

# Iowa Research Online

---

## Cryptic diversity and evolution in a genus of oak-gall-associated parasitoid wasps

Sheikh, Sofia Iqbal

<https://iro.uiowa.edu/esploro/outputs/graduate/Cryptic-diversity-and-evolution-in-a/9984124170702771/filesAndLinks?index=0>

---

Sheikh, S. I. (2021). Cryptic diversity and evolution in a genus of oak-gall-associated parasitoid wasps [University of Iowa]. <https://doi.org/10.17077/etd.005990>

---

<https://iro.uiowa.edu>  
Free to read and download  
Copyright 2021 Sofia Iqbal Sheikh  
Downloaded on 2024/05/04 12:00:48 -0500

---

CRYPTIC DIVERSITY AND EVOLUTION IN A GENUS OF OAK-GALL-ASSOCIATED  
PARASITOID WASPS

by

Sofia Iqbal Sheikh

A thesis submitted in partial fulfillment  
of the requirements for the Master of Science  
degree in Integrated Biology in the  
Graduate College of  
The University of Iowa

August 2021

Thesis Committee: Andrew A. Forbes, Thesis Supervisor  
Bin Z. He  
John M. Logsdon

Copyright by  
Sofia Iqbal Sheikh  
2021  
All Rights Reserved

## ACKNOWLEDGEMENTS

I would like to thank my advisor, Dr. Andrew Forbes, for providing me with numerous opportunities to grow into a scientist. I am lucky to have worked with such a supportive mentor, whose contagious enthusiasm for science has taught me how to maintain excitement about a project even when it isn't working as planned. I also owe a huge thanks to past and current members of the Forbes lab, and in particular Dr. Robin Bagley (comrade), Anna Ward, Alaine Hippee, Benjamin Trendle, and Sarah DeLong-Duhon, for the molecular benchwork training, insightful conversations, humor, and friendship. I am grateful to the Forbes lab collectively for treating science like a communal exercise and not a competition, the frequent discussions about science education and equity, and the many attempts at lab-pet keeping (with mixed results).

I would like to thank my committee members Dr. Bin He and Dr. John Logsdon for always being willing to chat all things molecular evolution, bioinformatics, and phylogenetics. Additional faculty members who helped enrich my graduate school experience include Dr. Steve Hendrix for his plant knowledge and thought-provoking questions during lab meetings, and Dr. Maurine Neiman for the opportunity to do science outreach. I would like to also thank the department overall for opportunities to engage with science/academia beyond my own research.

A huge shout-out goes to my parents and small army of siblings who have been incredibly supportive of me long before and throughout graduate school. I am also immensely grateful to my friends, both old and new, for their support during challenging times and excitement during good times, and for listening to me talk about tiny wasps. Finally, a big thank you goes to collaborators in the gall and parasitoid research world, including Dr. Jean-Yves Rasplus, who generously shared unpublished sequencing data to be used in this study.

## ABSTRACT

Parasites tend to be host specialists. While a generalist strategy confers access to more potential hosts, the evolution of habitat-specific adaptations may improve fitness on one or a few similar hosts, leading to specialized host-associations. This can be especially true when host niches vary across multiple ecological axes that may be relevant for successful parasitism. One example of such hosts are oak galls, which are induced by gall-forming cynipid wasps, and exploited by parasitic insects. Oak galls are highly diverse across several dimensions including their external and internal morphology, phenology, and chemistry. Such variability among gall traits should theoretically drive parasites toward specialization. It is therefore unexpected that some oak-gall-associated parasite species are described as attacking many different galls. The Hymenopteran parasitoid species *Ormyrus labotus* attacks galls of >65 cynipid gallformers, with rearing records spanning more than 65 host galls associated with a diverse set of oak tree species and plant tissues, making it an apparent generalist with an unusually large host range. In this work, I used an integrative approach pairing molecular tools (barcoding and reduced representation genomic sequencing) with morphological and ecological data to test whether *O. labotus* is truly a single generalist species, and how host use has evolved in the *Ormyrus* genus broadly. I find evidence that a few *Ormyrus* species, including *O. labotus*, are complexes of many specialist species, each with a much more restricted host range. Investigating cryptic diversity is crucial for accurate species estimates, improving our understanding of how lineages diversify, and designing effective biocontrol strategies against invasive species, like many galling wasps.

## PUBLIC ABSTRACT

Parasitism, wherein one organism exploits another, is the most common life history strategy in all of life. Few organisms lack parasites, and many parasites tend to have life histories strongly associated with just one or a few similar hosts – i.e., they are specialists. The success of parasites as measured by their species richness and abundance is often attributed to this extreme level of specialization. Among parasitic organisms, parasitic insects, and in particular parasitoid wasps (which lay their eggs in other insects and are lethal to the host), are incredibly diverse but most species have not been studied in detail. Oddly for parasites, many parasitoid species are described as being associated with a large variety of hosts; in other words, they are apparently generalists (an evolutionary strategy wherein multiple, and often ecologically and chemically dissimilar, hosts can be exploited). However, because few of these generalists have been studied in any great detail, it is possible that many supposed generalist species might consist of several specialized and morphologically cryptic lineages. In this thesis, I investigated one such supposed generalist parasitoid, *Ormyrus labotus*, and other wasps in the genus *Ormyrus* associated with oak gall wasps. Oak gall wasps are a hyper-diverse group of herbivorous insects that induce abnormal structures (galls) on oak trees. Oak galls vary considerably in their ecology, morphology, and chemistry. Despite these differences, *O. labotus* is described as attacking 65 species of oak gall wasps. To test whether *O. labotus* is one generalist species, I used a molecular approach in combination with morphological and ecological data and found evidence for 35-36 species within several previously named species of *Ormyrus*, including 19-20 species matching the description of *O. labotus*. This study, alongside several other recent studies, highlight the need to investigate cryptic diversity to better estimate species diversity and understand the ecological interactions that structure host use by parasites.

## TABLE OF CONTENTS

LIST OF TABLES .....	vi
LIST OF FIGURES .....	vii
INTRODUCTION .....	1
Evolution of specialized life histories .....	2
Oak gall system .....	4
METHODS .....	8
Collections and rearing .....	8
COI barcoding approach .....	9
Ultra-conserved elements (UCEs) approach .....	11
RESULTS .....	16
COI barcoding .....	16
UCEs .....	17
Synthesis .....	18
DISCUSSION .....	26
Host ranges and host shifts .....	27
Life history evolution .....	31
The importance of hidden specialists .....	33
Conclusion .....	35
REFERENCES .....	45
APPENDIX .....	55

## LIST OF TABLES

Table 1: Summary of a few previous studies that used an integrative approach to investigated putative generalists.....	41
Table 2: <i>Ormyrus labotus</i> host associations.....	42
Table 3: Average percentage divergence in mtCOI sequences between clades fitting the morphological description of <i>Omyrus labotus</i> .....	44
Table A1: ID numbers and collection information for <i>Ormyrus</i> included in the COI project.. ...	58
Table A2: ID numbers and collection information for <i>Ormyrus</i> included in the UCE project... ..	62
Table A3: Summary of NGS sequencing data.....	67

## LIST OF FIGURES

Figure 1: Simplified web of interactions in the oak gall system.....	36
Figure 2: A glimpse of oak gall morphological diversity.....	37
Figure 3: Map of all unique collection regions represented in the COI study.....	38
Figure 4: Combination of molecular (COI), morphological, and ecological data used to develop species hypotheses for <i>Ormyrus</i> specimens).....	39
Figure 5: UCE maximum likelihood tree.....	40
Figure A1: Bayesian COI tree.....	55
Figure A2: Maximum likelihood COI tree. ....	56
Figure A3: ASAP species delimitation results.....	57

## INTRODUCTION

Understanding the processes that generate biodiversity is a major focus of evolutionary biology. What drives one lineage to become many? One approach to this question is to investigate systems or evolutionary lifestyles that are especially diverse, like parasitism. Parasitic life history strategies have evolved several times and are the most common lifestyle among multicellular organisms (Price 1980, Windsor 1998, Poulin and Morand 2004, Weinstein and Kuris 2016). Parasitism appears frequently in insects: the guild of phytophagous (plant-feeding) and other parasitic insects (such as parasitoids) constitutes close to half of all insect species (Price 1980, Janz and Wahlberg 2006). Parasitic insects are thus uniquely positioned to provide insights into the mechanisms implicated in the origins and maintenance of diversity.

For parasitic insects exploiting a host, there are a variety of abiotic and biotic dimensions (like predators, competition, or plant chemical defenses) that comprise the host environment. Intuitively, such challenges should favor a “jack of all trades” approach – i.e., a generalist parasite that can switch between multiple hosts as needed. However, the opposite is commonly observed; the great diversity of parasitic insects is largely a function of their restricted host ranges (Price 1980). While host species (animals, plants, fungi, even other parasites) are exploited by numerous parasitic insect species, many of those parasites are specialized on just one or a few hosts (Price 1977, Poulin 1992, Price 2002). One hypothesis for why increased specialization tends to evolve among parasites is because new adaptations that increase performance on some host species can consequently reduce the same parasite’s ability to successfully attack other hosts. In other words, fitness tradeoffs favor specialized life histories; the opportunity cost incurred by a restricted diet is offset by improved fitness on just one or a few similar hosts (Jaenike 1990, Fox and Morrow 1981, Agarwal *et al.* 2010). Given the

complexity of host environments, multifarious selection may exist for combinations of traits that help maximize fitness within the context of one host/environment (Futuyma 1988, Nosil & Harmon 2009). The accumulation of host-specific traits can lead to genetic signatures of divergence between closely related populations adapting to ecologically distinct hosts. This divergence is termed host-associated differentiation, or HAD (Stireman *et al.* 2005), and can eventually result in reproductively isolated sister species (Abrahamson *et al.* 2008).

### ***Evolution of specialized life histories***

The biology of parasitic insects and the ways in which they interact with their hosts and larger environments is conducive to the development of specialized host-associations. For example, for many phytophagous insects, plant volatile compounds serve as chemosensory cues for both locating preferred habitats and avoiding less favorable ones (Berenbaum and Feeny 2008). However, the number of host/nonhost signals that can be processed by an insect is neuronally constrained, thus likely limiting the evolution of broad host preferences that require processing more plant-stimuli in search of a host than generalists (Bernays *et al.* 1994, Bernays 2001). In support of this idea, several studies have demonstrated that specialized parasites pay selective attention to host cues and spend less time searching for a host (Janz and Nylin 1997, Bernays and Mikenberg 1997, Egan and Funk 2006). Additionally, polyphagous feeding strategies (wherein multiple different hosts are used) increase variation and complexity in resources used, which can increase the likelihood of selecting a lower quality host. Choosing a lower quality host can weaken the relationship between oviposition preference and offspring performance (Singer 1972, Craig *et al.* 1989, Craig and Itami 2008). This relationship is defined by the degree to which the ovipositing choice of a female (including the host species, host genotype, plant tissue, etc.) corresponds with the success of her offspring on that host (Courtney

1981). Offspring success is driven by nutrients, chemical defenses of the host, predation, and similar features defining the suitability of a host for early stages of development. Evidence on the magnitude of correlation between preference and performance is equivocal, although in general host-specialist females show stronger preference for more suitable, higher quality hosts (Gripenberg *et al.* 2010).

In addition to navigating a plethora of sensory cues in heterogeneous environments, parasitic insects with short adult lifecycles are under strong selection to efficiently find mates (Jaenike 1990). Mating often occurs on or near the host for many parasitic insects, therefore as the number of potential hosts increases the probability of encountering potential mates may decrease. This implies counterbalancing selection for restricted host ranges (Rodhe 1979). Finding mates co-occurring on a host plant also requires a degree of synchrony between the phenology of the parasite and host – that is, the developmental timeframe of the host can be relevant for the lifecycle of the parasite that exploits it. This synchrony plays a crucial role in parasite fitness (e.g., van Asch and Visser 2007) and has been shown to be important in the evolution of new host-associated populations, perhaps the progenitors of new specialist species (Komatsu and Akimoto 1995, Forbes *et al.* 2009). Asynchrony, or phenological mismatching between the lifecycle of a parasite and its host has consequences for its reproductive success (Visser and Gienapp 2019). Additionally, escape from competition, drift (Gompert *et al.* 2014, Hardy *et al.* 2016), and standing genetic variation for traits involved in novel host use (Futuyma *et al.* 1995, Forister *et al.* 2007) have all been proposed to explain the tendency toward specialization in parasitic insects.

Despite all the above, many insect parasites with several of the qualities that should favor specialization (i.e, that mate on or near their hosts, use chemical cues to locate hosts, attack

temporally ephemeral hosts, etc.) appear to act as generalist species. Moreover, within some insect genera, some species are described as apparent specialists while others are described as using many hosts, despite few other obvious differences in their general biology, the type of hosts they attack, or other dimensions of their niche. This could portend a real and meaningful difference among congeners, but such situations might instead signal that a generalist species is not a generalist at all (Acs *et al.* 2010, Nicholls *et al.* 2018). Most insect species, after all, were originally described solely based on morphological traits, such that the taxonomist who placed too little weight on variation in a particular character or had chosen a genus where morphology was often unhelpful or misleading, might not have captured differences relevant to actual reproductive isolating barriers. With the advent of molecular ecological studies of parasitic insect species carefully reared from known hosts, there have been several striking examples of putative generalists revealed to instead consist of two or more enigmatic specialists (Table 1).

### ***Oak gall system***

One example of a system where one might expect to, and where we do, find abundant specialization is among the oak gall wasps and their associated natural enemies (Figure 1). Oak gall wasps (Hymenoptera: Cynipidae: Cynipini) are a diverse tribe of herbivorous wasps that induce highly structured growths (galls) on oak trees. The gall structure serves as both shelter and a food source for the gall wasp larva(e) (Ronquist *et al.* 2015). There are over 700 described species of oak gall wasps in North America alone (Stone *et al.* 2002) and most of them are specialized gallers of just one or a few closely related oak tree species, such that the tree species, along with the appearance and location on the tree of the gall itself, is often sufficient to identify the species of wasp responsible for the gall (Weld 1959, Stone *et al.* 2002). Such tight associations between gall wasps and specific host oak species indicate that plant volatiles may be

involved in tree-host recognition at multiple trophic levels (Germinara *et al.* 2011), or that oak chemistry influences either female preference for oviposition or offspring performance, or both (Abrahamson *et al.* 1998, Abrahamson *et al.* 2003). In turn, tree-host cues aid parasites in search of their gall host (Germinara *et al.* 2011) and have been shown to restrict the host ranges of parasitoids (Askew *et al.* 2013).

Though galls putatively offer gall wasp larvae protection from predators (Ronquist *et al.* 2015, Bailey *et al.* 2009), a taxonomically diverse community of parasitoid wasps is commonly associated with most galls (Forbes *et al.* 2016). Parasitoid larvae feed on the developing gall wasp, resulting in its death. The parasitoids often have life histories closely linked with the gall wasp, and/or morphological adaptations that appear essential to overcoming certain gall defensive traits, and/or rearing records that apparently closely track the oak tree species on which the host gall is induced (Ward *et al.* 2020). Such high levels of specialization should come as no surprise. Galls occurring on specific tissues of specific tree-host species and at discrete times during the growing season compose distinct spatiotemporal niches. Galls growing on different tissues are known to release distinct volatiles, which may serve as host-searching cues for parasitoids with different volatile preferences (Hayward and Stone 2005).

In addition to plant/gall chemistry, the high interspecific morphological variation among oak galls may play a role in pattern searching by parasitoid enemies, as well as their respective abilities to parasitize the gall (Bailey *et al.* 2009). Gall morphological features range from external traits (for example, size, toughness, spines, or nectar secretion) to internal structures (such as the number of chambers or presence of radiating fibers) (see Figure 2 for examples). Some research suggests that the community of parasites associated with a gall may drive phenotypic innovations of the gall to reduce mortality of the developing galler(s) inside (Price

1987). One hypothesis for why galls have such wide morphological variation, such as spikes or fuzz, is that these traits evolve as a tactic for escaping natural enemies (Stone and Schönrogge 2003). Under this “enemy-escape” hypothesis, gall morphology is considered a trait under selection, with the acquisition of defensive traits providing a period of relief from, or reduction in, natural enemies. While natural enemies are expected to eventually “catch up” to each new iteration of a gall, the enemy escape hypothesis has received support as a viable explanation for observed diversity in oak gall phenotypes (Bailey *et al.* 2009). For a parasitoid, adaptations that allow it to overcome specific defensive morphological traits would likely also restrict the host range of galls that it can parasitize.

Despite the many apparent hurdles to a parasitoid successfully acting as a broad generalist on oak galls, many parasitoids are nevertheless described as such (Washburn and Cornell 1981, Askew 2006, Askew 2013, Ferracini *et al.* 2018). For example, *Torymus flavipes* (Hymenoptera: Chalcidoidea: Tormyridae) is described as having 38 host galls associations, and another species in the same genus, *Tormyrus auratus*, is reported to emerge from 41 gall species (Askew *et al.* 2013). However, both these ultra-generalists, and many others like them, have not been interrogated using a combination of molecular and ecological tools, such that their description as host generalists relies solely on a shared morphology and little else. In this work, I investigate one such apparent generalist parasitoid with 65 named oak gall hosts (Hanson 1992, Table 2), *Ormyrus labotus* (Hymenoptera: Chalcidoidea: Ormyridae). If *O. labotus* and similarly described parasites really are generalists, we might learn what quirks of biology have allowed for their cosmopolitan nature among so many closely related specialist congeners. On the other hand, if they are complexes of specialists this will comport with expectations about parasitic insect diversity. It will further suggest, among other things, that the species richness of described

parasitic insects – already touted as the largest group of Earth’s animals – is an undercount. Discerning the truth is of interest whatever the outcome: understanding patterns of host-use for both specialists and generalists is useful for designing effective biocontrol strategies that take advantage of parasite host-ranges to regulate pests (Nicholls *et al.* 2018). Additionally, clarifying the putative axes along which lineages specialize and which components play crucial roles in species diversification will improve our understanding of how parasites evolve.

I hypothesize that *Ormyrus labotus* is a complex of several species, each with a much smaller host range than is currently described for this taxon. *Ormyrus labotus* is described as attacking gall wasps from a broad range of tree habitats, gall morphologies, and seasons. The presence of multiple and highly variable niche dimensions that are apparently navigated by this generalist parasitoid make it ideal for addressing the hypothesis that species like *O. labotus* might be a complex of several species. Most other species within the genus *Ormyrus* show considerably smaller host ranges than *O. labotus*. For example, *O. unifasciattrpennis* has been reared from just three gall species. *Ormyrus crassus* has only one known host (Hanson 1987, Hanson 1992). *Ormyrus hegei* has five recorded host-associations, which largely share morphology (woody) and tree organ (stem) on which the gall is induced.

In this study, I use molecular tools in conjunction with morphological and ecological data to test whether *O. labotus* is truly an exceptional generalist, and, if not, how host ranges may have evolved in this genus of parasitoids. As opposed to defining species under any one of the three major species concepts, I am testing for the presence of one or a few versus multiple species using a combination of approaches.

## METHODS

### *Collections and rearing*

Between August 2015 and September 2019, I, other members of the Forbes lab, and several other collaborators, collected cynipid galls from various oak species across the continental United States. We recorded the date of collection, the geographical location, and tree host from which galls were collected. The species of gall was determined based on tree host, plant tissue, and gall morphology (Weld 1957, 1959, Weld 1960). Where the gall species could not be immediately determined, we documented a description of the morphology and specific plant tissue upon which the gall was found. We assigned a unique number to represent a collection (representing date, location, tree host, and species of gall), and stored the gall(s) from that collection in an individual container kept in an incubator (SANYO Electric Co. Ltd, Osaka, Japan). The incubator mimicked the external environment in terms of temperature, humidity, and light/dark cycles. We checked the incubator daily and removed any emergent insects for storage in 95% ethanol. We also recorded the collection number and emergence date, or date the insect was removed from the cup, whichever occurred first. Finally, we used taxonomic keys to identify each non-galler insect to the genus level (Goulet 1993). For wasps in the genus *Ormyrus*, specimens used in this study were keyed to the species level using Hanson's genus key (1992) and based on a pinned specimen, photographs, or both. I chose a set of 69 specimens (including already published sequences) reared from a diverse set of gall hosts and locations (Figure 3) that all keyed to *O. labotus* as well as representatives of other *Ormyrus* species reared from our collections (Appendix Table A1).

For all wasps used in molecular work, I documented morphology for each specimen. I first captured the body profile by stacking photographs taken with a Canon EOS 60D camera and

a Canon MP-E 65mm macro lens (Canon USA, Melville, NY), and processed in Zerene Stacker software (Zerene Systems LLC, Richland, WA). I then mounted a forewing on a microscope slide and photographed it using a Leica M125 stereomicroscope (Leica Inc., Switzerland).

