Systematics of *Zelus* Fabricius 1803 and Harpactorini (Hemiptera: Reduviidae: Harpactorinae)

PhD Dissertation Proposal

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INTRODUCTION

With close to 7,000 species, Reduviidae, the assassin bugs, are the second largest and one of the morphologically and ecologically most diverse families of true bugs (Hemiptera: Heteroptera) (Froeschner & Kormilev 1989, Maldonado 1990, Putshkov & Putshkov 1986-89, Schuh & Slater 1995, Weirauch 2008). Reduviids have fascinating biology including feeding specializations and parental care. Among the prey specializations are millipede-feeding in Ectrichodiinae (Giliomee 1985, Lawrence 1984), spider-hunting in the thread-legged bugs or Emesinae (Wignall & Taylor 2008, Wygodzinsky 1966), and blood-feeding in the Triatominae (Lent & Wygodzinsky 1979). Species of *Atopozelus* Elkins, *Occamus* Distant, *Pisilus* Stål, *Rhynocoris* Kolenati (Harpactorinae: Harpactorini), and *Ghinallelia* Wygodzinsky (Emesinae) exhibit parental care in various forms such as egg guarding or nymph carrying (reviewed in Tallamy *et al*. 2004).

Systematics of Reduviidae: Status, problems & needs – Despite the fascinating biology of Reduviidae, only few recent systematists work on the group and there is a need for systematic research employing modern methods and tools. Very few recent workers conduct comprehensive monographic taxonomic revisions. Modern phylogenetic analyses of subfamily relationships did not exist until Weirauch (2008) (but see Clayton 1990 for an unpublished cladistic analysis). Several subfamilies such as Saicinae (~138 spp.), Salyavatinae (~98 spp.), and Reduviinae (~1,000 spp.) are ill-defined and possibly paraphyletic or polyphyletic (Weirauch 2008; Weirauch & Munro, in press). Few studies have performed phylogenetic analyses within the subfamilies (but see Dougherty 1995, Hypsa *et al.* 2002).

In order to improve the systematics of Reduviidae, research on large-scale monographic systematic revisions, testing the monophyly of higher taxonomic groups, and phylogenetic analyses of relationships between and within subfamilies are much needed I aim to address some of these aspects as part of my PhD dissertation project objectives by conducting a monographic systematic revision of *Zelus* Fabricius 1803 (Harpactorinae: Harpactorini) and investigating the phylogenetics of selected genera of Harpactorini.

***Zelus* Fabricius 1803: Natural enemies, systematics & pheromones** – With 60 valid species and a total of ~75 estimated species, *Zelus* is one of the largest genera of Reduviidae (Hart 1972, Maldonado 1990). Its species are among the most frequently collected reduviids in the New World as indicated by the large number of specimens in museums (>10,000), but also judging from collecting experiences in our lab. It has high species diversity in the tropics (e.g., 21 spp. in Colombia versus 5 in the US). Species of *Zelus*, among several other genera of Harpactorinae (e.g., *Arilus* Hahn, *Sinea* Amyot & Serville, and *Montina* Amyot & Serville) have been explored and studied as natural enemies in the Americas (Cogni *et al.* 2002, Cohen & Tang 1997, reviewed in Hagen *et al*. 1999). Recently, males of *Zelus tetracanthus* Stål have been found to be attracted to aggregation pheromones of grain bostrichid beetles (Edde & Phillips 2006) and further research on the elucidation of pheromones of *Zelus* spp. will facilitate their use as natural enemies. To advance the research on *Zelus* spp. as natural enemies, reliable taxonomic information such as accurate species description and efficient identification keys is indispensable since misidentifications can arise easily for similar species (e.g, Curkovic *et al*. 2004)

Hart (1972) conducted a systematic revision of *Zelus* in his PhD dissertation with substantial taxonomic changes including new species, synonyms and combinations. This work remains largely unpublished (but sees Hart 1986 and 1987 for revisions of 20 North American, Northern Mexican and Caribbean species) and thus the taxonomic changes remain 'unavailable' to the scientific communities according to the rules of defined in the *International Code of Zoological Nomenclature*. Hart's (1972) work was valuable, and yet the current state of taxonomy of *Zelus* remains unsatisfactory. Hart (1972) did not provide habitus images and only documented male genitalic structures. His descriptions of external morphology were simplistic and sometimes inconsistent. The taxonomic key is 96-couplet long and difficult to use. Hart (1972) only examined three collections based in Latin American countries, where additional new species will most likely be discovered. Also, ~30% of museum specimens remain unidentified or misidentified (data collected from museum curators in March 2009). Furthermore, many important aspects for a modern monographic systematic revision are missing or inadequate in Hart (1972) primarily due to historical constraints. These include testing the monophyly and identifying the phylogenetic position of *Zelus*, constructing a species-level phylogeny of *Zelus* spp., and employing bioinformatics tools to document and disseminate taxonomic information. I thus propose to conduct a

modern systematic revision of *Zelus* based on >10,000 specimens, examine and evaluate taxonomic changes in Hart (1972), and produce a monograph of 75 species.

Harpactorini: Systematics & problems – *Zelus* belongs to the tribe Harpactorini as defined by Davis (1969), the largest tribe in the largest subfamily of the Reduviidae, Harpactorinae. Harpactorini currently comprise 289 genera and 2003 described species (Maldonado 1990, see also Brailovsky & Barrera 2004, Malipatil 1991, Melo 2008, etc. for new species and genera since 1990), i.e. 87% of the species diversity of Harpactorinae and 30% of that of Reduviidae (Maldonado 1990). Harpactorini contain some of the largest genera in Reduviidae such as *Sphedanolestes* Stål (181 spp.) and *Rhynocoris* Kolenati (118 spp.) from the Old World and *Zelus* Fabricius (60 spp.) from the New World (Maldonado 1990). I will focus on tackling two problems of the systematics of Harpactorini. First, this group is currently not defined by diagnostic characters, but distinguished from related groups based on the absence of characters of the other tribes (Davis 1969). This leads to an important question: are Harpactorini monophyletic? A recent phylogenetic study of Reduviidae based on morphology supported the monophyly of Harpactorini (Weirauch 2008). However, a preliminary molecular phylogeny of Reduviidae indicates the paraphyly of Harpactorini with respect to Rhaphidosomini (Harpactorinae) (Weirauch & Munro, in press). The second problem is that compared to other tribes of Harpactorinae (e.g., Apiomerini [156 spp], Diaspidiini [11 spp.], Maldonado 1990) Harpactorini are very large and a supra-generic classification within the tribe is lacking. As an initial effort to address these problems, I propose to construct a phylogeny of 70 representative genera of Harpactorini using molecular data. In the context of this phylogenetic framework of Harpactorini, I will test the monophyly of *Zelus,* and identify its phylogenetic placement.

Sticky trap predation in Harpactorinae and *Zelus* – While the monographic revision of *Zelus* and the phylogenetic study of Harpactorini address the core research topics in systematic entomology, utilizing the phylogenies to study character evolution will enrich the dimension of the project. I thus propose to study the evolution of sticky trap predation using endogenous sticky substances and associated morphological structures in *Zelus* and other Harpactorini. Many Harpactorinae seem to share an association with resin-producing plants or the use of sticky substances for prey capture, a phenomenon called sticky trap predation. Fourteen genera within Ectinoderini, Apiomerini and Harpactorini are known to inhabit plants that produce resins or sticky substances (Berénger & Pluot-Sigwalt 1997). Species of Ectinoderini, Apiomerini and Diaspidiini collect plant resins, which are smeared onto the legs and body and used to capture prey or glue eggs into a clutch (Choe & Rust 2007; Miller 1942, 1971; Roepke 1932; Usinger 1958; Weirauch 2005). Members of these three tribes are called resin bugs (Davis 1969). Resin-collecting has not been documented in members of the tribe Harpactorini.

By contrast, species of *Zelus* utilize an endogenous source of sticky substances. Four species of *Zelus*, *Zelus leucogrammus* (Perty), *Zelus longipes* (Linnaeus), *Zelus luridus* Stål, and *Zelus renardii* Kolenati are found to secret sticky substances from dermal glands on the front tibiae (Barth 1952, Weirauch 2006, Wolf & Reid 2001). The sticky secretions are retained by specialized tibial setae resembling the trichomes of sundew leaves (Fig. 5) (Weirauch 2006, Wolf & Reid 2001, Zhang, pers. obs.). Behavioral observations (Weirauch 2006; Law, pers. comm.; Zhang, pers. obs.) suggested that the sticky substances secreted onto the front tibiae assist the bugs in capturing prey. No studies have investigated homologous structures on the front tibiae of other genera of Harpactorini and the evolutionary origin of this phenomenon is therefore unknown. Only two anecdotal records are published: Readio (1927) mentioned secretory setae in *Pselliopus cinctus* (Fabricius) without documentation of the structures involved, and Cobben & Wygodzinsky (1975) speculated on the existence of sticky setae in *Cosmoclopius curacavensis* Cobben & Wygodzinsky.

Current hypotheses on tribal phylogenetic relationships suggest that the association with sticky plants and sticky substances may be plesiomorphic (ancestral) within Harpactorinae (Weirauch 2008, Weirauch & Munro, in press). Following from that hypothesis, resin collecting would appear to be plesiomorphic as well with a subsequent loss in the Rhaphidosomini-Tegeini-Harpactorini assemblage. Under this scenario, an endogenous production of sticky substances from glandular structures on forelegs in *Zelus* is likely a derived character. Morphological examinations of representative genera of Harpactorini are crucial to document sticky glands and structures associated with the sticky trap behavior. These characters will

then be optimized on the phylogenies of Harpactorini and *Zelus* and will result in a testable hypothesis on the evolution of sticky trap predation using endogenous sticky secretion within the group.

