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Myrmecicultoridae, a New Family of Myrmecophilic Spiders from the Chihuahuan Desert (Araneae: Entelegynae)

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ABSTRACT

The new genus and species *Myrmecicultor chihuahuensis* Ramírez, Grismado, and Ubick is described and proposed as the type of the new family, Myrmecicultoridae Ramírez, Grismado, and Ubick. The species is ecribellate, with entelegyne genitalia, two tarsal claws, without claw tufts, and the males have a retrolateral palpal tibial apophysis. Some morphological characters suggest a possible relationship with Zodariidae or Prodidomidae, but the phylogenetic analysis of six markers from the mitochondrial (12S rDNA, 16S rDNA, cytochrome oxidase subunit I) and nuclear (histone H3, 18S rDNA, 28S rDNA) genomes indicate that *M. chihuahuensis* is a separate lineage emerging near the base of the Dionycha and the Oval Calamistrum clade. The same result is obtained when the molecular data are combined with a dataset of morphological characters. Specimens of *M. chihuahuensis* were found associated with three species of harvester ants, *Pogonomyrmex rugosus, Novomessor albisetosis*, and *Novomessor cockerelli*, and were collected in pitfall traps when the ants are most active. The known distribution spans the Big Bend region of Texas (Presidio, Brewster, and Hudspeth counties), to Coahuila (Cuatro Ciénegas) and Aguascalientes (Tepezalá), Mexico.

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INTRODUCTION

Many arthropods live as symbionts or commensals in ant nests, which constitute close, protected habitats with a stable provision of resources (Cushing, 1997, 2012). The colonization of subterranean ant nests is frequently associated with evolutionary changes due to reduced selection pressure on visual cues and senses, and increased pressure on chemical and tactile interactions with the ants (e.g., Dettner and Liepert, 1994; Elgar and Allan, 2004; Witte et al., 2008). One example of marked morphological transformation is the corinnid genus, *Attacobius* Mello-Leitão, associated with leaf-cutter ants (Roewer, 1935; Platnick and Baptista, 1995; Erthal and Tonhasca, 2001). With their pale brown uniform coloration and reduced eyes, they are so unlike regular corinnids that they have been placed in five families (Clubionidae, Liocranidae, Gnaphosidae, Prodidomidae, and Corinnidae). Arachnologists were so confused by *Attacobius* that not only was its family placement uncertain, but, as remarked by Bonaldo and Brescovit (2005), a handful of specimens produced no fewer than five generic names.

We describe here *Myrmecicultor chihuahuensis* Ramírez, Grismado, and Ubick, a new species and genus of a mysterious myrmecophilic spider collected from the Big Bend Region (Presidio, Brewster, and Hudspeth counties) of Texas, and near Cuatro Ciénegas in Coahuila, Mexico. This taxon puzzled nine spider taxonomists for over 15 years. The specimens were first discovered in pitfall traps in 1999 by Greg Broussard, a biology Master's student, while conducting a spider survey of the Dalquest Desert Research Station (DDRS). Broussard's major advisor, N.V.H., was unable to identify these specimens and suspected they represented a new species. The station is situated on the northeast corner of the Big Bend Ranch State Park and bisected by the Presidio/Brewster county line (Broussard and Horner, 2006; map 1). In 2008, David Lightfoot from the Museum of Southwestern Biology, University of New Mexico, observed several spiders on the surface of a nest of the harvester ant *Pogonomyrmex rugosus* Emery, near Cuatro Ciénegas, 335 km south of the DDRS site, with a similar Chihuahuan Desert habitat (fig. 11E, F). Lightfoot took several photographs, collected a small series, and showed them to Sandra Brantley, also at the Museum of Southwestern Biology. Brantley suggested they send them to N.I.P. at the American Museum of Natural History for identification, who recognized the specimens as the same spider species from the DDRS previously sent by N.V.H. Once the association with harvester ants was revealed, the annual collection of spiders at the DDRS more than doubled after setting pitfall traps a meter from the entrance of the ant nests.

Myrmecicultor chihuahuensis specimens have entelegyne female genitalia, a retrolateral tibial apophysis on the male palp, and the bases of the trichobothrial shafts bear an expansion covered by small bumps, all indicating they belong in the RTA clade (Griswold et al., 2005, Ramírez, 2014). Only two tarsal claws are present, and the tapeta of the posterior median eyes is characteristically oriented at 90°, a disposition considered typical of gnaphosids and related families by Homann (1952, 1971). This peculiar morphology is known to function as a sky compass in a gnaphosid species (Dacke et al., 1999), and may have the same function in a large clade of dionychan spiders (the "OMT clade"; see Ramírez, 2014). The species was included (as cf. Gnaphosoidea TEX) in a phylogenetic analysis of dionychan spiders using morphological data, but its relationships remained unresolved, with low support in the OMT clade, and its relationships and taxonomic associations changed with every subtle modification of the dataset (Ramírez, 2014: 331). With the addition of DNA sequence data, it became clear that the significance of the OMT character should be reevaluated. Wheeler et al. (2017) presented a phylogeny of spiders wherein most of the OMT clade appeared as monophyletic (labeled as Dionycha part A; see fig. 1), except for the Prodidomidae, which grouped as sister to the rest of Dionycha. Because prodidomids have typical gnaphosidlike tapeta in their posterior median eyes, this opened the possibility that the oblique tapeta represents a synapomorphy of all the Dionycha, later lost in the Dionycha part B.

Other than the eyes, the morphology of these spiders offers few clues as to their relationships. The long cheliceral fangs, the apical dorsal patch of chemosensory setae on the palpal tarsus and some details of the tarsus-metatarsus joint suggest a relationship with the subfamily Prodidominae, but their spinnerets, especially the anterior laterals, lack the specialization of those spiders, such as the elongated piriform-gland spigot bases flanked by long setae. On the other hand, the short posterior spinnerets of *M. chihuahuensis*, the strongly procurved anterior eye row, and the leg setae are similar to those of Zodariidae, a family frequently associated with ants (Jocqué, 1991; Pekár et al., 2011). However, *M. chihuahuensis* differs from zodariids in several characters: the major ampullate spigots of the anterior lateral spinnerets are situated laterally rather than centrally; the serrula on the endite is normally developed; and the legs lack a tibial process that fits on a metatarsal pouch (Ramírez et al., 2014; Jocqué and Henrard, 2015). In the present contribution, we analyze DNA sequences and morphological data to help determine the phylogenetic position of *M. chihuahuensis*. Although we did not arrive at a precise placement of this mysterious spider, we consider it time to make this remarkable species widely known.

MATERIAL AND METHODS

The format of description follows that of Ubick and Vetter (2005), except for macrosetae, as in Ramírez (2003). Morphological terms follow Ramírez (2014). Specimens are deposited in the collections of the American Museum of Natural History, New York (AMNH), the California Academy of Sciences Entomology, San Francisco (CAS), the Denver Museum of Nature and Science, Colorado (DMNS), the Museo Argentino de Ciencias Naturales "Bernardino Rivadavia," Buenos Aires (MACN-Ar), the Museum of Southwestern Biology, University of New Mexico, Albuquerque (MSBA), and Midwestern State University, Wichita Falls, Texas (MSU).

Samples were critical point dried and coated with gold-palladium for scanning electron microscopy (SEM) with a FEI-XL30 or Hitachi S-520 standard SEM, or a Hitachi-S4700 field emission SEM, all under high vacuum.

Morphological characters and outgroup were taken from Ramírez (2014), adding scorings for *Penestomus* (Penestomidae) and Zodariidae following Miller et al. (2010) and Ramírez et al. (2014); the morphology and combined data matrix are deposited in Zenodo (doi: 10.5281/zenodo.2648632). DNA sequences of six markers from the mitochondrial genome, 12S rDNA (12S), 16S rDNA (16S), cytochrome oxidase subunit I (COI) and the nuclear genome, histone

H3 (H3), 18S rDNA (18S), 28S rDNA (28S) were generated at the AMNH, using the primers and protocols provided in Wheeler et al. (2017). The new sequences are deposited in GenBank with accession numbers MK775712–MK775717. The taxon sampling was chosen to represent the major groups of the RTA clade, with a denser sampling of two groups sharing potential synapomorphies with *M. chihuahuensis* (zodarioids and prodidomids; see Introduction).

Sequences were aligned with MAFFT (Katoh and Standley, 2013) using the online platform at the European Bioinformatics Institute (http://www.ebi.ac.uk/Tools/msa/mafft/) with default parameters. Models for the six markers were selected using jModeltest (Darriba et al., 2012). The molecular data partition was then analyzed with MrBayes (Ronquist et al., 2012) using the CIPRES portal (Miller et al., 2010) for 20 million generations. The morphological data partition was analyzed under implied weights parsimony using TNT, with a concavity constant $k = 12$; k values between 3 and 30 were examined as well. The combined data partitions were analyzed with MrBayes, using the standard Mk model for the morphological partition, plus the sequence data partition as above.

