MOLECULAR EVOLUTION AND DIVERSIFICATION OF THE AUSTRALIAN CICADA GENUS *PAUROPSALTA* GODING AND FROGGATT, 1904 IN RESPONSE TO THE ARIDIFICATION OF AUSTRALIA

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BACKGROUND: Cicadas (Hemiptera: Cicadoidea) are excellent model organisms for the study of systematics and biogeography. Cicadas are widespread and present on all continents except Antarctica and are found in most habitats. Species can easily be identified using male genitalia e.g., [1] and/or song e.g., [2]. Songs also enhance our ability to collect distributional data. Cicadas have limited dispersal ranges due to a short-lived adult stage and usually a multi-year underground nymphal stage [3,4] resulting in highly structured populations. This structure facilitates phylogeographic reconstructions of historical events e.g., [2,5,6].

The tribe Cicadettini contains one-third of all cicada species and has its center of diversity in Australia. The genus *Pauropsalta* Goding and Froggatt, 1904 is the largest cicadettine genus in Australia with 36 nominal species, 13 of which are known from one or few specimens [7-9]. Molecular and song data suggest ~ 65 undescribed spp. may exist. Preliminary studies by Moulds (unpublished) suggest that *Pauropsalta* may comprise multiple genera. With such a large number of undescribed species and uncertain generic status, this genus begs for a systematic revision.

Pauropsalta are distributed throughout Australia, which makes this group ideal for the study of speciation and diversification. Preliminary molecular data, including many outgroup species, suggests that this genus originated about 10-20 MYA [10]. During this time, Australia was becoming drier and cooler as South America broke free of Antarctica and the Antarctic circumpolar current formed [11]. Over the next 20 Myr, the land that was once warm and wet [12] became increasingly dry and arid in the late Cenozoic. The end result of this process is one of the world's largest desert regions, comprising 70% of Australia.

Recent phylogenetic studies have shown Australian arid-adapted plants and animals have undergone considerable increases in diversification rates during this time of climate change. For example, gall-thrips [13], sclerophyllous plants [14], and various squamates [15-19] all show signs of increased diversification during the aridification of Australia. Increases in diversification rates have produced lineages with more species than expected under the null model of constant diversification [19]. This pattern of increased diversification during the aridification of Australia may be present in the genus *Pauropsalta*. The short internal branches of the COI phylogeny (Fig. 1) combined with the potential origination of the genus 10-20 MYA [10] suggest that this genus went through a rapid radiation while Australia was becoming arid. To fully answer this and related questions will require a phylogeny based on sound taxonomy and species sampling of all nominal species [20]. Both poor sampling and unsure taxonomy can lead to biases in diversification and biodiversity statistics [20,21]. My initial COI (~ 1500 bp) phylogeny supports the hypothesis that *Pauropsalta* consists of multiple genera/species once thought to be single taxa (Fig. 1). For example, *P. encaustica* may consist of multiple--possibly 15--species. A thorough morphological re-examination of *Pauropsalta* is needed before phylogeographic hypotheses can be tested.

OBJECTIVES: The objectives of this study are 1) conduct a Bayesian and parsimony morphological analysis of the taxa in my initial COI phylogeny to test whether the morphological data supports the existence of multiple genera, while formally describing those genera and species supported by morphological and molecular analyses; 2) continue to accumulate nuclear and mitochondrial sequence data to combine with the morphological data to create a robust combined data phylogeny; 3) reconstruct biogeographic ancestral states and calculate diversification indices using the combined data tree to identify where diversification events occurred; 4) date the nodes of the phylogenetic tree to determine the timing of diversification events and approximate when the cooling of Australia caused lineages in the east to become separated from those in the west by looking at east-west bifurcations in the phylogeny.

MATERIALS AND METHODS: I currently have sequenced 339 specimens for the 3' end of COI (~800bp), 140 specimens for the entire COI locus (~1500bp), and 60+ specimens for elongation factor-1a (~2500bp). The COI phylogeny I have created will be used as a guide for the description of genera and species; the presence of both mitochondrial DNA, nuclear DNA and songs will help identify potential hybrids. Discrete binary morphological characters will be scored as "present" or "absent", while multistate characters will be

