Cycad Cone Beetles: a DNA Study
-TCS Grant Proposal

ABSTRACT
A preliminary study of the mitochondrial gene 16S-rRNA in cycad cone beetles, indicates that it has great potential for use in taxonomic studies of cycad pollinators. Individual species of beetles suspected as being pollinators in different cycad species can be discriminated and identified based on the DNA sequence of this gene (ie. “DNA barcoding”). A phylogeny of cycad beetles can be constructed on this DNA data. Financial support is requested from The Cycad Society (TCS) to fund further DNA analysis and to help collect new beetle specimens from Asia for study.

INTRODUCTION
Cycads are an ancient group of plants with high priority in conservation from both a scientific and a biodiversity perspective (Donaldson 2003). Once thought to be wind pollinated, studies in recent years (Norstog et al. 1986, Tang 1987, Donaldson 1997, Terry 2001, Hall et al. 2004, Kono & Tobe 2007, Procheș & Johnson. 2009) have clearly established that living cycads are pollinated by insects. Each cycad species or species group appears to be dependent on its own specialized pollinator(s) for reproduction. Consequently, an understanding of cycad insect pollination systems is critical in the formulation of conservation plans for cycads. Currently, ecological studies on cycad pollination in India, China, Mexico and other countries is impeded by the poor understanding of the taxonomy of cycad pollinators. This is a grant proposal to The Cycad Society to help fund a DNA study of cycad beetles in Asia and the New World. DNA studies, along with ongoing morphological studies, will facilitate the recognition and description of beetles associated with cycad cones. This taxonomic work will provide a foundation for field workers to explore cycad pollination in more detail and thus determine which insects play the most critical roles in cycad pollination.

PROJECT MANAGERS
(CV’s attached)

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MATERIALS AND METHODS

The study systems

Cycad beetles in three families containing at least 9 or more genera are the focus of this study:

Family: Erotylidae:
- New World: *Pharaxonotha, Planismus* & at least 1 other undescribed genus
- Asia: 2 undescribed genera

Family: Belidae
- New World: *Rhopalotria & Parallocorynus*

Family: Curculionidae
- Asia: *Tychiodes* & at least 2 other genera

DNA analysis

The focus of this study is a single gene, named 16S-rRNA, which is located in the mitochondria of all multi-cellular organisms on earth. The gene has been demonstrated to be highly variable in most animal groups and is commonly used to decipher the evolutionary relations within groups of related genera and among species in species groups (Giribet et al. 2001). For techniques in sequencing this gene see (Kambhampati and Smith. 1995. Preliminary study by the project managers of this grant has shown that this gene is extremely useful in distinguishing cycad beetles found on different cycad species from different countries and islands. The individual DNA sequences of each beetle species appears to be unique and may be used in “DNA barcoding” – that is each cycad beetle can be identified solely on the basis of the DNA sequence of the 16S-rRNA gene.

Research Objectives for November 2010 – December 2011

1) **Continue to sequence the 16S-rRNA gene for cycad beetles:** Beetle samples are continuously being submitted by workers at various institutions including: University of Panama, Instituto de Ecologia (Mexico), SERBO (Mexico), School of Basic and Applied Sciences (India), University of Guam, MBC (FL, U.S.A.), etc... for analysis. The extraction, amplification, and sequencing of DNA require chemicals which must be continuously replenished.

2) **Conduct insect collecting expedition to Thailand:** Nong Nooch Tropical Garden has agreed to host and assist an expedition to collect fresh cycad cone insects in Thailand for Jan-Feb 2011. Funds are needed for airfare, lodging and other logistical expenses.

3) **Scientific meetings and field collections in China:** During CYCAD2011, which will be held in China in December 2011 at Fairy Lake Botanical Garden in the city of Shenzhen, results of this research will be presented and research relationships will be pursued.
   
   **A) Slide presentation and scientific paper:** a powerpoint presentation and scientific paper based on this research will be submitted to the cycad community at the CYCAD2011 meetings and in the proceedings based on these meetings.

   **B) Research relationships will be developed with Chinese researchers:** We will pursue research opportunities with various researchers and institutions in China and other Asian countries during the meeting. The objective is to engage a wider number of scientists in collecting cycad beetles from a broader range of countries in Asia and surrounding areas.
AMOUNT OF FUNDING AND GENERAL BREAKDOWN OF EXPENDITURES

A private corporation, SeqGen Inc., specializing in the maintenance of DNA sequencing equipment, has offered to fund a grant of $7000 or more to the project managers for this research. SeqGen Inc. wishes to submit this funding to the project managers through TCS. These funds will be disbursed in two parts: an initial grant of $2000 to be disbursed in 2010 or as soon as possible and a second grant of $5000 to be disbursed in 2011 based on a review of publication(s) or other evidence of progress. The project managers are not requesting more funds than what has been pledged, however, if TCS wishes to add funds to this amount it will be gratefully accepted. An approximate breakdown of how these funds will be spent is given in the table below.

