

# **Article**



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# A new species in the spider family Myrmecicultoridae (Arachnida, Araneae) and evidence of myrmecophagy in the family

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#### **Abstract**

A new species in the unusual myrmecophagous family Myrmecicultoridae is described. This new species, *Myrmecicultor pueblaensis* **sp. nov.** shares several unique morphological features with the one other species in the family, *M. chihuahuensis* Ramírez, Grismado, and Ubick 2019. A multi-locus phylogenetic analysis of six molecular markers from the mitochondrial (12 S rDNA, 16S rDNA, cytochrome oxidase subunit I) and nuclear (18S rDNA, 28S rDNA, histone H3) genomes also places this new species as sister to *M. chihuahuensis*. In addition, the new species is a myrmecophage and shows similar hunting behavior to *M. chihuahuensis*. We hypothesize that additional species of this unusual family may be distributed throughout the desert ecosystems of southern Mexico in association with various species of ants.

**Key words:** Taxonomy, phylogenetics, morphology, ant symbiosis

#### Introduction

The spider family Myrmecicultoridae was established in 2019 by Ramírez et al. (2019). Until now it included a single species, Myrmecicultor chihuahuensis Ramírez, Grismado, and Ubick. Phylogenetic analysis based on mitochondrial and nuclear loci placed this family as a separate lineage emerging near the base of the Dinonycha and Oval Calamistrum clade. Myrmecicultoridae (and its monotypic species) is characterized by the following: two tarsal claws without claw tufts; procurved eye rows with the posterior median eyes (PME) oblong and with noticeable tapeta; the presence of a retrolateral tibial apophysis (RTA) on the male pedipalp; long fangs; a dorsal patch of setae on the palpal cymbium of males and on the pedipalp of females; a high clypeus; and a serrula on the endites of males and females. Subsequent research exploring the natural history and biology of this monotypic species determined that these spiders are myrmecophages (Cushing et al. 2022), hunting ants in a manner similar to that documented for spiders in the family Zodariidae (Pekár 2002; Cushing & Santangelo 2002).

From 8 – 9 July 2024, the authors were leading a field expedition to the desert habitat of the southern Mexican state of Puebla. While headlamping at night, each of the authors noticed groups of adult and juvenile spiders actively hunting on two different trails. This collective activity was so unusual that both authors independently collected multiple spiders (adults and immatures). Ants were also seen on the trails in the vicinity of these spiders. The first author (PEC) watched as one of the spiders approached an ant from behind, rushed forward, bit the rear leg of the ant, retreated, and waited for the venom to take effect. This myrmecophagous hunting behavior was identical to that reported by Cushing *et al.* (2022) for *M. chihuahuensis*. It was further determined that this species had similar morphological characters mentioned above for *M. chihuahuensis*. The morphological resemblance to this previously described species but the evident difference in epigynal and palpal structures led the authors to suspect the existence of a second species in the family Myrmecicultoridae, which is herein described under the name *Myrmecicultor pueblaensis* sp. nov..

#### **Materials and Methods**

#### Material used

A total of 12 spiders were collected in Mexico, state of Puebla, 10 Km west of Zapotitlán Salinas at 18.33510° N, 97.51850° W, 1587 m elev. These 12 specimens included two males, five females, and five juveniles. One juvenile was destructively sampled for molecular sequencing. One male is designated (below) as the holotype, one female paratype is designated as the allotype, and the remaining specimens collected at this site are designated as additional paratypes. The holotype is deposited in the arachnology collection at the Universidad Nacional Autónoma de México (UNAM) with unique identifier, UID, #CNAN-Ar011706); all paratypes are deposited in the arachnology collection at the Denver Museum of Nature and Science (DMNS). The female allotype is separated from the rest of the paratypes in a sub-vial. The paratypes at DMNS have been assigned the unique identifier DMNS ZA.46195 and the collecting data (provenance, date, collector) recorded in the Ecdysis database (https://ecdysis.org/index.php) and pushed out to other data portals such as GBIF (https://www.gbif.org/).

# Molecular sequencing

To determine the phylogenetic position of *M. chihuahuensis*, Ramírez *et al.* (2019) used both morphological character analysis as well as multi-locus DNA sequencing. Therefore, to test the hypothesis, suggested by the morphological similarity, that the Puebla specimens represent a new species in the family Myrmecicultoridae, we sequenced the following loci (also used by Ramírez *et al.* 2019): 12S rDNA (12S), the 3' end of the 16S rDNA (16S), and cytochrome oxidase subunit I (COI) from the mitochondrial genome and 18S rDNA (18S), the D3 large subunit ribosomal RNA 28S rDNA (28S), and a variable fragment of the histone H3 (H3) protein-coding gene from the nuclear genome (Table 1).