treated as "unordered" (= non-additive). Continuous characters will be transformed to binary or multistate characters using gap weighting [22]. Character polarity will be determined using outgroups [23, 24], e.g., *Gudanga aurea*, *Birrima castanea*, and *Gramenotigrina bowensis* identified by a tribal-level phylogenetic study [10].
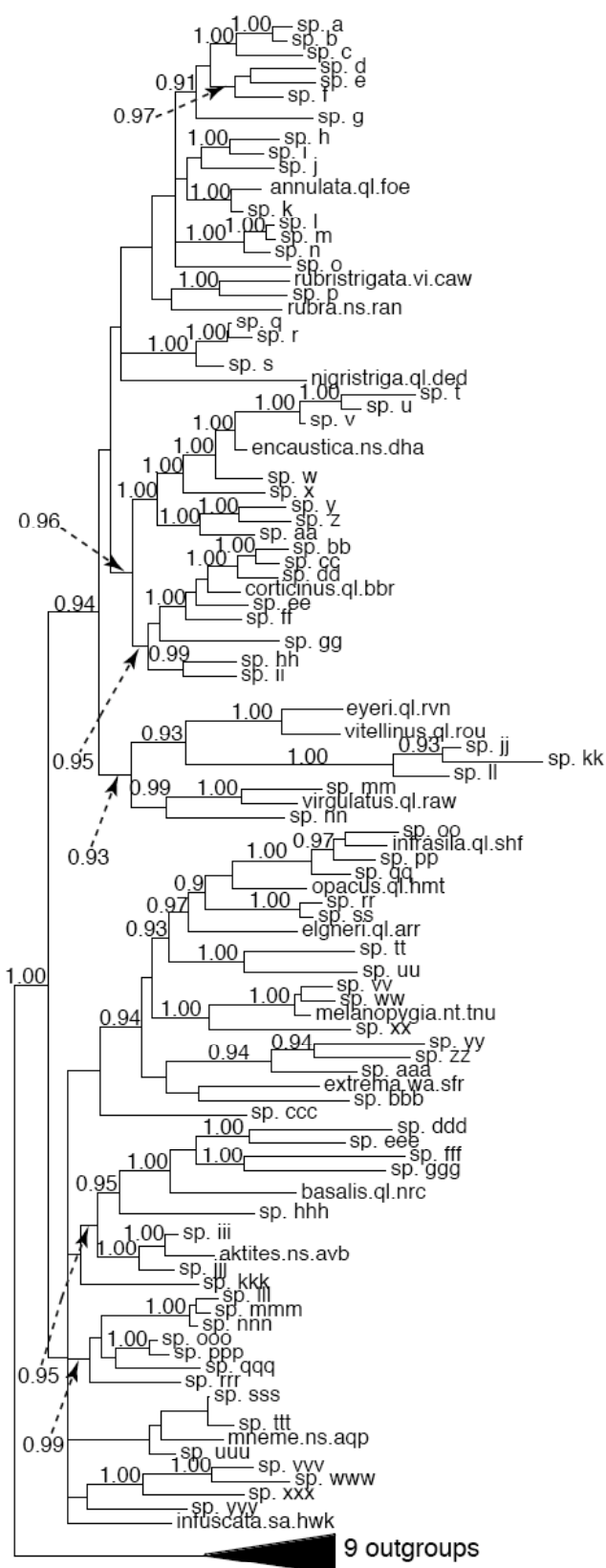
Cladograms will be generated using parsimony and Bayesian inference. Parsimony cladograms will be estimated in PAUP* [25] with branch support assessed using bootstrapping [26]. Bayesian inference will be implemented in MrBayes 3.1.2 [27, 28] using the Mk1 model [29] for morphological data with branch support calculated using posterior probabilities after burn-in. Molecular models of sequence evolution will be explored using the Akaike Information Criterion in Modeltest [30] and Bayesian posterior predictive simulation in PuMA [31]. Bayesian stochastic inferred ancestral biogeographic ranges will be estimated on the combined data tree with species ranges from new species descriptions and those previously described using SIMMAP [32]. Diversification rate shifts will be identified using SymmeTREE [33] and LASER [34], while diversification indices will be calculated using R programming libraries [35, 36]. Lastly, if the data do not fit the molecular clock model, then a relaxed clock approach will be implemented in BEAST [37].

SIGNIFICANCE: This proposed study represents the first contemporary generic revision of the genus *Pauropsalta* and will greatly expand the study of Ewart [7], which primarily reviewed Queensland species and described ten new species from this region. In addition, it will add to the limited number of Australian arid zone diversification studies focusing on insects. It will include descriptions of new species and possibly new genera, a key to all species and genera, and phylogenetic and diversification analyses of mitochondrial and nuclear genes. Taxonomy is the basis for all biological sciences and the foundation on which biological systems are described. The information preserved in taxonomic classifications of *Pauropsalta* species and genera will plays a fundamental role in determining the tempo and mode in which this diverse group of cicadas radiated throughout Australia.

The information arising from the generic revision will be used to explore various macroevolutionary aspects of this genus. For example, I will use species distributions to reconstruct biogeographic ancestral character states to explore how the distribution of this group changed as Australia became arid. Ecological niche modeling, which uses species distributions, will also be explored to determine if the current distribution of species can be explained by environmental factors such as rainfall, temperature, and soil type. The phylogeny, which will include all new and established species, will be used to estimate diversification rates in response to the aridification of Australia. In addition, the morphological data compiled for this study may give insight into how these cicadas have adapted to climate change since the Miocene. Lastly, the species and generic

Figure 1. Bayesian consensus phylogram of COI partitioned by codon position. Posterior probabilities ≥ 0.90 are shown.

descriptions will provide a better estimate of cicada diversity in Australia. Currently, ~ 240 spp. are described, while it is estimated that 500 spp. are undescribed. There are only three active cicada taxonomists currently describing species of Australian cicadas, one is a graduate student who is revising the species complex of *P. annulata* of Queensland and the other two are retired professionals; I am communicating with all three.

