

Annual Report

Systematics of Eucharitidae

The first year of the grant has gone extremely well. I have been able to resolve the biology and phylogenetic position of *Gollumiella*, which was considered a crucial taxon in the initial proposal. We have complete 28S-D2&D3 sequence for 140 taxa, and of these we have 67 sequenced for 18S-E23. We are also looking into COI (about 20 reactions PCR'ed but not yet sequenced) and EF-1alpha. The results are highly concordant with ant host relationships and behaviors associated with reaching the ant host.

Collecting trips were made in peninsular Malaysia in August, Australia in February and March, and locally throughout this past summer. Additional new collections of eucharitids in alcohol for sequencing were supplied by Lubomir Masner (South America and South Africa), Mike Sharkey (Colombia), Terry Erwin (Ecuador canopy fogs), Brian Fischer (Madagascar), and Chris Burwell (Australia). The most recent results were presented at the meetings of the International Hymenopterist's Society in San Diego (December) and Beijing (July) and the University of California, Davis (February). In July, the final version of "A Revision of the Genera of Eucharitidae of the World (Hymenoptera: Eucharitidae)", in both a book and CD form, were sent to the printers through the American Entomological Institute Memoirs series. This work includes a morphological analysis of all 53 genera of Eucharitidae, and forms a solid basis for comparison to the molecular analyses.

The major findings are as follows:

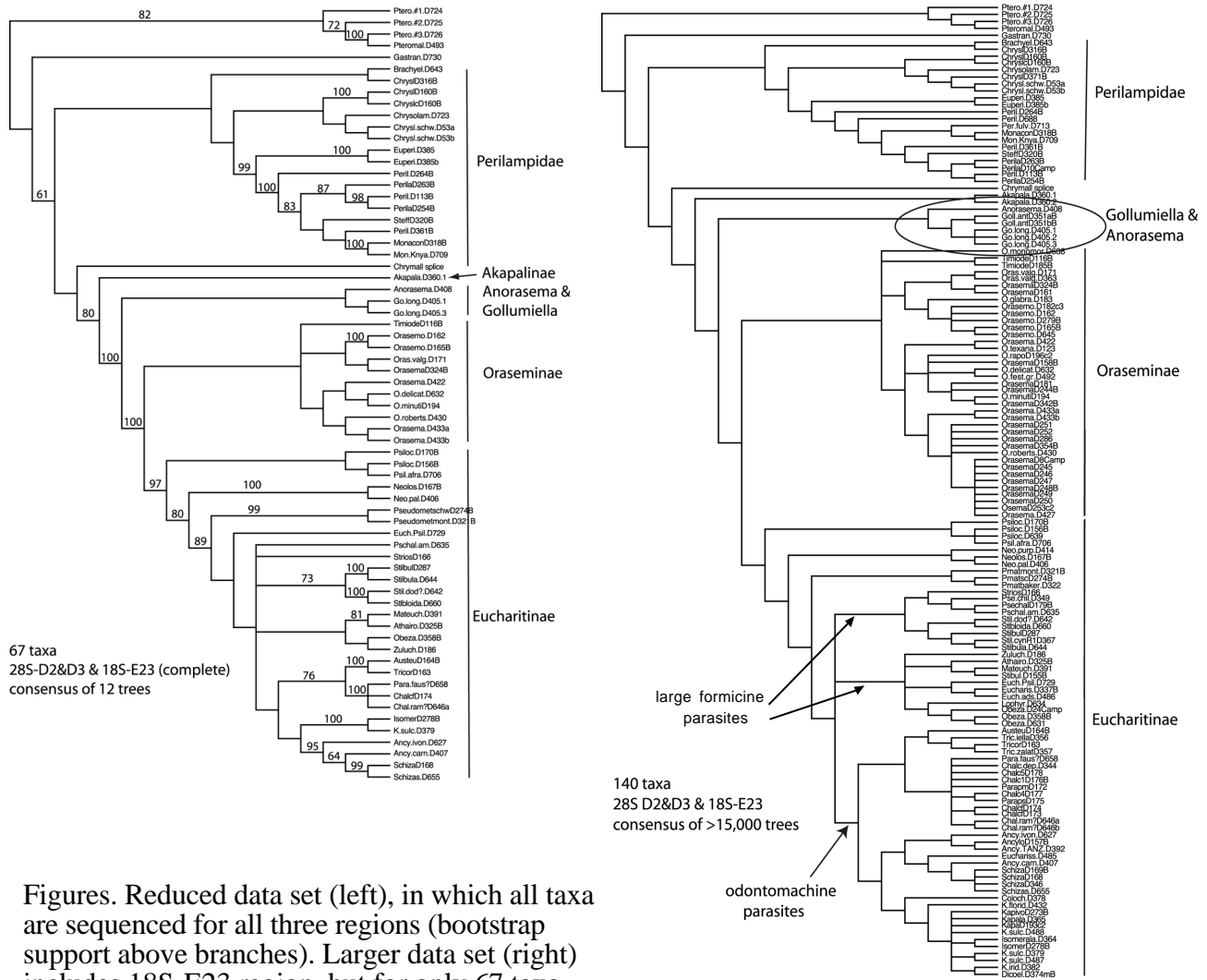
1) The Malaysia trip was very successful. In addition to several eucharitine genera, I was able to collect a single specimen of *Anorasema pallidipes* (potential sister group of *Gollumiella*), and *Gollumiella antennata*. This latter species was collected in large numbers on a single tree fern bush in the University of Malaya botanical gardens. Adults deposited large numbers of erect eggs at the base of leaf branches on the tree fern. The first-instar larvae are planidiaform, with a typical morphology for Eucharitidae. The host ant was *Paratrechina* (Formicinae), and the first instar larva was internal on the host ant larva (a behavior typical of Oraseminae and only *Pseudometagea* within the Eucharitinae). No thrips associations were observed. Morphological analyses always place *Gollumiella* and *Anorasema* as basal members of the Eucharitini within the Eucharitinae. Based on all rDNA transcript gene regions (28S-D2 & D3 and 18S-E23), independently or combined, *Gollumiella* (2 spp.) and *Anorasema* are sister groups and both are strongly supported as the outgroup of Oraseminae + Eucharitinae. Based on the distribution of traits in the other two subfamilies, this clearly places Formicinae and internal parasitism in the first instar as ancestral traits for the entire family.