OBJECTIVES & APPROACHES of the dissertation research

1. Conduct a monographic systematic revision of *Zelus* Fabricius 1803.

A. Produce a monograph of *Zelus* for ~75 species based on ~10,000 specimens and disseminate taxonomic information through online open-access resources.

B. Construct a species-level phylogeny within *Zelus* using ~80 morphological characters for all species and 6 genes for 30 species, and build a sub-generic classification.

2. Construct a phylogeny of selected genera of Harpactorini.

A. Test the monophyly of Harpactorini.

B. Generate a framework for a phylogeny-based supra-generic classification within Harpactorini.

C. Test the monophyly and identify the phylogenetic placement of *Zelus* within Harpactorini.

3. Study the evolution of sticky trap predation based on comparative morphology of associated structures in *Zelus* and Harpactorini.

A. Document tibial glands and associated structures in species groups of *Zelus*.

B. Investigate homologous structures on the front tibiae of representative genera of Harpactorini.

C. Test hypotheses on the evolution of sticky trap predation within *Zelus* and the Harpactorini.

4. Investigate pheromones in *Z. tetracanthus* and *Z. renardii* (optional).

A. Conduct bioassays to investigate the presence of pheromones and the nature of the pheromones in *Z. tetracanthus* and *Z. renardii*.

B. Identify chemical structures of pheromones in *Z.tetracanthus* and *Z. renardii* (in collaboration with Dr. Millar).

OBJECTIVE 1: Monographic systematic revision of Zelus

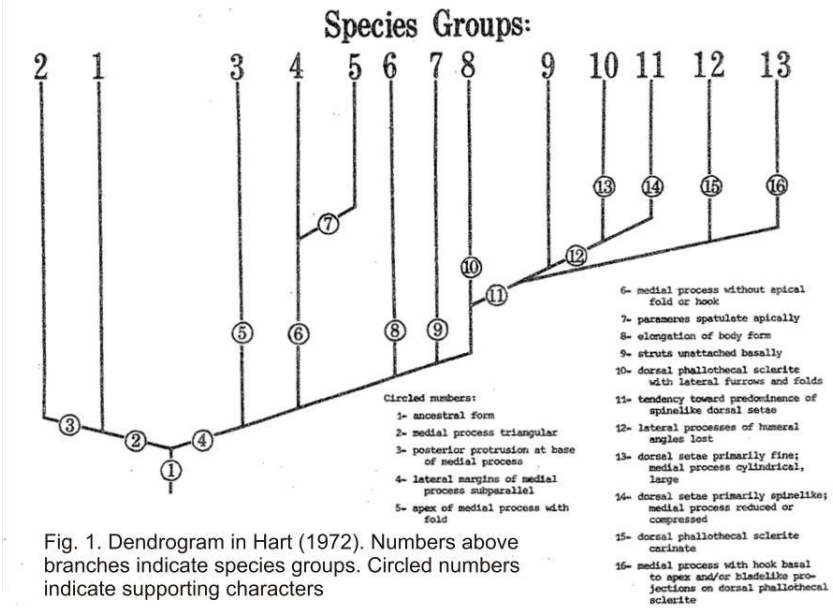

Fig. 1. Dendrogram in Hart (1972). Numbers above branches indicate species groups. Circled numbers indicate supporting characters

Background: Taxonomic history: Hart (1972, pp. 4-14) provided a comprehensive literature review of the taxonomic history of *Zelus* prior to 1972. It was characterized by what he called 'extreme fluctuations' since the generic limit of *Zelus* had been very unstable (cf. Maldonado 1990). In Hart (1972), there were descriptions of 25 new species, 26 new synonyms, and 7 species were removed from *Zelus*. This work remained largely unpublished. There has been little taxonomic activity on *Zelus* after Hart (1972). Hart later in two small publications (1986, 1987) covered 20 species in North America, Northern Mexico and the West Indies, including 4 of the new species proposed in his dissertation. Coscaron *et al*. (2002) and Melo *et al.* (2005) documented immature stages of *Zelus*

leucogrammus (Perty) and *Zelus longipes* (Linnaeus), respectively. Jadin *et al.* (2002) described a new species, *Zelus josephpaulusi*. In my view, the generic placement of this species in *Zelus* is doubtful. The median process of the pygophore of the males is bifurcating, whereas it is undivided in all other species of *Zelus* (Hart 1972). Forero (2003) provided records and distribution maps of *Z. longipes* in Colombia. Gil-Santana (2008) added *Zelus versicolor* (Herrich-Schäffer) as a new record for Bolivia and documented the color polymorphism in females. If taxonomic changes made in Hart (1972) and Jadin (2002) are considered, 67 species may be included in *Zelus*. I expect to discover additional new species after examining a larger sample of specimens especially from Latin America, totaling the number of species of *Zelus* to about 75.

Species phylogeny and sub-generic classification: The taxonomic history of the sub-generic classification within *Zelus* was reviewed in Hart (1972). Stål (1862) recognized 3 subgenera based on pronotal armature. Hart (1972) claimed that this classification was 'superficial' since pronotal armature, in his view, was prone to convergence. Instead, he proposed 13 species groups and hypothesized their relationships as a hand-drawn dendrogram based on pre-conceived plesiomorphic and synapomorphic characters without justifications for the polarity of the characters (Fig.1). In this scheme, male genitalic structures such as the shape of the median process of the pygophore, the folding of the apex of the median process, and the dorsal phallothecal sclerite are important group-defining characters. Interestingly, Hart also suggested a tendency towards pronounced sexual dimorphism in *Zelus*. Rigorous phylogenetic analyses are needed to test Hart's proposals of species groups, species phylogeny and his hypothesis regarding the evolution of sexual dimorphism.

Preliminary results: I have obtained loans of more than 3,000 specimens from 7 museums including one in Costa Rica and one in Colombia. I have taken habitus images of 28 species (including several species with as of now unpublished names) and made them available online at 'Discoverlife' [http://www.discoverlife.org/mp/20q?search=Zelus]. I dissected and documented 10 species of male specimens of *Zelus* with the Auto montage GT Vision photographic system. Photos have been uploaded in 'MorphBank' [http://www.morphbank.net/Browse/ByImage/?tsn=107367] (Fig. 2). With help from an undergraduate assistant, about 400 specimens have been databased and the distribution map can be viewed through 'Global Mapper' at [http://www.discoverlife.org/mp/20m?act=make_map] (Fig. 3). I constructed a preliminary phylogeny (Fig. 4) of 11 species of *Zelus* representing 6 species groups as proposed in Hart (1972) based on 3 genes (16S, 28S D2, 28S D3-D5) using the parsimony criterion as implemented in TNT (Tree search using New Techonology, Goloboff *et al.* 2003). The monophyly of two species groups were recovered (groups 4 & 7 in Hart 1972). The monophyly of species group 11 was not recovered and the relationships between the species groups were very different from that in Hart (1972). The results (tree not shown) obtained from using the Maximum Likelihood approach (RAxML via CIPRES) differed significantly from both the parsimony results and Hart (1972) (tree not shown).

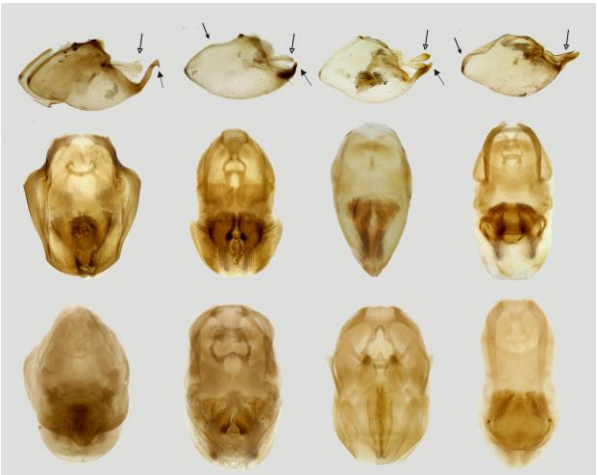

Fig. 2. Examples of male genitalic structures of *Zelus* spp. Top row: pygophore from left lateral view, arrows point to taxonomic characters. Second and third rows: phallus from dorsal view, anterior side facing up.

Fig. 3. Distribution map of *Zelus* spp. generated by 'GlobalMapper'.

Proposed research

Objective 1A: **Produce a monograph of ~75 species of Zelus.** My goals of this part of the monographic revision pertain to the α-taxonomy of *Zelus,* i.e. focus on species delimitation and description, taxonomic character documentation, specimen dabasing and geo-referencing, and generation of identification keys. Species delimitation and description: Hart (1972) used primarily male genitalic characters to delimit species. I will examine, document and describe both external morphology and male genitalic characters, and explore and document female internal and external genitalic structures that have shown to yield valuable taxonomic characters in other groups of Harpactorinae (e.g., *Apiomerus* Hahn; Forero, pers. com.). I will use the DELTA (DEscriptive Language for TAxonomy) software package (Dallwitz *et al*. 1993) to record taxonomic characters in matrix-form, and translate them into natural language descriptions as currently done in our lab (e.g., Zhang & Weirauch, in prep.). To avoid predisposed conception, I will initially sort specimens to species without consulting Hart (1972), form my hypotheses of species delimitation independently, and subsequently check my species hypotheses with the species treated in Hart (1972). Congruent species definitions between Hart (1972) and my own observations based on additional independent characters will provide corroboration for that species hypothesis. I will examine the discrepancies with close scrutiny, and adjust or change species hypotheses. Habitus images & taxonomic character documentation: I will provide habitus images in dorsal view of males and females of all species. I will document relevant taxonomic characters with images taken with Microtpics or GT-Vision systems and with illustrations when photography does not allow to capture important details. Scanning electron microscopy will be used to document ultra-structures such as gland-associated setae and cuticular surface structures. I will explore the use of confocal microscopy to document female internal genitalic structures. Habitus images will be uploaded on 'Discoverlife' [www.discoverlife.org] and morphological characters on 'Morphbank' [www.morphbank.org] to make the information easily accessible and allow for future online collaborative work. Databasing and geo-referencing specimens: I target to database and geo-reference ~5,000 specimens, i.e. half of the specimens on loan, using the PBI plant bug locality database funded by NSF. This excludes a significant proportion of the material examined (~5,000 specimens), but is justified by the fact that a large number of specimens will pertain to only a handful of species with largely redundant distributional information. The data is fed to www.discoverlife.org and the distribution maps of *Zelus* spp. are automatically generated through 'Global Mapper' (Fig. 3). Identification keys: The identification key in Hart (1972) is mainly applicable to males and difficult to use. To enhance efficiency, I will construct identification keys to both species groups of *Zelus* and individual species. I will adopt both conventional paper-compatible dichotomous keys and online interactive keys. The former is computer-independent, while the latter has higher efficiency and can easily be updated or corrected (Walter & Winterton 2007). I will make the online interactive keys available at www.discoverlife.org as currently done for the key to the genera of Apiomerini [http://www.discoverlife.org/mp/20q?guide=Apiomerini]. Taken all of the above together, I expect to produce a systematic monograph treating ~75 species with detailed and consistent descriptions and documentations of taxonomic characters.

