Interactions Between the Velvety Tree Ant *Liometopum occidentale* (Hymenoptera: Formicidae) and *Pseudacteon* Phorid Flies (Diptera: Phoridae)

A thesis submitted in partial fulfillment of the requirements

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ABSTRACT

Interactions Between the Velvety Tree Ant *Liometopum occidentale* (Hymenoptera: Formicidae) and *Pseudacteon* Phorid Flies (Diptera: Phoridae)

By
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Phorid fly parasitoids of ants are capable of decapitating their host during development and have been used as attempted biological control agents for imported fire ants. Little is known about the interactions between the native Velvety Tree Ant host, *Liometopum occidentale*, and its phorid fly parasitoids. This system is native to Southern California, occupying endangered habitats that have been in decline due to human development, changing weather patterns, and fierce competition from invasive species. To further understand the ecological significance and behavior of this system, I examined interactions of these parasitoids and the Velvety Tree Ants by observing and quantifying the behavioral displays exhibited in the presence of phorid fly parasitoids. To understand parameters of activity of the parasitoids, I measured temperature and time of day through five temporal periods encompassing the parasitoids’ season. Two different phorid species were found attacking *Liometopum occidentale*: *Pseudacteon californiensis* Disney and an undescribed species of *Pseudacteon* herein designated *Pseudacteon sp A*. Because *P. sp A* keys to the genus *Microselia* Schmitz, I carried out molecular analyses to determine the
phorids’ evolutionary relationships. Based on analysis of three gene regions, *Pseudacteon sp A* and *Pseudacteon californiensis* are sister-species relative to other *Pseudacteon* parasitizing *Solenopsis* fire ants. The status of some North American phorid species, currently assigned to *Microselia*, needs to be further investigated.
Parasitoids are parasitic organisms dependent on host organisms for their survival, ultimately resulting in the death of their host. The parasitic feeding stage in a parasitoid’s life cycle is its larval stage. In phorid flies of the genus *Pseudacteon*, females use their ovipositor to penetrate the host ant’s membranous weak points and insert an egg into its thorax or abdomen. Once the egg hatches, the phorid larva emerges and migrates internally to the head capsule of the ant host, consuming the hemolymph, muscle and nervous tissue and killing the host. This process usually results in the detachment of the ant host’s head capsule from the rest of the body. The phorid larva pupates inside the head capsule and completes its development there until it emerges as an adult phorid fly (Porter et al., 1995b).

During its larval stage, the parasitoid larva develops inside the host and builds up energy reserves before emerging as an adult. Little is known about the feeding and foraging behavior of phorid parasitoids of ants beyond this larval stage within the host organism. Studies have shown that some phorid parasitoids are able to feed as adults (Brown, 2000), with beneficial results on overall phorid longevity (Fadamiro et al., 2005). Once an adult *Pseudacteon* has emerged from the host, dispersal is generally limited, with most remaining within a few hundred meters of their emerging sites, but some outliers are able to travel a couple of kilometers. Some *Pseudacteon* species are known to disperse up to 74 km over a 3.5 year period (Farnum and Loftin, 2011).

Parasitic flies are organisms of particular interest as the family Phoridae parasitizes a broad range of insect species. The effects of this parasitism can range from
unapparent behavioral modifications in the some host organisms to more pronounced cases such as in honey bees, where phorid parasitism is a contributing factor to colony collapse (Core et al., 2012). Many phorid flies are highly host specific with each host ant species targeted by its own specific phorid fly (Porter et al., 1995a; Porter, 2000). Host specificity has been key in the attempted biological control of *Solenopsis* fire ant pests through the use of their natural phorid parasitoids (Folgarait et al., 2003). The high specificity of phorids has resulted in a physiological dependence with specific adaptations by the parasitoids to their ant hosts (Folgarait et al., 2002; Porter, 2000).

The behavioral modifications caused by phorid parasitism in ants has been the focus of several studies that attempt to understand the behavior, relationship, interactions, and adaptations between ant hosts and their phorid parasitoids (Feener and Brown, 1992; LeBrun and Feener, 2002; Lebrun, 2005). Studies have shown that parasitoids alter the community structure and interspecies interactions of ant hosts (Hsieh and Perfecto, 2012; LeBrun and Feener, 2002). In ants, research studies tried to measure the presence of modifications relating to interspecies interactions as a result of parasitism. For instance, Feener et al., (1981) discovered that *Apocephalus* shifted the competitive balance from its target ant host *Pheidole dentata* over to its competitor *Solenopsis texana*. This competitive shift was a result of *Apocephalus* triggering an apparent and measurable response that resulted in *Pheidole* abandoning resources when faced with the threat of parasitism.

Phorids parasitoids of ants are masters at utilizing host resources, and this efficiency may have contributed to their incredible success as parasitoids. Four major types of differential host use have been described by Brown (1999): temporal differences,
caste or size differences, host activity differences and body part differences. The
temporal differences seen in phorid parasitoids of ants include seasonal variation,
temperature variations and temporal variations. Temperature in particular, as it is tied to
climate patterns, has been known to influence activity patterns in some phorid species
(Folgarait et al., 2003, 2007b; Pesquero et al., 1996; Plowes et al., 2012).

Ant-phorid systems are abundant in nature and many have been studied to answer
specific ecological questions. Some of these questions can be answered and quantified by
studying an ant-phorid system, more specifically, they can be answered in the local
Southern California system of the Velvety Tree Ant, *Liometopum occidentale* and its two
phorid parasitoids *Pseudacteon californiensis* and *Pseudacteon sp A*. This ant-phorid
system can be found in the two national forests surrounding the Los Angeles basin. The
Angeles National Forest is located mostly within Los Angeles County, covering over
600,000 acres with elevation ranging from 1200 feet to over 10,000 ft (USDA, 2016a).
Los Padres National Forest occupies over one million acres spanning several counties in
California including Santa Barabara, Ventura, and Los Angeles County with elevation
ranging from sea level to over 8,000 feet (USDA, 2016b). These national forests provide
federally protected land with habitats that support and maintain a thriving ant-phorid
system whose behaviors and activity patterns can be studied and quantified.
CHAPTER 2

Species Diversity and Identification of *Pseudacteon* Phorid Flies Hosted by

*Liometopum occidentale*

INTRODUCTION

Among the approximately 20 families of parasitic flies (O’Hara, 2008), the Phoridae is of particular interest. Their attacks on ant hosts result in a wide range of responses, from little, if any response, to dramatic shifts in food retrieval and activity patterns (Elizalde and Folgarait, 2012). Many phorid flies are highly host specific with each host ant species targeted by its own specific phorid fly (Porter et al., 1995a; Porter, 2000). Host specificity has been key in the attempted biological control of *Solenopsis* fire ant pests through the use of their natural phorid parasitoids (Folgarait et al., 2003). This specificity has been observed in the United States native fire ant *Solenopsis geminata* and its associated phorid parasitoids. Although *Solenopsis geminata* has coexisted with the invasive ants *Solenopsis invicta* and *Solenopsis richteri* for decades, exposure of these closely related ants to the native phorids hosted by *S. geminata* has not resulted in a host switch (Folgarait et al., 2002). Although some parasitoids such as *Pseudacteon curvatus*, a parasitoid of *Solenopsis invicta*, are able to develop in closely related species like the native southern fire ant *Solenopsis xyloni*, rates of parasitism in these non-native hosts are two thirds lower. This lower viability of phorids in non-native hosts may be due to complications in early larval development (Porter, 2000). Host specificity gives rise to a physiological dependence with specific adaptations by the parasitoids to their ant hosts.
These adaptations allow the parasitoid to bypass the host’s physiological defenses and successfully complete their life cycle (Folgarait et al., 2002; Porter, 2000).

