



A kleptoparasitic beetle larva (*Hymenorus dorsalis*) exploits vertical division of labor in nests of the Florida harvester ant, *Pogonomyrmex badius*

Christina L. Kwapich¹ · Lauren Ross¹ · Isabel Zayas¹ · M. Andrew Johnston²

Received: 16 August 2024 / Revised: 10 December 2024 / Accepted: 12 December 2024
© International Union for the Study of Social Insects (IUSSI) 2024

Abstract

Pogonomyrmex badius workers divide transport tasks across subterranean nest space. After foragers deposit seeds and insects in shallow entrance chambers, younger workers transport these items downward to storage and brood chambers. We found that larvae of the beetle *Hymenorus dorsalis* exploit this temporary caching behavior, and intercept nearly all incoming insect prey before it is delivered to ant larvae. In field colonies, ~84% of beetle larvae consumed insects collected by host foragers. When beetles were absent, host seed consumption remained the same, but 31% more ant larvae fed on insects. In the lab, *H. dorsalis* readily fed on host brood, dead insects, seed-pulp, and seed coats. However, they could not open the smallest whole seeds collected by host ants. *Pogonomyrmex badius* nests in our study ranged from 40 to 270 cm deep, with 124 to 9,596 workers, and 0–31 beetles. Across populations, ~18 to 63% of nests were parasitized ($n = 97$ excavations, 219 beetles), and ~95% of *H. dorsalis* recovered were larvae. Beetle abundance was not correlated with worker number, but immature colonies were more likely to host beetles at one site. Colonies with <2000 workers also stored relatively more brood in the top 20 cm top of their nests, where beetle larvae co-occurred (avg. beetle depth = 7.60 cm). Smaller colonies may be more susceptible to brood predation by *H. dorsalis* and incur a greater per capita cost when parasitized. Alternatively, parasitized colonies may be smaller because of the high nutritional costs imposed by *H. dorsalis* larvae.

Keywords Tenebrionidae · Alleculinae · Demography · Kleptoparasitism · Nest architecture · Myrmecophile · Brood predator

Introduction

In ant nests, a strict identification and discrimination system acts as a filter that prevents foreign individuals from entering. Nevertheless, a considerable assortment of animals, called myrmecophiles, have evolved mechanisms to overcome ant defenses and invade ant nests. Some entice ants to carry them inside their nests with unique adoption and appeasement compounds, while others may attach themselves to incoming ant foragers, rely on brute force, mimic host recognition odors, or slip inside unnoticed due to size

or chemical insignificance. Once inside, parasitic myrmecophiles exploit the social food flow system of their hosts by consuming stored resources, eating host brood, or by soliciting regurgitated food directly from the mouths of the ants (Hölldobler and Kwapich 2022; Delgado and Palma 2024). Some parasitic myrmecophiles are able to penetrate into the deepest portions of nests, where they deposit their offspring directly into host nurseries. While many myrmecophiles become fully integrated into ant societies, others are ‘persecuted guests’ that must continuously and vigilantly avoid detection and attacks by their hosts (Parmentier et al. 2016).

Much like a coral reef, whole communities of interacting myrmecophiles operate within and around subterranean ant nests (Ivens et al. 2016; Parmentier and Claus 2019; Parmentier et al. 2020a, b; Kwapich 2021; Parmentier et al. 2021). Nests can extend several meters below the ground surface, and are organized into functional spaces such as brood chambers, food depots and granaries, soil backfill chambers, fungus gardens, compartments for mutualistic

✉ Christina L. Kwapich
Christina.Kwapich@ucf.edu

¹ Department of Biology, University of Central Florida, Orlando, FL 32816, USA

² Department of Entomology, Purdue University, West Lafayette, IN 47904, USA

associates, chambers for repletes, trash chambers, resin stockpiles, toilets, morgues, warming chambers, foraging and travel tunnels, airflow shafts, and more (Czaczkes et al. 2015; Parmentier et al. 2020a, b; Hölldobler and Kwapich 2022). How myrmecophiles partition meters-upon-meters of three-dimensional nest space in the field, what they eat, and how they interact with soil and natural architectural features remain central but overlooked details of myrmecophile biology that are rarely reported (Parmentier et al. 2023; Delgado and Palma 2024; Parmentier and Braem 2024; Parmentier et al. 2024). Most studies of myrmecophile behavior take place in two-dimensional lab observation nests oriented in a horizontal plane and do not address how myrmecophiles exploit host division of labor across vertical nest space. In addition to the challenges of directly observing myrmecophiles in natural soil nests, sporadic excavations rarely intersect the full demographic spread of ant host populations, making it difficult to assess the costs that myrmecophiles exact on ant colonies of different sizes in the field, or to calculate the likelihood of myrmecophile occurrence across host colony ontogeny. This problem is further exacerbated by the difficulty of excavating complete colonies in rocky and clay soils, and the rarity of many myrmecophile species, which may inhabit just a small portion of host nests in a population.

The associates of surface-dwelling army ants are better-cataloged and better-understood than those of soil dwelling ants, perhaps because their spatial relationships with hosts can be observed directly, and various life stages can be counted and collected (Rettenmeyer et al. 2011; von Beeren et al. 2016a,b; von Beeren and Tishechkin 2017). To uncover the full diversity of parasitic myrmecophiles interacting with ground-nesting ants, it is necessary to make numerous, chamber-by-chamber nest excavations, in age-structured populations, across multiple seasons. A comparison of host productivity in the presence and absence of parasitic myrmecophiles can reveal the demographic costs of hosting them, and the relative risk of infection for colonies of different sizes and life stages. While it is still not possible to directly observe myrmecophiles under many meters of soil, behavioral studies in the field nests can be approached using indirect methods, such as the introduction of dyed foods prior to excavation, or through marking host workers performing spatially partitioned tasks, which may intersect with the locations of myrmecophiles deeper within or around nests (Kwapich et al. 2017; Kwapich and Hölldobler 2019).

The Florida harvester ant, *Pogonomyrmex badius*, is a dimorphic seed harvesting ant found in open sandy soils located in sandhills and longleaf pine forests in the southern United States of America, East of the Mississippi River. The soft, quartz sand of the Southeastern Coastal Plain makes scientific research on *P. badius* feasible, as it is possible for one person to hand-excavate a mature colony, or several

immature colonies, in one day. Colonies produce a single, spiraling nest that can extend more than two and a half meters below the surface (Tschinkel 2004). Within nests, workers are vertically stratified by developmental age and labor role; with foragers at the top, and the greatest proportion of nurses, brood, the queen and newly eclosed callow workers at the bottom. Proportional allocation to foraging follows a strict, seasonal pattern that is conserved across years. Approximately 3.70% of foragers die per day, and each forager supports an average of 1.64 larvae, regardless of colony size or date during the active season (Tschinkel 2001; Kwapich and Tschinkel 2013, 2016). Colonies reach sexual maturity with 700 workers and attain a maximum size of approximately 11,000 workers, with a lifespan that may exceed 30 years (Smith and Tschinkel 2006; Tschinkel 2017).

Pogonomyrmex badius harvest seeds from the soil, but also rely heavily on scavenged insects. The trophic position of *P. badius* is intermediate between that of an insectivore and herbivore. Insect protein is fed preferentially to alate gynes and large-headed major workers, which have a higher $d^{15}N$ than minor workers (Smith and Suarez 2010). Large colonies store more than 300,000 individual seeds in granary chambers 40 cm to 100 cm below ground (Tschinkel 1999; Tschinkel and Kwapich 2016). Seeds less than 1.4 mm in width can be rapidly processed by the ants and fed to larvae at the bottom of the nest (Martyn et al. 2023). However, ants cannot open or mechanically process seeds > 1.40 mm in diameter, and instead wait for large seeds to germinate in storage chambers before gaining access. While a single large seed may have the nutritional value of 15 smaller seeds, the payoff for collecting large seeds is delayed, as germination may occur many months or even years after harvesting (Tschinkel and Kwapich 2016). When colonies are experimentally prevented from foraging, ant larvae die or are cannibalized (Smith 2007). This is likely because 70% of stored seeds are too large to open and are not accessible despite increased demand (Tschinkel and Kwapich 2016). Thus, the role of immediately available food items, like small seeds and scavenged insects, is of paramount importance to the survival of *P. badius* larvae. Scavenged insects, in particular, are essential for colony fitness, because of the disproportionate amount of protein needed to produce large gynes (Smith and Suarez 2010).

Pogonomyrmex badius nests may exceed depths of 270 cm, but incoming foragers only deposit seeds and scavenged insects in shallow entrance chambers, no deeper than 12 cm below the surface (Kwapich and Tschinkel 2013). These items are later carried downward to waiting larvae or to storage chambers by a different group of younger workers, called transfer workers (Tschinkel et al. 2015; Tschinkel and Hanley 2017). The vertical progress of items toward storage chambers can be tracked by presenting foragers with fluorescently

marked seeds at different intervals prior to hand excavating a nest. Marked seeds harvested by ants 15 min prior to excavation only appear in the top 5 cm of nest space. Ninety min after they are collected by foragers, most seeds remain in the top 20 cm. Marked seeds are most abundant in storage chambers in the middle or bottom half of nests between 4 and 48 h after harvesting (Tschinkel et al. 2015). Just as seeds are moved downward sequentially, ants carry excavated sand and trash to the surface in a series of steps (Rink et al. 2013; Tschinkel et al. 2015). In effect, all incoming food and outgoing debris is temporarily cached in the uppermost chambers of every *P. badius* nest. More rarely, ant larvae and pupae appear in near-surface chambers on warm mornings. Thus, brood can also briefly reside at the top of the nest alongside incoming food and outgoing debris. This confluence of transient food, trash, and brood in the uppermost chambers of nests presents an opportunity that myrmecophiles could exploit.