### ***COI barcoding approach***

#### **Sequencing and phylogenetic reconstruction**

For barcoding, I sequenced a ~650bp segment of the mitochondrial COI gene (COI), which contains both conserved and variable regions, making it convenient for PCR amplification and detecting putative species (Herbert *et al.* 2003). Of the *Ormyrus* selected for COI sequencing, we extracted a small set using DNA using a DNeasy Blood and Tissue kit (Qiagen Inc., Valencia, CA). For the majority of specimens, I used a CTAB/PCI approach following the methods developed by Chen *et al.* (2010). For all extracted DNA samples, I amplified an ~650bp region of COI using either the following primer pairs: COI\_pF2: 5' ACC WGT AAT RAT AGG DGG DTT TGG DAA 3' and COI\_2437d: 5' GCT ART CAT CTA AAW AYT TTA ATW CCW G 3' (Kaarinen 2010), or an in-house designed forward primer, Orm\_2: 5' TRG GDG CTC CDG ATA TRG CW 3' paired with the COI\_2437d primer from Kaarinen *et al.* (2010). Sanger sequencing was done in both forward and reverse directions on an ABM 3720 DNA Analyzer (Applied Biosystems, Foster City, CA) in the University of Iowa's Roy J. Carver Center for Genomics. I used Geneious v9.1.8 (Biomatters, Inc., San Diego, CA) to prepare consensus sequences, and Geneious Alignment (a built-in aligning program) to generate and manually edit a multiple sequence alignment. In addition to the *Ormyrus* I sequenced for this study, I also used existing *Ormyrus* sequences from Genbank to increase representation of gall hosts/tree species/and locales in our (see accession numbers in Table A1), as well as 25 COI sequences of *Ormyrus rosae* (Zhang et al 2014). jModelTest2 (Darriba 2012) was used to test for

the best fitting substitution model for my dataset: GTR+I+G was selected. For phylogenetic reconstruction, I used MrBayes (v3.2.7, Ronquist *et al.* 2012), which ran two independent analyses using four MCMC chains (one cold, three hot) for 3,500,000 generations. I also used RaxML v8.2.12 (Stamatakis 2014) with 1000 bootstrap pseudoreplicates for phylogenetic reconstruction under the maximum likelihood criterion.

### **Species delimitation**

To develop working hypotheses about the number of species present within our sampling, I first delimited molecular species using two computational approaches. I used ASAP, which takes a multiple sequence alignment as input to search for a gap between inter- and intraspecific divergence, and then uses that to sort sequences into putative species groups (Puillandre *et al.* 2020). The second method was bPTP, a coalescence-based approach that uses a phylogenetic tree as input and estimates the probability of descendant branches being members of the same or different species at each node present in the tree by using branch lengths as a proxy as for substitutions (Zhang *et al.* 2013). I used the multiple sequence alignment generated in Geneious as input for ASAP, and the Bayesian tree as input for bPTP. For both programs, I used the default settings for all parameters, except for the substitution model in ASAP, which was changed from the default of Jukes-Cantor to Kimura-2-parameter. Finally, I combined the ASAP and bPTP species estimates with host information (gall species, gall morphology, tree host, plant tissue, and geography) to infer reproductively isolated clades that are supported by molecular and host-associated differences. The inclusion of ecological niche characters can be helpful for inferring potential reproductive barriers (for example, occupying niches that differ in their phenology, such that their occupants are temporally isolated from each other).

### ***Ultra-conserved elements (UCEs) approach***

The COI dataset indicated the presence of many cryptic specialist species (see results section), leading me to: 1) verify these findings with the use of additional genetic markers, and 2) ask which ecological axes might structure host-use via specialization of oak-gall-associated *Ormyrus*. Recent advances in high-throughput sequencing and genomic sampling methods have created new opportunities to trace the evolutionary history of a group of organisms. To that end, and in addition to our COI approach, we used a reduced representation genomic sequencing approach that targets thousands of shared ultra-conserved genomic elements (UCEs) (Bejerano 2004). Due to their high sequence conservation, UCE core regions are viable targets to capture out of genomic DNA, a process called target enrichment. DNA flanking the UCE region increases in variability with distance from that region, and this variability can be used for robust phylogenetic inference (Faircloth *et al.* 2012; examples: McCormack *et al.* 2011, Branstetter *et al.* 2017, Myers *et al.* 2019).

### **Taxon sampling**

To select specimens for inclusion in the UCE sequencing, I first picked representative individuals from the COI sampling that had  $\geq 1\text{ng}/\mu\text{l}$  concentration of DNA. Next, to account for collections not represented in the COI project, I sorted all *Ormyrus* emergences by the species of gall from which they emerged. For each gall species, I classified *Ormyrus* emergences by the tree host that the gall was collected from; if *Ormyrus* were reared from the same gall species collected from multiple tree-hosts, an individual representative of each unique tree-host was selected for this study. These first two steps enable us to better characterize host ranges and host use in our sampling. Next, to test for isolation by distance, I looked for variation in geographical location from which a particular gall species was collected. Lastly, I accounted for variation in emergence times – if multiple *Omyrus* emerged out of a particular gall species at distinct times

during the year (more than a few months apart), I included an *Ormyrus* for each timeframe. This allows for testing whether these individuals were of the same species and indicative of multiple generations per year. The availability of specimens from multiple temporal ranges across multiple gall species allows for inferences about different hosts the parasite may be using for each generation. Common features between galls used may indicate axes of host specialization. Using these criteria, I found a total of 135 *Ormyrus* reared from 60 unique gall species, across a total of 24 species of oak tree hosts. Of the 135 individuals, 56 are from the COI barcoding project.

### **Library preparation**

From a small subset of the *Ormyrus* included in this study, we extracted DNA using a DNeasy Blood and Tissue kit (Qiagen). For the remaining specimens, I used a CTAB/PCI approach following the methods developed by Chen *et al.* (2010). Next, depending on quantity available from the DNA extraction, I used 25-50 ng of input DNA to do a 2x bead clean using homebrew beads (we prepared beads using the Faircloth & Glenn Serapure protocol V2.2). After the bead clean, I followed the v5.19 KAPA HyperPlus Kit NGS library preparation protocol with minor modifications. I performed enzymatic fragmentation of the bead-cleaned DNA using reagents from a KAPA HyperPlus Kit for 15 minutes to obtain a mean fragment distribution of 300-500 bp. I followed the protocol for end-repair A-tailing, adapter ligation to barcode fragments, post-ligation cleanup, PCR amplification (10 cycles), and post-amplification cleaning. I departed from the default KAPA protocol by using a double-sided size select bead clean on the amplified library to capture only fragments within the 300-500 bp range (SPRIselect User Guide PN B24965AA protocol). First, I performed a 0.65x clean to eliminate small fragments. I then completed a .15x bead clean to eliminate large fragments, and a final 1.3x bead clean to elute

target fragments for the library into TLE buffer. Finally, I quantified each library using a Quant-it High Sensitivity Kit to establish that our libraries were within the correct fragment range

### **Target enrichment and sequencing**

After preparing libraries, I performed target capture and enrichment of UCE loci using the hym-v2 bait set, which contains 2590-targeted UCE loci (Branstetter 2017). To capture UCE loci, I followed the myBaits Hybridization Capture for Targeted NGS manual v5.0. First, I pooled 10-12 individual libraries at equimolar concentrations, with pooled libraries ranging from a total of 150 to 600ng input DNA. I then carried out a hybridization reaction for each pool at 65°C for 24 hours. In this reaction, first the pooled library DNA was denatured, and then exposed to blockers that hybridized to the adapters on the denatured DNA. After the blockers, I added complementary RNA baits bound to streptavidin beads. The baits hybridized to their target UCE loci, after which I used a magnet to capture the bait-target hybrids and wash away the non-target DNA. I resuspended the bead-bound libraries in the kit-provided buffer, followed by workflow B of the enriched library recovery step to remove the captured fragments from the beads. This involved incubating the suspension at 95°C for 5 minutes followed by magnetic pelleting of beads. The supernatant was collected and used for library amplification. Libraries were amplified for 14 cycles using the Q5 Hot Start High-Fidelity kit.

I used qPCR with three primer sets to determine whether a small, random subset of the hybridization reactions successfully enriched the pooled libraries for UCE loci. For each of the three loci, I ran three replicates of the enriched and unenriched (control) pools with 1 ng input DNA for each. For each replicate reaction, I ran a negative control reaction with no DNA. I used SYBR Green master mix to prepare each reaction and ran the plate on a Roche LC480 in the Carver Center for Genomics at the University of Iowa. I calculated the average crossing point

(Cp) across the three replicates for the enriched and unenriched pools, and then compared those averages for each primer to calculate  $\Delta C_p$ . The  $\Delta C_p$  by multiplied 1.78 to account for reaction efficiency (<https://www.ultraconserved.org/#protocols>). For this final value, a change between the enriched and unenriched pools greater than 50-fold indicated successful enrichment.

Additionally, I checked a subset of the hybridized libraries on an Agilent Model 2100 Bioanalyzer to ensure they maintained the correct fragment distribution after PCR. For sequencing, I pooled successfully hybridized pools at equimolar concentrations for paired-end sequencing on a NovaSeq 6000 at the University of Iowa IIHG Genomics Division.

### **Data processing and phylogenetic reconstruction**

The sequencing facility de-multiplexed raw reads: this process separates reads by the unique combination of indices per specimen assigned during the library preparation step. Using these de-multiplexed reads, I completed all bioinformatics step in the PHYLUCE v1.7.1 software package (Faircloth 2016). Following the PHYLUCE pipeline, I first used Fastqc to check read quality, and then used Illumiprocessor for adapter and quality trimming (Faircloth 2013). Next, of the PHYLUCE installed *de-novo* assemblers, I used Spades as it yielded the longest average contig length compared to Velvet and Abyss. I used the hym-v2 probe set (<https://www.ultraconserved.org>) to find and extract UCE loci within out datasets. Then, I used MAFFT to align each UCE locus across all *Ormyrus* individuals from which that locus was recovered, and constructed a 75% complete data matrix, meaning that a UCE locus must be found in at least 75% of all individual specimen to be included in downstream analysis. UCE loci that met this threshold were concatenated for phylogenetic reconstruction. I used the concatenated data matrix to build a phylogeny under the maximum likelihood criterion using IQ-TREE v2.1.3 with 1000 ultrafast bootstraps (Hoang *et al.* 2017), the TVM+F+R8 substitution

model based on ModelFinder (Kalyaanamoorthy *et al.* 2017), the “bnni” flag to reduce the chance of overestimating branch supports, and a Shimodaira-Hasegawa approximate likelihood-rate test (SH-aLRT) (Guindon *et al.* 2010) using 1000 replicates. UCE data from five taxa were used to determine an outgroup based on unpublished data from Dr. Jean-Yves Rasplus. The closest identified genus to *Ormyrus* is *Asparagobius* (Hymenoptera: Pteromalidae: Ormocerinae) based on unpublished results from the Rasplus lab, however this genus is considerably diverged from *Ormyrus*, therefore I used UCE data from *Asparagobius* to determine the most distant ingroup as the outgroup. Based on that, I reconstructed a phylogeny using my *Ormyrus* sequences with two *Ormyrus salmanticus* sequences provided by Dr. Jean-Yves Rasplus.

### **Species delimitation**

I estimated putative species from the UCE data based on a combination of results from COI phylogeny, SODA v1.0.1 (Rabiee and Mirarab 2020), and ecological data. I generated locus trees for SODA using IQ-TREEv2.1.3, which implements ModelFinder (Kalyaanamoorthy *et al.* 2017) for each locus and uses the result to build the respective tree. Species delimitation results from SODA tend to be congruent with other methods (Rabiee and Mirarab 2020). Like other MSC models, it has a false positive rate, although this can be reduced by inclusion of more loci (1752 loci used for this analysis). Additionally, since SODA produces species hypotheses based on gene trees, there were several polyphyletic groupings that may be due to incomplete lineage sorting or based on population structure within a species. Considering this, and the tendency for over splitting, I took a more conservative approach by grouping individuals into one species in cases where the SODA results produced polyphyletic groups in the ML tree. I also looked for congruence with COI groups, as most of the COI groups formed monophyletic clades in the UCE tree.

## RESULTS

### *COI barcoding*

The Bayesian (Figure A1) and ML (Figure A2) approaches produced similar tree topologies for the COI dataset, although the relationships among some of the youngest clades differ between inferred trees. Additionally, in older nodes, the RaxML (Figure A2) tree produced bifurcating events but with very low bootstrap values. Because our goal with the COI data was to detect putative species and not definitively resolve the evolutionary histories of those species, we generally did not focus on older nodes. Instead, we relied on the well-supported terminal groups to distinguish between genetically and ecologically distinct species.

Figure 4 synthesizes the Bayesian tree with the molecular species delimitation results, morphological identification, and ecological data used to determine the number of putative species in our sampling (represented by the corresponding clade numbers to the right of the tree). In most cases, bPTP and ASAP results (Figure A3) were congruent with each other and supported by host/ecological differences. There were four exceptions to this; in three cases (clades 15, 26 and 29; Figure 4), I used the more conservative estimate provided by ASAP, as bPTP can sometimes overestimate genetic differentiation due to geography, or slight population-level differences (Lou *et al.* 2018). Both delimitation methods split clade 32 into two species, however I believe this is unlikely to be the case given the strong similarities between ecological characters. Both groups use two of the same gall species (*Dryocosmus cinereae* and *Andricus quercusostensackenii*) on trees in the red oak section. Each group has one additional gall species different from the other, however both are highly morphologically similar, share phenology (May-June, Weld 1959), and are found on trees in the red oak section. It may be that these are actually different species, but such a conclusion will require additional study.

In total, there are five species that we were unable to determine a morphological identification for, two species that do not match any existing species descriptions, and 17-19 species that match the morphological description of *O. labotus*, with wide genetic divergence in COI (Table 3). Of these nineteen species, one (clade 37; Figure 4) is not associated with oak galls, however, absent of molecular barcodes or host information, *Ormyrus* reared from rose galls generally matches *O. labotus* (Hanson, 1992), and group with the *O. labotus* based on COI.

### *UCEs*

The average locus alignment length was 1007bp with a mean of 108.86 taxa. The 75% criteria (n=102 out of 137) yielded 1752 loci, and the final concatenated data matrix consisted of 2,092,783 columns, 312,466 parsimony-informative sites, 231,123 singleton sites, and 1,549,215 constant sites (see Table A3 for sequencing and loci summary per individual). Using the species delimitation approach described above, there are 34 species present in the UCE dataset with corresponding clade numbers in Figure 5. In several cases, I grouped individuals into one species where SODA split them into multiple polyphyletic species with overlapping gall hosts. In one instance SODA assigned two individuals to one species that may reflect two species (13 and 14; Figure 5). Both clades contain one individual each, however we were only able to confidently assign a morphological identification (*Ormyrus distinctus*) to one (14). This species is reported to occur in California, whereas the unidentified individual in clade 13 was reared from a gall collected in Florida. It may be that the currently reported distribution of *O. distinctus* does not reflect the true distribution – this inconsistency is observed for individuals that morphologically key to *O. nr turio* (see host range description below) – such that the *Ormyrus* wasp in clade 13 may have been correctly grouped into one species with the *O. distinctus* in clade 14.

## **Synthesis**

Host ranges for putative species identified in this study are provided below based on specimens found in clades in one or both phylogenies (Figures 4 and 5). A few species identified in the COI approach are not represented in the UCE study, and vice versa, in which case the relevant tree is indicated. For collection information for each specimen and clade see Table A1 (samples with COI sequenced) and Table A2 (samples with UCEs sequenced).

***Ormyrus distinctus*** is a complex of three putative species. The first species (clades 11 and 12) was reared from two leaf galls in Folsom, CA and Borrego Springs, CA. One was the spiny turban gall, *Cynips douglasii*, on valley oak [*Quercus lobata*], and the second *Andricus bakkeri*, a cup-like leaf gall on scrub oak. Both wasps emerged around the same time (late August to early September). The second species (clades 13 and 14) was also reared in September from two stem galls, *Disholcaspis quercusvirens* and *Disholcaspis simulata* on southern live oak [*Quercus virginiana*] in FL and scrub oak in Borrego Springs, CA, respectively. The third species (clade 14 – *nr distinctus*) was reared from an acorn gall that matches the description of *Andricus costatus* on sonoran scrub oak [*Quercus turbiellii*]. All tree hosts noted here are in the white oak section [*Quercus*], except for southern live oak [*Virentes*]. The three species matching the description of *O. distinctus* are not monophyletic in either phylogenies.

***Ormyrus dryohizoxeni*** (clade 15) was reared from two gall species collected in FL: *Belonocnema treatae*, a woody leaf gall on southern live oak [*Quercus virginiana*], and *Andricus quercusfoliatus*, a cell in elongated bud scales on sand live oak [*Quercus geminata*].

***Ormyrus reticulatus*** (clade 8) was reared from three gall species. One individual overwintered in *Disholcaspis quercusglobulus*, a round bullet stem gall on white oak [*Quercus alba* – white oak section] in Iowa City, IA, emerging in June. A second individual was reared from *Andricus quercuspetiolicola*, a midrib or petiole swelling also on white oak and emerging

in July. The third individual was reared in mid-June out of an unidentified red wrinkled gall found under a southern red oak tree [*Quercus falcata* – red oak section] in Paris, TN.

*Ormyrus thymus* (clade 2) is represented by one individual reared from a stem gall *Bassettia pallida* on sand live oak in Inlet Beach, FL. A single, enigmatic, host is recorded for this species – seeds of *Bucida cucides* (Combretaceae) in Belize, but adult wasps have previously been collected from Florida, California, and Georgia (Hanson 1992). This collection was previously reported in Weinersmith *et al.* (2020).

*Ormyrus nr turio* (clade 6) was reared from *Callirhytis flavipes*, a multi-cell midrib swelling on leaves of bur [*Quercus macrocarpa*] and swamp white oaks [*Quercus bicolor*] in Tiffin, IA and Iowa City, IA, respectively. A third specimen was reared from *Neuroterus saltarius*, a small spangle gall on leaves of bur oak. These wasps were morphologically closest to *O. turio*, a species previously recorded from only one stem gall host, *Bassettia ligni* in California (Hanson 1992).

*Ormyrus venustus* is a complex of four species. The first species (clade 3) is represented by two individuals. One overwintered, emerging in July from *Disholcaspis quercusmamma*, a woody stem gall on swamp white oak [*Quercus bicolor*] in Iowa City, IA. The second individual emerged in October from *Disholcaspis pedunculoides*, an acorn gall on sonoran scrub oak [*Quercus turbienlla*] in Rio Verde, AZ. The second species (clade 4) was reared from two gall hosts in Iowa City, IA: *Acraspis erinacei*, a leaf gall with spines on white oak, and *Amphibolips quercusostensackenii*, a round integral leaf gall with radiating fibers, on post oak [*Quercus stellata*]. The third species (clade 5) is represented by one individual reared from *Andricus quercuspetiolicola* in Austin, TX. The fourth species (clade 7–*nr venustus*) consists of two

individuals, both reared from the same collection of *Xanthothes eburneum*, a leaf gall on gambel oak [*Quercus gambelii*] collected in Show Low, AZ.

**Unidentified *Ormyrus*** are three species that I was unable to identify morphologically, either because they were males (generally females are used for identification) or because physical specimens were available for study and important characters were obscured in photos of those specimens used for genetic work. The first species (clade 6) was reared from two gall hosts: one an unidentified woolly leaf gall on sonoran scrub oak and gray oak [*Quercus grisea*], and the second a leaf gall in the *Atrusca* genus with internal radiating fibers. All galls from which this species of *Ormyrus* was reared were collected in Payson, AZ. The second species (clade 38; UCE tree) is represented by two individuals, both from the same collection of *Neuroterus irregularis*, an early spring leaf swelling on post oak [*Quercus stellata*] in Austin, TX. The third species (clade 16; COI tree) emerged from *Neuroterus saltarius*, a saucer-shaped leaf gall on white oak. The last unidentified clade in the COI tree (clade 23) is represented by a single individual that I was unable to morphologically ID. However, in the UCE tree, this individual groups into a species with five other *Ormyrus* wasps that morphologically key to *O. labotus* (clade 23; Figure 5, see description below).

