Combining Non-invasive DNA extraction with Next-Generation Sequencing to study California Gall Wasps

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Background

California oak gall wasps have intrigued researchers for decades due to their striking ability to induce vibrant growth of physically diverse structures from their host's tissue. The galls' primary function is to nourish the wasp's larvae, protecting them while they are most vulnerable to predation.[1] Most of the work done with gall wasps has focused on gall morphology, so very little is known about the wasps themselves. For example the underlying mechanism for gall formation is unknown, largely due to a lack of understanding of the phylogenetic relationships of oak gall wasps. This uncertainty in their taxonomy makes the California oak gall wasps an excellent candidate for research.[2]

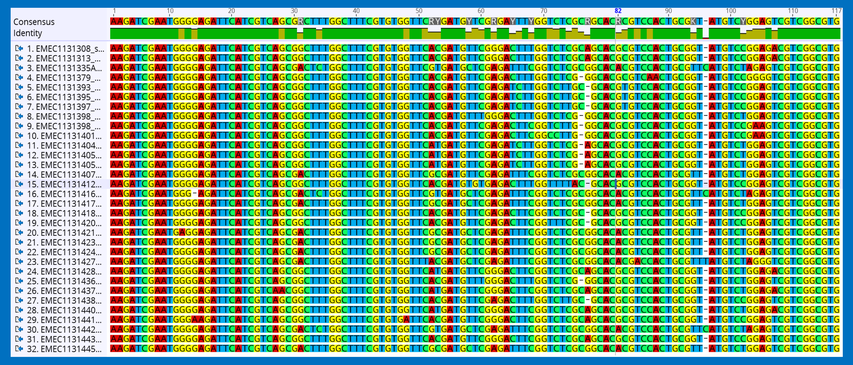
Examples of California oak galls – photos by Joyce Gross

Objectives

The Essig Museum of Entomology is home to one of the best collections of oak gall wasp species, including specimens collected over 100 years ago. This collection could greatly help illuminate problems in the California oak gall wasp phylogeny and taxonomy. Here, non-invasive DNA extraction techniques[3][4] are used to create a digital molecular dataset that will be available for public use. This dataset will be used to provide species-level identifications for specimens in the Essig Museum and other Natural History Museums as well as pave the way for future research regarding mechanisms of gall formation.

Methods

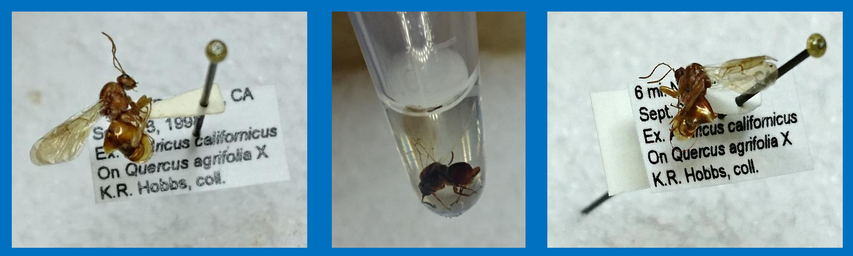
1) Genetic material was isolated from 111 identified and 42 unidentified oak gall wasp specimens
2) Three genetic loci were targeted for PCR amplification (28S, COI, and LWRh)
3) Amplified fragments were barcoded with unique primer tails
4) Barcoded fragments were sequenced using the Illumina MiSeq Next-Generation Platform sequencing
5) Specimens were re-pinned and returned to the Essig Museum
6) Sequences were aligned to estimate a molecular phylogeny
7) Results were compared to published records for verification and to provide species-level IDs when possible



28S sequence alignment for specimens from the Essig Museum

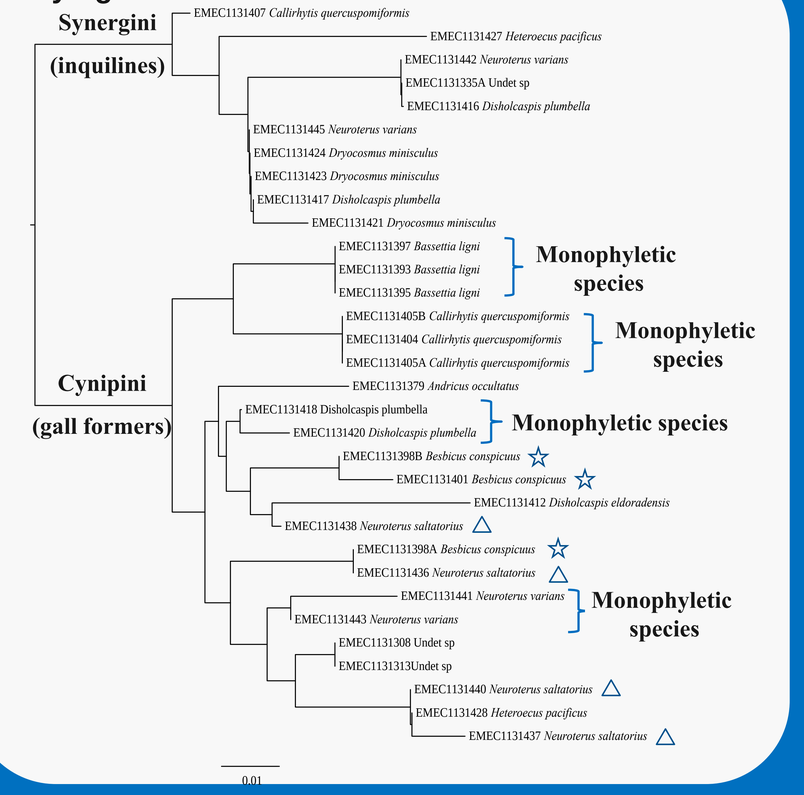
Results

1. Fragments of 28S, COI, and LWRh were amplified from 78, 27, and 124 specimens, respectively
2. Of those loci, only 28S sequenced successfully (32 specimens)
3. Ten specimens identified as gall formers, are actually inquilines
4. Only four species were reconstructed as monophyletic



Example of oak gall wasp specimen prior to DNA extraction (left), during (center), and after (right)

Phylogenetic Reconstruction Based on 28S



Future Research

1. Use the morphological specimens to verify some of the misidentifications noted in our analysis
2. Use the 28S results to identify specimens to genus, and then to design genus-specific primers for COI and LWRh
3. Analyze factors that might have influenced the success of amplification and sequencing (e.g. age, collection method, DNA concentration, etc.)

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References

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