Dozens of *Pogonomyrmex badius* colonies were fully excavated, and censused during our studies of ant forager allocation, demography, feeding, resource use, colony founding, and nest architecture between 2009 and 2014 (Kwapich and Tschinkel 2013; Tschinkel 2013; Tschinkel et al. 2015; Kwapich and Tschinkel 2016; Tschinkel and Kwapich 2016). During excavations, we found that the uppermost chambers of nests were often perforated with like-sized holes, loosely capped with soil. Beneath these holes were shallow tunnels, each occupied by a single larva, pupa, or teneral adult beetle belonging to the genus *Hymenorus* (family: Tenebrionidae, subfamily: Alleculinae). Although adult specimens of the beetle have been described, to our knowledge, details of the association between *P. badius* and *Hymenorus* sp. larvae have not previously been reported (but see Hölldobler and Kwapich 2022). The discovery of beetles during nest excavations was happenstance, but they were common enough that we could compare their occurrence across colonies of different sizes, record their spatial relationships with host demographic groups and behavioral castes, and map their location within host nest architecture, across multiple seasons, years, and populations. We determined the nature of the relationship between the beetles and their host ants by measuring feeding preferences, and the numbers of ant host larvae and beetle larvae that consumed portions of dyed food offered to field nests. Our study considers the costs of hosting *Hymenorus* sp. larvae for *P. badius* colonies of different sizes and describes how beetle larvae fit into the spatial and social world of the Florida harvester ant, alongside its many other guests.

Methods

Whole colony excavations

From 2009 to 2014, 66 *Pogonomyrmex badius* colonies were excavated in two neighboring sandhills in Florida's Apalachicola National Forest south of Tallahassee (30.35° N, –84.41° W), where more than 400 individually marked colonies occurred alongside the ants *Solenopsis geminata*, *Trachymyrmex septentrionalis*, *Forelius pruinosus*, *Pheidole adrianoi*, *Dorymyrmex bureni*, *Aphaenogaster floridae*, *Camponotus socius*, *Formica archboldi*, *Formica dolosa*, *Cyphomyrmex rimosus*, *Odontomachus brunneus*, and numerous less common species (Deyrup 2016). The site, known as Ant Heaven, was characterized by an over story of 50-year-old long leaf pine (*Pinus palustris*), a mid-story of turkey oak (*Quercus laevis*), occasional sand pines (*Pinus clausa*) and a ground covering of dwarf huckleberry (*Gaylussacia* spp.), prickly pear (*Opuntia* spp.), beard grass (*Andropogon* spp.), gopher apple (*Licania michauxii*), and catbrier (*Smilax* spp.). An additional 22 founding nests with queens, but no workers, were excavated 14, 35, or 45 days after mating flights in 2012. These nests did not include myrmecophiles and were excluded from analysis.

To make complete nest excavations, a large pit was hand excavated adjacent to each focal nest in the early morning before foraging workers departed. During excavations, subterranean chambers were revealed by making thin, horizontal slices into the nest area with a brick trowel. Exposed myrmecophiles, ants, seeds, and host brood were removed from their chambers by mouth aspirator and a Dewalt D500 vacuum cleaner, then sorted into separate boxes by depth and/or 20-cm increments and censused in the laboratory. Complete excavations yielded beetle larvae, pupae, and adults belonging to the genus *Hymenorus* ($n = 128$ beetles, 12 nests). Most nests also contained ant workers that were previously captured while foraging, marked with fluorescent ink (Gans brand), and released two to nine days prior to excavation. The number of marked foragers recovered inside nests, and their proportion at each depth was recorded for 48 nests during the active season (see Kwapich and Tschinkel 2013).

To determine if beetles were truly associated with the *P. badius* nests or occurred independently in unassociated soil, we also searched for beetles in the human-sized pits made during nest excavations, as well as fifteen 10 cm × 10 cm × 10 cm pits at distance of 10 cm, 50 cm and 100 cm from the margin of five *P. badius* nest mounds. Arrays were placed around 2 colonies with beetles, and 3 colonies without beetles, following colony excavations in June of 2014. To describe the thermal environment of host

nests, temperature-logging ibuttons were buried at Ant Heaven, and set to record temperature every 1.5 h from June 19th–October 10th, 2012, for a total of 16 temperature measures a day and 1,824 total recordings per logger. Loggers were placed in PVC capsules, strung together on a wire, and buried at depths of 5, 10, 20, 40, 80 and 160 cm in an open, unshaded area.

Targeted collecting

Based on the complete excavations described above, we determined that beetle larvae were always restricted to the upper-most chambers of *P. badius* nests. To obtain additional beetle specimens, we conducted targeted sampling by partially excavating colonies at additional sites in Orange County, Florida, USA, between March and July of 2024. Sites were located within longleaf pine dominated mesic flatwoods and scrubby flatwoods at the University of Central Florida Arboretum (UCF, 28.60° N, −81.20° W), and along a roadside near mesic flatwoods East of Orlando (28.61° N, −81.03° W). After systematically searching sandy open areas within the University of Central Florida's 400+ acre arboretum natural lands, 73 *P. badius* colonies were identified and mapped. We partially excavated 30 colonies by digging 15 cm deep pits that extended 1–4 cm past the margin of each colony's nest disc. Prior to excavation, we also measured the nest disc of each colony, which has been established as proxy for colony size and corresponding nest volume (Tschinkel 1998; Tschinkel 2017). Host colony location, nest disc diameter, and beetle number were recorded using the program ArcGIS Field Maps (Fig. 1).

A total of 68 *Hymenorus* larvae, pupae, and adults were recovered from 19 *P. badius* nests at UCF, and an additional 23 larvae and pupae were collected from one roadside colony East of Orlando, FL. All beetles were taken back to the lab for rearing and identification. In the laboratory, beetle larvae were used in food preference experiments (described below) or kept in damp sand and offered fruit flies and crushed seeds until they pupated. Like some other tenebrionids, they were reluctant to pupate when kept together with other beetle larvae (Tschinkel and Willson 1971).

Identification of beetles

Beetle identity was confirmed using a combination of published literature (Fall 1931; Bousquet et al. 2018), comparison with both type specimens via images (Museum of Comparative Zoology, Harvard University, Cambridge, MA, USA), and direct examination (United States National Museum of Natural History, Washington, D.C., USA and Purdue Entomological Research Collection, Purdue University, West Lafayette, IN, USA). Dissections were performed by softening adult beetles in warm distilled water, removing the abdomen and placing it in hot 10% potassium hydroxide until soft tissues were macerated and then transferred into acetic acid to neutralize the reaction. Images were taken using a Canon RP digital camera connected to a Laowa 25mm Ultramicro lens. Multiple images from subsequent focal planes were captured and stacked together using the Zerene Stacker software (ZereneSystems.com) and edited in Adobe Photoshop to create the final figures. Adults, pupae, and larvae from both Tallahassee (AH) and Orlando (UCF)

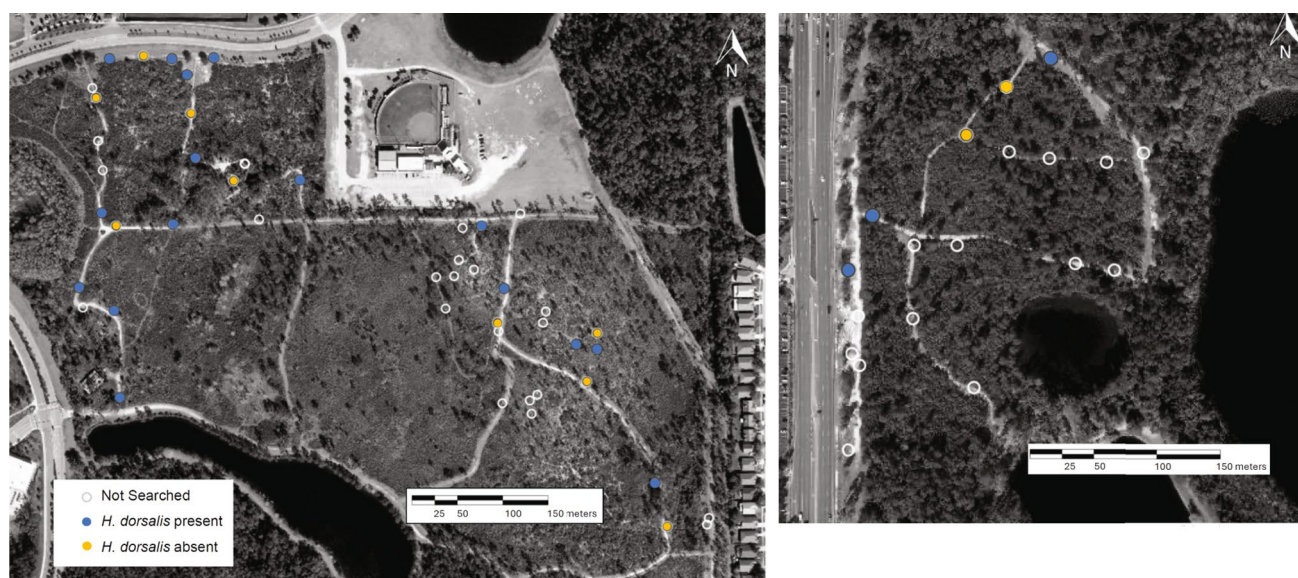


Fig. 1 A map of *Pogonomyrmex badius* colonies from the UCF Campus Arboretum, east of the T.R. Newman Nature Pavillion (left) and west of Lake Claire (right). The presence (blue) and absence (yellow) of *Hymenorus dorsalis* is indicated by dot color

sites were retained as vouchers, and deposited in the personal collections of M. Andrew Johnston (Lafayette, IN, USA), Christina L. Kwapich (Orlando, FL, USA), and M. Andrew Johnston (Lafayette, IN, USA), as well as the University of Central Florida Arthropod Collection (Orlando, FL, USA), and the Purdue Entomological Research Collection (West Lafayette, IN, USA).

