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The Life History Traits and Morphology of *Chaetodactylus krombeini*

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Dedication

My sisters Kimberly and Stephanie and my niece Rebecka.

“Don’t let the bastard grind you down.”

-The Handmaid’s Tale

ABSTRACT OF THE THESIS

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by

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Chaetodactylidae mites are kleptoparasites of solitary bees in the families Megachilidae and Apidea. *Chaetodactylus krombeini* is a North American pest most often associated with *Osmia lignaria*. Chaetodactylidae mites can cause up to 50% losses of bee offspring in nests and cause adult bees to develop into less efficient pollinators. Managed *Osmia* beekeepers need control methods for these parasites, however there is little known about *C. krombeini* life history making it difficult to efficiently decrease their harmful effects. Mite host preference is unclear as there are contradictory reports of male and female bees being the preferred host of the phoretic deutonymph. Host sex preference was examined by recording mite distribution within host nests. Mite distribution determined if phoretic deutonymphs dropped off hosts in female cells (back of nest) or male cells (middle and near nest entrance) during nest building. This experiment yielded low mite numbers within each nest, though mites were found in the back of nests regardless of host sex, possibly indicating mites have a positional

preference rather than a host sex preference. Heteromorphic deutonymphs of *C. krombeini* are hypothesized to be exclusively female. This hypothesis leads to additional hypotheses of *C. krombeini* requiring arrhenotokous parthenogenesis and oedipal mating to found mite populations. Sex determination assays of the heteromorphic deutonymphs were conducted in 2-week periods for each mite. Inert and phoretic deutonymphs were individually placed in microcentrifuge tubes with a pollen provision then sexed when mites developed into adults. Phoretic deutonymphs were allowed to overwinter with and without a bee host. Males and females developed from both morphs of the heteromorphic deutonymph. Male and female dispersal prior to mating may indicate sexual reproduction is required for this species. Scanning electron microscopy was used to examine *C. krombeini* morphology to gain insight into life history traits. Specimens of *C. krombeini* were prepared with ethanol and hexamethyldisilazane. Images will be useful to compare the morphology of *C. krombeini* to mites with known life history traits and to other species that are indistinguishable from *C. krombeini*. These experiments contribute to the knowledge of *C. krombeini*, helping future control efforts of this pest.

Table of Contents

List of Tables

Chapter 1

Chapter 2

Chapter 3

List of Figures

Chapter 1

Chapter 2

Chapter 3

Appendix A

Introduction

Crops pollinated by bees are an $18 billion industry in the US (Nowierski 2020). It can be difficult to sufficiently cross-pollinate orchard crops with honey bees alone, as they tend to continuously forage on one tree instead of moving from tree to tree, moreover wind and self pollination does not occur with many orchard crops (Torchio 1976; Brittain 2013). In addition, many orchard crops bloom in early spring when there is more extreme weather, which coincides with lower activity of managed honey bee colonies (Torchio 1976). Orchard crops also often have short blooming periods which, coupled with a large abundance of blooms per plant, contributes to the difficulty of sufficiently pollinating large scale orchard crops (Torchio 1976). Honey bees were predominantly used in the United States as pollinators for orchard crops until 1950, but with the unsustainable losses occurring in managed bee colonies (colony collapse disorder), there was a need to focus on other pollinators to ensure sufficient orchard crop production (Torchio 1976; Ellis et al. 2010; USDA 2022). In Japan, the solitary bee *Osmia cornifrons* (Radoszkowski) has been used to successfully pollinate commercial apple orchards (Torchio 1976). A related species, *Osmia lignaria* Say (the blue orchard bee), is native to the United States and Canada and has been used as a commercial orchard crop pollinator (Rust 1974).

Osmia lignaria are solitary bees in the family Megachilidae, tribe Osmiini (Rust 1974). They are distributed across the United States, excluding Florida, Hawaii, and Alaska, and are also found in parts of Southern Canada (Rust 1974). Female *O. lignaria* are ca. 11-13 mm long with a wing length of 7.6-9.2 mm, while males are ca. 9-10 mm in length with a wing length of 7-8.4mm (Rust 1974). Males have slightly longer antenna than females, and females have prongs that resemble horns on the lower half of their face (Bosch and Kemp 2001). Both male and female *O. lignaria* are a metallic blue/green/black color, with males having a tuft of white hair on the anterior ventral part of their heads resembling a mustache (Say 1837; Rust 1974).

Osmia lignaria adults emerge from cocoons in the early spring, typically in March-April, but may also emerge later due to environmental temperatures and elevation (Rau 1937; Levin 1966). Males often emerge first, ca. 1 to 3 days prior to females, due to protandrous emergence, but females can emerge at the same time as males (Rau 1937; Krombein 1962; Torchio and Tepedino 1980; Bosch and Kemp 2001). Mating occurs shortly after both sexes have emerged (Torchio and Tepedino 1980). *Osmia lignaria* females only mate once, while males will mate multiple times (Seidelman 2014). Female *O. lignaria* are no longer receptive to mating after copulation, and males perform a post-copulatory display that terminates responses to mating in females that have been inseminated (Seidelman 2014). Because males do not have any parental investment or provide mates with any resources, the only way to improve their fitness is to

copulate with multiple females (Seidelman 2014). Females are most receptive to mating advances from males when they first emerge and are less receptive as time passes (Seidelmann 2014). By the third day after female emergence, most females are not receptive to males' advances and refuse to mate (Seidelmann 2014). Females do not require mating to successfully produce offspring (Flander 1939). Female *O. lignaria* live for about five to six weeks after emergence, while males have a shorter lifespan and provide no contribution to nest development (Rau 1937; Torchio and Tepedino 1980). Fitness of females depends on how efficiently she can build and provision nests for her offspring, and the less time she spends on courtship and mating the more time she can allot to establishing nests (Seidelman 2014).

Within 1-2 days of mating, female *O. lignaria* begin to build their nests (Bosch and Kemp 2001). *Osmia lignaria* will readily build nests in most pre-bored horizontal burrows, including from man-made structures or abandoned nests of other Hymenoptera (Rau 1937). Most females prefer to nest near their natal nest; however, some females will disperse to other areas (Bosch and Kemp 2001). There is also a preference to nest near other active *O. lignaria*, resulting in nesting clusters (Bosch and Kemp 2001). When a nest location has been chosen by a female, she will perform a zigzag flight, which is hypothesized to help her memorize landmarks to assist in future nest location (Bosch and Kemp 2001).

Females will thoroughly clean out their selected nest prior to nest building (Rau 1937). When housekeeping of the nest is complete, females will start to bring in mud, held in the mandibles, to start lining the walls of the nest (Rau 1937). The female *O. lignaria* will build an initial partition, typically in the back of the nest cavity (Bosch and Kemp 2001). It can take about 10 trips to produce one partition (Bosch and Kemp 2001). After building the first partition, females will collect pollen and nectar to make a 'pollen provision'. Pollen is collected on setae on the ventral side of the abdomen (i.e., scopa) while the nectar is stored in the female's crop (Rau 1937; Bosch and Kemp 2001). Each female requires ca. 75 flower visits to gather a full load of pollen and nectar, and on average it takes ca. 25 loads of pollen and nectar to complete one provision (ca. 1,875 flowers per pollen provision) (Bosch and Kemp 2001). When females return to the nest cavity with their full provision load, they will walk to the last mud partition and regurgitate the nectar from their crop (Bosch and Kemp 2001). After regurgitating the nectar, she will exit the nest and turn around and reenter the nest backwards, walking to the same partition, scraping the pollen from her scopa with her back legs (Bosch and Kemp, 2001). This whole process is repeated about 15 to 35 times (Bosch and Kemp 2001). The female mixes the pollen and nectar together into a sloping cylindrical shape with a dry paste-like consistency (Rau 1937; Bosch and Kemp, 2001). The *O. lignaria* female will make a final foraging trip to collect only nectar, which is regurgitated onto the sloping end of the pollen provision (Bosch and Kemp 2001). Finally, the female will exit then re-enter the

nest backward and lay a sausage-shaped egg on the front of the newly constructed pollen provision (Rau 1937; Krombein 1962; Bosch and Kemp 2001). Following egg lay, the female will start collecting more mud to build another partition, sealing off the first cell and starting the next cell (Bosch and Kemp 2001). Mud partitions are very important for protecting offspring from parasites and predators that may infect adjacent cells within the nest (Krombein 1967; Bosch and Kemp 2001). In some wasp species, the texture of the partitions has been shown to help larvae to orient themselves toward the nest's entrance (Krombein 1967). The larvae will position their head toward the rough partition and away from the smooth partition in their cell, which prevents them from emerging as adults in the wrong direction and being unable to turn around because of their large size (Krombein 1967).

Blue orchard bees build their nest linearly with each cell containing one egg and one provision (Rau 1937; Bosch and Kemp 2001). Female *O. lignaria* build an average of 2.4 cells per day (Krombein 1962). Typically, one cell is left empty between the final provisioned cell and the nest entrance, AKA a vestibular cell, which is thought to prevent predators from entering provisioned cells (Levin 1966; Krombein 1967; Bosch and Kemp 2001). At completion of nest building, females will build a final partition at the nest opening that is thicker than other partitions within the nest (Rau 1937; Krombein 1967; Bosch and Kemp 2001).

Each nest has ca. 4-10 provisioned cells (Rau 1937; Bosch and Kemp 2001). A female can build 2-7 nests in her lifetime, with a sex ratio of about 1.5 to 2 males: 1 female offspring (Krombein 1962; Bosch and Kemp, 2001).

Hymenoptera are haplodiploid, and only mated females can produce offspring of both sexes (Flanders 1939). This characteristic allows mated females to choose the sex of their offspring (Rau 1937; Flanders 1939; Krombein 1962). Female offspring of *O. lignaria* are typically located at the back of the nest, while male offspring are located closer to the nest entrance (Rau 1937; Krombein 1962; Bosch and Kemp 2001). Cells for female offspring are generally larger and are provided with more pollen than those for males (Rau 1937; Krombein 1962; Levin 1966). Adult females are generally larger than males, which is consistent with females being provided with more food during development (Rau 1937; Levin 1966; Krombein 1967; Torchio 1980).

Osmia lignaria are univoltine, and eggs are produced in the spring of one year with adults emerging in the spring of the following year (Rau 1937; Krombein 1967; Bosch and Kemp 2000, 2001). Eggs hatch ca. one week after being laid and there are five larval stages (Bosch and Kemp 2001). The L1 larvae feed on egg juices and do not fully leave the chorion until molting into L2 larvae (Bosch and Kemp 2001). The L2-L4 instar feeds on the pollen provision (Bosch and Kemp 2001). The L5 instar will excrete feces (small blackish pellets) then will begin spinning its cocoon with silk from its salivary glands (Bosch and Kemp 2001). The three-layered cocoon takes 2-3 days to complete (Levin 1966).

Cocoons are ovoid-shaped, brownish, and have a nipple-like structure on the anterior end (Levin 1966; Bosch and Kemp 2001). The prepupa within the cocoon is dormant for 1-2 months in the summer, then will molt into a white pupa (Bosch and Kemp 2001). The pupa will start to blacken a few days after molting, though the wings are still not fully developed (Bosch and Kemp 2001). One month later the pupa will molt into an adult and overwinter until the following spring (Rau 1937; Krombein 1967; Bosch and Kemp 2001). Exposure to cold temperatures (3-5°C) is required during the overwintering period for adults to emerge in the spring, and adult *O. lignaria* will start to emerge as the temperature increases (Torchio 1976; Bosch and Kemp 2001).

Osmia lignaria are polylectic and will provision their nests with multiple pollen sources (Levin 1966; Torchio 1976; Bosch and Kemp 2000, 2001). *Osmia lignaria* are attracted to flowers in the Rosaceae family and have been found in various fruit trees including *Malus, Prunus,* and *Pyrus* (apples, plums, cherries, and pears) as well as in almond trees (Torchio 1981; Bosch and Kemp 2000). Both male and female *O. lignaria* are useful pollinators, although males only actively collect nectar (Torchio 1976, 1981; Bosch and Kemp 2001). Although *O. lignaria* will visit multiple floral sources, they tend to focus on repeatedly returning to a preferred host plant, which makes them an efficient cross pollinator, which is essential for orchard crops that do not self-fertilize (e.g., almonds, apples, apricots, cherries, and pears) (Torchio 1976; Bosch and Kemp 2001). *Osmia lignaria* will readily nest in man-made nesting materials, which can easily be

provided in orchards to establish bees near floral sources (Torchio 1976; Bosch and Kemp 2001). Although *O. lignaria* are solitary bees, they will gregariously nest (Torchio 1976; Bosch and Kemp 2001). The timing of adult emergence can be manipulated by up to 3-4 months to synchronize with the blooming period of the crop of interest (Torchio 1976, 1981). Another advantage for using *O. lignaria* to pollinate orchards is their ability to fly in inclement weather which often coincides with orchard crop blooms (Torchio 1976; Bosch and Kemp 2001). *Osmia lignaria* have also been shown to improve *Apis mellifera* movement between rows of trees, and increased pollination and fruit set are observed when both species are used in the same orchard (Brittain et al.2013).

Multiple parasites and predators negatively impact *Osmia* spp. (Krombein 1967; Bosch and Kemp 2001). One of the most important parasites of *Osmia* spp. are mites in the family Chaetodactylidae, specifically *Chaetodactylus krombeini* Baker of North America (Yamada et al. 1971; Krunić *et al*. 2001). Mites in the family Chaetodactylidae live in the nest cells of the families Megachilidae and Apidae, specifically in the genera of *Osmia* and *Lithurgus* (Krombein 1962, 1967; Eickwort 1994; Klimov et al. 2016). Chaetodactylid mites are associated with their host permanently, requiring the host for food, harborage, and dispersal (Krombein 1962; Eickwort 1994; Klimov et al. 2016).

Chaetodactylus krombeini is most often associated with the solitary bee *Osmia lignaria* (Rau 1937; Krombein 1962, 1967; Klimov et al. 2016). *Chaetodactylus krombeini* are kleptoparasitic mites that infest cells within the

nests of *Osmia* spp. and feed on the pollen provisions provided to *Osmia* larvae (Krombein 1962, 1967; Krunić et al. 2005; Bosch and Kemp 2001). It is hypothesized that *C. krombeini* specifically feeds on the nectar in *Osmia* pollen provisions (Krombein 1962). This parasitic relationship within the bee nests harms the bee offspring in multiple ways: mites will consume the pollen provision meant for the developing bee offspring causing bee offspring to develop into a small adult will low fecundity, or mites will directly feed on and kill the bee offspring (van Lith 1957; Krombein 1962; Bosch and Kemp 2001; Krunić et al. 2005; Bosch and Kemp 2001). One advantage to killing the bee offspring is it allows the mites to consume the entire provision without competition (Eickwort 1994). However, killing the host may cause mites to be trapped in the nests and not have a route to a new food source for future generations (Krombein 1962).

Chaetodactylus krombeini use multiple pathways for development to the adult stage based on different environmental conditions (Baker 1962; Krombein 1962; Bosch and Kemp 2001; Qu et al. 2003; Klimov et al. 2016). Early season infested cells with plenty of food will allow a mite life cycle of Egg, Larva, Protonymph, Tritonymph, and Adult (Krombein 1962). Cells with a scarce food supply, low humidity, mite overcrowding, or synchronous life stage with host may produce two facultative life cycles: Egg, Larva, Protonymph, Phoretic Deutonymph or Egg, Larva, Protonymph, Inert Deutonymph (Krombien 1962, 1967; Knülle 1987; Eickwort 1994; Bosch and Kemp 2001; Park et al. 2008; Krunić et al. 2005). The inert deutonymph is adapted to stay in the cell without

any food until a new bee reuses and provisions that nest in the next season (Krombein 1962). The phoretic deutonymph is adapted to attach to an eclosed adult bee for dispersal to a new nest (Krombein 1962). In 1962 and 1967, Krombein stated that inert deutonymphs will transform into phoretic deutonymphs when an adult *Osmia* is present in the cell, although this has not been documented elsewhere. The heteromorphic deutonymph stage allows *C. krombeini* to establish a new population in two ways: the phoretic deutonymph can only continue the next generation in the nest of the same host species it originated from, but it is possible for inert heteromorphic deutonymphs to continue the next generation with other host species that reuse the nest it is occupying (Krombein 1962; Bosch and Kemp 2001).

Mite reproduction can occur with a mated pair or through arrhenotokous parthenogenesis (Krombein 1962; Krunić 2005; Rożej-Pabijan and Witaliński 2018). Closely related species (*C. osmiae*) have been verified to be haplo-diploid, with females being diploid and males being haploid (Rożej-Pabijan and Witaliński 2018). Astigmatid mites can perform spermatogenesis with or without meiotic division, and when meiotic division does not occur arrhenotoky takes place and haploid males are produced (Rożej-Pabijan and Witaliński 2018). Both heteromorphic morphs of the deutonymph stage have been hypothesized to be females only (Krombein 1962; Krunić et al. 2005). If this theory is correct, combined with the ability to produce male offspring with arrhenotokous parthenogenesis, in the event that only one heteromorphic deutonymph invades

a host cell it would be possible for one mite to found a new population (Krombein 1962; Krunić et al. 2005; Rożej-Pabijan and Witaliński 2018). When a heteromorphic deutonymph is in a host cell, it will morph into a tritonymph, then a sexually mature adult, then produce an unfertilized egg (Krombein 1962; Krunić et al. 2005). The unfertilized egg would then develop into an adult male which can mate with its mother to produce both male and female offspring (Krombein 1962; Krunić et al. 2005); this is known as oedipal mating (Adamson and Ludwig 1993).

In a newly constructed nest cell that is provided with sufficient food, protonymphs will skip the heteromorphic deutonymph life stage and morph directly into tritonymphs (Krombein 1962; Krunić et al. 2005). During the development cycle of the host offspring, Chaetodactylidae mites can produce about 10 generations within a cell, although the number of generation and breeding time is dependent on the amount of food available, moisture content, and temperature (Krombein 1962; Krunić et al. 2005). In closely related chaetodactylids, females have been observed to have a high oviposition rate and can produce about 3 to 15 eggs per day, producing between 137 to 515 eggs in their whole lifetime depending on temperature and duration of oviposition day (Chmielewski 1993; Qu et al. 2003). As with many astigmatid mites, *C. krombeini* has a short generation time (Krombein 1962; OConnor 1994, 2009; Krunić et al. 2005). Chaetodactylid embryonic development lasts between 3 to 10 days followed by the larval stage (4-7 days), the protonymph stage (4-9 days), and the

tritonymph stage (4-7 days) (Chmielewski 1993). Astigmatid adults often have a short life; adult males of Chaetodactylidae live 32-83 days and adult females live 28 to 154 days (Chmielewski 1993; OConnor 1994). Although chaeltodactylids have been shown to be haplo-diploid and capable of producing male offspring with unfertilized eggs, it has also been reported that copulation is required to produce offspring in some species (Chmielewski 1993; OConnor 1994; Rożej-Pabijan and Witaliński 2018). Within 3 to 4 days after cells are provisioned by the female bee, both male and female mites are present (Krombein 1962). Female mites will usually lay their eggs on the wall of the cell furthest from the pollen provision (Krombein 1962).

