class each on cultivation and diversity for undergraduate horticulturists after I return to RBGE

What has not been achieved

- Gain practical experience in all aspects of cultivation of a worldwide representation of cycads in different growing conditions in both outdoor and protected conditions
- Learn about approaches to public interpretation of the cycads in botanic gardens

7. Expenditure and cost in NY and MBC

New York and Miami (5th of June-14th August 2011)	
Reason	Expense (£)
Flight (Heathrow-New York-Miami-Heathrow)	£798
Baggage fee (NY-Miami)	£30
Visa	£50
Accommodation	
Deposit	£500
Arrival deposit (\$300)	£186.08
Rent (at \$33=£20.87 per night total 50 nights)	£1,043.50
Dinning Services Charge (\$4.25)	£132
Technology fee (\$1.25)	£62.50
Living costs (food etc. for 70 nights at £20 perday)	£1,400
Gifts	
RBGE branded mug (x2)	£8
Guide books (x4)	£24
Subway & Buses	£250
Trip to Heathrow	£60
Overnight stray	£74
Hotel to terminal 5(two way)	£30
AT&T Sim (Mobile phone for USA) Pay as you go sim	£70

8. Bibliography

- Bakhtiar, Y, D. Miller, T. Cavagnaro, and S. Smith. "Interaction between two arbuscular mycorrhizal fungi and fungivorous nematodes and control of the nematode with fenamifos." *Applied Soil Ecology*, 2001: 107-117.
- C.I.T.I.E.S. "Review of Significant Trade Cycads." *Convention in trade of endangered species of flora and fauna*. November 2003. www.cities.org (accessed October 26, 2011).
- C.I.T.I.E.S, Convention on International Trade in Endangered Species of Wild Fauna and Flora. *What is CITES?* 2011. www.cities.org (accessed April 2, 2011).
- Goloboff, P. A., J. S. Farris, and K. C. Nixon. *TNT. Computer program distributed by the authors*. 2004. www.zmuc.dk/public/phylogeny/TNT (accessed October 19, 2011).
- Griffith, Patrick M., Michael A. Calonje, Dennis Wm. Stevenson, E. Husby Chad, and Damon P. Little. "Time, Place and Relationships: Cycad Phenology in a Phylogenetic and Biogeographic Context Tiempo, Espacio Y Relaciones: Fenología De Cicadas en un Contexto Filogenético Y Biogeográfico." *Publication pending*, 2011: 1-32.
- Hill, K D, R Osborne, and D W Stevenson. "The World List of Cycads [La Lista Mundial De Cícadas ." *7th International Conference on Cycad Biology*. New York: New York Botanic Gardens Press New York , 2007. 453-483.
- IUCN, The World Conservation Union, and Cycad Specialist Group SSC. "Status Survey and Conservation Action Plan." *Cycads Status Survey and Conservation Action Plan*, 2003: 1-97.
- Jones, David L. *Cycads of The World*. Washington D.C.: Smithsonian Institution Press, 2002.
- Lund, U., and C. Agostinelli. *Circular statistics*. 2007. cran.r-project.org (accessed October 19, 2011).
- Montgomery Botanical Center . *Living Plant Collections*. August 12, 2011. www.montgomerybotanical.org (accessed August 12, 2011).
- Norstog, K J, and T J Nicholls. *The Biology of the Cycads*. New York: Cornell University Press, 1997.
- Oracle. *MySQL*. 2011. <http://www.mysql.com/> (accessed October 20, 2011).
- Stevenson, Dennis Wm., interview by James Clugston. *Montgomery Botanical Center Collection* (December 1, 2010).

