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SHORT COMMUNICATION

Living with the enemy: behavioral study of *Myrmecicultor chihuahuensis* Ramírez, Grismado & Ubick (Araneae: Myrmecicultoridae)

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Abstract. The spider, *Myrmecicultor chihuahuensis* Ramírez, Grismado & Ubick 2019 is a myrmecophage. In an earlier study, we found that the spider's cuticular hydrocarbon (CHC) profile overlapped with that of its prey. In the present study, we photographed nests of *Novomessor* ants to determine whether these spiders live inside the ant nest with their prey or in the vicinity of the colony. We set up two 35 mm cameras over two main entrances of one nest of *N. albisetosus* (Mayr 1886) such that images would be captured of the ant colony surface (including the entrances) every 15 – 60 sec over five consecutive nights. These images included five showing *M. chihuahuensis* directly at one of the nest entrances investigating dead ants. The spider was not seen away from the entrance. This study provides evidence suggesting that this myrmecophage may live inside the colony with the ants it eats.

Keywords: *Novomessor*, *Myrmecicultor*, *Pogonomyrmex*, myrmecophile, symbiont
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The spider *Myrmecicultor chihuahuensis* Ramírez, Grismado, & Ubick (Myrmecicultoridae) was described in 2019 (Ramírez et al. 2019). This species has only been collected in the vicinity of ant nests in the genera *Novomessor* and *Pogonomyrmex*, specifically, in pitfall traps or on the surface of nests of *N. albisetosus* (Mayr, 1886), *N. cockerelli* (André, 1893), and *P. rugosus* Emery, 1895. David Lightfoot (pers. comm.) observed *M. chihuahuensis* on the surface of a *P. rugosus* colony near Cuatro Ciénegas, Mexico, whose colony entrance had been plugged (likely by the ants) (see Fig. 4). Lightfoot observed the spiders as well as a lone ant contacting pebbles plugging the colony entrance as if trying to re-gain entrance to the nest.

Cushing et al. (2022) provided evidence that this spider is a myrmecophage. The hunting strategy is very similar to that of *Zodarion* spp. (Zodariidae) (Couvreur 1990a, b; Pekár & Křál 2002). As with species of *Zodarion*, *M. chihuahuensis* attacks the ant from the rear, bites the rear legs one or more times, retreats until the ant is paralyzed from the venom, re-approaches, bites the ant behind the head, and carries the ant away to feed (Cushing et al. 2022). In addition, *M. chihuahuensis* uses the dead ant as a shield when a live ant from the colony approaches. This "shielding" behavior has been noted in other myrmecophagous spiders in the family Thomisidae (Piza 1937; Bristowe 1941; Oliveira & Sazima 1984; Castanho & Oliveira 1997) as well as in *Z. rubidum* Simon, 1914 (Couvreur 1990a). In the 2022 paper, we also presented evidence that *M. chihuahuensis* had a cuticular hydrocarbon (CHC) profile similar to that of the ants (Cushing et al. 2022). Our co-author in that earlier publication, Adrian Brückner, from the California Institute of Technology, also carried out a CHC comparison of *M. chihuahuensis* and another species of *Novomessor*, *N. cockerelli*, with which the spider is also associated. Fig. 1 presents a comparison of all three CHC profiles. The CHC profile of *N. cockerelli* was not included in the 2022 publication. The similarity of the CHC profile of the spider and the latter species of *Novomessor* is striking and suggests that the spider may be able to acquire colony specific CHCs depending on the ants being attacked.

PEC's lab traveled to Dalquest Desert Research Station (DDRS) located in the Chihuahuan Desert in Texas, United States to carry out this project. This field station is operated by Midwestern State University (MSU). Previously, we traveled to DDRS 9–12 June 2015 and 20–24 October 2019 to excavate *N. albisetosus* nests to determine whether the spiders lived inside the nest chambers. During these earlier trips, we dug adjacent to or down into the mound of four *N. albisetosus* nests and collected adult and juvenile spiders from the excavation pit of three of these four nests (Cushing et al. 2022 and unpubl. field notes). In no instance did we observe spiders inside nest chambers. However, these nests are located in a deep slot canyon in dry washes and arroyos. The sandy soil in this entire canyon area has been entirely re-worked by flooding. Thus, the underground nest chamber patterns are unpredictable since the ants shift the location of chambers as large boulders and other obstructions are encountered. In addition, the chambers themselves collapse when exposed. Because of this, although spiders were found in the excavation pits, we were unable to determine if they were falling into the pits as chambers were exposed or from burrows or retreats in the direct vicinity of the nests.