When mites exhibit a heteromorphic deutonymphs stage, which occurs between the protonymph and tritonymph stages, generation time is typically much longer (Chmielewski 1993; Krunić et al. 2005). The deutonymph stage is the life stage in which mites will enter winter diapause (Chmielewski 1993; Krunić et al. 2005). The heteromorphic deutonymphs are completely morphologically different from nymphal stages that proceed and succeed this life stage (Walter and Krantz 2009). This facultative stage helps ensure the survival of astigmatid mites, as they often live in temporary habitats with transitory food sources (Knülle 1987; Oconnor 1994). The highly modified heteromorphic deutonymph forms not only allows for dispersal and diapause, but also allows for survival in harsh environments (Krombien 1962; OConnor 1994, 2009; Krunić et al. 2005).

Inert heteromorphic deutonymphs stay encased within the protective cast skin of protonymphs (Baker 1962; Krombein 1962; Krunić et al. 2005). Inert deutonymphs are smooth, round, have four pairs of greatly reduced legs, and are immobile (Baker 1962; Klimov et al. 2016). This morph has the capacity to lay dormant in this life stage for several months to years while waiting for a new bee host with similar nesting behavior (Krombein 1962; Eickwort 1994; Krunić et al. 2005). Phoretic deutonymphs are more sclerotized than other life stages and have a propodosomal and hysterosomal shield that gives them a brownish color (Baker 1962; Krombein 1962; Bosch and Kemp 2001; Fain and Pauly 2001). The first three pairs of legs have recurved empodial claws, while the last pair of legs have long whip-like setae (earning them the nickname “hairy-toed mite”) (Baker 1962; Bosch and Kemp 2001). Phoretic deutonymphs also have a suctorial plate posteriorly on their ventral side (Baker 1962; Krombien 1962). These morphological adaptations likely aid with phoresy (van Lith 1957; Krombein 1962; Eickwort 1994; OConnor 2009). When phoretic deutonymphs attach to adult bees they are most often located on the posterior thorax and anterior abdomen, with some mites randomly dispersed through the bee’s body (Krombein 1962; Eickwort 1994). Phoretic mites are often in locations that are not groomed by their host, they will pursue these locations instead of being removed from other locations on the host's body (Eickwort 1994). In literature there is disagreement regarding if males or females are the preferred host sex of *C. krombeini* (Krombien 1962; McKinney and Park 2013). Males *Osmia* spp. do not provision

nests, only interacting with females for courtship and mating during their short lives (Rau 1937; Krombein 1962; Bosch and Kemp 2001). Mites do not transfer from male to female during copulation, or the transfer is negligible, making male *Osmia* spp. an unreliable host for the location of new habitats for *C. krombeini* (Krombein 1962; Eickwort 1994; McKinney and Park 2013).

In 1937, Rau described two *Osmia lignaria* males being covered by layers of mites and later finding a female that was similarly covered with mites in the family *Tyroglyphidae*. In 1967 Krombein wrote that Rau was most likely actually describing *C. krombeini* (Rau 1937; Krombein 1962). Krombein (1962) observed that very few females were infested with mites, and that the females that had mites on them had very few mites. Further observations by Krombein (1962) showed that males were more often infested with phoretic deutonymphs and when males were infested, they had more mites per bee. Krombein (1962) believed the reason for male hosts being much more infested than female hosts was because of *O. lignaria*'s skewed sex ratio (1 female: 2 males) and because males emerged from nests before females. However, McKinney and Park (2013) observed that mites preferred to infest female cells which were located in the back of the nests. Krombein (1962) explains that mites that infest the innermost cells would likely die if their host died before becoming an adult, which would

leave mites trapped in the cell. He further explained that mites need to infest cells in the middle or closer to nest opening to be provided with a host that phoretic deutonymphs can attach to as they chew their way out of the nest (Krombein 1962).

Krombein (1962) pointed out the opportunities for parasitism for both phoretic and inert heteromorphic deutonymphs. Phoretic deutonymphs will maintain a close relationship with their original host species while inert deutonymphs will parasitize any bee species that used the pre-existing nest. *Osmia* spp. in natural nests may come in contact with both morphs of heteromorphic deutonymphs, spreading mite infestation to multiple *Osmia* species native to a region but also to other cavity nesting bees (Krombein 1962). Though the negative effects of mite infestations to other bee species are not well-studied, control of Chaetodactylidae in commercial nests will likely aid bee conservation efforts.

Minimizing the number of mites in bee nests is particularly difficult due to both parasite and host being arthropods and treatments meant to kill the parasite may also have deleterious effects to the host (Sekita and Yamada 1993). Currently mite control is limited to managed bee nests, but this can have a positive impact on wild bee health as mite dispersal from managed bees is likely. Replacing nesting material by using paper or clear straws and removing infested cells will help reduce mite populations (Sekita and Yamada 1993; Bosch and Kemp 2001). Heat treatments of infested nests when pre-pupae bees are in

diapause have also been shown to be effective at reducing the numbers of Chaetodactylid mites (Sekita and Yamada 1993; Bosch and Kemp 2001). Nests exposed to 30-32℃ for 30-40 days at 70% RH, killed up to 100% of mites, and the longer the mites were exposed to high temperatures the greater the mortality rate. Bee survival surpassed 80% throughout high temperature exposure (Sekita and Yamada 1993). In commercial settings, phoretic deutonymphs are typically the more problematic morph. That is because the inert deutonymphs can be managed using multiple strategies such as the use of paper straws in nests, flaming nests after bee cocoons have been removed (to burn residual mites), the use of chemicals in nests, or heat treatment of nests as mentioned above (Sekita and Yamada 1993; Bosch and Kemp 2001; Jim Watts, personal communication).

Control methods of cocoons include removing loose cocoons from nesting material instead of maintaining whole nests does not allow for emerging bees to walk through infested cells, thereby minimizing the number of mites an adult bee will encounter, minimizing the number of mite infestation in new bee nests (Sekita and Yamada 1993; Bosch and Kemp 2001). This method is time consuming and loose cocoons can increase the dispersal of female bees, but it is also a very effective method for controlling mite populations (Sekita and Yamada 1993; Bosch and Kemp 2001). The use of endosulfan, an organochlorine, can be effective for mite control in managed populations (Sekita and Yamada 1993; Bosch and Kemp 2001). Endosulfan is not toxic to the bee host and treating nesting material with 60 ppm endosulfan helped minimize the mortality of host

eggs caused by mites (Sekita and Yamada 1993; Bosch and Kemp 2001). Overwintering cocoons can also be submerged in 0.007% solution of endosulfan for three minutes then dried to kill mites (Krunić et al. 2001). Another method to remove mites from overwintering cocoons is to blow them off with pressurized air after they have been stripped from nesting material (Stutzman, personal communication). However, if mite removal from cocoons is unsuccessful phoretic deutonymphs can be spread among properties easily, especially as their brownish coloration and small size allow phoretic deutonymphs to be mostly undetectable to the naked eye when attached to a similarly colored cocoon. Park et al. (2008) established that *C. krombeini* can sufficiently disperse from nest to nest by walking. Their ability to disperse increases especially during commercial transportation, as mites are able to move easily among cocoons when they are shipped from commercial growers to farmers or hobbyists in other regions or states. This, paired with arrhenotokous parthenogenesis, could lead to mite infestations spreading more rapidly in commercial nests than in nature. Control of *C. krombeini* in commercial settings will contribute to bee conservation but will more importantly increase crop pollination and improve crop yield where commercial *Osmia* spp. are used (Torchio 1976; Bosch and Kemp 2001).

Chaetodactylus krombeini have been said to prefer both male and female bee hosts, with male positions in nest making it possible for multiple hosts to pass through infestations and females providing a clearer route to suitable resources. Determining a preference in host sex could help control efforts

towards this kleptoparasite, making it clear where pesticides should be efficiently applied in nests. *Chaetodactylus krombeini* heteromorphic deutonymphs have also been hypothesized to only be female and founding nests through arrhenotokous parthenogenesis. If *C. krombeini* heteromorphic deutonymphs are not exclusively female this could help establish an economic threshold within nests, making it unnecessary to treat nests with chemicals if it is unlikely that few mites could establish infestations if both sexes are required to produce offspring. Many life history traits of *C. krombeini* are not clear, making scanning electron microscope (SEM) images useful for determining functions of specific morphology of these mites. Learning the function of different appendages of this species could also give insight into their behavior. This research is necessary for clarifying the life history of *C. krombeini*, which will lay the foundation for further research in mite control in solitary bee nests.

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Chapter 1

Chaetodactylus krombeini Host Sex Preference

Abstract

Chaetodactylus krombeini (Acari: Chaetodactylidae) is considered one of the most important pests to *Osmia* solitary bees because their infestations increase bee mortality and morbidity. As a kleptoparasite mite within solitary bee nests, *C. krombeini* feed on pollen provisions intended for bee offspring. Previous literature has contradictorily reported that *C. krombeini* have a preferred host sex of both male and female *Osmia* bees. By examining mite distribution within bee nest blocks, we aimed to determine if *C. krombeini* exhibit any preferences based on host sex or position of larvae within *Osmia lignaria* nests. This was examined using large tents in which commercially acquired *Osmia* were permitted to forage and reproduce. Few bee nests were infested with mites, likely due to low initial mite infestations (2021-2022) and low *O. lignaria* populations (2022-2023). However, in parasitized nests, mites were found infesting the first nest cells in the back of nests regardless of the sex of the bees, suggesting a mite positional preference within nests.

Introduction

Osmia lignaria (Hymenoptera: Megachilidae), are solitary bees native to North America that are often raised commercially for use as orchard crop pollinators (Rust 1974). *Osmia lignaria* make their nests linearly in preexisting cavities and provide provisions of nectar and pollen in mud partitioned nest cells for each egg (Rau 1937). Provisioning *O. lignaria* typically build their nests with female offspring in the back of the nest, and male offspring located towards the middle and nest entrance (Rau 1937; Krombein 1962, 1967; McKinney and Park 2013). Hymenoptera are haplo-diploid, and females determine the sex of their offspring by choosing which egg to fertilize with stored sperm from their spermatheca (Flanders 1939).

Chaetodactylus krombeini are kleptoparasitic mites that infest partitioned cells within the nests of *Osmia* spp. and consume the pollen provisions meant for developing bee larvae (Krombein 1962, 1967). Infestations in these solitary bee nests can lead to smaller, less efficient pollinators as well as 20-50% bee mortality when humidity and temperatures are high (32.5˚C) (Bosch and Kemp 2001; Krunić et al. 2001; Ahn et al. 2016; J. Watts, personal communication, December 2021). *Chaetodactylus krombeini* has six life stages: egg, larva, protonymph, heteromorphic deutonymph, tritonymph, and adult (Fig. 1.1) (Krombein 1962). The heteromorphic deutonymph stage is facultative; mites may enter either an inert form or a phoretic form depending on environmental conditions (Krombein 1967; Krunić et al. 2005). Most life stages of *C. krombeini*

are present throughout bee development, but the formation of heteromorphic deutonymphs begins when conditions within the nest start to decline, likely due to a shortage of food, low humidity, or mite overcrowding (Krombein 1962; Knülle 1987; Krunić et al. 2001). The inert deutonymph will remain in the abandoned bee nest, waiting for a new bee to use the cavity (Krombein 1962). The phoretic deutonymph will attach to emerging adult bees as they leave the nest and will drop off into the nest cell of a new nest when the adult bee starts provisioning for their offspring (Krombein 1962). When both inert and phoretic deutonymphs are provided with new food resources in a bee nest they will continue development, morphing into a tritonymph and then an adult (Krombein 1962).

Previous studies contradictorily report that phoretic deutonymphs will preferentially infest cells containing either male or female bee offspring (Krombein 1962; Park et al. 2008; McKinney and Park 2013). Rau (1937) described two *O. lignaria* adult males covered by layers of mites, and later in these observations found an adult female that was similarly covered with *Tyroglyphidae* mites. Krombein (1967) wrote that Rau was most likely describing *C. krombeini.* Krombein (1962) observed that very few adult female bees were infested with mites, and when they were, infestation levels were low. Further observations by Krombein (1962) described the pattern of adult male bees being more often infested with phoretic deutonymphs, and when infested, having more mites per bee relative to females. Based on these observations, Krombein (1962) hypothesized that male hosts were more infested than female hosts because *O.*

lignaria have a skewed sex ratio (1 female: 2 males) and males emerge from nests before females. However, McKinney and Park (2013) observed that mites preferred to infest female host cells, which were in the back of the nests.

Mites that primarily infest female host nest cells would have a clearer path to a new habitat with a food source for future generations, whereas male host cells would be a poor host choice because they do not build nests with pollen provisions (Rau 1937; Bosch and Kemp 2001; Krombein 1962). Krombein (1962) explained that mites that infest the innermost nest cells would likely not survive if their bee host died before emerging as an adult, which would leave mites trapped in the nest cell. He further suggests that mite infestations should occur in nest cells in the middle or front of the nest so that phoretic deutonymphs can attach to host bees as they emerge from the nest (Krombein 1962). The preference for a male host nest cell is argued to be ideal because, even if the host dies within the nest cell, more bee offspring are likely to have to travel through the infested middle and front nest cells during emergence (Krombein 1962). However, female nest cells are thought to be the preferred infestation site as female adult hosts provide a more predictable route to new pollen provisions compared to male adult hosts (McKinney and Park 2013). Male *O. lignaria* are not thought to pass on mites during courtship or mating, which makes male bees an unclear path for future generations of *C. krombeini* (Krombein 1962). Other mites, such as mites from the genus *Rhinoseius*, use phoresy to transfer from flowers to a flying host to new suitable resources (Colwell and Naeem 1994; Park et al. 2008). In one

study *C. krombeini* phoretic deutonymphs were not observed to transfer between hosts via flowers (Park et al. 2008). However, the study tested blueberry flowers only, which have a smooth downward facing floral surface. Other orchard blossoms, such as apples, almonds, and cherries may offer a more suitable surface for mites and should also be tested.

This study evaluated the host sex preference of *C. krombeini* by examining mite distribution in *O. lignaria* nests with both male and female offspring and in male-only nests. Hymenoptera are haplo-diploid, therefore unmated females produce only male offspring while mated females can produce both male and female offspring (Flanders 1939). Therefore, the sex of *O. lignaria* offspring was manipulated by allowing one bee treatment group to mate (male and female offspring) and keeping one bee treatment group unmated (male offspring only).

Materials and Methods

Mite Colony

Chaetodactylus krombeini mites were collected in October of 2021 and 2022 from multiple commercial *Osmia lignaria* nests in Washington and Oregon. At UCR, mite populations were sorted by collection site and maintained in 1.5 µL microcentrifuge tubes in an environmental chamber (VWR Model No: 2005 and Fisher Scientific Model No: 146E) set to 26°C. Each tube was filled with 100 to 250 µL of a stock mixture of 60% honey bee pollen, 16% honey, 20% DI water,

and 4% fructose (pollen provision). Each 1.5 µL tube was sprayed with a 0.1% dilution of food grade fungicide (methyl paraben, CAS: 99-76-3) to prevent mold from forming after the pollen provision was added. This food would last for multiple *C. krombeini* generations. Every 2 weeks mites from an established tube population would be transferred in groups of 10 into new tubes with a fresh pollen provision. Mite colonies were maintained this way from November 2021 to June 2023, with new field-collected mites added each spring.

Osmia lignaria

In 2021, *O. lignaria* cocoons were purchased from Foothill Bee Ranch (Foresthill, Placer County, CA) and Northwest Pollination (Canby, Clackamas County, OR) in November 2021. *Osmia lignaria* cocoons were maintained at 4°C from November 2021 to March of 2022. During the overwintering period, cocoons were separated into males and females by size. Male cocoons are typically 10-12 mm in length compared to female cocoons at 12-14 mm in length (Bosch and Kemp 2001). Cocoons were moved to containers at room temperature (23°C ± 1°C) on March 21, 2022. As *O. lignaria* emerged, sex was confirmed and they were moved in one of three small rearing cages (BugDorm-2S120, W60 x D60 x H60 cm) in the laboratory: unmated females only, females and males, and excess males. Each rearing cage was provisioned with sugar water and a pollen

source. Bees from the laboratory were released ca. 1 week after the first bee emergence into coordinating field tents: male and female bees into mated treatment tents and unmated female bees into the unmated treatment tents.

In 2022, *O. lignaria* cocoons were purchased from Northwest Pollination (Canby, Clackamas County, OR) in November 2022. Cocoons produced from the 2021 field trial were also used. Because cocoon size was a poor indicator of adult sex, cocoons were sorted into individual clear gel capsules (XPRS Nutra size: 000) then maintained at 4°C for overwintering until April 13, 2023. As bees emerged within the gel capsules, they were sexed and released into coordinating tents in the field (as above) at the end of each day for 9 days.

Field Trials

Twelve 4.25 m^3 tents were set up in a field at the University of California Riverside's Agricultural Operations. Tents were numbered and placed in two rows ca. 3.7 meters apart, with each cage ca. 2.4 meters apart (Fig. 1.2). Each pair of tents was randomly assigned a bee treatment (mated or unmated). Each tent contained two or three observation nest blocks with 26 nesting galleries each (Fig. 1.3). Observation nest blocks measuring 29.5 cm x 18 cm x 38 mm were made from untreated Douglas fir 2" x 8" lumbar and nesting galleries were cut to ca. 7.5 mm depth with a router. Each nest block was burned to create a unique charred pattern on the nest blocks to aid in *O. lignaria* nest location when provisioning nest cells. Transparency film was attached to the nesting gallery

sides of each nest block with glue (Aleene's Original Tacky Glue, USA). Observation nest blocks were covered with black waterproof poster board held in place by two large rubber bands. This allowed observers to periodically check nest formation in the nesting galleries. Each tent contained two (2022) or three (2023) observation nest blocks, placed in nest stands facing a southeast direction, nest stand legs were coated with Tanglefoot (Tangle-Trap, Paste Formula, Form 97-40) to deter ants (Fig. 1.4). Nest blocks were reused in 2023 after removing the plastic film, burning the nesting galleries, and replacing it with new plastic film. Nest cells were numbered from back to front, with the back of the nest starting at nest cell #1 (Fig. 1.5).

In 2022, each tent contained 15 pots of *Phacelia tanacetifolia* (lacy phacelia), one pot of Salvia, and three buckets of cut *Brassica nigra* (black mustard) for adult *O. lignaria* foraging. One of the three buckets of *B. nigra* was changed out every 3 days in each tent to ensure that there was sufficient pollen and nectar available for adult bees. Irrigation was supplied in each tent and plants were watered every day for ca. 5-10 minutes depending on the daily temperature. Watering also ensured a mud source for *O. lignaria* nest partitioning.