Objective 1B: Species phylogeny within *Zelus* & sub-generic classification. I target to construct a species-level phylogeny within *Zelus* using 80 morphological characters for all species and 6 genes for 30 species, and build a sub-generic classification. Phylogeny based on morphological characters: I will assemble at least 80 morphological characters, i.e. 40 external, 30 male genitalia, and 10 female genitalia. Male genitalic structures harbor a substantial amount of characters that appear to be useful for phylogenetic analysis: e.g., the shape of the dorsal phallothecal sclerite, the median process of the pygophore (Fig. 2), and the structures of the parameres vary between species of *Zelus* and were used to characterize species groups in Hart (1972). Phylogeny based on molecular data: I target to sequence 6 genes, i.e. 16S, 28S D2, 28S D3-D5, COI, Cytochrome B, and H3 (Histone 3)/Wg (Wingless) and have so far obtained the first 3 genes for 11 species of *Zelus*. Sub-generic classification: I will test and revise the species groups proposed in Hart (1972) with both the morphological and the molecular phylogenies. I will provide diagnoses and identification keys to the species groups.

Approaches: Specimens: Museum collections: I will take on loan more than 10,000 specimens from museums worldwide. The emphasis will be on collections based in Latin American countries that Hart did not examine. I have so far obtained loans of ~4,000 specimens representing ~40 species from 7

museums. I will be working in the Smithsonian National Museum of Natural History for 10 weeks and the American Museum of Natural History for 2-3 weeks during the winter and spring quarters of 2010 with a Smithsonian Fellowship just awarded to me. Part of this research time will be devoted to sorting specimens of *Zelus*, taking habitus images, and databasing and geo-referencing selected specimens. Specimens: Field collections: *Targeted countries*: During the coming summer and academic quarters, I target to collect *Zelus* spp. in Mexico (July 2009, 17 days, funding secured [~3,000]), French Guiana (3 weeks, funding secured [NSF-PEET; 3,000-4,000]), Colombia (May 2010, 3 weeks, funding secured [van den Bosch scholarship, $5,000]), and during an OTS (Organizations of Tropical Studies) field course in Costa Rica in August 2010 (3 weeks, funding secured [NSF-PEET]). The four targeted countries have exceptionally high diversity of *Zelus* (Hart 1972, Maldonado 1990), but also Harpactorini more broadly. *Collecting and export permits, and logistics*: I am aware of policies regarding collecting and exporting/importing specimens in both the U.S. and foreign countries. Our lab has established local contacts in Mexico (Dr. Harry Brailovsky), Colombia (Dr. Carlos Sarmiento), and with a French national (Dr. Jean-Michel Berénger). We have accumulated alcohol specimens of 11 species of *Zelus*, and I aim on collecting 20-25 additional species during the proposed field work. *Collection methods*: *Zelus* spp. are diurnal and inhabit vegetation (Hart 1972, Bérenger & Pluot 1997). I will employ a variety of methods comprising sweep netting, beating vegetation, and searching with eyes that are effective for catching *Zelus* spp. based on our experiences. *Preservation methods*: Specimens collected in the field will be kept in 95%-100% alcohol and then transferred to isopropylene glycol, a non-toxic, non-flammable material suitable for transportation on airplanes. Previous trials in our lab have confirmed that material collected and transported in this way maintain to generate good sequences for several genes.

Databasing and georeferencing: Specimen locality data will be entered in the web-based database developed during the PBI project on Plant Bugs [http://www.research.amnh.org/pbi/databases/locality_database.html] funded by NSF. The output of specimen data is flexible, and includes specimen records, lists of host or prey organisms, and coordinates that can be used to plot distribution maps. Data are publicly available through 'Global Mapper' at www.discoverlife.org (Fig. 3). The specimen database allows for targeted downloading of localities that lack coordinates; those localities can then be geo-coded using software such as 'Geolocate' and 'Google Earth'. Matrix labels that uniquely identify each specimen (USIs) will be attached to the specimens. With an estimate of 3 minutes per specimen, it will amount to about 500 hours to enter the proposed 5,000 specimens, a workload that can be achieved in a 3-year period. The specimen database will also be used to keep track of voucher specimens for DNA extractions as currently done for other reduviid alcohol specimens in our lab.

Descriptive taxonomy: *Species description*: I will use the DELTA software to prepare consistent species descriptions for the relatively large genus *Zelus*. The software is able to translate characters as a matrix into natural language descriptions. Formatting can be manipulated easily and consistency can be achieved. *Taxonomic character documentation*: I will use the Microptics-USA system and the Automontage GT-Vision micro-imaging system for acquiring habitus and structural-detail images of *Zelus* as is done in our lab (e.g., Weirauch 2005, 2006, 2007; Zhang & Weirauch, in prep.). I will also provide illustrations to complement light microscopical imaging, SEM, confocal microscopy and histological techniques (where appropriate; e.g. Schuh, Weirauch, Henry & Halbert 2008). The equipment for these approaches is present in our lab or on campus (SEM, confocal, histology facilities). I will use 'CorelDraw' and 'PhotoShop' image processing software to make plates of publication quality.

Morphological phylogenetic characters: The character matrix will be exported from DELTA in NEXUS format that can be manipulated for phylogenetic analyses. I will focus on discrete morphological characters for all species of *Zelus* and a comprehensive sample of outgroups. Coding (binary and multistate, non-additive) will follow standard procedures as was done for 162 characters in Weirauch (2008). The utility of continuous characters will also be explored.

Molecular data: *DNA extraction, amplification and sequencing*: DNA will be extracted using the DNesay kit. PCR will be performed with a Fisher Scientific Thermo Cycler and sequencing using the Applied Biosystems 3730xl DNA Sequencer at the UCR core facility. A right hind leg or tibia will be removed from the specimen for extraction. All extracted specimens will be vouchered, matrix coded, and

entered in the PBI Plant Bug specimen database. *Sequence editing and quality controls*: Sequencher 4.8 will be used to edit and assemble sequences. All protein-coding genes will be checked to make sure they are translatable, and all sequences will be checked against the Genbank database using the BLAST function to monitor contaminations. *Alignment*: Protein-coding genes are usually easily aligned with Clustal-W. I will explore different options of multiple sequence alignment for ribosomal genes (MAFFT, T-Coffee, Jalview, ProbCons) and further investigate secondary structure alignment models for rDNA data (Gillespie *et al*. 2005, Gillespie *et al*. 2006). I will also explore direct optimization (Wheeler 1996) as implemented in POY version 4.0 (Varón et al. 2007), a program that allows for parsimony and maximum likelihood approaches (Wheeler 2006).

Phylogenetic analytical methods: Both separate and combined analyses of the morphology and molecular data sets will be performed. The morphological data set will be analyzed using the parsimony criterion as implemented in the free software TNT (Goloboff *et al.* 2003). The molecular data set will be analyzed using a variety of methods (parsimony, maximum likelihood, and Bayesian) implemented in several programs or online portals (TNT, RAxML via CIPRES, MrBayes). For the combined morphological and molecular analysis I will use TNT and POY (for parsimony analyses) and Bayesian approaches (MrBayes [Huelsenbeck and Ronquist 2001], BaliPhy [Suchard & Redelings 2006]). I will examine data set congruence (ILD tests, Farris *et al*. 1995; MRI, Wheeler *et al*. 2006), calculate branch support using nonparametric bootstrap analysis (Felsenstein 1985), Bremer support (Bremer 1988), and partitioned Bremer support (Baker & DeSalle 1997).