The pitfall traps used for collecting consisted of two 18 oz. durable plastic cups, one inside the other. Using a gardening hand trowel, holes were dug to a depth that allowed the stacked cups to be inserted into the ground so that the rim of the upper cup was at ground level. Holes were backfilled, and soil packed around the cups. This design allowed removal of the upper cup while the lower cup remained in the ground to preserve the integrity of the hole. Approximately 300 ml of Sierra Antifreeze® (propylene glycol) was poured into the upper cup. The propylene glycol served as both a killing and preserving fluid, which allowed the traps to be collected approximately every 30 days. The traps were covered with flat stones rested on smaller stones to give 1 cm clearance, allowing insects and spiders free access to the trap. On collection dates, the upper cup was removed, and its contents strained through a fine mesh tea strainer. Specimens were stored in 95% ethanol and transported to the laboratory for separation and identification. The samples were sorted and stored in 70%, 95%, or 100% ETOH, the last group targeted for DNA analysis. A total of 140 traps were used in the collecting period from 1999 to 2016: 20 total traps were set in 1999 and 2000; from 2006–2016, 10 traps were set each year. In 2008, the association of the spiders with the ants was established and from 2009 to 2016, the traps were set approximately 1 m from ant-nest entrances, yielding larger numbers of spiders in the traps than previously caught when the traps were set randomly in the habitat. A total of 147 specimens were collected from these pitfalls, including 64 males, 37 females, and 46 immatures. The months of December to April were not sampled on a regular basis because the specimens were not found in the cooler months of the year, seemingly related to the inactivity of the ants.

From 9–12 June 2015, P.E.C., N.V.H., and two students excavated two *Novomessor albisetosis* (Mayr) nests near Alamo Spring (N 29.555″, W 103.785°, elevation 1137 m). The nest sites were in the South Fork of the Alamo de Cesario Creek, which normally is a dry creek bed, but may contain a large amount of water with rains of an inch or more. These nests of *N. albisetosis* were unlike those of *Pogonomyrmex,* which occur in more stable soils, in that the nest chambers did not branch off a single centralized underground runway, as is characteristic of the nests of many seed harvesting ant species. Rather, in *N. albisetosis* habitat, underground rocks ranging in size from 10 kg down to pea size forced the ants to spread the nest chambers out from a generally central area in a more horizontal fashion. Visual observations were made in the ant chambers using a flashlight, the loose contents taken from the nest were screened through a 6.25 mm wire mesh screen onto a white cloth, and organisms picked from the cloth with a camel hair brush.

SYSTEMATICS

The analysis of the sequence data partition (fig. 1) recovered the main clades of entelegyne spiders as in the comprehensive analyses of target sequence data by Wheeler et al. (2017). This is expected, as the analysis is based on the same data with the addition of sequences of *M. chihuahuensis*. The main clades are also compatible with the new analysis of transcriptomic data by Fernández et al. (2018). According to this analysis, *M. chihuahuensis* belongs to the RTA clade, sister to the Oval Calamistrum clade plus Dionycha.

The analysis of the morphological partition (fig. 2A) did not recover many of the higher groups, accentuating the problems found in the morphological analysis of Ramírez (2014), probably due to the shallow taxon sampling. In particular, the Oval Calamistrum clade and the sparassids all fall within dionychans, and *Penestomus* is far from zodarioids. The morphological data place *M. chihuahuensis* close to zodarioids, but with low support. As expected by the disorienting morphology of *M. chihuahuensis*, no clear family assignation arises from this data partition. The analysis of the combined data partitions (fig. 2B) basically reproduced the larger sequence data partition.

Given that the independently obtained transcriptomic data (Garrison et al., 2016; Fernández et al., 2018) largely agrees with our target sequence analysis, the molecular data partition appears to be a more reliable indicator of relationships in this dataset, compared to the morphology partition. Our phylogenetic analysis indicates that *M. chihuahuensis* is a member of the RTA clade, but it is not nested in any of its main groups, namely the dionychans, the Oval Calamistrum clade, or the zodarioids. For this reason, we create a new family.

The four putative morphological synapomorphies grouping *M. chihuahuensis* with Dionycha + the Oval Calamistrum clade are rather unconvincing: patellar indentation I–II width: narrow; apical ventral tarsal cuticle sclerotization: entire sclerotized; inferior tarsal claw I: absent; cymbium dorsal chemosensory patch: present (chars. 108, 131, 134, and 324 of Ramírez, 2014). All present several reversals and convergences in other taxa (consistency indices of 0.20, 0.09, 0.17, and 0.10, respectively). In particular, the absence of the inferior claw in *M. chihuahuensis* is probably an independent loss, because several members of the Oval Calamistrum clade possess three claws.

Myrmecicultoridae Ramírez, Grismado, and Ubick, new family

Type Genus. *Myrmecicultor*, new genus.

Diagnosis. Ecribellate entelegyne with two tarsal claws, without claw tufts, males with retrolateral palpal tibial apophysis, six spinnerets in a group, and female palpal claw well developed. It resembles zodariids in the procurved eye rows and the short posterior lateral spinnerets, but can be distinguished by having a serrula on the endites and the major ampullate gland spigots situated laterally rather than centrally. It resembles prodidomines in the procurved posterior eye row and the obliquely oriented tapetum on the posterior median eyes, but can be distinguished from those by having unmodified piriform gland spigots without flanking setae.

Myrmecicultor Ramírez, Grismado, and Ubick, new genus

Type Species. *Myrmecicultor chihuahuensis*, new species.

Etymology. The generic name is a combination of *myrmex* (ancient Greek for "ant") and *cultor* (Latin for worshiper, follower), and refers to the association between these spiders and their host ants. Gender masculine.

Diagnosis. By the characters of the family.

Description. See under the species description.

Myrmecicultor chihuahuensis Ramírez, Grismado, and Ubick, new species

Figures 1–12, map 1

Cf. Gnaphosoidea TEX, Ramírez, 2014 (provisional name in phylogenetic analysis).

Types. Male holotype (CAS 9067289; temporary preparations CJG-1504, 1508), from the United States: Texas, *Brewster Co.*, Dalquest Desert Research Station (DDRS), Big Sandy #4, N 29°33′05.7″, W 103°47′38.3″, 1232 m, Oct. 16–Nov. 1, 2014, N.V. Horner, propylene glycol pitfall traps, in 100% ETOH. Male and female paratypes (CAS 9067288), DDRS, Big Sandy #3, N 29°33′04.2″, W 103°47′38.3″, elev. 1232 m, Sept. 24–Oct. 15, 2014, N.V. Horner, in 100% ETOH. Female paratype (CAS 9067293; temporary preparation CJG-1505), DDRS, below Alamo #4, N 29°33′20.00″, W 103°47′04.7″, 1143 m, Oct. 16–Nov. 1, 2014, N.V. Horner, propylene glycol pitfall traps, in 100% ETOH. Female paratype (AMNH [ARANP19], temporary preparations CJG-620, 632, MJR-1382–1383), DDRS, Sandy Canyon Sept. 22–Oct. 4, 2005, N.V. Horner and G.H. Broussard, pitfall covered, in propylene glycol.

Etymology. The specific epithet is an adjective referring to the Chihuahuan Desert, where most specimens were collected.

Diagnosis. By the characters of the family.

Male (holotype): Total length 2.89. Carapace length 1.47, width 1.20. Clypeus 0.26 (at AME), 0.19 (at ALE). Eye sizes and interdistances: AME 0.13, ALE 0.10, PME 0.13, PLE 0.09, AME-AME 0.06, AME-PME 0.08, AME-ALE 0.02, PME-PME 0.03, PME-PLE 0.07, ALE-PLE 0.02, AER 0.39, PLE-PLE 0.03, PER 0.43. Leg lengths: Leg I: total 4.70 (femur 1.55, patella 0.56, tibia 1.32, metatarsus 1.27, tarsus broken); Leg II: 4.98 (1.32 0.56 1.10 1.18 0.78); Leg III: 4.43 (1.19 0.49 0.97 1.13 0.65); Leg IV: 5.70 (1.47 0.56 1.29 1.55 0.83). Leg formula 4123. Color in alcohol: Carapace whitish, black in eye region; sternum whitish. Abdomen uniformly whitish both dorsally and ventrally. Legs also whitish, but distal podomeres slightly darker. Live specimens darker, with pale