In 2023, each tent contained 16 pots of lacy phacelia and 3 buckets of black mustard. After two weeks, the buckets of black mustard were replaced with *Brassica napus* (canola), with one bucket of canola replaced every 3 days.

In both 2022 and 2023, treatment groups were separated in a random pairwise fashion across the two rows of field tents. Purchased *O. lignaria* cocoons were naturally infested with *C. krombeini* mites. In 2022, *O. lignaria* were exposed to ca. 50 mites (mixed age and sex) in each vial prior to release into tents. In 2023, ca. 100 mites (phoretic deutonymphs) were introduced to *O. lignaria* in the gel capsules. In spring 2022 *O. lignaria* were brought to the field on March 30, 2022, in ventilated vacuum vials and in spring 2023 *O. lignaria* were brought to the field in plastic containers (separated by treatment group) on April 15, 2023, contained bees were then placed on nest stands, and allowed to fly out into tents. In 2022, 12-14 females were released in each unmated tent and 23-26 females were released in each mated tent, with one male released per female. In 2023, 11-12 females were released in each tent, with one male released for every female in the mated tents. Nest blocks were checked every other day for bee nest building, bee developmental stages, and for detectable mites within the nest cells of nests. Nest blocks were brought to the lab after ca. 8 weeks and bee development and mite presence was recorded until all bees pupated in the lab (2022: 9 weeks and 4 days; 2023: 6 weeks and 2 days). Any remaining adult bees were caught and euthanized.

In both seasons after all bees pupated, nest blocks were deconstructed. The transparency film was removed one nesting gallery at a time with a razor blade. All cocoons were removed and placed in clear gel capsules (XPRS Nutra

size: 000) that were labeled with the tent, nest block, nesting gallery, and nest cell it was collected from. Any living mites were collected and placed in microcentrifuge tubes to be reared in the lab.

Statistics

Statistical analyses were conducted in SAS (SAS Institute Inc., Cary, NC, 2012, v. 9.4). A Kruskal-Wallis test (PROC NPAR1WAY) was performed to evaluate the effect of mite presence or absence on bee emergence. A generalized linear model (Proc GLM) was used to examine the effect of mite presence or bee sex on adult bee length. A t-test (Proc TTEST) was used to compare observed bee length (by sex) to expected average adult bee length.

Results

2021-2022:

Bees in all tents (n = 12) successfully built nests for bee offspring in 2022. In 2022, 346 nest cells were provisioned, 286 cocoons were produced, and 168 adult bees emerged (Table 1.1). All bees that emerged as adults were male. Five of the 118 remaining cocoons contained dead larvae or white pupa (the life stage between prepupa and black pupa). Only three bee offspring were female (all in tent 6), and they all died inside their cocoons. At the end of the field season ants started attacking nests, killing seven larvae in tents 2, 6, 7, 10, 11, and 12. An additional seven larvae were found dead in nests (Table 1.1).

Eight of the 300 nest cells with bee offspring contained mites, and seven of those eight nest cells were in nest cell #1 (back of the nest) with one observation of mites in nest cell #2 (Fig. 1.5). Seven of the eight mite-infested nest cells were in tent 6 with one mite-infested nest cell in tent 2; both tents held mated females. Two of the eight nest cells that contained mites were occupied by female bee offspring, with most infestations occurring in male nest cells. In all nest cells where mites were observed, bees died as adults before emerging from cocoons. There was a significant effect of mite infestation on bee emergence (χ^2=9.67, df=1, P=0.0019), with a decrease in bee emergence when mites were present.

Bee length (head posterior to abdomen anterior) was measured for all adult offspring whether or not they emerged from the cocoons, except for 51 male adults that were saved for 2023 field trials. The two females that occupied nest cells with mites measured 8.32 mm and 8.87 mm, which is below the reported average size of female *O. lignaria* (11-13 mm long) (Rust 1974). Males with mites measured 6.75 mm, 6.78 mm, 7.55 mm, 7.87 mm, 8.43 mm, and 8.63 mm and were also found to be below the average adult size (9-10 mm) (Rust 1974). The length of the bees cannot be compared to other studies of *O. lignaria* performed in tents, as it is more common to measure intertegular span (the length between wing attachment to the thorax) to estimate bee size in solitary bees (Cane 1987).

2022-2023:

Bees in 11 of 12 tents successfully produced cocoons, with tent 1 only producing one pollen provision and no offspring. In 2023, 194 nest cells were provisioned, 147 cocoons were produced, with only two dead larvae and one dead egg observed in nest cells (Table 1.1). Tents 11 and 12 produced the majority of cocoons, 51 and 27, respectively. Cocoons produced in 2023 were dissected in September 2023 to determine host sex preference of *C. krombeini*, therefore making emergence data unavailable. Cocoon dissections revealed a total of 107 male bees and 32 female bees produced. There were also three dead larvae, three white pupae, and two black pupae that could not be sexed.

Mites were found in 11 nest cells of nests in tents 4, 7, 9, and 10. Eight of the 11 nest cells with mites were located in nest cell #1; three were located in nest cell #2 (Fig. 1.5). Of the 11 nest cells with mites, only 2 nest cells contained a cocoon. Dissections showed that nests cells with mite infestations produced one male bee offspring and one female bee offspring. One mite-infested nest cell in tent 9 contained the remains of bee chorion (Fig 1.6). The one infested nest cell in tent 7 only contained phoretic deutonymphs and no other life stages; there was no sign of bee offspring (Fig. 1.7).

Bee lengths were measured on all sexed dissected cocoons. The female bee offspring that was produced in the presence of mites measured 9.10 mm, which is below the average length for female O. lignaria (11-13 mm) (Rust 1974). The male bee offspring produced in the presence of mites measured 7.55 mm, also below the average length (9-10 mm) (Rust 1974).

Discussion

All mite infestations observed in both seasons were found in the back of nests, in cells #1 and #2. Although mites were in the back of the nests where female bees are typically found, most of the bee offspring were males in 2022. In 2023, mite infestations were observed on one male and one female bee host. The observed prevalence of mites only in the back of nests suggests that there is a mite positional preference rather than a host sex preference. This might be due to *Osmia* hosts having a short life span, and mites needing to leave their host as soon as possible to have the opportunity to start new populations.

In spring 2022, very few nest cells across tents and treatments were mite infested. The small number of mite infestations is likely due to not introducing enough phoretic deutonymphs to bees before they were released in the tents. Previous literature suggests that phoretic deutonymphs are easily produced when there is a loss of humidity, shortage of food, and overcrowding of conspecifics in the nest cell (Krombein 1962; Knülle 1987). However, this was not easily replicated under lab conditions (see Appendix A). Under lower humidity

conditions mites desiccated or the food source became moldy leading to mite death. A small number of phoretic deutonymphs were only produced in one microcentrifuge tube that was left undisturbed for 2 months and the food source did not become moldy. Phoretic deutonymphs were not commonly found on the purchased cocoons either, likely due to beekeepers actively removing cocoons from mite infested nest cells and removing phoretic mites from cocoons prior to shipping to customers (Sekita and Yamada 1993; Bosch and Kemp 2001; Kari Stutzman, personal communication October 2022).

In spring 2023, there were a greater number of nest cells (n = 11) infested with mites, though this is mostly due to a damaged nest. Mites in nest block 9C (spring 2023) were able to move among nest galleries 15, 16, 17 and 18 due to the transparency film coming away slightly from the nest block. This damage also allowed a spinning larva to fall out of its nest cell and into another gallery, but the larva was still able to complete the cocoon.

Several challenges arose during the experiment that made it more difficult to draw conclusions about mite preferences. Mites only persisted in three of the eight nest cells that were infested in spring 2022, possibly due to the heat in Riverside, CA, which reached up to 35°C during the experiment. Heat treatments are used as an effective control method for Chaetodactylid mites, with temperatures of 30-32°C shown to eradicate an entire mite population in bee nests (Sekita and Yamada 1993; Bosch and Kemp 2001). There were 3 nest blocks in each tent in spring 2023 because of the expectation of releasing more

females in the field due to improved bee rearing methods. However, there were few adult female *O. lignaria* available because no females emerged from the 2022 field season. We purchased 700 *O. lignaria* from a beekeeper, but > 40% of these bees were *O. cornifrons*, which could not be used in this experiment as they are not native to this region. Only 136 female bees were released among the 12 tents. There were very few females to build nests in spring 2023, which in turn caused a low mite count despite introducing phoretic mites to all bees prior to release.

It was often difficult to detect early mite infestations in the provisioned cells as the bees often placed the pollen provision against the transparency film of the nest blocks, blocking all observations within a nest cell. This made it unclear when nest cells were initially infested and when nest cells had continuous infestations. Mite infestations were only apparent when mite levels were high and mites could be seen walking across the transparency film (Fig. 1.8).

Ant attacks caused the 2022 spring experiment to end early as they were destroying nest cells that could have been shown to have mites present after nest blocks were deconstructed. There were also ant attacks in multiple nests in spring 2023, however there were no offspring lost. As a precaution in spring 2023, nest blocks were taken to the lab to prevent further ant attacks and potential offspring losses. Adult bee survival was low in 2023, which may have

been impacted by other arthropods (i.e., spiders) or accidental death by Tanglefoot (introduced to deter ants). Earwigs were another insect pest that entered provisioned nests, likely scavenging for pollen provisions in nest cells that were not yet partitioned.

There was an uneven number of female bees released across the two treatments in spring 2022. More female bees were released in mated tents as cocoon measurements proved to be inaccurate in sexing bees. Many males and females were mixed, and if any female bee came in contact with a male bee she was included in the mated treatment.

Bee emergence decreased when mites were present in the nest cell. However, there was a low sample size with only eight cells infested with mites. Future studies with more widespread infestations in nests will likely provide a more robust sample size for more accurate analysis.

Three previous studies have made three different conclusions on the location of *C. krombeini* in *Osmia* spp. nests: phoretic deutonymphs were found in larger numbers on adult male *O. lignaria* than on *O. lignaria* females (Krombein 1962), phoretic deutonymphs were observed to unload in the middle of *O. cornifrons* nests (Park et al. 2008), and mite infestations were found in the back of *O. cornifrons* nests where female *O. cornifrons* nest cells are located (McKinney and Park 2013). Mite infestations in female *Osmia* nest cells provide a clear route for mite survival, but if the female bee dies before emergence mites will likely become trapped in the back of a nest (Krombein 1962). Male *Osmia*

nest cells are located in the middle or front of the nest, so even if the male offspring dies other bees will have to pass through its nest cell to leave the nest, allowing mites to attach to bees as they leave the nest (Krombein 1962). However, if the male bee survives and becomes a host to phoretic deutonymphs he may not provide a route to a new habitat for future mite generations (Krombein 1962). The evidence from these previous works does not clearly explain where the mites are likely to be located in the nest, or if there is a difference in location of mites among *Osmia* spp. nests.

The goal of this study was to determine if there was a host sex preference of *C. krombeini* when they are carried into *O. lignaria* nests. Unfortunately, this field experiment resulted in low mite numbers in spring 2022 and low bee numbers in spring 2023. However, mite infestations that were observed were in the back of the nest regardless of the sex of the bee offspring. This suggests that mites have a positional preference within nests. Better understanding of mite infestations is important for control of mites in managed solitary bee nests. If mites have a positional preference for the back of nests, nests can be more easily targeted with pesticides. This can minimize the amount of pesticides placed in the environment and in bee nests if chemical applications are only needed in a specific location. Future research should examine the cues that

stimulate *C. krombeini* to drop off the bee host and what cues cause them to attach to a host. It may be possible to use this information to determine if there are ways to make a host unattractive, thereby protecting bees from mite infestations.

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Table 1.1. Number of provision nest cells, cocoons, sex of bee, and mite infestation produced in each year of study.

	Total cells provisioned	Cells with bee offspring	Dead immatures	Cocoons Produced	Died in Cocoon			Emerged adults		Cells with detectable mites	% of mite infested cells
					Immature	Male	Female	Male	Female		
2021-2022	346	300	14	286	5	107	6	168	0	8	2.3%
2022-2023	194	149	3	147	8	107*	32*	--	--	11	5.7%

*All adult offspring from 2022-2023 were considered to have died in cocoons due to all cocoons being dissected for bee sexing.

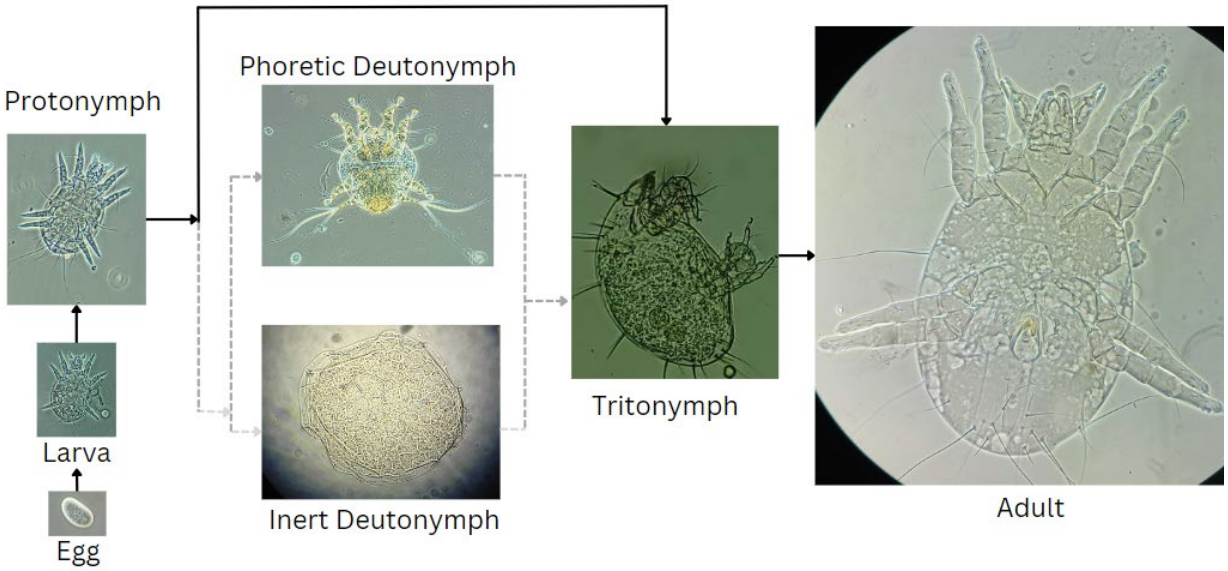


Figure 1.1. Developmental pathway of *Chaetodactylus krombeini*. Solid black lines indicate a normal life cycle under ideal nest cell conditions. Grey dashed lines indicate the facultative life cycle when the heteromorphic deutonymph life stage is induced with poor nest cell conditions (e.g., mite overcrowding, low humidity, low food quantity/quality, or synchronous to host life cycle). The phoretic deutonymph stage is specialized for phoresy on the bee host, while the inert deutonymph is a dormant stage that survives in the absence of a bee host.

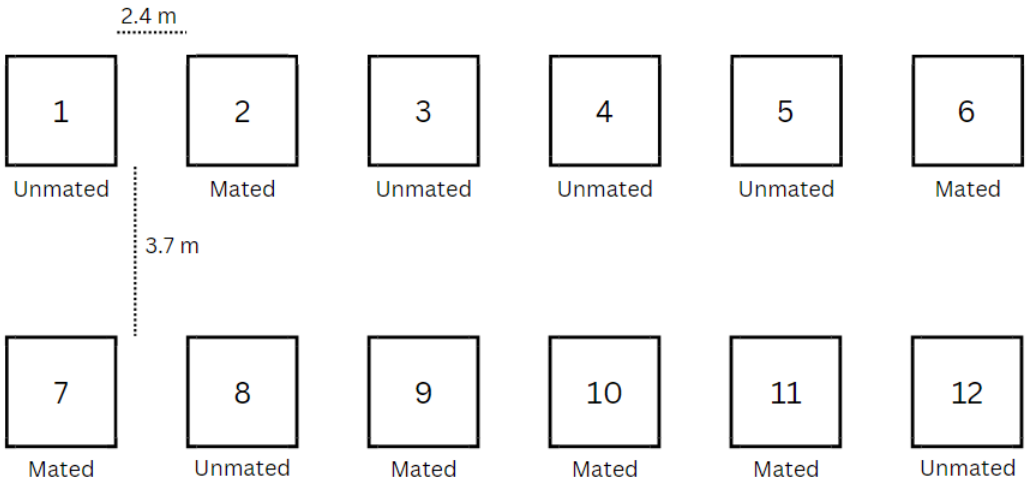
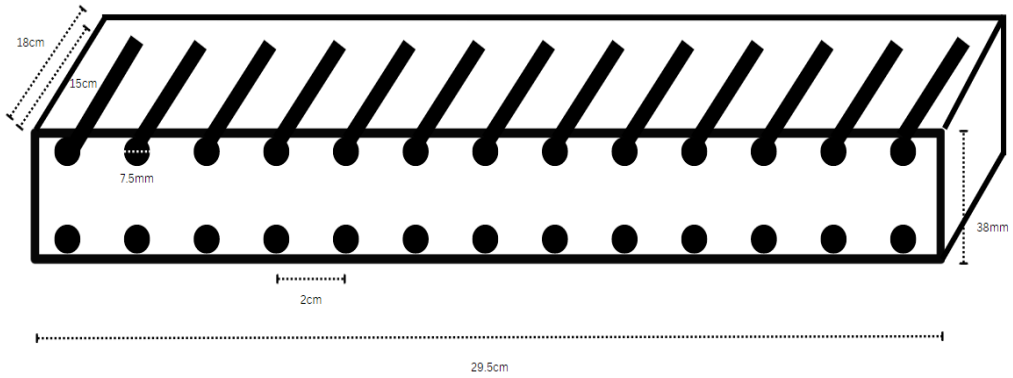


Figure 1.2. Layout of treatment tents used in spring 2022 and spring 2023. Each pair of tents was randomly assigned to the treatment group (mated or unmated female *O. lignaria*).

A



B

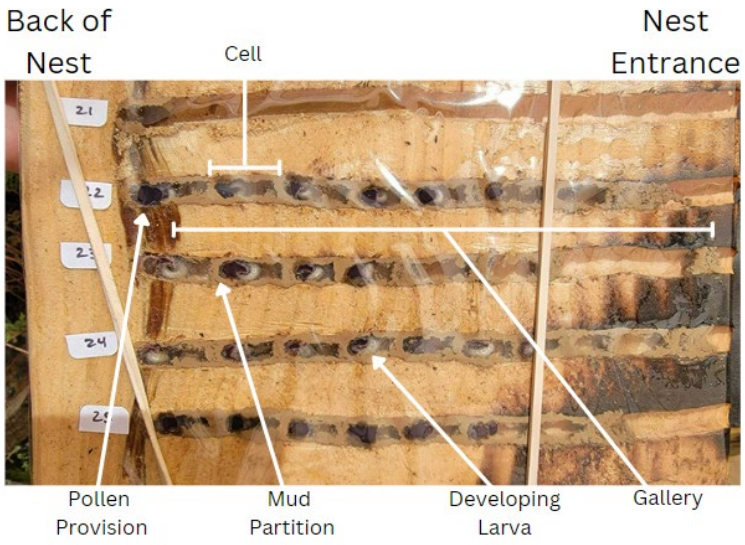


Figure 1.3. (A) A schematic of the bee nest blocks used throughout the study. *Osmia* nest blocks measured 29.5 cm x 18 cm x 38 mm and were crafted from untreated Douglas fir. Each side of the nest block had 13 nesting galleries, with 26 total nesting galleries (longitudinal cavities) available per nest block. Each tent had two (spring 2022) or three (spring 2023) nest blocks for provisioning bees. (B) Nest block from study showing nest cell, gallery, mud partition, pollen provision, developing larvae, and the orientation of the nest from back to entrance.