In the present study, we hoped to determine whether this species of myrmecophage lives with the ants it hunts (inside the colony) or whether the spiders come upon the surface of the active ant nest at night to hunt, living in the general vicinity of the ant nest, but not inside the nest. From work we did October 2020, we determined that in the lab, the spiders emerged from the circular retreat (see fig. 3 in Cushing et al. 2022) about one hour after sunset (Cushing pers. obs. & field notes). In order to document the behavior of wild *M. chihuahuensis* spiders, we traveled to DDRS 11–16 October 2022. We were unable to observe nests directly at night because previous work by MSU scientists indicated that these slot canyons are active game trails regularly used by mountain lions and black bears (NVH pers. comm.). Because of this, the university discourages researchers from working in the canyons at night. Instead, we set up a photographic system over a nest of *Novomessor albisetosus* at N 29.55139°, W 103.79248°, 1228 m elev. Pitfall traps placed in the vicinity of this nest previously by NVH had yielded many specimens of *M. chihuahuensis* over the years (Ramírez et al. 2019). This was the same nest from which the live females used in the behavioral bioassay reported in Cushing et al. (2022) were collected.

The DMNS photographer (RMW) set up two cameras: a Nikon D800 and a Nikon D850, each with a Micro NIKKOR (Nikon) 60 mm