**TABLE 1.** Primer sequences used in the molecular analysis and associated GenBank numbers.

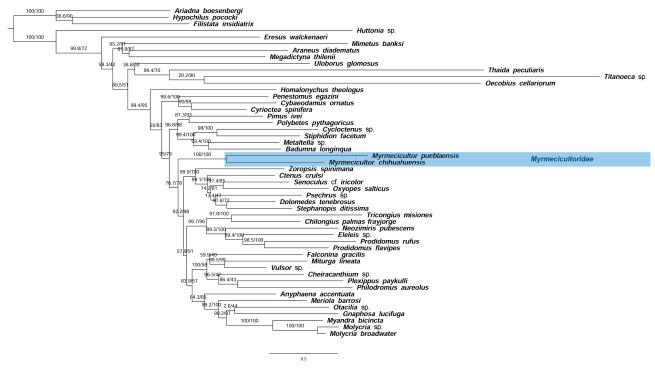
| Gene     | Name     | Sequence (5'-3')          | GenBank#s | References             |
|----------|----------|---------------------------|-----------|------------------------|
| COI      | LCO1490  | GGTCAACAAATCATAAAGATATTGG | PV448916  | Folmer et al. (1994)   |
|          | HCO2198  | TAAACTTCAGGGTGACCAAAAATCA |           | Folmer et al. (1994)   |
| 12S rDNA | 12S-ai-F | AAACTAGGATTAGATACCCTATTAT | PV492139  | Köcher et al. (1989)   |
|          | 12S-bi-R | AAGAGCGACGGGCGATGTGT      |           | Köcher et al. (1989)   |
| 16S rDNA | 16Sar    | CGCCTGTTTATCAAAAACAT      | PV492140  | Simon et al. (1994)    |
|          | 16Sbr    | CTCCGGTTTGAACTCAGATCA     |           | Simon et al. (1994)    |
| 18s rDNA | 18S-1F   | TACCTGGTTGATCCTGCCAGTAG   | PV449816  | Carranza et al. (1996) |
|          | 18S-5R   | CTTGGCAAATGCTTTCGC        |           | Carranza et al. (1996) |
| 28s rDNA | 28Sa     | GACCCGTCTTGAAACACGGA      | PV449817  | Nunn et al. (1996)     |
|          | 28Sbout  | CCCACAGCGCCAGTTCTGCTTACC  |           | Prendini et al. (2005) |
| Н3       | H3af     | ATGGCTCGTACCAAGCAGACVGC   | PV454979  | Colgan et al. (1998)   |
|          | H3ar     | ATATCCTTRGGCATRATRGTGAC   |           | Colgan et al. (1998)   |

For the molecular analysis, we destructively sampled one juvenile of the new species for the molecular analysis using a standard Qiagen Blood and Tissue DNA Extraction Kit (Qiagen, Germany) according to the manufacturer's instructions in the Genomics Lab at the DMNS. Gene fragments were amplified using the polymerase chain reaction (PCR). For each locus, we used the protocol outlined in Cushing *et al.* (2015) or in Wheeler *et al.* (2017). All PCR mixes contained 15 µl DreamTaq, 10.1 µl nuclease-free water, 1.2 µl each of forward and reverse primers, and 2 µl of genomic DNA. Nuclease-free water was used as the negative control in each run. For all primer sets (see Table 1 for primer sequences), the PCR amplification protocol was 95°C for 4 min followed by 40 cycles of 95°C for 30 s,

49°C for 30 s and 72°C for 1 min plus a final extension step of 72°C for 3 min. The COI PCR product was sequenced in both directions at EtonBio (Durham, North Carolina, USA). All remaining loci were sequenced in both directions at Quintara Biosciences (Cambridge, Massachusetts, USA). Double-stranded sequences were assembled using the Geneious version 2024.0.2 program package. BLAST analysis was performed to compare the sequences with those in the NCBI GenBank. All sequences generated for this study were deposited in GenBank.