Figure 1.4. Nest blocks (arrow) in a yellow nest stands. Each tent contained one nest stand which held all the nest blocks. The nest stands were set up facing Southeast in each tent. Tanglefoot was coated on all four legs of the nest stand to help prevent ants from attacking nests.

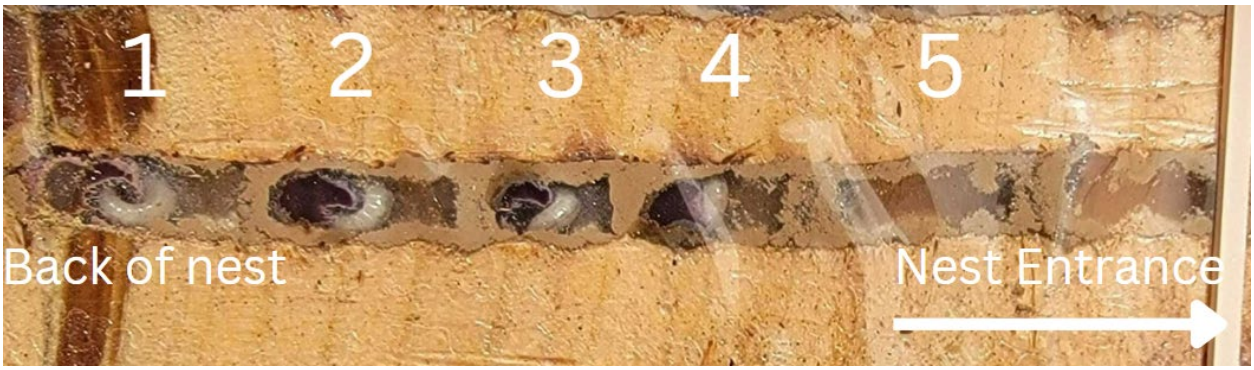


Figure 1.5. Nest gallery with numbered nest cells, starting with nest cell #1 in the back of nests and counting up toward the nest entrance. Female bee offspring are often located in the back of nests, with male bee offspring located in the middle and near the nest entrance.

Figure 1.6. Pollen provision from mite-infested nest cell with bee chorion remains. Mites were observed in this nest cell with a healthy-looking *Osmia* egg. As the mite infestation grew there was no sign of a developing bee larva. When the nest was dissected the bee chorion was discovered still lodged in the pollen provision. Mites likely killed the egg to prevent competition over the food source.

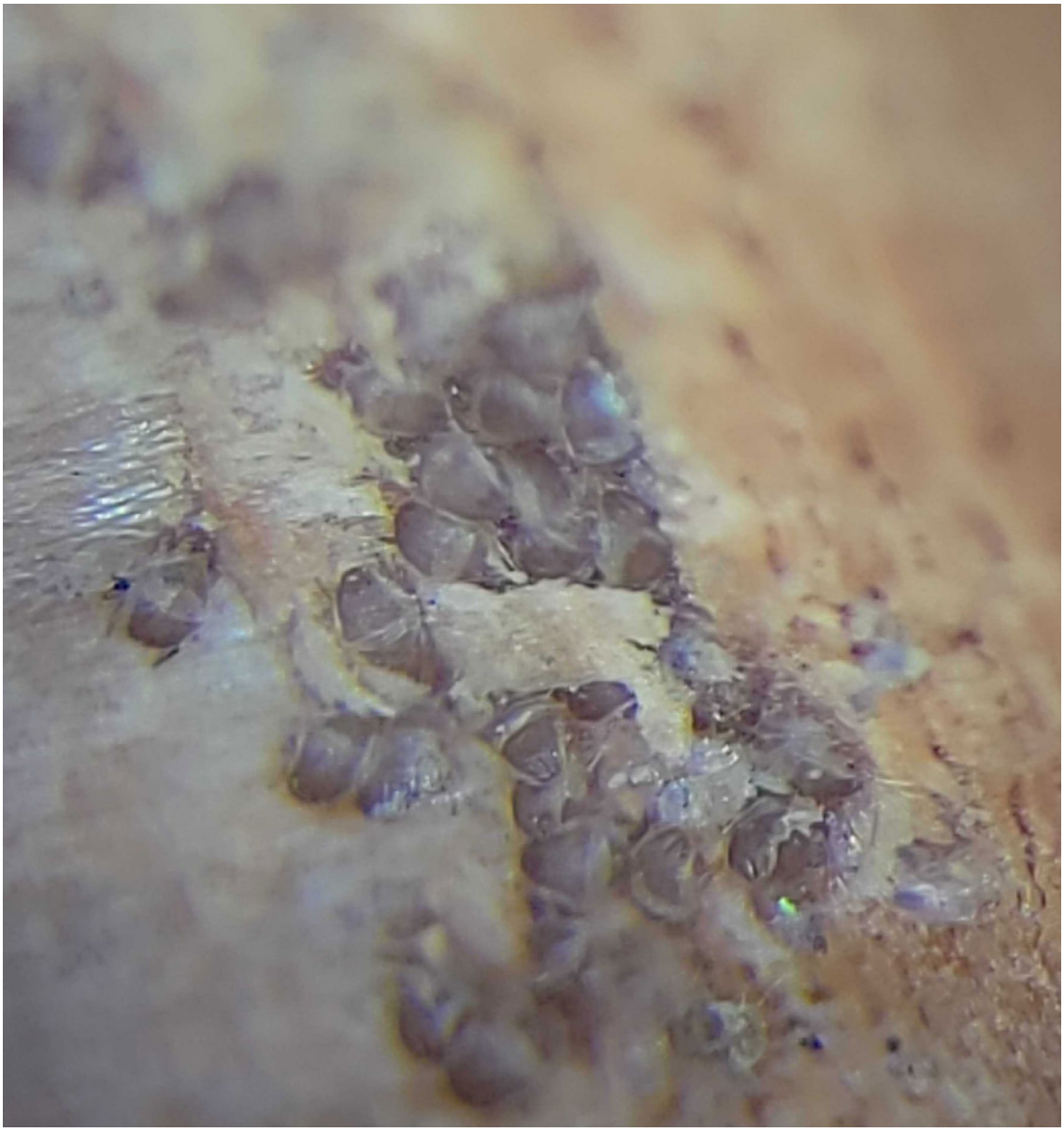


Figure 1.7. A phoretic deutonymph infestation in a provisioned *Osmia* nest cell. This infestation was from spring 2023, in a mated tent (tent 7). This is from nest cell #1, and there was no evidence of an egg or larva in this nest cell. This nest cell was never partitioned with mud by the mother bee. Phoretic deutonymphs were not active when the nest was deconstructed, and no other mite life stages were visible in the nest.

Figure 1.8. *Chaetodactylus krombeini* in an *Osmia lignaria* observation nest. Circles show where mites are walking on the transparency film. This infestation was from a mated tent (tent 6) from spring 2022, in nest cell #1. Mites that are circled are likely adult females because they were easily visible on the transparency film.

Chapter 2

Chaetodactylus krombeini Heteromorphic Deutonymph Sex Determination

Abstract

Chaetodactylus krombeini (Acari: Chaetodactylidae) are mites that parasitize solitary bee nests and eat pollen provisions intended for developing bee larvae. Mites in the family Chaetodactylidae have a facultative life stage, the heteromorphic deutonymph, that is used for dispersal to new habitats with food resources. The heteromorphic deutonymph has two forms: a regressive, inert morph that remains stationary in the host nest until a new bee reuses the nest and provides a provision for its offspring, and a phoretic form that is a highly specialized morph adapted for attachment to an adult bee, which it uses to disperse to a new nest site. Both forms of the heteromorphic deutonymph of *C. krombeini* have been hypothesized to be only female, having the ability to produce male offspring that develop from unfertilized eggs (arrhenotoky) and then mating with their sons to produce male and female offspring (oedipal mating). This hypothesis was based on a comparison to unrelated mites that are also associated with solitary bees, however this hypothesis has not been tested with *C. krombeini*. This study was conducted to determine if all heteromorphic deutonymphs are only female. Inert and phoretic deutonymphs were isolated under various conditions and development was observed into adulthood. These

assays showed 32-38% of deutonymphs developed into adult males. These results shed new light on the survival and reproductive strategies of *C. krombeini* and question the role of true arrhenotoky in founding new mite populations.

Introduction

Chaetodactylus krombeini is a kleptoparasitic mite species that is an obligate parasite in solitary bee nests, specifically bees within the families Apidae and Megachilidae (Krombein 1962; Klimov et al. 2016). These mites feed on pollen provisions intended for developing bee larvae, causing bees to develop into smaller adults which negatively impacts bee size and fecundity (Krombein 1962; Bosch and Kemp 2001). Chaetodactylid mites have also been reported to attack and kill developing bee larvae and eggs, destroying 20-50% of *Osmia* nests (van Lith 1957; Krombein 1962; Krunić et al. 2001; Jim Watts, personal communication December 2021). Several astigmatid mite species, including *C. krombeini*, possess a facultative heteromorphic deutonymph life stage, often referred to as a hypopus (Knülle 1987; Houck and OConnor 1991). The heteromorphic deutonymph is morphologically distinct from the other nymphal life stages (Walter and Krantz 2009). This facultative stage helps ensure the survival of astigmatid mites, as they often live in temporary habitats with transitory food sources (Knülle 1987; OConnor 1994). The heteromorphic deutonymph life stage can be precipitated by multiple genetic and environmental conditions, such as low food quality, reduction of humidity, mite overcrowding, or synchronous life stage with its host (Krombein 1962; Knülle 1987). The formation of the

heteromorphic deutonymph is controlled by genotype-environment interactions, where the quality of the diet initiates a switch mechanism to activate the development of the facultative life stage, as seen with other Astigmatan mites (*Lepidoglyphus destructor*) (Knülle 1987). The two morphs of the heteromorphic deutonymph of *C. krombeini* consist of an inert form that stays behind waiting for a new host to reuse the nests, and a phoretic form that attaches to bees as they emerge from their nests as adults (Krombein 1962). The inert form has the opportunity to move to a new host species when founding new populations, while the phoretic form allows this mite species to maintain the same host species when founding new populations (Krombein 1962).

Chaetodactylus krombeini and other closely related mite species have been hypothesized by some to only produce female heteromorphic deutonymphs, therefore requiring the use of arrhenotokous parthenogenesis, the ability to produce male offspring from unfertilized eggs, followed by oedipal mating (Adamson and Ludwig 1993) to start new populations (Krombein 1962; Krunić et al. 2005; McKinney and Park 2013). Oedipal mating is a strategy where unmated female mites lay an unfertilized (haploid) egg which hatches into a male. The female mite then mates with her son and lays fertilized eggs which will hatch into females (diploid). Arrhenotokous parthenogenesis has been observed in other mites including *Macrocheles muscaedomesticae* (Farahi et al. 2018), *Ornithonyssus sylviarum* (McCulloch and Owen 2012), *Tetranychus urticae* (Tuan et al. 2016), and the related *C. osmia* (Rożej-Pabijan and Witaliński 2018).

However, studies have shown that other Chaetodactylids produce both male and female heteromorphic deutonymphs (Qu et al. 2003). Having both sexes found a population may avoid negative effects on progeny survival and fertility caused by inbreeding depression (Charlesworth and Charlesworth 1987). This study was conducted to clarify if heteromorphic deutonymphs are only female, or if both sexes can develop from this facultative life stage.

Materials and Methods

Mite Colony Maintenance

Chaetodactylus krombeini mites were collected from commercial *Osmia lignaria* nests in Oregon and Washington and shipped to UCR. Mite populations were sorted by collection site and maintained in 1.5 µL microcentrifuge tubes in an environmental chamber (VWR Model No: 2005 and Fisher Scientific Model No: 146E) set to 26°C ± 0.5°C. In addition to mites, each microcentrifuge tube was filled with 100 to 250 µL of a stock mixture of 60% honey bee pollen, 16% honey, 20% DI water, and 4% fructose. Each 1.5 µL microcentrifuge tube was sprayed with a 0.1% dilution of food grade fungicide (methyl paraben, CAS: 99-76-3) to prevent mold from forming after pollen provisions were added (for colony mites only, as pollen provisions in experiments were discarded after 2 weeks). For colony maintenance, this food would last for multiple *C. krombeini* generations.

Osmia spp.

Osmia lignaria cocoons were purchased from Northwest Pollination (Canby, Clackamas County, OR) in November 2022. Cocoons were shipped overnight and kept in a 4°C refrigerator from November 2022 to April of 2023 for overwintering. *Chaetodactylus krombeini* phoretic deutonymphs were placed on all purchased cocoons (allowing mites to overwinter with host), then cocoons were individually placed in clear gel capsules (XPRS Nutra size: 000) for accurate sexing when bees emerged before being returned to a 4°C refrigerator. Cocoons were moved to containers at room temperature (23°C ± 1°C) on April 13, 2023. A high proportion of the purchased cocoons contained *O. cornifrons,* an introduced orchard bee commonly found in the eastern US. This species was sent by accident and could not be used in the field experiments. As *Osmia cornifrons* emerged, they were freeze-killed. After bees were dead (both *O. cornifrons* and *O. lignaria* males), they were mounted on a pin and phoretic deutonymphs were removed from the bee under a microscope to be used in the sex determination experiment (Fig. 2.1).

Inert Deutonymphs

Inert deutonymphs were collected from different field *Osmia* nesting sites for each of the eight two-week sex determination assays. For each assay, one inert deutonymph was placed into each 1.5 µL microcentrifuge tube (n = 10 mites total) with ca. 50 µl pollen provision as described above (Table 2.1). Every 1-3

days for 14 days each tube was examined under a dissection microscope and mite life stage (inert deutonymph, tritonymph, or adult) was recorded. Mites that molted into adults were sexed by observation of ovipore (female) or aedeagus (male) (Fig. 2.2). All mites were maintained in an environmental chamber at 26°C ± 0.5°C during the assay.

Phoretic Deutonymphs Overwintered with Host

Phoretic deutonymphs were removed from field collected mite populations and placed in 1.5 µL microcentrifuge tubes, ca. 300 per tube. Bee cocoons were then placed in microcentrifuge tubes with phoretic mites to allow mites to attach to cocoons. Cocoons and phoretic deutonymphs were held in a 4°C refrigerator to overwinter from November 2022 to April 2023. In April 2023, phoretic deutonymphs were collected from eclosed adult bees. Ten adult bees were randomly selected, placed in a freezer for at least 30 minutes to kill the bee, then mounted on a pin under a microscope to remove mites (Fig. 2.1). There was no observation of differences in infestations between bee sex and bee species.

Only visible phoretic deutonymphs were removed using forceps or small disposable mascara wands (Micro Eyebrow Brush with Cap, CANIPHA, Amazon.com), and all other life stages of mites present were discarded. Phoretic deutonymphs were placed individually into microcentrifuge tubes with ca. 50 µL of pollen provision (as above). Sample size of mites was dependent on how many phoretic deutonymphs were attached to each adult bee. Every 2-5 days for

14 days tubes were examined and mite life stage (phoretic deutonymph, tritonymph, or adult) was recorded. Mites that molted into adults were sexed by observation of ovipore or aedeagus (Fig. 2.2) and then freeze-killed. All mites were maintained in an environmental chamber at 26°C ± 0.5°C during the assay.

Phoretic Deutonymph Overwintered without Host

Phoretic deutonymphs were removed from field collected nests and placed in 1.5 µL microcentrifuge tubes in the absence of bee cocoons and placed in a refrigerator at 4°C in November 2022 to overwinter without a host. Mites were removed from 4°C refrigerators on May 11, 2023, to remove them from overwintering simulated conditions. One phoretic deutonymph was placed in each tube with ca. 50 µL of pollen provision; a total of 128 phoretic deutonymphs were examined. For two-weeks every 2-5 days tubes were examined and mite life stage (phoretic deutonymph, tritonymph, or adult) was recorded. All mites were maintained in an environmental chamber at 26°C± 0.5°C during the assay.

Statistics

Statistical analyses were conducted in VassarStats (Lowry 2023). A Binomial Probabilities test was performed to compare the observed percentage of males that emerged from the heteromorphic deutonymphs to expected probabilities in mite populations that use sexual, arrhenotoky, and pseudo-arrhenotoky reproduction strategies.

Results

Inert Deutonymphs

Inert deutonymphs developed into adults from seven of the eight sites examined (Table 2.1). Both male and female adult mites developed from inert deutonymphs. Of the 80 inert deutonymphs that were examined, 32 developed into adults, 12 were male and 20 were female, with 3 inert deutonymphs only making it to the tritonymph stage before dying (Table 2.1; Fig. 2.3). Female mites were observed to develop from seven of the eight sites, with up to 100% of the adult mites being female. However, males were also seen in most sites, only missing from two of the eight sites. In two sites more male adults emerged than females, with one site having 2 males to 1 female and another site having 4 males to 1 female. Only one site did not have any mites develop into tritonymphs or adults.

The observed inert deutonymphs sex ratio was compared to the average sex ratio (females to males) of populations that use sexual reproduction (1:1), arrhenotoky (9:1), and pseudo-arrhenotoky (7:3) (Nagelkerke and Sabelis 1998). The proportion of males that developed from inert deutonymphs (k=12, n=32) was compared to each mode of reproduction: sexual (z-ratio=-1.24, P=0.053), arrhenotoky (z-ratio=4.89 P=0.000027), and pseudo-arrhenotoky (z-ratio=0.73, P=0.096).

Phoretic Deutonymphs Overwintering with Host

Both male and female adults developed from phoretic deutonymphs that overwintered with their bee host (Table 2.1). Of the 249 phoretic deutonymphs examined, 19 developed into adults, 6 were males and 13 were female, and only 1 phoretic deutonymph morphed into a tritonymph before dying (Table 2.2; Fig 2.3). The remaining 230 phoretic deutonymphs either died within the two-week assay or did not develop past the deutonymph stage during the assay.

The observed proportion of males that developed from phoretic deutonymphs that overwintered with a host (k=6, n=19) was compared to the different modes of reproduction: sexual (z-ratio=-1.38, P=0.052), arrhenotoky (z-ratio=2.75, P=0.0069), and pseudo-arrhenotoky (z-ratio=-0.1, P=0.19).