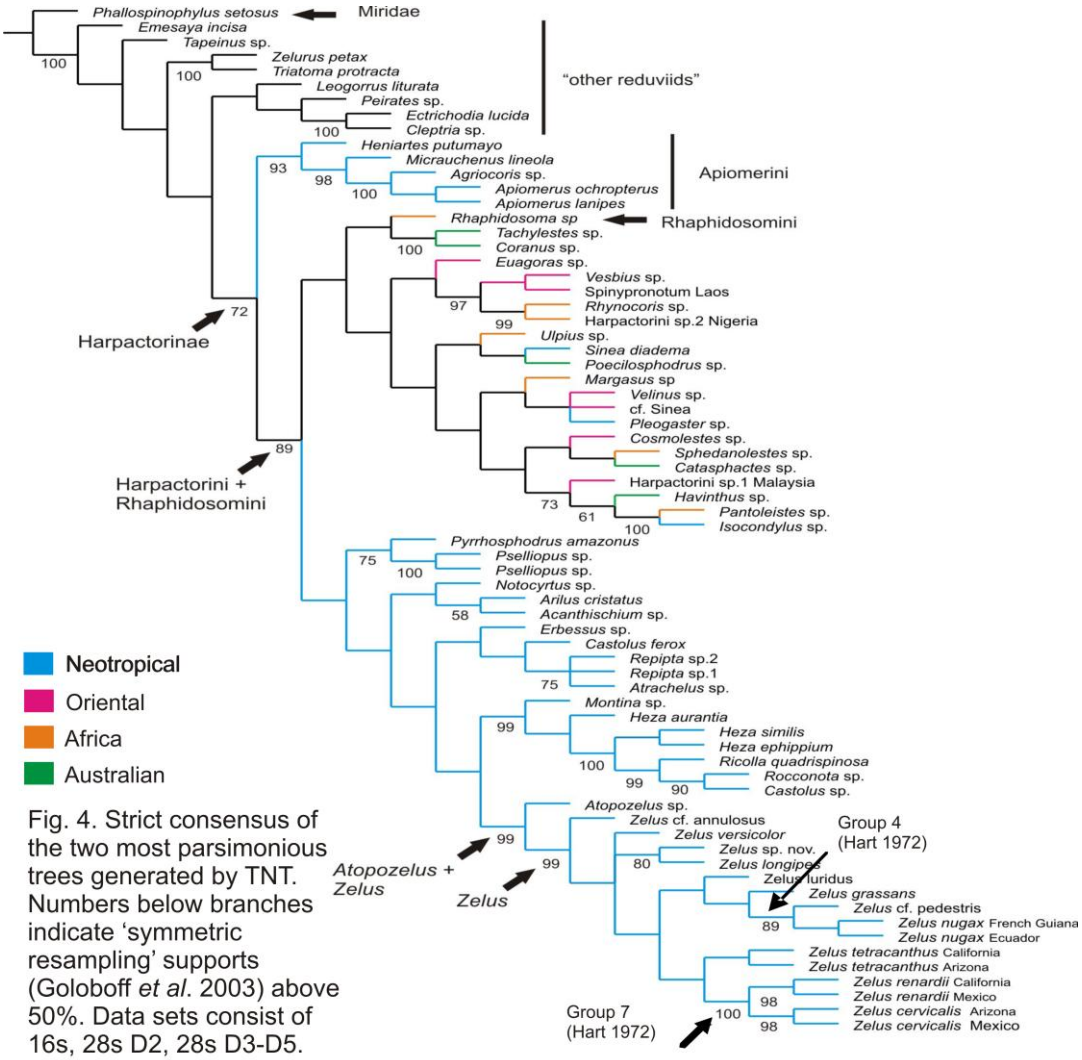

Fig. 4. Strict consensus of the two most parsimonious trees generated by TNT. Numbers below branches indicate 'symmetric resampling' supports (Goloboff *et al*. 2003) above 50%. Data sets consist of 16s, 28s D2, 28s D3-D5.

OBJECTIVE 2: Genus-level phylogeny of Harpactorini

Background

Systematic history and monophyly of Harpactorini: The name Harpactorini was originally proposed in Amyot & Serville (1843). Stål (1859) used the name to refer to members of Reduvina, the equivalence of a collection of several tribes of Harpactorinae as defined in Davis (1969) that have an anterior mesopleural tubercle, the plica. Zelini, another name created in Amyot & Serville (1843) was used to refer to members of the Harpactorinae without the plica. Stål stopped using these two names later. Subsequent authors (e.g., Miller, Villiers, Davis) did not use the plica as a character for tribal delimitation or supra-generic classification. The modern concept of Harpactorini contains members with and without the plica.

The monophyly of Harpactorini has not been rigorously tested. While the other 5 tribes of Harpactorinae can be defined by putative synapomorphies, Harpactorini are a group distinguished primarily on the basis of absence of characters of other tribes (Davis 1969). The monophyly of Harpactorini was recovered in Weirauch (2008), although there was no discussion of supporting characters. The validity of the result was limited because the study only examined 5 species of Harpactorini and did not include Rhaphidosomini (Harpactorinae), a possible sister taxon. In a later molecular phylogenetic analysis that had a larger taxon sample and included a species of Rhaphidosomini, Harpactorini were paraphyletic with respect to that species (Weirauch & Munro, in press).

Classification within Harpactorini: Subdivisions within Harpactorini have been proposed, but the groupings were largely arbitrarily based on superficially similar structures that do not necessarily reflect phylogenetic relationships. They were sometimes done on a regional basis, and consistency was lacking between authors. Distant (1904) used 11 'divisions' to classify the species of Harpactorinae which included what is now considered as Harpactorini, Rhaphidosomini and Tegeini in his monograph, *The Fauna of British India, including Ceylon and Burma*. The taxonomic category, 'division' is not currently used in zoological nomenclature, and the 'divisions' in Distant (1904) are a supra-generic category roughly comparable to tribes. Distant (1904) did not specify which of the divisions were created by him, but apparently at least 9 were new names. Most divisions were based on a few superficially similar structures. Several groups were monotypic. Subsequent workers seldom adopted these proposals. The most recent catalogue of Reduviidae, Maldonado (1990) treated 3 non-Harpactorini tribes of Harpactorinae as separate families and Harpactorini along with another two tribes comprised the Harpactorinae. He did not explicitly list a tribal classification within Harpactorinae or the name Harpactorini, nor did he discuss the supra-generic classification within Harpactorini. In the 6-volume keys to the Heteroptera of the Far East of the former U.S.S.R., Vinokurov *et al*. (1988) did not use any tribal classifications for Harpactorinae, presumably due to the small number of genera present. Hsiao *et al*. (1981) in a monograph of Chinese heteropterans listed the Rhaphidosomini as a subfamily and the rest of the Harpactorinae were essentially all members of Harpactorini since other tribes were not present or found in China then. Within the Harpactorinae *sensu* Hsiao *et al.* (1981), eight tribes were included in the key to the genera. The grouping was very similar to that of Distant (1904) and the number of genera included was larger. Hsiao *et al*. (1981) did not state the original source of the classification, but presumably it was based on Distant (1904). Having used this key to identify some reduviid specimens from Thailand and Southeast Asia in general, I found it indeed quite effective and efficient to have the subdivisions.

The existing schemes or proposals have been primarily applied to the Old World fauna. The New World Harpactorini have not been subjected to classification schemes as that of Distant (1904). Champion (1898) in the *Biologia Centrali Americana* did not employ a classification scheme for Harpactorini (as Harpactorinae therein). Proposals and delimitations of genera in the New World and the Old World to a large extent seemed to be independently carried out, leaving the possibility of creating synonyms. A number of the New World genera of Harpactorini share highly similar characters with some of the Old World counterparts. The New World *Sinea* is similar to the Old World *Irantha* and *Scipinia* and several other genera in having conspicuous spines on the front femora, a character found in only a few genera. Several genera from both the Old World and the New World have a pair of large spines behind

the antennae and they also have similarly slender body forms. It thus interesting and important to ask the question: are these similar characters results of shared common deep ancestry (i.e., date back to Pangea) or are they independent derivations in the New World and in the Old World? Questions like this have important implications on the classification of Harpactorini.

Monophyly, phylogenetic placement, and sister group of *Zelus*: The monophyly of *Zelus* appears to be relatively well supported. Hart (1972) defined Zelus by putative synapomorphic characters such as the antennal segment I & II subequal in length, the fore and hind femora subequal in length and width, and the pygophore with an undivided median process. Hart (1972) removed several species from *Zelus*, implying that the delimitation of *Zelus* had not stabilized and membership changes would be necessary. Hart (1972) proposed *Atopozelus* together with another unpublished genus as the sister group to *Zelus*, and *Ischnoclopius* as the next closest relative. Hart (1972) also hypothesized that no Old World harpactorine genera might be closely related to *Zelus*. Except for two recent publications (Weirauch 2008; Weirauch & Munro, in press.), there has not been any study on the phylogenetic placement of *Zelus* in Harpactorini.

Preliminary results: I have expanded the molecular data sets of Harpactorini in Weirauch & Munro (in press) by more than twice, i.e., thirty six genera or 51 species are sampled for 3 genes, 16s, 28s D2, 28s D3-D5. In both the parsimony (Fig. 4) and maximum likelihood (tree not shown) analyses, a species of Raphidosomini rendered Harpactorini paraphyletic. *Coranus* sp. + *Tachylestes* sp. are the sister clade to that species. Including more species of *Coranus* and *Tachylestes* and other potentially closely related species is needed. With exceptions of 3 genera nested within the Old World samples, the Neotropical Harpactorini form a clade (as indicated in blue in Fig. 4). This is an indication of deep biogeograhic split dating back to pre-Gondwana and reconsiderations of generic delimitation might be necessary. No distinct biogeographic structures are observed for the Harpactorini fauna in the Old World (Fig. 4). The sampling of the Old World Harpactorini is inadequate to test the existing subdivisions and no clear structure could be teased out.. Morphological examination is yet to be carried out to investigate the morphological evidence for this grouping. Eleven species of *Zelus* were sampled, and its monophyly is recovered with strong support. *Zelus* is nested within the exclusively Neotropical clade. *Atopozelus* is the sister taxon to *Zelus*, a result congruent with the hypotheses in Hart (1972). *Ischnoclopius* and the other unpublished new genus were not included.

Proposed research

Objective 2A: Test the monophyly of Harpactorini. To test the monophyly of Harpactorini, I will improve the sampling of Rhaphidosomini and Harpactorini and include representatives of the Tegeini that had not been included previously in the molecular phylogeny. For the selection of genera of Harpactorini, I will focus on the ones possibly more closely related to Raphidosomini than to the rest of Harpactorini (e.g., *Coranus* spp., *Tachylestes* spp.).