## Phylogenetic analysis

We deconstructed the original matrix built by Ramírez *et al.* (2019) to incorporate our fresh samples. The loci were reassembled using Mesquite 3.81 (Maddison and Maddison, 2023). The consensus sequences for individual loci were aligned in the online version of MAFFT (Katoh *et al.* 2019). The final data matrix comprised 6468 aligned bp with 50 terminals with 3191 distinct patterns, 2068 parsimony-informative, 885 singleton, and 3514 constant sites, after editing by eye. The phylogenetic relationships were reconstructed from the aligned data set with Maximum Likelihood (ML) as optimality criterion using IQ-TREE2 (version 2.4.0) software, in which ModelFinder automatically selected the appropriate molecular model for each partition (Kalyaanamoorthy *et al.* 2017) and calculated ultrafast bootstrap approximation (Hoang *et al.* 2018). Selected molecular model included: TIM2+F+I+G4: 12s; GTR+F+I+G4: 16s; TIM2e+I+G4: 18s; TIM3+F+I+G4: 28s; GTR+F+I+G4: COI, GTR+F+I+G4: COI\_1; TIM+F+I+G4: COI\_2; K3Pu+F+G4: COI\_3; TPM2+I+G4: H3; GTR+F+G4: H3\_1; JC+I: H3\_2; SYM+G4: H3\_3. For the protein coding genes COI and H3, the stop codons were minimized with Mesquite (Maddison & Maddison 2023).



**FIGURE 1.** Phylogenetic tree resulting from the analysis of six genetic markers on the optimality criterion of ML best score -88631.383. The blue box indicates the circumscription of the family Myrmecicultoridae with the known species *M. chihuahuensis* and *M. pueblaensis* **sp. nov.** The topology shown here is identical to that obtained by Ramirez *et al.* (2019). Branch support on the tree indicates SH-like aLRT/Ultrafast bootstrap values.

### **Imaging**

We used a Keyence VHS high resolution digital imaging system with proprietary stacking software to compare the morphology of *M. chihuahuensis* with the new species. Because the molecular phylogenetic analysis clearly placed the new species in a clade with *M. chihuahuensis* and because we had limited adult specimens to work with (2 males and 5 females), we did not review the complete dataset of 397 morphological characters used in

Ramírez et al. (2019); rather, we focused on assessing and imaging characters that could be seen under light microscopy and that were either unique to the family Myrmecicultoridae when compared to other members of the same phylogenetic clade (see Ramirez et al. 2019, fig. 2A) or distinct for this newly discovered species. In the parsimony analysis of morphological data included in Ramirez et al. (2019, fig. 2 A), Myrmecicultoridae shared a clade with Homalonychidae (Homalonychus theologus Chamberlin 1924) and with two species of Zodariidae (Cybaeodamus ornatus Mello-Leitão 1938 and Cyrioctea spinifera (Nicolet 1849)). Morphological characters assessed and compared between the two species of the genus Myrmecicultor are listed in Table 2.

**TABLE 2.** Morphological characters assessed for the new species and comparison with *Myrmecicultor chihuahuensis*. AME = Anterior Median Eye; PME = Posterior Median Eye. See Ramírez 2014 for descriptions of these characters.

| Character   | M. chihuahuensis                                       | Myrmecicultor pueblaensis sp. nov.   |  |
|---|--|--|--|
| Clypeus   | High   | High   |  |
| Anterior eye row curvature in anterior view                 | Notably procurved                                      | Notably procurved  |  |
| Posterior eye row curvature in dorsal view                  | Notably procurved                                      | Notably procurved  |  |
| ALE black cup   | Eye with silvery tapetum on top of the dark background | Eye with silvery tapetum on top of<br>the dark background; ALE only partly<br>surrounded by black pigment. |  |
| Eyes surrounded by black pigment                            | Eyes mostly surrounded by black pigment                | Eyes mostly surrounded by black pigment  |  |
| PME lens curvature  | Convex   | Flattened  |  |
| PME tapeta symmetry axes                                    | Orthogonal to each other                               | Orthogonal to each other   |  |
| Fovea   | Dark, longitudinal                                     | Dark, longitudinal   |  |
| Carapace shape  | Highest in cephalic region                             | Highest in cephalic region   |  |
| Cheliceral boss   | Absent   | Absent   |  |
| Cheliceral promarginal teeth                                | One small tooth  | No teeth   |  |
| Cheliceral retromarginal teeth                              | No teeth   | One retromarginal tooth and proximal denticle (diastema between)   |  |
| Fang length   | Very long with shaft serrula                           | Short with shaft serrula   |  |
| Labium length/width ratio                                   | Wider than long  | Wider than long  |  |
| Endites obliquely depressed                                 | Not depressed  | Depressed  |  |
| Endite ventral distal macrosetae                            | Absent   | Present  |  |
| Endite shape  | As wide as long  | Longer than wide   |  |
| Serrula on endites  | Present  | Present  |  |
| Serrula rows  | Single row   | Single row   |  |
| Serrula width   | Wide, bordering apex                                   | Wide, bordering apex   |  |
| Female palpal tarsus dorsal chemosensory setae distribution | In a defined patch                                     | In a defined patch   |  |
| Male palpal tarsus dorsal chemosensory setae distribution   | In a defined patch                                     | In a defined patch   |  |
| Palpal claw teeth   | Many teeth in a row                                    | Many teeth in a row  |  |
| Sternum posterior end profile                               | Convex   | Convex   |  |
| Sternum shape   | Heart shaped   | Shield shaped  |  |
| Pointed extension sternum between coxae IV                  | Yes  | Yes  |  |
| Precoxal triangles in female                                | Absent   | Present, fused to sternum  |  |
| Trochanter IV length  | Less than 1.5 X length of trochanter III               | Less than 1.5 X length of trochanter III for males; greater than for females                               |  |
| Preening brush  | Legs III & IV  | Legs III & IV (less developed on LIV)  |  |