      SCHEDULE: This proposal represents a broad outline of my proposed graduate research plan. Currently, I am a second year PhD student at the University of Connecticut in the Simon Lab. This summer I will be spending 10+ weeks in Australia working with Dr. Max Moulds of the Australian Museum. He hosts one of the three largest cicada collections in the world, which houses ~5600 *Pauropsalta* specimens and ~52000 total cicada specimens. Under the expert tutelage of Dr. Moulds I will conduct a complete morphological revision of *Pauropsalta* (Objective 1) and anticipate publishing the results in a peer-reviewed journal Fall 2009. The rest of 2009 and 2010 I anticipate accruing additional sequence data to fulfill Objectives 2,3, and 4, while using the data to apply for the NSF Doctoral Dissertation Improvement Grant.

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BUDGET AND JUSTIFICATION

10+ weeks living stipend in Cairns, Australia = \$14/day x 10.5 weeks = \$1,029
Roundtrip flight to Cairns, Australia = \$2,500
Total Funding Requested = \$1000

Living expenses: Dr. Max Moulds has offered to host me in his home for the entire 10.5 weeks. The estimated \$1,000 will cover groceries for the 10.5 week period plus a contribution towards utility costs (Note: the U.S. State Department estimates per diem cost for meals and incidentals at \$93/day, compared to my \$14/day).

Airline ticket: \$2,500 (the current approximate going rate). The ticket was purchased using frequent flyer miles donated by Chris Simon.

ADDITIONAL FUNDING SOUGHT THROUGH OTHER SOURCES

1. Ecology and Evolutionary Biology Departmental Grant, University of Connecticut: requested \$110.53 to cover airport and ticketing fees. *Submitted February 27, 2009.*

2. Ernst Mayer Grant, Harvard University, Museum of Comparative Zoology: will request \$1000 to cover illustration costs. *Due April 15, 2009.*

3. NSF-Doctoral Dissertation Improvement Grant: will request \$12000 to cover lab costs (e.g., sequencing, reagents, etc.) and a collecting trip to Australia. *Due November 29, 2009.*

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Ph.D. Student, Entomology, Department of Ecology and Evolutionary Biology,
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M.S., Marine Biology & Marine Environmental Science, NSU Oceanographic Center,
Nova Southeastern University, Dania Beach, FL, 2007.

B.S., Environmental Science, Department of Biology,
Muhlenberg College, Allentown, PA, 2000.

RESEARCH EXPERIENCE:

University of Connecticut, Department of Ecology and Evolutionary Biology
Research Assistant, January – May 2008, January – May 2009
NSF-Systematic Biology and Biodiversity Inventory: Systematics and biogeography of Australian Cicadetinni and their relatives worldwide.

Pennsylvania State University/United States Department of Agriculture – APHIS
Lab Technician, August 2006 – August 2007
Determined population structure and phylogeography within and among *Anastrepha* spp. (Diptera: Tephritidae) of economic importance using microsatellites and mtDNA.

PUBLICATIONS (INCLUDING SUBMITTED AND IN PREPARATION):

Owen, C.L., C.G. Messing, G.W. Rouse, M.S. Shivji. 2009. Using a combined approach to explain the morphological and ecological diversity in *Phanogenia gracilis* Hartlaub, 1893 (Echinodermata: Crinoidea) *sensu lato*: two species or intraspecific variation? Marine Biology, *Accepted*.

Owen, C.L., C.G. Messing, G.W. Rouse, M.S. Shivji. Using mtDNA and morphology to define species boundaries among three closely related crinoids, *Submitted*.

Ruiz, R., **C.L. Owen**, B.A. McPheron, D.B. Thomas. Phylogeographic analysis of Mexican fruit fly (Diptera: Tephritidae) using mitochondrial DNA sequence data, *In Prep*.

ORAL AND POSTER PRESENTATIONS:

Owen, C.L., C.G. Messing, G.W. Rouse, M.S. Shivji. 2006. Delimiting species of crinoids: comparing new mtDNA phylogenies with current species designations and reevaluating overlapping morphological characters. Oral presentation. 12th International echinoderm conference, Durham, New Hampshire.

Ruiz, R., B.A. McPheron, A. Tu, E. Deutsch, **C.L. Owen**, M. Aluja, J. Pinero. 2006. Sequence-based analysis of population structure in *Anastrepha obliqua*. Poster presentation. 7th International symposium on fruit flies of economic importance/6th Meeting of the working group on fruit flies, Salvador, Bahia, Brazil.

PROFESSIONAL SOCIETIES: Society of Systematic Biologists, Society for the Study of Evolution