Exploring the Co-evolution of Burying Beetles and Their Phoretic Mites

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Abstract

Phoretic mites and their hosts, burying beetles (*Nicrophorus*), represent a unique relationship that has evolved alongside the behaviors of both organisms. Both the beetles and mites rely on carrion for sustenance and breeding. The key difference between the two is that the mites have very low mobility due to their size. On the other hand, burying beetles can cover kilometers searching for a carcass to breed on, as the mites hitch a ride in the process. Through this study, we dove deeper into how this relationship evolved by examining how phoretic mites choose a host, specifically *N. investigator* and its associated mite complex. We examined any differences in host preference throughout the beetle's life cycle and whether the sex of the host affects the mites' behavior, while controlling for body size and species. Mites were counted on beetles at four stages of maturity to understand their preferences. Alongside these observational studies, we also carried out a paired-choice test to directly observe the mites choosing a host. To remove and count mites, we made use of pressurised carbon dioxide to anesthetize and remove any mites. We found a significant positive relationship between beetle size and mite load at all life stages, including the larval stage. We also found that, on average, female beetles had higher levels of infestation compared to males in wild-caught populations. This is the first time these trends have been observed on *N. investigator*, and much more research needs to be done in order to better understand the relationship between *Nicrophorus* and their phoretic mites.

Introduction

Burying beetles (*Nicrophorus*) breed and feed on freshly dead animals. They are found across the Northern Hemisphere, and their name reflects their unique behavior. When a pair of beetles or a single female finds a corpse acceptable for use as a breeding resource, the beetles will bury the carrion before shaping it into the perfect den or "crypt" for their larvae to grow (Milne and Milne, 1976; Potticarry et al., 2023). Burying beetles are obligate carrion breeders, meaning that locating and burying a carcass is required for them to breed (Smith and Merrick, 2001; Wilson and Knollenberg, 1987). Nearly all species of burying beetle have co-evolved with species of phoretic mites, which "hitch rides" on the beetles to reach carcasses, where they also feed and breed (Nehring et al., 2017). Based on this close relationship, the fitness of these phoretic mites is directly affected by their ability to select a successful beetle host (Springett, 1968). Deuteronymph mites either depart the crypt with the adult beetles or remain with the larva, emerging from the crypt attached to the newly pupated adult beetles. Thus, the mite life cycle is highly aligned with the beetles, with the beetles moving to their next food source and continuing their life cycle (Schwarz and Koulianos, 1998).

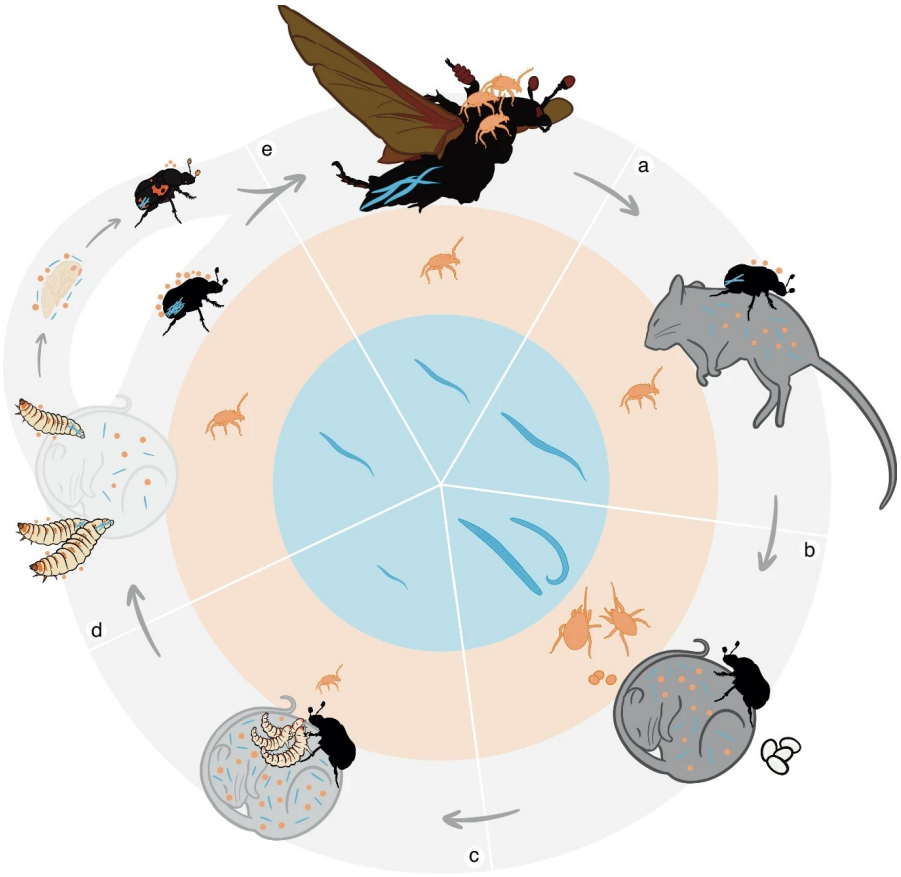

Figure 1: Illustrates the highly connected lifecycles of the burying beetles alongside their phoretic mites and nematodes. Illustration created by Megan M.Y. Chang (Lee et al. 2026).

The existence of such a close relationship between the mites and beetles is interesting from a co-evolution and life history standpoint (Wilson and Knollenberg 1987, Knee et al. 2012, Nehring et al. 2017). Understanding the evolution of behaviors in animals tells us about an organism's place in an ecosystem and the selective pressures that shaped them. For us to better understand how the mite's phoretic behaviors have evolved, we must understand the intricacies of the interactions between the two species and which factors may increase or decrease the benefits of the relationship that drove the co-evolution of these unique behaviors in the first place. There has been extensive past research into the unique symbiotic behavior, seeking to understand what a mite may consider when choosing a host (Bajerlein et al., 2025) and even how the mite's presence affects the beetles (Nagel et al., 2025; Lee et al., 2026).

While there has been extensive research into mite behaviors, much of the existing work has been conducted on the phoretic mites found on burying beetle species in Europe, such as *Nicrophorus vespilloides,* a very common subject for burying beetle studies (De Gasperin et al, 2015; Bajerlein et al., 2026). These studies have looked into which factors (such as beetle size, sex, sexual maturity) a mite "considers" when choosing a *Nicrophorus vespilloides* host. A 2026 paper found that male *Nicrophorus vespilloides* have a greater mite burden than females, claiming that size had no effect (Bajerlein et al., 2026). Other past studies have found conflicting results, claiming that mites do select for size when presented with two male *Nicrophorus investigator* beetles (Grossman and Smith, 2008). Research into the

dispersal behaviors of the mites using *Nicrophorus vespilloides* found that the deuteronymphs prefer sexually mature beetles to immature ones (Schwarz and Muller, 1991). It is important to note that there has been no research into the reproductive stage or male vs. female preferences on the mites found on *Nicrophorus investigator*, though there have been findings related to *N. vespilloides, N. vespillo, N. humator,* and *N. interruptus*. Comparisons among related species, especially those less studied, will add to existing findings about the mite-host relationship. When conducting research on *Nicrophorus*, which have diverse and complex behaviors and life histories (especially overwintering stage), it is important not to generalize among species. While all burying beetles share some common behaviors, there are many differences in their breeding, parental habits, and life cycles, and it would be incorrect to generalize their behaviors, much less those of a related organism such as their phoretic mite symbiotes. Comparisons among species and the mite's host choice can tell us about how this relationship may have evolved. If we can understand why a mite may choose one beetle over another, we can better understand the factors that drive this symbiotic relationship and what each species gains (or loses) from the interaction.

For this study, I will be focusing mainly on *Nicrophorus investigator* and its associated complex of phoretic mites. This species of burying beetle is found in Colorado, where the study will be conducted, and has received far less investigation than *Nicrophorus vespilloides.* I want to understand if the mites associated with *Nicrophorus investigator* have a preference for either sex of beetle in each stage of their interactions. The stages I will be focusing on include sexually mature beetles searching for a carcass, emerging parent beetles after breeding, and newly eclosed beetles emerging from the soil after overwintering as pre-pupae. I hypothesized that phoretic mites will prefer male burying beetles due to their increased chance for multiple broods, especially on beetles emerging after mating. I also predicted that mites will select larger larvae on which to overwinter, emerging with larger adults. When faced with a choice between size or sex, the decision may depend on reproductive condition. Comparing the mite infestations on males and females in each stage of their life history could shed light on what the mites value in a host and if that preference changes over the course of a beetle's life cycle. Through this, we can gain insight into how this symbiotic relationship evolved, and if there are any differences in the mite interactions between *Nicrophorus investigator* compared to other species of *Nicrophorus.*

Materials and methods

Collecting and measuring beetles at different life stages: All adult beetles were placed into individual containers, measured (pronotum width, mm), and weighed (g) before their mites were counted. I measured each beetle's pronotum, the hardened plate on an insect's back directly behind the head, at its widest point and weighed the beetle on a balance (OHAUS ScoutPro, 120x0.001g), allowing us to later compare the level of infestation to size as well as sex. The sex of the beetle was determined based on the characteristic brush-like front legs on the male beetles. Similar comparisons have been made in previous studies, which have found that when given the choice between two males, the mites prefer the larger host, most likely due to the increased relative fitness of a larger male beetle (Grossman and Smith, 2008). We took this into account when creating matched breeding pairs (see below). Each beetle in a pair had a pronotum width within 0.3mm (one standard deviation) of each other.

Overwinter emergers:

I sampled newly eclosed adult beetles as they emerged from the soil after overwintering. These beetles were acquired from the overwinter plots located behind the Gothic Research Center and the aspen forest plot. In the summer of 2025, 162 larvae were placed into the overwinter cans in Gothic. Of the 162 larvae, 32 of them emerged, making up our overwinter data points. Each beetle was collected in its own vial before counting mites.

Wild-caught adults (pre-breeding):

I also captured 200 wild beetles from 26 traps set around the Gothic Valley. These traps consist of a metal can filled with soil and some added water. When setting the traps, we add either a dead deer mouse (*Peromyscus maniculatus*) or a chicken leg to attract the beetles. The can was then covered with a metal mesh with a small hole at the center. Once the beetles enter the trap, they cannot crawl out of the concave lid (Grossman and Smith, 2008). We checked the traps and collected beetles every other day for the extent of the collection period, which ran from June 19th to July 29th, 2026. This allowed beetles to enter the traps during their active daytime hours before the traps were emptied. Each beetle was collected in its own vial before counting for mites.

Adult beetles and larvae after breeding:

To identify any preferences the mites had at the larval (in preparation for overwintering) and adult post-breeding life stages, we set up a breeding experiment. We paired similar-sized (+/- 1 s.d.) newly emerged and/or wild-caught beetles in 20 pairs to breed in a lab setting (the outdoor cage in the GRC building) on dead *Peromyscus maniculatus (collected under Scientific License from CPW, salvage permit to R. Smith).* Pairs of beetles had their mites removed and were bred in small plastic containers with about 8 cm of substrate locally-sourced soil to bury in. Along with the beetles, we added 10 deuteronymph mites to each container, allowing them to attach to the two beetles however they choose. After breeding, both the male and female beetles emerged from the burrow, leaving the larvae (and any associated mites) to overwinter in the soil.. We checked each container every morning, making sure to collect any beetle within 24 hours of its emergence. The beetles were determined to have left the crypt if they were observed on the surface twice in a row. When each parent beetle emerged into the container, we measured the beetle and counted the mites. Along with measuring the infestation of the parent beetles, we also counted mites on the larvae once they were fully grown and had left the carcass.