Beetle diet and location in the field nests

In a previous study, we (CLK) determined that *P. badius* foragers deposit seeds in shallow nest chambers. These seeds are later moved downward over hours and days by a different group of ants (Tschinkel et al. 2015). Unlike whole seeds, the treatment of scavenged insects and processed seeds in *P. badius* nests has not been reported. To determine how insect prey moves through the nest and who consumes it, we offered crushed seeds and insects to 7, immature field colonies at Ant Heaven, then excavated them after 24 h. Colonies ranged in size from 124 to 770 workers, and 4 nests contained a total of 57 *H. dorsalis*. Six colonies were sampled during the peak of annual activity in June of 2014, and June of 2012. The 7th colony was sampled in February of 2012, when colonies were not active beyond the nest disc, and lacked brood. By checking both beetle and ant larva guts for dye, we were able to determine if beetles intercepted food before it was delivered to host larvae. It should be noted that *Pogonomyrmex* species do not perform adult-to-adult trophallaxis, so any evidence of feeding by associated beetles was accomplished by direct consumption.

Twenty-four hours before excavation, field colonies were offered (A) insect protein consisting of mashed termites (*Reticulitermes flavipes*) and tabanid flies (*Diachlorus ferrugatus*) ($n=3$ colonies), or (B) a mixture of crushed seeds harvested from chambers in *P. badius* nests ($n=1$ colony), or (C) a buffet of both mashed insects and crushed seeds ($n=3$ colonies). Prior to offering them to colonies, equal portions of insects and/or crushed seeds (by volume) were packed into separate, 5 ml weigh boats, then soaked in either methylene blue dye or fluorescent pink rhodamine B dye for 30 min. The food items were then dumped on to a paper towel, washed clean with deionized water, and dried on the hood of a car. The dry, dyed seed pulp and/or insects were then offered to colonies in weigh-boats placed on the margin of each focal nest mound. All items were readily collected by the ants and taken inside their subterranean nests within the hour that they were presented ($n=7$ fed colonies, 4 with beetle larvae).

One day after dyed food items were offered to colonies, each nest was hand-excavated, and the guts of recovered beetle and ant larvae were checked for the presence of dye by visual examination (under standard room and UV lights), or dissection when no dye was obvious through the yellowish

cuticle ($n=57$ beetle larvae). When present in ant larvae, dye was always visible through the more translucent cuticle (under standard room and UV light), and its presence or absence could be scored in the gut and meconium without dissection ($n=755$ ant larvae). Methylene blue and rhodamine B were chosen to dye the crushed seeds and insects because they readily bind to those food items and turn the guts of both ant and beetle larvae bright blue or fluorescent pink, respectively (Fig. 2). When both dyes are present together, the blue dye is visible to the naked eye, while the rhodamine B fluoresces pink under UV light. By varying which dye we used for each food type, we could be sure that neither dye made food sources unattractive or less likely to be consumed by the beetles.

Food preference assays in the lab

Whole seed consumption: To determine if beetle larvae were capable of opening and feeding on whole seeds, we placed 10 larvae in individual, 5-cm \times 4-cm plastic cylinders filled 20% with sieved, damp sand. After 12 h, we added 3 whole seeds belonging to each of three of the species that ants commonly collect and can mechanically process (1–1.40 mm wide): deer tongue grass (*Dichanthelium clandestinum*), hairy lens grass (*Paspalum setaceum*), and cottonweed (*Froelichia floridana*) (Tschinkel and Domínguez 2017). After 24 h, seeds were counted and inspected under a stereoscope to look for signs of damage or consumption. After the individual trials, the same 10 beetle larvae were combined in a single plastic sandwich box, half-filled with sand, and offered a total of 15 whole seeds, 5 of each plant species. As before, after 24 h, seeds were counted and inspected under a stereoscope to look for signs of damage or consumption. Because larvae did not consume dye in these trials (and did not consume the seeds), they were recombined with our stock of *H. dorsalis* larvae and retained in the lab to be used in subsequent choice tests.

Insect and crushed seed consumption: In the field, beetle larvae showed a strong preference for one of the food items offered. To further investigate this result, 22 beetle larvae from a single colony were brought to the lab and separated into individual 5-cm \times 4-cm cylinders, filled 20% with sieved, damp sand. After approximately 12 h, each cylinder was provisioned with two freeze-killed and crushed termites and two fruit flies soaked in dye ($n=12$), or an equal volume of crushed seeds belonging to 3 species commonly collected by *P. badius*: *D. clandestinum*, *P. setaceum*, and *F. floridana* ($n=10$). We alternated the dyes used for seeds and insects such that each beetle larva received either insects dyed with rhodamine B dye ($n=6$), insects dyed with methylene blue ($n=6$), crushed seeds dyed with rhodamine B dye ($n=5$), or crushed seeds dyed with methylene blue ($n=5$). After 24 h,



Fig. 2 The left panel shows *Hymenorus dorsalis* larvae that have consumed insects dyed with fluorescent rhodamine B, under standard (top), and UV light (bottom). The right panel shows an *H. dorsalis* larva that consumed insects dyed with methylene blue

we scored consumption by visual assessment of dye in the guts of beetle larvae under standard and UV light.

Brood consumption: During excavations, we occasionally found healthy host brood in the uppermost chambers of host nests where beetles also occur. To determine if beetle larvae would consume host brood given the opportunity, we separated beetle larvae from 3 source colonies into individual 5 cm × 4 cm cylinders, filled 20% with sieved, damp sand. After approximately 12 h, each cylinder was provisioned with one live *P. badius* pupa or pharate pupa ($n = 11$). To control for the effects of isolating pupae from adult ants, we also placed 10 ant pupae and 3 pharate pupae in separate cylinders with damp sand and inspected them for any signs of damage or rot that might have occurred due to handling or exposure after 24 h. We scored consumption by assessing presence, absence, or damage to each live ant pupa offered.

Brood vs. insect choice test: To determine if beetle larvae would choose to eat host brood preferentially, we next presented a different group of beetle larvae with dead insects and live *P. badius* pupae simultaneously. Beetle larvae were placed into separate cylinders, filled 20% with sieved, damp sand ($n = 14$). After 12 h, we added one living *P. badius* pupa, and an equivalent volume of dead insects crushed and soaked in methylene blue on the surface of the sand (2 fruit flies, 2 termites, $n = 14$). We scored consumption by visual assessment of dye in the guts of beetle larvae after 24 h under standard and UV light, and by the presence, absence, or damage to the live ant pupa offered. During the choice assay, two of the beetle larvae molted, and were given an

extra day with food items. Of these, one made a choice, while the other did not feed. For unknown reasons a 2nd beetle larva also did not feed, reducing our sample to 12 trials.

Analysis

We collected 219 beetles from 32 unique colonies during our study. To determine if colony size was a good predictor of the presence or absence of *H. dorsalis*, we used binomial Generalized Linear Models with a logit link function to model the effects of nest disc diameter at the UCF Arboretum (a proxy for worker number, GLM1), or total worker number at Ant Heaven (GLM2), on the presence or absence of beetles in nests. Next, we used Poisson GLMs with log link functions to model the effects of nest disc diameter (GLM3), or worker number (GLM4), on the number of *H. dorsalis* (counts) per nest.

Adult *H. dorsalis* can fly and must seek food outside of ant nests after eclosing, so we didn't have an a priori reason to think that colonies neighboring parasitized nests would be more likely to host beetles based on proximity and direct dispersal. Together with the scarcity of parasitized nests and different distances between nests, we were unable to include host spatial relationships in our models. However, we did compare maximum nest disc diameter between 19 colonies with *H. dorsalis*, and 11 colonies without *H. dorsalis* in the UCF Arboretum (student's *t* test, independent by groups), and the proportion of mature and immature nests parasitized at Ant Heaven, based on worker counts made

during complete excavations. *Pogonomyrmex badius* colonies reach sexual maturity with 700 workers, but we scored colonies of up to 901 workers as “immature” to account for worker production that might occur after mating flights each June. Mature colonies in our sample had between 1,075 and 9,656 workers.

Finally, we used linear regressions to compare colony demographic and nest attributes at Ant Heaven. Lab trials examining preference for insects vs. live ant pupae were assessed using a Chi-squared goodness of fit test, which assumed that each food item had an equal likelihood of being eaten alone or together with the other item (33.33%). We excluded two beetles that did not eat ($n = 12$ trials included). All analyses, data management, and graphical representations of data were made with Statistica version 13.5 integrated with ‘R’ version 4.3.1. Colony and nest attribute data are publicly available (<https://doi.org/10.6084/m9.figshare.27937791.v1>).

Results

Myrmecophile species identity

Diagnosis

Hymenorus dorsalis Schwarz, 1878. Adult 5–7 mm, bicolored dorsally, with head, prothorax, elytral base and venter rufus, apical two thirds of elytra piceus, anterior femora simple, not

expanded, antennomere 3 subequal in length to 4, antennomeres 6–8 more than twice as long as wide. Male with eyes somewhat narrowly separated, the distance between the eyes 1/5 to 1/3 the width of a single eye, tarsal claws of front legs each with 12–15 pectinations beneath.

Description of male Terminalia

Sternite VIII apparently forming two separate, symmetrical sclerotized plates; each plate longer than ventrite VII, with anterior strut bearing mesal recurved process anteapically, broadly triangularly exiting mesally at roughly mid-length, posterior lobe strongly produced, evenly arcuate laterally, and bearing a tuft of 5–10 setae at apex. Spicule plates (sensu Doyen 1966, sternite IX sensu Campbell 1971) connected anteriorly as flattened processes, forming cup-shaped capsule posteriorly, each spicule plate with oblique lateral sclerotized baculum near widest portion of capsule (Fig. 3) (Doyen 1966; Campbell 1971).

Remarks

The typical form of this bicolored species of *Hymenorus* is fairly distinctive among its congeners. The reddish head, pronotum, and elytral humeri contrasted with the piceus apical two thirds of the elytra is a dorsal habitus only shared with *H. semirufus* Fall, 1931 and *H. dichrous* Blatchley, 1919. *Hymenorus semirufus* is a smaller species with a narrower body, recorded as 4.2–5.25 mm in length (5.25–7 mm

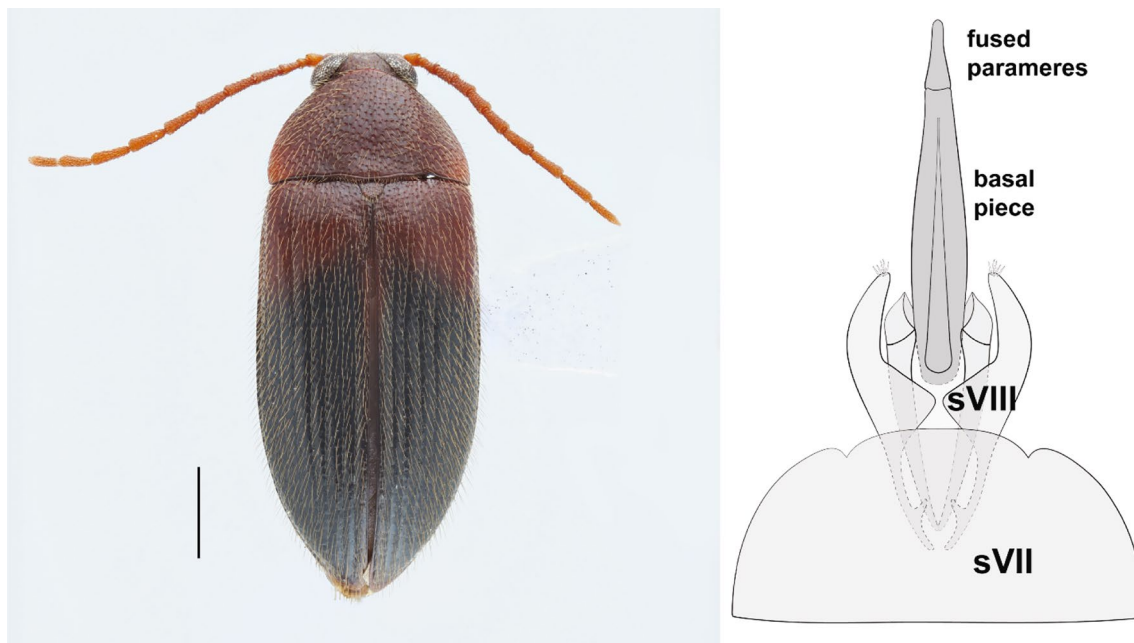


Fig. 3 Adult male *H. dorsalis* with 1 mm scale bar (left). Drawing of male *H. dorsalis* terminalia (right)

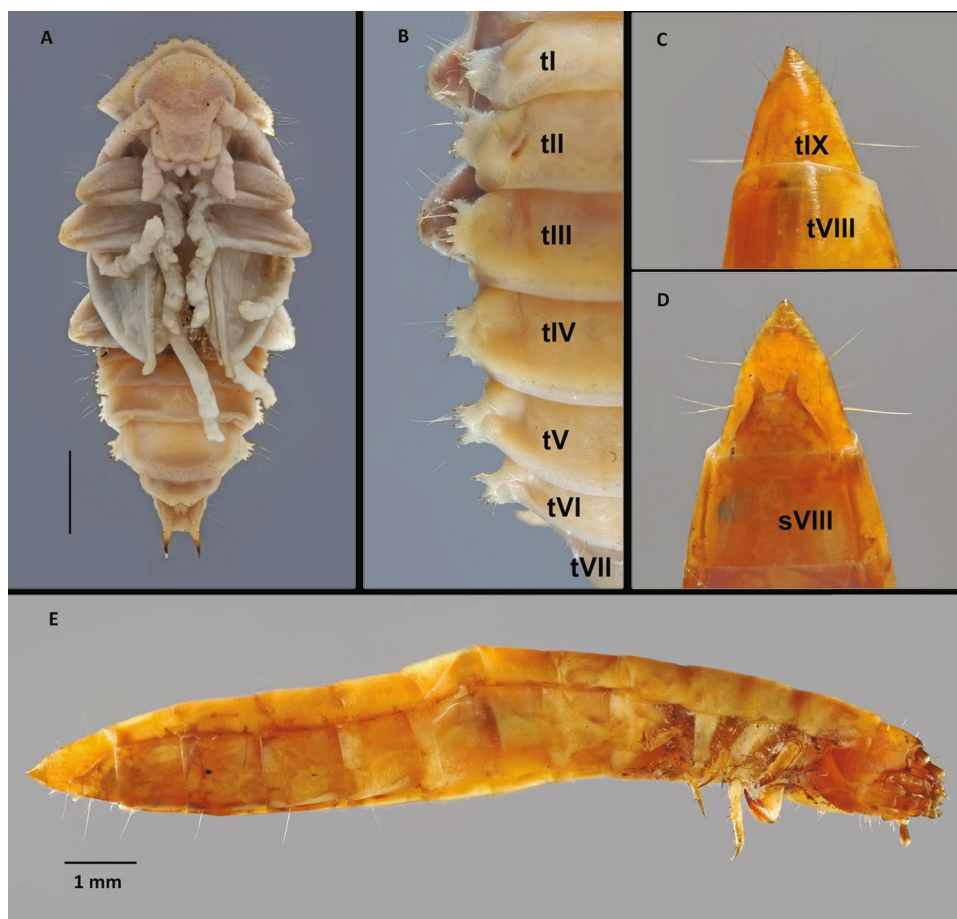
for *H. dorsalis*) and the male has 23–24 teeth on each tarsal claw of the front legs (12–15 in *H. dorsalis*) and has its eyes separated by 1/4 to 1/2 their own width (1/5 to 1/3 in *H. dorsalis*). *Hymenorus dichrous* is similar in size and dorsal coloration to *H. dorsalis* but differs by having eyes in the male separated by 5/7 to 1 × their own width (1/5 to 1/3 in *H. dorsalis*) and the antennae are shorter and more robust with the third antennomere short and closer in size to the second antennomere (third longer and similar in length to the fourth in *H. dorsalis*).

Species identifications for the specimens used in this study proved very difficult because initial samples consisted of teneral adults excavated from pupal cells, or preserved shortly after eclosing from pupae from Ant Heaven (Tallahassee, FL). In these specimens, the apical two thirds of the elytra were rufus and either very similar or slightly lighter in color to the rest of the body, thereby obscuring the identification as the distinctly bicolored *H. dorsalis*. However, subsequent bicolored specimens from Orlando, FL were reared out, which had longer to mature and harden. Fall (1931) mentioned having seen one specimen of *H. dorsalis* from North Carolina which was “nearly unicolorous” which may support our observations here.

Other than the dorsal coloration, all characters were shared among all specimens examined, including the male terminalia.

The immature stages (Fig. 4) are similar in nature to the general descriptions made by earlier workers. The mature larva (Fig. 4. Plate C-E) measures 12–13 mm in length and agrees with the description of *H. obscurus* Say by Wickham (1896) and the illustrations of *Hymenorus* sp. by Böving and Craighead (1931). Abdominal segment nine (Fig. 4, Plate C) is conical in shape and lacks urogomphi, as in the rest of the subfamily. Abdominal segment X (Fig. 4, Plate D) is modified into a pygopod which terminates in two tubular projections which seem to be hidden at rest underneath segment IX. The pupa is 6–7 mm in length and also agrees with earlier descriptions for the genus by Wickham (1876) and Steiner Jr (1995). The pupa (Fig. 4. Plate A) bears lateral gin traps (sclerotized processes which grind against each other as the pupa wiggles when disturbed and are presumed to defend from predators and parasites) on abdominal tergites I–VII which each possess 4–6 large, sclerotized spines laterally and a row of short spines on the anterior margin (Fig. 4. Plate B). These structures may help separate species within the genus.

Immature stages of *Hymenorus dorsalis*. Labels refer to abdominal tergites and sternites, scale bars = 1 mm. **A** pupa, ventral habitus; **B** pupa gin traps in dorsal view; **C** larva, abdominal apex, dorsal view; **D** same, ventral view; **E** mature larva, lateral habitus



Notes on behavior

Hymenorus dorsalis larvae exposed during nest excavations were mobbed by ants. The ants displayed aggressive behaviors including gaped mandibles, biting, and curled abdomens. In response, the larvae thrashed but were overwhelmed and killed quickly. When separated from soil, *H. dorsalis* larvae were active and fast moving. They traveled in both the forward and in reverse direction over large distances in smooth laboratory trays or dishes (Supp. Video 1). When offered sand, *H. dorsalis* larvae quickly burrowed in and made long tunnels with frequent connections to the surface.

Hymenorus dorsalis pupae were also very responsive and writhed and thrashed when touched (Supp. Video 2). It is assumed this is a defensive behavior shared with other tenebrionid beetles, where the sharpened points of the gin traps grind against each other to ward off would-be predators (Steiner Jr 1995), though it may also attract ants purposefully. As we excavated the top of a nest in spring 2024, disturbed ants began evacuating with larvae and alate pupae that were warming at the top of the nest. One *P. badius* worker was observed carrying an intact, *H. dorsalis* pupa during the evacuation of ant sexual brood (Supp. Video 3).

Occurrence and location within host nests

In our study population, *Pogonomyrmex badius* nests were between 40- and 270-cm deep, with an average depth of 132 cm (SD=53) and a median depth of 134 cm ($n=62$ nests measured). Complete nest excavations at Ant Heaven revealed that the uppermost chamber floors were sometimes dotted with small holes, loosely capped with soil. Beneath these holes were shallow tunnels, each occupied by a single larva, pupa, or teneral adult beetle. *Hymenorus dorsalis* occurred at an average depth of 7.60 cm (SD=8.70), most commonly between 1 and 10 cm ($n=126$), and in one nest, near a chamber at 28 cm ($n=2$ larvae). Beetles were never found deeper in host nests, in surrounding soil, or independent of *P. badius* colonies. From mid-June and to mid-October 2012, temperatures 5 cm below the surface averaged 27.50° C (SD=3.70) and up to 40° C. Temperatures 10 cm below the surface were less variable, at an average of 27.90° C (SD=2.80) and up to 37° C. Within these uppermost chambers, *H. dorsalis* experience the hottest and driest conditions across the vertical length of any *P. badius* nest and are subject to inundation by water during afternoon thunderstorms, which occur almost daily during Florida's subtropical summer.

Within the uppermost chambers of host nests, *H. dorsalis* lived alongside host foragers, and frequently with host larvae and pupae. An average of 92% (SD=20.50) of marked host foragers recovered within nests were found in the top 20 cm ($n=48$ nests, Max. 512 marked foragers). Based

on Lincoln-index mark-recapture data (see Kwapich and Tschinkel 2013), within parasitized nests, there were an estimated 8 to 255 total foragers per beetle, and between 5 and 271 competing host larva per beetle ($n=10$ colonies with both host brood and beetles). Although most brood were stored at the bottom of nests, 66.70% of colonies housed at least one ant larva or pupa in the top 20 cm (32 of 48 colonies). A median of 1.7% and up to 89% of ant larvae and pupae per colony were recovered in the top 20 cm of nests (avg. 12.10%, SD=20.80). When present, more than half of brood in the top 20 cm were larvae (avg. 66.90%, SD=27% larvae). Given their location in the uppermost chambers of host nests, all *H. dorsalis* would have access to freshly collected items deposited by incoming host foragers, and in more than half of nests sampled, they would also have access to between 1 and 544 individual host larvae and pupae.

Occurrence across sampling sites

Beetle occurrence with host colonies differed between field sites: 18.10% of nests at our Ant Heaven site contained *H. dorsalis*, compared to 63.30% nests at our more southerly, UCF Arboretum site. Parasitized colonies hosted between 1 and 31 individuals (avg.=6.84, SD=8.21; median=3). Most *H. dorsalis* recovered across sites were larvae (94.52%), followed by pupae (3.65%), and teneral adults (1.83%), suggesting that *P. badius* nests act as nurseries and pupation sites for the beetles, but that adults do not remain in nests after eclosing ($n=219$ beetles, 32 colonies). Often, larvae belonging to multiple size classes, and presumably instars, were present within the same nests. When teneral adults were found during excavations, a strong odor of quinones was detectable, and adults discharged their defensive secretions when handled, leaving a lasting odor on the skin. Overall, beetle larvae were present in nests excavated from February to November, pupae were collected from late April through late July, and adults occasionally from May–July.

At the UCF Arboretum, nest discs ranged from 21 to 75 cm in width (avg. 42.14 cm, SD=15.17; median=40 cm), representing colonies with <500 workers to >7,000 workers. Among the 30 colonies excavated in this population, nest disc diameter was a significant predictor of the presence of *H. dorsalis* (binomial GLM1, Wald=2.78, $p=0.036$). At the UCF Arboretum, colonies parasitized by beetles were on average slightly larger (average disc size 46.73 cm, SD=15.48) than colonies without beetles (average disc size 33.09 cm, SD=13.89) ($T_{28}=-2.41$, $p=0.022$). However, both mean values for disc diameter fall within the 'intermediate' size class defined by Tschinkel (2017) and correspond to sexually mature colonies, with average colony sizes of approximately 1,500 to 4,000 workers. The total number of *H. dorsalis* per nest did not correspond to nest disc diameter (UCF: Poisson GLM3, Wald=1.59, $p=0.21$, one outlier

removed, $n=29$), nor to the actual number of host workers in fully censused colonies with beetles at Ant Heaven ($r = -0.011$, Poisson GLM4, Wald = 0.20, $p = 0.65$, $n = 12$).

At Ant Heaven, censused colonies ranged in size from 124 to 9,596 workers, with an average of 1,946 (SD = 1,855) workers and a median size of 1,312 workers ($n = 66$). Of these, beetles were found in 12 colonies made up of between 407 and 3,950 workers. Colony size was not a significant predictor of *H. dorsalis* presence at Ant Heaven (GLM2, Wald = 1.25, $p = 0.26$), and an equal number of mature (6) and immature (6) colonies were parasitized. Unlike our UCF Arboretum site, a greater proportion of immature colonies at Ant Heaven hosted beetles. Just 14.6% of 41 mature colonies hosted *H. dorsalis*, while 24% of 25 immature (< 901 workers) did. Despite the presence of queens, brood, and foraged seeds, no beetles were recovered inside any of 22 founding nests excavated 14 to 45 days after mating flights in 2012. All founding nests lacked workers but contained both founding queens and brood.

Beetle diet in the field

Twenty-four h after offering dyed insect corpses and crushed seeds to *P. badius* field colonies, there was evidence of these items inside the guts of *H. dorsalis* larvae, ant larvae, and several ant callows (one nest). Of the 7 *P. badius* field colonies offered dyed food items, 6 nests contained ant larvae, and 4 nests contained a total of 57 *H. dorsalis* larvae. Of the 6 nests that received dyed insects, 4 contained *H. dorsalis* larvae. Together, 83.70% of beetle larvae collected from these field nests consumed dyed insects collected by host workers. Beetle larvae only consumed crushed seeds when insects were not offered simultaneously (8 of 14 beetle larvae, 1 winter colony, no host brood). In total, 77.2% of *H. dorsalis* larvae in field nests consumed either dyed insects or crushed seeds 24-h after food items were brought into the nest by ant foragers. Compared to *H. dorsalis*, ant larvae ate more seeds and more food types simultaneously. When colonies were offered dyed, crushed seeds, 59% of all ant larvae fed on seeds, and 14.5% had evidence of both seeds and insect prey in their guts at the same time. Uneaten, dyed insects were found 2 cm below the surface in a single, unparasitized nest with just 124 workers (total nest depth, 40 cm). Uneaten, dyed seed pulp was found in 4 colonies (2 parasitized), in chambers between 2 and 30 cm deep, including two seed-storage chambers.

Beetle diet in the lab

Hymenorus dorsalis larvae were not able to access whole seeds belonging to any of the three species offered, even though they rank among the smallest seeds collected by *P. badius* foragers and are easily opened by the ants ($n = 10$).

When the same beetles were placed together in a group of ten, they removed or damaged the loose outer layer of the seed coats of all deer tongue grass seeds offered (five of 15 seeds) but were still unable to open and access the interior of any whole seed. When presented separately, all *H. dorsalis* larvae consumed either dyed insects ($n = 12$), or crushed seeds ($n = 10$), and 90.90% consumed *P. badius* pupae or pharate pupae ($n = 11$). In some trials, all parts of the host pupa were consumed, but two beetles left pieces of hollowed pupal gasters and heads behind. The ten control pupae and three pharate pupae kept on damp sand without workers or beetles, remained intact and did not succumb to mold or damage from moisture or handling over the same 24-h period. This confirms that the damage to host brood observed in the presence of *H. dorsalis* larvae was caused by consumption. When dead insects and live ant pupae were offered to *H. dorsalis* simultaneously, 12 of 14 larvae ate one or both items, and two did not feed. Of those that fed, 16.7% (2) consumed both insects and live pupae, 58.3% (7) consumed insects only, and 25% (3) consumed only live ant pupae. Given equal access, and excluding larvae that did not feed, *H. dorsalis* were equally likely to consume dead insects and live ant pupae when offered simultaneously (X^2 (df = 2, $n = 12$) = 3.5, $p = 0.17$, expected = 4).

Cost of hosting beetle larvae

When present, beetle larvae consumed large quantities of scavenged insects at the expense of host larvae. For example, in one of our field nests 22 of 23 (96%) beetle larvae consumed dyed insects, while just 3 of 180 (1.6%) ant larvae present in the same nest did. In another field nest, 12 of 18 (67%) beetle larvae consumed dyed insects, while just 8 of 178 (4.5%) ant larvae did. In the absence of *H. dorsalis*, a greater proportion of ant larvae accessed scavenged insects. For example, 58 of 64 (91%) ant larvae in one field nest had evidence of dyed insect parts in their guts when beetle larvae were absent. On average, 37.2% of ant larvae accessed scavenged insects when beetles were absent, and just 6.30% of ant larvae accessed insect protein when beetles were present, a decrease of 31% ($n = 3$ colonies with, 3 without beetles). The disproportionate consumption of insect prey by beetle larvae did not seem to influence seed consumption by host ant larvae. In nests offered a combination of crushed seeds and insects, an average of 52% of ant larvae ate crushed seeds when beetles were absent (2 nests), and 56% consumed crushed seeds when beetles were present (1 nest).

There was a significant, positive relationship between worker number and nest depth, and between the worker number and the number of larvae and pupae per *P. badius* nests sampled in our study (Fig. 5). On average, colonies with fewer than 2,000 workers housed a greater portion

Pogonomyrmex badius colony and nest attributes

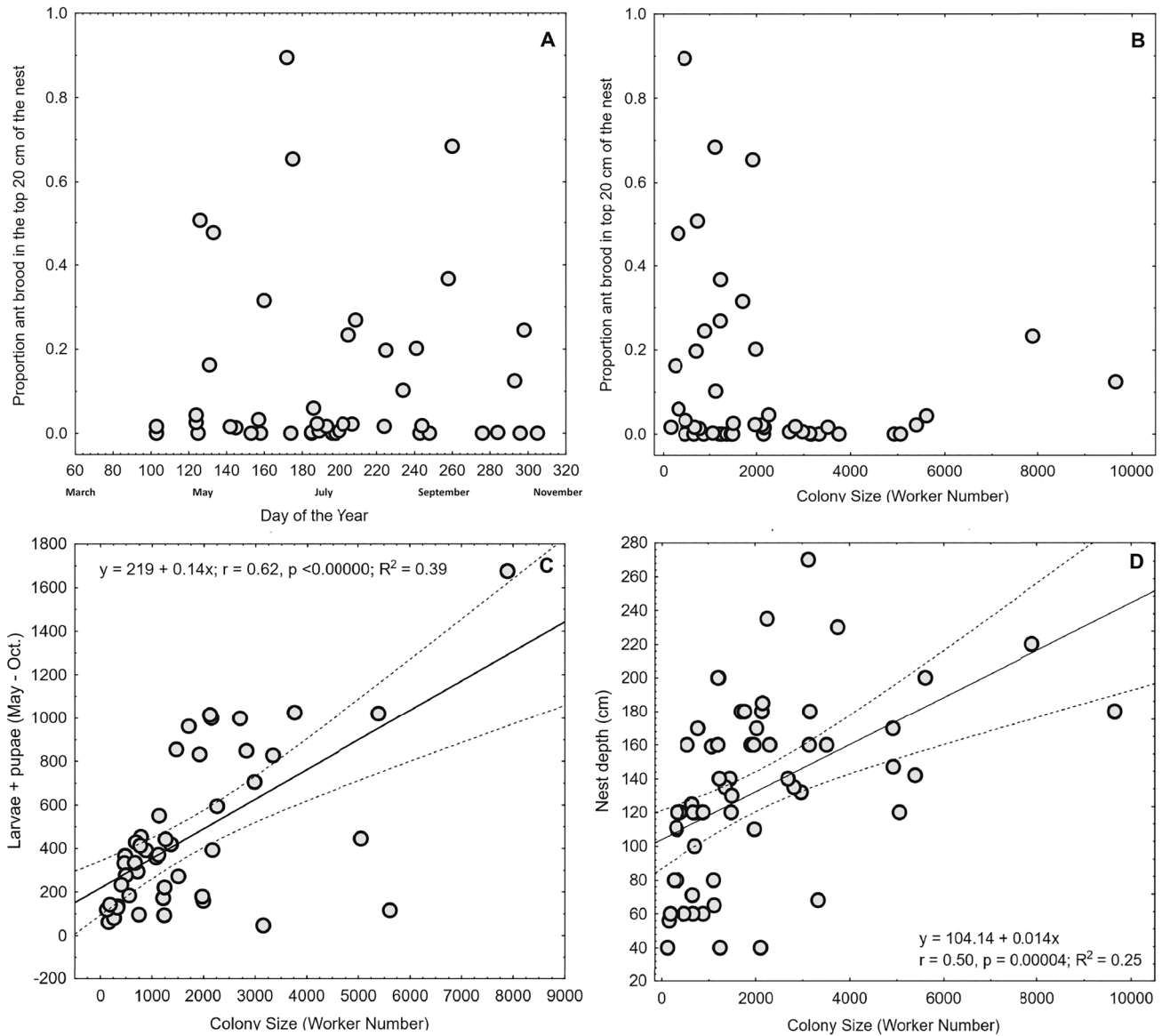


Fig. 5 **A** Throughout the year (March–Nov.), *P. badius* colonies housed varying proportions of brood in nest chambers <20 cm below the surface, where parasitic *H. dorsalis* are also found. **B** Colonies with fewer than 2,000 workers more frequently stored a higher portion of their brood in the uppermost nest chambers ($n=48$). **C** From

May to October, there was a positive relationship between colony size and the number of larvae and pupae per nest ($n=46$). **D** Nest depth was also positively correlated with colony size throughout the year (bands are 95% confidence intervals, $n=62$)

of their brood in the uppermost nest chambers (18%) than colonies with between 2,001 and 10,000 workers (2.9%) ($T_{46} = 2.63$, $p = 0.012$, $n = 48$ colonies). The tendency to store brood in superficial chambers may be attributable to the comparatively shorter nests excavated by small colonies (Fig. 5). In effect, *P. badius* brood in colonies with fewer than 2,000 ants are likely more susceptible to consumption by *H. dorsalis*, because of increased spatial overlap.

Discussion

Hymenorus dorsalis larvae are kleptoparasites and brood predators that exploit the tendency of *P. badius* foragers to temporarily cache food in the uppermost chambers of nests before it is shuttled downward by transport workers, across several meters of nest space. These beetles exploit a unique spatial and social niche within *P. badius* nests and societies. Although larvae are attacked by host ants when exposed, and do not appear to be tended by or socially integrated with

their hosts, *P. badius* nests serve as nurseries and pupation sites for *H. dorsalis*. Larvae are dietary generalists that preferentially consume scavenged insects and host brood when available, but also feed on processed seeds and seed coats opportunistically. They cannot open even the smallest whole seeds collected by host workers, and must act quickly to feed on incoming prey, as items are carried to storage chambers by transfer workers beginning 90 min collection (Tschinkel et al. 2015; Tschinkel and Kwapich 2016). Our findings suggest that *H. dorsalis* may steal the ‘lion’s share’ of incoming insects prey scavenged by *P. badius* foragers. The preferential consumption of foraged insects by *H. dorsalis* may have measurable fitness consequences for parasitized colonies, because developing alates require a greater proportion of insect protein than minor workers (Smith and Suarez 2010).

Hymenorus dorsalis larvae readily consume live ant brood in the lab, and frequently co-occur with piles of host brood in the uppermost chambers of field nests. Actual brood predation rates in the field are uncertain, as larvae and pupae may be well defended by host workers. Likewise, under natural circumstances, processed seeds are unlikely to appear within the upper nest regions occupied by *H. dorsalis*, and are predicted to be a less meaningful part of *H. dorsalis* diet than outbound seed chaff and waste. While host corpses would also be a likely food source if present, our previous work suggests that worker mortality is restricted almost entirely to the forager population, and that foragers die afield from extrinsic sources of mortality, rather than intrinsic ones (Kwapich and Tschinkel 2016). Likewise, nearly all *P. badius* workers marked in autumn survive the winter to begin work the following spring (Kwapich and Tschinkel 2013), and during nest excavation, dead workers were never found on nest discs or the interior chambers of *P. badius* nests.

Pogonomyrmex badius colonies begin foraging in March or April and produce brood from April to October, before entering a period of winter inactivity from October to March. Despite the lack of freshly foraged goods and host brood, *H. dorsalis* still appeared in winter nests, beneath shallow chambers. It is possible that these winter beetle larvae subsist on waste deposited in superficial nest chambers throughout the winter, or that cold temperatures decrease their metabolic demands. In either case, *H. dorsalis* within the top 10 cm of nests are subject to the most extreme high (40 °C) and low temperature across the annual cycle.

Several questions remain about *H. dorsalis* and its relationship with *P. badius*: What trophic position do kleptoparasitic larvae attain relative to omnivorous *P. badius* host workers and alates (Smith and Suarez 2010; Parmentier et al. 2024)? Do adult females oviposit inside host nests or in external chaff piles? If so, what cues allow them to orient toward host colonies, and what behavioral strategies allow them to overcome ant defenses during oviposition?

If females oviposit outside *P. badius* nests, how do beetle larvae enter or intercept ant nests undetected? We never encountered *H. dorsalis* eggs but found a mixture of beetle larvae and pupae in nests, including larvae belonging to different size classes. This suggests that multiple females oviposit in host colonies, or that differential feeding affects development rate within sibling cohorts.

Colony size and the cost of hosting *Hymenorus dorsalis*

At Ant Heaven, *H. dorsalis* were slightly more likely to appear in the nests of immature colonies than the largest, mature colonies. This may represent a difference in nest detectability and preference by *H. dorsalis*, or it could suggest that colonies with fewer workers are less vigilant and more susceptible to invasion. There is at least one more alternative: Over time, colonies with *H. dorsalis* may suffer demographic costs that could ultimately decrease the number of new workers produced. Colony size and age are not necessarily correlated in *Pogonomyrmex badius* populations (Tschinkel 2017), and the presence of costly parasites could be one reason why. In the lab, *H. dorsalis* larvae sometimes took several months to pupate, even when separated from other larvae at 26° C, and after consuming enormous quantities of fruit flies, crushed seeds, and host brood each week. The strict, isometric relationship between forager number and larva number across *P. badius* colonies of all sizes (Kwapich and Tschinkel 2013) is likely disrupted by the presence of rapacious *H. dorsalis* larvae.

Given the lower absolute number of foragers and incoming food in small *P. badius* nests, and the larger size and apparent appetites of the beetle larvae, the costs of hosting *H. dorsalis* may be substantial for smaller colonies. This is exacerbated by the tendency of smaller colonies to excavate relatively shallow nests, and to store a greater proportion of their larvae and pupae in the top 20 cm of nest space where beetles also occur. We did not find a difference in the number of *H. dorsalis* in colonies of different sizes, which could be due to earlier emergence and departures by adults resulting from a single clutch, or differences in the original number larvae. Although competition with host larvae is lower in small colonies (fewer host larvae), there are also fewer foragers bringing in resources, which could increase reliance on host brood as a food source. In turn, because small host colonies produce fewer total larvae and pupae than large colonies, each brood item lost represents a greater proportion of the immature population in the nest. The loss of 1 larva out of 10, for example, is more substantial than the loss of 1 larva out of 1,000.

The costs of hosting kleptoparasitic beetles is well known in other ant species. For example, larvae of the parasitic beetles *Lomechusa pubicollis* and *Lomechusoides strumosus*

(Staphylinidae: Aleocharinae) have been shown to receive significantly more food than host larvae (Hölldobler 1967; Hölldobler et al. 2018). Likewise, Wasmann (1920) found a strong correlation between aberrant worker morphology and a decline in the production of alates in colonies that house *L. strumosus*. Although the exact cause of the aberrant development of workers and decline in production of sexuals is not known, given that the beetles are diverting food from the colony, it is likely this phenomenon is caused by starvation of the ant larvae (Hölldobler and Kwapich 2017).

The genus *Hymenorus*

Little is known about the myrmecophilous habits of beetles in the genus *Hymenorus* (subfamily: Alleculinae), though adults are often found on flowers or in leaf litter. *Hymenorus inquilinus* Casey, 1891 adults are known generically from “ant nests” in California (Blaisdell 1892; Fall 1931) and adults of undetermined species of *Hymenorus* were collected from the refuse piles of *Atta mexicana* nests in Sonora, Mexico (MAJ personal observation), and the nest of a wood-nesting *Phiedole* species in Florida, USA (CLK personal observation). *Hymenorus* larvae have been collected in duff held within tree holes (W.E. Steiner, personal communication to MAJ).

Other members of the family Tenebrionidae are commonly associated with the trash and chaff piles amassed by seed harvesting ants in arid climates (Slobodchikoff 1979; McIntyre 1999). *Oochrotus unicolor*, for example, is specially adapted to life in *Messor barbarus* waste dumps, where it consumes discarded seeds and receives minimal aggression from host workers. *Oochrotus unicolor* occasionally accepts dead insect prey, but in contrast to *H. dorsalis*, does not feed on host brood when offered (Parmentier et al. 2020b). Most harvester-ant-associated tenebrionids consume discarded seed husks and vegetal matter in ant debris piles, though some consume host corpses, and at least one species (*Pimelia integra*) preys on live *M. bouvieri* workers. (Sánchez-Piñero and Gómez 1995). *Scaurus uncinus* is likewise unusual, because it does not consume seeds or plant matter associated with host nests. Instead, adults of both sexes enter *M. barbarus* nests using ‘brute force’ (often at the expense of their own appendages) to feed on ant eggs, larvae, pupae, as well as dead ants and insect prey cached by the ants. Females deposit eggs inside host nests, though both eggs and *S. uncinus* larvae are attack by ants when detected (Delgado and Palma 2024).

A central question in myrmecophile biology concerns how free-living arthropod lineages transitioned from the exterior of ant nests to the interior of ant nests over evolutionary time. It is possible that subterranean kleptoparasitism in *H. dorsalis* is a behavior derived from a more generalized, detritivore ancestral condition, whereby interactions

with external trash piles led to a specialized relationship within the upper portions of ant nests (depot chambers), as has been suggested in other myrmecophilous beetle species (Hölldobler and Kwapich 2019; Delgado and Palma 2024). Likewise, foragers of some ant species, like *M. barbarus*, temporarily cache large numbers of seeds outside of their nest entrances, creating an opportunity for kleptoparasites to intercept them externally (Reyes and Fernández Haeger 1999). If ants were to move external depot sites inside, over evolutionary time, it is possible that existing kleptoparasites could follow. Of course, the larvae of soil dwelling or burrowing arthropod lineages could also intercept ant nests laterally, without having to pass through the chemo-tactile ‘filter’ at the well-defended nest entrance.

Partitioning of nest space by the myrmecophiles of *Pogonomyrmex badius*

Colonies of *Pogonomyrmex badius* host a small but diverse group of myrmecophile species, each specializing on a particular region of the nest or foraging trail. On the surface, myrmecomorphic gnaphosid spiders (*Micaria delicatula*) travel along *P. badius* foraging routes (Hölldobler and Kwapich, 2022). The assassin bug *Apiomerus crassipes* (Hemiptera: Reduviidae) waits outside the nest entrance and picks off departing *P. badius* foragers early in the morning, (Morrill 1975), while the false widow spider *Asagena* (= *Steatoda*) *fulva* (Theridiidae) appears at midday to capture returning foragers in the same location (Hölldobler 1970; Hölldobler and Wilson 1970; Hölldobler 1971). Inside the nest itself, small white springtails (Collembola) blanket the floors of shallow waste and food-depot chambers. These chambers and surrounding soil are also home to *H. dorsalis*, larvae of an unidentified elaterid beetle, silverfish, and brightly-colored larvae of the myrmecophagous antlion, *Brachynemurus nebulosus* (Bahls and Deyrup 1988).

Midway down the vertical length of each *P. badius* nest, commensal entomobryid, *Pseudosinella rolfsi*, and isotomid springtails graze on fungus and waste in granaries (Porter 1985). A 2 mm long spider, *Masoncus pogonophilus*, feeds on them exclusively. The spider is only found in the nests of *P. badius* (Porter 1985; Cushing 1995a; Cushing 1995b). Farther below the granaries, ant eggs, larvae and pupae are kept in brood chambers (though brood occasionally appear near the surface). Transfer workers carry processed seed pulp and the remaining portions of insects not scavenged by *H. dorsalis* to these deep chambers, where they are placed on the upturned bellies of waiting larvae. The incoming insect protein delivered to larvae is a likely source of mermithid worm infections, common in *P. badius* adults (CLK pers. obsv. In Hölldobler and Kwapich 2022). Brood chambers are also home to mites from four different families (Families:

Belbidae, Uropodidae, Laelaspidae and Rhodacaridae) (Porter 1985).

Hymenorus dorsalis larvae occupy a unique spatial and social niche within Florida harvester ant nests. Together with *H. dorsalis*, the small but varied community of myrmecophiles found with *P. badius* specializes on every aspect of host foraging behavior, morphology, transport of resources, circadian rhythms, and nest architecture. This constellation of host traits differs from that of the dozens of other subterranean ant species occupying the same forests, each with its own hidden wellspring of biodiversity.

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1007/s00040-024-01014-w>.

Acknowledgements CLK is grateful to Walter R. Tschinkel for his support, contributions to fieldwork, and important conversations during this study. We would also like to acknowledge financial support from the University of Central Florida, field assistance from UCF students Nevin Persaud and Jackson Talmadge in spring of 2024. We would also like to thank G Pérez-Lachaud, JP Lachaud, and two anonymous reviewers for improving the manuscript. Work was carried out in the Apalachicola National Forest under US Forest Service permits APA583 and APA56302 and with permission from the University of Central Florida Arboretum (permit: R-2024-23).

Data Availability Colony and nest attribute data are publicly available on FigShare: <https://doi.org/10.6084/m9.figshare.27937791.v1>

Declarations

Conflict of interest The authors declare no competing interests related to this work.

References

- Bahls P, Deyrup M (1988) A habitual lurking predator of the Florida harvester ant. *Advances in Myrmecology*, Leiden, New York:547–551
- Blaisdell FE (1892) Notes on some Californian Cistelidae. *Zoe* (1890–1906) 3(3):252
- Bousquet Y, Thomas DB, Bouchard P, Smith AD, Aalbu RL, Johnston MA, Steiner WE (2018) Catalogue of Tenebrionidae (Coleoptera) of North America. *ZooKeys* 728:1
- Böving AG, Craighead FC (1931) An illustrated synopsis of the principal larval forms of the order Coleoptera. Brooklyn Entomological Society
- Campbell JM (1971) A revision of the Alleculidae (Coleoptera) of the West Indies. *Memoirs Entomol Soc Canada* 103(S81):7–140
- Cushing PE (1995) Description of the spider *Masoncus pogonophilus* (Araneae, Linyphiidae), a harvester ant myrmecophile. *J Arachnol* 23:55–59
- Cushing PE (1995b) Natural history of the myrmecophilic spider, *Masoncus pogonophilus* Cushing, and its host ant, *Pogonomyrmex badius* (Latreille). Dissertation. University of Florida
- Czaczkes TJ, Heinze J, Ruther J (2015) Nest etiquette—where ants go when nature calls. *PLoS ONE* 10(2):e0118376
- Delgado JA, Palma RL (2024) Entry by brute force: An unusual behaviour displayed by *Scaurus uncinus* (Coleoptera: Tenebrionidae), to access nests of *Messor barbarus* (Hymenoptera: Formicidae) in Spain. *Euro J Entomol* 121:324–340
- Deyrup M (2016) *Ants of Florida: identification and natural history*. CRC Press
- Doyen JT (1966) The skeletal anatomy of *Tenebrio molitor* (Coleoptera: Tenebrionidae). Page 103. The skeletal anatomy of *Tenebrio molitor* (Coleoptera: Tenebrionidae). *BioOne*
- Fall H (1931) The North American species of *Hymenorus* (Coleoptera: Alleculidae). *Trans Am Entomol Soc* (1890–57(2):161–247
- Hölldobler B (1967) Zur Physiologie der Gast-Wirt-Beziehung (Myrmecophilie bei Ameisen. I. Das Gastverhältnis der Ateomes- und Lomechusa- Larven (Col. Staphylinidae) zu Formica (Hym Formicidae). *Zeitschr Vergl Physiol* 56(1):1–21
- Hölldobler B (1970) *Steatoda fulva* (Theridiidae), a spider that feeds on harvester ants. *Psyche* 77(2):202–208
- Hölldobler B (1971) Homing in the harvester ant *Pogonomyrmex badius*. *Science* 171(3976):1149–1151
- Hölldobler B, Kwapich CL (2017) *Amphotis marginata* (Coleoptera: Nitidulidae) a highwayman of the ant *Lasius fuliginosus*. *PLoS ONE* 12(8):e0180847. <https://doi.org/10.1371/journal.pone.0180847>
- Hölldobler B, Kwapich CL (2019) Behavior and exocrine glands in the myrmecophilous beetle *Dinarda dentata* (Gravenhorst, 1806)(Coleoptera: Staphylinidae: Aleocharinae). *PLoS ONE* 14(1):e0210524
- Hölldobler B, Kwapich CL (2022) The guests of ants: how myrmecophiles interact with their hosts. Belknap Press of Harvard University Press, Cambridge, MA
- Hölldobler B, Kwapich CL, Haight KL (2018) Behavior and exocrine glands in the myrmecophilous beetle *Lomechusoides strumosus* (Fabricius, 1775) (formerly called *Lomechusa strumosa*) (Coleoptera: Staphylinidae: Aleocharinae). *PLoS ONE* 13(7):e0200309. <https://doi.org/10.1371/journal.pone.0200309>
- Hölldobler B, Wilson EO (1970) Recruitment trails in the harvester ant *Pogonomyrmex badius*. *Psyche* 77(4):385–399
- Ivens ABF, von Beeren C, Blüthgen N, Kronauer DJC (2016) Studying the complex communities of ants and their symbionts using ecological network analysis. *Annu Rev Entomol* 61:353–371. <https://doi.org/10.1146/annurev-ento-010715-023719>
- Kwapich CL (2021) Green anole (*Anolis carolinensis*) eggs associated with nest chambers of the trap-jaw ant *Odontomachus brunneus*. *Southeast Nat* 20(4):N119–N124. <https://doi.org/10.1656/058.020.0409>
- Kwapich CL, Gadau J, Hölldobler B (2017) The ecological and genetic basis of annual worker production in the desert seed harvesting ant, *Veromessor pergandei*. *Behav Ecol Sociobiol* 71(8):110
- Kwapich CL, Hölldobler B (2019) Destruction of spiderwebs and rescue of ensnared nestmates by a granivorous desert ant (*Veromessor pergandei*). *Am Nat* 194(3):395–404. <https://doi.org/10.1086/704338>
- Kwapich CL, Tschinkel WR (2013) Demography, demand, death, and the seasonal allocation of labor in the Florida harvester ant (*Pogonomyrmex badius*). *Behav Ecol Sociobiol* 67(12):2011–2027
- Kwapich CL, Tschinkel WR (2016) Limited flexibility and unusual longevity shape forager allocation in the Florida harvester ant (*Pogonomyrmex badius*). *Behav Ecol Sociobiol* 70(2):221–235. <https://doi.org/10.1007/s00265-015-2039-1>
- Martyn TE, Kwapich CL, Kline A, Gornish ES (2023) Granivorous ants prefer small and unprotected seeds—implications for restoration in arid ecosystems. *Restor Ecol* 31(2):e13759. <https://doi.org/10.1111/rec.13759>
- McIntyre NE (1999) Use of *Pogonomyrmex occidentalis* (Hymenoptera: Formicidae) nest-sites by tenebrionid beetles (Coleoptera: Tenebrionidae) for oviposition and thermoregulation in a temperate grassland. *Southw Naturalist* 44(3):379–382
- Morrill W (1975) An unusual predator of the Florida harvester ant. *J GA Entomol Soc* 10:50–51