Two **unknown species of *Ormyrus*** were present in my sampling that did not fit any existing species descriptions (Hanson 1992). The first species (clade 10) was reared from two gall hosts: *Andricus quercusfoliatus*, a bud gall on two species of live oak southern live oak in Hammond, Citrus, and Lithia Springs, FL, and sand live oak in St. Teresa, Water Road, and Ochlocknee, FL; and *Callirhytis quercusclavigera*, a spring stem gall on scarlet oak [*Quercus coccinea*] in Gainesville, FL. All five *Ormyrus* from *A. quercusfoliatus*, overwintered in the gall, with four emerging the following March-April (see Table A2). The second unknown species

(clade 21; COI tree) emerged from two unidentified leaf galls on sand laurel oak [*Quercus hemisphaerica*] in Florida.

*Ormyrus labotus* is a complex of 19-20 putative species, which I refer to by their clade assignments in Figures 4 and 5:

Clade 9 emerged from three gall species: *Andricus quercusstrobianus*, a cluster of stem galls on swamp white oak, *Acraspis villosa*, a spiny leaf gall also on bur oak, and *Andricus quercuspetiolicola*, a midrib/petiole swelling on swamp white oak. All galls were collected in Iowa City, IA, and *Ormyrus* from *Acraspis villosa* overwintered, emerging the following summer after the gall was induced. Wasps in this clade were the only “*Ormyrus labotus*” wasps that did not group in the larger *labotus* clade (Figures 4 and 5).

Clade 18 (COI tree) was reared from *Andricus pattoni*, a woolly midrib cluster on the leaf of a post oak [*Quercus stellata*] in Peducah, KY, and from *Bassettia pallida*, a stem swelling on sand live oak in FL. Given the shared gall host, *Andricus pattoni*, it may be that this clade and clade 23 (UCE tree; Figure 5) represent one species, however no representative from this clade is present in clade 23, therefore additional sequencing is needed to determine whether both clades constitute one species.

Clade 19 was represented by nine individuals. Three were reared from *Andricus dimorphus*, a midrib cluster gall on bur [*Quercus macrocarpa*] and dwarf chinquapin [*Quercus prinoides*] oaks. The individual reared from *Philonix nigra* on the leaf of dwarf chinquapin oak, which shares general morphology with *A. dimorphus*. These four *Ormyrus* individuals underwent diapause – that is, they overwintered in the gall. Additional hosts for this putative species include *Neuroterus noxiosus* and *Andricus chinquapin*, two integral leaf galls on swamp white, and

*Andricus nigricens* and *Neuroterus saltarius*, two detachable leaf galls on swamp white oak collected in Iowa City, IA.

Clade 20 was reared from *Callirhytis pigra*, a midrib leaf swelling on red oak [*Quercus rubra*] in Nashville, TN. Clade 22 was also reared from *Callirhytis pigra*, but on black oak [*Quercus velutina*] in Vestal, New York, and with individuals in both clades emerging a month and half apart from each other, suggesting some degree of temporal isolation. These individuals also do not group together in either phylogeny.

Clade 23 was reared from three gall species: *Andricus pattoni*, *Andricus robustus*, and *Andricus biconicus* on post oak [*Quercus stellata*] in Paducah, KY and St. Louis, MO. All three galls share phenology and occur on the same plant tissue (former two on the midrib, and the last one on the petiole).

Clade 24 (COI tree) was represented by a single individual emerging from an unidentified leaf vein swelling on pin oak [*Quercus palustris*] in Iowa City, IA.

Clade 25 was reared from four gall species. One was an unidentified spangle leaf gall on post oak in St. Louis, MO. There were two other spangle leaf galls that develop in early fall, *Phylloteras volutellae* and *Phylloteras pocoulum*, on swamp white oak leaves in Iowa City, IA. The fourth gall species was *Andricus chinquapin*, a small, spring, integral leaf gall on swamp white oak in Iowa City, IA.

Clade 26 was reared from *Andricus quercuspetiolicola* and *Disholcaspis quercusglobulus* on post oak. The former is a midrib or petiole swelling, while the latter is a bullet stem gall. One other gall host also occurs in this species: *Dryocosmus floridensis*, a bud gall on laurel oak [*Quercus laurifolia*] collected in Gainesville, FL.

Clade 27 was reared from *Andricus quercuslanigera*, a woolly leaf gall, and *Disholcaspis quercusvirens*, a stem gall, on southern live oak in Kyle, TX and Gainesville, FL, respectively.

Clade 28 wasps (COI tree) were reared from *Belonocnema treatae*, a woody spherical leaf gall, on two species of live oak in Houston, Lake Jackson, and Ingleside, TX. Clade 29 was also reared from *B. treatae* and a second gall, *Andricus quercusfoliatus*, both on live oaks in FL and MS. These wasps are separated into two species in Figure 4 because they differ by an average of 10.3% in COI sequence (Table 3), and clade 29 wasps have a distinct striped patterning on their lateral metasoma, earning them the moniker “tiger-morphs” in our working group. However, it may be that both clades are one species with genetic divergent COI haplotypes due to geography. Since no *Ormyrus* from clade 28 have UCE sequences, I cannot conclude whether these clades constitute one or two species.

Clade 30 was reared from three gall species: *Andricus quercuspetiolicola* on bur and swamp white oak, *Callirhytis seminator* (detachable woolly stem gall) on white oak, and *Loxaulus mammula* (branch swelling) on white oak. All galls listed for this species are spring galls collected from Tiffin and Iowa City, IA, with emergences all concentrated in June-July.

Clade 31 includes six gall hosts *Andricus quercussingularis*, *Amphibolips quercusostensackenii*, *Amphibolips quercusrugosa*, *Dryocosmus cinereae*, *Dryocosmus quercuspalustris*, and *Dryocosmus quercusnotha* across 5 species of red oaks in IA. All galls used in this clade are integral leaf galls with similar internal defensive traits (either a rolling cell or radiating fibers internally – see Figure 2).

Clade 32 was reared from 7 gall species, across 5 oak species (2 and 3 in the white and red oak sections, respectively) from IA, IL, PA, WI, and WV. The gall species include: *Andricus quercusfrondosus* (bud gall with bracts), *Melikaiella ostensackenii* (parenchyma thickening),

*Callirhytis quercusgemmaria* (nectar-secreting stem gall), *Callirhytis quercusoperator* (woolly catkin gall), *Disholcaspis quercusmamma* (bullet stem gall), *Acraspis macrocarpae* (textured ellipsoid leaf gall), and unidentified leaf vein swelling. In the UCE tree this clade splits into three paraphyletic groups (32a-32c; Figure 5), with an additional host, *Disholcaspis quercusglobulus*, in clade 32a.

Clade 33 consists of two individuals from the same gall species, *Callirhytis quercuscornigera*, a horned woody stem gall on red and pin oaks in MO.

Clade 34 was reared from six gall species, *Acraspis macrocarpae*, *Acraspis erinacei* (spiny leaf gall), *Amphibolips quercusinanis* (empty oak apple), *Andricus quercusflocci* (woolly leaf gall), *Callirhytis quercusfutilis* (integral leaf blister), and *Philonix nigra* (globular with felt). All galls listed but *C. quercusfutilis* are detachable leaf galls, and on two species of white oak, except for *A. quercusinanis* on red oak [*Quercus rubra*].

Clade 35 was reared from two leaf galls, *Acraspis erinacei* and *Acraspis pezomachoides* (textured ellipsoid) on white oaks in IA, KY, PA, and NY. In the UCE tree, this clade has one individual that groups with the sister clade (36) in the mtCOI tree. This wasp was reared from *Acraspis prinoides* on pin oak. An individual from the same collection is also present in clade 36 of the UCE tree, potentially indicating incomplete lineage sorting or introgression between these two sister clades.

Clade 36 was reared from six galls: *Acraspis macrocarpae* (like *A. pezomachoides* but on bur oak), *Acraspis prinoides*, *Acraspis villosa* (like *A. erinacei* but on bur oak), *Andricus foliaformis* (midrib leaf swelling with bracts on bur oak), *Callirhytis flavipes* (midrib swelling on bur oak), and *Melikaiella ostensackeni* (parenchyma thickening on red oak). Galls were collected in IA and IL.

Clade 39 (UCE tree) was reared from *Melikaiella ostensackeni*, *Callirhytis scitula*, *Dryocosmus quercuspalustris*, and an unidentified raised vein gall on three species of red oaks in Iowa City, IA and Hannibal, MO. All four galls are leaf swellings of variable sizes.

Clade 40 (UCE tree) is represented by one individual collected from *Andricus quercustrobianus* on swamp white oak in Iowa City, IA. Another individual from this collection is found in clade 9.

## DISCUSSION

From the results of my COI barcoding approach, I conclude that the supposed generalist *O. labotus* is in fact a complex of several species, each with a far narrower host range than had previously been reported (Hanson 1992; Noyes 2021). The combined molecular and ecological analysis yielded 35-36 putative species present across all samples, including 17-18 species nested within larger clades of wasps that all ran to *O. labotus* in the Hanson (1992) key (Figures 4 and 5). Two other species matching *O. labotus* and not represented in the COI set were found in the UCE analysis. Additionally, multiple cryptic species were present in two other previously named species, *O. distinctus* and *O. venustus*. Both species, like *O. labotus*, have historical emergence records from a large variety of oak galls without any obvious unifying ecological themes among their hosts that might restrict the types of galls they can attack, like shared gall morphology and plant organ, or a shared gall wasp host genus.

In further support of the conclusion that the COI “species” are real biological units, I also found strong congruence between species groupings in the COI and UCEs phylogenies. In all but three minor instances, individuals that group into one monophyletic species in the COI approach also group together in the UCE approach, indicating that the cryptic species discovered here are in fact reproductively isolated from each other. The lack of well-supported resolution at more basal nodes of the COI gene trees (Figure 4) could indicate that these species are recently diverged. This is also reflected in the UCE tree, which has relatively short branches between species in the larger *O. labotus* clade (Figure 5, marked by arrow).

Lineage divergence can occur due to reproductive isolation evolving when populations are geographically isolated or due to differential selection in the context of different ecologies, or both (Funk 1998, Powell *et al.* 2013, Vidal *et al.* 2019). Since most of our collection efforts were

biased toward the Midwestern US, there are many clades with *Ormyrus* individuals reared from different galls collected in close geographic proximity to one another. Moreover, there are several clades where *Ormyrus* collected from distant locales form one species (for examples, see clades 3, 8, 15, 29, 35, 40; Figure 5), indicating that gene flow is not strongly restricted by distance, but rather by ecology (host associations). This strengthens the case that these are reproductively isolated species and is an indication that the associations that these wasps have with their hosts may have been important in their diversification.

### ***Host ranges and host shifts***

Given that specialist parasites require host-specific traits to ensure success, a host shift (use of a new or alternative host) can introduce divergent natural selection upon traits that improve fitness on this new host plant (Dres and Mallet 2002, Vertacnik 2017). This can lead to the evolution of reproductive isolation between individuals using the alternative host and conspecifics using the ancestral host, a crucial step along the speciation continuum (Diehl and Bush 1984, Powell *et al.* 2013). Thus, host specialization and host shifting underpin important mechanisms of diversification within phytophagous insects (Matsubayashi *et al.* 2010, Forbes *et al.* 2017, examples: pea aphids, Via and Skillman 2000; cotton leafhoppers, Antwi *et al.* 2015). Because plant-feeding insects are themselves often host to rich communities of parasites, diversification in plant-feeding insects can create new niches for their parasites to exploit and in which to adapt, resulting in stepwise diversification (Stireman *et al.* 2005, Stireman *et al.* 2006, Abrahamson and Blair 2008, Feder and Forbes 2010).

Oak gall wasp species richness is positively associated with oak tree host diversity (Cornell and Washburn 1979). For example, there are ~40 species of oak in the Western Palearctic with 140 associated gall wasp species. In comparison, the Nearctic has ~300 oak

species with ~700 species of gall wasps (Stone *et al.* 2002). In the Nearctic, climatic and soil variation promoted increased oak diversification (Hipp *et al.* 2018), in turn, expanding the number of ecological niches available for oak gall wasps. For this reason, authors have hypothesized that there remains vast undiscovered diversity in oak-gall associated natural enemies in the Nearctic oak gall system (Stone *et al.* 2002, Lobato-Vila and Pujade-Villar 2017). This prediction is borne out by our findings – previously it was thought that there were just 16 Nearctic *Ormyrus* species (Hanson 1992), however we find many more species present from a relatively small sampling of gall wasp species, including 18-20 species (Figures 4 and 5) among the single *O. labotus* species. Because our sampling of North American *Ormyrus* was haphazard in its approach, omitting many gall wasp hosts and under sampling some geographic regions, many additional species may yet remain to be discovered. Despite this, each *Ormyrus* species shows host-associated structure in both COI and UCEs phylogenies, and *Ormyrus* speciation events seem universally correlated with changes in host association.

Most *Ormyrus* species in our study (26 out of 34 clades in Figure 5) are apparently restricted to gall hosts occurring on trees species within only one tree section, and more than half of the *Ormyrus* species were reared from just three or fewer gall species. Further, in several clades that had larger host ranges relative to their congeners (four to seven host associations compared to three or fewer), I find examples where common adaptive axes might define the types of galls that they can attack. For example, in one *Ormyrus* species (clade 19; Figure 5), while six gall species are used across three different species of white oaks, five of these are midrib leaf clusters (the sixth is a small leaf gall). Further, three of the host galler species for clade 19, *Andricus nigricens*, *Andricus dimorphus*, and *Philonix nigra*, greatly overlap in phenology, thus potentially acting as “temporal islands” for that this species of *Ormyrus*.

While commonalities can be identified between host traits across the host range of any given *Ormyrus* species, an equally interesting question is whether *Ormyrus* shifts to new hosts are correlated with changes in particular niche dimensions, while other are more likely to be conserved (e.g., host galler species, gall morphology or phenology, or tree species). The UCE phylogeny allows for a comparison of host ecologies between sister taxa, however I caution that the phylogeny presented in this study (Figure 5) represents just one possible evolutionary history of *Ormyrus*. Future analyses, including a comparison between concatenated vs coalescent tree building approaches, different partitioning schemes, and tests for biological processes that can result in incongruence between phylogenies produced by different methods (like rapid radiations, incomplete lineage sorting, introgression, etc.) will be important to resolve a best-supported tree. Nevertheless, well-supported relationships between closely related *Ormyrus* in Figure 5 may provide clues about how these species diversified.

Across older nodes in the UCE tree (Figure 5), corresponding mainly to species other than the *O. labotus* complex, it appears that *Ormyrus* rarely shifted onto galls occurring in different tree sections, except for the occasional shift between white and live oaks. This latter may constitute a relatively minor change in tree habitat compared to shifts between the white/live and red oak section, given that live oaks [*Virentes*] are closely related section to white oaks [*Quercus*] (Hipp *et al.* 2018).

Host shifts among the *Ormyrus labotus* complex are not strongly associated with any one particular niche dimension. Across this large group of many species, I find examples of shifts between tree sections, tree species, gall morphologies, and cynipid taxa. Between a few closely related clades, several aspects of host ecology are conserved with shifts in just one niche dimension (at least among those measured in this study); for example, clades 27 and 29 (Figure

5) reflect shifts in the morphology of galls attacked, while the tree section, and to some extent tree hosts, of the gall hosts remain unchanged. Conversely, there are examples of shifts along multiple host dimensions; for example, clade 23 and 40 (Figure 5) reflect differences in both tree section and morphology of hosts. Galls in clade 23 are all textured leaf clusters on post oaks [*Quercus stellata* – white oak section], whereas gall hosts of clade 40 are all integral swellings on three species of red oaks. This suggests a potential role for tree chemistry and gall morphology in leading to, or maintaining, reproductive isolation between these two sister clades. Overall, patterns of host shifting within the larger *O. labotus* clade resist any single generalization.

Though this study is the largest study of *Ormyrus* diversity since Hanson (1992), future studies should sample *Ormyrus* more heavily across the US and beyond – having additional representatives from each putative species will further improve accuracy in determining host ranges. With the addition of more individuals, or additional undetected species, and considering other host dimensions, we may find that one type of host-shift occurs more commonly than others. Alternatively, a more complete sampling might support my current hypothesis that there is no one consistent pattern or single host dimension that is paramount to how *Ormyrus* have diversified. One explanation for this is that diversification is driven by ecological opportunity, such that the fitness value of an alternative niche, for example one that might be temporarily free from conspecific competition or predators, is sufficient to drive specialization on a newly encountered/available host. Individuals can be exposed to new potential hosts, for example via oviposition mistakes (Larsson and Ekblom, 1995), and successful colonization can occur if successive generations survive and mate on the new host. Additionally, if individuals encounter a new host that resembles their existing host in some trait (for example, but not restricted to, gall

morphology), then they may be pre-adapted to survive on the new host. Because different *Ormyrus* species specialize on a unique suite of many niche dimensions, and more than one axes may be involved in the evolution of restricted host ranges, variable trends emerge in how they have diversified across potential gall hosts.

### ***Life history evolution***

The co-occurrence of different parasitic species on the same host is likely involved in driving the evolution of variable life strategies, which allow for small-scale resource partitioning (Harvey *et al.* 2014). For many of the putative species discovered here, there is little overlap in gall host ranges; 38 out of 60 gall wasp host species in this study are used by only one specialist species of *Ormyrus*. However, there are a few notable instances where multiple species of *Ormyrus* share one or a few gall hosts. For example, two species that match the description of *O. labotus* (clade 31 and 40; Figure 5) both use the gall *Dryocosmus quercuspalustris*, but wasps using that specific gall host in clade 31 emerged in June, while the clade 40 wasp emerged in November. Similarly, two other putative species that key to *O. labotus* (clade 30 and 26; Figure 5) use *Andricus quercuspetiolicola*, however clade 30 wasps using this gall host emerged in the summer (June-July), whereas those in clade 26 emerged in September. These two examples of *Ormyrus* sharing gall hosts indicate the importance of temporal isolation, a form of reproductive isolation which can evolve as a niche-partitioning strategy in parasitoid communities (Harvey *et al.* 2014). Interestingly, *A. quercuspetiolicola* is also used as a host by *O. reticulatus* (clade 8; Figure), with emergence times that overlap with the *Ormyrus* in clade 30. Similarly, *Melikaiella ostensackeni* is exploited as a gall host by three *Ormyrus* species (clade 40, 32c, 36; Figure 5) with overlapping emergence times. This could be an example of three species using the same host or could indicate specialization on different resources in the gall – i.e., attack of an inquiline

or other parasitoid larvae as opposed to gall wasp larvae. That *Ormyrus* may sometimes act as hyperparasitoids (Fig 1, panel C), a possibility that has been suggested by Hanson (1992) and noted in a careful rearing study in which both an *Ormyrus* sp. and cynipid gall wasps emerged from one *Andricus singularis* gall (Brookfield 1972). *Ormyrus* may thus be a rewarding genus for future studies of the maintenance of reproductive isolation between closely related taxa in shared ecological niches, as well how multi-trophic communities of natural enemies are structured and regulated by host resources.

In addition to variable resource-partitioning strategies, I find evidence for differences in two related life history traits: voltinism (the number of generations per year) and within-species variation in diapause. Emergence patterns and host ranges in some clades suggest bivoltine life histories. For example, in clade 34 two gall hosts, *C. quercusfutilis* and *A. quercusinanis*, share phenology (spring/early summer galls) relative to the other galls used by this species (*Acraspis erinacei*, *Philonix nigra*, and *Andricus quercusflocci* – all spines/fuzzy leaf galls on white oaks in the fall). For this putative species, as well as others demonstrating bimodal host ranges in terms of gall phenology, additional rearing data is needed to conclude how many generations occur throughout the year.

In several putative species one or a few individuals from the same collection underwent an overwintering diapause while others did not. For instance, clade 26 (Figure 5) has 2 individuals from same collection (*Andricus quercuslanigera* on *Quercus virginiana* in Kyle, TX), one of which emerged in November of the same year the gall was induced, whereas the other individual overwintered in the gall, emerging the following April. Phenotypic plasticity in diapause and bet-hedging strategies have been observed within single species in the face of both predictable and unpredictable environmental variation (Le Lan *et al.* 2021). Such plastic

responses in emergence times may create population-level differences in voltinism as well (He *et al.* 2010). Aphid parasitoids have been shown to produce a mix of diapausing and non-diapausing phenotypes among their offspring if environmental or host-associated cues are only partially predictable (Tougeron *et al.* 2019). Similarly, several oak galling wasps demonstrate bet-hedging, wherein some portion of their progeny undergo prolonged diapause. Some parasitoids may also undergo diapause to remain synchronized with their host (Doutt *et al.* 1976, Corley *et al.* 2004), and such life history strategies could be relevant for successful biocontrol efforts (Ferracini *et al.* 2014).