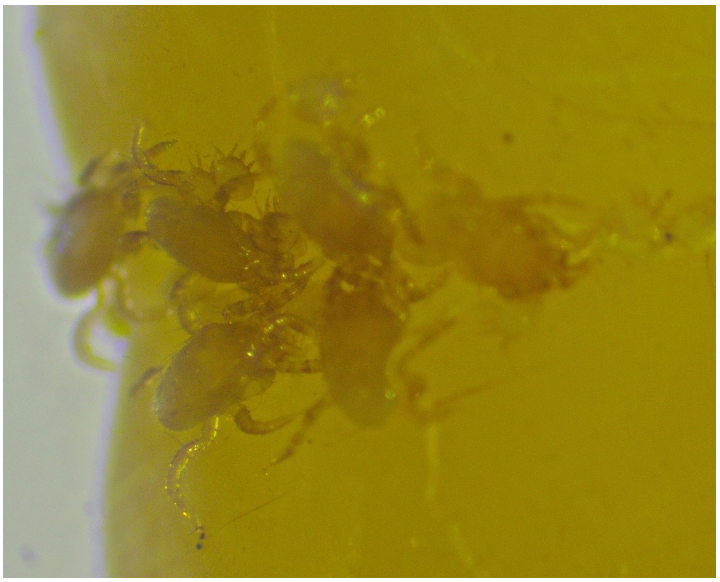

Image 1: Image taken under a microscope of *Poecilochirus* mites clinging to a *N. investigator* larva.

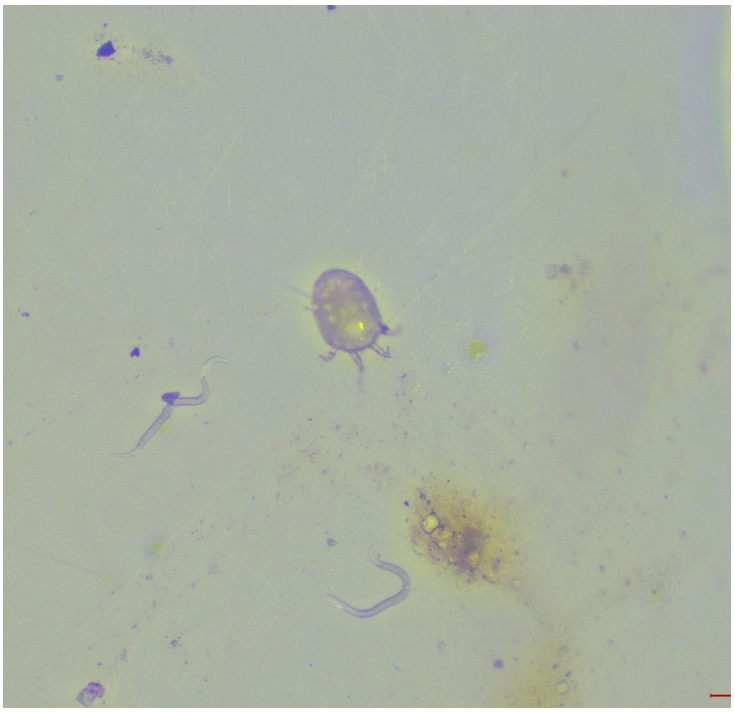

Image 2: Image of a larval *Poecilochirus* mite alongside phoretic nematodes taken under a dissecting scope.

Removing and Counting mites

Each beetle was held in its own container. I removed the mites using a steady stream of pressurized CO_2, temporarily stunning them, allowing them to fall into a Petri dish filled with 2ml of 70% isopropyl alcohol. This process was also aided by needle-point tweezers, allowing any remaining mites to be removed by hand. The CO_2 was also blown under the elytra to remove any mites hiding beneath. The

alcohol quickly kills the mites, allowing for easy counting. To collect live mites, we used the same process but replaced the isopropyl alcohol with water, allowing us to transfer the live mites into a container after they were counted for later use.

It is important to note that when collecting and counting mites, I did not attempt to discern the species within the complex, as the species look nearly identical and are extremely difficult to identify. For this study, we are just looking at the behaviors of the mite complex as a whole, meaning that species identification was unnecessary. That being said the most likely species found on the *N. investigator* is *Poecilochirus carabi.* We collected vouchers of both the beetles and their mites for any future studies that would look into my findings, allowing for future investigation and replication of this experiment.

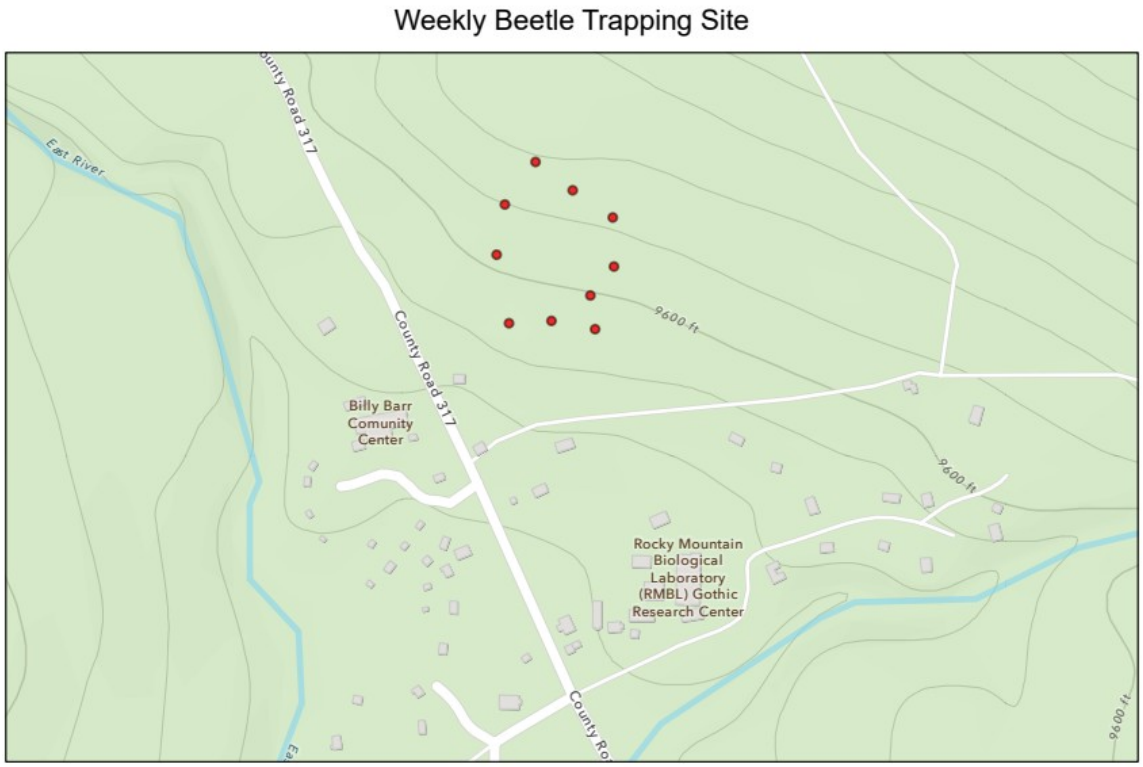

Figure 2. Map of the "Weekly Aspen" site and all of the traps used to catch wild beetles.

Pairwise choice tests among adults

Along with the life stage tests, we conducted a series of pairwise choice tests. For these tests, two beetles (one male, one female, or both of the same sex) of approximately equal size, and also pairs of unequal size (male or female larger by at least one s.d.) were placed into a container about 3.5 cm wide and 5.7 cm tall. Along with the beetles, we added 10 *P. carabi* to the container. We used 10 mites because this is within the range found on wild-caught beetles and will reveal any association patterns between the hosts (Grossman and Smith). After 5 minutes, the beetles were removed from the container, and their mites were removed and counted using a stream of carbon dioxide. After counting was complete, both

beetles and mites were returned to their individual holding containers for further testing, though no repeat beetles were used in choice trials.

We used Jamovi and Microsoft Excel to create figures and perform statistical analysis. This not only helped us to visualize our results but also determined if any of our findings were significant.

Results

Mite Loads on Wild-Caught Beetles

For wild-caught beetles that had their mites counted in the lab, we found a significant positive relationship between the pronotum size of the beetle and mite load ($t = 3.54$, $df = 272$, $p < 0.001$), Fig. 3. Along with the increase in mite load with size, we also found that wild-caught female beetles had significantly more mites compared to their male counterparts. The mean mite count for wild-caught female beetles was 16.5, while the mean for males was only 12 ($t = -3.16$, $df = 272$, $p = 0.002$), Fig. 3.

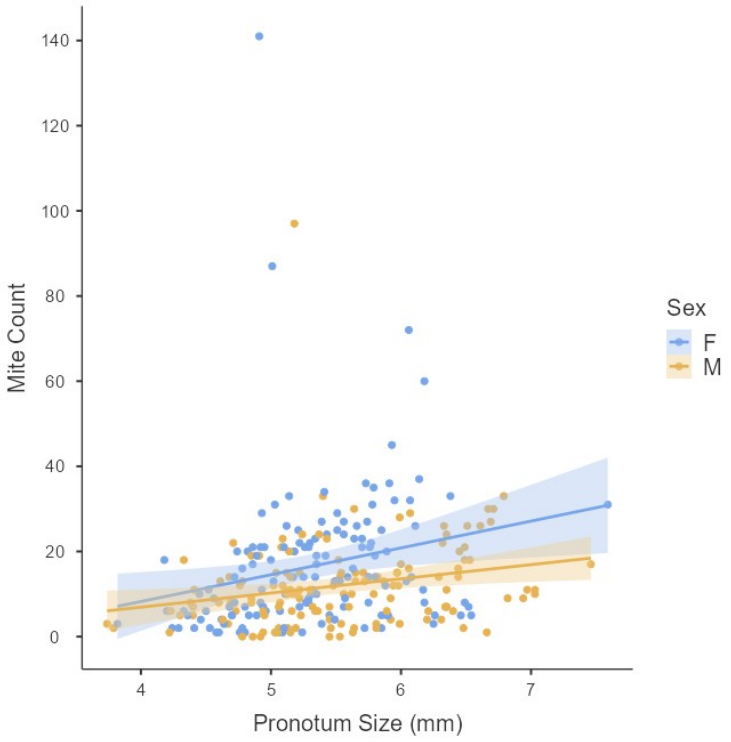

Figure 3. The figure shows the mite count for each wild-caught beetle compared to its pronotum size (n = 272). Trend lines for males and females are illustrated independently to show the difference in mite loads between sexes. All mite data for this figure were collected in the lab using CO_2 removal methods.