There are some instances where there has been a host shift as seen in the parasitic fly *Apocephalus borealis*, whose natural host is *Bombus vosnesenskii*, the Bumble Bee, but has recently expanded its range to include the Honey Bee, *Apis mellifera*. (Core et al., 2012). The evolutionary history of phorids is under constant research, and when it comes to species host jumps and the physiological ability of phorids to parasitize non-native hosts, the prevailing consensus is that this ability is likely limited to closely related organisms. This is true of ant parasitoids studied in the *Solenopsis* complex, as survival of phorids reared from non-closely related hosts is significantly decreased (Folgarait et al., 2002; Porter, 2000). The genus *Apocephalus* has been of particular interest in this research for its plasticity and ability, particularly when it comes to the specialized subgenus *Mesophora*, to parasitize a range of insects and other arthropods including bees, wasps and spiders. This broad range of host organisms makes *Mesophora* key to understanding the evolutionary history and mechanisms of host jumps in phorid parasitoids. In the genus *Apocephalus*, ants are considered the primitive host as the majority of phorid parasitoids in this genus are hosted by ants. *Mesophora* includes a phorid species, *A. mortifer*, which can be reared from Cantharoid beetles, a host that is extremely divergent from ants (Brown, 1996).

Phorids as a group have a rich evolutionary history dating back a hundred million years (Solórzano Kraemer et al., 2011). Phorid parasitoids of ants have been incredibly successful, with mass species radiation across a diverse range of hosts (Brown, 1999). Additionally, phorid diversity has been attributed in part to differential host use, whereby
multiple species parasitize a single ant species but differentiate in their use of host resources. Differential host use has been so successful in some cases that a single host individual is able to host multiple larva from different species (Brown, 1999). Phorids are incredibly diverse with hundreds of described species and hundreds more yet to be discovered. The study of ant-phorid systems allows us to gain a better understanding of the evolutionary history and relationships between phorid parasitoids and their ant hosts. Additionally, studying these ant-phorid systems allows us to examine the evolutionary relationships between the phorids themselves. In this chapter I have described the species diversity of phorid parasitoids hosted by *Liometopum occidentale* and carried out molecular analyses to increase our understanding of the evolutionary relationship between the different phorid species in this system and the possible implications for other closely related species.

MATERIALS AND METHODS

Velvety Tree Ants, *Liometopum occidentale* and their associated phorid fly parasitoids were collected from July to September of 2015 from two main sites, Placerita Canyon State Park (34.38°N, 118.46°W) in the Angeles National Forest and the Chuchupate Campground (34.78°N, 119.00°W) in the Los Padres National Forest (Figure 1). Tuna placed on an index card was used to locate the ants. Nests were found by following foraging ants back to the trees that housed their nests. *Liometopum occidentale* was mainly found near streams or areas with lush vegetation and oak trees in both national forests. In the absence of oaks, the Velvety Tree Ant was also found using various species of pine trees.
Phorid flies were collected at tuna bait stations that attracted their hosts. Two species of phorids were encountered, *Pseudacteon californiensis* Disney and an undescribed species, referred to here as *Pseudacteon sp A*. These two phorids differ in ovipositor morphology, wing patterns, and features of the tarsal segments. I identified 289 total phorids and placed them in the two species groups using ovipositor morphology (Figure 2). I examined the species for evidence of hybridization via intermediate morphological features. Dr. Brian Brown, Curator of Entomology at the Los Angeles County Natural History Museum, identified the phorid species. These species identifications were further confirmed through molecular analyses. I measured a subsample of 24 phorids, 12 phorids from each species with a Dino-Lite AM4815ZT Digital Microscope, taking measurements for overall body length and ovipositor length. I used a t-Test to test for significant differences in body length and ovipositor morphology between the two species.

*Pseudacteon sp A* specimens were collected at Placerita Canyon Park and *Pseudacteon californiensis* specimens were collected from Chuchupate Campground for molecular analyses. DNA extractions used a FastDNA SPIN Kit Protocol from MP Biomedicals (Solon, Ohio) modified as follows. A 2 mL microcentrifuge tube with O-ring caps filled with ~40 beads of Lysing matrix D was prepared, to which 800 µL of CLS-TC Cell Lysis Solution for insects was added. Four phorids of the same species and from the same site were added to the tube. This mixture was homogenized with the FastPrep instrument included with the kit at speed 15 (1900 cycles per minute) for 2 minutes. Alternatively a Fisher Vortex Genie 2 was used at speed 8 for 20 minutes to achieve the same results. Homogenized tubes were centrifuged at 14,500 rpm for 30 minutes to
precipitate phorid remains. Supernatant (~700 µL) was transferred to a 2 mL microcentrifuge tube and an equal volume of Binding Matrix was added. Tubes were gently inverted at room temperature for 5 minutes to mix. Half of the suspension was transferred to a SPIN Filter and centrifuged at 14,500 rpm for 3 minutes. The catch tube was emptied and the remaining suspension added to the SPIN Filter and centrifuged as before. The catch tube was emptied again, then 500 µL of prepared SWES-M was added, and the pellet resuspended gently using the force of the liquid from the pipet tip. This mixture was centrifuged at 14,500 rpm for 3 minutes and the contents of the catch tube discarded. Without any addition of liquid, the tube was centrifuged a second time at 14,500 rpm for 3 minutes to ensure that all the ethanol had been eluted. The catch tube was replaced with a new, clean tube. DNA was eluted by gently resuspending the Binding Matrix above the SPIN filter in 100 µL of DES. The tube was capped and incubated for 5-10 minutes at 55°C in a water bath. The tube was then centrifuged at 14,500 rpm for 3 minutes to bring eluted DNA into the clean catch tube and the SPIN filter was discarded. A Nanodrop 2000c from ThermoFisher Scientific (Waltham, Massachusetts) was used to measure DNA concentrations of each tube extracted to calculate the required PCR volumes.

Three universal Pseudacteon primers developed by the Brackenridge Field Laboratory at the University of Texas, Austin were used for the phylogenetic analyses. The custom primers were ordered from Invitrogen and diluted to 100 pmol/µL for use in the PCR reactions. The primers were CO1 (Cytochrome oxidase I), 28S (ribosomal 28s), and LepWG (WG or Wingless). The CO1_fwr sequence was 5’–CAA CAY TTA TTA TTY TTY GG–3’ and the CO1_rev sequence was 5’–TCC ATT GCA CTA ATC TGA TTY TTY GG–3’.
The 28s_fwr sequence was 5’– GCG AAC AAG TAC CGT GAG GG –3’ and the 28s_rev sequence was 5’– TAG TTT ACC ATC TTT CGG GTC –3’.

The LepWG_fwr sequence was 5’– GAR TGY AAR TGY CAY GGY ATG TCT GG –3’ and the LepWG_rev sequence was 5’– ACT ICG CAR CAC CAR TGG AAT GTR CA –3’.

Polymerase chain reaction (PCR) amplifications for 25 µL reactions were carried out with the following recipe. For one reaction: 5 µL 5x One Taq DNA Polymerase Buffer, 0.5 µL 10 mM dNTP mix, 0.5 µL Forward Primer, 0.5 µL Reverse Primer, 0.25 µL One Taq DNA Polymerase, 13.25 µL of Nuclease Free H2O. 1-5 µL (100 ng) DNA extract. Nuclease-free water was added after the addition of the DNA extract to complete the 25 µL reaction. Thermocycler conditions were initial denaturing at 94°C 5 min, 30 cycles of 30 sec denaturing at 94°C, 1 min annealing at 60°C, 1.45 min extension at 68°C, followed by a final extension of 5 min at 68°C.

A 1% agarose solution was prepared using 100 mL 1x TAE and 1g agarose. The 1% agarose solution was heated for 45 seconds in a microwave and allowed to cool to the touch. 5 µL of red dye was added for every 100 mL of agarose solution and poured to create a 1% agarose gel. 10 µL total volume was added to each well consisting of 3 µL of water, 2 µL of a 6x loading dye, and 5 µL of the PCR sample. Invitrogen’s (ThermoFisher Scientific, Waltham, Massachusetts) 1kb plus DNA ladder was used. Gel electrophoresis was performed for 70 minutes at 100 Volts.

PCR purification and DNA sequencing was performed by Laragen, Inc (Culver City, California). I cleaned up the raw sequences and conducted the molecular analyses using Geneious R9 (Biomatters Inc, Newark, New Jersey) (Figures 3, 4 and 5). I analyzed CO1, 28S and WG for single nucleotide polymorphisms (SNPs) and CO1 and WG for...
amino acid substitutions. Phylogenetic trees were generated in Geneious using the Brackenridge Field Laboratory phorid fly database for each of the sequenced genes. The individual gene trees were generated using Neighbor-Joining, the Tamura-Nei genetic distance model, and resampled with 1000 replicates that were used to create a consensus tree for each gene. A consensus tree of all three genes with each species represented by at least 2 out of 3 genes, CO1, 28s and WG, was generated using Neighbor Joining and Bootstrapped 10,000 times.