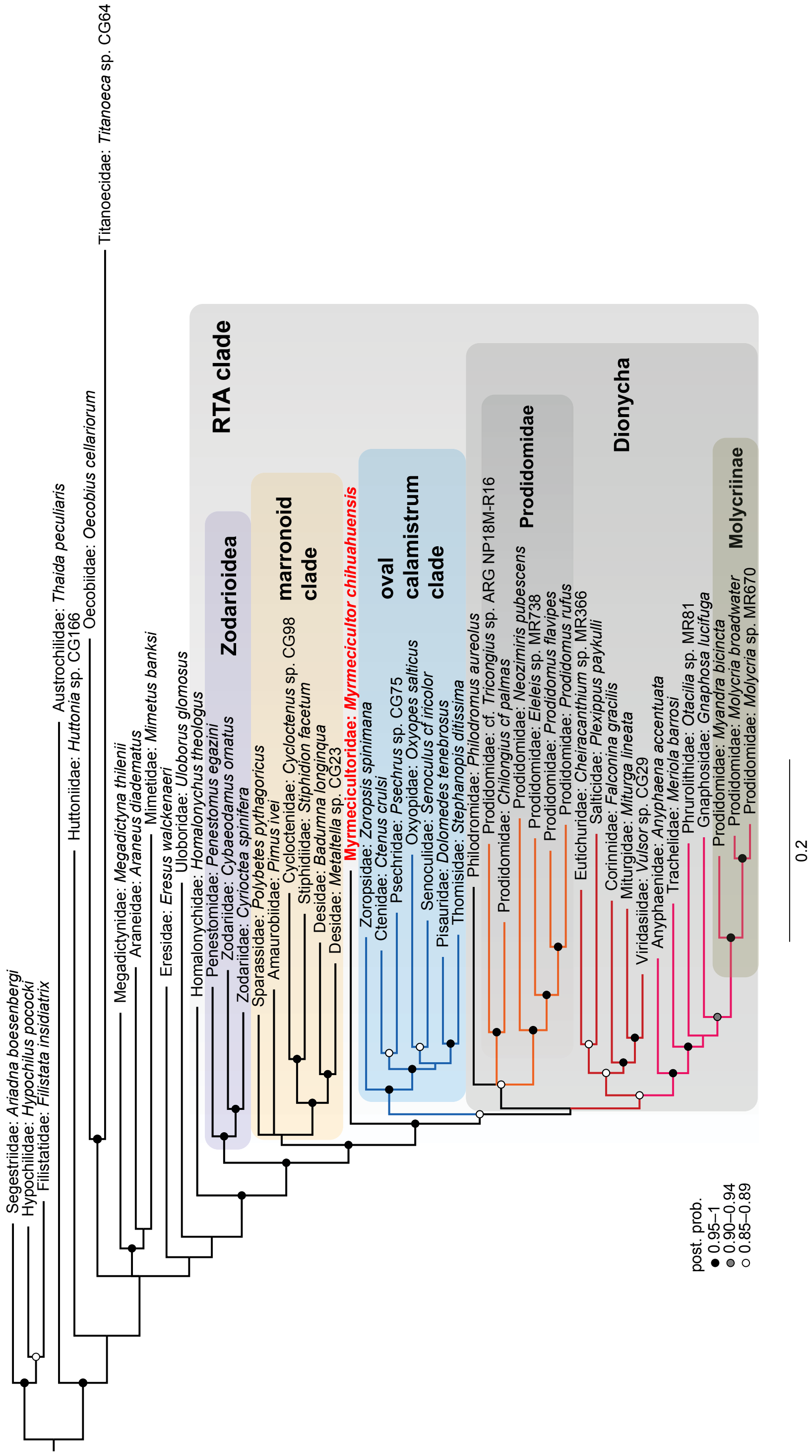


FIG. 1. Phylogenetic tree obtained from the Bayesian analysis of sequence data from six markers in the mitochondrial (12S rDNA, 16S rDNA, cytochrome oxidase subunit I) and nuclear (histone H3, 18S rDNA, 28S rDNA) genomes. Circles on nodes indicate support values.

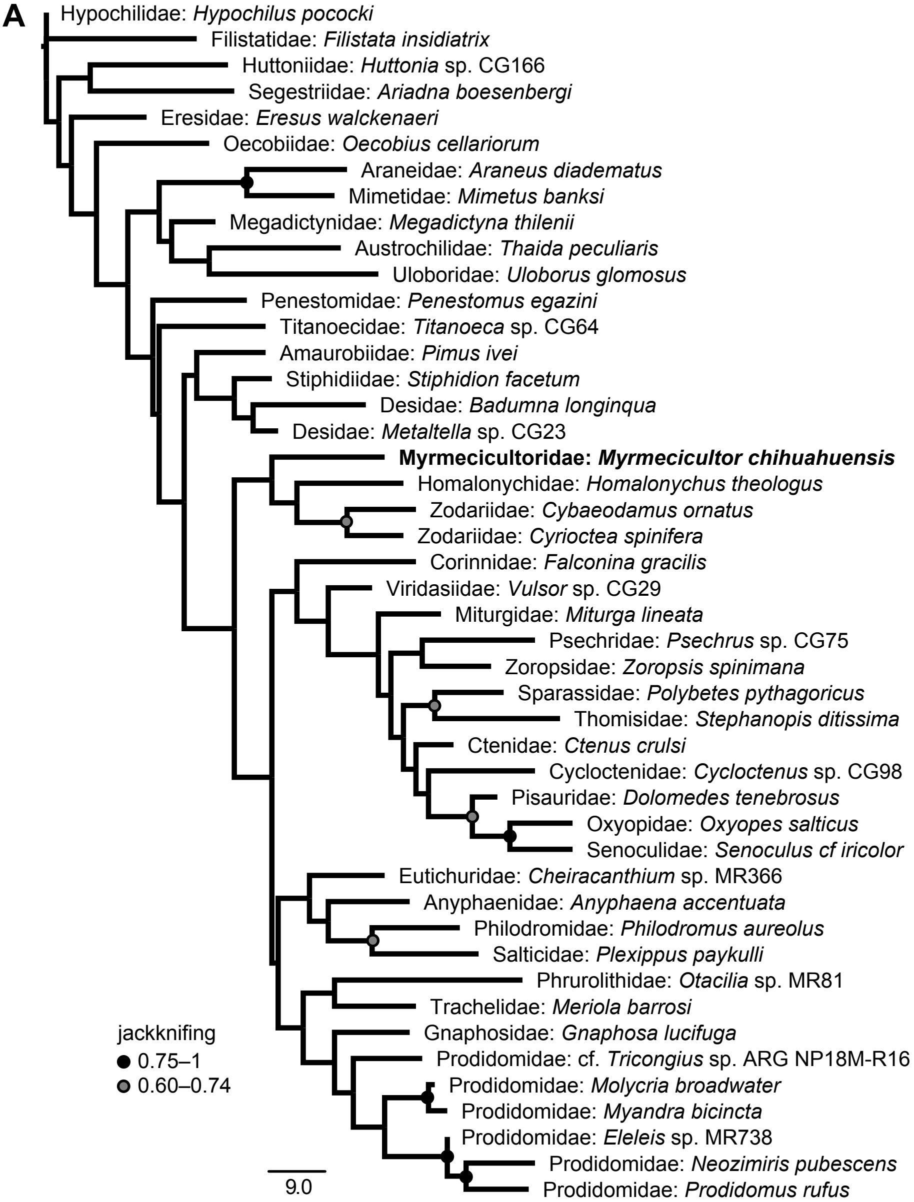


FIG. 2. **A.** Phylogenetic tree obtained from the parsimony analysis of morphological data under implied weights; the tree shown under concavity constant *k* = 12 is also the majority-rule consensus of trees obtained with values of *k* between 3 and 30. **B.** Phylogenetic tree obtained from the Bayesian analysis of sequences and morphological data. Circles on nodes indicate support values.

brown coloration (fig. 11F). Vestiture: Carapace almost glabrous, chelicerae with few needlelike setae at promargin. Sternum with marginal setae, some setae on posterior extension. Abdomen dorsum with two types of setae, mostly needlelike, with some thickened setae scattered irregularly; appendages densely clothed with thick setae, but with few spines (see below); metatarsi III

and IV with distal preening brush of plumose setae. Carapace broadly oval in dorsal view, highest in front of fovea; thoracic fovea narrow, dark longitudinal line. Eyes mostly surrounded by black pigment, contrasting sharply with pale carapace (fig. 4D); AME largest, most convex, lateral eyes subequal, partly surrounded by black pigment, PME nearly rectangular, with oblique tapeta at 90° angle. AER procurved in dorsal view, PER strongly procurved in dorsal view; both eye rows procurved in anterior view. Clypeus high, about twice ALE diameter (fig. 4C). Chelicerae as in female. Sternum heart shaped, anteriorly truncate, with posterior pointed extension between coxae IV, with marginal setae. Precoxal triangles absent. Labium trapezoidal, wider than long; endites quadrate, with serrula on anterior margin. Abdomen lacking dorsal scute. Epiandrum with two spigots (observed in KOH-digested specimen). Respiratory system consisting of two book lungs and wide tracheal spiracle slightly separated from spinnerets, giving origin to four simple, unbranched tracheae, all limited to abdomen, gradually attenuated. Spinnerets superficially as in female, but not examined with SEM; PMS apparently lacking spigots (as observed with stereomicroscope). Leg spines: I: femur d1-1-0, p1-0-0, metatarsus v2ap; II: femur 1-1-0, p1 (very small)-0-0, metatarsus v2ap; III: femur d1-1-0, tibia v2ap, metatarsus v2ap (below preening brush); IV: femur d1-1-0, tibia v2ap, metatarsus v2ap (below preening brush). Palp: femur with 1-1 dorsal macrosetae, lacking ventral process; tibia cup shaped, with RTA forming procurved concavity with retrolateral dorsal small, pointed protrusion; cymbium with dorsoapical patch of chemosensory setae (fig. 9G) situated in a cavity, dorsal surface with widened bipectinate setae (probably scales) (fig. 9F), and seven trichobothria in two longitudinal rows. Copulatory bulb: subtegulum simple, visible from retrolateral side; tegulum simple bearing spermophore without loops; median apophysis articulated, small hook in retrobasal position; two conductors (C1 and C2; figs. 9D, 10D), latter larger than former, more sclerotized, coiled, anteriorly directed. Embolus articulated, bent at right angle to retrolateral side; tip widened.