We also observed a significant increase in mite load throughout the summer, indicating an increase in the overall population ($t = 6.4$, $df = 272$, $p < 0.001$), Fig 4. Along with lab-counted beetles, data collected visually from census beetles showed a similar trend, with increasing beetle counts between the first and second census periods ($F = 9.56$, $df = 581$, $p = 0.002$). Along with a matching temporal pattern, we also observed a significant positive relationship between pronotum size and mite count ($F = 33.87$, $df = 581$, $p < 0.001$), aligning with the trends observed in our lab-counted data.

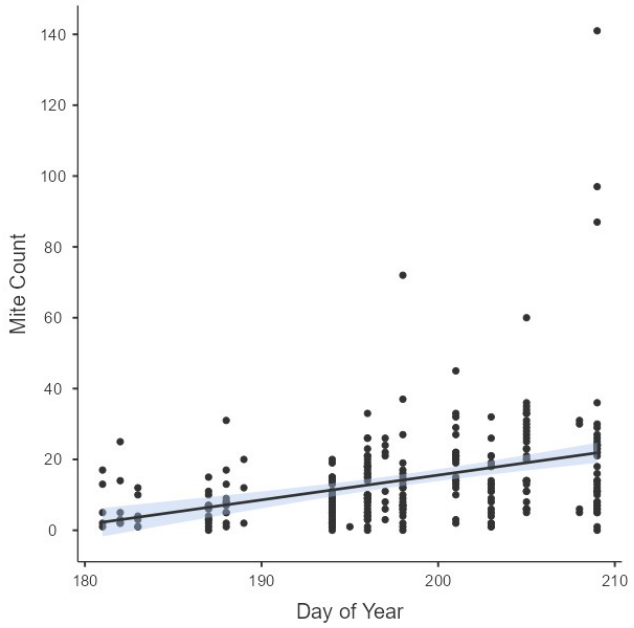

Figure 4. The figure shows the mite count for each lab-counted beetle plotted against the day it was caught ($n = 272$). Shows an overall increase in mite count throughout the study period, possibly indicating an overall increase in population.

Pairwise Choice Trials

The choice trials we conducted found no significant difference in preference for any of our three treatments. This was most likely due to the small number of replicates (10 per treatment), reducing our statistical power (Binomial exact test, $p > 0.1$). This being said, we did observe a trend similar to what we saw in our wild-caught beetles, indicating that mites may prefer a female beetle if both beetles are similar in size (Fig 5).

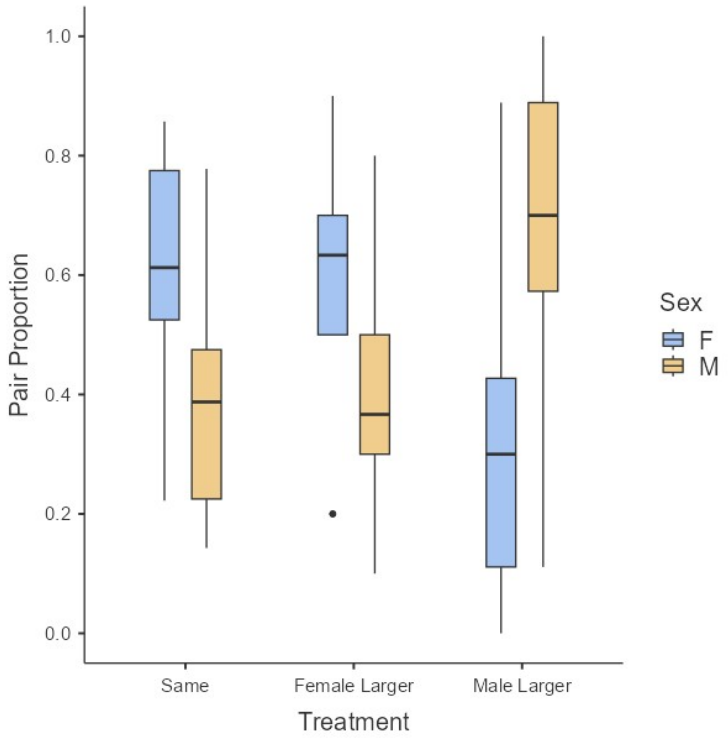

Figure 5. Displays the proportion of the 10 initial mites on the male vs. female for each treatment group. The visual trend indicates that size was the main deciding factor for both treatments, with one larger beetle, while sex was more important when the beetles were matched for size, even though these results are not statistically significant.

Duration of Brood Care and Mite Loads of Beetles Exiting the Crypt

We found that the male beetle exited the crypt on average 3.84 days before the female ($t = -5.66$, $df = 36$, $p < 0.001$). We also found a significant positive relationship between the day the beetle was removed from the crypt and the proportion of mites on the individual ($t = 2.69$, $df = 38$, $p = 0.011$), Fig 6. This indicates that the later a beetle exits the crypt, the more mites it will have. Trending similarly to this, we found that the second beetle to leave had a marginally significantly larger proportion of mites compared to the first to leave ($t = -2.298$, $df = 35$, $p = 0.069$). Based on this trend and the finding that male beetles tend to leave earlier, we found that female beetles exiting the crypt had a marginally significantly higher proportion of mites compared to males ($t = -2.00$, $df = 36$, $p = 0.053$), Fig 7.