RESULTS

Two species of phorid flies were found ovipositing on the Velvety Tree Ants based on identifications by Dr Brian Brown and confirmed by molecular analyses using three amplified *Pseudacteon* species genes. The species identified were *Pseudacteon californiensis* and a new species *Pseudacteon sp A*. There were distinct morphological differences between the species, most notable ovipositor morphology, used here to identify the species (Figure 2). *Pseudacteon sp A* was slightly larger than *P californiensis* but the difference was not significant (Figure 6). There was a difference in ovipositor length between the species, with *P. californiensis* exhibiting significantly larger ovipositors than *P. sp A* (t = 4.82, df = 22, p < 0.001, Figure 7). Both *Pseudacteon californiensis* and *Pseudacteon sp A*, show similar profiles on the amplified regions. The amplified mitochondrial Cytochrome Oxidase I (CO1) regions were ~850bp. The amplified nuclear gene Wingless (LepWG or WG) regions were ~650bp. The amplified nuclear gene 28S regions were ~620bp. The CO1 species sequences had 88 single nucleotide polymorphisms (10.33% divergence) and 6 amino acid substitutions (2.12%
divergence). The WG sequences had 8 single nucleotide polymorphisms (1.23% divergence) and 8 amino acid substitutions (3.70% divergence). The 28S sequences had 9 single nucleotide polymorphisms (1.45% divergence). The single nucleotide polymorphisms and amino acid substitutions for each gene are listed in Figures 8-12. The three single gene phylogenetic trees, using CO1, WG and 28S (Figures 13-15), and a concatenated tree of all three genes (Figure 16), place *Pseudacteon sp A* as a sister taxa to the already established *Pseudacteon* clade, and a sister species of *Pseudacteon californiensis*.

DISCUSSION

The studies of ant-phorid systems have been the cornerstone of phorid related behavioral studies for decades. Studies of the intricate interactions between these organisms have expanded our collective behavioral and natural history knowledge of phorids and ants (Brown and Feener, 1991; Folgarait et al., 2003; LeBrun and Feener, 2002). The study of *Pseudacteon* phorids has been instrumental in expanding this knowledge base and there have been extensive studies showing the impact these parasitoids have on their hosts. *Pseudacteon* phorids have been of particular interest in ecological efforts to contain invasive species via natural biological controls, particularly that of the imported fire ants, *Solenopsis invicta* and *Solenopsis richteri* (Folgarait et al., 2003). *Pseudacteon* is a rich genus of phorids, with its richness attributed to the radiation of *Solenopsis* in South America, where most of the described *Pseudacteon* phorids and the majority of *Solenopsis* ant species are found (Folgarait et al., 2007b). The ability of these diverse phorids to use a combination of visual, chemical, acoustic and olfactory
cues to locate and hone in on their hosts for oviposition make these parasitoids potentially effective self sustaining biological control agents (Brown and Feener, 1991; Chen et al., 2012; Mathis and Tsutsui, 2016; Sharma and Fadamiro, 2013; Witte et al, 2010).

In this study I found two *Pseudacteon* species of phorids attacking the Velvety Tree Ant, *Liometopum occidentale*. The initial classification of the new *Pseudacteon sp A* phorid left an open question as to which genus it should be placed in. Based on morphological features it belongs to the genus *Microselia* Schmitz, of which the only identified North American species is *Microselia texana* (Brown, pers. comm., July 17, 2015). Morphologically, the species are similar in size, with *P. sp A* being slightly but not significantly larger than *P. californiensis*. The two species had different ovipositor morphology, which was the main structure I used to separate the species. *P. californiensis’s* ovipositor is significantly larger and more pronounced than the one seen in *P. sp A*.

The molecular analyses of the species also showed a difference between the species even while using the highly conserved genes – CO1, WG and 28S. For the CO1 gene sequences, there were a striking 88 SNPs between the species representing a 10.33% divergence between them. The encoded amino acids however only showed 6 AA substitutions, representing a 2.12% divergence in the CO1 gene between the species, showing that although the amplified regions showed a high number of SNPs, most were silent mutations that did not significantly alter the encoded primary structure. The WG gene showed 8 SNPs representing a 1.23% divergence, however unlike in the CO1 gene, each one of these WG SNPs resulted in an amino acid substitution, representing a 3.70% divergence in the primary structure between the species. The 28S gene was only analyzed
for SNPs since it is a ribosomal RNA component and not translated. There were 9 SNPs for the 28S gene between the species, representing a 1.45% divergence in the sequence.

The phylogenetic trees generated for each gene place *Pseudacteon sp A* among taxa of the already established *Pseudacteon* clade, and a sister species of *Pseudacteon californiensis*. The tree generated for the WG gene shows both *Pseudacteon sp A*, initially though to be part of *Microselia*, and *Microselia texana* within the *Pseudacteon* clade. Although this could point to the New World *Microselia* belonging to *Pseudacteon*, the reliability of the available GenBank sequences for *Microselia texana* has been called into question due to inconsistent sequence reproduction attempts (Plowes, pers. comm., May 24, 2016). If further molecular analyses and sequences of *Microselia texana* support the *M. texana* GenBank sequences, this could resolve the question of whether *Microselia texana*, which *Pseudacteon sp A* resembles morphologically, also belong to *Pseudacteon*, as the phylogenetic evidence in this study suggests. Furthermore, Brown (1992) already suggested that at least some New World species of *Microselia* should be transferred to *Pseudacteon* based on morphology.

A concatenated consensus tree generated for all three genes shows the close relationship of *Pseudacteon sp A* to *Pseudacteon californiensis* and the *Pseudacteon* clade. The combined phylogenetic tree represents at least two of the three genes, CO1, 28S, and WG, for each species. The sequences extracted from *Pseudacteon sp A*, molecular analyses and the generated phylogenetic trees support the hypothesis that this new species belongs to *Pseudacteon*, despite its morphological features resembling *Microselia*. Based on morphological differences, consistent phylogenetic consensus
support, overlapping habitats, activity ranges, and shared host species with *Pseudacton californiensis*, this new phorid parasitoid was referred to as *Pseudacteone sp A*.

Phorid parasitoids of ants are generally species specific, with this host specificity resulting in a specific physiological dependence by the parasitoid, where it is only able to successfully parasitize and develop in the host it is adapted to or very closely related species (Folgarait et al., 2002; Porter, 2000). The Velvety Tree Ant, like other ant species (Brown, 1999; Plowes et al., 2012), has to deal with two simultaneous parasitoids, *Pseudacteon californiensis* and *Pseudacteone sp A*. This gives rise to some interesting questions regarding the evolutionary history of the phorids and their evolutionary relationship with *Liometopum occidentale*. Phorid parasitoids’ impact on ant communities can be seen in the evolutionary arms race between phorids and their ant hosts (Porter et al., 2004). This relationship results in phorids bypassing host physiological defenses, and the ants attempting to evade parasitism through behavioral modifications.

The presence of two phorid parasitoids in this local ant-phorid system is of particular interest and allows us to study the ecological implications of a two-phorid system on a native ant host. The presence of two parasitoids targeting the same ant could have come about via a species jump or have been a result of species radiation by the initial parasitoid. The specificity of phorid parasitoids may suggest that species radiation may be the more recent cause of phorid species diversification and host adaptation, but species jumps do occur and have been studied recently (Brown, 1996; Core et al., 2012). While *Apocephalus* displays the type of host jumps we’ve come to expect, between closely related Hymenoptera in the case of bees and bumble bees, the jump to such a
divergent group as the Cantharoid beetles shows that jumps to less closely related species do occur and may be more common over large evolutionary time scales (Brown, 1996). This is an interesting area of future research to gain a better understanding of the evolutionary history of a family that dates back a hundred million years (Solórzano Kraemer et al., 2011). In the case of *Pseudacteon californiensis* and *Pseudacteon sp A*, the closely related branches in the phylogenetic trees may imply that these two phorids may have recently, evolutionarily speaking, radiated into two different species, leaving *Liometopum occidentale* with two parasitoids.
INTRODUCTION

The behavioral modifications caused by phorid parasitism in ants has been the focus of several studies that attempt to understand the behavior, relationship, interactions, and adaptations between ant hosts and their phorid parasitoids (Feener and Brown, 1992; LeBrun and Feener, 2002; Lebrun, 2005). Studies have shown that parasitoids alter the community structure and interspecies interactions of ant hosts (Hsieh and Perfecto, 2012; LeBrun and Feener, 2002). In ants, research studies tried to measure the presence of modifications relating to interspecies interactions as a result of parasitism. For instance, Feener et al., (1981) discovered that Apocephalus shifted the competitive balance from its target ant host Pheidole dentata over to its competitor Solenopsis texana. This competitive shift was a result of Apocephalus triggering an apparent and measurable response that resulted in Pheidole abandoning resources when faced with the threat of parasitism.