Phoretic Deutonymphs Overwintering without Host

None of the 128 phoretic deutonymphs that overwintered without a host developed into tritonymphs or adults. All died within the two-week assay, indicated by mites becoming immobile and desiccating.

Discussion

This study showed that *C. krombeini* heteromorphic deutonymphs are not exclusively female. Development to the adult life stage was seen in both inert and phoretic deutonymphs of *C. krombeini* in these experiments, however it was much more prevalent in the inert form. Only phoretic mites that overwintered with

a host (male or female bee) developed into adults, but still very few (<8%) successfully developed into adult mites. Inert deutonymphs are a much more regressive form than the highly specialized phoretic deutonymph, and it is possible that inert deutonymphs only need increased humidity caused by a new food source to provide the necessary cue to continue development (Krombein 1962). There may also be less energy needed to morph from an inert deutonymph to a tritonymph than needed to morph from a phoretic deutonymph to a tritonymph.

Phoretic deutonymphs may require multiple cues to initiate further development, having more sensory organs than the inert deutonymph (Chapter 3). Mite development in host associated phoretic deutonymphs may also have been hampered by damage endured during removal from the host with forceps. Of the eight sites examined for inert deutonymph sex determination, one site did not have any development past the deutonymph stage. This is likely due to the formation of mold in field collected tubes. Mold formation began in the remaining pollen provision that was shipped with mite samples, which eventually spread to the mites that were in contact with molding pollen provision, killing all mites.

Researchers have previously hypothesized that heteromorphic deutonymphs of *C. krombeini* are all female, and because of this it was thought that mite reproduction in new nests was by arrhenotokous parthenogenesis followed by oedipal mating (Krombein 1962; Krunić et al. 2005). This hypothesis was originally developed by Krombein (1962) who compared *C. krombeini* life

history traits to Histiostoma mites associated with Halictid bees, which had been observed to lay eggs that only develop into males. However, the male offspring were not observed to mate with their mothers (Krombein 1962). Other closely related Chaetodactylid mites have been shown to be haplo-diploid and capable of arrhenotokous parthenogenesis (Rożej-Pabijan and Witaliński 2018). However, arrhenotokous parthenogenesis may not be a necessary or primary survival strategy because both male and females are present in both dormant and dispersal morphs of *C. krombeini* heteromorphic deutonymphs. Previous studies have observed that copulation was required for egg production in *C. osmiae*, which was shown to be haplo-diploid in 2018 (Chmielewski 1993; Rożej-Pabijan and Witaliński 2018). In the current study, following the two-week assays some females were not placed in the freezer and were instead kept for observation. These unmated females did not produce eggs after up to 3 weeks of observation. Some of these unmated females were then placed together to see if the presence of other females would initiate oviposition as a strategy to avoid inbreeding depression, but females still did not lay any eggs. In a host preference field experiment (Chapter 1), there was a single adult female mite found in a provisioned cell that survived through the season, but she did not found a colony in the nest. While it is possible that field nest conditions were not ideal for reproduction, this may suggest that male mites or copulation is required for egg lay.

While further study is needed, statistical analyses comparing the observed sex ratio of *C. krombeini* with expected sex ratios of different modes of reproductions showed that in adults developing from both inert and phoretic deutonymphs had no significant difference in sex ratios when compared to sexual reproduction and pseudo-arrhenotoky, however there was a significant difference in the sex ratio when compared to arrhenotoky. This further suggests that *C. krombeini* do not use arrhenotoky as their primary mode of reproduction and that these mites may use sexual reproduction or pseudo-arrhenotoky to reproduce. Mites that disperse before mating often disperse as groups instead of individually (Mitchell 1970). *Chaetodactylus krombeini* inert and phoretic deutonymphs are often observed in groups on adult bee hosts or in bee nests, increasing the number of founders introduced to new nests (Krunić et al. 2005; JH, personal observation). *Chaetodactylus krombeini* that disperse on hosts as a group likely drop off together in a new nest. Inert deutonymphs from field collections and phoretic deutonymphs that overwintered with adults were both found in groups (Chapter 1), giving mites that found new populations a higher chance to reproduce sexually. Both sexes being present in both morphs of the heteromorphic deutonymphs also provides evidence that copulation is likely required for mites to produce offspring. Mites dispersing in groups, evidence that copulation is required for reproduction, having haplo-diploid sex determination, and mites from this study producing 63% and 68% females in the inert and phoretic deutonymph, respectively, suggests that *C. krombeini* may use pseudo-

arrhenotoky as their primary reproductive strategy. In pseudo-arrhenotoky males are produced from fertilized eggs but become haploid after the paternal chromosomes are inactivated (Sabelis and Nagelkerke 1988). This reproduction strategy is often seen in Phytoseiidae mites, with the overall production of daughters observed to be 69-76% (Nagelkerke and Sabelis 1998). Further studies should investigate if other Chaetodactylid mites use arrhenotokous parthenogenesis or pseudo-arrhenotoky. The requirement of copulation to reproduce could lead to control methods for these parasites.

In summary, *C. krombeini* males and females can develop into heteromorphic deutonymphs, making their primary mode of reproduction unclear. Further research is needed to understand how life stage transitions are regulated, including what genes are involved and their expression in response to different stimuli. This may give insight into what is required for both morphs of heteromorphic deutonymphs to develop. Corente and Knülle (2003) stated that the hypopus-inducible period occurred during late larval to early protonymphal life stages, and production of heteromorphic deutonymphs is a response to these life stages experiencing a nutrition deficiency. This information can be used to determine if all late larvae to late protonymphs are capable of morphing into heteromorphic deutonymphs if they are placed in environments with low food

quality. Understanding survival strategies of these nest parasites, such as mode of reproduction and what genes are involved in life stage transitions, could provide information on their environmental tolerances, leading to more effective control strategies.

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Table 2.1: Inert Deutonymph Sex Determination. Location of collection site of mite infestations in commercial *Osmia* nests. Ten inert deutonymphs were collected from each site. Only mites that developed through the adult stage could be sexed. The number of each mite life stage and sex of the adults is reported.

Site	Tritonymph	# of Adults	Female	Male
Renton, King County, Washington	4	4	3	1
Molalla, Clackamas County, Oregon	3	3	1	2
Kenmore, King County, Washington	5	5	4	1
Colton, Clackamas County, Oregon	0	0	0	0
Molalla, Clackamas County, Oregon	6	6	6	0
Kent, King County, Washington	7	6	3	3
Renton, King County, Washington	5	5	1	4
Kent, King County, Washington	3	3	2	1

Table 2.2: Phoretic Deutonymph Sex Determination. Phoretic deutonymphs were collected from different *Osmia* spp. and sexes. Only mites that developed through the adult stage could be sexed. The number of each mite life stage and sex of the adults is reported.

Bee Species	Bee Sex	Deutonymphs	Tritonymphs	Adults	Female	Males
O. cornifrons	Male	16	0	0	0	0
O. cornifrons	Male	8	0	0	0	0
O. cornifrons	Male	41	11	10	7	3
O. cornifrons	Male	41	0	0	0	0
O. lignaria	Male	27	1	1	1	0
O. cornifrons	Male	9	2	2	1	1
O. cornifrons	Female	45	0	0	0	0
O. cornifrons	Female	10	4	4	2	2
O. cornifrons	Female	6	1	1	1	0
O. cornifrons	Female	46	1	1	1	0

Figure 2.1. *Osmia lignaria* male mounted on pin with phoretic deutonymphs (arrow) attached to posterior thorax.

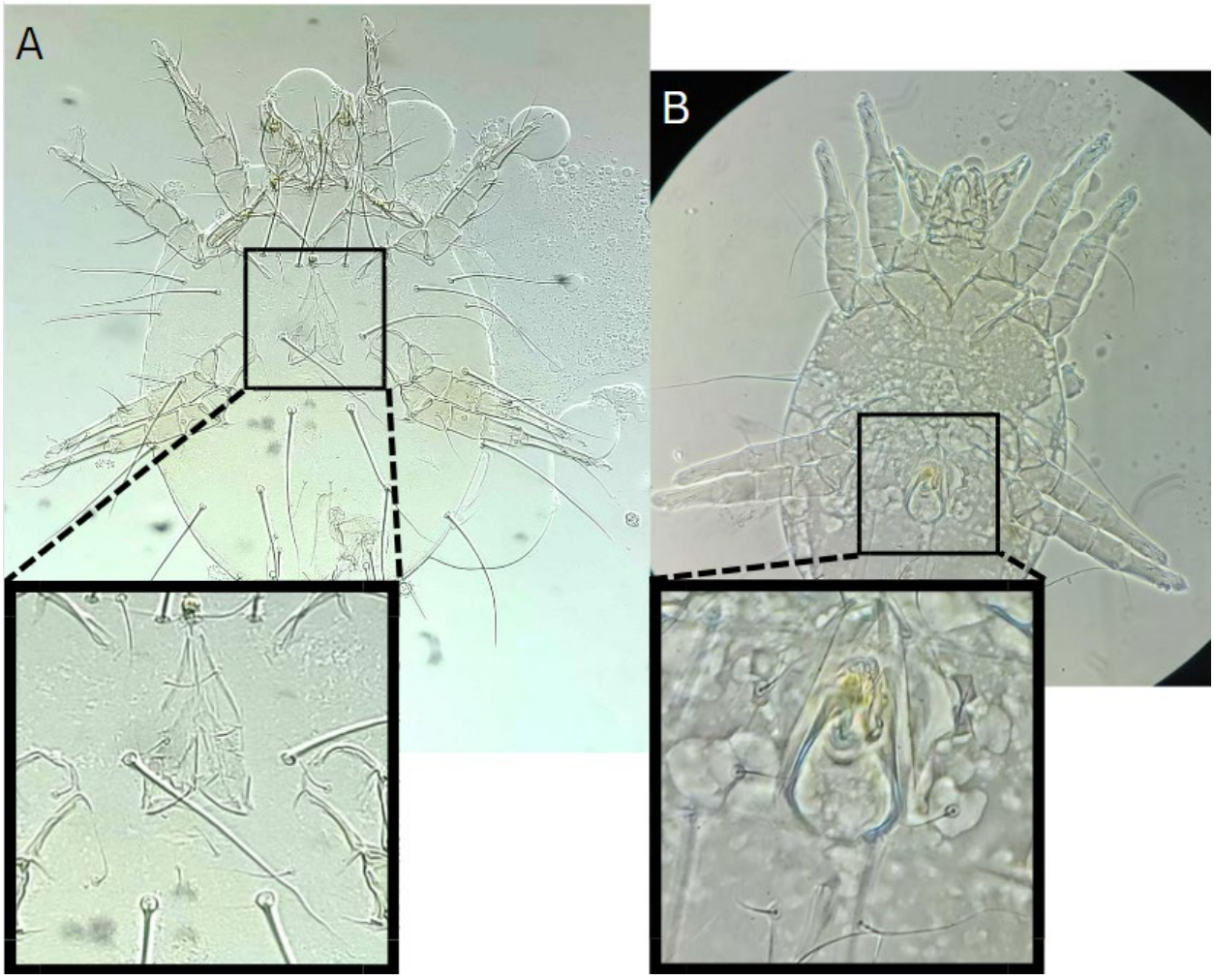

Figure 2.2. *Chaetodactylus krombeini* female (A) and male (B) adults were cleared, and slide mounted in PVA mounting medium (100×). Genitalia are distinct on the ventral side of both sexes, with a cleavage-like ovipore present on females and a dark colored aedeagus present on males.

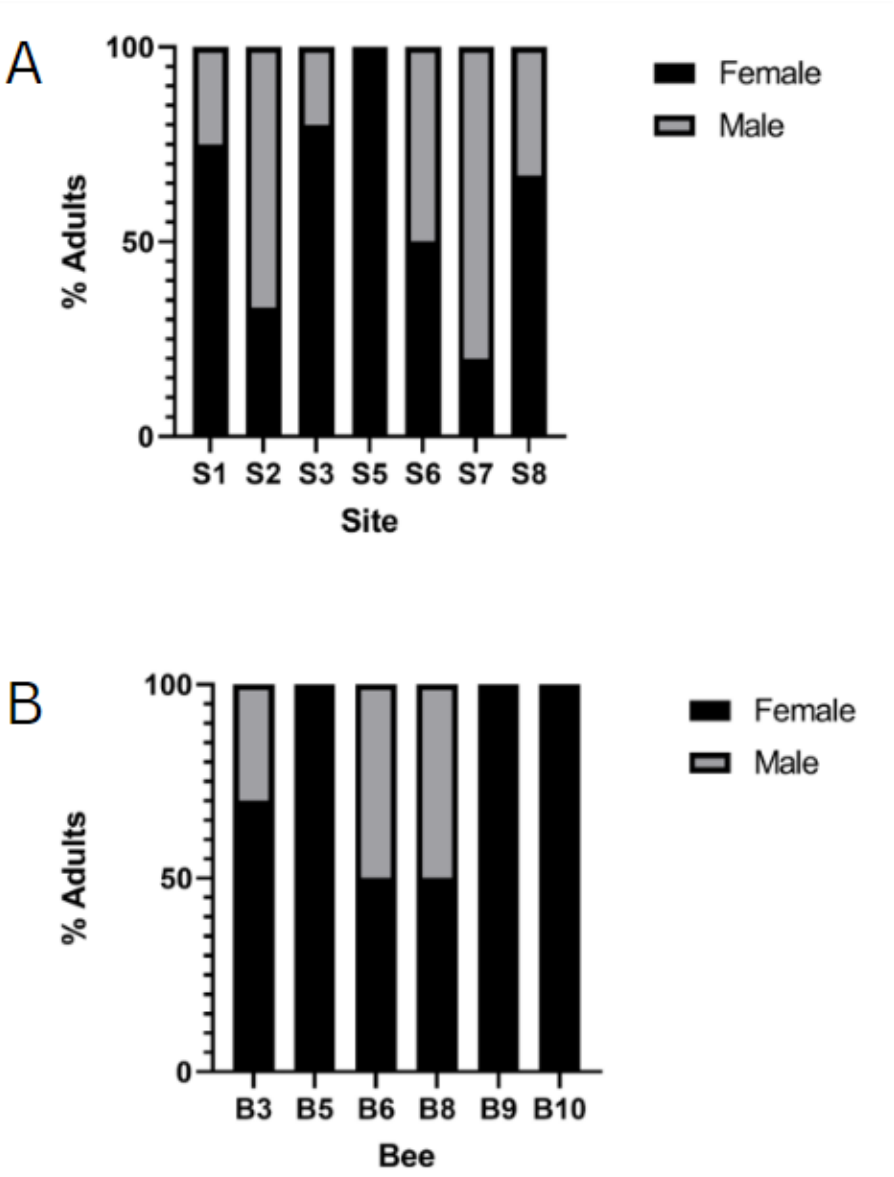

Figure 2.3. Percentage of each sex at emergence. Inert deutonymphs percentage of each sex that developed into adults at each sample site (A). Phoretic deutonymph percentage of each sex that developed into adults from individual host bee (B).

Chapter 3

Chaetodactylus krombeini Morphology Imaging with Scanning Electron Microscope

Abstract

Chaetodactylus krombeini (Acari: Chaetodactylidae) mites are closely associated with solitary bees in the genus *Osmia*. These mites depend on their bee host for harborage, food resources, and dispersal. Infestations of *C. krombeini* have been shown to increase morbidity and larval mortality. The life history traits of *C. krombeini* are not well understood, thereby complicating control efforts of this mite in managed bee nests. Scanning electron microscope (SEM) images can provide more understanding on the morphology of *C. krombeini*, helping determine the purpose of appendages, and how morphology may influence behavior. These detailed images may also provide further information during mite identification.

Introduction

Chaetodactylus krombeini is a kleptoparasitic mite species that lives exclusively in the nests of solitary bees (Krombein 1962). Mites in the family Chaetodactylidae feed on the pollen provisions of solitary bees, specifically in the families of Megachilidae and Apidae (Krombein 1962; Klimov et al. 2016). These pollen provisions are placed in nests and are meant for the developing bee offspring (Krombein 1962). C*haetodactylus krombeini* has six life stages: egg,

larva, protonymph, facultative heteromorphic deutonymph, tritonymph, and adult (Krombein 1962). When there is enough food and humidity in nests there are only five life stages present: egg, larva, protonymph, tritonymph, and adult (Krombein 1962). The heteromorphic deutonymph only appears when there is lower humidity, low quantity of food, or mite overcrowding (Krombein 1962; Knülle 1987). The heteromorphic deutonymph life stage is used to ensure survival, locating new suitable habitats for continued development, and producing new populations (Krombein 1962). The phoretic deutonymph actively disperses by attaching to adult bees emerging from a nest. The inert deutonymph will stay behind and remain dormant in the nest to infest the next bee that reuses the nest (Krombein 1962).

Chaetodactylidae mites are the most important pest to *Osmia* spp. (Krunić et al. 2001). Mites can decrease adult bee size, making them less efficient pollinators, and kill developing bee offspring, causing losses in nests of 20-50% (van Lith 1957; Krombein 1962; Bosch and Kemp 2001; Krunić et al. 2001; Jim Watts, personal communication December 2021). To develop effective control methods against these mites, more of their life history traits need to be clearly understood.

Many life history traits of *C. krombeini* are not known or well understood. Often, traits are hypothesized based on comparisons to other mites (Krombein 1962). Morphology can provide insight into many of the less understood traits such as mating strategies, feeding habits, or dispersal capabilities. For example,

it is hypothesized that *C. krombeini* feed on nectar in the pollen provisions, making no use of the pollen (Krombein 1962, Bosch and Kemp 2001). The mites *Euseius stipulatus* and *Amblyseius similoides* use pollen as a food source, and *Pneumolaelaps longanalis* feed on nectar and the pollenkit (sticky coating of pollen surface) of pollen grains (Royce and Krantz 1989; Flechtmann and McMurtry 2009). Comparing the chelicerae morphology of *C. krombeini* to mites with known feeding habits may shed light on *C. krombeini* food preferences.

Clear morphological characters can also help with mite taxonomy. The phoretic deutonymph of *C. krombeini* is indistinguishable from other Chaetodactylidae phoretic deutonymphs that are associated with *Osmia pumila*, which can be coinfested with multiple species (OConnor and Klimov 2009). Scanning electron microscope (SEM) photos of phoretic deutonymphs' propodosomal and hysterosomal shields, empodial claws, or suctorial attachment plate may show differentiating characteristics between *C. krombeini* and other phoretic deutonymphs found in *O. pumila* nests, facilitating mite identification when compared.

Understanding morphology also provides insight into behavior as appendages are needed for tasting, perceiving the environment, aggression, and mating (Walter and Proctor 2013). Detailed high magnification images of different *C. krombeini* life stages may provide information on specific morphological characteristics for future functional studies. Here, we present SEM images of

egg, larva, protonymph, inert and phoretic deutonymph, tritonymph, and male and female adults of *C. krombeini* collected from *O. lignaria* and *O. cornifrons* nests and reared under laboratory conditions.