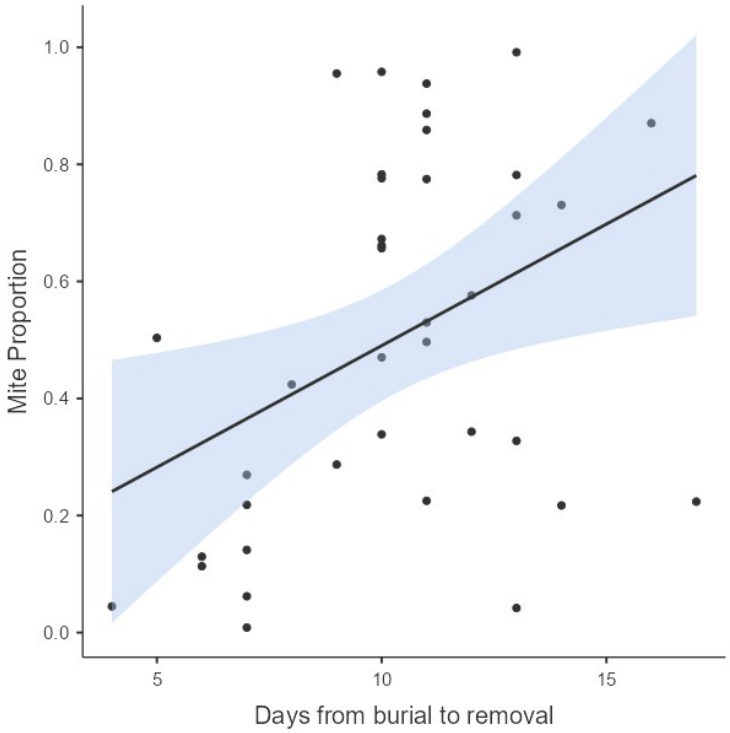

Figure 6. Shows the proportion of mites on an emerging adult plotted against the number of days from burial to removal. The line of best fit shows a positive trend indicating that the proportion of mites on a given beetle is predicted to be larger the later it leaves the crypt.

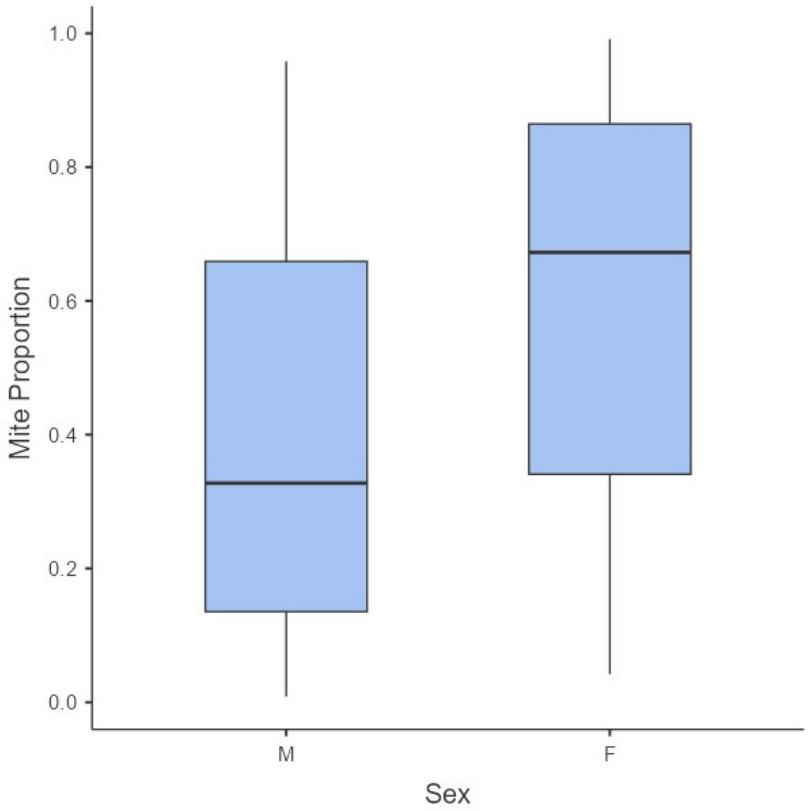

Figure 7. This box plot shows the difference in proportion of mites between male and female parents exiting the crypt. The marginal significance of this difference is most likely due to the wide variation in data, though the trend points towards females having a higher proportion of mites compared to males.

Larval Mites

When counting mites on fully-developed larvae from our lab broods, we found there was a significant positive relationship between larval mass and mite count ($t = 3.968$, $df = 79$, $p < 0.001$), Fig 8.

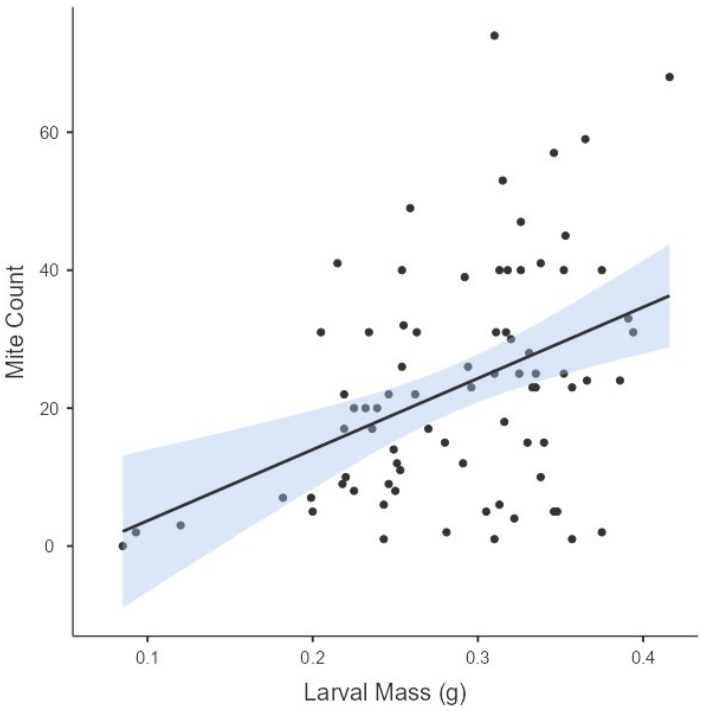

Figure 8. Shows mite count graphed against larval mass (n = 79). The line of best fit shows the significant positive relationship between the two.