Some of the more noticeable behavioral responses to phorid parasitism are gaster flagging and hiding in Solenopsis fire ants. Gaster flagging is the movement of the abdomen up and down in the air in a vibrating motion, the purpose of which is to spread venom as a defense mechanism (Obin and Vander Meer, 1985). In Liometopum occidentale, the defensive posturing is slightly different but still noticeable. The Velvety Tree Ants’ defensive behavior involves a portion of the ants ceasing their foraging activity, standing tall in a vertical position with their mandibles raised in the air. During
this display, some worker ants will continue to forage while others stand guard (LeBrun and Feener, 2002). This defense mechanism is notably distinguishable from normal foraging behavior in the absence of phorids, and in other ant species, has resulted in significant alterations to the host’s defense and foraging behavior (Elizalde and Folgarait, 2012).

Apparent behavioral modifications of the ant host, and more specifically a change in competitive interactions as a result of parasitism, has further been examined in different ant species, particularly in the South and Western United States. Subsequent studies have further linked phorid activity to behavioral modifications in ant species, often causing a shift in ecological balance as a result of the host organisms’ defensive behavior in reaction to the phorids (Elizalde and Folgarait, 2012; Plowes et al., 2012, 2011). The modified behavior seen in ants was readily apparent in the interactions by Apocephalus sp 8 and Pheidole diversipilosa. In these ant-phorid interactions there was a marked change in foraging behavior exhibited by the parasitized ant, P. diversipilosa. Foraging activity in this ant species decreased in excess of 90% when phorids were present. Being the dominant ant species in the ecosystem, a change in foraging behavior induced by A. sp 8 resulted in a turnover of resources from P. diversipilosa to its competitor, the Formica gnava ant (LeBrun and Feener, 2002). As ecosystem engineers, ants mediate a number of interactions between other organisms. A shift in species dominance induced by parasitism has the potential to alter the overall balance of an ecosystem and the organisms dependent on it (Hsieh and Perfecto, 2012).

The community interactions mediated by a dominant species in an ecosystem are often responsible for maintaining that ecosystem’s species balance. A shift in species
dominance for any reason has the potential to result in the emergence of a different species hierarchy in the affected ecosystem (Hsieh and Perfecto, 2012). Phorids as a result of their parasitism can cause remarkable changes in their host populations. Drastic shifts in species balance have been examined in the interactions between *Azteca instabilis*, a phorid-parasitized ant and *Azya orbiger*, a coffee berry borer. Although *A. instabilis* doesn’t directly compete with *A. orbiger*, a retreat of *A. instabilis* from the natural ecosystem caused by *Pseudacteon* parasitism leads to an explosion in *A. orbiger* populations, triggering a massive shift in the balance of the present species. This leads to declines among organisms preyed upon by *A. orbiger* and protected by *A. instabilis*. This drastic shift leads to billions of dollars in economic damage to coffee industries as a result of the parasitized ant’s inability to mediate the community interactions in the presence of phorid parasitism (Hsieh and Perfecto, 2012). In this chapter I describe the behavioral response of *Liometopum occidentale* to *Pseudacteon* phorid attacks, the specificity of that response and the phorid effect on ant foraging patterns.

MATERIALS AND METHODS

Behavioral interactions were observed between two phorid fly species and their ant host, the Velvety Tree Ant, in the Open Space areas surrounding the Placerita Canyon State Park (34.38°N, 118.46°W) in the Angeles National Forest (Figure 1). Tuna bait stations were placed at the base of Coast Live Oak trees to attract the ants. Bait stations consisted of redwood board cutouts measuring 0.25 x 3.5 x 5 inches with water based tuna placed on top. The hard redwood boards allowed ants to forage on the top surface of the board where they would be exposed to phorid flies.
Behavioral experiments occurred from July to August of 2014. Bait stations were placed in the afternoon on a given test day, adjacent to ant nests. Phorids arrived between late afternoon and the early evening hours, approximately from 3 to 6 pm. The arrival time fluctuated from around thirty minutes after bait placement on a cool day to hours on a hot day, where the temperatures did not begin to decline until the evening hours. Behavioral experiments were not run when ambient temperature exceeded 32°C in the evening or when wind speed exceeded 32 km/hr, as phorid activity was absent or reduced under these conditions.

Velvety Tree Ants were allowed to forage on the tuna baits while awaiting the arrival of the phorid flies. Ant behavior was observed in the absence of phorids and recorded with Nikon D3300 DSLR and Nikon D40X DSLR cameras. Ant foraging and defense behavior in the absence of phorids was recorded every four minutes until phorid arrival and every two minutes thereafter. The time interval for recording was at least forty minutes before the first oviposition event and forty minutes after. The arrival of the phorid flies could be confirmed by the noticeable response by the ants. After the first attempt at oviposition event by the phorids, images were taken every two minutes to document ant foraging and defense behavior in the presence of the flies. It was not possible to distinguish between the two species of phorids in video footage and still images. Ant behavior on the recorded still images was categorized in two ways, foraging or defense. A quadrant of the bait station was selected for each behavioral experiment and counted at each time interval.

To quantify ant host defensive behavior before and after phorid oviposition attempts, the selected quadrant was tallied for the number of ants showing a defensive
posture relative to the total number of ants on the quadrant. This yielded a proportion of ants defending at every time interval before and after oviposition events. An equation was derived for each trial based on the defense proportion in the intervals before the first oviposition event, defined as the projected defense trend. A second equation was derived based on the defense proportion in the intervals after the first oviposition event, defined as the phorid effect for host defensive behavior. The difference between the phorid effect line in defense behavior and the projected defense trend is a quantifiable difference in the defense proportion due to phorid attacks, defined as the phorid induced deviations. These phorid induced deviations at every data point were averaged for every trial and standardized. A t-Test was conducted of the average standardized deviations in defense proportion to determine whether the phorid oviposition events result in a significant alteration of ant defense behavior.

Ant foraging behavior was quantified by counting the total number of ants present in the selected quadrant at each time interval. The number of ants at every time interval before the first oviposition attempt were used to derive an equation of the projected ant foraging activity in the absence of phorids. The number of ants at every interval after the first oviposition attempt were used to derive the phorid effect on ant foraging behavior. The difference between these two equations at every interval after the first oviposition attempt gives us the phorid induced deviations in foraging behavior. These deviations were averaged for every trial and standardized. A t-Test was conducted of the average standardized deviations in ant foraging activity to determine whether the phorid oviposition events result in a significant alteration of ant foraging activity.
RESULTS

The phorid parasitoids in this study, *Pseudacteon californiensis* and *Pseudacteon sp A*, induced significant behavioral modifications in the Velvety Tree Ant as a result of their presence. Ant behavior in response to phorid fly presence was categorized in two ways, foraging or defense (Figure 17). *Liometopum occidentale* changed its body posture in the presence of phorid flies. In the absence of phorids the ants foraged at the tuna bait stations, gathering resources, and taking them back to the nest (Figures 17A & 17B). Body posture of ants shifted when phorid flies arrived at the bait stations and attempted oviposition (defined as an oviposition event). Ants exhibited a defensive body posture that involved the ant standing on its hindlegs with its mandibles open (Figures 17C & 17D). The proportion of ants showing a defensive posture increased and the proportion of ants foraging decreased in the presence of phorid flies.