### ***The importance of hidden specialists***

Why does it matter that cryptic clades of small parasitic insects might often be lumped together as a false generalist? One evident reason is that it obscures how species interact with their hosts, confounding, for example, biocontrol strategies. Parasitic insects include many forest and agricultural pests, and their parasitoids are used as biocontrol agents (Waage *et al.* 1982). Prior to this study, if *O. labotus* was considered as a control for invasive galling wasp, it might fail given the actual restricted host ranges of each putative species. Beyond economic reasons, host range data is commonly used to ask synthetic questions about the relationship between host specialization and diversification (Winkler and Mitter 2008, Armbruster and Muchhala 2009, Novotny *et al.* 2012; Ebel *et al.* 2015; Forbes *et al.* 2017). Conclusions arising from such work are highly dependent on both correct species delimitation and the completeness of host range investigation.

Incomplete understanding of host ranges might also hinder our ability to study actual generalists, when they do occur. There are good arguments for why some parasitic species may settle on a generalist approach (e.g., Futuyma 1988). Compared to occupying a narrow

ecological distribution, a broader niche allows for diet mixing between different life stages and a nutritionally balanced diet (Bernays and Minkenberg 1997). Generalism also confers the ability to bet hedge against changing environments by maintaining access to alternative hosts (Egan and Funk 2006). However, a higher standard of evidence is needed to call species a generalist, that is, molecular studies should corroborate host-association and natural history records across the geographical range of a putative generalist (Loxdale 2016). The work presented here and by others (Table 1) suggest that abundant cryptic diversity may frequently be grouped into a single apparent specialist. This trend is relevant for how we select insect systems to infer the ecological conditions and genetic/morphological tools that enable generalist lifestyles. Functional studies, behavioral assays, morphometric analyses, and transcriptomic work with the potential to elucidate the processes that result in different feeding strategies require *true* generalists to compare against their closely related specialist counterparts.

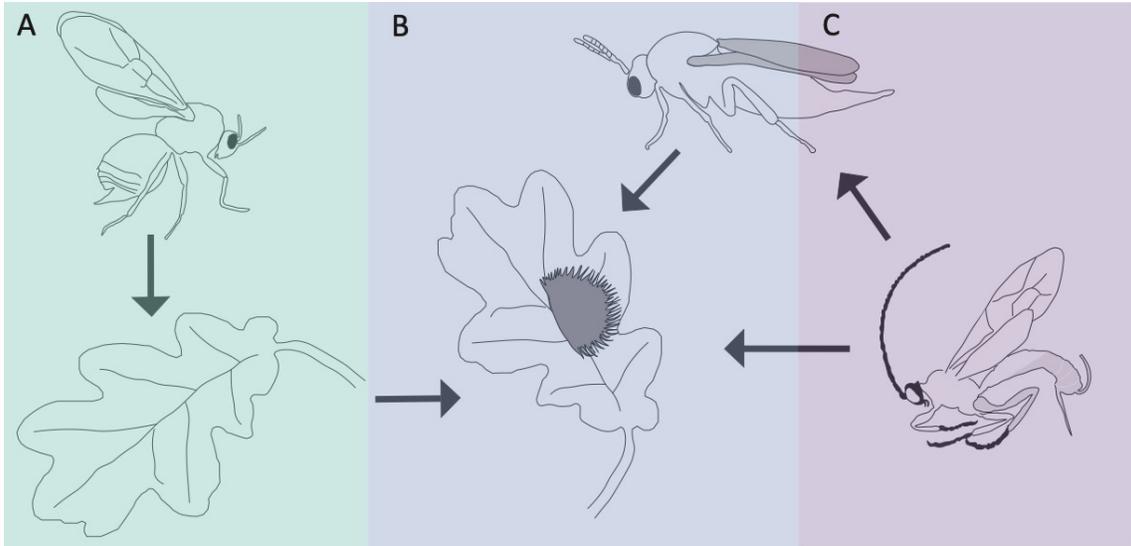
Finally, in this current conservation crisis, work to refine our understanding of biodiversity has become even more critical. Across the tree of life, different species differ in how they interact with their environments, such that a species that is lost from a system cannot be easily substituted with even a closely related species. This necessitates concerted efforts towards both documenting existing diversity and understanding how it evolves. Integrative taxonomic efforts regularly find new species even within large taxa (ex: Fennessy *et al.* 2016), though, some of the most species-rich groups are also among the most understudied. In particular, parasitic wasps are likely the most species-rich group in class Insecta (Noyes 2012, Forbes *et al.* 2018), but are also among the most resistant to taxonomic classification due to the “taxonomic impediment” of too few taxonomists and too many species (Taylor 1983, Giangrande 2003). This problem is further exacerbated by an increasing lack of funding for taxonomic work, such

that there are fewer trained experts to investigate hyper-diverse systems. Thus, funding for integrated taxonomic efforts should be prioritized in the biological sciences.

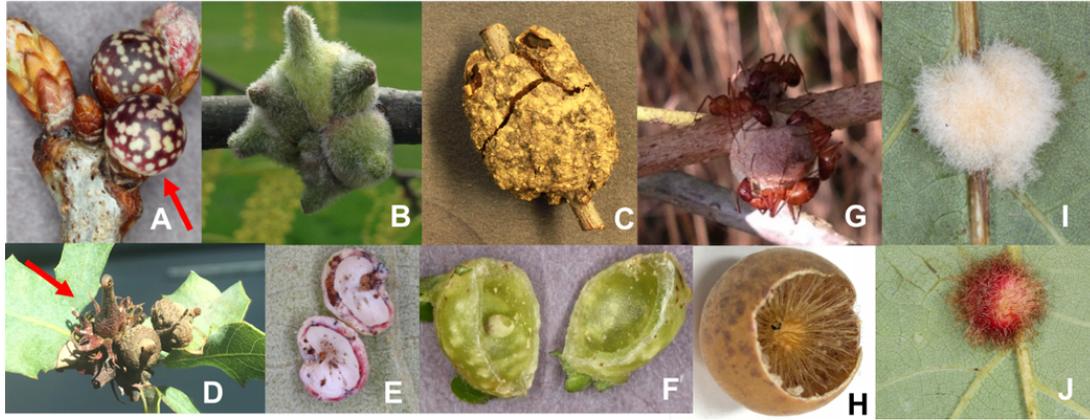
### ***Conclusion***

Integrative approaches as used here allow for estimating species richness within a sample (e.g via COI barcoding and ecological information) and for identifying the relevance of different niche dimensions in the evolutionary history of extant taxa (UCEs/genomic sampling methods). The combination of two molecular approaches, ecological data, and morphological information yielded a total of 35-36 putative species of *Ormyrus*, with 19-20 species of *Ormyrus labotus*. This study, alongside several others (Table 1), demonstrates a recurring pattern of detecting an array of host-specialized diversity within a supposed generalist. Such studies also highlight that, for various considerations (from economic to basic science), we must work to increase accuracy in detection of reproductively isolated species and description of their respective host ranges and ecologies.

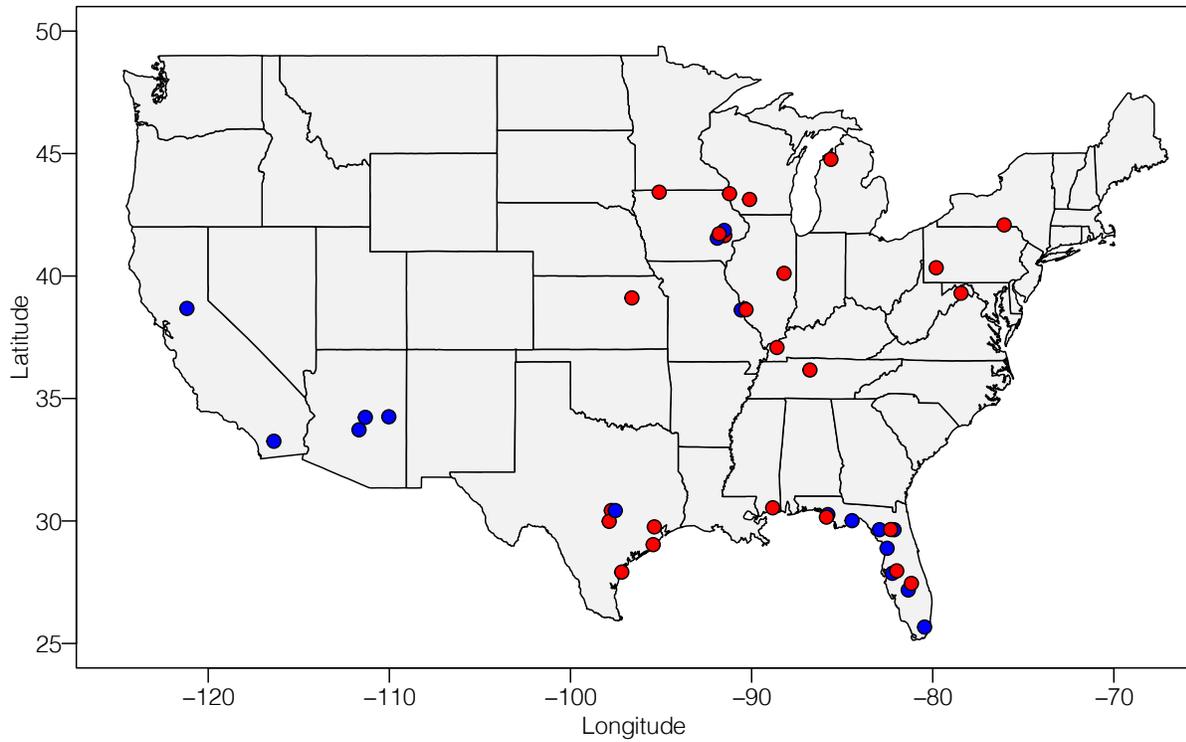
**Figure 1:** Simplified web of interactions in the oak gall system. Panel A shows interactions at the first insect trophic level, between host plant (specific oak species and tree organ) and herbivore (cynipid wasp), resulting in the production of a gall. In Panel B, the gall is subsequently attacked by parasitoids (lethal to host), inquilines (feed on gall tissue), and other natural enemies, constituting interactions at a second trophic level. Panel C indicates interactions at a third trophic level, where hyperparasitoids use the gall to target larvae of wasps shown in A and B.



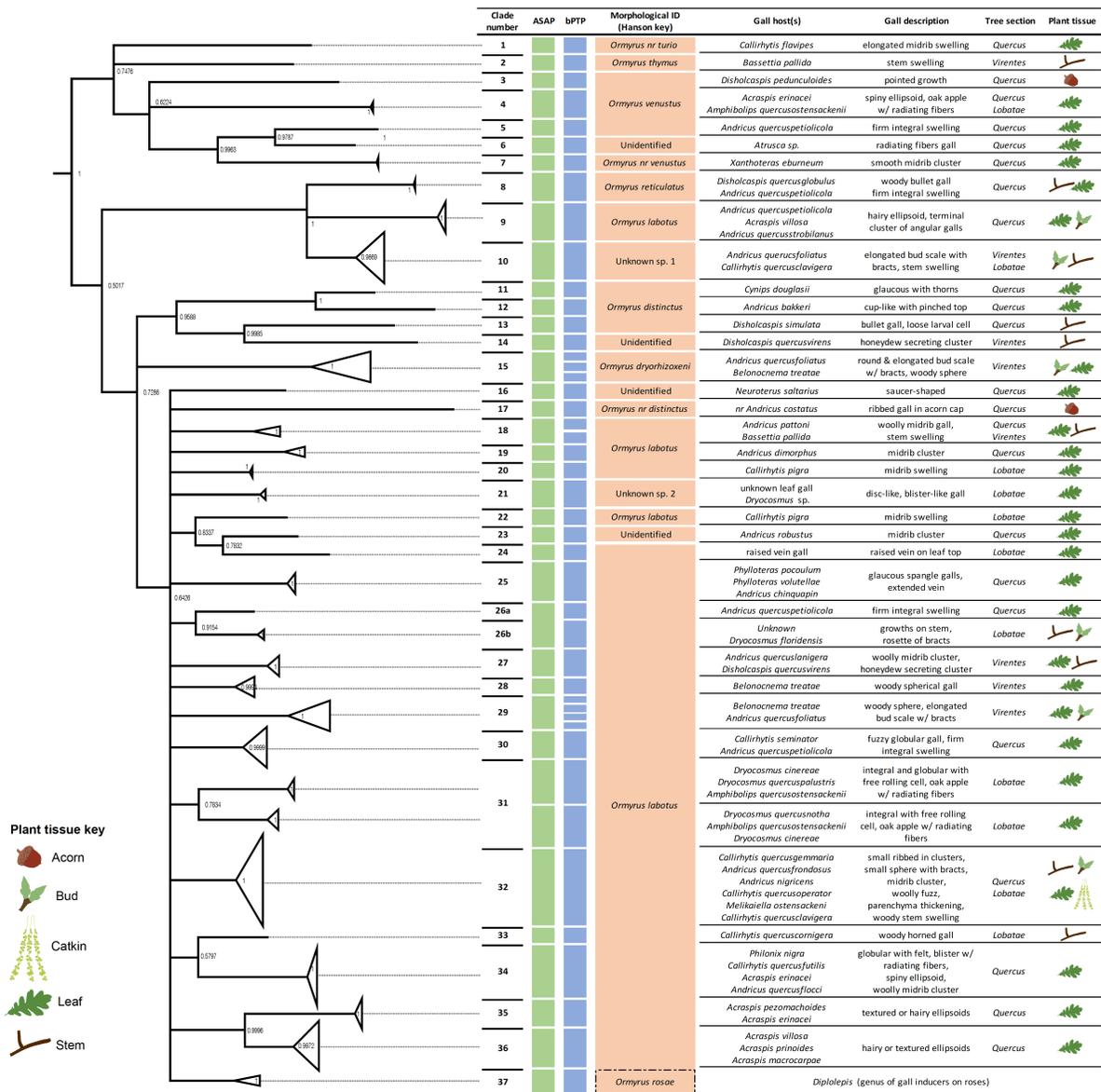
**Figure 2:** A glimpse of oak gall morphological diversity. Galls occur on many tissues, such as buds, stems, branches, acorns, and leaves (A-E), and display features such as free internal space (F), nectar secretion (G), radiating fibers, or a fuzzy texture surrounding the larval chamber(s) (I, J). Figure adapted from Anna KG Ward.



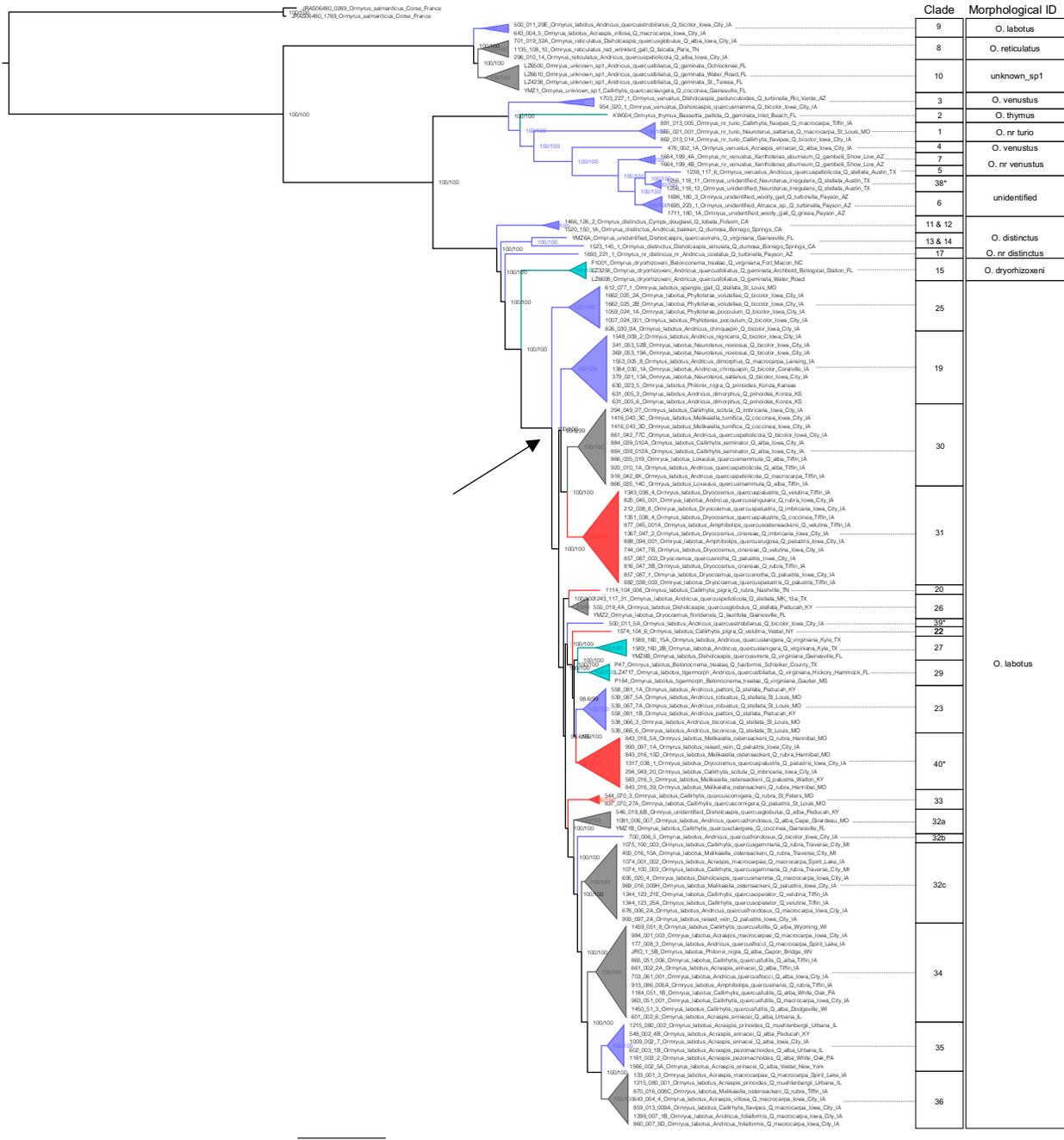
**Figure 3:** Map of all unique collection regions represented in the COI study. Each dot represents one city or locale from which at least one collection yielded an *Ormyrus* used in this study. Red dots indicate unique regions from which a gall collection resulted in a wasp that keyed out to *Ormyrus labotus*. Blue dots indicate unique geographic regions from which a gall collection reared an *Ormyrus* species other than *O. labotus* based on morphology. See Appendix Table A1 for full collection information. *Ormyrus* sequenced for UCEs were reared from galls collected in similar locales, with the addition of a few sites in KY, MO, and PA (Appendix Table A2).



**Figure 4:** Combination of molecular (COI), morphological, and ecological data used to develop species hypotheses for *Ormyrus* specimens included in the phylogeny. The clade numbers represent putative species based on the three datasets together. To the furthest left is the COI Bayesian tree, with nodes collapsed based on our species hypotheses (one exception to this is clade 31, which is collapsed based on species delimitation results). The two columns to right of the clade number indicate species assignments based on the ASAP and bPTP delimitation approaches, respectively. The next column shows the morphological identification of individuals within the corresponding clade based on existing species descriptions. Columns to the right of the morphological ID summarize ecological data for each clade and indicate traits of the gall host(s).



**Figure 5:** UCE maximum likelihood tree collapsed by putative species, with clade numbers corresponding to groupings in the COI tree (Figure 4). Clade numbers with asterisks indicate putative species that were not represented in the COI tree. Clade colors are based on tree section; purple indicates that all *Ormyrus* in that respective clade were reared from gall hosts that all galls collected on an oak tree in the white oak section [*Quercus*], blue indicates live oaks [*Virentes* – closely related section to *Quercus*], red indicates red oak section [*Labotae*], and gray indicates galls collected on trees in the more than one oak section. The black arrow indicates the ancestral node of the species in this study that match the morphological description of *O. labotus* (in addition to clade 9).



**Table 1:** Summary of a few previous studies that used an integrative approach to investigated putative generalists and found the presence of several specialist lineages with restricted host-ranges relative to the original numbers of host associations.

Reference	System	Family	Original number of hosts	Number of cryptic species/lineages	Number of hosts attacked by each newly discovered cryptic species	Hosts
Hambäck <i>et al.</i> , 2013	<i>Asecodes lucens</i>	Hymenoptera: Braconidae	5	3-5	1-3	Parasitoid of chrysomelid beetles
Dickey <i>et al.</i> , 2015	<i>Scirtothrips dorsalis</i>	Thysanoptera: Thripidae	>100	9	1-20	Parasite of plants
Forbes <i>et al.</i> , 2009	<i>Diachasma alloeum</i>	Hymenoptera: Braconidae	3	3	1	Apple maggot complex
Ward <i>et al.</i> , 2020	<i>Synergus oneratus</i>	Hymenoptera: Cynipidae	11	5	2-4	Inquilines of oak galls
Wood, 1980	<i>Enchonopa binotata</i>	Hemiptera: Membraciade	16	11	1-5	Treehoppers on various plants
Knee <i>et al.</i> , 2012	<i>Uroobovella nova</i>	Mesostigmata: Urodinychidae	14	5	1-9	Mites on burying beetles
Mills and Cook, 2014	<i>Apiomorpha minor</i>	Hemiptera: Eriococcidae	18	9	1-7	Gall inducers on Eucalyptus
Hebert <i>et al.</i> , 2004	<i>Astraptes fulgerator</i>	Lepidoptera: Hesperiidae	>38	10	1-11	Caterpillars feeding on leaves
Leppänen, <i>et al.</i> , 2014	<i>Pontania viminalis</i>	Hymenoptera: Tenthredinidae	9	14-15	1-3	Gall inducers on Salix
Simth <i>et al.</i> , 2006	Belvosia Woodley07	Hymenoptera: Tachinidae	25	8	1-8	Parasitic flies on caterpillars
Simth <i>et al.</i> , 2006	Belvosia Woodley04	Hymenoptera: Tachinidae	7	4	3-4	Parasitic flies on caterpillars
Simth <i>et al.</i> , 2006	Belvosia Woodley03	Hymenoptera: Tachinidae	6	3	1-4	Parasitic flies on caterpillars
Condon <i>et al.</i> , 2014	Bellopius morph9	Hymenoptera: Braconidae	5	5	1-2	Parasitoids of tephritid flies

**Table 2:** *Ormyrus labotus* host associations. An asterisk next to a name in the first column indicates a gall species from which we also reared an *Ormyrus labotus* included in this study.

**Hanson 1992:**

*Acraspis alaria*  
*Acraspis erinacei*\*  
*Acraspis gemula*  
*Acraspis macrocarpae*\*  
*Acraspis pezomachoides*\*  
*Acraspis prinoides*\*  
*Acraspis quercushirta*  
*Acraspis villosa*\*  
*Amphibolips gainesi*  
*Amphibolips nubilipennis*  
*Amphibolips quercusoelebs*  
*Amphibolips quercusspongifica*  
*Amphibolips tinctoriae*  
*Andricus cinnamomeus*  
*Andricus coronus*  
*Andricus fullawayi*  
*Andricus ignotus*  
*Andricus pattoni*\*  
*Andricus quercusflocci*\*  
*Andricus quercusfoliatus*\*  
*Andricus quercuslanigera*\*  
*Andricus quercusostensackenii*\*  
*Andricus quercuspetiolicola*\*  
*Andricus quercussingularis*\*  
*Andricus tecturnarum*  
*Belonocnema treatae*\*  
*Callirhytis clavula*  
*Callirhytis cornigera*\*  
*Callirhytis elongata*  
*Callirhytis favosa*  
*Callirhytis flavipes*\*  
*Callirhytis gallaestriatae*  
*Callirhytis infuscata*  
*Callirhytis lanata*  
*Callirhytis pedunculata*  
*Callirhytis pulchra*  
*Callirhytis quercusfutilis*\*  
*Callirhytis quercusgemmaria*\*

**New in this study:**

*Andricus biconicus*  
*Andricus chinquapin*  
*Andricus dimorphus*  
*Andricus nigricens*  
*Andricus robustus*  
*Andricus quercusfrondosus*  
*Andricus quercusstrobilanus*  
*Bassettia pallida*  
*Callirhytis pigra*  
*Callirhytis quercusclavigera*  
*Disholcaspis quercusvirens*  
*Dryocosmus cinereae*  
*Phylloteris pocoulum*  
*Phylloteris volutellae*

## Table 2 - continued

*Callirhytis quercusmedullae*  
*Melikaiella ostensackeni*\*  
*Callirhytis quercusoperator*\*  
*Callirhytis quercuspunctata*  
*Callirhytis quercusscitula*  
*Callirhytis quercussimilis*  
*Callirhytis seminator*\*  
*Callirhytis tubicola*  
*Callirhytis tumifica*\*  
*Callirhytis vaccinii*  
*Disholcaspis cinerosa*  
*Disholcaspis spongiosa*  
*Dryocosmus kuriphilus*  
*Dryocosmus quercusnotha*\*  
*Dryocosmus quercuspalustris*\*  
*Loxaulus humilis*  
*Loxaulus quercusmammula*\*  
*Neuroterus exiguus*  
*Neuroterus floccosus*  
*Neuroterus quercusbatatus*  
*Neuroterus quercusirregularis*  
*Neuroterus quercusverrucarum*  
*Philonix fulvicollis*  
*Philonix nigra*\*  
*Xanthoteras eburneum*\*  
*Xanthoteras politum*  
*Xanthoteras quercusforticorne*

**Table 3:** Average percentage divergence in mtCOI sequences between clades fitting the morphological description of *Omyrus labotus*.

	clade 9	clade 18	clade 19	clade 20	clade 22	clade 24	clade 25	clade 26	clade 27	clade 28	clade 29	clade 30	clade 31	clade 32	clade 33	clade 34	clade 35
clade 9																	
clade 18	13.34																
clade 19	14.71	10.02															
clade 20	13.53	7.35	9.63														
clade 22	13.91	9.23	8.55	9.38													
clade 24	14.6	9.88	11.8	9.41	8.97												
clade 25	13.95	8.45	9.63	8.27	8.22	9.84											
clade 26	13.48	8.08	8.39	6.45	9.08	10.02	8.55										
clade 27	13.84	9.2	11.23	7.84	7.67	8.22	9.37	9.14									
clade 28	14.81	8.54	11.81	7.15	10.27	9.8	9.22	6.59	8.75								
clade 29	15.78	10.52	12.77	10.69	11.49	11.63	10.05	9.94	11.19	10.27							
clade 30	14.61	8.56	8.59	8.19	7.84	10.86	8.56	8.04	9.04	7.58	10.74						
clade 31	14.53	8.93	8.73	8.17	8.98	10.42	8.65	8.27	8.69	7.94	11.55	8					
clade 32	14.38	7.76	9.54	6.65	9.14	10.84	9.61	7.15	8.33	7.22	10.39	8.03	8.44				
clade 33	13.72	8.34	8	7.11	7.41	8.8	7.87	7	8.62	8.47	10.38	7.68	7.54	6.73			
clade 34	14.92	11.3	9.87	8.41	8.13	10.3	10.32	8.79	9.77	8.57	13.01	8.17	8.51	9.27	6.78		
clade 35	15.61	9.54	10.92	10.28	12	12.39	9.88	10.13	11.53	10.82	12.59	10.48	10.54	10.11	10.66	12.05	
clade 36	15.46	9.91	11.59	10.33	9.73	10.67	10.24	9.82	10.26	10.36	12.75	9.07	10.9	9.87	10.29	11.23	8.39

## REFERENCES

- Abrahamson, W. G., Hunter, M. D., Melika, G., & Price, P. W. (2003). Cynipid gall-wasp communities correlate with oak chemistry. *J. Chem. Ecol.*, *29*, 209–223.
- Abrahamson, W. G., Melika, G., Scrafford, R., & Csóka, G. (1998). Gall-inducing insects provide insights into plant systematic relationships. *American Journal of Botany*, *85*(8), 1159–1165.
- Abrahamson, W. G., Blair, C. P. (2008). Sequential radiation through host-race formation: Herbivore diversity leads to diversity in natural enemies. In *Specialization, Speciation and Radiation: The Evolutionary Biology of Herbivorous Insects*; Tilmon, K.J., Ed.; University of California Press: Berkeley, CA, USA, pp. 188–202.
- Ács, Z., R. J. Challis, P. Bihari, M. Blaxter, A. Hayward, G. Melika, G. Csóka, Z. Péntzes, J. Pujade-Villar, J. L. Nieves-Aldrey. (2010). Phylogeny and DNA barcoding of inquiline oak gall wasps (Hymenoptera: Cynipidae) of the Western Palaearctic. *Mol. Phylogenet. Evol.*, *55*, 210–225.
- Agrawal AA, Conner JK, Rasmann S. (2010). Trade-offs and negative correlations in evolutionary ecology. In *Evolution After Darwin: the First 150 Years*. Edited by: Levinton J, Futuyma D, Eanes W, Bell M. Sunderland: Sinauer Associates, 243-268.
- Antwi, J. B., Sword, G. A., & Medina, R. F. (2015). Host-associated differentiation in a highly polyphagous, sexually reproducing insect herbivore. *Ecology and evolution*, *5*(13), 2533–2543.
- Armbruster, W. S., & Muchhala, N. (2009). Associations between floral specialization and species diversity: cause, effect, or correlation? *Evolutionary Ecology*, *23*(1), 159-179.
- Askew, R. R., Melika, G., Pujade-Villar, J., Schönrogge, K., Stone, G. N., & Nieves-Aldrey, J. L. (2013). Catalogue of parasitoids and inquilines in cynipid oak galls in the West Palaearctic. *Zootaxa*, *3643*(1), 1–133.
- Askew R. R, Plantard O, Gómez JF, Hernández-Nieves M, Nieves-Aldrey JL. (2006). Catalogue of parasitoids and inquilines in galls of Aylacini, Diplolepidini and Pediastidini (Hym., Cynipidae) in the West Palaearctic. *Zootaxa*, *1301*, 1–60.
- Bailey, R., Schönrogge, K., Cook, J. M., Melika, G., Csóka, G., Thuróczy, C., & Stone, G. N. (2009). Host Niches and Defensive Extended Phenotypes Structure Parasitoid Wasp Communities. *PLoS Biology*, *7*(8), e1000179.
- Bejerano, G. (2004). Ultraconserved Elements in the Human Genome. *Science*, *304*(5675), 1321–1325.

- Berenbaum, M. R., & Feeny, P. P. (2008). Chemical Mediation of Host-Plant Specialization: The Papilionid Paradigm. In *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects*; Tilmon, K.J., Ed.; University of California Press: Berkeley, CA, USA, pp. 2–19.
- Bernays, E.A. (1997). Feeding by caterpillars is dangerous. *Ecological Entomology*, *22*, 121–123.
- Bernays, E. A., Bright, K. L., Gonzalez, N., & Angel, J. (1994). Dietary Mixing in a Generalist Herbivore: Tests of Two Hypotheses. *Ecology*, *75*(7), 1997–2006.
- Bernays E.A & Minkenberg O. (1997). Insect herbivores: different reasons for being a generalist. *Ecology*, *78*, 1157–1169.
- Bernays, E. A. (2001). Neural limitations in phytophagous insects: Implications For Diet Breadth And Evolution Of Host Affiliation. *Annual Review of Entomology*, *46*(1), 703–727.
- Branstetter, M. G., B. N. Danforth, J. P. Pitts, B. C. Faircloth, P. S. Ward, M. L. Buffington, M. W. Gates, R. R. Kula, and S. G. Brady. (2017). Phylogenomic insights into the evolution of stinging wasps and the origins of ants and bees. *Curr. Biol.*, *27*, 1019–1025.
- Brookfield, J.F. (1972). The inhabitants (Hymenoptera: Cynipidae, Chalcidoidea) of the cynipidous galls of *Quercus borealis* in Nova Scotia. *Can. Entomol.* *104*, 1123–1133.
- Chen, H., Rangasamy, M., Tan, S. Y., Wang, H., & Siegfried, B. D. (2010). Evaluation of five methods for total DNA extraction from Western Corn Rootworm Beetles. *PLoS one*, *5*, e11963.
- Condon, M. A., Scheffer, S. J., Lewis, M. L., Wharton, R., Adams, D. C., & Forbes, A. A. (2014). Lethal Interactions Between Parasites and Prey Increase Niche Diversity in a Tropical Community. *Science*, *343*(6176), 1240–1244.
- Corley, J. C., Capurro, A. F., & Bernstein, C. (2004). Prolonged diapause and the stability of host–parasitoid interactions. *Theoretical Population Biology*, *65*(2), 193–203.
- Cornell H. V., Washburn J. O. (1979). Evolution of the richness area correlation for cynipid gall wasps on oak trees. A comparison of two geographic areas. *Evolution*, *33*, 257–74.
- Courtney, S. P. (1981). Coevolution of pierid butterflies and their cruciferous foodplants. III. *Anthocharis cardamines* (L.). Survival, development and oviposition on different hostplants. *Oecologia*, *51*, 91–96.
- Craig, T. P., Itami, J. K., & Price, P. W. (1989). A Strong Relationship Between Oviposition Preference and Larval Performance in a Shoot-Galling Sawfly. *Ecology*, *70*(6), 1691–1699.

- Craig, T. P., & Itami, J. K. (2008). Evolution of preference and performance relationships. In *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects* (pp. 20-28). University of California Press.
- Darriba, D., Taboada, G. L., Doallo, R., & Posada, D. (2012). jModelTest 2: more models, new heuristics and parallel computing. *Nature Methods*, 9(8), 772–772.
- Dickey, A. M., Kumar, V., Hoddle, M. S., Funderburk, J. E., Morgan, J. K., Jara-Cavieles, A., Shatters, R. G. Jr., Osborne, L. S., & McKenzie, C. L. (2015). The Scirtothrips dorsalis Species Complex: Endemism and Invasion in a Global Pest. *PLOS ONE*, 10(4), e0123747.
- Diehl, S. R., and G. L. Bush. (1984). An evolutionary and applied perspective of insect biotypes. *Annu Rev Entomol.*, 29, 471–504.
- Doutt RL, Annecke DP, Tremblay E. (1976). Biology and host relationships of parasitoids. In: Huffaker CB, Messenger PS (eds) *Theory and practice of biological control*. Academic Press, New York, USA, pp 143–163.
- Drès, M., and J. Mallet. (2002). Host races in plant–feeding insects and their importance in sympatric speciation. *Philos Trans R Soc Lond B Biol Sci.*, 357, 471–492.
- Ebel, E. R., DaCosta, J. M., Sorenson, M. D., Hill, R. I., Briscoe, A. D., Willmott, K. R., & Mullen, S. P. (2015). Rapid diversification associated with ecological specialization in Neotropical *Adelpha* butterflies. *Molecular Ecology*, 24(10), 2392–2405.
- Egan, S. P., & Funk, D. J. (2006). Individual advantages to ecological specialization: insights on cognitive constraints from three conspecific taxa. *Proceedings of the Royal Society B: Biological Sciences*, 273(1588), 843–848.
- Feder, J. L., & Forbes, A. A. (2010). Sequential speciation and the diversity of parasitic insects. *Ecological Entomology*, 35, 67–76.
- Faircloth, B. C. (2016). PHYLUCE is a software package for the analysis of conserved genomic loci. *Bioinformatics*, 32, 786–788.
- Faircloth, B. C., McCormack J. E., Crawford, N. G., Harvey, M. G., Brumfield, R. T., Glenn, T.C. (2012). Ultraconserved elements anchor thousands of genetic markers spanning multiple evolutionary timescales. *Systematic Biology*, 61, 717–726.
- Faircloth, B.C. (2013). Illumiprocessor: A trimmomatic wrapper for parallel adapter and quality trimming. <http://dx.doi.org/10.6079/J9ILL>.
- Ferracini, C., Gonella, E., Ferrari, E., Saladini, M. A., Picciau, L., Tota, F., Pontini, M., & Alma, A. (2014). Novel insight in the life cycle of *Torymus sinensis*, biocontrol agent of the chestnut gall wasp. *BioControl*, 60(2), 169–177.

- Ferracini, C., Bertolino, S., Bernardo, U., Bonsignore, C. P., Faccoli, M., Ferrari, E., Lupi, D., Maini, S., Mazzon, L., Nugnes, F., Rocco, A., Santi, F., & Tavella, L. (2018). Do *Torymus sinensis* (Hymenoptera: Torymidae) and agroforestry system affect native parasitoids associated with the Asian chestnut gall wasp? *Biological Control*, *121*, 36–43.
- Fennessy, J., Bidon, T., Reuss, F., Kumar, V., Elkan, P., Nilsson, M. A., Vamberger, M., Fritz, U., & Janke, A. (2016). Multi-locus Analyses Reveal Four Giraffe Species Instead of One. *Current Biology*, *26*(18), 2543–2549.
- Forbes, A. A., Powell, T. H. Q., Stelinski, L. L., Smith, J. J., & Feder, J. L. (2009). Sequential Sympatric Speciation Across Trophic Levels. *Science*, *323*(5915), 776–779.
- Forbes A.A., Hood G.R., Hall M.C., Lund J., Izen R., Egan S. P., & Ott J. R. (2016). Parasitoids, hyperparasitoids, and inquilines associated with the sexual and asexual generations of the gall former, *Belonocnema treatae* (Hymenoptera: Cynipidae). *The Annals of the Entomological Society of America*, *109*, 49-63.
- Forbes, A. A., Devine, S. N., Hippee, A. C., Tvedte, E. S., Ward, A. K. G., Widmayer, H. A., & Wilson, C. J. (2017). Revisiting the particular role of host shifts in initiating insect speciation. *Evolution*, *71*(5), 1126–1137.
- Forister, M. L., Ehmer, A. G., & Futuyma, D. J. (2007). The genetic architecture of a niche: variation and covariation in host use traits in the Colorado potato beetle. *Journal of Evolutionary Biology*, *20*(3), 985–996.
- Fox, L. R., & Morrow, P. A. (1981). Specialization: Species property or local phenomenon? *Science*, *211*, 887-893.
- Funk, D. J. (1998). Isolating a role for natural selection in speciation: Host adaptation and sexual selection in *Neochlamisus bebbianae* leaf beetles. *Evolution*, *52*(6), 1744–1759.
- Futuyma, D. J., Keese, M. C., & Funk, D. J. (1995). Genetic constraints on macroevolution: the evolution of host affiliation in the leaf beetle genus *Ophraella*. *Evolution*, *49*(5), 797–809.
- Futuyma, D. J., & Moreno, G. (1988). The evolution of the ecological specialization. *Annual Review of Ecology and Systematics*, *19*, 207-234.
- Germinara, G. S., De Cristofaro, A. & Rotundo, G. (2011). Chemical Cues for Host Location by the Chestnut Gall Wasp, *Dryocosmus kuriphilus*. *J Chem Ecol.*, *37*, 49–56.
- Giangrande, A. (2003). Biodiversity, conservation, and the 'Taxonomic impediment' Aquatic Conservation: *Marine and Freshwater Ecosystems*, *13*(5), 451–459.

- Gompert, Z., Comeault, A. A., Farkas, T. E., Feder, J. L., Parchman, T. L., Buerkle, C. A., & Nosil, P. (2013). Experimental evidence for ecological selection on genome variation in the wild. *Ecology Letters*, *17*(3), 369–379.
- Goulet H., Huber J. T. (eds). (1993). *Hymenoptera of the world: An identification guide to families*. Agriculture Canada, Canada.
- Gripenberg, S., Mayhew, P. J., Parnell, M., & Roslin, T. (2010). A meta-analysis of preference-performance relationships in phytophagous insects. *Ecology Letters*, *13*(3), 383–393.
- Guindon, S., Dufayard, J.-F., Lefort, V., Anisimova, M., Hordijk, W., & Gascuel, O. (2010). New Algorithms and Methods to Estimate Maximum-Likelihood Phylogenies: Assessing the Performance of PhyML 3.0. *Systematic Biology*, *59*(3), 307–321.
- Hanson, P. (1987). *Systematics and host associations of Ormyrus species (Hymenoptera: Chalcidoidea)*. Oregon State University.
- Hanson P. (1992). The Nearctic species of Ormyrus Westwood (Hymenoptera: Chalcidoidea: Ormyridae). *Journal of Natural History*, *26*, 1333–1365.
- Hardy, N. B., Peterson, D. A., & Normark, B. B. (2016). Nonadaptive radiation: Pervasive diet specialization by drift in scale insects? *Evolution*, *70*(10), 2421–2428.
- Harvey, J. A., Snaas, H., Malcicka, M., Visser, B., & Bezemer, T. M. (2014). Small-scale spatial resource partitioning in a hyperparasitoid community. *Arthropod-Plant Interactions*, *8*(5), 393–401.
- Hayward A, Stone G. N. (2005). Oak gall wasp communities: ecology and evolution. *Basic and Applied Ecology*, *6*, 435–443.
- He, X. Z., Wang, Q., Walker, J. T. S., Rogers, D. J., & Lo, P. L. (2010). A sophisticated life history strategy in a parasitoid wasp: Producing univoltine and multivoltine phenotypes in a local population. *Biological Control*, *54*(3), 276–284.
- Hebert, P. D. N., Cywinska, A., Ball, S. L., & deWaard, J. R. (2003). Biological identifications through DNA barcodes. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, *270*(1512), 313–321.
- Hebert, P. D., Penton, E. H., Burns, J. M., Janzen, D. H., & Hallwachs, W. (2004). Ten species in one: DNA barcoding reveals cryptic species in the neotropical skipper butterfly *Astraptes fulgerator*. *Proceedings of the National Academy of Sciences*, *101*(41), 14812–14817.
- Hipp, A. L., Manos, P. S., González-Rodríguez, A., Hahn, M., Kaproth, M., McVay, J. D., Avalos, S. V., & Cavender-Bares, J. (2018). Sympatric parallel diversification of major oak clades in the Americas and the origins of Mexican species diversity. *New Phytologist*, *217*(1), 439–452.

- Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q., & Vinh, L. S. (2017). UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Molecular Biology and Evolution*, 35(2), 518–522.
- Jaenike, J. (1990). Host specialization in phytophagous insects. *Annual Review of Ecology and Systematics*, 21, 243-273.
- Janz N, & Nylin S. (1997). The role of female search behaviour in determining host plant range in plant feeding insects: a test of the information-processing hypothesis. *Proc. R. Soc. B.* 264, 701–707.
- Janz, N., Nylin, S., & Wahlberg, N. (2006). Diversity begets diversity: host expansions and the diversification of plant-feeding insects. *BMC Evolutionary Biology*, 6(1), 4.
- Kaartinen, R., Stone, G. N., Hearn, J., Lohse, K., Roslin, T. (2010). Revealing secret liaisons: DNA barcoding changes our understanding of food webs. *Ecological Entomology*, 35, 623-638.
- Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A., & Jermini, L. S. (2017). ModelFinder: fast model selection for accurate phylogenetic estimates. *Nature Methods*, 14(6), 587–589.
- Komatsu, T., & Akimoto, S. (1995). Genetic differentiation as a result of adaptation to the phenologies of individual host trees in the galling aphid *Kaltenbachiella japonica*. *Ecological Entomology*, 20(1), 33–42.
- Larsson, S., & Ekbom, B. (1995). Oviposition Mistakes in Herbivorous Insects: Confusion or a Step Towards a New Host Plant? *Oikos*, 72(1), 155.
- Le Lann, C., van Baaren, J., & Visser, B. (2021). Dealing with predictable and unpredictable temperatures in a climate change context: the case of parasitoids and their hosts. *Journal of Experimental Biology*, 224(Suppl\_1).
- Leppänen, S. A., Malm, T., Värri, K., & Nyman, T. (2014). A comparative analysis of genetic differentiation across six shared willow host species in leaf-and bud-galling sawflies. *PloS one*, 9(12), e116286.
- Lobato-Vila, I., and J. Pujade-Villar. 2017. Description of five new species of inquiline oak gall wasps of the genus *Synergus* Hartig (Hymenoptera, Cynipidae: Synergini) with partially smooth mesopleurae from Mexico. *Zool. Stud.* 56, 1–28.
- Loxdale, H. D., & Harvey, J. A. (2016). The ‘generalism’ debate: misinterpreting the term in the empirical literature focusing on dietary breadth in insects. *Biological Journal of the Linnean Society*, 119(2), 265–282.

- Luo, A., Ling, C., Ho, S. Y. W., & Zhu, C.-D. (2018). Comparison of Methods for Molecular Species Delimitation Across a Range of Speciation Scenarios. *Systematic Biology*, 67(5), 830–846.
- Matsubayashi, K. W., Ohshima, I., & Nosil, P. (2010). Ecological speciation in phytophagous insects. *Entomologia Experimentalis et Applicata*, 134(1), 1–27.
- McCormack, J. E., Faircloth, B. C., Crawford, N. G., Gowaty, P. A., Brumfield, R. T., & Glenn, T. C. (2011). Ultraconserved elements are novel phylogenomic markers that resolve placental mammal phylogeny when combined with species-tree analysis. *Genome Research*, 22(4), 746–754.
- Mills, P. J., & Cook, L. G. (2014). Rapid chromosomal evolution in a morphologically cryptic radiation. *Molecular Phylogenetics and Evolution*, 77, 126–135.
- Minh, B. Q., Schmidt, H. A., Chernomor, O., Schrempf, D., Woodhams, M. D., von Haeseler, A., & Lanfear, R. (2020). IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Molecular Biology and Evolution*, 37(5), 1530–1534.
- Myers, E. A., R. W. Bryson, Jr., R. W. Hansen, M. L. Aardema, D. Lazcano, and F. T. Burbrink. (2019). Exploring Chihuahuan Desert diversification in the gray-banded kingsnake, *Lampropeltis alterna* (Serpentes: Colubridae). *Mol. Phylogenet. Evol.*, 131, 211–218.
- Nicholls, J. A., K. Schönrogger, S. Preuss, and G. P. Stone. (2018). Partitioning of herbivore hosts across time and food plants promotes diversification in the *Megastigmus dorsalis* oak gall parasitoid complex. *Ecol. Evol.*, 8, 1300–1315.
- Nosil, P., & Harmon, L. (2009). Niche dimensionality and ecological speciation. In R. Butlin, J. Bridle, & D. Schluter (Eds.), *Speciation and Patterns of Diversity* (Ecological Reviews, pp. 127–154). Cambridge: Cambridge University Press.
- Noyes 2012. An ordinate fondness of beetles, but seemingly even more fond of microhymenopteran! *Hamuli*, 3, 5–8.
- Noyes, J.S. 2021. Universal Chalcidoidea Database. World Wide Web electronic publication. <http://www.nhm.ac.uk/chalcidoids>
- Novotny, V., Miller, S. E., Hrcek, J., Baje, L., Basset, Y., Lewis, O. T., Stewart, A. J. A. & Weiblen, G. D. (2012). Insects on plants: explaining the paradox of low diversity within specialist herbivore guilds. *American Naturalist*, 179, 351–362.
- Poulin, R. (1992). Determinants of host-specificity in parasites of freshwater fishes. *International Journal for Parasitology*, 22(6), 753–758.

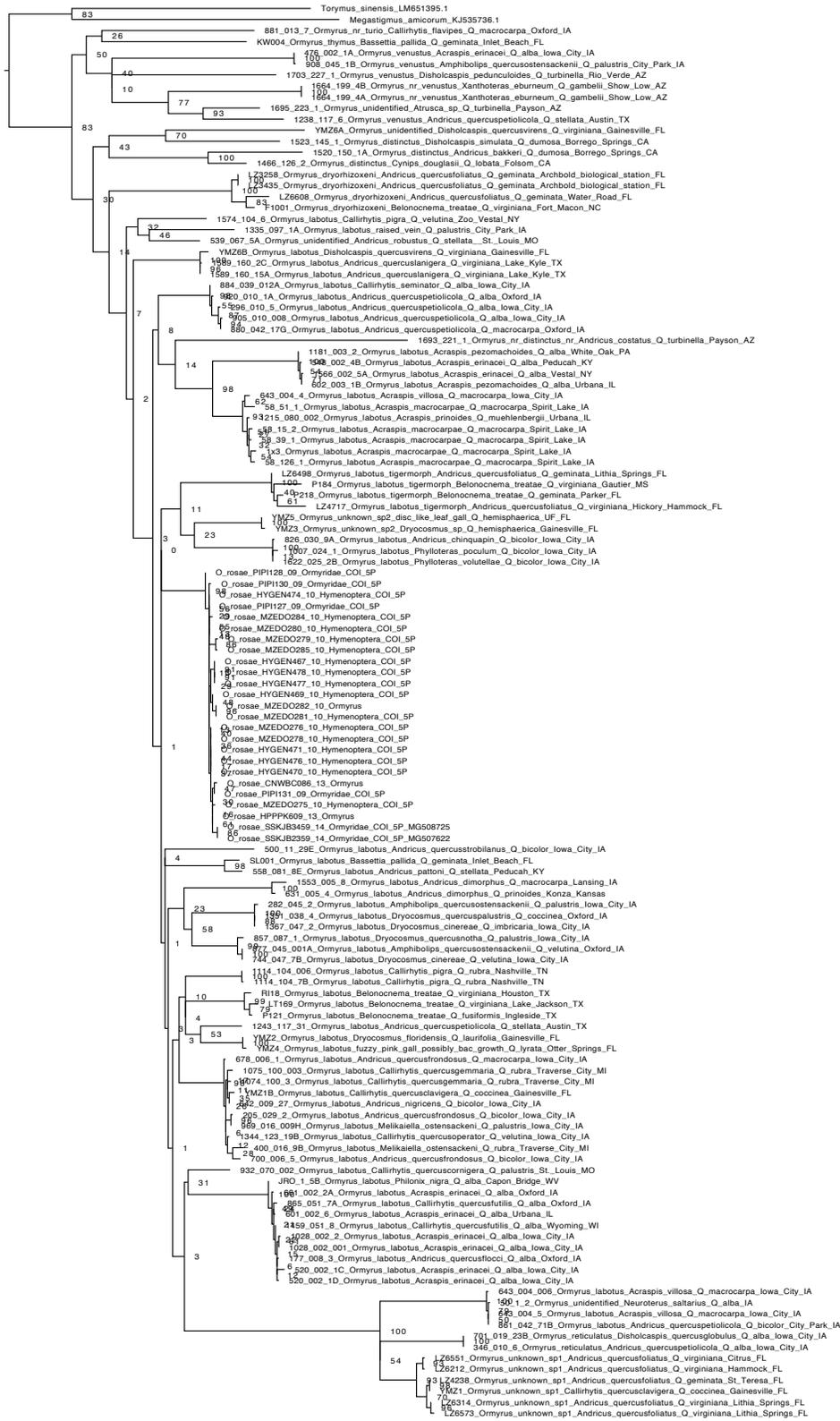
- Poulin R, & Morand, S. (2004). *Parasite Biodiversity*. Smithsonian Institution Press, Washington, D.C.
- Powell, T. H., G. R. Hood, M. O. Murphy, J. S. Heilveil, S. H. Berlocher, P. Nosil, and J. L. Feder. (2013). Genetic divergence along the speciation continuum: the transition from host race to species in *Rhagoletis* (Diptera: Tephritidae). *Evolution* 67, 2561–2576.
- Price, P. W. (1977). General concepts on the evolutionary biology of parasites. *Evolution*, 31(2), 405–420.
- Price PV. (1980). *Evolutionary Biology of Parasites*. Princeton University Press, Princeton.
- Price, P.W. (2002). Species interactions and the evolution of biodiversity. Pp. 3–26 in O. Pellmyr and C. Herrera, eds., *Plant-animal interactions*. Blackwell Science, Oxford, U.K.
- Puillandre, N., Brouillet, S., & Achaz, G. (2020). ASAP: assemble species by automatic partitioning. *Molecular Ecology Resources*, 21(2), 609–620.
- Rabiee, M., & Mirarab, S. (2020). SODA: multi-locus species delimitation using quartet frequencies. *Bioinformatics*, 36(24), 5623–5631.
- Rohde, K. (1979). A Critical Evaluation of Intrinsic and Extrinsic Factors Responsible for Niche Restriction in Parasites. *The American Naturalist*, 114(5), 648–671.
- Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D. L., Darling, A., Höhna, S., Larget, B., Liu, L., Suchard, M. A., & Huelsenbeck, J. P. (2012). MrBayes 3.2: Efficient Bayesian Phylogenetic Inference and Model Choice Across a Large Model Space. *Systematic Biology*, 61(3), 539–542.
- Ronquist, F., Nieves-Aldrey, J. L., Buffington, M. L., Liu, Z., Liljeblad, J., & Nylander, J. A. A. (2015). Phylogeny, evolution and classification of gall wasps: The plot thickens. *PLoS ONE*, 10, 1-40.
- Singer, M. C. (1972). Complex components of habitat suitability within a butterfly colony. *Science*, 176, 75-77.
- Smith, M. A., Rodriguez, J. J., Whitfield, J. B., Deans, A. R., Janzen, D. H., Hallwachs, W., & Hebert, P. D. (2008). Extreme diversity of tropical parasitoid wasps exposed by iterative integration of natural history, DNA barcoding, morphology, and collections. *Proceedings of the National Academy of Sciences*, 105(34), 12359-12364.
- Stireman III, J. O., J. D. Nason, and S. B. Heard. (2005). Host-associated genetic differentiation in phytophagous insects: general phenomenon or isolated exceptions? Evidence from a goldenrod-insect community. *Evolution*, 59, 2573–2587.

- Stireman, J. O., Nason, J. D., Heard, S. B., & Seehawer, J. M. (2006). Cascading host-associated genetic differentiation in parasitoids of phytophagous insects. *Proceedings. Biological sciences*, 273(1586), 523–530.
- Stamatakis, A. (2014). RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics*, 30, 1312–1313.
- Stone, G. N., Schönrogge, K., Atkinson, R. J., Bellido, D., & Pujade-Villar, J. (2002). The population biology of oak gallwasps (Hymenoptera: Cynipidae). *Annual Review of Entomology*, 47, 633–668.
- Stone, G. N., & Schönrogge, K. (2003). The adaptive significance of insect gall morphology. *Trends in Ecology and Evolution*, 18, 512–522.
- Taylor, RW. (1983). Descriptive taxonomy: past, present, and future. In *Australian Systematic entomology: a bicentenary perspective*, Highley E, Taylor RW (eds). CSIRO: Melbourne; 93–134.
- Tougeron, K., Brodeur, J., van Baaren, J., Renault, D. and Le Lann, C. (2019). Sex makes them sleepy: host reproductive status induces diapause in a parasitoid population experiencing harsh winters. *Peer Community Ecol.*
- van Asch, M., & Visser, M. E. (2007). Phenology of Forest Caterpillars and Their Host Trees: The Importance of Synchrony. *Annual Review of Entomology*, 52(1), 37–55.
- Vertacnik, K. L., & Linnen, C. R. (2017). Evolutionary genetics of host shifts in herbivorous insects: insights from the age of genomics. *Annals of the New York Academy of Sciences*, 1389(1), 186–212.
- Via, S., Bouck, A. C., & Skillman, S. (2000). Reproductive isolation between divergent races of pea aphids on two hosts. II. Selection against migrants and hybrids in the parental environments. *Evolution*, 54(5), 1626–1637.
- Vidal, M. C., Quinn, T. W., Stireman, J. O., III, Tinghitella, R. M., & Murphy, S. M. (2019). Geography is more important than host plant use for the population genetic structure of a generalist insect herbivore. *Molecular Ecology*, 28(18), 4317–4334.
- Visser, M. E., & Gienapp, P. (2019). Evolutionary and demographic consequences of phenological mismatches. *Nature Ecology & Evolution*, 3(6), 879–885.
- Waage, J., Greathead, D., Brown, R., Paterson, R., Haskell, P., Cook, R., & Krishnaiah, K. (1988). Biological Control: Challenges and Opportunities. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, 318(1189), 111-128.

- Ward, A. K. G., Sheikh, S. I., & Forbes, A. A. (2020). Diversity, Host Ranges, and Potential Drivers of Speciation Among the Inquiline Enemies of Oak Gall Wasps (Hymenoptera: Cynipidae). *Insect Systematics and Diversity*, 4(6).
- Washburn, J. O., & Cornell, H. V. (1981). Parasitoids, Patches, and Phenology: Their Possible Role in the Local Extinction of a Cynipid Gall Wasp Population. *Ecology*, 62(6), 1597–1607.
- Weld L. H. 1957. Cynipid Galls of the Pacific Slope. Privately printed, Ann Arbor, MI.
- Weld L. H. 1959. Cynipid galls of the Eastern United States. Privately printed, Ann Arbor, MI.
- Weld L. H. 1960. Cynipid galls of the Southwest. Privately printed, Ann Arbor, MI.
- Weinstein, S. B., & Kuris, A. M. (2016). Independent origins of parasitism in Animalia. *Biology Letters*, 12(7), 20160324.
- Windsor, D. A. (1998). Most of the species on Earth are parasites. *International Journal for Parasitology*, 28, 1939–1941.
- Winkler, I.S. & Mitter, C. (2008). The phylogenetic dimension of insect-plant interactions: a review of recent evidence. *Specialization, Speciation, and Radiation: The Evolutionary Biology of Herbivorous Insects* (ed. K. Tilmon), pp. 240–263. University of California Press, Berkeley, CA, USA.
- Zhang, J., P., Kapli, P., Pavlidis, and A. Stamatakis. (2013). A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, 29, 2869–2876.
- Zhang, Y., Gates, M., & Shorthouse, J. (2014). Testing species limits of Eurytomidae (Hymenoptera) associated with galls induced by *Diplolepis* (Hymenoptera: Cynipidae) in Canada using an integrative approach. *The Canadian Entomologist*, 146(3), 321-334.

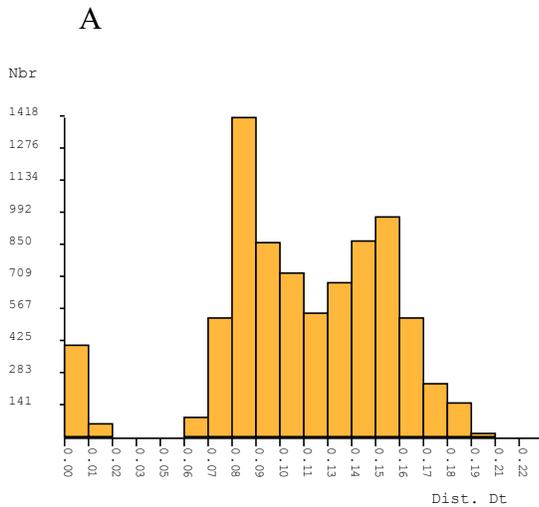


Figure A2: Maximum likelihood COI tree; nodes labeled with bootstrap.



0.07

**Figure A3:** ASAP species delimitation results. ASAP is a hierarchical clustering algorithm that uses a multiple sequence alignment to search for a barcode gap (A), which indicates the limit between intraspecific (left of the gap) and interspecific genetic distances (right of the gap). For every partition (the assignment of all sequences into groups, with the low end of this range 1, such that all sequences belong to one group, and the high end is the total number of sequences in the MSA), ASAP assigns two scores, one is a p-value (probability of panmixia – lower p-values correspond to better partitions), and the other is a relative gap width (W). The combination of those values is used to assign an ASAP score and rank to each partition, with ten shown in B.



**B**

Nb of species	asap-score	P-val (rank)	W (rank)	Threshold dist.	Text
40	1.00	● 5.64e-03 (1)	2.24e-03 (1)	0.054368	<a href="#">list csv</a>
* 41	4.00	● 2.12e-01 (5)	1.89e-03 (3)	0.043194	<a href="#">list csv</a>
38	4.50	● 1.80e-01 (3)	1.47e-03 (6)	0.057059	<a href="#">list csv</a>
40	5.50	● 5.71e-01 (9)	2.17e-03 (2)	0.055444	<a href="#">list csv</a>
* 46	7.50	● 3.38e-01 (7)	8.32e-04 (8)	0.022183	<a href="#">list csv</a>
* 47	10.50	● 6.21e-01 (10)	2.31e-04 (11)	0.017233	<a href="#">list csv</a>
* 43	11.00	● 8.22e-01 (17)	1.68e-03 (5)	0.029325	<a href="#">list csv</a>
102	13.50	● 1.82e-01 (4)	8.43e-05 (23)	0.001592	<a href="#">list csv</a>
104	14.50	● 7.49e-01 (14)	1.30e-04 (15)	0.001519	<a href="#">list csv</a>
32	15.50	● 3.94e-02 (2)	4.41e-05 (29)	0.067244	<a href="#">list csv</a>

**Table A1:** ID numbers and collection information for *Ormyrus* included in the COI project. IDs with an asterisk indicate sequences that were previously sequenced and published. Clade numbers correspond to putative species assignments based on COI barcoding (Figure 4). Gall collections were made by various members of the Forbes lab (including Andrew A Forbes, Anna KG Ward, Alaine C. Hippee, Eric Tvedte, Sara Devine, Joseph Verry, and Will Carter), and collaborators including Miles Zhang, Jim Ott, Robert Busbee, Linyi Zhang, Glen Hood, Scott Egan, and Kirsten Prior.