Female (paratype CAS 9067293): Total length 2.73. Carapace length 1.26, width 1.09. Clypeus 0.13 (at AME), 0.07 (at ALE). Eye sizes and interdistances: AME 0.14, ALE 0.09, PME 0.12, PLE 0.09, AME-AME 0.05, AME-ALE almost touching, AME-PME 0.06, PME-PME 0.02, PME-PLE 0.06, ALE-PLE 0.03, AER 0.37, PER 0.41. Palpus and leg lengths: Palpus: 1.35 (0.39 0.21 0.29 0.46); Leg I: 4.86 (1.33 0.51 1.14 1.09 0.79); Leg II: 4.27 (1.12 0.45 0.97 1.02 0.71); Leg III: 3.91 (1.03 0.40 0.84 1.03 0.61); Leg IV: 5.04 (1.28 0.49 1.16 1.35 0.75). Leg formula 4123. Leg spines: Palp: tarsus d1-1; I: femur d1-1-0, p1 (very small)-0-0; metatarsus v1ap; II: femur 1-1-0; metatarsus v1ap; III: femur d1-1-0, metatarsus v1ap (below preening brush); IV: femur d1-1-0, tibia v1ap, metatarsus v1ap (below preening brush, fig. 6B). Tarsal organ (observed on leg II) as simple pit, not domed (fig. 7C). Patellar indentation narrow, leading to retrolateral lyriform organs (fig. 7A). Tarsal step opposing metatarsal sensory organ with irregular, mainly longitudinal ridges (fig. 6G). Two tarsal claws, pectinate, without claw tuft or any other adhesive setae (fig. 6E, F); only two large setae with many barbs oriented distally, but without expanded tips. Trichobothrial shaft with expanded base covered by bumps, hood with several proximal transverse ridges (fig. 7B), on single row on leg tarsi. Palpal claw well developed, with teeth, tarsus apically with dorsal cavity containing a patch of chemosensory setae (fig. 5F, G). Color and general morphology (fig. 3) as in male. Vestiture as in male, widened bipectinate setae as that of male cymbium present on prolateral sides of palpal tibia and tarsus. Chelicerae slightly expanded anteriorly, anterior surface (fig. 5B, D) with row of rake setae and row of whisker setae parallel to fang, one promarginal whisker seta large (the promarginal escort seta), weak promarginal mound in front of fang base, promargin with one very small tooth (seen in male digested with KOH), retromargin without teeth, retromarginal escort seta present (socket observed, fig. 5E). Cheliceral fang very long, falcate, flat, with shaft serrula, venom duct opening present (fig. 5B–E). Genitalia: epigynum as single sclerotized plate with unpaired, shallow median concavity (fig. 3E). Posterior margin sinuous, with two rounded cavities (fig. 10F). Copulatory openings not visible, probably near central depression. Copulatory ducts fused anteriorly, with tortuous lumen, leading to simple spermathecae, copulatory duct and spermatheca together in uniformly sclerotized, solid piece (fig. 10F). Spinnerets small, clustered together, arising from slightly projecting base (fig. 8). ALS with basal articles conical, adjacent to each other, distal articles crescent-shaped, sclerotized, ectal area with setae (fig. 8C); seven piriform gland spigots tightly grouped, with shafts well defined from base, two major ampullate gland spigots with shafts larger than those of piriforms; no setae associated with piriform bases. PMS, PLS much shorter than ALS. PMS conical, with single spigot with thick shaft, probably cylindrical gland spigot. PLS with basal spigot with thick shaft, probably cylindrical gland spigot, and four smaller ones, probably aciniform gland spigots.

Additional Material Examined. **MEXICO: Coahuila:** *Municipio de Cuatrociénegas de Carranza*: Cuatrociénegas Protected Area, ca. 9.7 km S Cuatrociénegas, N 26°54′31.896″, W 102°07′6.718″, Sep. 23, 2011, D.C. Lightfoot, 3♂ (MSBA 51822). **Aguascalientes:** *Municipio de Tepazalá:* 12 km (7.5 mi) N Tepezalá, Aug. 30, 1965, W. Gertsch, R. Hastings, 3♂ (AMNH; discovered by N.I.P. in May of 2019 while sorting unidentified specimens). **U.S.A.: Texas:** *Hudspeth Co.:* Indio Mountains Research Station (UTEP), N 30°46′34.7″, W 105°01′09.3″, Jul. 28–Aug. 30, 2017, N.V. Horner, 3♂, 1 juv. (CAS 9063001). *Presidio Co.:* Big Bend Ranch State Park: Ojito Adentro, N 29°29.5′, W 104°03.7′, Oct. 14, 2000, under rock, P.W. Hyder, 3♀ (AMNH 1256). DDRS Camp, N 29°33′25.91″, W 103°47′37.95″, 1266 m (4154 ft), propylene glycol pitfall trap [PGPT], G.H. Broussard: Sep. 5, 1999, 1♂ (AMNH); Sep. 15, 2000, 1♀ (AMNH). White Canyon Rd. #6, N 29°33′46.9″, W 103°48′03.2″, 1273 m (4177 ft), PGPT, N.V. Horner: Aug. 15–Sep. 9, 2015, 1♂ (MSU); Oct. 4–20, 2015, 1♀ (MSU). *Brewster Co.:* Below Alamo Springs, N 29°33′15.6″, W 103°47′08.4″ to N 29°33′17.8″, W 103°47′11″ to N 29°33′20″, W 103°47′04.7″, 1121–1158 m (3742–3798 ft), PGPT, N.V. Horner: May. 30–Jun. 30, 2014, 8 juv. (MSU), 7 juv. (CAS 9067292); Jul. 18–Aug. 17, 2012, 4♂ (CAS 9076507); Jul. 4–Aug. 12, 2013, 3 juv. (CAS 9067290, 9067291); Jul, 30–Sep. 2, 2014, 7♂ (MSU); Aug. 15–Sep. 9, 2015, 3♂ (MSU); Aug. 24–Oct. 1, 2016, 3♂, 1♀ (MACN-Ar 38650), 1♂ (MACN-Ar 38649); Sep. 2–27, 2007, 1♂ (AMNH); Sep. 21–Oct. 6, 2007, 1♀ (CAS 9076536); Sep. 14–Oct. 9, 2010, 1♀ (CAS 9076511); Sep. 10–Oct. 3, 2015, 8♂, 3♀ (MSU); Oct. 7–Nov. 5, 2012, 1♂, 1♀ (CAS 9076528); 1♀ (CAS 9076530); Oct. 16–Nov. 1, 2014, 1♀(CAS 9067293); Oct. 21–Nov. 21, 2015, 2♀ (MACN-Ar 38651, 38663); Main Canyon, N 29.55512°, W 103.78561°, 1137 m, inside ant nest, P.E. Cushing, N.V. Horner, K. Sanko, C. Dowling, 10–11 Jun 2015, 1♂ (DMNS ZA.40328), 11 Jun 2015, 1 juvenile (DMNS ZA.40329); same, N 29.55506°, W 103.78536°, 12 Jun 2015 (DMNS ZA.40330). *Brewster and Presidio Counties*: Sandy Canyon, [N 29°33′07″, W 103°47′36″, 1215 m (3988 ft)], PGPT, G.H. Broussard: Oct. 7, 1999, 2♂ (AMNH); Sep. 15, 2000, 2♂, 1♀ (CAS 9076539, ♂ used for SEM); Big Sandy Canyon, N 29°33′07.38″, W 103°47′37.51″ to N 29°33′01.2″, W 103°47′38.1″, 1207–1260m (3960–4133 ft), PGPT, N.V. Horner: May. 23–Jun. 16, 2010, 1 juv. (CAS 9076513, 1♂ used for SEM); May. 30–Jun. 30, 2014, 1♂, 1♀, 4 juv. (MSU); Jun. 27–Jul. 13, 2009, 1 juv. (AMNH); Jun. 17–Jul. 22, 2010, 1 juv. (CAS 9076510); Jun. 14–Jul. 9, 2011, 1 juv. (CAS 9076519); Jun. 21–Jul. 17, 2012, 6 juv. (CAS 9076522, 9076526); Jun. 25–Jul. 24, 2016, 4 juv. (MSU); Jul. 14–Aug. 22, 2009, 4♂ (AMNH); Jul. 23–Aug. 10, 2010, 2 juv. (CAS 9076512); Jul. 10–Aug. 12, 2011, 1 juvenile (CAS 9076532); Jul. 18–Aug. 17, 2012, 3♂ (CAS 9076516), 1♂, 1♀, 3