The shift in the slope, or trajectory of defense proportion after the first phorid oviposition event is defined as the phorid effect. This shift can be quantified in the form of phorid-induced deviations (see example in Figure 18), defined as the difference between the phorid effect and the projected defense trend pre-oviposition event. The phorid-induced deviations from each of the 14 trials were averaged. A t-Test of the average standardized deviations for all trials showed a significant increase in ant defense behavior due to phorid attacks ($n = 14$, $t = 5.151$, $p < 0.001$, df = 13).

In addition to the measured phorid effect on host defensive behavior, there was also a quantifiable significant phorid effect on overall ant foraging behavior (see example in Figure 19). The projected ant foraging activity and trajectory saw a significant reversal and decline after the arrival of phorid flies and the subsequent oviposition events. The
phorid effect on ant foraging activity resulted decline in overall ant foraging activity. \( (n = 14, t = 3.233, p < 0.003, df = 13) \).

Overall, in the 14 trials conducted, phorid fly attacks on the Velvety Tree Ant resulted in a 28% average decrease in overall foraging activity from projected values (Figure 20). In the absence of phorid attacks, ant workers normally have a stable base level of defense present at the stations. The phorid attacks resulted in an average increase in the proportion of ants defending of 22% in addition to the ants’ projected base level of defense (Figure 20).

DISCUSSION

The behavioral modifications seen in *Liometopum occidentale* are a result of phorid parasitism in the species. I found significant alteration of ant behavior in the presence of phorid flies, similar to that first noted by Feener and Brown (1992). The first change seen in ant behavior after phorid oviposition attempts is the shift from foraging behavior to a defensive posture in the targeted and surrounding ants on the bait. This defensive behavior is triggered after a single oviposition attempt and is likely mediated by ant alarm pheromones. The defensive behavior, where a portion of the workers stand on their hind limbs and snap their mandibles in the air confers protection on the workers exhibiting that behavior as well as those still foraging (Elizalde and Folgarait, 2012). The defending workers spread evenly throughout the baits and allow the foragers to continue gathering resources. The phorids are often able to evade the defending workers with their quick oviposition attempts and target mainly distracted workers, usually those that are foraging. Defending nearby workers however were seen targeting these incoming phorid
attacks on the foragers, at times resulting in a successful capture with their open mandibles, therefore eliminating the threat of oviposition from that particular phorid. This defensive behavior and the elevated proportions of the ants defending at the baits persisted as long as the phorids remained.

Overall foraging behavior was also significantly altered by phorid attacks, resulting in a significant decline in the number of ants remaining at the baits after phorid oviposition attempts. The main phorid effect on foraging could be seen in the significant trajectory change in overall ant presence with a downward slope after the arrival of phorids. Persistent phorid ovipositions resulted in a significantly steeper decline trajectory in the study than more temporally spread out attacks, suggesting that higher persistent phorid pressure may suppress foraging activity altogether, resulting in onerous repercussions for the overall health of the ant colony when it comes to competitive interactions with other organisms (Feener and Brown, 1992; LeBrun and Feener, 2002). This critical mass of phorid activity however was never achieved in this study and there were never sufficient phorid attacks to completely eliminate ant foraging at any given time. The predicted total abandonment behavior was observed during the course of this study in preliminary surveys on the local southern fire ant, *Solenopsis xyloni* when interacting with its parasitoid *Pseudacteon amuletum*. A single phorid oviposition attempt in *S. xyloni* was able to trigger a cascade of defensive behavior that resulted in the workers abandoning the resource altogether and not returning as long as phorids remained present. The Velvety Tree Ants, however, through their persistent defense behavioral alteration were able to more effectively protect their workers and continue foraging, albeit at a significantly reduced presence.
The statistical model used to quantify the phorid effect on *L. occidentale* uses projected values based on pre-oviposition data and real values post-oviposition event. This allows me to quantify the change in trajectory overtime as it relates to defense behavior and overall foraging activity. While there are limitations to this model, primarily the fact that ant activity is not perpetually linear as defense and foraging numbers eventually plateau, this was not an issue in my behavioral experiments involving short time scales. Ant activity, if extrapolated far beyond the time scope of my behavioral studies, may be able to fit curved or quadratic models better than the linear models I used in this study. This however was not possible in this study, as attempting to extrapolate a curve or quadratic function from limited data resulted in the program being unable to determine when the equation should level, in the end resulting in a relatively linear equation. Because of the difficulty and unreliability of fitting alternate models to my data, I decided to use a linear equation to project ant activity, as it related to defense and foraging, over limited time scales – in this study, 40 minutes before oviposition and 40 minutes after.

Once I decided to use a linear equation to show the phorid effect on their ant hosts, the choice was which value to use to show this effect. Using a change in slope could also show there is a phorid effect and the data would be significant. However the limitations of a change-in-slope approach is in the inability to quantify the magnitude of the change. While this approach would undoubtedly prove there was an effect, I felt it was necessary to show the magnitude of that effect. Alternatively, showing the difference in magnitudes between the slopes could have also accomplished something similar, but
this would essentially measure the magnitude between two fitted linear models to show the overall phorid effect.

The model I eventually settled on (examples seen in Figures 18 and 19), in my view, allows for a more conservative approach by using projected values only as needed, and using all the real recorded data otherwise available to quantify the phorid effect. Using this model, I am able to incorporate the irregular swings in ant activity patterns, whether positive or negative, over time, what I’ve called phorid-induced deviations. These deviations, when averaged across all the behavioral trials give us a conservative estimate of the effect that *Pseudacteon californiensis* and *Pseudacteon sp A* have on their host, *Liometopum occidentale*. Having to deal with two parasitoids, the behavioral modifications seen in *L. occidentale* are a balancing attempt to protect the species from both parasitism and competitors. This balancing act results in a tradeoff for both. The defense seen in host organisms as a result of parasitism are an attempt to mitigate the damage, while allowing the colony to continue foraging as is seen in *Liometopum occidentale*. 
INTRODUCTION

Phorid parasitoids of ants are an extremely successful group, with a rich and long evolutionary history that includes over 300 described species and an extensive range of interactions with other organisms (Brown, 1999). Though extremely host specific, there are instances where several phorid species can coexist in a single host (Brown, 1999). This ability is particularly important to phorids as the number of suitable hosts are often limited by virtue of being too small for successful parasitism, too inaccessible to phorid parasitoids or any number of reasons that would make them unsuitable hosts (Brown, 1999). Given the large impact that phorids can have on entire ecosystems (Hsieh and Perfecto, 2012; LeBrun and Feener, 2002), it is of particular ecological importance to study and understand the natural history, species distribution, activity patterns and differential host use of phorids in local systems.

Brown (1999) has stated that phorids are masters of differential host use, and this efficiency in utilizing host resources may have contributed to their incredible success as parasitoids. Four major types of differential host use have been described by Brown (1999): temporal differences, caste or size differences, host activity differences and body part differences. The temporal differences seen in phorid parasitoids of ants include temperature and temporal variations. Temperature in particular, as it relates to climate patterns, has been known to influence activity patterns in some phorid species (Folgarait
et al., 2003, 2007b; Pesquero et al., 1996; Plowes et al., 2012). Weather patterns for instance, are uncontrolled in the phorids’ natural environment, and phorid presence in prior studies has been found to slightly correlate with warm and wet seasons, with extremely dry seasons generally resulting in a sharp decrease in phorid activity (Folgarait et al., 2003, 2007b; Plowes et al., 2012) Large amounts of rainfall however may also contribute to a decrease in phorid activity (Plowes et al., 2012).

Studies in Argentina have shown substantial overlap in phorid activity ranges, particularly in temporal, seasonal and time of day variation (Folgarait et al., 2007a). Additionally, some phorid species such as *Pseudacteon nocens*, a fire ant parasitoid, are able to modulate their temperature preferences by moving in and out of the sun depending on the ambient temperature (Folgarait et al., 2007a, 2007b). For instance, in cooler temperatures, *P. nocens* has been observed moving into sunlight, and moving back to shaded areas as temperatures rise (Folgarait et al., 2007b). Among the species surveyed by Folgarait et al., (2007b), there was a significant difference in species presence between light and shaded areas, with phorid species classified as “temperate” largely being found in full shade while those classified as “hotter” were found mostly in the sun.

The study of temporal differences among phorid species has largely been centered on the selection of biological controls for invasive *Solenopsis* fire ants (Folgarait et al., 2003, 2007a, 2007b). The variations in interspecies activity patterns have been used to select good candidates for release as biocontrols (Folgarait et al., 2003). An effective biocontrol would be able to provide sustained pressure to the host species year-round, a factor that must be taken into account given that many phorids have seasonal activity patterns (Brown, 1999).
In this local Southern California system hosted by *Liometopum occidentale*, little is known about the activity patterns of *Pseudacteon californiensis* and *Pseudacteon sp A*. The Velvety Tree Ants are generally present in these areas year-round as the mild Southern California weather variations do not significantly limit their activity in the winter, though activity is most noticeable in warmer seasons. The limiting factor in a study of ant-phorid activity patterns is often the phorids themselves. To gain a better understanding of this system, the phorids, and their behavior, I describe the activity patterns of *Pseudacteon californiensis* and *Pseudacteon sp A* in regard to time of day, temperature, temporal periods and site preference.

**MATERIALS AND METHODS**

The effect of temperature on activity of phorid flies was assessed by sampling ants and phorids in the summer of 2015 at the Placerita Canyon State Park (34.38°N, 118.46°W) in the Angeles National Forest and the Chuchupate Campground (34.78°N, 119.00°W) in the Los Padres National Forest (Figure 1). Tuna bait stations were established at the base of Coast Live Oak trees to attract ants, and subsequently the phorid flies that associate with them. Temperature data was recorded at each site sampled using an Etekcity Lasergrip 774 Digital Infrared Thermometer. Temperature recordings took place from July through September of 2015, beginning when ambient temperature was 15°C. Both temperature and time of day was noted for every phorid oviposition event (i.e., when at least one phorid fly attempted to oviposit on an ant). Temperature data was recorded until phorid activity ceased.
In addition to using tuna baits at sites sampled for ants and phorids, full body extracts (ant pheromones) from Velvety Tree Ants were placed next to the tuna baits. Ant pheromone extracts were prepared with the method provided by the Brackenridge Field Laboratory. The aim of creating ant pheromone extracts was to separate pheromones from the main sections of the body: alarm pheromones mainly found in the head section, foraging trail pheromones mainly found in the gaster, and other intermediate and neutral pheromones found in the thorax. The basic formula used was one ant section per 50 µL of extract to be prepared. To create the extract, ants were frozen to incapacitate them and then sectioned. Twenty ants, divided into their major body parts (head, thorax or abdomen), were placed in a 1.5 mL centrifuge tube with 1 mL of 100% hexanes. Two sets of nitrile gloves were used when handling the hexanes under a fume hood. A 0.5 mL pestle was used to grind the ant sections for 1 minute. The tubes were centrifuged for 1.5 minutes at 10,000 rpm causing the separation of the ant section crushed remains and the dissolved pheromones in the hexane. The supernatant was transferred over to a labeled vial and stored. Full body ant pheromone extracts were prepared by placing eight entire ants into 1.2 mL of 100% hexanes. This was consistent with the general formula of 50 µL of extract prepared from one ant section and took into account the fact that a full ant body includes three sections. The same procedure used for the section extracts was followed. Ant bodies were ground for 1 minute, tubes centrifuged for 1.5 minutes at 10,000 rpm, and the supernatant extracted and transferred to a labeled vial and stored.

Phorids were collected and brought back to the lab using a home-made aspirator design provided by the Brackenridge Field Laboratory. The aspirator consists of a 2 mL microcentrifuge tube with the conical bottom cut off. A fine mesh material was fused to
the bottom by melting part of the tube with a Bunsen burner and placing the tube on the fine mesh. A hole was made on the o-ring cap with a small soldering iron to place a wet cotton swab to provide moisture for the captured phorids during transport. The microcentrifuge tubes were attached to vinyl tubing and used to aspirate the phorids attracted to the pheromone baits while they were attacking the ants.

Descriptive statistics were generated for phorid attack temperature data and phorid attack time of day data. Pearson’s correlation was used to determine whether there was a significant correlation between ambient temperature and time of day for phorid oviposition event frequency. T-tests were used to analyze significant differences in the species’ activity patterns. Analysis of Variance tests were conducted to determine whether there was significant variation in phorid attack temperature and phorid attack time based on temporal period (July – September). A G-test of independence was used to determine whether *Pseudacteon sp A* and *Pseudacteon californiensis* were site dependent.

RESULTS

There was a significant correlation between temperature and time of day for phorid fly activity (r = 0.45, p < 0.001, df = 263). The median temperature for phorid activity was 19.0°C (range 15.39°C – 25.72 °C) for the combined species (Figure 21). There was a significant difference in phorid activity temperature by site (t = 8.73, df = 185, p < 0.001). The median activity temperature for Chuchupate was 21.0°C (range 15.67°C – 25.72°C), and 18.17°C (range 15.39°C – 23.28°C) for Placerita (Figure 22). Phorid activity varied temporally and the flies were most active in late July and early
August (Figure 23). There was significant variation between temporal periods (July – September) in combined phorid attack temperature (ANOVA $F = 15.48$, $p < 0.001$, df = 4, 260) (Figure 24).

The median time for phorid activity was 0913 (range 0715 – 2140) for the combined species (Figure 25). There was a significant difference in phorid activity time by site ($t = 10.34$, df = 109, $p < 0.001$). The median activity time for Chuchupate was 1046.50 (range 0805 – 2140), and 850 (range 0715 – 1024) for Placerita (Figure 26). There was significant variation between temporal periods (July – September) in combined phorid attack time (ANOVA $F = 2.72$, $p < 0.03$, df = 4, 260) (Figure 27). Overall, phorid oviposition was influenced by ambient temperature and time of day, with most of the oviposition events being concentrated in the early morning hours during cooler temperatures (Figures 28 and 29).

The region of the ant’s body where the phorids oviposited was recorded during temperature and time recordings. All 265 oviposition events of both combined phorid parasitoid species targeted the gaster of *Liometopum occidentale*. Species distribution of *Pseudacteon californiensis* and *Pseudacteon sp A* was found to be site dependent ($G = 214.01$, df = 1, $p < 0.001$). The Placerita State Park site in the Angeles National Forest was dominated by the new species, *Pseudacteon sp A*, representing 91.85% of all 184 samples collected. The Chuchupate Campground in the Los Padres National Forest was dominated by *Pseudacteon californiensis*, representing 90.48% of all 105 samples collected (Figure 30).
DISCUSSION

Like most other living organisms, phorids need a good and stable environment with sufficient resources in which to thrive. The ant hosts, *Liometopum occidentale* appear to be a bit more resilient than their parasitoids in times of drought based on the observations of this study where ants would keep foraging even in temperatures nearing 40ºC. Generally speaking, in order to find a good source of phorid flies, a humid environment with a nearby water source or ample rain is necessary. Phorids are heavily dependent on their hosts for the completion of their lifecycle and the long-term viability of the local phorid populations. This study shows that there is some plasticity in phorid behavior to cope with changing weather patterns and overall variation in temporal periods throughout the phorid activity seasons.

The activity range seen in the summer of 2015 field season shows that *Pseudacteon sp A* and *Pseudacteon californiensis* are able to concentrate their activity at temperatures that are favorable to them, in this study a median combined temperature of 19.0°C (range: 15.39°C – 25.72 °C). While there was a significant difference in the average activity temperature by site, the temperature ranges of both sites were similar – Chuchupate (range 15.67°C – 25.72°C) and Placerita (range 15.39°C – 23.28°C). Additionally there was a broad spread in the activity times for the combined species (range 0715 – 2140) with a significant difference between the sites – Chuchupate (range 0805 – 2140), and Placerita (range 0715 – 1024). The broad spread in the activity times and extreme difference between sites but overlapping temperature ranges shows that time of day may not be the prevailing factor in determining phorid activity. Regardless of what time of day the phorids were attacking at, activity was still concentrated at cooler
temperatures. These cooler temperatures were usually only found in the early morning hours in the Placerita Canyon site, but were more common during later hours of the day at the higher elevation site of Chuchupate. This variation in ambient temperature between the sites may explain the difference in spread of time of day activity seen in the phorids.

Phorid temperature activity studies in central Texas have found that phorid activity is absent when temperatures dip below 20ºC in the fall (Folgarait et al., 2003). This however is in contrast to our study and a contributing factor to the shut down of phorid activity could be due to temporal variations, as I saw a decline in the fall despite days with favorable conditions. A different *Pseudacteon* study in Argentina found that phorid activity ceased when temperatures dipped below 14ºC, which is 6ºC lower than the Texas study (Folgarait et al., 2003). These adaptations and sensory mechanisms in these species are remarkable and can result in variation of activity time from one day to the next depending on ambient temperature (Folgarait et al., 2003, 2007b).

The species distribution in my study shows a significant dependence on site for each phorid species. The new species, *Pseudacteon sp A* dominated the baits at the Angeles site and *Pseudacteon californiensis* at the Los Padres site. It isn’t possible to draw any conclusions based on two sites of study, however it is possible that elevation may play a role in species segregation. The elevation at the Chuchupate Campground was 1903m, which was much higher than the 472m elevation at the Placerita Canyon Park site. While temperature ranges were similar between the two sites, other factors may play a role in species dominance at each of the sites. An interesting area of future study would examine the role of elevation in species preference for these two *Pseudacteon* phorids.
The noted similarities in the temperature ranges between the two sites raise interesting questions regarding differential host use. Although the data is not divided up by species since this could not be determined in the field when the temperature and time recordings were made, each site is dominated by one of the species – *P. sp A* in Placerita, and *P. californiensis* in Chuchupate. Given the significant dominance seen at each site, the temperature and time of day data is largely representative of the species that dominates that particular site. In other systems where multiple phorid species attack a single host species, there is often a division of resources between the species through differential host use (Brown, 1999). This differential host use can often be seen in the temporal differences in regard to phorid activity between species. In this study however, though there was a significant difference in the average activity temperature, the overall temperature ranges largely overlapped. Additionally, the activity differences in time of day could largely be explained by favorable temperature variations in any given day. The body part used for oviposition – the gaster of *Liometopum occidentale*, was also identical between the species.

Given the similarities in attack behavior and overlapping activity ranges for *Pseudacteon californiensis* and *Pseudacteon sp A*, there doesn’t appear to be differential host use in this system if we strictly follow the currently described types of differential host use (Brown, 1999). In other systems, this division provides phorids efficiency in the utilization of host resources as there is less of a need to compete directly for resources and instead specialize to exploit a particular time, temperature, activity, or physiological niche in the form of different body parts (Brown, 1999). The lack of this division of host resources seen in this system is particularly interesting given that *Pseudacteon*
occidentale and Pseudacteon sp A have otherwise nearly identical activity patterns. There could be other factors that allow both species to exploit these similar temperature ranges without directly competing with each other. Elevation for instance, might be the prevailing factor that allows this activity overlap without direct competition between the species, though further studies are needed to verify this. The findings of this study provide multiple avenues for future research. The study of ant-phorid systems, their behavior, diversity, and activity ranges increase our understanding of the evolutionary and natural history of the species. In regard to differential host use, these findings could indicate the existence of a fifth type based on elevation gradients in species with significant overlap like Liometopum occidentale.
CHAPTER 5

CONCLUSIONS

The intricate natural balance between the phorid parasitoids and their ant hosts play a role in the overall health of many ecosystems and can have wide ranging implications on other organisms within this system (Hsieh and Perfecto, 2012). The natural interactions taking place between the phorid parasitoids and their hosts can have negative or positive repercussions for the ecosystem as a whole. In some cases, phorid parasitism may offset the balance of competitive interactions, essentially causing a shift in the keystone species in that ecosystem. The presence of phorid parasitism may additionally have negative repercussions by causing an alteration of behavior in mutualistic interactions resulting in a collapse of the sensitive species balance and the rise of unintended pests in the ecosystem. The presence of phorids however may also be a net positive for the overall system, such as when it comes to the control of invasive species and pests as has been the case with invasive fire ants (Hsieh and Perfecto, 2012; LeBrun and Feener, 2002; Lebrun, 2005; Mottern et al., 2004; Plowes et al., 2011). Overall, the interactions between phorids and their hosts can have wide ranging implications for not just the species involved, but the ecosystem as a whole.

When it comes to the ant-phorid system of *Liometopum occidentale* and its two phorid parasitoids, *Pseudacteon californiensis* and *Pseudacteon sp A*, the implications may be even more far reaching. *Liometopum occidentale*, is under siege by environmental factors, declining natural habitat due to human development and invasive species. This is a local species that nests in trees, mainly oaks that are found near streams and tends to persist at low elevations in unique environmental niches. Additionally,
*Liometopum occidentale* is particularly sensitive to decreases in relative humidity and increases in temperature (Hoey-Chamberlain and Rust, 2014). This preferred habitat leaves the ants dependent on the stability of the overall ecosystem. Long droughts that may dry up these streams eventually have a destabilizing effect on these ecological niches, the stability of the local *Liometopum occidentale* populations, and by extension, the viability of *Pseudacteon californiensis* and *Pseudacteon sp A*. The declining natural habitats in urban areas leaves these local species vulnerable, leading to a rise in invasive species and a decline of the native species (Vonshak and Gordon, 2015). Even outside of the urban areas in these protected enclaves, I have observed that *Liometopum occidentale* still finds itself under siege by the ever-increasing range of invasive species such as *Linepithema humile*, the Argentine ant. A competitive disadvantage that is made more prominent during phorid attacks, as a competitor with less environmental and interspecies pressure may in the end be able to displace the Velvety Tree Ants in their selective habitat. *Liometopum occidentale* and its two phorid parasitoids, *Pseudacteon californiensis* and *Pseudacteon sp A* are all local species in Southern California. The role these species play in the overall ecosystem is unknown and the decline of this system via competitive interactions, phorid induced behavioral modifications or climate change may in the end be detrimental to the stability of the ecosystems these species inhabit.


APPENDIX

Figure 1. The Velvety Tree Ant, *Liometopum occidentale* and its associated phorid parasitoids were collected from two main study sites: Chuchupate Campground in the Los Padres National Forest (Top Left) and Placerita Canyon Park in the Angeles National Forest (Bottom Right).
Figure 2. Ovipositor morphological differences between *Pseudacteon sp A* (Top) and *Pseudacteon californiensis* (Bottom).
Figure 3. Sequence alignment of CO1 gene for *Pseudacteon californiensis* and *Pseudacteon sp A*.

<table>
<thead>
<tr>
<th>Sequence Alignment</th>
<th>Sequence Length</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO1 P californiensis</td>
<td>683 bp</td>
</tr>
<tr>
<td>CO1 P sp A</td>
<td>683 bp</td>
</tr>
</tbody>
</table>

**Identities:** 739/852 (86%), **Positives:** 740/852 (88%), **Gaps:** 16/852 (2%)
Figure 4. Sequence alignment of 28S gene for *Pseudacteon californiensis* and *Pseudacteon sp A*.
Figure 5. Sequence alignment of WG gene for *Pseudacteon californiensis* and *Pseudacteon sp A*.
Figure 6. Mean body length for *Pseudacton sp A* and *Pseudacteon californiensis* with standard errors. \( t = -1.42, \text{df} = 23, p = 0.1698 \).

Figure 7. Mean ovipositor length for *Pseudacton sp A* and *Pseudacteon californiensis* with standard errors. \( t = 4.82, \text{df} = 22, p < 0.001 \).
Figure 8. CO1 Single nucleotide polymorphisms shown with Base pair location and nucleotide variations for *Pseudacteon californiensis* and *Pseudacteon sp A*. Ambiguous nucleotides representing both species are shown in the consensus column. Percent species difference represents the percentage of nucleotide variations between species relative to the total nucleotide sequence length.