Gollumiella and *Anorasema* also lack a stalked egg as is typical of most Oraseminae and Eucharitinae (and all basal members). In *Gollumiella*, the egg has a peculiar anchor that is used to insert the tip of the egg into the plant tissue; a behavior first described by C.P. Clausen in 1940.

2) The Australian trip was focused on collecting *Akapala*. This genus consistently is placed by morphology and the molecular data as the sister group of (*Gollumiella*+*Anorasema*) + Eucharitidae s.s. (Oraseminae + Eucharitinae). With Chris Burwell of the Queensland Museum, we collected extensively at Mt Isa (the site of a collection of > 25 individuals on a *Eucalyptus* trunk) and Alice Springs. No *Akapala* were found even though conditions appeared to be perfect (very lush); however, I was able to collect several other genera for sequencing and new behavioral information. Chris Darling of the Royal Ontario Museum (Toronto) also tried to collect specimens of *Akapala* a few weeks before us in Mt Isa but was not successful.

3) The sequencing has gone very well. 18S-E23 was complimentary to the 28S-D2 and D3 data, and the resulting tree is strongly supported for the basal associations, using the smaller data set in which all taxa have information for all three genes (see figure). The combined 28S-D2&D3 and 18S-E23 (for 67 of the 140 taxa) provides extremely good resolution within Eucharitinae, although because of conflicts in terminal groups, there is a large number of trees (>27,000). However, almost all the entire tree is resolved (see figure). The results support the monophyly of the large odontomachine (*Ectatomma*, *Odontomachus*, etc.) parasitoids. Within the Eucharitini, these results favor one of the two islands of trees that resulted from the morphological analyses. The large formicine parasitoids form two unresolved monophyletic clades within Eucharitini, although 89% of the trees favor monophyly of these two clades. Taxon sampling has proved crucial and we are looking into ways of analyzing the data to prove the necessity of more taxa over a complete sequence block.

4) Two undergraduates and one graduate student have been employed on this project. This fall, a new Master's degree student will start at UCR to work with me on the molecular systematics of the eucharitid clade parasitic on odontomachine ants.



Figures. Reduced data set (left), in which all taxa are sequenced for all three regions (bootstrap support above branches). Larger data set (right) includes 18S-E23 region, but for only 67 taxa.