Materials and Methods

All mites imaged were from a colony maintained at UCR. Dozens of specimens were prepared for SEM imaging in a variety of ways to achieve high quality images. Mites were killed in varying ethanol concentrations, by being placed in a freezer, or they were already dead in colony with no chemical preparation. Specimens dehydrated in an ethanol series started at 10, 40, 50, or 70%, increasing to 100% in 10% increments, each held for 20 minutes (Brown 1993; Heraty and Hawks 1998). In a fume hood, hexamethyldisilazane (HMDS) was placed to cover the specimens for 20 minutes, then removed and replaced with fresh HMDS just to cover the specimens for an additional 24 hours (Table 1) (Brown 1993; Heraty and Hawks 1998). All mite specimens were mounted to the specimen stub with conductive double-sided tape. Mite images were taken with a tabletop Hitachi TM4000PlusE Ⅱ Scanning Electron Microscope at the University of California, Riverside Core Microscopy Facility. Measurements were recorded to the nearest 1 μm from one specimen from each life stage. The voucher specimens were deposited in the Entomology Research Museum at the University of California, Riverside.

Results and Discussion

The egg is ovoid-shaped, having no visible distinguishing characteristics (Fig. 3.1); however, the outline of a developing mite within the egg can be observed in this image (Fig 3.2). Baker (1962) reported the *C. krombeini* egg to be 170-185 µm long and 110-120 µm wide. Using the SEM images, we measured the egg to be ca. 120 µm long and 85 µm wide (Fig. 3.2). The larva is distinguished by having only three pairs of legs and no genital valve (Fig. 3.2). The larva was reported to be 223-236 µm long; we found it to be ca. 240 µm long (Fig. 3.2) (Baker 1962). Both the protonymph and tritonymph have four pairs of legs and a genital valve with one set of setae and based on these images they only differed in their body size and tritonymphs having a visible anterior coxal apodeme (Fig. 3.2). The genital valve in other mite species (e.g., superorder Acariformes) have an increasing pair of setae on the genital valve as mite life stage progresses (e.g., protonymph one set of setae, deutonymph two sets of setae, and tritonymph three sets of setae) (Krantz and Walter 2009). When slide mounted, the protonymph and tritonymph stages are indistinguishable under a compound microscope unless they are side by side for size comparison. The protonymph was reported to be 319-414 µm long; we found it to be ca. 300 µm (Fig. 3.2) (Baker 1962). The tritonymph was reported to be 408-427 µm long; we found it to be ca. 385 µm long (Fig. 3.2) (Baker 1962). The inert deutonymph has a small pore at its posterior end, has only two visible reduced pairs of legs at the anterior end, and has no mouthparts (Fig. 3.3). The inert deutonymph has a very

regressive form, which likely requires little energy to develop into from a protonymph. The inert deutonymph possibly requires very little energy to develop into a tritonymph as well. If the development into and out of an inert deutonymph is at a low energy cost to mites, this could allow for the potential for a long diapause lasting for months to possibly years (Knülle 1987; Krunić et al. 2005). The inert deutonymph was reported to be 306-325 µm long and 287-300 µm wide; we found it to be ca. 290 µm long and ca. 230 µm wide (Fig. 3.3) (Baker 1962).

The phoretic deutonymph has many distinguishing characteristics that set it apart from all other life stages (Fig. 3.3). The phoretic deutonymph has large recurved empodial claws on the first three pairs of legs to aid in attachment to a bee host; they also have long setae extending from its fourth pair of legs (Fig. 3.3 and Fig. 3.4). There is also a suctorial attachment plate on the ventral posterior end of the phoretic deutonymph, used for attachment to a host during phoresy (Fig. 3.4 and Fig. 3.5). The mouthparts are greatly reduced on the phoretic deutonymph and are completely different compared to the mouthparts of the other life stages (Fig. 3.4 and Fig. 3.6). The phoretic deutonymph is specifically adapted for phoresy and to survive in harsher environments outside the nest (Eickwort and Houck 1994). It is not clear when the phoretic deutonymph drops off its host in new nests, but female *Osmia lignaria* will begin to build their nests within 1-2 days of mating and will build about 2.4 nests per day, so mites likely

need to stay attached to their host for at least 2 days (Krombein 1962; Bosch and Kemp 2001). The phoretic deutonymph was reported to be 306-331 μm long; we found it to be ca. 300 μm long (Fig. 3.3) (Baker 1962).

Male and female adults are distinguishable by their reproductive organs on the ventral side and having setae differing in lengths and texture (barbed or smooth) on their dorsal side (Fig. 3.7 and Fig. 3.8) (Klimov and OConnor 2008). Gravid females are often more robust than males, but the two sexes are difficult to tell apart when viewing them from the dorsal side with a compound microscope. The adult female was reported to be 542-669 μm long; we found it to be ca. 450 μm long (Fig. 3.4) (Baker 1962). The male was reported to be 453-478 μm long; we found it to be ca. 460 μm long (Fig. 3.4) (Baker 1962). All feeding stages (larvae, protonymphs, tritonymphs, and adults) have similar mouth parts including chelicerae set above the mouth opening and pedipalps set under the chelicerae at the bottom of the mouth opening (Fig. 3.7). All image measurements were done on one specimen of each life stage, as there was poor quality of most images due to specimen shriveling.

Most life stages of the mites imaged here measured ca. 2-29% smaller than previous reports, however the larva and males measured with in the expected range (Baker 1962). The size difference from what Baker (1962) observed and what we observed may disagree because of the precision of tools we used to measure each life stage of *C. krombeini*. The SEM machine vacuum

may also have caused some change in the mite size, or there might be a size difference due to our lab reared mites being fed on an artificial diet (honey bee collected pollen, honey, DI water, and fructose).

The chelicerae of *C. krombeini* did not show close similarities to *E. stipulatus* or *A. similoides*. *Chaetodactylus krombeini* have their chelicerae set above their mouth opening, with one short, fixed digit and one short movable digit on each chelicera. Both *E. stipulatus* and *A. similoides* have thinner and more elongate chelicerae than *C. krombeini*, but they also have one fixed and one movable digit on their chelicerae. *Euseius stipulatus* and *A. similoides* were observed to feed on pollen by holding it above the hypostome with one retracted chelicera while the other chelicera is protracted (Flechtmann and McMurtry 2009). A few rapid movements of both chelicerae cause the pollen grain to collapse allowing the inner content to be removed (Flechtmann and McMurtry 2009). Comparison of *C. krombeini* to *P. longanalis* showed more similarities, both having short mandibular like chelicerae with one fixed digit and one movable digit on each chelicera. *Pneumolaelaps longanalis* feed on nectar coated bumble bee pollen, and mites were observed to feed on pollen by holding the nectar coated pollen grain near the hypostome with palpi, rotating the pollen grain with the chelicerae and palpi to remove the nectar coating along with the surface pollenkit that is incorporated with the exine, consuming both nectar and pollenkit

(Royce and Krantz 1989). Further research should examine *Osmia* pollen provisions before and after mites have fed on them with SEM imaging to see if *C. krombeini* are able to puncture the exine and feed on the intine.

The SEM preparation methods for specimens did not produce consistent results. We tested several starting concentrations of ethanol to try to keep mites from shriveling when placed under vacuum, however starting with lower concentrations of ethanol did not protect the mites at vacuum (Table 3.1). The specimens that withstood the vacuum best during imaging were mites that died in mite colonies and were directly placed in the SEM without any preservation with ethanol or HMDS (Table 3.1). This is possibly due to *C. krombeini* being soft bodied, causing the introduction of chemicals to be damaging to their exoskeleton. For best image results, we found that astigmatid mites should not be preserved, but should either be freshly dead or still alive when they are placed at vacuum to ensure the integrity of the mites' body throughout imaging. The phoretic deutonymph should only be placed under vacuum for imaging when this life stage is still alive, as in our experience this will ensure that mite appendages are stretched out and observed clearly. When the phoretic deutonymph is killed prior to being placed in vacuum the mite legs will curl under the ventral side of the mite and the idiosoma will bend slightly over the legs.

We found the tabletop Hitachi TM4000PlusE Ⅱ SEM to be easy to operate and more accessible than traditional SEM imaging machines that often limit the view of the specimen or require specimens to be gold-coated. The

tabletop SEM allows specimens to be easily and quickly adjusted when a different view was desired. The tabletop SEM only required a slight adjustment to the stub height when changing from the mite to the bee host, adding to the simplicity and efficiency of imaging with this tool.

Future research should take SEM images of other Chaetodactylidae mites for comparison to distinguish closely related mites from one another. This rapid process for identification could be used prior to molecular identification as it takes less than an hour to produce images. Furthermore, few Chaetodactylidae mites have barcodes available for comparison. Future research should compare SEM images of *C. krombeini* to mites with known life history traits and determine if morphology is similar, as comparisons may reveal functions of appendages and behavioral traits. Using the power of the SEM to analyze mite morphology and behavior may uncover clues on how to better control these parasites.

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Table 3.1. Ranking of ethanol dilutions series based on quality of images produced.

Ethanol Concentration	Rank Quality of Images
70% EtOH + HMDS	Lowest
50% EtOH + HMDS	Lowest
40% EtOH + HMDS	Lowest
10% EtOH + HMDS*	Inconclusive**
10% EtOH***	Moderate
No chemical treatment	Best

*Mites killed by freezing prior to chemical preparation.
**Due to mites' small body size and the number of chemical transfers with pipette, all mites were accidentally discarded with waste chemicals. No images were produced with this low dilution series.
***No increase of ethanol concentration, mites only preserved in 10% ethanol dilution.

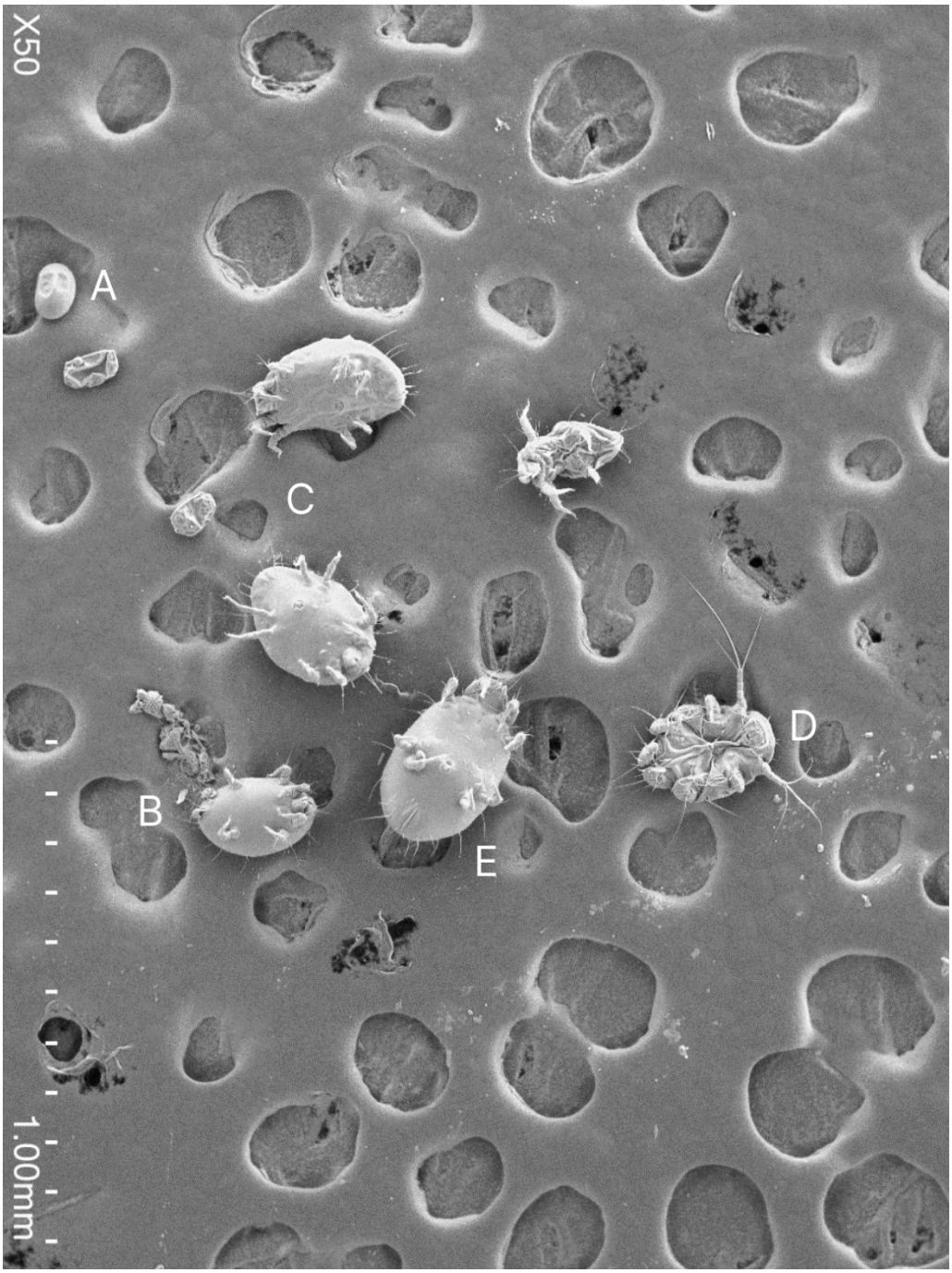

Figure 3.1. All immature stages of *Chaetodactylus krombeini*, excluding inert deutonymph. (A) egg (B) larva (C) protonymph (D) phoretic deutonymph (E) tritonymph, (50×).

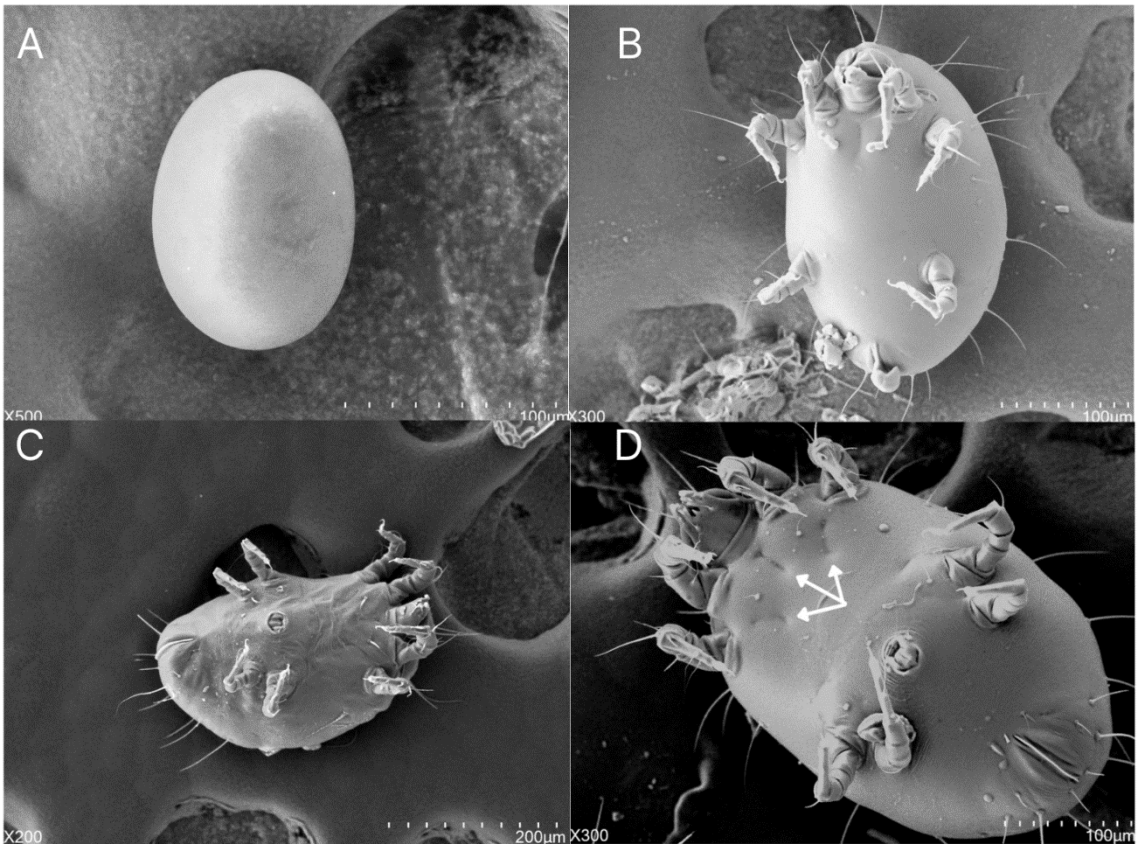

Figure 3.2. (A)*Chaetodactylus krombeini* egg (500×), (B) larva (300×), (C) protonymph (200×), and (D) tritonymph (300×). The anterior coxal apodema (white arrows) and larger size distinguishes the tritonymph from the protonymph stage.

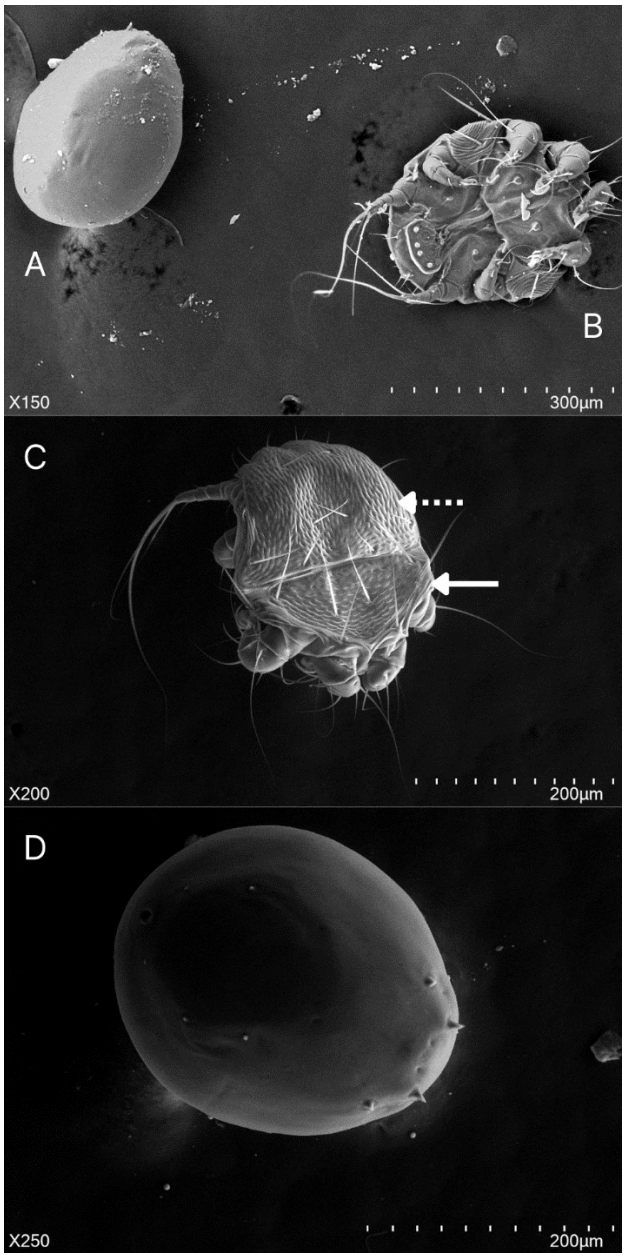

Figure 3.3. Heteromorphic deutonymphs of *Chaetodactylus krombeini.* (A and D) The inert deutonymph has highly reduced appendages and is nonfeeding. They are adapted for a long dormant period that occurs between one bee host generation vacating a nest, and the next generation reusing the nest the following season (150× and 250×, respectively). (B) Ventral view of phoretic deutonymph (150×). (C) Dorsal view of phoretic deutonymph showing the propodosomal (solid arrow) and hysterosomal shields (dashed arrow). The phoretic deutonymph is highly sclerotized and adapted for harsher environments outside the nest and on the bee host. (200×).