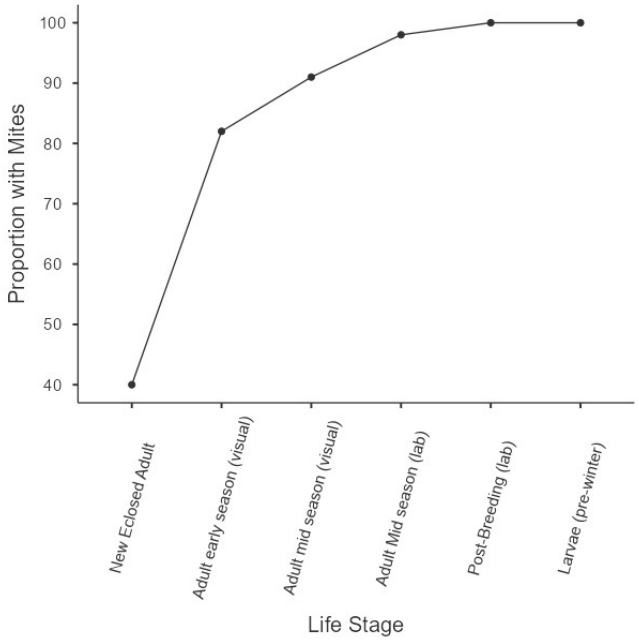

Figure 9. The x-axis shows the life stages of the beetles throughout the course of their summer lifecycle plotted against the proportion of beetles with mites. (Visual) and (Lab) indicate the method used for counting mites. Once the beetles were in their post-reproductive phase, 100% of them had some mites.

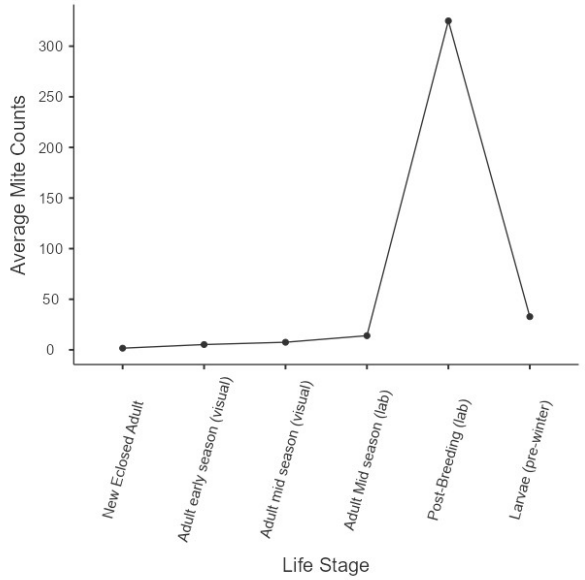

Figure 10. The x-axis shows the life stages of the beetles throughout the course of their summer lifecycle plotted against the average mite count for each group. (Visual) and (Lab) indicate the method used for counting mites. The spike in the mite population is from post-reproductive beetles emerging from their crypts.

Discussion

We found that the mites, when attaching to a *N. investigator* beetle at any stage in its life cycle, select for larger host individuals. The larger the beetle, the more of an ideal host it becomes, as seen in Figure 3. We suspect this is due to the increased physical advantage that comes with size. This physical advantage is seen in flight (Smith et al. 2000), overwintering success (Smith 2002), and securing, burying, and protection of the carcass from both inter- and intraspecific competitors. This, in turn, allows the associated mites into the crypt where they can reproduce safely underground before emerging with the parents or overwintering with the larvae. This result is consistent with past findings for other *Nicrophorus* species such as *Nicrophorus vespilloides* (Bajerlein et al. 2025). It is possible that selection for size only occurs for some *Nicrophorus* species, as another study found that size was not a significant predictor for infestation on *N. humator, N. interruptus, or Nicrophorus vespillo* (Bajerlein et al. 2026). For the phoretic *Poecilochirus* mites found on *N. investigator* to successfully reproduce, they must maintain contact with a beetle with high future reproductive potential. Without the burying beetle, these mites have little to no chance of reaching a carcass on which they can reproduce. Because this is so vital, host selection is extremely important for these mites, and they seem to prefer a range of different qualities in a host.

While it could be speculated that a larger beetle just provides more room for mites to occupy, with some beetles having hundreds of mites, it is unlikely that space is the restricting factor on mite load, as they can disperse themselves across the beetle's surface easily. We still observe the same trends at

lower levels of infestation (6-20 mites), indicating that there are other factors contributing to host preference besides space.

Sex is another factor besides host size that we found contributes to host preference. On our wild-caught beetles, we found there were significantly more mites on female beetles compared to males. This trend was also partially reflected in the choice trial trends (Figure 5) and in the post-reproductive adult population. This finding was contrary to our original prediction that males would have more mites. This finding is also distinct from past research indicating that male *N. vespilloides*, along with other *Nicrophorus* species, tend to have more mites than females (Schwarz and Muller, 1991; Bajerlein et al., 2026). This difference could be due to a variety of reasons, one of which is the distinct life cycles of these species. *N. vespilloides* is bivoltine, meaning that its behavior and life history are very different from those of *N. investigator.* Because *N. investigator* overwinters in the resting larval/pre-pupal stage rather than as an adult, the behavior of the adults is very different from that of other species. This likely affects the behavior of the associated mite complex as they spend their entire life cycle with a host, meaning they must adapt their own life history to match. It is also possible that the complex of mites found on *N. investigator* takes longer to breed, meaning more of them will end up on the later-emerging parent.