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<tr>
<th>Initial $2000</th>
<th>Estimated</th>
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<tr>
<td>Expendable chemicals &amp; equipment for DNA extraction, amplification and sequencing for 2010 &amp; early 2011</td>
<td>$700</td>
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<td>Visas for Thailand (1-2 persons)</td>
<td>$100</td>
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<tr>
<td>Round trip airfare (partial cost for 1-2 persons) to Thailand during Jan-Feb (pollination period for most native Cycas)</td>
<td>$1200</td>
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<tr>
<th>2nd part of grant $5000</th>
<th>Estimated</th>
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<tr>
<td>Expendable chemicals &amp; equipment for DNA extraction, amplification and sequencing for late 2011 &amp; early 2012</td>
<td>$1300</td>
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<tr>
<td>Lodging and travel expenses in Thailand (partial cost for 1-2 persons)</td>
<td>$1500</td>
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<tr>
<td>Round trip airfare to China for CYCAD2011 (partial cost for 1-2 persons)</td>
<td>$1200</td>
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<tr>
<td>Registration, lodging &amp; per diem at CYCAD2011 (partial cost for 1-2 persons)</td>
<td>$1000</td>
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HOW THE PROJECT FITS THE INTERESTS OF TCS

This project pursues fundamental scientific knowledge on the reproduction of cycads, which fits into the TCS goal of supporting scientific research in cycads. Furthermore, the results of this research will enable graduate students and other researchers to accurately study and evaluate cycad pollinating beetles in the field. Information gained on these insects will allow conservation workers to incorporate conservation strategies for pollinating insects into those for cycads as a whole. Conservation of cycad populations and insect pollinators is mutually dependent. TCS support for this research will thus benefit conservation. Scientific & popular articles and powerpoint presentations of this research will educate both the scientific and general cycad public.

LITERATURE CITED


CURRICULUM VITAE

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PERSONAL

Born 31 May 1960, Hong Kong
Citizenship: U.S.A.
Married, no children

EDUCATION

Bachelor of Arts degree in Biology with High Honors, University of California at Berkeley, June 1982.


AWARDS AND HONORS

Phi Beta Kappa, 1982.

Maytag Fellowship, University of Miami, 1982-1985.

PROFESSIONAL POSITIONS

United States Department of Agriculture, Officer 1988-1997,
ENTOMOLOGIST 1997-present.

Fairchild Tropical Garden, Research Associate (adjunct staff), 1988-2004.
Under this title I have served as:

-Montgomery Foundation/Fairchild Tropical Garden expeditions to Southeast Asia, coordinator and expedition member, 1994, 1995.

-4th International Conference on Cycad Biology, Organizing Committee Member (held May 1996 in Panzhihua, China).
IUCN Species Survival Commission, Cycad Specialist Group, Member 1995-present.


Smithsonian Tropical Research Institute, Panama, Research Assistant 1983.

**PUBLICATIONS**

*Papers in peer-reviewed scientific journals, proceedings, and other major publications*

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PERSONAL

Born 24 November 1969, China
Citizenship: China
Married, no children

EDUCATION

Ph.D, Agricultural Entomology, Graduate School of Chinese Academy of Agricultural Sciences, Beijing, China, June 1998. Dissertation: Genetic variations and migration of *Helicoverpa armigera*.

Master degree of Entomology, Henan Agricultural University, ZhengZhou, China, June 1995. Studies on coccid inhabiting parasitic wasps in Southern China


AWARDS AND HONORS


“Geotypes and Migration Activities of *Helicoverpa armigera*” won the first prize of Science and Technology Progress, awarded by Ministry of Agriculture of People’s Republic of China in 1999 (National level prize in China).
“Studies on Parasitic Wasps of Forest Insect Pests in Henan Province” won the third prize of Science and Technology Progress, awarded by Science and Technology Associated Committee of Henan province in 1999.

NEW INSECT SPECIES DESCRIBED

*Adelencyrtus brachycaudae* Xu & Shi  
*Adelencyrtus chinensis* Xu & Shi  
*Microterys hunanensis* Xu & Shi

PROFESSIONAL POSITIONS

UMass Genomics Resource Laboratory /Department of Plant, Soil, and Insect Science, University of Massachusetts. Lab Manager and Research Assistant professor. Aug, 2007-present.

Dr. Stephen Rich’s Laboratory. Department of Plant, Soil, and Insect Science, University of Massachusetts. Research Associate, September 2005 – July 2007. Division of Infectious Diseases, Department of Biomedical Sciences, Tufts University, Research Associate, June 2004 – August 2005.

Department of Biology and Institute of Arthropodology and Parasitology, Georgia Southern University, Research Associate. September 2001 – May 2004.


State Key Laboratory of Plant Disease and Insect Pests, Chinese Academy of Agricultural Sciences. Research Entomologist, August 1998 – November 2000.

PUBLICATIONS

Peer review papers:


Books (co-author):

Presentations/abstract at professional meetings:


