

ISH Symposia ... *Continued*

Phylogeny of Microgastrinae: Taxon sampling in the age of NGS

James Whitfield (University of Illinois)

Phylogeny and biogeography of two cosmopolitan braconid wasp radiations: the doryctine tribes Rhaconotini and Spathiini

Alejandro Zaldívar-Riverón (Universidad Nacional Autónoma de México)

Sergey Belokobylskij (Zoological Institute of the Russian Academy of Sciences)

Rubi Meza-Lázaro (Universidad Nacional Autónoma de México)

Evolution and Biology of Chalcidoidea: Integrating Genomics, Fossils, Microbiomes and Natural History

By: John Heraty, University of California, Riverside, CA, USA

Our goal for this symposium at the International Congress of Entomology (ICE 2016) is to bring together researchers that specialize in phylogenetic, evolutionary and biological information on Chalcidoidea to present our current understanding of the group and to develop new integrative ways to address future studies in the group

Chalcidoidea (Hymenoptera) is one of the greatest of all insect radiations, with a tremendous taxonomic, biological and morphological diversity that is the product of many different evolutionary processes. We will integrate phylogenomic data, fossil data, and morphological data with evolutionary and biological processes to explain what we know about the evolution of this group, and what we see as priorities for future research.

Monday, September 26, 2016: 1:30 PM-5:30 PM

Presentation # 93111; 1:30 PM

Fossil chalcidoid wasps and the history of megadiversity

Lars Krogmann (lars.krogmann@smns-bw.de)¹, Ralph Peters², Roger A. Burks³ and

John M. Heraty³, ¹State Museum of Natural History, Stuttgart, Germany, ²Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany, ³University of California-Riverside, Riverside, CA

The evolutionary history of the parasitoid wasp superfamily Chalcidoidea is largely unknown mainly because their rich fossil record has never been comprehensively studied. Here, the first overview of the fossil history of Chalcidoidea is presented based on more than 1,000 fossils from Cretaceous, Eocene and Miocene ambers. Previously unrecognized specimens from Lebanese amber significantly increase the minimum age of the superfamily to about 130 million years. The phylogenetic relationships of these stem group representatives to extant Chalcidoidea are discussed. Chalcidoidea already exhibited a broad diversity in the Lower Cretaceous. Recently discovered fossils from Burmese amber (~99 mya) help dating the crucial transition from endo- to ectoparasitoidism. The minimum ages of further biological transitions are presented based on new fossil records of various chalcidoid families and subfamilies. The new fossils provide important calibration points for molecular dating studies and constitute a rich source of phylogenetically relevant morphological data that is revealed by digital microscopy and μ CT.

Presentation # 93112; 1:45 PM

The most ancestral genus of Mymaridae, with comments on generic relationships and evolution within the family

John Huber (John.Huber@agr.gc.ca), Natural Resources Canada c/o Canadian National Collection of Insects, Ottawa, ON, Canada

Several described genera of Mymaridae are the most ancestral group or groups within the family. *Macalpinia* and *Triadomerus* are Cretaceous fossils from Canadian amber and *Myanmymar* is from Burmese amber. *Eustochomorpha*, from Australia, is extant; it has not been found as fossils. Several other extant genera also have ancestral features and appear to be closely related to the Cretaceous fossils. All are loosely united by several plesiomorphies: female funicle 8-segmented, clava 2- or 3-segmented, and fore wing venation at

least half as long as wing length. Otherwise, they are quite different from one another, suggesting that Mymaridae had evolved and diversified well before 100 million years ago. No synapomorphy has yet been found to unite the genera. Two other Cretaceous fossils, *Carpenteriana* and *Enneagmus* appear to be more derived, due to loss of segments in the female antenna and tarsi; such losses occur in most extant genera. Morphological features of the ancestral genera are illustrated and compared to each other and to features found in Rotoitidae, arguably the next most ancestral family of Chalcidoidea, and Mymarommatidae, the sister group to Chalcidoidea. The relatively few reliable published host records show that species of Mymaridae parasitize a wide range of host families in at least six insect orders – Coleoptera, Diptera, Hemiptera, Hymenoptera, Odonata, Psocoptera – and several doubtful records also suggest Lepidoptera and possibly Thysanoptera. A likely reason for this wide host range is proposed.

Presentation # 93113; 2:00 PM

Phylogenomics, genomics, and our understanding of Chalcidoidea evolution

Ralph Peters (ralph_peters@hotmail.com), Zoologisches Forschungsmuseum Alexander Koenig, Bonn, Germany

Phylogenomics simply means inferring a phylogeny with data that represent a large proportion of the genome. This should – in theory – result in a robust phylogenetic tree that mirrors the real evolutionary branching patterns. Based on this tree, it is possible to trace innovations and modifications over time, i.e., to understand the evolution of a taxonomic group. Accordingly, we put a lot of effort on the generation of phylogenomic trees and the generation of data on character complexes whose modifications we can trace. In Chalcidoidea, we have a dated 48-species transcriptome-based tree. Although it will need many more taxa to be included for the full picture, this tree already allows for some inferences on the evolution of the group. Results show that all early branching lineages of Chalcidoidea are egg parasitoids and that convergent evolution of some rather obvious phenotypic characters has happened more frequently than previously thought. The phylogenomic

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dataset will be largely extended by ongoing projects that use the latest NGS methods. The extended dataset will allow for more fine-scaled inferences and, eventually, also new classifications. Furthermore, we have sequenced an additional four genomes of chalcid species. Now, Chalcidoidea rank among the best-covered taxa in terms of sequenced genomes in insects. In upcoming years, tracing genomic modifications and linking them to the observed morphological or biological diversity or to species-richness-patterns will become a new field of research that is no longer restricted to a few model species but can be done on a broader scale, for example, in Chalcidoidea.

Presentation # 93109; 2:15 PM

There is more than one way to jump: Eupelmidae, Encyrtidae and the interplay between phylogenomics and morphology

James Woolley (jimwoolley@tamu.edu), Texas A&M University, College Station, TX

It has long been thought that evolution of a complex of morphological characters associated with jumping locomotion is characteristic of a monophyletic group, including Eupelmidae, Tanaostigmatidae and Encyrtidae. These characters, studied most intensively in Eupelmidae, include expansion of the acropleuron to comprise most of the surface of the mesopleuron, together with expanded mesopleural-mesopleural muscles that provide the mechanical force for jumping. Associated adaptations include modifications to the prepectus, mesotibial spur, and mesobasitarsus. The structure of the mesopleuron is similar in Encyrtidae and Tanaostigmatidae and species in both families are powerful jumpers, leading to the hypothesis that all three families form a monophyletic clade. However, these relationships have been difficult to demonstrate with molecular data. Although these three families formed a monophyletic group in a recent, comprehensive analysis using only morphological data, Encyrtidae were not related to Eupelmidae+Tanaostigmatidae when both nuclear (28S D2-D5) and mitochondrial (18S) ribosomal sequences were added to the analysis. The results of analysis of

transcriptomes of exemplar taxa of Chalcidoidea reported by Ralph Peters in this symposium now provide strong evidence that Eupelmidae, Tanaostigmatidae and Encyrtidae are unrelated to one another. If true, each family has acquired jumping behavior independently. This provides an opportunity to re-examine the character systems associated with jumping behavior using a process of reciprocal illumination. These character systems will be explored to determine the features common to all three families, and the characters themselves will be re-examined in the context of related taxa to determine how the convergence has occurred.

Presentation # 93114; 2:30 PM

Figs and wasps arm-in-arm: An 80 million year walk in the jungle

Jean-Yves Rasplus (rasplus@supagro.inra.fr) and Astrid Cruaud, INRA - Centre de Biologie pour la Gestion des Populations, Montferrier-sur-Lez, France

The obligate mutualism between fig trees (*Ficus* species, Moraceae) and their pollinating wasps (Agaonidae, Hymenoptera) stands among the few outstanding cases of plant-insect diversification. Our recent findings strongly suggest that the fig-pollinator mutualism represents an extreme case among plant-insect interactions of coordinated dispersal and long-term co-diversification (Cruaud et al. 2012, *Syst. Biol.*, 61:1029-1047). Phylogenetic analyses for figs and fig wasps (200 spp. each) under relaxed molecular clock assumptions suggested the Cretaceous diversification of crown groups and contemporaneous divergence for nearly half of all fig and pollinator lineages. Event-based co-phylogenetic analyses further supported the co-diversification hypothesis. Recently, we proposed the inclusion of Sycophaginae, an unclassified subfamily of non-pollinating fig wasps, within Agaonidae based on several morphological apomorphies (Heraty et al. 2013, *Cladistics*, 29(5) : 466–542). In our talk, we will present the molecular estimates of a global phylogeny of Agaonidae *sensu lato*, based on ca 500 species and ~6 kb of DNA sequence data. We will investigate the biogeographical histories of the major clades and compare their timing of radiation and dispersion with

available data on *Ficus*. We will compare these results to those inferred from RAD-seq (shallower nodes) and exome capture (deeper nodes) data sets.

Presentation # 93115; 2:45 PM

Unjumbling the jumbled trichogrammatids: NGS to the rescue

Astrid Cruaud (cruaud@supagro.inra.fr)¹, Geraldine Bout², Guenaëlle Genson³ and Jean-Yves Rasplus¹, ¹INRA - Centre de Biologie pour la Gestion des Populations, Montferrier-sur-Lez, France, ²UMR ISA INRA / UNSA / CNRS, Sophia Antipolis, France, ³INRA - UMR 1062 CBGP (INRA/IRD/CIRAD/SupAgro), Montferrier-sur-Lez, France

Trichogramma are minute chalcid wasps that are egg parasitoids of ten insect orders (mainly Lepidoptera). Several species are commercialized for the control of crop pests of major economic importance. Nevertheless, experts agree that the taxonomy of the genus needs improvement. Morphological identification is difficult for non-specialists and molecular characterization is only partially and heterogeneously carried out, which may hamper the efficiency of biocontrol programs. Many species of *Trichogramma* are described as highly polyphagous. However, so called “generalists” may be complexes of stenophagous species with uncertain reproductive isolation. Finally, infections by facultative symbionts can influence individual fitness, reproductive isolation and ecological specialization, but there is little data on most symbionts associated with *Trichogramma* species. Classical molecular markers (COI, ITS2) are not informative enough to decipher species complexes, identify potential hybridization between strains, understand the genetic characterisation of phenotypic traits important for biocontrol success. A change of scale is necessary and Next Generation Sequencing brings solutions. In this talk we will present how we managed to tackle the major obstacle of the small size of trichos to obtain ca 120,000 RAD-tags of 100 bp each along the genome of every specimen we analysed. We will show that these unprecedented data sets open new avenues to decipher relationships among species complexes with high confidence and identify genetic flow. In an attempt to elucidate

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the causes of reproductive barriers, we will analyse our results in the light of the microbiome of each strain obtained with deep sequencing of multiple amplicons of the bacterial 16S rRNA.

Break; 3:00 PM

Presentation # 93118; 3:15 PM

What exactly is an aphelinid anyway?

Andrew Polaszek (A.Polaszek@nhm.ac.uk), Natural History Museum, London, England

Despite being one of the smaller families in terms of currently valid species, the chalcidoid family Aphelinidae is relatively diverse both biologically and morphologically. The composition of the family has varied greatly in terms of included genera, as well as in its tribal and subfamilial classification. Two genera, *Aberus* and *Cales*, were recently elevated to family status (Azotidae, Calesidae). The history of Aphelinidae classification is reviewed briefly, and the pros and cons of splitting the group and erecting monogeneric families, are discussed. Clearly molecular sequence data are contributing very significantly to the clarification of both deep and shallow relationships within the group, but how do we provide even a relatively stable classification as new technologies reveal new patterns?

Presentation # 93123; 3:30 PM

The role of the symbiont Cardinium in shaping the ecology and evolution of Encarsia

Marco Gebiola (marco.gebiola@gmail.com)¹, Suzanne E. Kelly¹, Peter Hammerstein², Massimo Giorgini³ and Martha Hunter¹, ¹University of Arizona, Tucson, AZ, ²Institute for Theoretical Biology, Humboldt-Universität zu Berlin, Berlin, Germany, ³National Research Council of Italy, Portici, Italy

The *Encarsia pergandiella* (Hymenoptera: Aphelinidae) species complex includes parasitoids of whiteflies that vary in reproductive mode and endosymbiotic bacterial infection, and

that are widely used as biological control agents against key agricultural pests such as *Bemisia tabaci* and *Trialeurodes vaporariorum*. Two members of this complex are *E. gennaroi*, uninfected by facultative symbionts, and *E. suzannae*, infected by the symbiont *Cardinium*, that causes the reproductive incompatibility known as cytoplasmic incompatibility (CI). *Encarsia gennaroi* and *E. suzannae* are morphologically and genetically differentiated, yet they are still able to mate and produce some hybrid offspring. Theory shows that unidirectional CI in conjunction with nuclear incompatibilities in the other direction can cause speciation, yet there are few empirical examples that support this scenario, and the general importance of CI in speciation remains controversial. Using *E. suzannae* and *E. gennaroi*, we performed laboratory mating preference and crossing experiments, and also examined competitive outcomes following a secondary contact scenario in population cage and greenhouse experiments. We aimed to 1) assess the role of CI-inducing *Cardinium* and genetic isolation mechanisms in reproductive isolation and competitive interactions of *Encarsia*, and 2) evaluate if and how the interaction of such species affects the biological control of a key agricultural pest. The results support asymmetric pre- and post-zygotic isolation between these species consistent with reproductive isolation of the two species evolving in the context of CI.