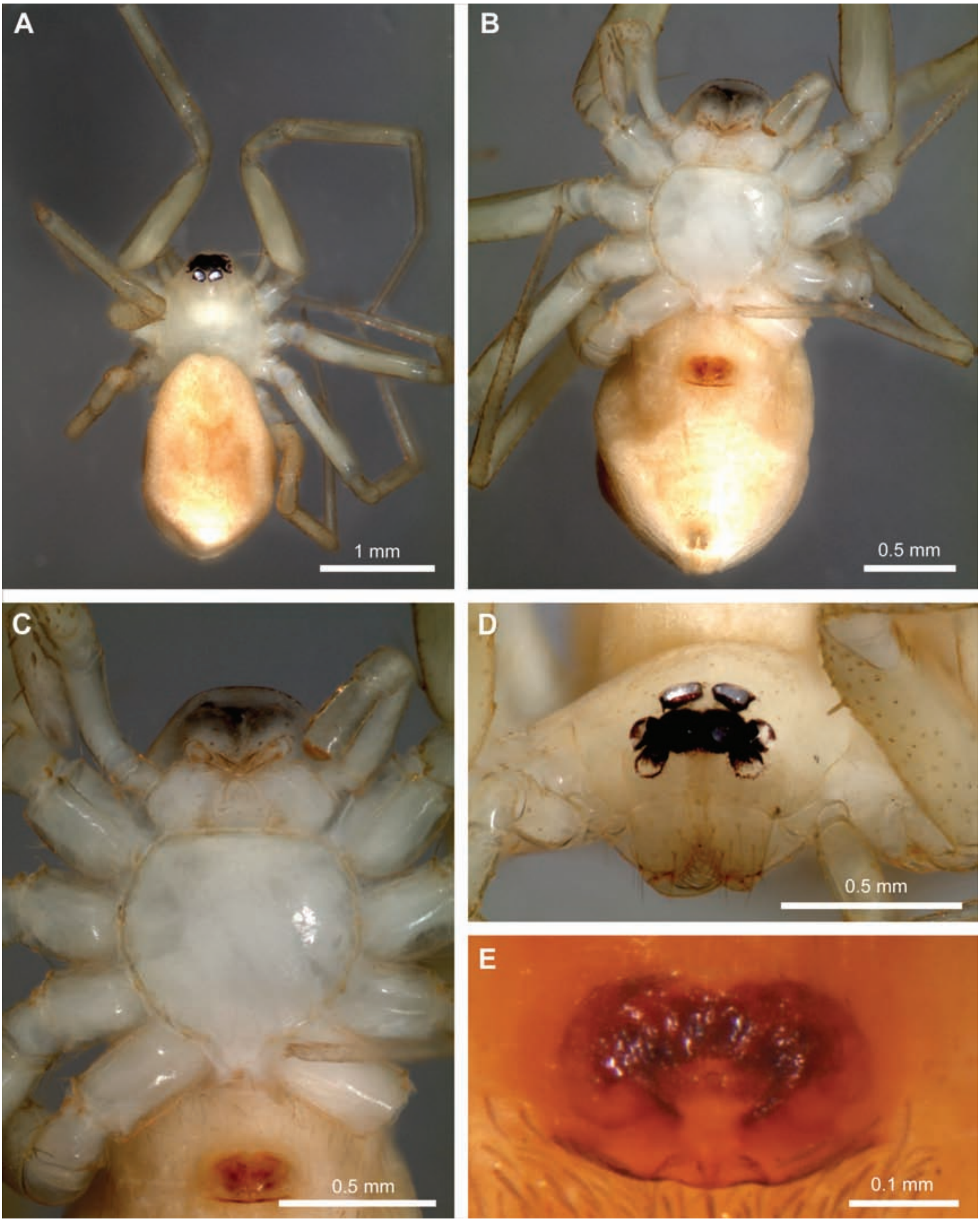


FIG. 3. *Myrmecicultor chihuahuensis*, new species, female structures. **A.** Habitus, dorsal view. **B.** Same, ventral view. **C.** Prosoma, ventral view. **D.** Same, anterior view. **E.** Epigyne, ventral view. (**A–D**, paratype CAS 9067293; **E**, AMNH [ARANP19]).

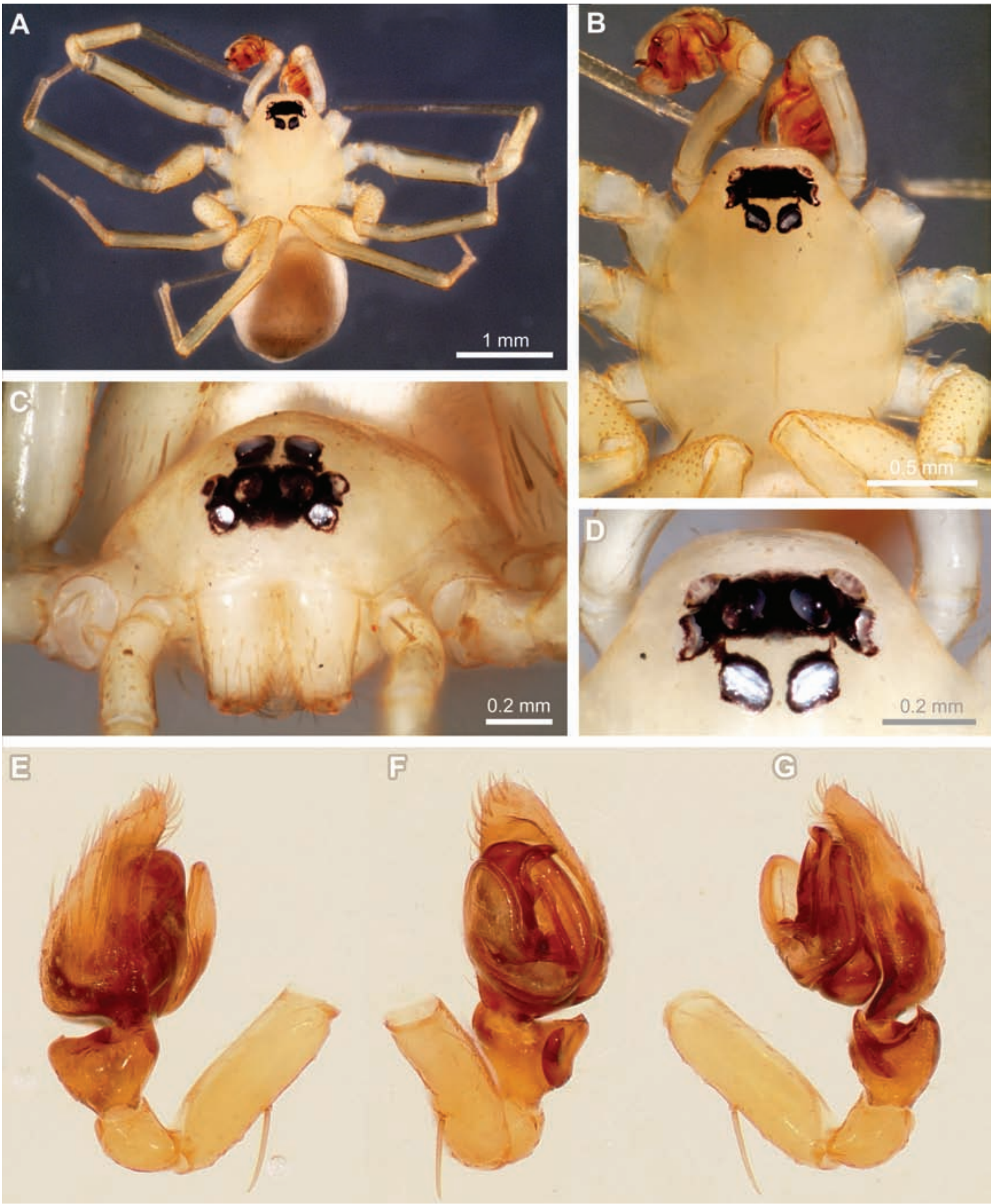


FIG. 4. *Myrmecicultor chihuahuensis*, new species, male structures. **A.** Habitus, dorsal view. **B.** Prosoma, dorsal view. **C.** Same, anterior view. **D.** Eyes, dorsal view. **E.** Right palp, mirrored image, prolateral view. **F.** Same, ventral view. **G.** Same, retrolateral view. (**A–D**, holotype; **E–G**, CAS 9076539).

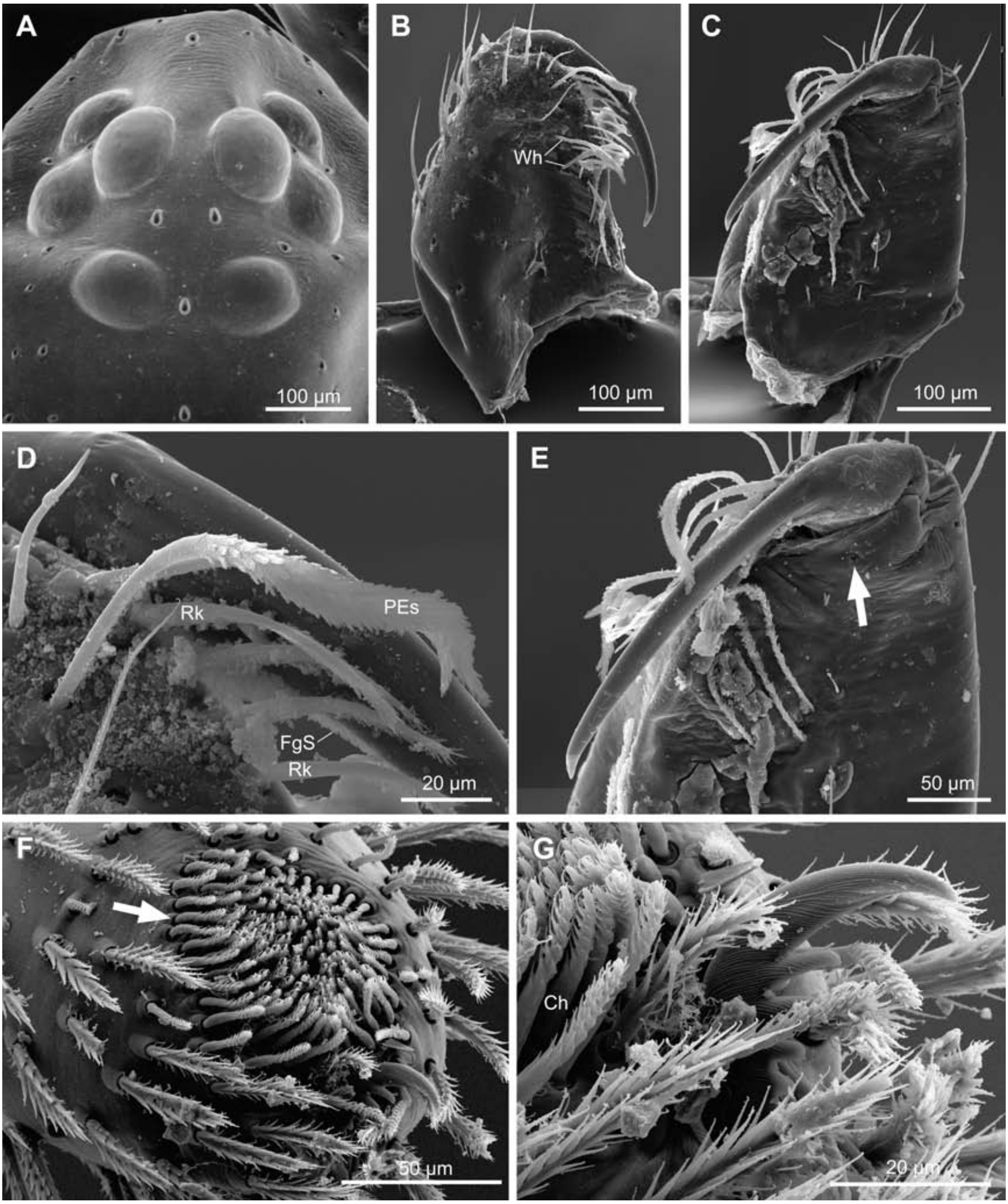


FIG. 5. *Myrmecicultor chihuahuensis*, new species, female structures. **A.** Eyes, dorsal view. **B.** Left chelicera, anterior view. **C.** Same, posterior view. **D.** Same, detail of promarginal setae and fang, anterior view. **E.** Fang and retromargin, posterior view, arrow to socket of retromarginal escort seta. **F.** Palpal tarsus tip, dorsal apical view, arrow to patch of chemosensory setae. **G.** Same, detail of claw and chemosensory setae. Abbreviations: Ch = chemosensory seta; FgS = fang shaft serrula; PEs = promarginal escort seta; Rk = promarginal rake seta; Wh = cheliceral whisker seta.

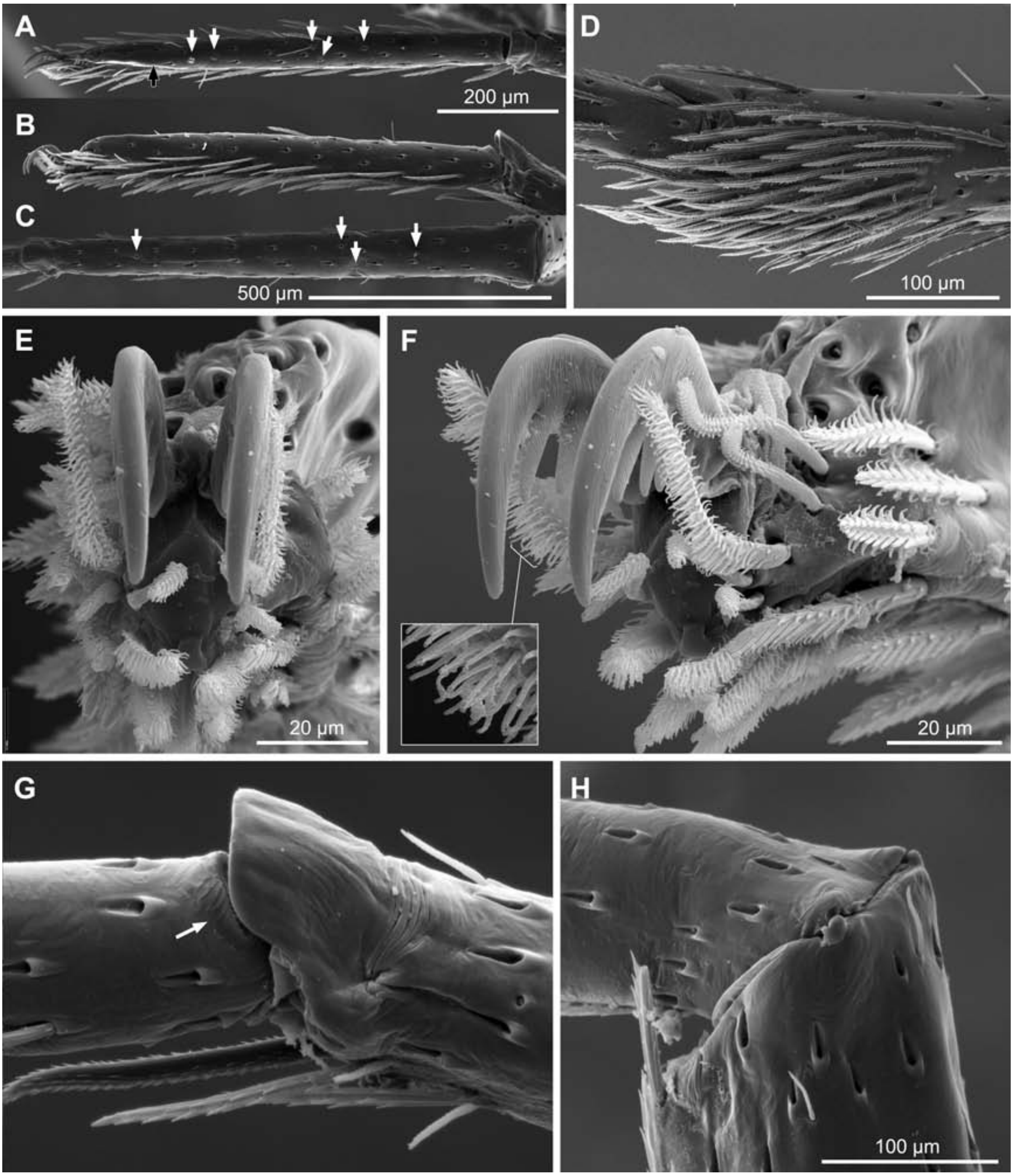


FIG. 6. *Myrmecicultor chihuahuensis*, new species, female structures. **A.** Left tarsus II, dorsal view, white arrows point to trichobothrial sockets, black arrow to tarsal organ. **B.** Same, retrolateral view. **C.** Metatarsus II, dorsal view, white arrows point to trichobothrial sockets. **D.** Tip of metatarsus III showing preening brush. **E.** Left claws II, apical view. **F.** Same, retrolateral apical view, inset to barbs of setae, showing unexpanded tips. **G.** Metatarsus-tarsus II articulation, arrow to irregular longitudinal ridges on tarsal step. **H.** Tibia-metatarsus II articulation.

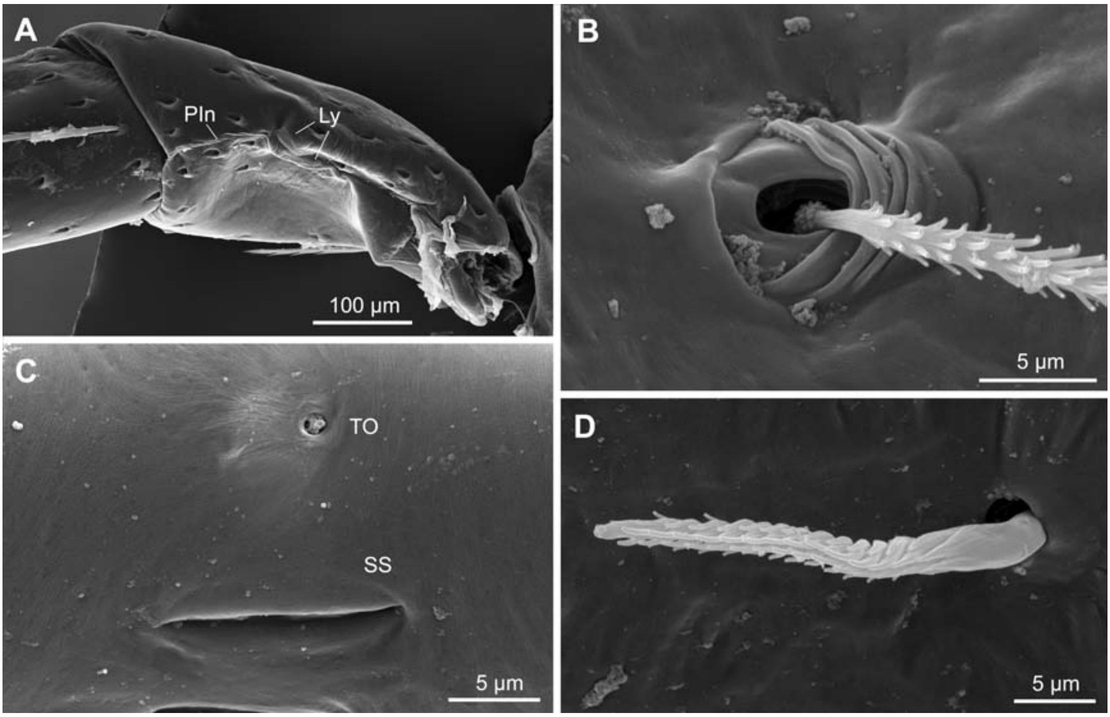


FIG. 7. *Myrmecicultor chihuahuensis*, new species, female structures of left leg II. **A.** Patella, retrolateral view. **B.** Trichobothrium on metatarsus. **C.** Tarsal organ and slit sensilla. **D.** Chemosensory seta on metatarsus. Abbreviations: PIn = patellar indentation; Ly = lyriform organ; TO = tarsal organ; SS = slit sensillum.