Objective 2B: Towards a frame work of classification within Harpactorini. I will sample representatives from all proposed subdivisions of Harpactorini *sensu* Distant (1904) and Hsiao *et al*. (1981). I target to sample 70 genera of Harpactorini covering the widest possible morphological diversity and geographical range, representing about a quarter of Harpactorini's generic diversity. I will sample several species for large genera and achieve ~100 species altogether. The resulting phylogeny will be compared with the various existing subdivisions and revisions of the classification will be suggested or made.

Objective 2C: Monophyly of *Zelus*, sister group, and phylogenetic placement. To test the monophyly of *Zelus*, the taxon sampling will focus on, but not be restricted to the New World Harpactorini. The phylogenetic placement of *Zelus* will also be investigated. I will identify the sister group to *Zelus* and other closely related genera, which however, will depend upon availability of material for molecular work.

Approaches: Alcohol specimens: Currently, our lab has assembled alcohol specimen collections from Madagascar, Nigeria, South Africa, Thailand, Malaysia, and various countries in the Americas. Including the already sequenced 36 genera, more than 50 genera can be readily selected from our current collection. With upcoming field trips described above, we will obtain additional specimens of Harpactorini, mostly from the New World. Field trips conducted by other members of our lab will target Southeast Asian

countries, China, and Africa. Collaborators and contacts from the US and foreign countries such as Japan, Denmark, China, Australia, and Singapore will also help with accruing additional material from their own collections or future field trips. I foresee difficulty in obtaining specimens of Tegeini, the unrepresented tribe, which only occur in Southeast Asia and Australia. Field trips to that region by other members of the lab and contacts in Singapore and Australia might offer the opportunity to secure specimens. Molecular data: I will explore 5 genes to build the phylogeny. Gene samples will cover nuclear and mitochondrial ribosomal genes (28s, 12s), nuclear protein-coding gens such as H3 (Histone 3) and Wg (Wingless) and mitochondrial protein-coding genes such as COI or Cytochrome b. Such a combination of genes will provide resolution at different levels. Methods of DNA extraction, amplification, sequencing, editing, and sequence alignment have been described previously. Phylogenetic analyses: Methods of phylogenetic analyses will largely follow that described in the species phylogeny of *Zelus* for molecular data.

OBJECTIVE 3: Evolution of Sticky trap predation in *Zelus* and Harpactorini

Background: Diverse predation strategies are found in Reduviidae. Many reduviids have modifications of the front legs for prey capture. Simple modifications are those in the Peiratinae, where the fore femora are strongly dilated, presumably giving them strong grasping powers. The Emesinae, members of which include spider-web-dwelling and spider-hunting species, have characteristically elongated fore coxae and ongoing research in our lab is investigating the pretarsal structures that allow them to walk on spider webs. Some phymatines have their femur and tibia modified to form a chela-like structure (e.g., C*arcinocoris castetsi* Handlirsch).

Members of the subfamily Harpactorinae do not seem to possess exaggerated modifications on the front legs. Most species have regular cylindrical or in a few cases, spinous or hairy front legs. It is thus interesting to investigate what predatory strategies this large group of assassin bugs employs. Members of three tribes, Ectinoderini, Apiomerini, and Diaspidiini collect plant resins with front legs and use them to capture prey. The tribes Rhaphidosomini and Tegeini appear to be highly specialized predators, with the former probably probing their long labium into crevices or holes in search for prey that would be usually inaccessible (Maldonado 1990) and the latter feeding on termites. Nothing peculiar is known of the predatory strategies of the members of Harpactorini. However, a few recent studies shed light on this topic. Weirauch (2006) and Wolf & Reid (2001) documented glandular cells on the epidermis of front tibiae of *Zelus luridus* and *Zelus longipes*. The tibial dermal glands open to the cuticular surface of the tibiae, and secrete sticky substances. Associated structures are the 'sundew hairs/setae' that resemble the trichomes of the leaves of the sundew flowers (Fig. 5), and other specialized setae such as the pad-like setae whose significance is not unknown. The sundew hairs presumably function in retaining the sticky substances.

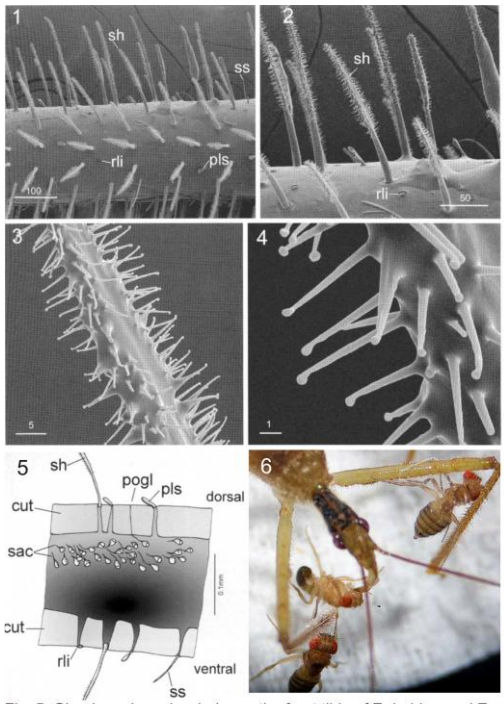

Fig. 5. Glands and sundew hairs on the front tibia of Z. *luridus*. and Z. *renardii* preying on *Drosophila* sp. 1-4, sundew hairs, overview and close-up. 5, schematic drawing of integument of front tibia showing gland cells and associated structures. 6, Drosophilids stuck on the fore legs of *Z. renardii*. Abbr: rli-ring like invagination, pls-pad like setae, sh-sundew hairs, ss-simple setae. (1-5 from Weirauch 2006)

Other than *Zelus*, anecdotal records of Harpactorini mentioned secretory setae in *Pselliopus cinctus* (Fabricius) (Readio 1927) and *Cosmoclopius curacavensis* Cobben & Wygodzinsky (Cobben & Wygodzinsky 1975). Many museum specimens of Harpactorini are also frequently observed to appear to be coated with sticky substances on the legs or body, indicating the presence of an endogenous source of sticky secretions.

Both the use of an exogenous and endogenous source of sticky substances for prey capture are called sticky trap predation, although the term does not imply homology. My study of the sticky trap predation phenomenon is targeting at explorations of the presence and distributions of structures associated with production and dissemination of

endogenous secretions in Harpactorini. Also, I will continue documenting the morphological structures of additional species of *Zelus*, which mostly remain unexamined.

Proposed research

Objetive 3A: Document foreleg morphology of *Zelus*. To investigate whether all the species of *Zelus* possess homologous structures on forelegs and document the variation of such structures, I will target at least one representative species of the 13 species groups proposed by Hart (1972). The work can then be expanded to a larger selection if deemed worthwhile based on preliminary examinations.

Objective 3B: Explore and document glandular structures in Harpactorini. Few examinations of glandular structures on the forelegs outside *Zelus* exist (Weirauch, pers. comm.). Many questions remain to be answered. The first question I ask is: is an endogenous secretion of sticky substances on the front legs found in other Harpactorini beyond *Zelus*? To answer this question, I will sample ~70 genera of Harpactorini that represent high morphological, geographical, and phylogenetic diversity. The sampling will be focusing on, although not restricted to the genera in the generic phylogenetic analysis of Harpactorini so that characters can be mapped on the phylogeny. Another question is: besides forelegs, are there other structural sources of sticky substances in Harpactorini? I will examine specimens that appear to be coated with sticky substances to identify structural sources of the substances such as dermal glands.

Objective 3C: Test hypotheses of sticky trap predation in *Zelus* and Harpactorini. Results of the morphological examinations of *Zelus* and Harpactorini can be mapped on the genus-level phylogeny of Harpactorini to result in testable hypotheses of the evolution of sticky trap predation. Specifically, I would like to investigate the phylogenetic origin of endogenous secretion of sticky substances in Harpactorini. Important questions are, however, not restricted to: is there a single phylogenetic origin or are there multiple origins of endogenous secretions in Harpactorini and are all glandular structures involved in predation found on forelegs or are there phylogenetic patterns of their locations?

Approaches: Taxon sampling: Initially, I will quickly examine 70 genera of Harpactorini to investigate the presence of possible homologous structures on the forelegs or similar gland on other parts of the body. The number of genera that require detailed structural documentations will depend upon the number of positive cases. Our lab and UCR museum have at least 50 genera of Harpactorini. A fellowship awarded to me for working in the Smithsonian National Museum of Natural History for 10 weeks in 2010 will grant me access to one of the largest reduviid collections in the US. I will be mainly devoting the research time there on the explorations of glandular structures of Harpactorini. Comparative morphology: Light microscopy will be used for observing and documenting glandular structures on the forelegs. The right front tibia will be excised, cleared in KOH (10%), stained with Chlorazol black, placed on a glass slide, and examined with a compound microscope. Photos will be taken or illustrations made to document the glands and associated structures. Scanning electron microscopy will be used to document setae and other minute integumental structures such as gland pores. Character mapping on phylogeny: Characters will be mapped on the genus-level phylogeny of Harpactorini that will also include the species level phylogeny of *Zelus*. To reconstruct ancestral states and infer character transformations, both parsimony optimization (MacClade) and probabilistic approaches (SIMMAP, DIVA) will be explored.