It is important to note that while we did observe some female preference for mites, we are still unsure exactly what a mite senses when it selects a host. The mites may be picking up on chemical signals that help to identify the sex of their host, or they may be reacting to behavioral differences between the male and female (Schwarz and Muller, 1991)

Another trend that we observed was the increase in overall mite loads throughout the study period, as seen in Figure 4. We know that the mite population greatly increases when they are allowed to breed with a buried carcass, with the average mite load increasing from 14 to over 300. It is likely that these mites then disperse themselves throughout the beetle population at carrion sites (Schwarz and Koulianos 1998), resulting in a smaller but consistent average mite load across the wild population by mid-summer (Figure 9).

When counting mites on the larvae from our lab-bred brood boxes, we found a similar size trend to adult beetles, with larger larvae having significantly more mites compared to smaller ones. This is most likely due to mites selecting for increased fitness as larval survival rates increase with size (Smith, 2002). The fitness of the mites is directly linked to the larvae, as they will not have the chance to emerge in the following summer if the larvae die, most likely resulting in their death as well. This means that picking a suitable overwinter host is essential for the fitness of the mites.

Based on the mite preference we found for *N. investigator* compared to existing research on other species such as *N. vespilloides*, it is clear that the preferences and behavior of each associated mite complex have shifted and evolved alongside their preferred host. The altered life histories of the different species could have easily led to the divergence in host selection preference and behavior, as different traits reflect high fitness depending on the species. Future experiments under a more controlled setting may provide insight into what factors are driving this choice. A comparative study between a bivoltine and univoltine species could also help to identify any differences in deuteronymph behavior based on the host species and their behavior.

References

Aspects of the Relationship Between Burying Beetles, Necrophorus Spp. and the Mite, Poecilochirus necrophori Vitz on JSTOR. (n.d.). . https://www.jstor.org/stable/2957?casa_token=Lwp2xakFboYAAAAA%3AJLLc4MbhYMoBQuct5yYn0idQDn8y3qmGFG-kX65cU3cPEmCAuG4Kd8ZoOV7nAkxZ_b7ciRSaBqI26fChsIwjgSDZ27alJceUuNRim7-4azvptA5Z7_yM&seq=1.

Bajerlein, D., P. Zduniak, A. Wyszyńska, E. Baraniak, M. Przewoźny, T. Grzegorczyk, and A. Urbański. 2025. Influences of carrier sex, body size, and time on the symbiotic interaction between Nicrophorus vespilloides and the Uroobovella nova mite species complex. Scientific Reports 15:19823.

Bajerlein, D., P. Zduniak, A. Wyszyńska, E. Baraniak, M. Przewoźny, T. Grzegorczyk, and A. Urbański. 2026. Males have a greater mite burden than females, and size does not matter: species- and sex-specific infestation patterns of mites (Uropodina) on burying beetles (Nicrophorus spp.). Frontiers in Zoology 23:9.

De Gasperin, O., A. Duarte, and R. M. Kilner. 2015. Interspecific interactions explain variation in the duration of paternal care in the burying beetle. Animal Behaviour 109:199–207.

Grossman, J. D., and R. J. Smith. 2008. Phoretic Mite Discrimination Among Male Burying Beetle (Nicrophorus investigator) Hosts. Annals of the Entomological Society of America 101:266–271.

Knee, W., F. Beaulieu, J. H. Skevington, S. Kelso, and M. R. Forbes. 2012. Cryptic species of mites (Uropodoidea: Uroobovella spp.) associated with burying beetles (Silphidae: Nicrophorus): The collapse of a host generalist revealed by molecular and morphological analyses. Molecular Phylogenetics and Evolution 65:276–286.

Lee, Y.-H., W.-J. Lin, M.-T. Tsai, B. Lan, Y.-L. Chu, J.-I. Yang, and S.-J. Sun. 2026. Context-dependent indirect effects mediate ecological transitions between parasitism and mutualism. Communications Biology 9:706.

Milne, L. J., and M. Milne. 1976. The Social Behavior of Burying Beetles. Scientific American 235:84–89.

Nagel, L., J. M. Kendrick, and F. Bonier. 2025. Phoretic mites do not affect burying beetle reproductive success, despite eliminating fly competitors. Ecological Entomology 50:351–359.

Nehring, V., J. K. Müller, and N. Steinmetz. 2017. Phoretic Poecilochirus mites specialize on their burying beetle hosts. Ecology and Evolution 7:10743–10751.

Schwarz, H. H., and S. Koulianos. 1998. Review. When to leave the brood chamber? Routes of dispersal in mites associated with burying beetles. Experimental & Applied Acarology 22:621–631.

Smith, R. (n.d.). Effect of larval body size on overwinter survival and emerging adult size in the burying beetle, Nicrophorus investigator. https://cdnsciencepub.com/doi/epdf/10.1139/z02-151.

Smith, R. J., A. Hines, S. Richmond, M. Merrick, A. Drew, and R. Fargo. 2000. Altitudinal Variation in Body Size and Population Density of Nicrophorus investigator (Coleoptera: Silphidae). Environmental Entomology 29:290–298.

Smith, R., and M. Merrick. 2001. Resource availability and population dynamics of Nicrophorus investigator, an obligate carrion breeder. Ecological Entomology 26:173–180.

The-dispersal-behaviour-of-the-phoretic-mite-Poecilochirus-carabi-Mesostigmata-Parasitidae-adaptation-to-the-breeding-biology-of-its-carrier-Necrophorus-vespilloides-Coleoptera-Silphidae.pdf. (n.d.).

Wilson, D. S., and W. G. Knollenberg. 1987. Adaptive indirect effects: the fitness of burying beetles with and without their phoretic mites. Evolutionary Ecology 1:139–159.