<i>Ormyrus</i> ID number	Clade	Gall Host	Tree Host	Collection Location	Date collected	Date emerged
881_013_7	1	<i>Callirhytis flavipes</i>	<i>Quercus macrocarpa</i>	Oxford, IA	6/8/2017	7/6/2017
KW004*	2	<i>Bassettia pallida</i>	<i>Quercus geminata</i>	Inlet Beach, FL	8/1/2015	
1703_227_1	3	<i>Disholcaspis pedunculoides</i>	<i>Quercus turbinella</i>	Rio Verde, AZ	9/17/2019	10/1/2019
476_002_1A	4	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Iowa City, IA	8/11/2016	8/25/2016
908_045_1B	4	<i>Amphibolips quercusostensackenii</i>	<i>Quercus palustris</i>	City Park, IA	6/16/2017	6/27/2017
1238_117_6	5	<i>Andricus quercuspetiolicola</i>	<i>Quercus stellata</i>	Austin, TX	4/12/2018	6/4/2018
1695_223_1	6	<i>Atrusca</i> sp.	<i>Quercus turbinella</i>	Payson, AZ	9/17/2019	11/20/2019
1664_199_4A	7	<i>Xanthoterax eburneum</i>	<i>Quercus gambelii</i>	Show Low, AZ	9/16/2019	11/25/2019
1664_199_4B	7	<i>Xanthoterax eburneum</i>	<i>Quercus gambelii</i>	Show Low, AZ	9/16/2019	11/25/2019
701_019_23B	8	<i>Disholcaspis quercusglobulus</i>	<i>Quercus alba</i>	Iowa City, IA	4/12/2017	5/25/2017
643_004_006	9	<i>Acraspis villosa</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	9/27/2016	7/23/2017
643_004_5	9	<i>Acraspis villosa</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	9/27/2016	5/20/2017
346_010_6	8	<i>Andricus quercuspetiolicola</i>	<i>Quercus alba</i>	Iowa City, IA	6/24/2016	6/30/2016
861_042_71B	9	<i>Andricus quercuspetiolicola</i>	<i>Quercus bicolor</i>	City Park, IA	6/7/2017	7/5/2017
500_11_29E	9	<i>Andricus quercusstrobilanus</i>	<i>Quercus bicolor</i>	Iowa City, IA	8/18/2016	9/8/2016
LZ6212	10	<i>Andricus quercusfoliatus</i>	<i>Quercus virginiana</i>	Hammock, FL	12/15/2018	3/4/2019
LZ6551	10	<i>Andricus quercusfoliatus</i>	<i>Quercus virginiana</i>	Citrus, FL	12/17/2018	4/15/2019
LZ6314	10	<i>Andricus quercusfoliatus</i>	<i>Quercus virginiana</i>	Lithia Springs, FL	12/16/2018	3/15/2019
LZ6573	10	<i>Andricus quercusfoliatus</i>	<i>Quercus virginiana</i>	Lithia Springs, FL	12/16/2018	4/19/2019
LZ4238	10	<i>Andricus quercusfoliatus</i>	<i>Quercus geminata</i>	St. Teresa, FL	12/11/2017	9/26/2018
YMZ1	10	<i>Callirhytis quercusclavigera</i>	<i>Quercus coccinea</i>	Gainesville, FL	11/3/2019	
1466_126_2	11	<i>Cynips douglasii</i>	<i>Quercus lobata</i>	Folsom, CA	7/31/2018	8/19/2018
1520_150_1A	12	<i>Andricus bakkeri</i>	<i>Quercus dumosa</i>	Borrego Springs, CA	8/5/2018	9/2/2018

**Table A1 – continued**

1523_145_1	13	<i>Disholcaspis simulata</i>	<i>Quercus dumosa</i>	Borrego Springs, CA	8/5/2018	9/13/2018
YMZ6A	14	<i>Disholcaspis quercusvirens</i>	<i>Quercus virginiana</i>	Gainesville, FL		
LZ3258	15	<i>Andricus quercusfoliatus</i>	<i>Quercus geminata</i>	Archbold biological station, FL	12/14/2017	4/5/2018
LZ3435	15	<i>Andricus quercusfoliatus</i>	<i>Quercus geminata</i>	Archbold biological station, FL	12/14/2017	4/12/2018
LZ6608	15	<i>Andricus quercusfoliatus</i>	<i>Quercus geminata</i>	Water Road, FL	12/19/2018	4/24/2019
F1001*	15	<i>Belonocnema treatae</i>	<i>Quercus virginiana</i>	Fort Macon, NC		
50_1_2	16	<i>Neuroterus saltarius</i>	<i>Quercus alba</i>	IA		
1693_221_1	17	<i>nr Andricus costatus</i>	<i>Quercus turbinella</i>	Payson, AZ	9/17/2019	9/30/2019
558_081_8E	18	<i>Andricus pattoni</i>	<i>Quercus stellata</i>	Peducah, KY	9/3/2016	9/18/2016
SL001*	18	<i>Bassetia pallida</i>	<i>Quercus geminata</i>	Inlet Beach, FL	8/1/2015	
631_005_4	19	<i>Andricus dimorphus</i>	<i>Quercus prinoides</i>	Konza, Kansas	9/24/2016	11/6/2017
1553_005_8	19	<i>Andricus dimorphus</i>	<i>Quercus macrocarpa</i>	Lansing, IA	10/6/2018	8/7/2019
1114_104_7B	20	<i>Callirhytis pigra</i>	<i>Quercus rubra</i>	Nashville, TN	8/25/2017	9/15/2017
1114_104_006	20	<i>Callirhytis pigra</i>	<i>Quercus rubra</i>	Nashville, TN	8/25/2017	9/14/2017
YMZ5	21	disc like leaf gall	<i>Quercus hemisphaerica</i>	UF, FL	3/20/2020	
YMZ3	21	<i>Dryocosmus sp</i>	<i>Quercus hemisphaerica</i>	Gainesville, FL		
1574_104_6	22	<i>Callirhytis pigra</i>	<i>Quercus velutina</i>	Zoo, Vestal NY	10/17/2018	10/29/2018
539_067_5A	23	<i>Andricus robustus</i>	<i>Quercus stellata</i>	St. Louis, MO	9/2/2016	9/18/2016
1335_097_1A	24	raised vein	<i>Quercus palustris</i>	City Park, IA	5/16/2018	6/10/2018
826_030_9A	25	<i>Andricus chinquapin</i>	<i>Quercus bicolor</i>	Iowa City, IA	6/1/2017	6/17/2017
1007_024_1	25	<i>Phylloteras poculum</i>	<i>Quercus bicolor</i>	Iowa City, IA	7/14/2017	8/3/2017
1622_025_2B	25	<i>Phylloteras volutellae</i>	<i>Quercus bicolor</i>	Iowa City, IA	9/4/2019	9/17/2019
1589_160_2C	27	<i>Andricus quercuslanigera</i>	<i>Quercus virginiana</i>	Lake Kyle, TX	11/1/2018	11/12/2018
1589_160_15A	27	<i>Andricus quercuslanigera</i>	<i>Quercus virginiana</i>	Lake Kyle, TX	11/1/2018	4/3/2019
YMZ6B	27	<i>Disholcaspis quercusvirens</i>	<i>Quercus virginiana</i>	Gainesville, FL		
1243_117_31	26a	<i>Andricus quercuspetiolicola</i>	<i>Quercus stellata</i>	Austin, TX	4/12/2018	9/9/2018
YMZ2	26b	<i>Dryocosmus floridensis</i>	<i>Quercus laurifolia</i>	Gainesville, FL		

**Table A1 – continued**

YMZ4	26b	fuzzy pink gall possibly bac growth	<i>Quercus lyrata</i>	Otter Springs, FL	1/23/2020	
RI18*	28	<i>Belonocnema treatae</i>	<i>Quercus virginiana</i>	Houston, TX		
LT169*	28	<i>Belonocnema treatae</i>	<i>Quercus virginiana</i>	Lake Jackson, TX		
P121*	28	<i>Belonocnema treatae</i>	<i>Quercus fusiformis</i>	Ingleside, TX	11/1/2015	Nov-15
LZ6498	29	<i>Andricus quercusfoliatus</i>	<i>Quercus geminata</i>	Lithia Springs, FL	12/16/2018	4/8/2019
LZ4717	29	<i>Andricus quercusfoliatus</i>	<i>Quercus virginiana</i>	Hickory Hammock, FL	12/15/2018	12/26/2018
P184*	29	<i>Belonocnema treatae</i>	<i>Quercus virginiana</i>	Gautier, MS	10/16/2015	Oct-15
P218*	29	<i>Belonocnema treatae</i>	<i>Quercus geminata</i>	Parker, FL	10/15/2015	Oct-15
920_010_1A	30	<i>Andricus quercuspetiolicola</i>	<i>Quercus alba</i>	Oxford, IA	6/20/2017	6/21/2017
296_010_5	30	<i>Andricus quercuspetiolicola</i>	<i>Quercus alba</i>	Iowa City, IA	6/6/2016	6/20/2016
880_042_17G	30	<i>Andricus quercuspetiolicola</i>	<i>Quercus macrocarpa</i>	Oxford, IA	6/8/2017	6/27/2017
905_010_008	30	<i>Andricus quercuspetiolicola</i>	<i>Quercus alba</i>	Iowa City, IA	6/15/2017	
884_039_012A	30	<i>Callirhytis seminator</i>	<i>Quercus alba</i>	Iowa City, IA	6/9/2017	6/29/2017
282_045_2	31	<i>Amphibolips quercusostensackenii</i>	<i>Quercus palustris</i>	Iowa City, IA	5/27/2016	6/12/2016
877_045_001A	31	<i>Amphibolips quercusostensackenii</i>	<i>Quercus velutina</i>	Oxford, IA	6/8/2017	6/21/2017
1367_047_2	31	<i>Dryocosmus cinereae</i>	<i>Quercus imbricaria</i>	Iowa City, IA	5/24/2018	6/9/2018
744_047_7B	31	<i>Dryocosmus cinereae</i>	<i>Quercus velutina</i>	Iowa City, IA	5/9/2017	6/5/2017
857_087_1	31	<i>Dryocosmus quercusnotha</i>	<i>Quercus palustris</i>	Iowa City, IA	6/7/2017	6/12/2017
1351_038_4	31	<i>Dryocosmus quercuspalustris</i>	<i>Quercus coccinea</i>	Oxford, IA	5/19/2018	6/9/2018
642_009_27	32	<i>Andricus nigricens</i>	<i>Quercus bicolor</i>	Iowa City, IA	9/27/2016	8/18/2017
678_006_1	32	<i>Andricus quercusfrondosus</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	3/29/2017	4/28/2017
205_029_2	32	<i>Andricus quercusfrondosus</i>	<i>Quercus bicolor</i>	Iowa City, IA	4/14/2016	5/21/2016
700_006_5	32	<i>Andricus quercusfrondosus</i>	<i>Quercus bicolor</i>	Iowa City, IA	4/12/2017	5/4/2017
YMZ1B	32	<i>Callirhytis quercusclavigera</i>	<i>Quercus coccinea</i>	Gainesville, FL	11/3/2019	
1075_100_003	32	<i>Callirhytis quercusgemmaria</i>	<i>Quercus rubra</i>	Traverse City, MI	8/13/2017	10/25/2017
1074_100_3	32	<i>Callirhytis quercusgemmaria</i>	<i>Quercus rubra</i>	Traverse City, MI	8/13/2017	9/4/2017
1344_123_19B	32	<i>Callirhytis quercusoperator</i>	<i>Quercus velutina</i>	Iowa City, IA	5/19/2018	6/9/2018
969_016_009H	32	<i>Melikaiella ostensackeni</i>	<i>Quercus palustris</i>	Iowa City, IA	7/6/2017	7/26/2017

**Table A1 – continued**

400_016_9B	32	<i>Melikaiella ostensackeni</i>	<i>Quercus rubra</i>	Traverse City, MI	7/12/2016	7/12/2016
932_070_002	33	<i>Callirhytis quercuscornigera</i>	<i>Quercus palustris</i>	St. Louis, MO	6/29/2017	7/31/2017
520_002_1C	34	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Iowa City, IA	8/19/2016	8/20/2016
520_002_1D	34	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Iowa City, IA	8/19/2016	8/20/2016
601_002_6	34	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Urbana, IL	9/6/2016	9/29/2016
661_002_2A	34	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Oxford, IA	10/18/2016	5/13/2017
1028_002_001	34	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Iowa City, IA	7/25/2017	7/26/2017
1028_002_2	34	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Iowa City, IA	7/25/2017	8/4/2017
177_008_3	34	<i>Andricus quercusflocci</i>	<i>Quercus alba</i>	Oxford, IA	4/2/2016	5/12/2016
865_051_7A	34	<i>Callirhytis quercusfutilis</i>	<i>Quercus alba</i>	Oxford, IA	6/8/2017	6/28/2017
1459_051_8	34	<i>Callirhytis quercusfutilis</i>	<i>Quercus alba</i>	Wyoming, WI	7/28/2018	8/11/2018
JRO_1_5B	34	<i>Philonix nigra</i>	<i>Quercus alba</i>	Capon Bridge, WV	8/14/2019	9/1/2019
548_002_4B	35	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Peducah, KY	9/3/2016	9/5/2016
1566_002_5A	35	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Vestal, NY	10/17/2018	6/8/2019
1181_003_2	35	<i>Acraspis pezomachoides</i>	<i>Quercus alba</i>	White Oak, PA	9/9/2017	11/12/2017
602_003_1B	35	<i>Acraspis pezomachoides</i>	<i>Quercus alba</i>	Urbana, IL	9/6/2016	9/10/2016
58_51_1	36	<i>Acraspis macrocarpae</i>	<i>Quercus macrocarpa</i>	Spirit Lake, IA	8/1/2015	8/12/2015
58_15_2	36	<i>Acraspis macrocarpae</i>	<i>Quercus macrocarpa</i>	Spirit Lake, IA	8/1/2015	8/8/2015
58_39_1	36	<i>Acraspis macrocarpae</i>	<i>Quercus macrocarpa</i>	Spirit Lake, IA	8/1/2015	8/6/2015
1x3	36	<i>Acraspis macrocarpae</i>	<i>Quercus macrocarpa</i>	Spirit Lake, IA	7/13/2015	7/17/2015
58_126_1	36	<i>Acraspis macrocarpae</i>	<i>Quercus macrocarpa</i>	Spirit Lake, IA	8/1/2015	8/6/2015
1215_080_002	36	<i>Acraspis prinoides</i>	<i>Quercus muehlenbergii</i>	Urbana, IL	9/11/2017	10/12/2017
643_004_4	36	<i>Acraspis villosa</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	9/27/2016	6/21/2017

**Table A2:** ID numbers and collection information for *Ormyrus* included in the UCE project. The clade numbers correspond with clades in the COI project (see Table A1), except for ones with an asterisk, which indicates a clade that was not represented in the COI tree.

<i>Ormyrus</i> ID number	Clade	Gall Host	Tree Host	Collection Location	Date collected	Date emerged
862-013-014	1	<i>Callirhytis flavipes</i>	<i>Quercus bicolor</i>	Iowa City, IA	6/7/2017	7/7/2017
881-013-005	1	<i>Callirhytis flavipes</i>	<i>Quercus macrocarpa</i>	Tiffin, IA	6/8/2017	7/4/2017
565-021-001	1	<i>Neuroterus saltarius</i>	<i>Quercus macrocarpa</i>	St Louis, MO	9/2/2016	9/6/2016
KW004	2	<i>Bassetia pallida</i>	<i>Quercus geminata</i>	Inlet Beach, FL	8/1/2015	
1703-227-1	3	<i>Disholcaspis pedunculoides</i>	<i>Quercus turbinella</i>	Rio Verde, AZ	9/17/2019	10/1/2019
954-020-1	3	<i>Disholcaspis quercusmamma</i>	<i>Quercus bicolor</i>	Iowa City, IA	6/30/2017	7/14/2017
476-002-1A	4	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Iowa City, IA	8/11/2016	8/25/2016
1238-117-6	5	<i>Andricus quercuspetiolicola</i>	<i>Quercus stellata</i>	Austin, TX	4/12/2018	6/4/2018
1695-223-1	6	<i>Atrusca</i> sp.	<i>Quercus turbinella</i>	Payson, AZ	9/17/2019	11/20/2019
1696-180-3	6	woolly gall	<i>Quercus turbinella</i>	Payson, AZ	9/17/2019	12/10/2019
1711-180-1A	6	woolly gall	<i>Quercus grisea</i>	Payson, AZ	9/17/2019	12/10/2019
1664-199-4A	7	<i>Xanthoteras eburneum</i>	<i>Quercus gambelii</i>	Show Low, AZ	9/16/2019	11/25/2019
1664-199-4B	7	<i>Xanthoteras eburneum</i>	<i>Quercus gambelii</i>	Show Low, AZ	9/16/2019	11/25/2019
701-019-32A	8	<i>Disholcaspis quercusglobulus</i>	<i>Quercus alba</i>	Iowa City, IA	4/12/2017	6/5/2017
296-010-14	8	<i>Andricus quercuspetiolicola</i>	<i>Quercus alba</i>	Iowa City, IA	6/6/2016	7/18/2016
1135-108-10	8	red wrinkled gall	<i>Quercus falcata</i>	Paris, TN	8/27/2017	6/15/2018
643-004-5	9	<i>Acraspis villosa</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	9/27/2016	6/21/2017
500-011-29E	9	<i>Andricus quercusstrobilanus</i>	<i>Quercus bicolor</i>	Iowa City, IA	8/18/2016	9/8/2016
LZ4238	10	<i>Andricus quercusfoliatus</i>	<i>Quercus geminata</i>	St. Teresa, FL	12/11/2017	9/26/2018
LZ6610	10	<i>Andricus quercusfoliatus</i>	<i>Quercus geminata</i>	Water Road, FL	12/19/2018	4/24/2019
LZ6500	10	<i>Andricus quercusfoliatus</i>	<i>Quercus geminata</i>	Ochlocknee, FL	12/19/2018	4/8/2019
YMZ1	10	<i>Callirhytis quercusclavigera</i>	<i>Quercus coccinea</i>	Gainesville, FL	11/3/2019	
1466-126-2	11	<i>Cynips douglasii</i>	<i>Quercus lobata</i>	Folsom, CA	8/5/2018	8/19/2018
1520-150-1A	12	<i>Andricus bakkeri</i>	<i>Quercus dumosa</i>	Borrego Springs, CA	8/5/2018	9/2/2018

**Table A2 – continued**

1523-145-1	13	<i>Disholcaspis simulata</i>	<i>Quercus dumosa</i>	Borrego Springs, CA	8/5/2018	9/13/2018
YMZ6A	14	<i>Disholcaspis quercusvirens</i>	<i>Quercus virginiana</i>	Gainesville, FL		
LZ3258	15	<i>Andricus quercusfoliatus</i>	<i>Quercus geminata</i>	Archbold Biological Station, FL	12/14/2017	4/5/2018
LZ6608	15	<i>Andricus quercusfoliatus</i>	<i>Quercus geminata</i>	Water Road	12/19/2018	4/24/2019
F1001	15	<i>Belonconema treatae</i>	<i>Quercus virginiana</i>	Fort Macon, NC		
1693-221-1	17	<i>nr Andricus costatus</i>	<i>Quercus turbinella</i>	Payson, AZ	9/17/2019	9/30/2019
1548-009-2	19	<i>Andricus nigricens</i>	<i>Quercus bicolor</i>	Iowa City, IA	10/2/2018	10/14/2018
1384_030_1A	19	<i>Andricus chinquapin</i>	<i>Quercus bicolor</i>	Coralville, IA	6/5/2018	10/29/2018
1553-005-8	19	<i>Andricus dimorphus</i>	<i>Quercus macrocarpa</i>	Lansing, IA	10/6/2018	8/7/2019
631-005-3	19	<i>Andricus dimorphus</i>	<i>Quercus prinoides</i>	Konza KS	9/24/2016	9/21/2017
631-005-6	19	<i>Andricus dimorphus</i>	<i>Quercus prinoides</i>	Konza KS	9/24/2016	11/12/2017
341-053-52B	19	<i>Neuroterus noxiosus</i>	<i>Quercus bicolor</i>	Iowa City, IA	6/24/2016	7/11/2016
369-053-19A	19	<i>Neuroterus noxiosus</i>	<i>Quercus bicolor</i>	Iowa City, IA	6/29/2016	7/19/2016
379-021-13A	19	<i>Neuroterus saltarius</i>	<i>Quercus bicolor</i>	Iowa City, IA	7/6/2016	7/30/2016
630-023-5	19	<i>Philonix nigra</i>	<i>Quercus prinoides</i>	Konza Kansas	9/24/2016	9/13/2017
1114-104-006	20	<i>Callirhytis pigra</i>	<i>Quercus rubra</i>	Nashville, TN	8/25/2017	9/14/2017
1574-104-6	22	<i>Callirhytis pigra</i>	<i>Quercus velutina</i>	Vestal, NY	10/17/2018	10/29/2018
538-066-3	23	<i>Andricus biconicus</i>	<i>Quercus stellata</i>	St Louis, MO	9/2/2016	9/27/2016
538-066-6	23	<i>Andricus biconicus</i>	<i>Quercus stellata</i>	St Louis, MO	9/2/2016	9/23/2016
558-081-1A	23	<i>Andricus pattoni</i>	<i>Quercus stellata</i>	Peducah, KY	9/3/2016	9/7/2016
558-081-1B	23	<i>Andricus pattoni</i>	<i>Quercus stellata</i>	Peducah, KY	9/3/2016	9/7/2016
539-067-5A	23	<i>Andricus robustus</i>	<i>Quercus stellata</i>	St Louis, MO	9/2/2016	9/18/2016
539-067-7A	23	<i>Andricus robustus</i>	<i>Quercus stellata</i>	St Louis, MO	9/2/2016	9/22/2016
826-030-9A	25	<i>Andricus chinquapin</i>	<i>Quercus bicolor</i>	Iowa City, IA	6/1/2017	6/17/2017
1059-024-1A	25	<i>Phylloteris pocoulum</i>	<i>Quercus bicolor</i>	Iowa City, IA	7/14/2017	8/27/2017
1007-024-001	25	<i>Phylloteris pocoulum</i>	<i>Quercus bicolor</i>	Iowa City, IA	7/14/2017	8/3/2017
1662-025-2A	25	<i>Phylloteris volutellae</i>	<i>Quercus bicolor</i>	Iowa City, IA	9/4/2019	9/17/2019