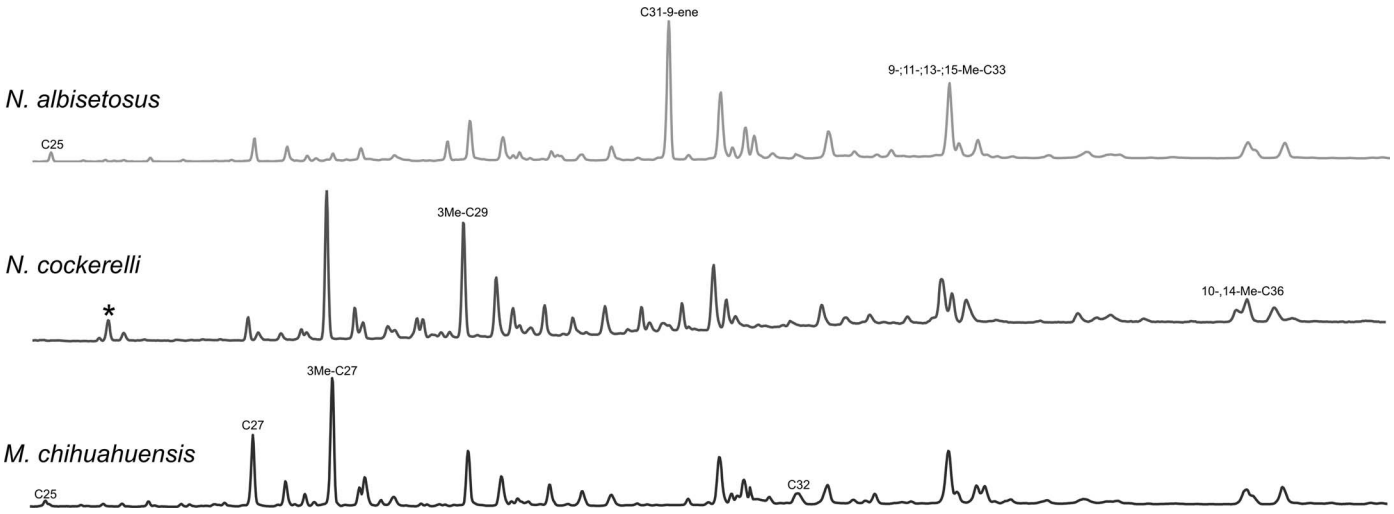


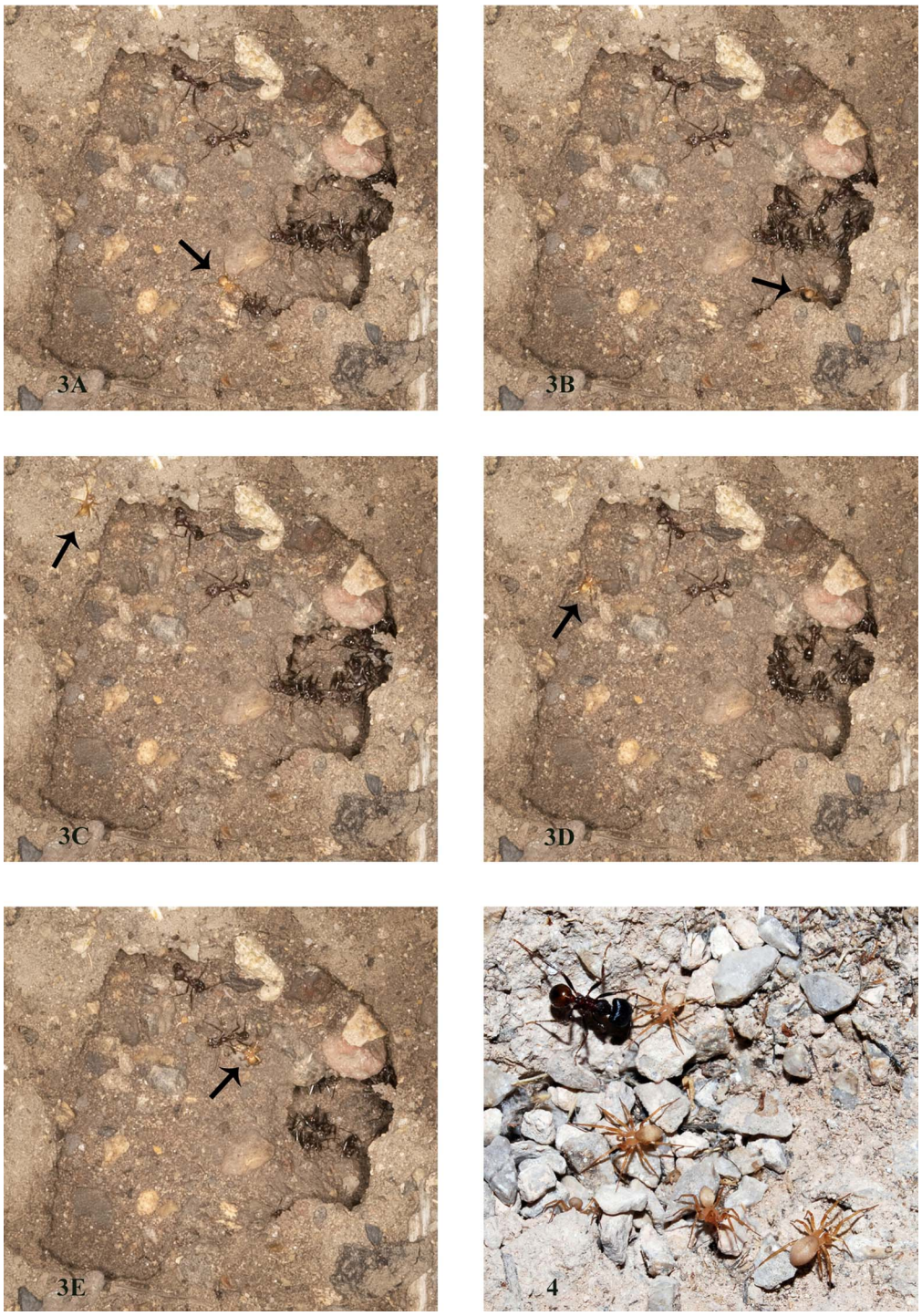
Figure 1.—Cuticular hydrocarbon (CHC) profiles of the spider *Myrmecicultor chihuahuensis* and two *Novomessor* ant species, *N. albisetosus* and *N. cockerelli* with which the spider is associated.