.....continued on the next page

TABLE 2. (Continued)

| Character                         | M. chihuahuensis | Myrmecicultor pueblaensis sp. nov. |
|-----------------------------------|------------------|------------------------------------|
| RTA position                      | Medial           | Apical                             |
| RTA with canal                    | Present          | Present                            |
| Cymbium dorsal chemosensory patch | Present          | Present                            |
| Prey-catching web                 | Absent           | Absent                             |
| Wrap-bite attack                  | Bite first       | Bite first                         |
| Orb web architecture              | NA               | NA                                 |
| Ant hunting behavior              | Present          | Present                            |
| Ant shielding behavior            | Present          | Unknown                            |

#### **Abbreviations**

Abbreviations used in the taxonomic section include: AER, Anterior Eye Row; ALE, Anterior Lateral Eye; PER, Posterior Eye Row; PME, Posterior Median Eye; and RTA, Retrolateral Tibial Apophysis (of the palp). Roman numerals are used to designate legs from anterior to posterior (I, II, III, IV) or leg segments from proximal to distal (ibid).

### Results

The analysis of the sequence data placed this new species as sister to *M. chihuahuensis* in the family Myrmecicultoridae with strong branch support (Fig. 1). The morphological characters the new species shares with *M. chihuahuensis* include the following (Table 2 and Figs. 2-6): a noticeably high clypeus (Figs. 3A, B); a narrow, dark longitudinal fovea (Figs. 2A - D); procurved AER and PER (Figs. 2 & 3); ALE with silvery tapetum on top of a dark cup (Fig. 3); PME tapeta orthogonal to each other (Figs. 2A - D); similarly shaped sternum in both species with pointed extensions between coxae IV (Figs. 2 E, F; although the anterior sternal plate of the new species is more rounded); no cheliceral boss; labium wider than long; presence of a serrula on the anterior apex of the endites of males and females (Fig. 6A, white arrows); a distinct patch of setae on the male dorsoapical cymbium and female palpal tarsus (Figs. 5G, 5H, 6C); two tarsal claws without claw tufts; female palpal claws with many teeth in a row; six reduced, tightly grouped spinnerets (Fig. 6B); metatarsi III & IV with a preening brush; males with a RTA (Figs. 5G, H); and a well-developed female palpal claw. In addition, both species are myrmecophages with similar hunting strategies.

# **Taxonomy**

Myrmecicultor pueblaensis Cushing and González-Santillán, sp. nov.

Figures 2B, 2D, 2F, 3B, 4B, 4D, 4F, 5D, 5E, 5F, 5H, 6

*Type series*. Male holotype (UNAM CNAN-Ar011706); female allotype and additional paratypes (DMNS ZA.46195). All specimens collected in Mexico, Puebla, 10 Km west of Zapotitlán Salinas, N 18.33510°, W 97.51850°, 1587 m elev.; 9 July 2024; collected by authors while headlamping at night from 20:00 – 23:30 hrs. This desert habitat is within the Poblano-Oaxaca Semiarid Region (Abd El-Ghani *et al.* 2017).

*Etymology*. The specific epithet is an adjective referring to the state of Puebla, Mexico. This is, thus far, the only locality from which these spiders have been collected.