**Table A2 – continued**

1662-025-2B	25	<i>Phylloteras volutellae</i>	<i>Quercus bicolor</i>	Iowa City, IA	9/4/2019	9/17/2019
612-077-1	25	spangle_gall	<i>Quercus stellata</i>	St Louis, MO	9/2/2016	9/7/2016
555-019-4A	26	<i>Disholcaspis quercusglobulus</i>	<i>Quercus stellata</i>	Peducah, KY	9/3/2016	9/21/2016
1243-117-31	26	<i>Andricus quercuspetiolicola</i>	<i>Quercus stellata</i>	MK 15a, TX	4/12/2018	9/9/2018
YMZ2	26	<i>Dryocosmus floridensis</i>	<i>Quercus laurifolia</i>	Gainesville, FL		
1589-160-15A	27	<i>Andricus quercuslanigera</i>	<i>Quercus virginiana</i>	Kyle, TX	11/1/2018	4/3/2019
1589-160-2B	27	<i>Andricus quercuslanigera</i>	<i>Quercus virginiana</i>	Kyle, TX	11/1/2018	11/12/2018
YMZ6B	27	<i>Disholcaspis quercusvirens</i>	<i>Quercus virginiana</i>	Gainesville, FL		
P47	29	<i>Belonocnema treatae</i>	<i>Quercus fusiformis</i>	Schleiker County, TX	10/15/2015	Oct-15
LZ4717	29	<i>Andricus quercusfoliatus</i>	<i>Quercus virginiana</i>	Hickory Hammock, FL	12/15/2018	12/26/2018
P184	29	<i>Belonocnema treatae</i>	<i>Quercus virginiana</i>	Gautier, MS	10/16/2015	Oct-15
861-042-77C	30	<i>Andricus quercuspetiolicola</i>	<i>Quercus bicolor</i>	Iowa City, IA	6/7/2017	7/7/2017
918-042-8K	30	<i>Andricus quercuspetiolicola</i>	<i>Quercus macrocarpa</i>	Tiffin, IA	6/20/2017	6/29/2017
920-010-1A	30	<i>Andricus quercuspetiolicola</i>	<i>Quercus alba</i>	Tiffin, IA	6/20/2017	6/21/2017
294-049-27	30	<i>Callirhytis scitula</i>	<i>Quercus imbricaria</i>	Iowa City, IA	6/6/2016	7/1/2016
884-039-010A	30	<i>Callirhytis seminator</i>	<i>Quercus alba</i>	Iowa City, IA	6/9/2017	6/29/2017
884-039-012A	30	<i>Callirhytis seminator</i>	<i>Quercus alba</i>	Iowa City, IA	6/9/2017	6/29/2017
866-035-019	30	<i>Loxaulus quercusmammula</i>	<i>Quercus alba</i>	Tiffin, IA	6/8/2017	6/14/2017
866-035-14C	30	<i>Loxaulus quercusmammula</i>	<i>Quercus alba</i>	Tiffin, IA	6/8/2017	6/29/2017
1416-043-3C	30	<i>Melikaiella tumifica</i>	<i>Quercus coccinea</i>	Iowa City, IA		7/3/2018
1416-043-3D	30	<i>Melikaiella tumifica</i>	<i>Quercus coccinea</i>	Iowa City, IA		7/3/2018
888-094-001	31	<i>Amphibolips quercusrugosa</i>	<i>Quercus palustris</i>	Iowa City, IA	6/9/2017	7/2/2017
877-045-001A	31	<i>Amphibolips quercusostensackenii</i>	<i>Quercus velutina</i>	Tiffin, IA	6/8/2017	6/21/2017
825-045-001	31	<i>Andricus quercusingularis</i>	<i>Quercus rubra</i>	Iowa City, IA	6/1/2017	6/15/2017
1367-047-2	31	<i>Dryocosmus cinereae</i>	<i>Quercus imbricaria</i>	Iowa City, IA	5/24/2018	6/9/2018
744-047-7B	31	<i>Dryocosmus cinereae</i>	<i>Quercus velutina</i>	Iowa City, IA	5/9/2017	6/5/2017
816-047-3B	31	<i>Dryocosmus cinereae</i>	<i>Quercus rubra</i>	Tiffin, IA	5/25/2017	6/6/2017
1343-038-4	31	<i>Dryocosmus quercuspalustris</i>	<i>Quercus velutina</i>	Tiffin, IA	5/25/2017	6/9/2018

**Table A2 – continued**

1351-038-4	31	<i>Dryocosmus quercuspalustris</i>	<i>Quercus coccinea</i>	Tiffin, IA	5/19/2018	6/9/2018
212-038-8	31	<i>Dryocosmus quercuspalustris</i>	<i>Quercus imbricaria</i>	Iowa City, IA	4/26/2016	6/18/2016
882_038_003	31	<i>Dryocosmus quercuspalustris</i>	<i>Quercus palustris</i>	Tiffin, IA	6/8/2017	6/12/2017
857-087-1	31	<i>Dryocosmus quercusnotha</i>	<i>Quercus palustris</i>	Iowa City, IA	6/7/2017	6/12/2017
857-087-003	31	<i>Dryocosmus quercusnotha</i>	<i>Quercus palustris</i>	Iowa City, IA	6/7/2017	7/3/2017
544-070-3	33	<i>Callirhytis quercuscornigera</i>	<i>Quercus rubra</i>	St Peters, MO	9/2/2016	5/21/2018
837-070-27A	33	<i>Callirhytis quercuscornigera</i>	<i>Quercus palustris</i>	St Louis, MO	6/3/2017	6/29/2017
601-002-6	34	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Urbana, IL	9/6/2016	9/29/2016
661-002-2A	34	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Tiffin, IA	10/18/2016	5/13/2017
984-001-003	34	<i>Acraspis macrocarpa</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	7/11/2017	8/3/2017
913-086-005A	34	<i>Amphibolips quercusinanis</i>	<i>Quercus rubra</i>	Tiffin, IA	6/20/2017	7/3/2017
177-008-3	34	<i>Andricus quercusflocci</i>	<i>Quercus macrocarpa</i>	Spirit Lake, IA	4/2/2016	5/12/2016
703-061-001	34	<i>Andricus quercusflocci</i>	<i>Quercus alba</i>	Iowa City, IA	4/12/2017	5/2/2017
1184-051-1B	34	<i>Callirhytis quercusfutilis</i>	<i>Quercus alba</i>	White Oak, PA	9/9/2017	9/12/2017
1450-51-3	34	<i>Callirhytis quercusfutilis</i>	<i>Quercus alba</i>	Dodgeville, WI	7/28/2018	8/7/2018
1459_051_8	34	<i>Callirhytis quercusfutilis</i>	<i>Quercus alba</i>	Wyoming, WI	7/28/2018	8/11/2018
865-051-006	34	<i>Callirhytis quercusfutilis</i>	<i>Quercus alba</i>	Tiffin, IA	6/8/2017	6/26/2017
963-051-001	34	<i>Callirhytis quercusfutilis</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	7/4/2017	7/6/2017
JRO-1-5B	34	<i>Philonix nigra</i>	<i>Quercus alba</i>	Capon Bridge, WV	8/14/2019	9/1/2019
548-002-4B	35	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Peducah, KY	9/3/2016	9/5/2016
1009-002-7	35	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Iowa City, IA	7/14/2017	11/6/2017
1566-002-5A	35	<i>Acraspis erinacei</i>	<i>Quercus alba</i>	Vestal, New York	10/17/2018	6/8/2019
602-003-1B	35	<i>Acraspis pezomachoides</i>	<i>Quercus alba</i>	Urbana, IL	9/6/2016	9/10/2016
1181-003-2	35	<i>Acraspis pezomachoides</i>	<i>Quercus alba</i>	White Oak, PA	9/9/2017	11/12/2017
1215-080-002	35	<i>Acraspis prinoides</i>	<i>Quercus muehlenbergii</i>	Urbana, IL	9/11/2017	10/12/2017
643-004-4	36	<i>Acraspis villosa</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	9/27/2016	5/20/2017
870-016-008C	36	<i>Melikaiella ostensackeni</i>	<i>Quercus rubra</i>	Tiffin, IA	6/8/2017	6/27/2017
133-001-3	36	<i>Acraspis macrocarpa</i>	<i>Quercus macrocarpa</i>	Spirit Lake, IA	3/12/2016	5/23/2016

**Table A2 – continued**

1215-080-001	36	<i>Acraspis prinooides</i>	<i>Quercus muehlenbergii</i>	Urbana, IL	9/11/2017	10/4/2017
1399-007-1B	36	<i>Andricus foliaformis</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	6/15/2018	6/18/2018
860-007-9D	36	<i>Andricus foliaformis</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	6/7/2017	6/18/2017
859-013-009A	36	<i>Callirhytis flavipes</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	6/7/2017	6/26/2017
500-011-5A	39	<i>Andricus quercusstrobilanus</i>	<i>Quercus bicolor</i>	Iowa City, IA	8/18/2016	8/20/2016
546-019-6B	32a	<i>Disholcaspis quercusglobulus</i>	<i>Quercus alba</i>	Peducah, KY	9/3/2016	9/27/2016
1081-006-007	32a	<i>Andricus quercusfrondosus</i>	<i>Quercus alba</i>	Cape Girardeau, MO	8/24/2017	9/10/2017
YMZ1B	32a	<i>Callirhytis quercusclavigera</i>	<i>Quercus coccinea</i>	Gainesville, FL	11/3/2019	
700-006-5	32b	<i>Andricus quercusfrondosus</i>	<i>Quercus bicolor</i>	Iowa City, IA	4/12/2017	5/4/2017
400-016-10A	32c	<i>Melikaiella ostensackeni</i>	<i>Quercus rubra</i>	Traverse City, MI	7/12/2016	8/7/2016
969-016-009H	32c	<i>Melikaiella ostensackeni</i>	<i>Quercus palustris</i>	Iowa City, IA	7/6/2017	7/26/2017
1047-001-002	32c	<i>Acraspis macrocarpae</i>	<i>Quercus macrocarpa</i>	Spirit Lake, IA	8/13/2017	8/31/2017
678-006-2A	32c	<i>Andricus quercusfrondosus</i>	<i>Quercus macrocarpa</i>	Iowa City, IA	3/29/2017	4/29/2017
1074-100-003	32c	<i>Callirhytis quercusgemmaria</i>	<i>Quercus rubra</i>	Traverse City, MI	8/13/2017	9/4/2017
1075-100-003	32c	<i>Callirhytis quercusgemmaria</i>	<i>Quercus rubra</i>	Traverse City, MI	8/13/2017	10/25/2017
1344-123-21E	32c	<i>Callirhytis quercusoperator</i>	<i>Quercus velutina</i>	Tiffin, IA	5/19/2018	6/9/2018
1344-123-25A	32c	<i>Callirhytis quercusoperator</i>	<i>Quercus velutina</i>	Tiffin, IA	5/19/2018	6/13/2018
695-020-4	32c	<i>Disholcaspis quercusmamma</i>	<i>Quercus macrocarpa</i>	Iowa city IA	4/9/2017	5/10/2017
993-097-2A	32c	raised vein	<i>Quercus palustris</i>	Iowa City, IA	7/11/2017	6/10/2018
1256-118-11	38*	<i>Neuroterus irregularis</i>	<i>Quercus stellata</i>	Austin, TX	4/12/2018	5/25/2018
1256-118-13	38*	<i>Neuroterus irregularis</i>	<i>Quercus stellata</i>	Austin, TX	4/12/2018	5/28/2018
583-016-5	40*	<i>Melikaiella ostensackeni</i>	<i>Quercus palustris</i>	Walton, KY	9/5/2016	9/14/2016
843_016_15D	40*	<i>Melikaiella ostensackeni</i>	<i>Quercus rubra</i>	Hannibal, MO	6/3/2017	7/4/2017
843-016-39	40*	<i>Melikaiella ostensackeni</i>	<i>Quercus rubra</i>	Hannibal, MO	6/3/2017	11/25/2017
843-016-5A	40*	<i>Melikaiella ostensackeni</i>	<i>Quercus rubra</i>	Hannibal, MO	6/3/2017	6/16/2017
294-049-20	40*	<i>Callirhytis scitula</i>	<i>Quercus imbricaria</i>	Iowa City, IA	6/6/2016	6/22/2016
1317-038-1	40*	<i>Dryocosmus quercuspalustris</i>	<i>Quercus palustris</i>	Iowa City, IA	5/15/2018	11/12/2018
993-097-1A	40*	raised vein	<i>Quercus palustris</i>	Iowa City, IA	7/11/2017	6/19/2018

**Table A3:** Summary of NGS sequencing data. The second column indicates the number of reads belonging to each individual wasp, the third column indicates the number of contigs from *de novo* assemblies.

Lab specific ID	Raw reads	Contigs	Average contig length (bp)	Number of UCE loci	Avg coverage per UCE locus	Lab specific ID	Raw reads	Contigs	Average contig length (bp)	Number of UCE loci	Avg coverage per UCE locus
1007_024_001	1219026	29112	421	2000	160	558_081_1A	598879	15382	416	1811	85
1009_002_7	526040	11746	472	1702	68	558_081_1B	625981	13824	464	1695	94
1059_024_1A	971844	19632	424	1804	137	565_021_001	749165	17915	451	1886	86
1047_001_002	960673	22806	397	1842	145	583_016_5	806069	18787	423	1689	109
1074_100_003	1045629	23708	404	1842	155	601_002_6	788347	16786	438	1960	97
1075_100_003	756639	21688	469	1928	77	602_003_1B	683221	15185	434	1813	92
1081_006_007	912295	29990	428	1944	97	612_077_1	613081	15590	478	1346	60
1114_104_006	1230673	25617	400	1906	167	630_023_5	940220	28109	444	1960	98
1135_108_10	1450493	35571	364	1903	140	631_005_3	1135466	26580	408	1972	135
1181_003_2	1435031	31796	428	1922	178	631_005_6	1163834	23734	458	1917	152
1184_051_1B	663950	16555	480	1807	80	643_004_4	508491	11888	451	1849	62
1215_080_001	1385378	26873	432	1855	204	643_004_5	527394	9303	496	1848	74
1215_080_002	641304	16363	423	1767	94	661_002_2A	795485	26117	414	1923	88
1238_117_6	1597620	33211	383	1832	197	678_006_2A	799326	16621	479	1912	89
1243_117_31	916427	26259	480	1911	88	695_020_4	2349875	36988	379	2008	307
1256_118_11	779567	24376	434	1916	77	700_006_5	2009052	21060	334	1903	285
1256_118_13	774987	19970	419	1887	93	701_019_32A	906273	15903	411	1953	123
1317_038_1	782036	16168	484	1838	81	703_061_001	644319	15553	413	1863	96
133_001_3	787675	27009	447	1896	85	744_047_7B	929807	20091	430	1836	130
1343_038_4	571959	13827	461	1836	70	816_047_3B	614420	13850	455	1879	74
1344_123_21E	1078785	21027	489	1865	113	825_045_001	1026152	22167	411	1849	141
1344_123_25A	1703773	52946	439	1917	167	826_030_9A	1363863	23447	371	1892	194
1351_038_4	835328	18766	400	1821	126	837_070_27A	1386735	28454	421	1961	164
1367_042_2	647915	14683	436	1938	82	843_016_15D	681848	13744	495	1703	80
1384_030_1A	4287195	5304	291	1248	495	843_016_39	1580616	25299	510	1814	183

**Table A3 – continued**

1399_007_1B	1105808	25431	424	1948	138	843_016_5A	1521336	27088	468	1884	186
1416_043_3C	791291	24519	464	1895	83	857_087_003	1005053	23193	388	1801	155
1416_043_3D	930301	34766	383	1906	97	857_087_1	751550	16779	457	1892	91
1450_51_3	483655	11269	465	1715	70	859_013_009A	986665	40289	374	1930	108
1459_051_8	811711	17596	470	1780	102	860_007_9D	468842	12731	385	1723	76
1466_126_2	492019	12351	457	1795	61	861_042_77C	679473	19037	356	1807	101
1520_150_1A	507533	16329	443	1866	56	862_013_014	923832	25820	448	1883	111
1523_145_1	847219	14948	372	1891	119	865_051_006	658950	14961	455	1821	75
1548_009_2	712543	17568	462	1874	91	866_035_019	851873	22001	441	1895	99
1553_005_8	683264	15114	457	1886	73	866_035_14C	730077	16186	442	1719	116
1566_002_5A	1979100	60864	432	1921	187	870_016_008C	837016	18073	397	1965	126
1574_104_6	1148016	23054	371	1906	160	877_045_001A	1944805	55714	435	1880	179
1589_160_15A	970879	23085	416	1802	144	881_013_005	650938	17335	467	1791	70
1589_160_2B	1042322	22557	373	1965	163	882_038_003	1473594	3514	322	1083	196
1622_025_2A	736649	18014	480	1830	87	884_039_010A	784674	18704	379	1895	107
1662_025_2B	922316	18615	426	1879	132	884_039_012A	1260341	25495	376	1770	177
1664_199_4A	960131	21154	457	1816	121	888_094_001	398092	10471	433	1597	66
1664_199_4B	1701805	32485	410	1899	229	913_086_005A	534014	12830	418	1821	84
1693_221_1	1760732	53457	444	1863	164	918_042_8K	1053484	20634	444	1828	133
1695_223_1	1056527	25970	444	1865	123	920_010_1A	836183	20052	398	1847	124
1696_180_3	648909	17016	440	1876	78	954_020_1	614280	21682	380	1901	76
1703_227_1	585386	13382	450	1850	68	963_051_001	723154	16890	469	1856	82
1711_180_1A	456531	11927	460	1785	50	969_016_009H	687609	16602	456	1766	86
177_008_3	1175595	23461	399	1918	160	984_001_003	1001765	19938	471	1826	126
212_038_8	928242	19849	447	1935	109	993_097_1A	932245	19724	448	1789	133
294_049_20	2338532	74306	393	2011	215	993_097_2A	806412	18716	423	1889	94
294_049_27	1106939	24817	407	1836	170	LZ4717	400954	10841	429	1604	69
296_010_14	672486	12679	405	1898	100	LZ3258	703946	15723	484	1825	75
341_053_52B	643230	17360	428	1762	100	F1001	925992	22685	424	1901	121

**Table A3 – continued**

369_053_19A	908835	20121	390	1853	140	JRO_1_5B	688821	15765	418	1881	99
379_021_13A	735842	16897	461	1858	89	KW004	597888	13935	421	1869	76
400_016_10A	727266	18185	413	1808	110	LZ4238	845443	21146	406	1957	111
476_002_1A	1214201	30556	392	1944	110	LZ6500	1495894	6154	343	1477	180
500_011_29E	599716	11748	387	1912	91	LZ6608	1214206	22787	406	1916	168
500_011_5A	772368	19957	388	1914	104	LZ6610	124397	1298	352	617	21
538_066_3	422528	17520	384	1798	50	P184	793293	24558	446	1946	83
538_066_6	1398982	31049	406	1952	164	P47	663731	14590	411	1907	101
539_067_5A	797900	20331	449	1880	98	YMZ1	1091851	35138	353	1936	114
539_067_7A	966704	20474	397	1947	140	YMZ1B	1188437	31438	409	1872	80
544_070_3	1133491	23169	423	1890	149	YMZ2	1062008	21257	468	1905	103
546_019_6B	665285	17172	432	1740	107	YMZ6A	1161832	22339	387	1861	150
548_002_4B	803143	16767	480	1858	83	YMZ6B	759223	16102	447	1775	111
555_019_4A	1001403	21333	465	1903	114						