Figure 9. CO1 Amino acid substitutions shown with Base pair location for *Pseudacteon californiensis* and *Pseudacteon sp A*. Percent species difference represents the percentage of amino acid substitutions between species relative to the total encoded amino acids for the sequence.
Table 1. WG SNP

<table>
<thead>
<tr>
<th>BP Location (CONSENSUS)</th>
<th>P. californiensis</th>
<th>P. sp A</th>
<th>Consensus</th>
</tr>
</thead>
<tbody>
<tr>
<td>334</td>
<td>G</td>
<td>C</td>
<td>S</td>
</tr>
<tr>
<td>349</td>
<td>T</td>
<td>G</td>
<td>K</td>
</tr>
<tr>
<td>367</td>
<td>A</td>
<td>C</td>
<td>M</td>
</tr>
<tr>
<td>403</td>
<td>T</td>
<td>C</td>
<td>Y</td>
</tr>
<tr>
<td>412</td>
<td>A</td>
<td>G</td>
<td>R</td>
</tr>
<tr>
<td>436</td>
<td>A</td>
<td>G</td>
<td>R</td>
</tr>
<tr>
<td>460</td>
<td>C</td>
<td>T</td>
<td>Y</td>
</tr>
<tr>
<td>544</td>
<td>G</td>
<td>A</td>
<td>R</td>
</tr>
</tbody>
</table>

Total Sequences Length: 651
Percent species difference: 1.229

Table 2. WG AA Substitution

<table>
<thead>
<tr>
<th>BP Location</th>
<th>P. californiensis</th>
<th>P. sp A</th>
<th>Consensus</th>
</tr>
</thead>
<tbody>
<tr>
<td>333-335</td>
<td>Arginine</td>
<td>Threonine</td>
<td>R/T</td>
</tr>
<tr>
<td>348-350</td>
<td>Isoleucine</td>
<td>Serine</td>
<td>I/S</td>
</tr>
<tr>
<td>366-368</td>
<td>Aspartic acid</td>
<td>Alanine</td>
<td>D/A</td>
</tr>
<tr>
<td>402-404</td>
<td></td>
<td></td>
<td>S/F</td>
</tr>
<tr>
<td>411-413</td>
<td></td>
<td></td>
<td>N/S</td>
</tr>
<tr>
<td>435-437</td>
<td></td>
<td></td>
<td>H/R</td>
</tr>
<tr>
<td>459-461</td>
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<td></td>
<td>T/I</td>
</tr>
<tr>
<td>543-545</td>
<td></td>
<td></td>
<td>R/Q</td>
</tr>
</tbody>
</table>

Total Amino Acids: 216
Percent species difference: 3.704

Figure 10. WG Single nucleotide polymorphisms shown with Base pair location and nucleotide variations for *Pseudacteon californiensis* and *Pseudacteon sp A*. Ambiguous nucleotides representing both species are shown in the consensus column. Percent species difference represents the percentage of nucleotide variations between species relative to the total nucleotide sequence length.

Figure 11. WG Amino acid substitutions shown with Base pair location for *Pseudacteon californiensis* and *Pseudacteon sp A*. Percent species difference represents the percentage of amino acid substitutions between species relative to the total encoded amino acids for the sequence.
Figure 12. 28S Single nucleotide polymorphisms shown with Base pair location and nucleotide variations for *Pseudacteon californiensis* and *Pseudacteon sp A*. Ambiguous nucleotides representing both species are shown in the consensus column. Percent species difference represents the percentage of nucleotide variations between species relative to the total nucleotide sequence length.

<table>
<thead>
<tr>
<th>28S SNP</th>
<th>BP Location (CONSENSUS)</th>
<th>P. californiensis</th>
<th>P. sp A</th>
<th>Consensus</th>
</tr>
</thead>
<tbody>
<tr>
<td>124</td>
<td>124</td>
<td>A</td>
<td>T</td>
<td>W</td>
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<td>W</td>
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<td>138</td>
<td>138</td>
<td>A</td>
<td>C</td>
<td>M</td>
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<td>144</td>
<td>144</td>
<td>T</td>
<td>G</td>
<td>K</td>
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<td>156</td>
<td>T</td>
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<td>Y</td>
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<td>R</td>
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<tr>
<td>580</td>
<td>580</td>
<td>A</td>
<td>G</td>
<td>R</td>
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</tbody>
</table>

SNPs: 9
Total Sequences Length: 621
Percent species difference: 1.449
Figure 13. Phylogenetic tree of mitochondrial gene, CO1, showing node consensus support percentages of *Pseudacteon californiensis* and *Pseudacteon sp A* to the *Pseudacteon* clade and other phorid genera.
Figure 14. Phylogenetic tree of nuclear gene, LepWG, showing node consensus support percentages of *Pseudacteon californiensis* and *Pseudacteon sp A* to the *Pseudacteon* clade, *Apocephalus*, and the currently available *Microselia texana* GenBank sequence.
Figure 15. Phylogenetic tree of nuclear gene, 28S, showing node consensus support percentages of *Pseudacteon californiensis* and *Pseudacteon sp A* to the *Pseudacteon* clade and other phorid genera.
Figure 16. Analysis of single concatenated data set of CO1, 28S and WG showing node consensus support percentages of *Pseudacteon californiensis* and *Pseudacteon sp A*, to the *Pseudacteon* clade and other phorid genera. Each species is represented by at least 2 of the three genes: CO1, 28S, WG.
Figure 17. Behavior of Velvety Tree Ants before and after phorid fly attacks. Normal foraging at bait station in the absence of phorid flies (A, B). Defensive behavior at bait station in the presence of phorid flies (C, D).

Figure 18. Representative trial of the proportion of Velvety Tree Ants defending at a foraging station over an 80 minute period. Time zero (0) is the first oviposition event. The proportion of ants defending at the bait station were recorded 40 minutes before and 40 minutes after the first oviposition event. Projected Defense shows expected ant defense proportion in the absence of phorid flies. Phorid effect is the change in the proportion of ants defending after the first oviposition event. Phorid induced deviation is the difference between the projected proportion of ants defending for every data point and the actual proportion of ants defending after the initial phorid attack. The deviations in each of 14 trials that were conducted were averaged. A t-Test of the average standardized deviations for all trials showed a significant increase in ant defense behavior due to phorid attacks ($n = 14, t = 5.151, df = 13, p < 0.001$).
Figure 19. Representative trial of Velvety Tree Ant abundance at a foraging station over an 80 minute period. Time zero (0) is the first oviposition event. The number of ants present at the bait station were recorded 40 minutes before and 40 minutes after the first oviposition event. Projected Foraging shows expected ant activity in the absence of phorid flies. Phorid effect is the change in ant foraging activity after the first oviposition event. Phorid induced deviation is the difference between the projected ant presence for every data point and the actual recorded ant presence after the initial phorid attack. The deviations in each of 14 trials that were conducted were averaged. A t-Test of the average standardized deviations for all trials showed a significant decrease in ant foraging activity due to phorid attacks (n = 14, t = 3.233, df = 13, p < 0.003).
Figure 20. Mean *Liometopum occidentale* Defense Increase proportion and Foraging Decline proportion as a result of phorid attacks across all 14 behavioral trials shown with standard errors.
Figure 21. Frequency of combined phorid fly oviposition events by temperature (°C) for all temporal periods.

Figure 22. Frequency of phorid fly oviposition events by temperature shown for each site.
Figure 23. Frequency of combined phorid fly oviposition events by temperature (°C) from early July (n=12), late July (n=132), early August (n = 81), late August (n=30) and early September (n = 10).
Figure 24. Mean phorid fly attack temperature at every temporal period with standard deviations for each temporal period (One-Way ANOVA, $F= 15.48$, df= 4,260, $p < 0.001$).
Figure 25. Frequency of combined phorid oviposition events by time of day showing attacks concentrated in the morning hours (n = 265). Median time for phorid activity was 0913 (range 0715 – 2140).

Figure 26. Frequency of phorid oviposition events by time of day shown for each site.
Figure 27. Mean phorid fly attack time at every temporal period with standard deviations for each temporal period (One-Way ANOVA, $F = 2.72$, $df = 4,260$, $p < 0.03$).
Figure 28. Scatterplot showing distribution of combined phorid oviposition events by temperature (°C) and time from early July to early September (n = 265).
Figure 29. Scatterplot showing distribution of phorid oviposition events by temperature (°C) and time for each site (n = 265).
Figure 30. Phorid species distribution based on site showing strong site dependence ($G = 214.01$, df = 1, $p < 0.001$).