Presentation # 93126; 3:45 PM

Genomics and adaptation in Wolbachia, an obligate reproductive parasite in Trichogramma wasps

Amelia Lindsey (alind005@ucr.edu)¹, John H. Werren², Stephen Richards³ and Richard Stouthamer¹, ¹University of California, Riverside, CA, ²University of Rochester, Rochester, NY, ³Baylor College of Medicine, Houston, TX

Wolbachia is a maternally inherited symbiont, known for altering host reproduction to select for female offspring. There is great interest in introducing *Wolbachia* with favorable phenotypes into myriad target hosts. Unfortunately, little is known about the genomic changes that occur as *Wolbachia* adapts to new insects. In *Trichogramma* wasps, infected

populations evolve a dependency on *Wolbachia*-induced parthenogenesis for the production of female offspring. We can use this relationship to identify genomic signatures of adaptation. We present the genome sequence for the obligate *Wolbachia* strain wTpre, infecting *Trichogramma pretiosum*. After comparison to 16 other *Wolbachia* genomes, we show that wTpre has the most unique genes of any arthropod-infecting *Wolbachia*. Comparisons of genomic sequence show the majority of these unique genes are genes that have been truncated through nonsense and frameshift mutations, representing 20% of the genome. To test whether or not wTpre has maintained adaptive ability after these extensive gene truncations, we have developed a system to challenge wTpre with novel *Trichogramma* hosts, and track adaptation. Initially, novel hosts produced fewer females, and a significant proportion of intersexes, resulting from incomplete modification by *Wolbachia*. Over time, *Wolbachia* adapted to the new hosts, seen as recovery of parthenogenesis. We will employ genome re-sequencing to identify the mutations responsible for adaptation in wTpre and contrast them with the mutations responsible for the aforementioned gene truncations. Knowledge of the genomic changes that occur during *Wolbachia* adaptation will be used to inform the transfer of *Wolbachia* to target insects for vector- and biological control.

Presentation # 93129; 4:00 PM

Genetics and genomics of Aphelinus - keeping ahead in the parasitism race

Keith R. Hopper (khopper@udel.edu), USDA - ARS, Newark, DE

Differences in parasitism among potential host species can provide strong selection for divergence and speciation in parasitic Hymenoptera. However, little is known about the evolution or genetic architecture of host specificity. Here we report research on the genomics and genetics of host specificity in *Aphelinus* species. We have sequenced, assembled, and annotated the genomes and transcriptomes of twelve *Aphelinus* species in five complexes. Using coding sequences, we developed a robust phylogeny, onto which we mapped parasitism of seven diverse species of aphids. For some aphids, parasitism was

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phylogenetically conserved, with closely related parasitoids showing similar levels of parasitism. For other aphids, parasitism diverged between closely related parasitoids, consistent with host-driven speciation. To explore the genetic architecture of differences in host specificity, we crossed and backcrossed *Aphelinus atriplicis*, which readily parasitizes *Diuraphis noxia*, with *Aphelinus certus*, which rarely parasitizes this aphid. Using genetic markers from reduced-representation genomic libraries, we mapped quantitative trait loci (QTL) affecting parasitism of *D. noxia*. We found eight QTL (six of which interacted in their effects) that explained ~40% of the variation in parasitism among backcross females. We compared the genomes and transcriptomes of these parasitoid species to find genes that diverged in sequence or expression, and we tested whether divergent genes mapped to QTL affected parasitism of *D. noxia*. We identified 14 divergent genes that mapped to parasitism QTL or significantly affected parasitism by themselves. These are among the first results on the genetic architecture of host specificity in parasitic wasps.