OBJECTIVE 4: Explorations of pheromones in *Zelus* (optional)

Background: Knowledge of pheromones has important implications in systematics. Sex pheromones may function in mate recognition and reproductive isolation via a variety of mechanisms (e.g., Lanier & Wood 1975, Löfstedt 1991). Phylogenetic studies have looked into the evolution of pheromones along a lineage of insects (Cognato 1997, Löfstedt & Kozlov 1997). Heteropteran bugs are known for having diverse kinds of scent glands and secreting a wide range of chemicals including pheromones (Aldrich 1988, Millar 2005). Currently, pheromone studies in Reduviidae are heavily biased towards Triatominae presumably for their medical importance. Sex pheromones, aggregation pheromones, alarm and defensive compounds and responses to host odors in Triatominae have been studied and reviewed in Cruz-Lopez *et al*. (2001). Pheromone studies outside Triatominae are sparse. An aggregation pheromone was found in males of *Pristhesancus plagipennis* Walker (James 1994), a member of Harpactorini and studied as a biological control agent in Australia. The attraction of *Z. tetracanthus* to pheromones of

bostrichid beetles (Edde and Phillips 2006) suggests a possible sex pheromone or aggregation pheromone in this species similar to the bostrichid beetle aggregation pheromone. I propose to investigate pheromones in two species of *Zelus*, *Z. tetracanthus* and *Z. renardii*. Both occur abundantly in Southern California and Arizona. It is understood that this part of the research is optional for my PhD project, however, will be highly valuable if could be successfully carried out.

Preliminary results: Pilot studies in Dr. Millar's lab have not been able to resolve the nature of the pheromones. Both males and females of *Z. tetracanthus* seemed to be responsive to a pheromone source. The preliminary studies also showed some conflicting results.

Proposed Research

Objective 4A. **Investigate the presence and the nature of the pheromones in *Z. tetracanthus* and *Z. renardii*.** I will be performing behavioral bioassays to investigate the presence of pheromones and their natures, i.e., sex pheromone or other kinds of the pheromones. In addition, I will also determine how long it takes for the adults to reach sexual maturity after emergence.

Objective 4B. Identify the chemical structure of the pheromones in *Z. tetracanthus* and *Z. renardii*. In collaboration with Dr. Millar, I will isolate the pheromones and identity the chemical compositions and structures of them.

Approaches: Live specimen cultures: I have been maintaining cultures of *Z. tetracanthus* and *Z. renardii* collected from Southern California or Arizona in large quantities. They appear to develop and reproduce readily in laboratory conditions. Behavioral assays: A Y-tube olfactometer assay will be used. To determine how long it takes for the adults to reach sexual maturity, virgin males and females will be allowed to mate after different days of emergence, e.g., 1 day, 3 days, and 5 days. I will evaluate the rate of successful mating as an indicator of sexual maturity. Other data such as lengths of pre-copulation and copulation time will also be recorded. Behavioral bioassays will only be performed with the specimens that are sexually matured. Virgin males and females will be kept separately before the assays. Air will be drawn through the olfactometer by a vacuum pump connected to a flow meter. Each arm of the Y-tube will be connected a container and the pheromone source specimen will be placed in that container. The specimen making the choice will be allowed 15 minutes to walk in the Y-tube. A choice is considered made when it walks pass half of one arm and stays there. Different combinations of sexes will be used. Initially, 10 replicates for each of the combinations will be performed. Additional replicates will be done if needed to achieve statistical significance. Isolation and structure determination: This part of the research will be carried out in close collaboration with Dr. Millar and standard analytical methods will be employed.

BROADER IMPACT

My dissertation project will have broader impact on various aspects. The monographic systematic revision of *Zelus* will provide basic and critical taxonomic information for using *Zelus* spp. in natural enemy research and future evolutionary and ecological studies. This project will be exemplary for future monographic revisions in Reduviidae and Heteroptera in general. The use of online open-access resources will accelerate dissemination of taxonomic information and make results available to a broad audience. The molecular markers used or explored in this project can be used or tested in future projects. The four components of my dissertation project inter-relate each other in a very coherent manner. The monographic revision of *Zelus* will be the focus of the project and deals with primarily alpha-taxonomy. The generic phylogeny of Harpactorini investigates higher level systematics and will be used to infer the evolution of sticky trap predation, the third objective of my dissertation research. The exploration of pheromones of Zelus expands the dimension of the project and will contribute to the use of *Zelus* spp. as natural enemies.

TIMELINE

The targeted project completion time is **June 2012**, approximately 3 years after I advance to PhD candidacy.

Aug-Dec 2009:

- Obtain loans of ~10,000 specimens of *Zelus*

- Sort all specimens to morpho-species
- Database 1,000 specimens
- Descriptions of 15 species
- Morphological phylogenetic characters and phylogeny of 15 species
- Behavioral assays of *Z. renardii* and *Z. tetracanthus*, and chemical analyses

Jan-Jul 2010:
- Field trips to French Guiana and Colombia (2 months)
- 10 weeks at the Smithsonian – examine 70 genera of Harpactorini to document foreleg structures
- Hemiptera field workshop in Costa Rica (3 weeks)
- Molecular phylogeny of additional 20 genera of Harpactorini (total 55) with 4-5 genes

Aug-Dec 2010:
- Descriptions of additional 15 species (total 30) of *Zelus*
- Database another 1,000 specimens of *Zelus* (total 2,000)
- Morphological characters and phylogeny for the additional 15 species of *Zelus* (Total 30 spp.)

Jan-June 2011:
- Descriptions of additional 15 species of *Zelus* (total 45)
- Database another 1,000 specimens (total 3,000)
- Morphological characters and phylogeny of the additional 15 species of *Zelus* (total 45 spp.)
- Expand the molecular phylogeny of *Zelus* to 20 species for 4-6 genes
- Molecular phylogeny of additional 15 genera of Harpactorini (total 70) with 4-5 genes

Jul-Dec 2011:
- Descriptions of additional 15 species of *Zelus* (total 60)
- Database another 1,000 specimens (total 4,000)
- Morphological characters and phylogeny of the additional 15 species of *Zelus* (total 60 spp.)

Jan-Jun 2012:
- Descriptions of additional 15 species of *Zelus* (total 75)
- Database another 1000 specimens (total 5,000)
- Morphological characters and phylogeny of the additional 15 species of *Zelus* (total 75 spp.)
- Write dissertation

EXPECTED OUTCOME

I expect to publish the dissertation as well as other side-projects as several peer-reviewed publications and also present the research at conferences and meetings.

Publications:

1. Side-project: systematic revision and cladistic analysis of 10 species of Malagasy peiratine assassin bugs. Target journal: *Systematic Entomology*. (**near completion**)
2. Molecular genus-level phylogeny of Harpactorini. Target journal: *Molecular phylogenetics and evolution*
3. Comparative morphology of fore leg structures of species of *Zelus* and Harpactorini. Target journal: *Biological Journal of the Linnean Society* or *American Museum Novitates*
4. Monographic revision of *Zelus* without the species phylogeny (alpha-taxonomy). Target journal: *Bulletin of the American Museum of Natural History*
5. The species phylogeny of *Zelus*. Target journal: *Systematic Entomology*.
6. Pheromones of *Zelus*. Target journal: *Journal of Insect Behavior* or *Journal of Chemical Ecology* (optional)

Conferences & Meetings:

2009, 2010, 2011 – ESA annual meetings

2010 – International Heteropterists Society Meeting

2012 – International Congress of Entomology conference

One or several of the Willi Hennig Society annual meetings

REFERENCES

Aldrich, J. R., 1988. Chemical Ecology of the Heteroptera. *Annual Review of Entomology*, **33**:211-238

Amyot, C. J.-B., and A. Serville. 1843. *Historie naturelle des insects Hémiptères*. Librairie Encyclopédique de Roret, Pairs. 675 pp.

Barth, R. 1952. Dei Fangdruse an den Beine von *Zelus* (*Diplocodus*) *leucogrammus* (Perty). *Zoolog. Jahrbiicher* **73:** 323-336

Baker, R. H., and R. DeSalle. 1997. Multiple sources of character information and the phylogeny of Hawaiian drosophilids. *Systematic Biology* **46**: 674-698.

Bérenger, J-M and D. Pluot-Sigwalt, 1997. Relations privilégées de certain Heteroptera Reduviidae prédateurs avec les végé taux. Premier cas connu dun Harpactorinae phytophage. (Special relationships of certain predatory Heteroptera Reduviidae with plants. First known case of a phytophagous Harpactorinae). *C.R. Acad. Sci. Paris Sci.* **320**:1007-1012.

Brailovsky, H. and Barrera, E. 2004 Especies nuevas de *Pselliopus* (Hemiptera: Heteroptera: Reduviidae: Harpactorinae) de México (Five new species of *Pselliopus* from Mexico). *Anales del Instituto de Biología, Universidad Nacional Autónoma de México, Serie Zoología* **75**(1): 313-330.

Bremer, K. 1988. The limits of amino acid sequence data in angiosperm phylogenetic reconstruction. *Evolution* **42**: 795-803.

Champion, G. C. 1897-1901. Insecta: Rhynchota (Hemiptera - Heteroptera) 2. In: *Biologia Centrali-Americana (Zoologia)* (F. D. Godman & O. Salvin, eds): i-xvi, 1-416. London [1897: 1-32; 1898: 33-192; 1899: 193-304; 1900: 305-344; 1901: i-xvi, 345-416].

Choe, D.-H. and M. K. Rust., 2007. Use of Plant Resin by a Bee Assassin Bug, *Apiomerus flaviventris* (Hemiptera: Reduviidae). *Annals of the Entomological Society of America*, **100**: 320-326.

Clayton, R. A. .1990. *A Phylogenetic Analysis of the Reduviidae (Hemiptera: Heteroptera) with Redescription of the Subfamilies and Tribes.* Dissertation, The George Washington University, Washington D.C., 191 pp.

Cobben, R. H., 1968. *Evolutionary Trends in Heteroptera. Part I. Eggs, Architecture of the Shell, Gross Embrylology, and Eclosion*. Centre for Agricultural Publishing and Documentation, Wageningen, Netherlands. 475 pp.