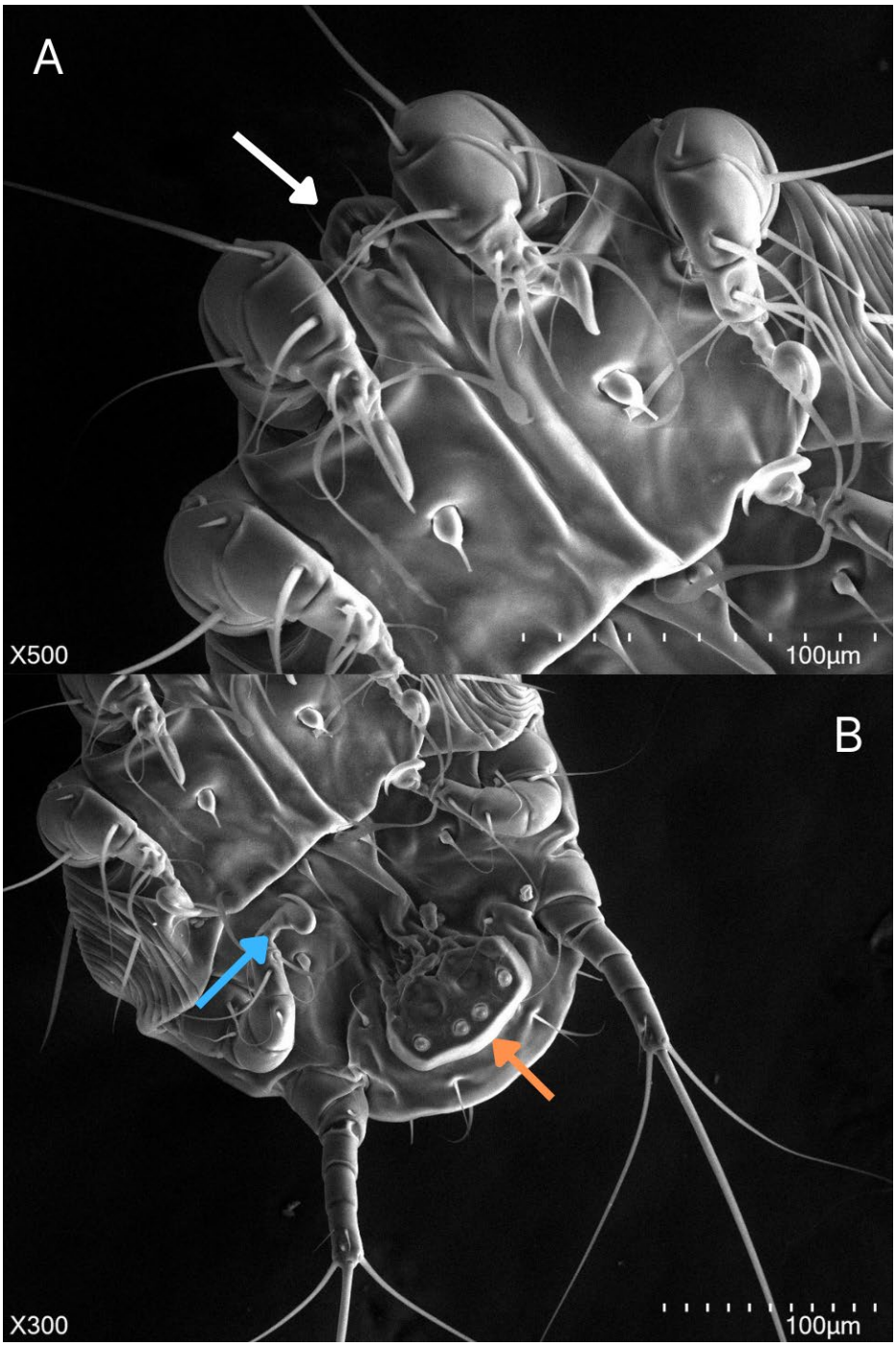

Figure 3.4. The ventral view of phoretic deutonymph of *Chaetodactylus krombeini* showing (A) the reduced mouth parts (white arrow), (500×) (B) the recurved empodial claw (blue arrow), and suctorial plate (orange arrow) (300×). These modifications are adaptations for phoresy and for overwintering with the bee cocoon without food resources.

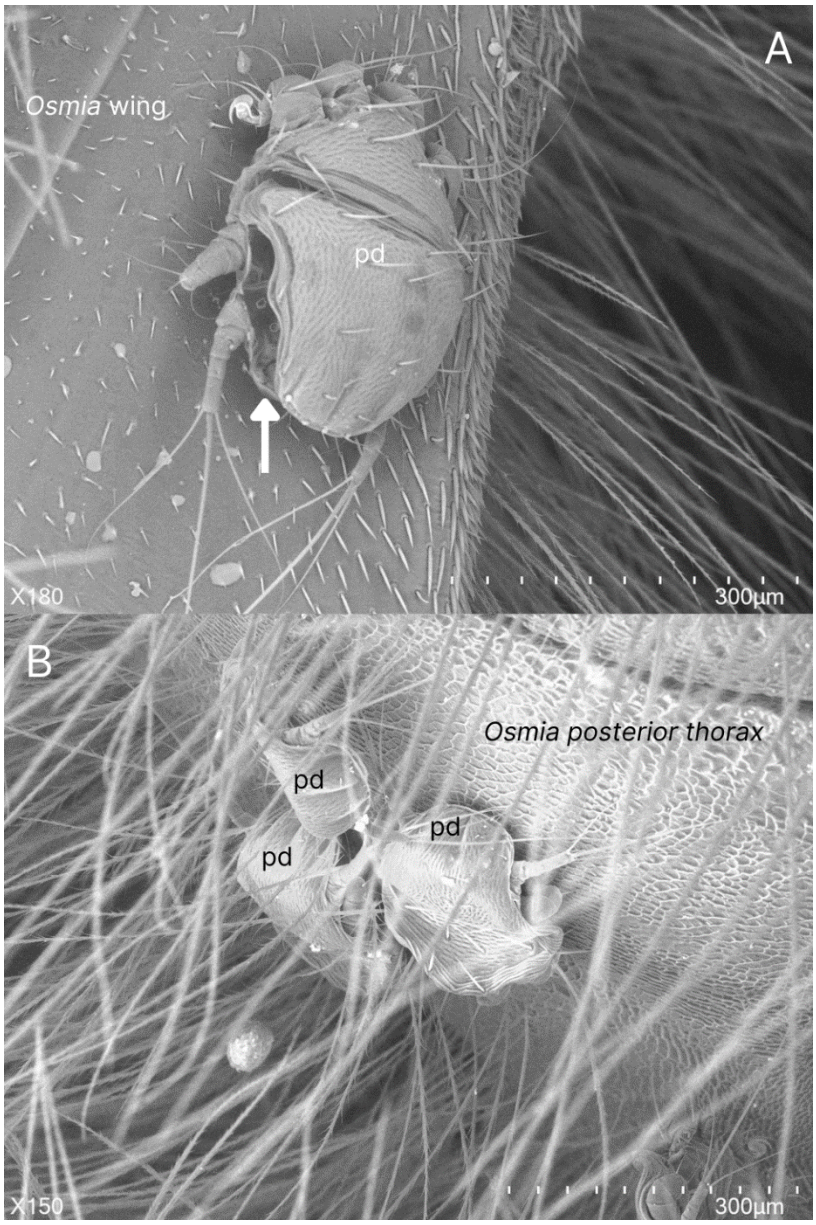

Figure 3.5. *Chaetodactylus krombeini* phoretic deutonymphs on an *Osmia* host. (A) The cast skin of phoretic deutonymph on an *Osmia* wing indicated by the exit cavity of the tritonymph (arrow) (180×). (B) Three phoretic deutonymphs on the posterior thorax of an *O. lignaria* male (150×). Phoretic deutonymphs can be found on the posterior thorax and in locations that are hard to reach when the host is grooming (e.g., between the wings on the thorax and abdomen). Phoretic deutonymphs are often found in groups on the host, as pictured. It is not unusual for bees to harbor tens of phoretic deutonymphs. *pd* phoretic deutonymph.

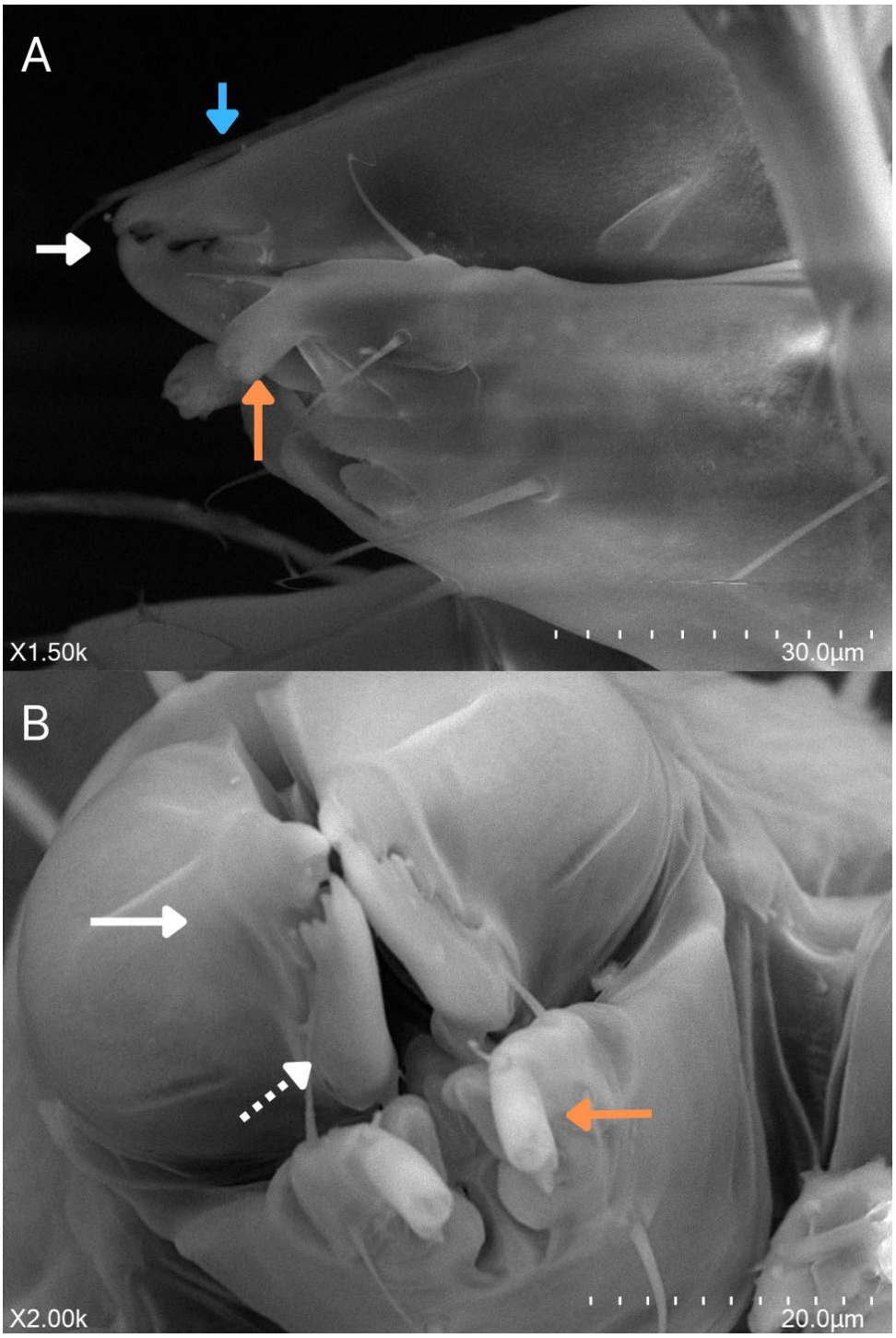

Figure 3.6. Mouthparts of *Chaetodactylus krombeini* (A) adult female (1.5k×) and (B) tritonymph, (2.00k×). The pedipalps (orange arrows) are below the chelicerae. The chelicerae (solid white arrows) have a fixed digit (blue arrow) and a movable digit (dashed white arrow).

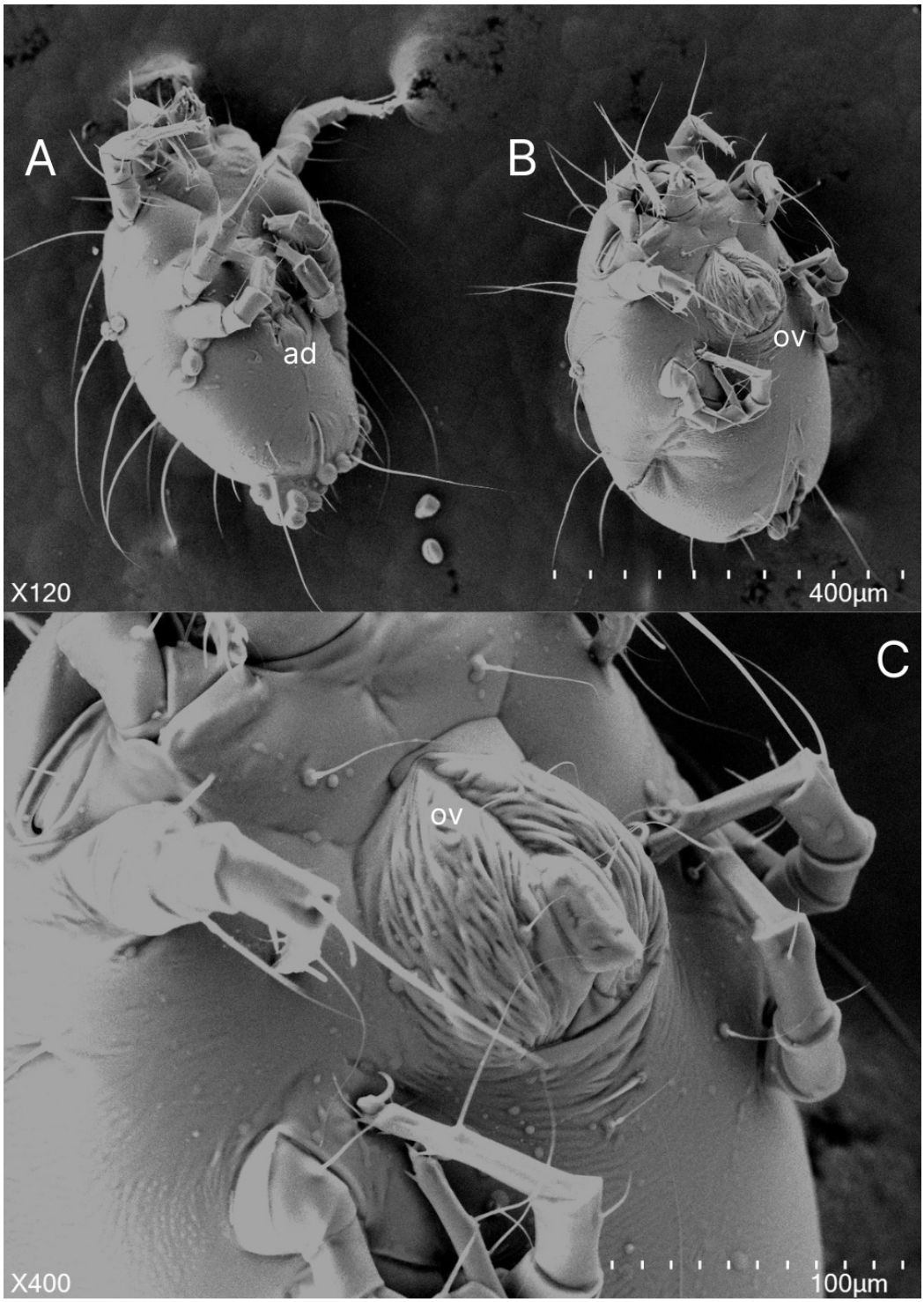

Figure 3.7. Ventral view of the adult male (A) and adult female (B) of *Chaetodactylus krombeini*, (120×). (C) Ovipore of adult female *C. krombeini*, (400×). *ad* aedeagus, *ov* ovipore.