- Parmentier T, Boeckx P, Bonte D, De Laender F (2023) You are what your host eats: the trophic structure and food chain length of a symbiotic community are coupled with the plastic diet of the host ant. *J Anim Ecol* 92(10):2028–2038. <https://doi.org/10.1111/1365-2656.13994>
- Parmentier T, Braem S (2024) Structural variation of ant nests mediates the local distribution and abundance of an associate. *Entomol Exp Appl* 172(7):626–635. <https://doi.org/10.1111/eea.13429>
- Parmentier T, Claus R (2019) A dazzling number of beetles (Coleoptera) in a hibernating nest of red wood ants, *Formica Rufa* Linnaeus (Hymenoptera: Formicidae). *Coleopt Bull* 73(3):761–764
- Parmentier T, Claus R, De Laender F, Bonte D (2021) Moving apart together: co-movement of a symbiotic community and their ant host, and its importance for community assembly. *Mov Ecol* 9(1):25. <https://doi.org/10.1186/s40462-021-00259-5>
- Parmentier T, De Laender F, Bonte D (2020) The topology and drivers of ant–symbiont networks across Europe. *Biol Rev* 95(6):1664–1688. <https://doi.org/10.1111/brv.12634>
- Parmentier T, Dekoninck W, Wenseleers T (2016) Survival of persecuted myrmecophiles in laboratory nests of different ant species can explain patterns of host use in the field (Hymenoptera: Formicidae). *Myrmecol News* 23:71–79
- Parmentier T, Gaju-Ricart M, Wenseleers T, Molero-Baltanás R (2020) Strategies of the beetle *Oochrotus unicolor* (Tenebrionidae) thriving in the waste dumps of seed-harvesting *Messor* ants (Formicidae). *Ecol Entomol* 45:583–593. <https://doi.org/10.1111/een.12832>
- Parmentier T, Molero-Baltanás R, Valdivia C, Gaju-Ricart M, Boeckx P, Łukasik P, Wybouw N (2024) Co-habiting ants and silverfish display a converging feeding ecology. *BMC Biol* 22(1):123. <https://doi.org/10.1186/s12915-024-01914-0>
- Porter SD (1985) *Masoncus* spider: a miniature predator of Collembola in harvester ant colonies. *Psyche* 92(1):145–150
- Rettenmeyer CW, Rettenmeyer ME, Joseph J, Berghoff SM (2011) The largest animal association centered on one species: the army ant *Eciton burchellii* and its more than 300 associates. *Insectes Soc* 58(3):281–292. <https://doi.org/10.1007/s00040-010-0128-8>
- Reyes J, Fernández Haeger J (1999) Sequential co-operative load transport in the seed-harvesting ant *Messor barbarus*. *Insectes Soc* 46:119–125
- Rink WJ, Dunbar JS, Tschinkel WR, Kwapich CL, Repp A, Stanton W, Thulman DK (2013) Subterranean transport and deposition of quartz by ants in sandy sites relevant to age overestimation in optical luminescence dating. *J Archaeol Sci* 40(4):2217–2226. <https://doi.org/10.1016/j.jas.2012.11.006>
- Sánchez-Piñero F, Gómez JM (1995) Use of ant-nest debris by darkling beetles and other arthropod species in an arid system in south Europe. *J Arid Environ* 31(1):91–104
- Slobodchikoff CN (1979) Utilization of harvester ant debris by tenebrionid beetles. *Environ Entomol* 8(4):770–772. <https://doi.org/10.1093/ee/8.4.770>
- Smith C, Tschinkel W (2006) The sociometry and sociogenesis of reproduction in the Florida harvester ant *Pogonomyrmex badius*. *J Insect Sci* 6(1):32
- Smith CR (2007) Energy use and allocation in the Florida harvester ant, *Pogonomyrmex badius*: are stored seeds a buffer? *Behav Ecol Sociobiol* 61(9):1479–1487
- Smith CR, Suarez AV (2010) The trophic ecology of castes in harvester ant colonies. *Funct Ecol* 24(1):122–130. <https://doi.org/10.1111/j.1365-2435.2009.01604.x>
- Steiner W Jr (1995) Structures, behavior and diversity of the pupae of Tenebrionidae (Coleoptera). *Biology, phylogeny, and classification of Coleoptera: papers celebrating the 80th birthday of Roy A. Crowson* 1:504–539
- Tschinkel WR (1998) Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*: worker characteristics in relation to colony size and season. *Insectes Soc* 45(4):385–410. <https://doi.org/10.1007/s000400050097>
- Tschinkel WR (1999) Sociometry and sociogenesis of colony-level attributes of the Florida harvester ant (Hymenoptera: Formicidae). *Ann Entomol Soc Am* 92(1):80–89. <https://doi.org/10.1093/aesa/92.1.80>
- Tschinkel WR (2001) Sociometry and sociogenesis of colonies of the harvester ant, *Pogonomyrmex badius*: distribution of workers, brood and seeds within the nest in relation to colony size and season. *Ecol Entomol* 24:222–237. <https://doi.org/10.1046/j.1365-2311.1999.00184.x>
- Tschinkel WR (2004) The nest architecture of the Florida harvester ant *Pogonomyrmex badius*. *J Insect Sci* 4:21. <https://doi.org/10.1093/jis/4.1.21>
- Tschinkel WR (2013) Florida harvester ant nest architecture, nest relocation and soil carbon dioxide gradients. *PLoS ONE* 8(3):e59911. <https://doi.org/10.1371/journal.pone.0059911>
- Tschinkel WR (2017) Lifespan, age, size-specific mortality and dispersion of colonies of the Florida harvester ant *Pogonomyrmex badius*. *Insectes Soc* 64(2):285–296
- Tschinkel WR, Domínguez DJ (2017) An illustrated guide to seeds found in nests of the Florida harvester ant *Pogonomyrmex badius*. *PLoS ONE* 12(3):e0171419. <https://doi.org/10.1371/journal.pone.0171419>
- Tschinkel WR, Hanley N (2017) Vertical organization of the division of labor within nests of the Florida harvester ant *Pogonomyrmex badius*. *PLoS ONE* 12(11):e0188630. <https://doi.org/10.1371/journal.pone.0188630>
- Tschinkel WR, Kwapich CL (2016) The Florida harvester ant, *Pogonomyrmex badius*, relies on germination to consume large seeds. *PLoS ONE* 11:e0166907
- Tschinkel WR, Rink WJ, Kwapich CL (2015) Sequential subterranean transport of excavated sand and foraged seeds in nests of the harvester ant *Pogonomyrmex badius*. *PLoS ONE* 10:e0139922
- Tschinkel WR, Willson CD (1971) Inhibition of pupation due to crowding in some tenebrionid beetles. *J Exp Zool* 176(2):137–145. <https://doi.org/10.1002/jez.1401760203>
- von Beeren C, Maruyama M, Kronauer DJC (2016) Community sampling and integrative taxonomy reveal new species and host specificity in the army ant-associated beetle genus *Tetradonia* (Coleoptera, Staphylinidae, Aleocharinae). *PLoS ONE* 11(11):e0165056. <https://doi.org/10.1371/journal.pone.0165056>
- von Beeren C, Maruyama M, Kronauer DJC (2016) Cryptic diversity, high host specificity and reproductive synchronization in army ant-associated *Vatesus* beetles. *Molec Ecol* 25(4):990–1005. <https://doi.org/10.1111/mec.13500>
- von Beeren C, Tishechkin AK (2017) *Nymphister kronaueri* von Beeren & Tishechkin sp. nov., an army ant-associated beetle species (Coleoptera: Histeridae: Haeteriinae) with an exceptional mechanism of phoresy. *BMC Zool* 2(1):3. <https://doi.org/10.1186/s40850-016-0010-x>
- Wasmann E (1920) *Die Gastpflege der Ameisen*. Verlag Gebrüder, Bornträger, Berlin
- Wickham H (1896) Description of the larvae of some heteromorous and rhyngophorus beetles. *J NY Entomol Soc* 4(3):118–124

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.