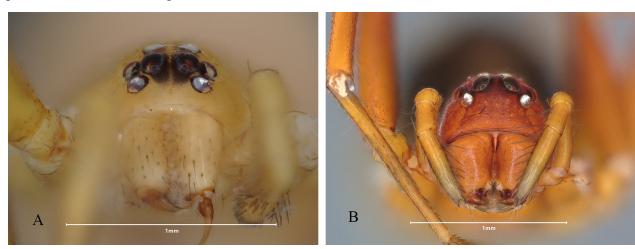
Diagnosis. The two species of Myrmecicultor differ in the following morphological characters (Table 2, Figs. 2–5): PME lens convex in M. chihuahuensis and flattened in M. pueblaensis sp. nov.; cheliceral basal transverse ridge is absent in M. chihuahuensis and present in M. pueblaensis sp. nov.; cheliceral promargin teeth are present in M. chihuahuensis and absent in M. pueblaensis sp. nov.; pronounced mound on the cheliceral retromargin is absent in M. chihuahensis and present in M. pueblaensis sp. nov.; the endites are not obliquely depressed in

M. chihuahuensis but are so in M. pueblaensis sp. nov. and the endites are only slightly longer than wide in M. chihuahuensis (more or less triangular in shape) whereas the endites of M. pueblaensis sp. nov. are distinctly longer than wide (compare Figs. 2 E and F); ventral distal macrosetae on the endites are absent in M. chihuahuensis whereas M. pueblaensis sp. nov. has a scattering of ventral distal macrosetae; the anterior margin of the sternum is straight in M. chihuahuensis and convex in M. pueblaensis sp. nov. (compare Figs. 2E and F); precoxal triangles on the female are absent in M. chihuahuensis and present (fused to the sternum) in M. pueblaensis sp. nov. (Figs. 2 E and F).



**FIGURE 2.** Dorsal habitus of *Myrmecicultor chihuahuensis* and *M. pueblaensis* **sp. nov.** and ventral views of promsoma. A) Habitus, female *M. chihuahuensis* (DMNS ZA.41786); B) Habitus, female *M. pueblaensis* **sp. nov.**; C) Habitus, male *M. chihuahuensis* (DMNS ZA.41786); D) habitus, male *M. pueblaensis* **sp. nov.**; E) *M. chihuahuensis* female sternum (DMNS ZA.44217); F) *M. pueblaensis* **sp. nov.** female sternum. Scale bars = 1 mm.

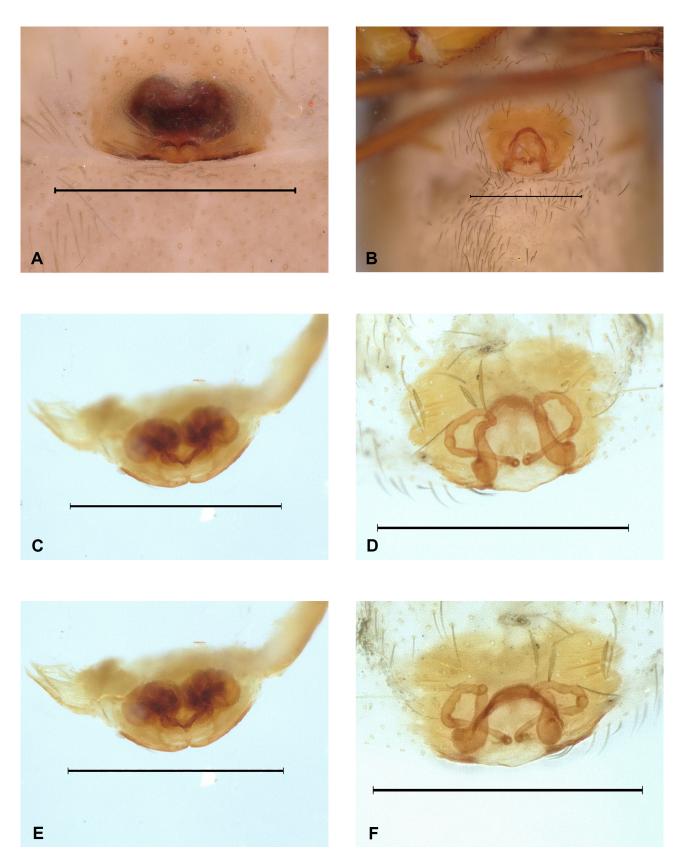
In addition, *M. chihuahuensis* has a broader and more medially positioned RTA in *M. chihuahuensis* whereas the RTA is more more elongate and more apically positioned in *M. pueblaensis* **sp. nov.** (Figs. 5G, H). The dorsal color pattern of the two species are also distinctive: *M. chihuahuensis* has a pale yellow carapace and pale abdomen lacking patterns; *M. pueblaensis* **sp. nov.** has a dark brownish-orange carapace and the abdomen is dark grey with three distinct white markings (Figs. 2 A-D). The fangs of *M. chihuahuensis* are long and thin whereas *M. pueblaensis* **sp. nov.** has much shorter fangs.



**FIGURE 3.** Anterior views of *Myrmecicultor chihuahuensis* and *M. pueblaensis* **sp. nov.** females (male aspects are similar to females for both species). A) Anterior view of *M. chihuahuensis* female showing the high clypeus and the procurved AME (DMNS ZA.44217); B) Anterior view of *M. pueblaensis* **sp. nov.** female.

Description. Ecribellate entelegyne spider with two tarsal claws, lacking claw tufts; males with RTA (Fig. 5H); with six spinnerets in a tight group (Fig. 6B); female palpal claw well developed with several teeth in a row; AME and PME recurved (Figs. 2B, 2D, 3 B); PME oblong, oval with well-developed obliquely oriented tapeta (Figs. 2B, D); serrula on anterior border of endites (arrow Fig. 6A); endites elongate and medially depressed; dorsal apical patch of setae on cymbium of males (Fig. 5H) and palp of females (Fig. 6C); and distinct, precoxal triangles fused to sternum (Fig. 2F). Chelicerae with one retromarginal tooth and denticle with large diastema between; promargin with no teeth; lightly sclerotized area beside three large bristle-setae on promargin (Fig. 6A); short fangs with serrated edge; serrula on edge of endites (Fig. 6A, white arrow); anterior part of fangs translucent with venom duct evident (Fig. 6A). Genitalia as illustrated in Figs. 4 and 5.

Male holotype (UNAM CNAN-Ar011706): All measurements in mm. Total length 2.47. Carapace broadly oval in dorsal view, highest in front of fovea. Carapace length 1.06; width 1.00. Clypeus high, almost twice ALE diameter. Clypeus height at AME 0.19; at ALE 0.17. Fovea a narrow, dark longitudinal line. Carapace highest in cephalic region. Sternum shield-shaped with pointed extension between coxae IV. Precoxal triangles faint but present. Marginal setae on sternum. Labium length 0.12; width 0.23. Endite length 0.4; width 0.19. Serrula on anterior margin of endites. Eyes: AME diameter 0.10, ALE diameter 0.10, PME diameter 0.09, PME length 0.11, PLE diameter 0.09. Eye inter-distances: AME-AME 0.07, AME-PME 0.05, AME-ALE 0.02, PME-PME 0.03, PME-PLE 0.08, ALE-PLE 0.04. AER 0.41. PER 0.48. Eyes ringed with black pigment; tapeta present in PME, PLE, and ALE. Leg I total 4.81 (femur 1.35, patella 0.40, tibia 1.16, metatarsus 1.19, tarsus 0.71). Leg II total 4.22 (femur 1.18, patella 0.38, tibia 1.00, metatarsus 1.04, tarsus 0.62). Leg III total 4.19 (femur 1.24, patella 0.38, tibia 0.87, metatarsus 1.10, tarsus 0.60). Leg IV total 5.65 (femur 1.62, patella 0.40, tibia 1.35, metatarsus 1.50, tarsus 0.78). Leg formula IV, I, II, III. Color: dorsal abdomen with two anterior large white dots and posterior white mark consisting partly of white flattened setae surrounded by dark greyish-black background (Fig. 2D). Additional flat white setae scattered on dorsum; ventral abdomen white; legs orange with femora slightly darker; carapace orange with scattered white setae, particularly on cephalon. All legs with single spine on proximal-dorsal surface of femora; no ventral spines. All legs with flattened, translucent setae on dorsum of femora. Metatarsi III & IV with preening brush (Fig. 6D). Thick, comb-like setae on all tarsi. Single trichobothria on distal dorsal metatarsi; several trichobothria on tarsi. Trochanter IV 1.3 times trochanter III. Palp as in Figs. 5D, E, F, H. Cymbium with dorsoapical patch of sensory setae (Fig. 5H). Conductor bifurcate at tip (Fig. 5D and embolus tube distinctly visible at tip (Fig. 5F).



**FIGURE 4.** Comparison of female genitalia of *M. chihuahuensis* (DMNS ZA.44217) and *M. pueblaensis* **sp. nov.** A) Female epigynum of *M. chihuahuensis*; B) Female epigynum of *M. pueblaensis* **sp. nov.**; C) Cleared dorsal epigynal structures of *M. chihuahuensis*; D) Cleared dorsal epigynal structures of *M. pueblaensis* **sp. nov.**; E) cleared ventral epigynal structures of *M. chihuahuensis*; F) cleared ventral epigynal structures of *M. pueblaensis* **sp. nov.** Scale bars = 500 μm.



**FIGURE 5.** Comparison of left palps of males. All views for *M. chihuahuensis* are of paratype DMNS ZA.41786; views for *M. pueblaensis* **sp. nov.** are of the holotype. A) Ventral view of *M. chihuahuensis*; B) Prolateral view of *M. chihuahuensis*; C) Retrolateral view of *M. chihuahuensis*; D) Ventral view male palp of *M. pueblaensis* **sp. nov.**; E) Prolateral view of *M. pueblaensis* **sp. nov.**; F) Retrolateral view of *M. pueblaensis* **sp. nov.**; G) RTA of *M. chihuahuensis* with distal setal patch visible (arrow); H) RTA of *M. pueblaensis* **sp. nov.** palpal tarsus with distal setal patch visible (arrow). Scale bars = 500 μm; Co = conductor; Em = embolus.



**FIGURE 6.** Additional diagnostic morphology of *M. pueblaensis* **sp. nov.** Female paratype (DMNS ZA.46195). A) Dorsal view of chelicerae, white arrow points to serrula visible on anterior edge of endites; B) Spinnerets; C) Patch of palpal setae on female pedipalp. Scale bars =  $500 \mu m$  for A-C. D) Preening brush of Leg III, scale bar =  $1 \mu m$ .

Female allotype (DMNS ZA.46195; largest female in lot, separated from other paratypes): Total length 3.68. Carapace length 1.5; width 1.29. Clypeus at AME 0.30; at ALE 0.21. Fovea a narrow, dark longitudinal line. Carapace highest in cephalic region. Sternum shield-shaped with pointed extension between coxae IV. Precoxal triangles distinct (Fig. 2F). Marginal setae on sternum. Labium length 0.18; width 0.28. Endite length 0.55; width 0.19. Serrula on anterior margin of endites. Eyes: AME diameter 0.13, ALE diameter 0.12, PME diameter 0.10, PME length 0.13, PLE diameter 0.09. Eye inter-distances: AME-AME 0.10, AME-PME 0.09, AME-ALE 0.03, PME-PME 0.06, PME-PLE 0.12, ALE-PLE 0.05. AER 0.50. PER 0.60. Tapeta present in PME, PLE, and ALE. Leg I total 5.87 (femur 1.59, patella 0.51, tibia 1.32, metatarsus 1.55, tarsus 0.90). Leg II total 5.26 (femur 1.46, patella 0.51, tibia 1.10, metatarsus 1.44, tarsus 0.75). Leg III total 5.09 (femur 1.37, patella 0.51, tibia 1.03, metatarsus 1.51, tarsus 0.67). Leg IV total 6.74 (femur 1.92, patella 0.52, tibia 1.60, metatarsus 1.88, tarsus 0.82). Leg formula IV,I,II,III. Color: dorsal abdomen with two anterior large white dots and posterior white mark consisting partly of white flattened setae surrounded by dark greyish-black background (Fig. 2B). Additional flat white setae scattered on dorsum; ventral abdomen white; legs orange with femora slightly darker; carapace orange with scattered white setae, particularly on cephalon. All legs with single spine on proximal-dorsal surface of femora; no ventral spines. All legs with flattened, translucent setae on dorsum of femora. Metatarsi III & IV with preening brush, although brush on IV is reduced. Thick, comb-like setae on all tarsi. Single trichobothria on distal dorsal metatarsi; several trichobothria on tarsi. Trochanter IV 1.94 times trochanter III. Female ventral and dorsal epigynal structures as in Figs. 4B, D, F: two small copulatory openings lead internally to copulatory tubes. Spermathecae widely separated (Figs. 4D, F).

*Male* paratype (DMNS ZA.46195; male placed in vial with female paratypes described below): Color, markings, setal characters, body shape, leg formula as for the holotype except leg femora not noticeably darker and pre-coxal triangles present but faint. Measurements as follows. Total length 2.56. Carapace length 1.17; width 0.98. Clypeus at AME 0.24; at ALE 0.16. Labium length 0.14; width 0.23. Endite length 0.39; width 0.15. Eyes: AME diameter 0.09, ALE diameter 0.09, PME diameter 0.10, PME length 0.11, PLE diameter 0.09. Eye inter-distances: AME-AME 0.09, AME-PME 0.04, AME-ALE 0.03, PME-PME 0.04, PME-PLE 0.10, ALE-PLE 0.02. AER 0.41. PER 0.48. Leg I total 4.93 (femur 1.40, patella 0.40, tibia 1.17, metatarsus 1.25, tarsus 0.71). Leg II total 4.19 (femur 1.12, patella 0.38, tibia 0.94, metatarsus 1.11, tarsus 0.64). Leg III 4.14 (femur 1.11, patella 0.39, tibia 0.83, metatarsus 1.19, tarsus 0.62). Leg IV total 5.47 (femur 1.56, patella 0.41, tibia 1.30, metatarsus 1.50, tarsus 0.70).