F2.8 lens with a ring flash attached to each lens. Each ring flash was connected to an extended battery pack. The photo area covered by each camera was 0.46 m x 0.33 m. Each camera was set above the most active nest entrances of this colony of *N. albisetosus*. The field of view of each camera encompassed both the active nest entrances as well as the surface of the nest mound (Fig. 2). We filmed from 18:16–01:44 October 11–12; from 17:00–21:00 during the evening of October 12; from 18:30–21:00 during the evening of October 13; from 18:30–21:00 the evenings of October 14 and 15. During the evenings of October 11 and 12, the cameras were set to take photos of the nest area at 1 minute (60 sec) intervals; during the nights of October 13 and 14, we reduced this to 30 sec intervals and reduced the time to every 15 sec during the last evening. This resulted in 755 images from October 11th (total from both cameras with one camera ending early due to a battery problem); 480 images from October 12th; 581 images from October 13th; 720 images from October 15th; and 1440 images from October 15th. This resulted in a total of 3,976 images of the nest surface over the five consecutive nights.

A spider of the same size and coloration as *M. chihuahuensis* was photographed in the depression of the most active entrance of the nest the evening of October 12. The spider was photographed approaching a dead ant at 19:53 directly at the nest entrance; at 19:54, the spider was photographed entering the nest entrance; at 19:55, the spider was photographed at the lip of the entrance; at 19:56, the spider was inside the depression; and at 19:57 the spider was photographed next to another dead ant in the depression of the nest entrance (Figs. 3A–E). During the five nights of filming, we never observed *M. chihuahuensis* on the surface of the nest away from the entrance and only saw the live spider during filming on October 12. We never saw it further than 5 mm from the depression of the nest entrance (Fig. 3C).

The similarity in color, size, and eye reflectance of the spider photographed October 12th and *M. chihuahuensis* (compare Fig. 3 with Fig. 4) is compelling. The adjacency of the photographed spider with ants, particularly dead ants, also strongly suggests this to be *M. chihuahuensis*. The fact that the spider never left the vicinity of the nest entrance; was not photographed away from the entrance, despite five nights of filming and nearly 4000 images surveyed; was

Figure 2.—Camera set-up over nests of *Novomessor albisetosus*; field of view includes main nest entrances as well as an extensive area surrounding the nest entrance (approximately 0.152 m^2 covered by each camera).



Figures 3–4.—Images of *Myrmecicultor chihuahuensis* in the direct vicinity of the nest entrance of a *Novomessor albisetosus* nest on October 12, 2022. (A) Spider approaching a dead ant; (B) Spider entering the nest entrance; (C) Spider at the lip of the entrance; (D) Spider inside the depression of the nest entrance; (E) Spider next to another dead ant in the depression of the nest entrance. Arrows point to the location of the spider; (4) Nest of *Pogonomyrmex rugosus* in the Chihuahuan Desert in Mexico with *Myrmecicultor chihuahuensis* on the surface with the ants. Photo by David Lightfoot, University of New Mexico, used by permission.

seen entering the nest; had been collected previously from the nest excavation pits; and has a CHC profile similar to species of *Novomessor* suggests that this spider is a myrmecophile as well as a myrmecophage, spending some or all of its life cycle inside the nest chambers of the ant nest. The observations by David Lightfoot of spiders contacting small pebbles plugging the nest entrance of a *P. rugosus* nest also support these findings.

Intriguing questions remain regarding the biology of this spider including: If it is living inside ant nests, how does it associate with two very different genera of ants (*Pogonomyrmex* and *Novomessor*)

without being attacked? What are the juveniles eating? How does it locate and become integrated into new colonies? How does it acquire CHCs from the hosts? Are CHCs even required for nest integration? Hölldobler & Kwapich (2022) indicate that CHC matching, or "identity theft" as they refer to this phenomenon in myrmecophiles, is not the only mechanism for myrmecophiles—even those that are also myrmecophages—to become integrated into ant colonies. Von Beeren et al. (2012) showed that myrmecophilous spiders isolated from their hosts lost their CHC mimetic compounds but were still able to reintegrate back into host colonies. We hope to continue our investigations of the natural history of this interesting myremcophage to address some of these questions.

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