juv. (CAS 9076518); Jul. 30–Sep. 3, 2014, 2 juv. (MSU); Aug. 15–Sep. 3, 2006, 1♀ (MSU); Aug. 29–Sep. 19, 2008, 1♀ (CAS 9076523); Aug. 10–28, 2010, 3♂ (MSU); Aug. 29–Sep. 13, 2010, 1♀ (MSU); Aug. 13–Sep, 14, 2011, 2♂, 1♀ (CAS 9076533, 9076540, 9076537); Aug. 24–Oct. 1, 2016, 1♂ (MSU), 1♀ (MACN-Ar 38636); Sep. 22–Oct. 4, 2005, 1♀ (AMNH); Sep. 26–Oct. 10, 2006, 1♀ (MSU); Sep. 28–Oct. 9, 2008, 1♀ (CAS 9076535); Sep. 19–27, 2008, 2♂ (CAS 9076538); Sep. 14–Oct. 9, 2010, 2♂, 2♀ (CAS 9076509, 9076514, 9076517); Sep. 15–Oct. 19, 2011, 1♂, 2♀ (CAS 9076520, 9076525, 9076527); 24 Sep–15 Oct 2014, 4♂, 1 juv. (MSU), 1♂, 1♀ (CAS 9067288); 9 Oct–2 Nov 2008, 1♂, 2♀ (AMNH); 4–14 Oct 2009, 1♀ (AMNH); 6 Oct–5 Nov 2012, 4♀ (CAS 9076521, 9076529, 9076534); 16 Oct–1 Nov 2014, 1♂, 1♀ (DMNS ZA.40327), 1♂ (CAS 9067289).

Distribution. Known only from the Big Bend region (Presidio, Brewster and Hudspeth counties) of Texas, and in Mexico from Cuatro Ciénegas, Coahuila, and Tepezalá, Aguascalientes (map 1).

NATURAL HISTORY

Specimens of *M. chihuahuensis* were associated with three species of harvester ants, *Pogonomyrmex rugosus, Novomessor albisetosis*, and *Novomessor cockerelli* (André). In DDRS, the most abundant host species was *N. albisetosis,* which to date is the only species of harvester ant found in the dry creek bed of the South Fork of the Alamo de Cesario Creek. *Pogonomyrmex rugosus*

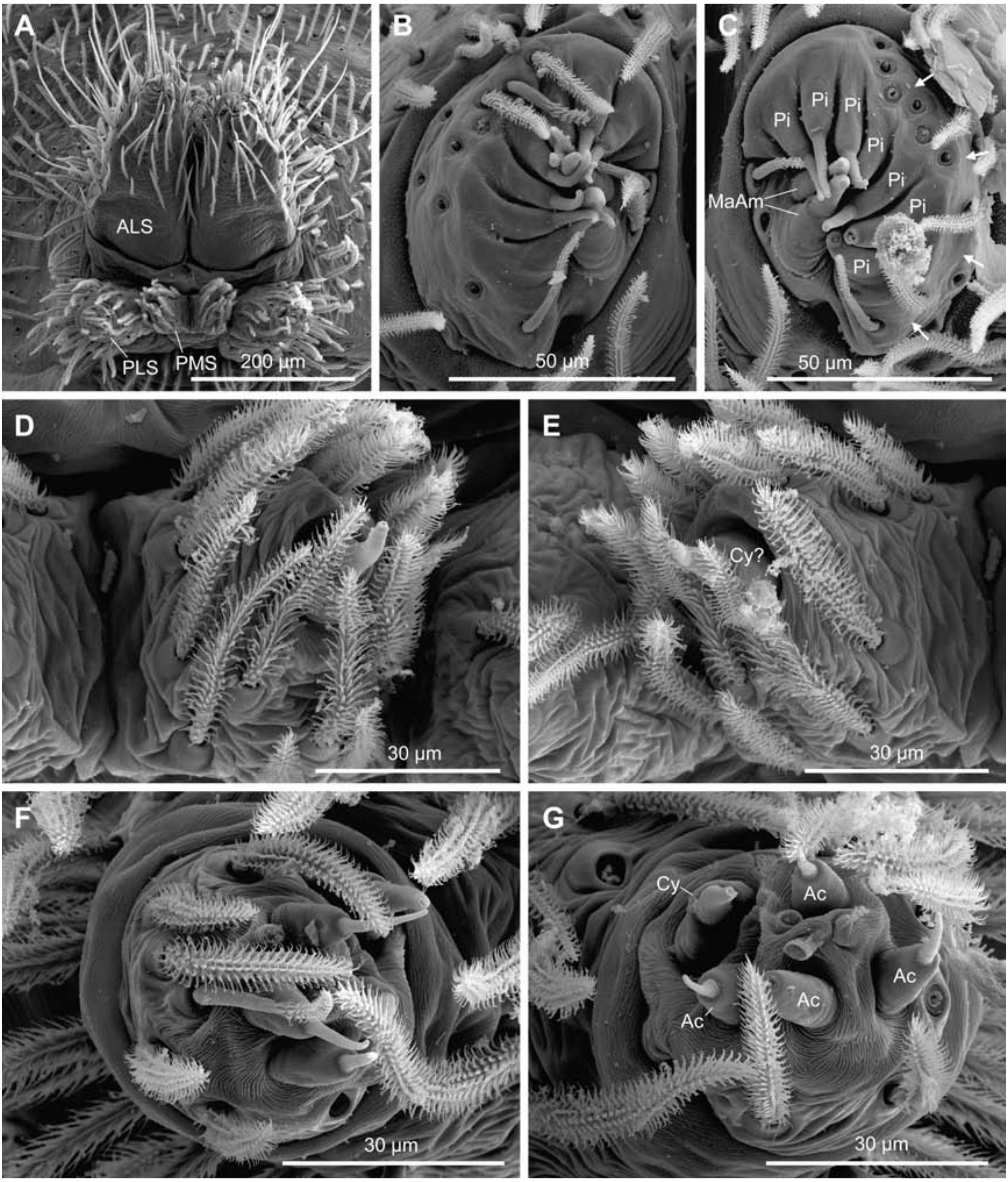


FIG. 8. *Myrmecicultor chihuahuensis*, new species, female spinnerets. **A.** Spinnerets, ventral-posterior view. **B.** Right ALS. **C.** Left ALS, arrows to sclerotized ectal area of distal article, bearing setae. **D.** Left PMS. **E.** Right PMS. **F.** Right PLS. **G.** Left PLS. Abbreviations: Ac = aciniform gland spigot; ALS = anterior lateral spinneret; Cy = cylindrical gland spigot; MaAm = major ampullate gland spigot; Pi = piriform gland spigot; PLS = posterior lateral spinneret; PMS = posterior median spinneret.