Female paratypes (DMNS ZA.46195, 4 females placed in with male paratype): Color, markings, setal characters, body shape, leg formula as for allotype except one female has both marginal setae on sternum as well as scattered setae on center area of sternum. Measurements as follows. Total lengths: 3.75, 3.20, 2.97, 2.87 (all females,  $\bar{x}$ including allotype = 3.29). Carapace lengths: 1.34, 1.32, 1.39 (all females,  $\bar{x} = 1.37$ ). Carapace widths: 1.22, 1.16, 1.10, 1.20 (all females,  $\bar{x}$  = 1.19). Clypeus at AME: 0.32, 0.25, 0.25, 0.27 (all females,  $\bar{x}$  = 0.28). Clypeus at ALE: 0.24, 0.17, 0.17, 0.20 (all females,  $\bar{x} = 0.20$ ). Labium lengths: 0.17, 0.17, 0.13, 0.17 (all females,  $\bar{x} = 0.16$ ). Labium widths: 0.29, 0.26, 0.32, 0.28 (all females,  $\bar{x} = 0.29$ ). Endite lengths: 0.44, 0.44, 0.49, 0.41 (all females,  $\bar{x} = 0.47$ ). Endite widths: 0.20, 0.19, 0.21, 0.20 (all females,  $\bar{x} = 0.20$ ). AME diameters: 0.13, 0.10, 0.10, 0.10 (all females,  $\bar{x} = 0.11$ ). ALE diameters: 0.10, 0.10, 0.11, 0.11 (all females,  $\bar{x} = 0.11$ ). PME diameters: 0.10, 0.10, 0.11, 0.08 (all females,  $\bar{x} = 0.10$ ). PME lengths: 0.15, 0.12, 0.13, 0.13 (all females,  $\bar{x} = 0.13$ ). PLE diameters: 0.11, 0.08, 0.10, 0.10 (all females,  $\bar{x} = 0.10$ ). AME-AME: 0.10, 0.08, 0.10, 0.10 (all females,  $\bar{x} = 0.10$ ). AME-PME: 0.07, 0.0.09, 0.07 (all females,  $\bar{x} = 0.08$ ). AME-ALE: 0.04, 0.04, 0.03, 0.03 (all females,  $\bar{x} = 0.03$ ). PME-PME: 0.03, 0.0.03, 0.05 (all females,  $\bar{x} = 0.04$ ). PME-PLE: 0.13, 0.12, 0.13, 0.11 (all females,  $\bar{x} = 0.12$ ). ALE-PLE: 0.06, 0.05, 0.04, 0.05 (all females,  $\bar{x} = 0.05$ ). AER: 0.59, 0.47, 0.48, 0.49 (all females,  $\bar{x} = 0.51$ ). PER: 0.59, 0.53, 0.52, 0.54 (all females,  $\bar{x} = 0.56$ ). Leg I totals: 5.51, 5.18, 4.98, 5.39 (all females,  $\bar{x} = 5.39$ ). Leg II totals: 4.86, 4.55, 4.55, 4.72 (all females,  $\bar{x} = 4.79$ ). Leg III totals: 4.77, 4.50, 4.40, 4.56 (all females,  $\bar{x} = 4.66$ ). Leg IV totals: 6.19, 5.75, 5.86, 6.03 (all females,  $\bar{x} = 6.11$ ).

Natural History: Spiders observed hunting in large groups made up of adult males, females, and juveniles in open areas of desert habitat. Ants seen in the vicinity with the spiders. Ants not collected or identified. One spider observed approaching ant from behind, biting rear leg, then retreating. This hunting behavior is essentially identical to the myrmecophagous behavior observed for M. chihuahuensis (Cushing et al. 2022). We suspect that the genus Myrmecicultor may be found throughout the desert habitats of southern Mexico and that additional species may be found in association with various species of ants as has been documented for M. chihuahuensis (Ramírez et al. 2019; Cushing et al. 2024).

#### **Data Availability Statement**

The sequence data for the mitochondrial and nuclear sequences have been uploaded to GenBank (https://www.ncbi.nlm.nih.gov/genbank/). The matrix, partition, and tree are available in Zenodo: https://zenodo.org/badge/DOI/10.5281/zenodo.15164897.svg (González-Santillán 2025).

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