Cobben, R. H., 1978. *Evolutionary Trends in Heteroptera. Pt. 2. Mouthpart-structures and Feeding Strategies*. Mededlingen Landbouwhogeschool 78-5. H. Veeman, Wageningen, Netherlands. 407 pp.

Cobben, R. & Wygodzinsky, P. 1975. The Heteroptera of the Netherlands Antilles – IX. Reduviidae Assassin Bugs). *Studies on the Fauna of Curaçao and other Caribbean Islands*, **48**: 1–62.

Cognato, A.I., S.J. Seybold, D.L. Wood, and S.A. Teale. 1997. A cladistic analysis pheromone evolution in Ips bark beetles (Coleoptera: Scolytidae). *Evolution* **51**: 313-318.

Cogni, R., A. V. L. Freitas, and B. F. Amaral Filho. 2002. Influence of prey size on predation success by *Zelus longipes* L. (Het., Reduviidae). *Journal of Applied Entomology*, **126**: 74-78.

Cohen, A. C. and R. Tang. 1997. Relative prey weight influences handling time and biomass extraction in *Sinea confusa* and *Zelus renardii* (Heteroptera: Reduviidae*). Environmental Entomology*, **26**: 559-565.

Cruz-Lopez, L., E. A. Malo, J. C. Rojas, and E. D. Morgan. 2001. Chemical ecology of triatomine bugs: vectors of Chagas disease. *Medical and Veterinary Entomology* **15**:351-357.

Curkovic, T., J. E. Araya, M. Baena, M.A. Guerrero. 2004. Presencia de *Zelus renardii* Kolenati (Heteroptera: Reduviidae) en Chile (Presence of *Zelus renardii* Kolenati (Heteroptera: Reduviidae) in Chile). *Boletin de la S.E.A*. Guerrero. 34: 163-165.

Dallwitz, M. J., Paine, T. A., and E. J. Zurcher. 1993. User's guide to the DELTA System: a general system for processing taxonomic descriptions. 4th edition. http://delta-intkey.com

Davis, N. T. 1969. Contribution to the morphology and phylogeny of the Reduvoidea. Part 4. The harpactoroid complex. *Annals of the Entomological Society of America* **62**: 74-94.

Distant, W.L. 1904. *The Fauna of British India, including Ceylon and Burma. Rhynchota (Heteroptera)*. London : Taylor & Francis Vol. II Pt 2 pp. 438

Dougherty, V. 1995. A review of the New World Ectrichodiinae genera (Hemiptera: Reduviidae). *Transactions of the American Entomological Society* **121**:173–225.

Edde, P. A. and T. W. Phillips., 2006. Field responses of nontarget species to semiochemicals of stored-product Bostrichidae. *Annals of the Entomological Society of America* **99**: 175-183.
Farris, J. S.; Kallersjo, M. and A. G. Kluge. 1995. Constructing a significance test for incongruence. *Systematic Biology* **44**: 570-572.
Felsenstein, J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**: 783-791.
Forero, D. 2003. Distributional records of *Zelus longipes* (Heteroptera: Reduviidae) in Colombia*. Entomólogo Bogotá* **31**(96): 4-9.
Froeschner, R.C. & N.A. Kormilev. 1989. Phymatidae or ambush bugs of the world: a synonymic list with keys to species, except *Lophoscutus* and *Phymata* (Hemiptera). *Entomography*, **6**: 1-76.
Gil-Santana, H. R.. 2008. New records, and nomenclatural and biological notes on Reduviidae (Hemiptera: Heteroptera) from Bolivia and Brazil. *Zootaxa*, **1785**: 43–53.
Gillespie, J.J., J.B. Munro, J.M. Heraty, M.J. Yoder, A.K. Owen and A. Carmichael. 2005. A secondary structural model of the 28S rRNA expansion segments D2 and D3 from chalcidoid wasps (Hymenoptera: Chalcidoidea): Implications for multiple sequence alignment and phylogeny reconstruction. *Mol. Biol. Evol.* **22**: 1593-1608.
Gillespie, J. J, Johnston, J.S., Cannone, J.J., and R. R. Gutell. 2006. Characteristics of the nuclear (18S, 5.8S, 28S and 5S) and mitochondrial (12S and 16S) rRNA genes of Apis mellifera (Insecta: Hymenoptera): structure, organization, and retrotransposable elements. *Insect Molecular Biology* **15**(5): 657-686.
Goloboff, P., Farris, J., and Nixon, K. 2003. T.N.T.: Tree Analysis Using New Technology [software; available online at www.zmuc.dk/public/phylogeny]
Hagen, K. S., N. J. Mills, G. Gordh, and J. A. Mcmurty. 1999. Terrestrial arthropod predators of insect and mite pests. Chapter 16. In: *Handbook of Biological Control*: *Principles and Application of Biological Control* (T. Bellows, *et al.* Eds.) Academic Press. pp. 382-504.
Hart, E. R. 1972. *A Systematic Revision of the Genus* Zelus *Fabricius (Hemiptera: Reduviidae)*. Ph.D dissertation. Texas A&M University.
Hart, E. R. 1986. Genus Zelus Fabricius in the United States, Canada, and Northern Mexico (Hemiptera: Reduviidae*). Annals of the Entomological Society of America*. **79**: 535-548.
Hart, E. R. 1987. The genus *Zelus* Fabricius in the West Indies (Hemiptera: Reduviidae). Annals of the Entomological Society of America **80**: 293-305.
Hsiao, T.-Y., S.-Z. Ren, L.-Y. Zheng, H.-L. Jing, H.-G. Zou, and S.-L. Liu. 1981. A Handbook for the Determination of the Chinese Hemiptera-Heteroptera, vol. 2. Science Press, Beijing. 654 pp., 85 pls. [In Chinese]
Huelsenbeck, J. P., and F. Ronquist. 2001. MRBAYES: Bayesian inference of phylogeny. *Bioinformatics* **17**: 54-755.
Hypsa, V., D. F.Tietz, J. Zrzavy, R. O. M. Rego, C. Galvao, J. Jurberg. 2002. Phylogeny and biogeography of Triatominae (Hemiptera : Reduviidae): molecular evidence of a New World origin of the Asiatic clade. *Molecular Phylogenetics and Evolution* 23: 447-457.
Jadin, Jean-Marie; Dahan, K., Jadin-Nyssens, M., 2002 Description morphologique et approche genetique d'un nouveau Reduviidae (Harpactorinae): *Zelus josephpaulusi*, n. sp. [Morphological description and genetic approach to a new Reduviidae (Harpactorinae): *Zelus josephpaulusi*, n. sp.] *Lambillionea* **102**: 3, Supplement: (Tome 2): 303-328
James D.G., Moore C.J., and J.R. Aldrich. 1994, Identification, synthesis, and bioactivity of a male-produced aggregation pheromone in assassin bug, *Pristhesancus plagipennis* (Hemiptera: Reduviidae). *J. Chem. Ecol.* **20**: 3281–95
Lanier, G. N., and D. L. Wood. 1975. Specificity of response to pheromones in the genus *Ips* (Coleoptera: Scolytidae). *J. Chem. Ecol.* **1**: 9-23
Lawrence R. F., 1984. *The centipedes and millipedes of southern Africa*. A. A. Balkema of Cape Town and Rotterdam, South Africa.

Lent, H. and P. Wygodzinsky., 1979. Revision of the Triatominae (Hemiptera, Reduviidae), and their significance as vectors of Chagas' disease. *Bulletin of American Museum of Natural History*, **163**: 125-520.

Löfstedt, C., and Kozlov, M.,1997. A phylogenetic analysis of pheromone communication in primitive moths, in: *Insect Pheromone Research–New Directions* Ed. by Cardé, R. T. & Minks, A. K, New York: Chapman & Hall, 1997

Löfstedt, C., W. M. Herrebout, & S. B. J. Menken. 1991. Sex pheromones and their potential role in the evolution of reproductive isolation in small ermine moths (Yponomeutidae). *Chemoecology* **2** (1): 20-28

Maldonado, J., 1990. Systematic catalogue of the Reduviidae of the World. *Caribbean Journal of Science*, Special publication No. 1, University of Puerto Rico, Mayagüez, Puerto Rico, 694 pp.

Malipatil, M.B. 1991. Two new genera of Harpactorinae (Hemiptera: Reduviidae) from Northern Australia *Australian Journal of Entomology* **30**(1): 83-88

Melo, M.C. 2008, New records of Peruvian Reduviidae (Heteroptera), with the description of a new species of *Tagalis* Stål 1860 (Saicinae). *Zootaxa* **1763:** 55–62.

Millar J. G. 2005. Pheromones of true bugs, pp. 37–84, in S. Schulz (ed.). *Topics in Current Chemistry 240. The Chemistry of Pheromones and Other Semiochemicals*. Springer, Berlin.

Miller, N.C.E. 1942. On the structure of the legs in Reduviidae (Rhynchota), *Proc. R. Entomol. Soc. London A* **17**: 49–58.

Miller, N.C.E. 1971. *The biology of the Heteroptera*, 2nd ed. E. W. Classey Ltd., Hampton, Middlesex, England.

Odhiambo, T. R. 1959. An account of parental care in *Rhinocoris albopilosus* Signoret (Hemiptera-Heteroptera: Reduviidae), with notes on its life history. *Proc. R. ent. Soc. Lond. (A).* 34: 175--185.

Parker, A. H. 1965. The maternal behaviour of *Pisilus tipuliformis* Fabricius (Hemiptera: Reduviidae). *Ent. exp. & appl.* 8" 13--19.

Putshkov, V. G. and P. V. Putshkov., 1986-1989*. A catalogue of the Reduviidae (Heteroptera) of the world*. Vinity, Lyubertsy. 6 volumes.