Presentation # 93130; 4:15 PM

Morphometrics and the description of cryptic species in the Chalcidoidea

Hannes Baur (baur.hannes@gmail.com), Natural History Museum Bern, Bern, Switzerland

Morphometric data play an important role in the differentiation of cryptic species, as they often can only be recognized by gaps in some quantitative characters. This is also the case for many groups of Chalcidoidea, where the use of distance measurements has a long tradition. For instance, it is quite common to include up to 30 body ratios in species descriptions of Pteromalidae, Eulophidae, Aphelinidae, and other families. Here I present multivariate ratio analysis (MRA), a relatively new set of statistical tools addressed to the practical needs of taxonomists. On the one hand, MRA goes far beyond the simple calculation of body ratios, as it allows the examination of covariation for multiple characters and taxa. This is done by modifying the raw data matrix, which is then

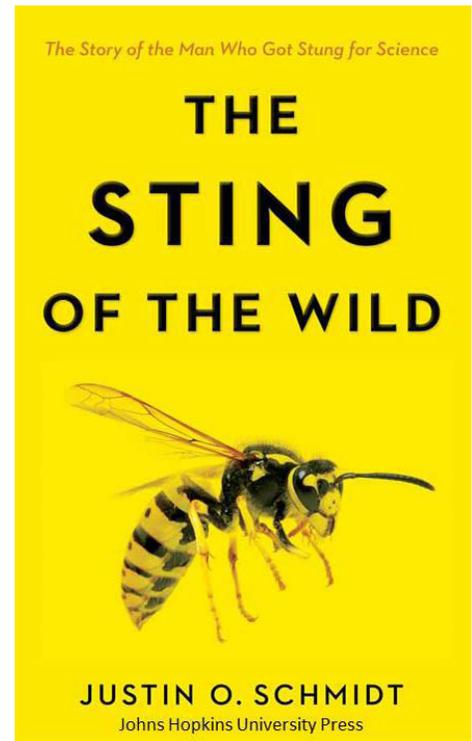
subjected to either a principal component analysis or a linear discriminant analysis. The analysis also allows a clear separation of variation in size and shape as well as an estimation of their correlation (allometry). On the other hand, the results of MRA can be interpreted in terms of body ratios. For instance, it is possible to extract the best separating ratios of two groups, which can be included in identification keys or descriptions. Such best ratios clearly improve the descriptive part of a taxonomic study. The application of MRA is demonstrated using species complexes of the genera *Pteromalus* and *Anisopteromalus*.

Presentation # 93132; 4:30 PM

Immature development of Chalcidoidea: Trends, functional ecology and comparison with other parasitoids

Alexander Gumovsky (entedon@gmail.com), National Academy of Sciences of Ukraine, Kiev, Ukraine; University of the Witwatersrand, Johannesburg, South Africa

The Chalcidoidea, a “megadiverse” parasitoid group, is characterized by high diversity in terms of host associations, life history traits and immature morphology. However, development remains unknown for many groups, and the only comprehensive reviews of larval morphology (Parker, 1924; Parker & Thomson, 1925) are outdated. Recently, molecular and combined research (Munro et al., 2011, Heraty et al., 2013) has produced a reasonably defensible phylogeny for the Chalcidoidea, which facilitates preliminary tracing of the evolutionary patterns of immature development. This report combines original data and critical literature review to generate an evolutionary scenario from mapping the available immature characters based on the likelihood (RAxML) tree reported by Heraty et al. (2013). The distribution pattern of these immature characters suggests that Chalcidoidea share, with other parasitoid groups, a number that are associated with certain modes of parasitism (for example, the head appendages used for siblicide within aggressive solitary endoparasitoids). However, some other characters (for example the serosa) also appear in distantly related and biologically dissimilar groups. Character mapping illustrated that larval morphology of Mymaridae



Upcoming book on Hymenoptera

By: Justin Schmidt, Southwestern Biological Institute, Tucson, AZ, USA

The Sting of the Wild is to be released in early May by Johns Hopkins University Press. It is a book entirely about Hymenoptera – well, ‘fess up time, actually some spiders, cicadas, flies, and such, are included; but they are mainly mentioned as food for Hymenoptera. The book is written about stinging Hymenoptera, why they sting, how their stings have changed their behavior and evolution, and how hymenopterous stings have changed our lives as human beings. The format is storytelling of adventures, historical accounts, natural history, and science told in a style designed for academic and curious readers of all stripes. Insects ranging from sweat bees and fire ants to harvester ants, yellowjacket wasps, tarantula hawks, cicada killers, mud daubers, and mutillid wasps, to honey bees and bullet ants are detailed. These insects are the star performers. They are the stars because their lives and stings are so fascinating and their lifestyles reveal amazing and diverse aspects of biology. A central theme throughout the writing is the pain caused by insect stings and how it affects humans and other animals alike. As a preview, people and toads respond