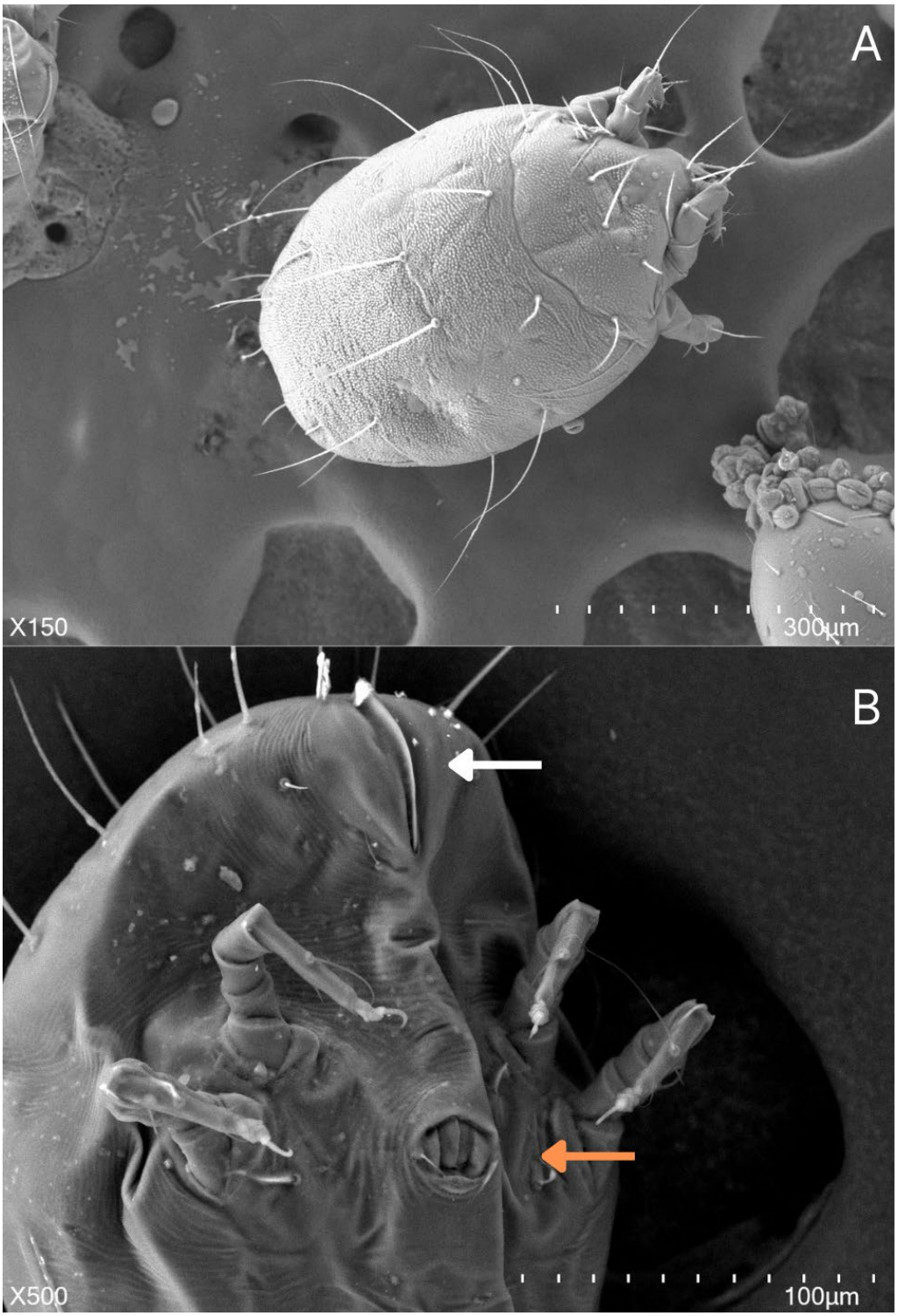

Figure 3.8. (A) The dorsal view of an adult female of *C. krombeini* (150×). (B) The ventral view of a protonymph of *C. krombeini* showing the anal slit (white arrow) and genital valve (orange arrow) (500×).

Conclusion

This research focused on the kleptoparasitic mite *Chaetodactylus krombeini* due to its harmful impact on managed *Osmia* bee nests, specifically of *Osmia lignaria* (Bosch and Kemp 2001; Krunić et al. 2001). *Chaetodactylus krombeini* increases mortality of bee offspring and can cause adult bees to develop into less efficient pollinators (van Lith 1957; Krombein 1962; Bosch and Kemp 2001; Krunić et al. 2005). Managed *Osmia* beekeepers need methods to control *C. krombeini* in *Osmia* nests, however, more needs to be understood about this mite's life history traits to develop effective control methods that will decrease mite presence in bee nests. Many of the life history traits of *C. krombeini* have been compared to other mites or have contradictory results reported. The phoretic deutonymph of *C. krombeini* has been reported to prefer hosts of both male and female *Osmia* bees (Krombein 1962; Park et al. 2008; McKinney and Park 2013). Infestations that are in the middle of bee nests or near the bee nests entrance, where male bee offspring are located, allows multiple emerging adult bees to pass through the mite infestation which thereby allows phoretic deutonymphs to attach to multiple bee hosts (Krombein 1962). Infestations that are in the back of bee nests, where female bee offspring are located, provides mites with female bee hosts which are clearer routes to new nests because female bees are the only sex that provision nests for offspring (Bosch and Kemp 2001; McKinney and Park 2013). Chapter 1 was conducted to determine if *C. krombeini* phoretic deutonymphs have a host sex preference.

This study showed that mites have a positional preference in *Osmia* nests as all mites were found to be in the back of nests regardless of bee host sex. However, in this study the mite numbers in bee nests were low and there were few female bee offspring produced, so these results should be confirmed.

Chaetodactylus krombeini heteromorphic deutonymphs were hypothesized to develop only into adult females which led Krombein (1962) to compare *C. krombeini* mating strategies to *Histiostoma* mites, which are associated with Halictid bees (Krombein 1962). Female mites were founding new mite infestations as heteromorphic deutonymphs, which led to the hypotheses that *C. krombeini* adult females use arrhenotokous parthenogenesis to produce male (haploid) offspring, then mate with their sons (oedipal mating) to produce female (diploid) offspring (Krombein 1962; Adamson and Ludwig 1993). In Chapter 2 heteromorphic deutonymphs were held individually until they developed into adults and could be sexed via morphology. Heteromorphic deutonymphs of *C. krombeini* developed into male and female adults from both the inert and phoretic deutonymph, which is contradictory to current opinion. This may indicate that copulation is required for offspring development. Both sexes disperse as phoretic deutonymphs on a host prior to mating. Dispersing in groups of four or greater provides a greater chance that a mating pair will come to be in the same nest cell and be able to found a new mite population (Mitchell 1970).

Also, 32-38% of heteromorphic deutonymphs developed into adult males, which further suggests that arrhenotoky is not used for reproduction (Nagelkerke and Sabelis 1998). We hypothesize that pseudo-arrhenotoky is the primary method of reproduction for C. krombeini, though further evidence is required.

Chaetodactylus krombeini and other Chaetodactylidae mites are often indistinguishable from each other based on morphology alone, making identification of mites difficult (OConnor and Klimov 2009). Clear understanding of *C. krombeini* morphology can provide insight into their behavior as appendages are needed for tasting, perceiving the environment, aggression, and mating (Walter and Proctor 2013). In Chapter 3, scanning electron microscope (SEM) images of *C. krombeini* were taken to provide detailed morphology for comparison to other mite species. The mouthparts of the feeding life stages of *C. krombeini* were compared to *Pneumolaelaps longanalis,* showing that they may similarly feed on a nectar coating of a pollen grain and possibly the pollenkit. SEM images also provided more detailed images of all the immature stages, making it easier to compare the protonymph to the tritonymph life stages.

Chaetodactylus krombeini proved to be a complicated organism with a complex development pathway, leading to more questions about its life history. A better understanding of mite positional preference in *Osmia* nests, especially under different environmental conditions, could lead to more targeted pesticide applications for mite control. Previous research that compared *C. krombeini* to other mite species showed similarities in reproductive strategies, which in turn

suggested that a small number of heteromorphic deutonymphs introduced to a bee nest could lead to substantial losses of bee offspring (Krombein 1962). However, the use of arrhenotoky was not observed with *C. krombeini* in this research and both sexes were shown to be capable of developing into heteromorphic deutonymphs. Additionally, dispersing or remaining dormant in groups as heteromorphic deutonymphs indicates *C. krombeini* likely requires copulation to reproduce and the observed mite sex ratio suggests the possibility of pseudo arrhenotoky (Mitchell 1970, Nagelkerke and Sabelis 1998). Further research should take a closer look at the reproductive strategy of *C. krombeini* and what genes are involved in life stage transitions and their expression in response to different stimuli. This will improve our overall understanding of *C. krombeini* life history and the development of Astigmatan hypopi generally. A thorough understanding of *C. krombeini* morphology will provide information about life history traits, likely leading to effective control efforts of these mites that may not require pesticides or harmful consequences to their bee host. Further research on *C. krombeini* is necessary to understand this mite species more clearly, as this mite has the capability to infest new bee nests and other bee species negatively impacting orchard crop yield (Krombein 1962; Torchio 1976).

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Appendix A

Introduction

A number of Astigmatan mites possess a facultative heteromorphic deutonymph life stage, often referred to as a hypopus (OConnor 1994). Heteromorphic deutonymphs often appear in mite populations when an ephemeral habitat is no longer suitable for continued population growth (Knülle 1987; OConnor 1994). Multiple environmental factors are said to trigger the heteromorphic deutonymph stage, such as: low humidity, low food quality or quantity, mite overcrowding, or synchronous with host life stage (Krombein 1962; Knülle 1987; OConnor 2009). The kleptoparasitic mite, *Chaetodactylus krombeini*, is permanently associated with their solitary bee hosts (Krombein 1962). *Chaetodactylus krombeini* live inside solitary bee nests feeding on food resources meant for developing bee offspring (Krombein 1962). When nest conditions start to deteriorate the facultative heteromorphic deutonymph life stage is induced (Krombein 1962). The inert deutonymph is a highly regressive morph that remains in the exoskeleton of the protonymph (Krombein 1962; Knülle 1987). The inert deutonymph stays behind in solitary bee nests waiting for nests to be reused by a new host, which will provide renewed food resources that will allow the continued development of the inert deutonymph (Krombein 1962).

The phoretic deutonymph is a highly specialized morph, possessing adaptations used for phoresy (Krombein 1962). The phoretic deutonymph attaches to adult bee hosts as the bee host emerges from nests, moving to locations on the bee host body that are not accessible to host when grooming (Krombein 1962; Eickwort 1994). When bee hosts begin building new nests for bee offspring, phoretic deutonymphs will drop off the bee host into new bee nests and continue development (Krombein 1962). When both inert and phoretic deutonymphs resume development in newly established nests they will develop into tritonymphs, then sexually mature adults, and will then found new populations within host nests (Krombein 1962; Eickwort 1994).

The hypopus induction stage of Astigmata mites lasts from late larval to early protonymphal stages (Corente and Knülle 2003). Producing inert deutonymphs in a lab setting was done by starving larvae and protonymphs of *C. krombeini*. This research aimed to produce phoretic deutonymphs to be used in life history assays of *C. krombeini* following hypotheses and methods by Krombein (1962) on *C. krombeini* and Knülle (1987) on *Lepidoglyphus destructor*. Here, we report multiple approaches that were unsuccessful at producing phoretic deutonymphs, suggesting that cues for initiating the phoretic deutonymph life stage likely differs across genera.

Materials and Methods

Mite Colony

Chaetodactylus krombeini mites were collected in October of 2021 and 2022 from multiple commercial *Osmia lignaria* nests in Washington and Oregon. At UCR, mite populations were sorted by collection site and maintained in 1.5 µL microcentrifuge tubes in an environmental chamber (VWR Model No: 2005 and Fisher Scientific Model No: 146E) set to 26°C. Each tube was filled with 100 to 250 µL of a stock mixture of 60% honey bee pollen, 16% honey, 20% DI water, and 4% fructose (pollen provision). Each 1.5 µL tube was sprayed with a 0.1% dilution of food grade fungicide (methyl paraben, CAS: 99-76-3) to prevent mold from forming after the pollen provision was added. This food would last for multiple *C. krombeini* generations. Every 2 weeks mites from an established tube population would be transferred in groups of 10 into new tubes with a fresh pollen provision. Mite colonies were maintained this way from November 2021 to June 2023, with new field-collected mites added each spring.

Low Humidity. Mites were placed into glass pipettes (Corning® 5.75-inch Pasteur Pipettes, Disposable, Bulk Pack, Non-Sterile, unplugged – Borosilicate; 23A00Q398), the pipette tip was plugged with modeling clay (Craftsmart® Plastalina Modeling Clay; 10062644), and the mites were provided with a lab made pollen provision (see above) and the end of the pipette was stopped with a

cotton ball (Fig. A.1). Pipettes with mites were kept in an environmental chamber (VWR Model No: 2005) set to 26°C. The cotton allowed the pollen provision to dry out (Fig. A.1), causing mites to be exposed to low humidity. Mites in pipettes desiccated with no production of heteromorphic deutonymphs.

No Food. Mite larvae and protonymphs (n = 15 total) were placed into a 1.5 µL microcentrifuge tube. Immature mites were left undisturbed for a week in the absence of food. When mites were examined, several mites had died, while three mites had developed into inert deutonymphs (Fig. A.2). No phoretic deutonymphs were produced by starvation.

Overcrowding. Mites were kept in glass pipettes (as above) in an environmental chamber set to 26°C (as above) and the pollen provision was replaced every three to five days to maintain adequate humidity in pipettes. Mites were not removed from pipettes, and populations were allowed to grow. No heteromorphic deutonymphs were produced in overcrowded populations; however, all mite life stages accumulated in the tip of the pipette and the population began to die.

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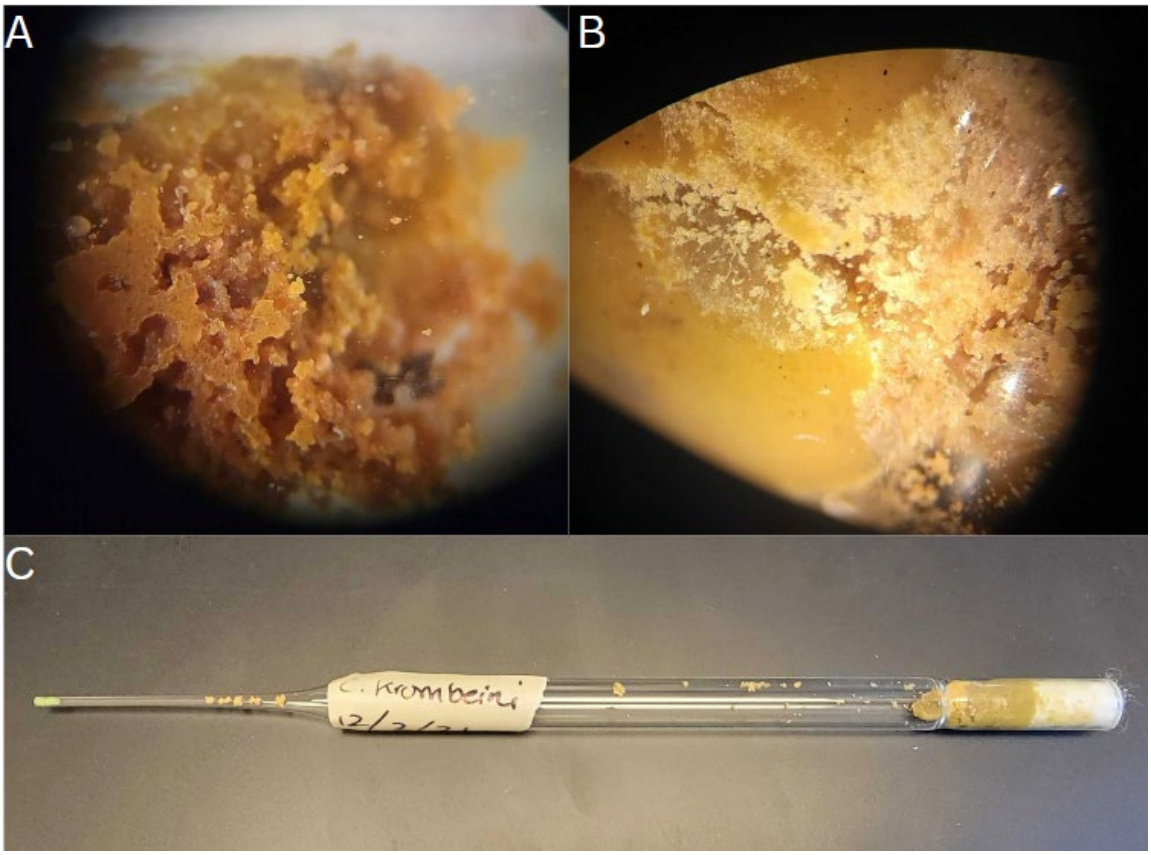

Figure A.1. Lab made pollen provision in glass pipette. (A) Pollen provision with a loss of moisture, indicated by its dark color and more granular appearance. (B) Normal pollen provision with adequate moisture, indicated by the light color and smoother globular appearance. (C) Pipette with mite population and pollen provision.

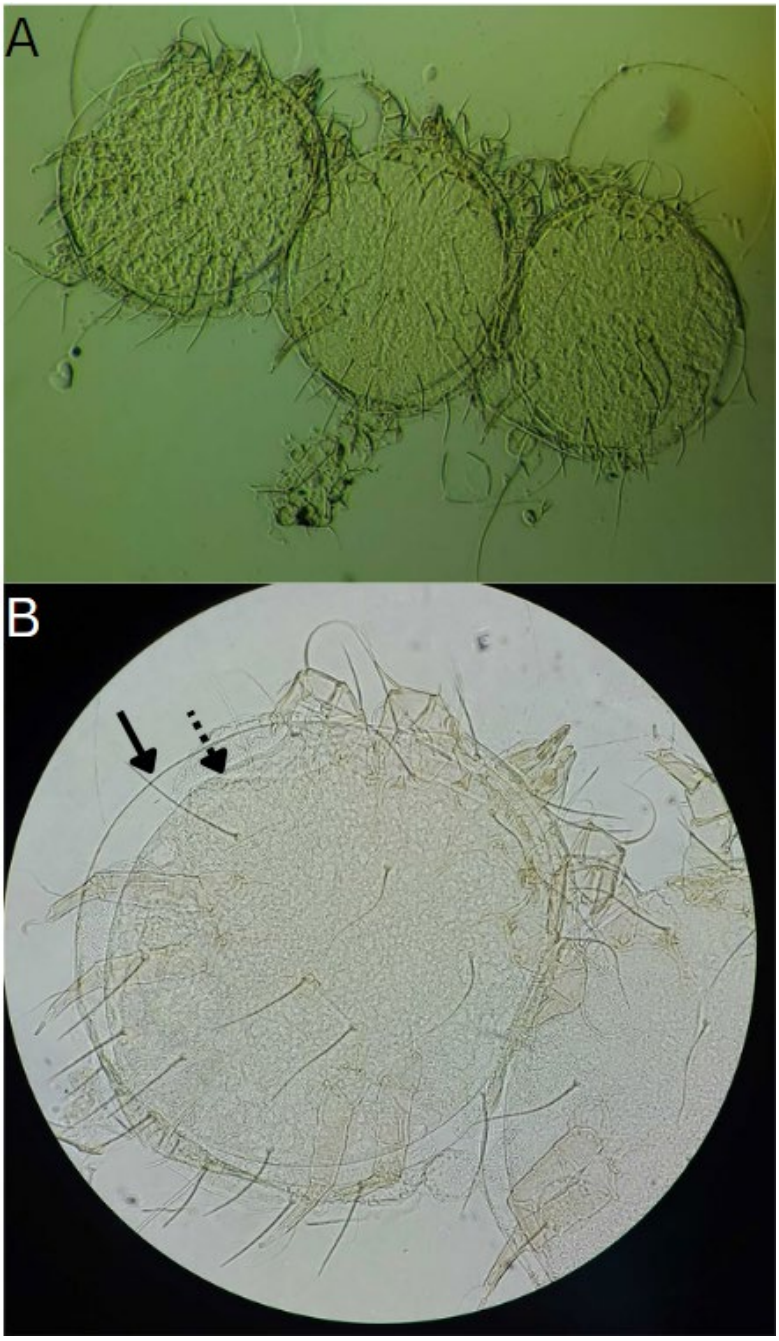

Figure A.2. *Chaetodactylus krombeini* inert deutonymphs cleared, and slide mounted in PVA mounting medium (A 100×) (B 400×). (A) Inert deutonymphs collected from microcentrifuge tube. (B) Inert deutonymph (solid arrow) protruding from protonymph exoskeleton (dashed arrow).