Readio, P.A. 1927 Studies on the biology of the Reduviidae of America north of Mexico, *Univ. Kansas Sci. Bull.* **17**: 5–291.

Roepke, W. 1932. Ueber Harzwanzen von Sumatra und Java. Miscellanea zoologica Sumatrana 68: 5 pp.

Suchard, M. A. and B. D. Redelings. 2006. BAli-Phy: simultaneous Bayesian inference of alignment and phylogeny. *Bioinformatics* **22**: 2047-2048.

Schuh, R.T. & Slater, J.A. 1995. *True bugs of the world (Hemiptera: Heteroptera): classification and natural history*. Comstock Pub. Associates, Ithaca, pp. i-xii, 1-336.

Stål, C. 1859. Hemiptera species novas descriptsit. Konglika svenska Fregattens Eugenies Resa mokring Jorden. III. *Zoologi, Insecter.* **1859**:219-298

Stål, C. 1862. Hemiptera Mexicana enumeravit specieque novas descripsit. *Stett. Entomol. Ztg.* **23**: 437-462

Tallamy D. W. 2004. Revisiting Paternal Care in the Assassin Bug, *Atopozelus pallens* (Heteroptera: Reduviidae) *Journal of Insect Behavior* **17** (4): 431-436

Usinger, R. L. 1958. Harzwanzen or “resin bugs” in Thailand. *Pan-Pac. Entomol.* **34**:52.

Varón, A., L. S. Vinh, I. Bomash, and W. C. Wheeler. 2007. POY 4.0 Beta 2398. American Museum of Natural History. [software; http://research.amnh.org/scicomp/projects/poy.php].

Villiers, A. 1948. *Faune de l'Empire Franfais. IX. Hdmiptdres Rdduviid~s de l'Afrique Noire.* Paris, Office de la Recherche Scientifique Coloniale, 489 pp.

Villiers, A. 1982. Hémiptères Reduviidae africains. Localisations et descriptions. 3. Harpactorinae Rhinocorini - - 11 p - 10 figs

Vinokurov, N. N., V. B. Golub, I. M. Kerzhner, E. V. Kanyukova, G. p. Tshernova. 1988. Homoptera and Hemiptera. *In:* P. A. Lehr (ed.), Keys to the Insects of the Far East of the USSR, Inst. Zool. 328 pp. [In Russian; trans. Ameind Publishing, New Delhi, 1988].

Walter D.E. and S.L. Winterton, 2007. Keys and the Crisis in Taxonomy: Extinction or Reinvention? *Annual Review of Entomology*, **52**: 193-208.

Weirauch, C. 2005. Pretarsal structures in Reduviidae (Heteroptera, Insecta). *Acta Zool*. (Stockholm) **86:** 91-110.
Weirauch, C. 2006. Observations on the sticky trap predator *Zelus luridus* Stal (Heteroptera, Reduviidae, Harpactorinae), with the description of a novel gland associated with the female genitalia. *Denisia* **19**: 1169-1180.
Weirauch, C. 2007. Revision and Cladistic Analysis of the *Polyozus* Group of Australian Phylini (Heteroptera: Miridae: Phylinae). *American Museum Novitates* **3590**: 60 pp.
Weirauch, C., 2008. Cladistic analysis of Reduviidae (Heteroptera: Cimicomorpha) based on morphological characters. *Systematic Entomology*. **33**: 229-274.
Weirauch, C. and G. Cassis. 2006. Attracting ants: The trichome in *Ptilocnemus lemur* (Heteroptera: Reduviidae: Holoptilinae) and novel glandular areas on the sternum. *Journal of the New York Entomological Society*. **114**: 28**-**37.
Weirauch. C., and Munro, J. A molecular phylogeny of Reduviidae based on ribosomal genes. *Molecular Phylogenetics and Evolution*. In press.
Wheeler, W. 1996. Optimization alignment: The end of multiple sequence alignment in phylogenetics? *Cladistics* **12**:1–9.
Wheeler, W. C. 2006. Dynamic homology and the likelihood criterion. Cladistics 22:157–170
Wheeler, W. C., M. J. Ramírez, L. Aagesen, and S. Schulmeister. 2006. Partition-free congruence analysis. *Cladistics*: **22**: 256-263.
Wignall A. E., and P. W. Taylor. 2008. Biology and life history of the araneophagic assassin bug *Stenolemus bituberus* including a morphometric analysis of the instars (Heteroptera, Reduviidae). *J Nat Hist* **42**:59–76
Wolf, K. W. and W. Reid. 2001. Surface morphology of legs in the assassin bug *Zelus longipes* (Hemiptera: Reduviidae): a scanning electron microscopy study with an emphasis on hairs and pores. *Annals of the Entomological Society of America* **94**: 457-461.
Wygodzinsky, P. W., 1966. A monograph of the Emesinae (Reduviidae: Hemiptera). *Bulletin of the American Museum of Natural History*, **133**: 1-614.
Zhang, G., and C. Weirauch. A systematic revision and cladistic analysis of Malagasy assassin bugs in the subfamily Peiratinae (Hemiptera: Reduviidae). In prep. Target journal: *Systematic Entomology*.

GUANYANG ZHANG – Curriculum Vitae

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A. PROFESSIONAL PREPARATION

09/2007-present, University of California, Riverside. Doctoral Graduate Student in Entomology (candidacy expected from Sept 2009).

07/2007 National University of Singapore, Singapore. Bachelor of Science in Life Sciences (Concentration in Biology).

B. PROFESSIONAL EXPERIENCE

09-12/2007 Teaching Assistant for *General Entomology*, University of California, Riverside.
09-10/2003 Tutor, Preparation Courses for the Biology Olympiad, National Junior College, Singapore.

C. PROFESSIONAL INTERESTS

Systematic entomology, more specifically systematics of Heteroptera and Reduviidae, and species discovery and philosophy of taxonomy. Ongoing projects comprise revisionary and cladistic work on Malagasy Peiratinae (Reduviidae), a monographic revision of *Zelus* Fabricius, and comparative functional morphology of forelegs and glands in relation to predatory behaviors in Harpactorini.

D. PUBLICATIONS

Zhang, G. 2009. Specimens versus sequences, *Science* **323** (5922): 1672 (Letter to the Editor).

Meier, R., and **Zhang, G.** DNA barcoding and DNA taxonomy: An assessment based on 4261 *COI* sequences for 1001 species in *Diptera Diversity: Status, Challenges and Tools* edited by Pape, T., Bickel, D., R. Meier. Brill Academic Publishers, 2009.

Meier, R., **Zhang, G.**, and Ali, F., 2008, The use of mean instead of smallest interspecific distances exaggerates the size of the "barcoding gap" and leads to misidentification, *Systematic Biology* **57** (5): 809-813.

Zhang, G., and Weirauch, C. A systematic revision and cladistic analysis of Malagasy assassin bugs in the subfamily Peiratinae (Hemiptera: Reduviidae). In prep. ~50 pp. Target journal: *Systematic Entomology*.

E. AWARDS & HONORS

06/2009-06/2010 Robert van den Bosch Scholarship in Biological Control (for the category systematics of natural enemies). **$5,000**.

02-04/2010 Smithsonian Graduate Student Fellow. Smithsonian Institution Fellowship Programs. $**6,000**. 10-week research on comparative functional morphology of Harpactorini and systematics of *Zelus*.

11/2008 UC Riverside Graduate Student Association Conference Travel Grant. **$300**.

11/2008 UC Riverside Entomology Graduate Student Association Travel Grant. **$100**.

09/2008. Best PhD Oral Presentation, Student Seminar Day, Department of Entomology, University of California, Riverside. $**75**.

06/2008-04/2009 California Desert Research Fund at the Community Foundation. *Survey of Kissing Bugs within the Inland Empire.* Co-awarded to Wei Song Hwang. $**2,883**.

09/2007-06/2008 Graduate Student Fellowship, University of California, Riverside.
08/2003-06/2007 University Scholars Programme, National University of Singapore.

F. CONFERENCES & PRESENTATIONS

02/2009 Heteroptera Synthesis Meeting, Riverside (sponsored by the Biodiversity Synthesis Center, Field Museum, Chicago & 'Encyclopedia of Life'): *Phylogenetic Analytical Methods: An Overview*

11/2008 Entomological Society of America Annual Meeting: *Malagasy assassin bugs (Heteroptera: Reduviidae): Association of dimorphic sexes and immature specimens with adults, synonymy of two genera & descriptions of four new species*

G. FIELD EXPERIENCE

Collecting trips (2006-2009): Arizona, California, Malaysia, and Mexico

H. LABORATORY SKILLS

Auto-Montage (GT-Vision) & Microptics imaging systems to obtain habitus and structural detail images of Reduviidae; SEM and confocal laser microscopy techniques; genitalic dissections; scientific illustrations; CorelDraw and PhotoShop software to assemble to edit images and make plates; organizing and uploading images to *Discover Life* and *MorphBank*; specimen databasing using the online PBI Plant Bug database; georeferencing of locality data; molecular work including extractions, PCR, sequence editing and alignment, data analysis using a range of phylogenetic programs.

I. COLLECTION MANAGEMENT EXPERIENCE

Sorting and identifying Reduviidae alcohol specimens from Colombia, Costa Rica, Ecuador, Madagascar, Mexico, Thailand, and the U.S. to subfamilies, genera or species; sorting and Identifying *Zelus* Fabricius dry and alcohol specimens; sorting Heteroptera dry specimens in the Entomology Research Museum @ UC Riverside to families; insect specimen preparation skills (mounting, labeling).

J. INFORMATION TECHNOLOGY EXPERIENCE

Contributions to the TaxonDNA program package [http://code.google.com/p/taxondna/]
Contributions to the Heteropteran Systematics @ UCR webpage [http://heteroptera.ucr.edu/]