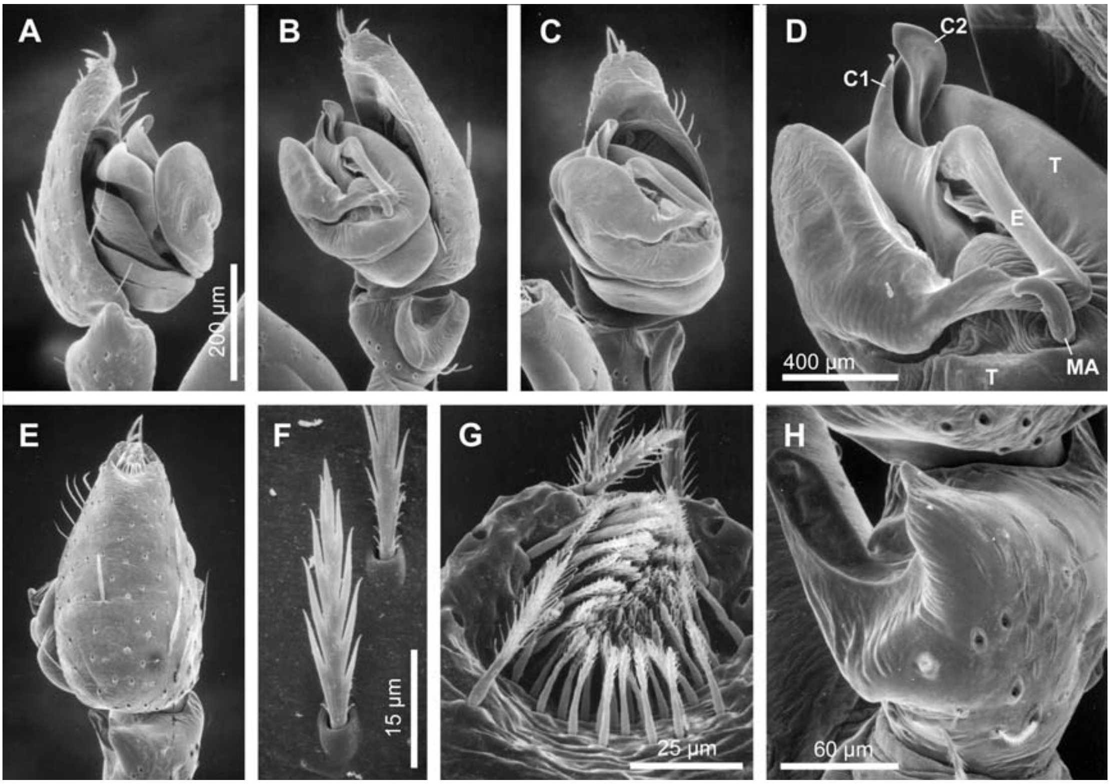


FIG. 9. *Myrmecicultor chihuahuensis*, new species, male left palp (CAS 9076539). **A.** Prolateral view. **B.** Same, retrolateral-ventral view. **C.** Same, ventral view. **D.** Same, detail of copulatory bulb, retrolateral-ventral view. **E.** Same, dorsal view. **F.** Same, detail of setae (probably scales). **G.** Tip of cymbium, detail of apical patch of chemosensory setae. **H.** Tibia and RTA, dorsal view. Abbreviations: C1, C2 = conductors; E = embolus; MA = median apophysis; T = tegulum.

and *N. cockerelli* were the hosts at higher elevations, where there was less moisture and a coarser soil type. The specimens collected in Hudspeth County were collected from the Indio Mountain Research Station operated by the University of Texas at El Paso. The host ant at this site was *N. cockerelli.* The specimens collected in Cuatrociénegas were on the surface of a *P. rugosus* nest. Two *M. chihuahuensis* were collected from one nest excavation pit and one from the other excavation pit. Because of the uncommon underground structure of these nests, it was not possible to determine whether the spiders were living inside nest chambers, although it is likely that they were.

DDRS Ant Habitats

The site known as White Canyon Road leads to the edge of the canyon, N 29°33′47.6″ W 103°48′09.8″, at an elevation of 1272 m where the ant *N. cockerelli* was found and only two spiders were collected. Dominant vegetation at this site included creosote bush (*Larrea tridentata),* cat claw (*Acacia neovernicosa*), and lechuguilla (*Agave lechuguilla*). The soil was poor, mostly desert pavement.

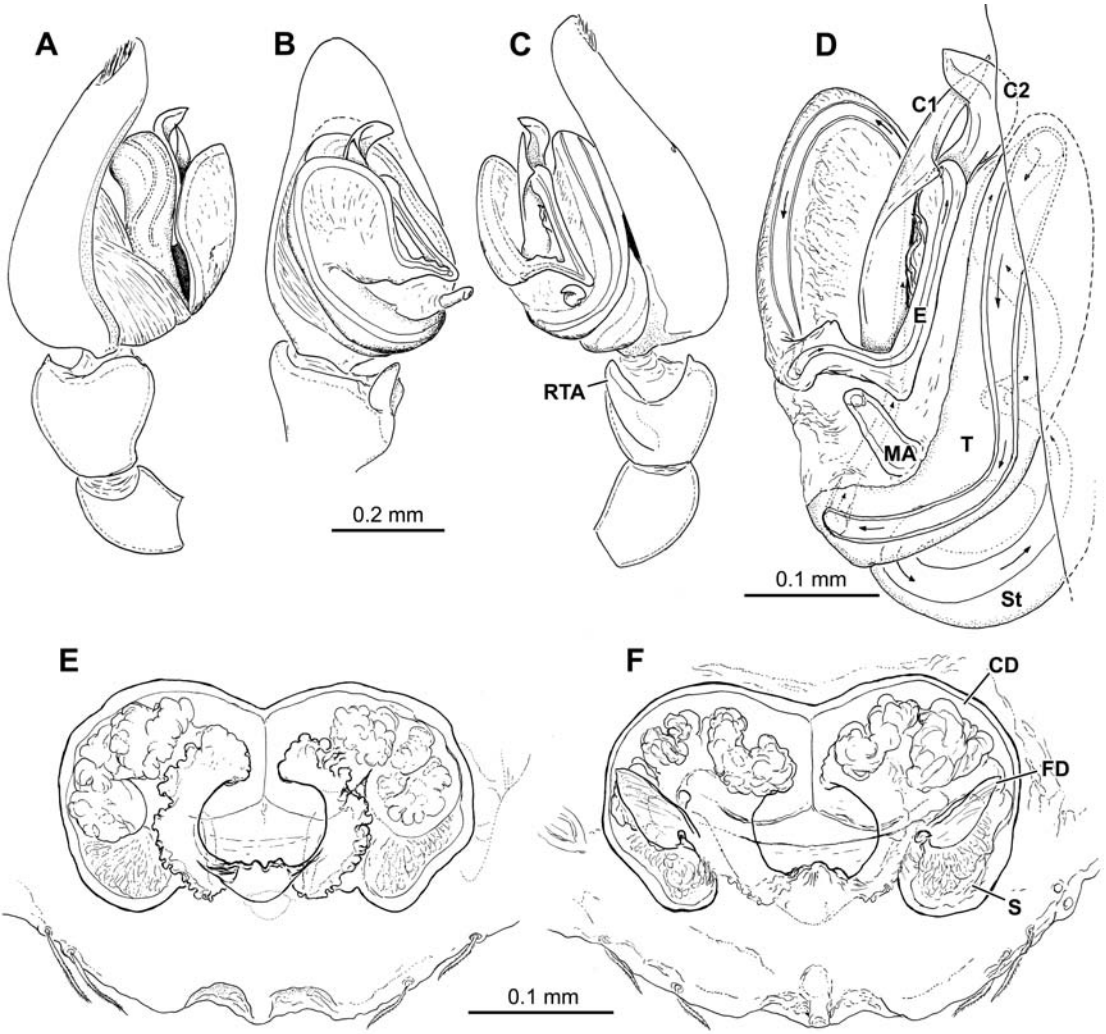


FIG. 10. *Myrmecicultor chihuahuensis*, new species, genitalia. **A.** Left male palp, prolateral view. **B.** Same, ventral view. **C.** Same, retrolateral view. **D.** Same, detail of copulatory bulb, cleared. **E.** Female genitalia, ventral view, cleared. **F.** Same, dorsal view. (**A–E**, CAS 9067289; **E–F**, AMNH [ARANP19]). Abbreviations: C1, C2 = conductors. CD = copulatory duct; E = embolus; FD = fertilization duct; MA = median apophysis; RTA = retrolateral tibial apophysis; S = spermatheca; St = subtegulum; T = tegulum.

An area known as the Camp Site was next to the canyon edge, N 29°33′25.2″ W 103°47′33.5″, at an elevation of 1276 m. This was the primary site for the ant *P. rugosus*. Few spiders were collected from this site. The dominant vegetation was creosote bush (*Larrea tridentate),* tarbush (*Vachellia vernicosa*), and lechuguilla (*Agave lechuguilla*). Some soil was present with mixed desert pavement.

The creek bed in the canyon, N 29°33′18.8″ W 103°47′08.4″, at an elevation of 1118.6 m, was the primary site for the ant *N. albisetosis.* The vast majority of the spiders were collected near nests of this species. This site was characterized by mixed vegetation dominated by

FIG. 11. Nests and habitats of harvester ants and *Myrmecicultor chihuahuensis*, new species **A.** South Fork of Alamo De Cesario Creek. **B.** Dry creek (1118 m), habitat of *Novomessor albisetosis* which requires adequate moisture and soil depth. **C.** Habitat of *Novomessor cockerelli* (1272 m), which requires sparse vegetation and less moisture. **D.** Mound nest of *Pogonomyrmex rugosus* (1276 m), soil mixed with desert pavement (small stones) and limited moisture. **E.** Nest of *P. rugosus* with 10 specimens of *M. chihuahuensis* (arrows). **F.** Same nest, spiders beside a host worker of *P. rugosus*; inset to a female. **G.** Excavation of *N. albisetosis* nest. (E–F, photos by D. Lightfoot, University of New Mexico).

desert willow (*Chilopsis linearis*), desert walnut (*Juglans major)*, false willow or Roosevelt weed (*Baccharis salicina),* and scattered oak *(Quercus sp.)* The soil was composed of loose sand dropped by running water in the creek bed, mixed with small to large stones.

The symbiotic relationship between the spider and the three species of harvester ants is not known at present. Observation proves that the spider is not a mimic of the ant, and the ant appears to tolerate the spider. Based on size difference, it appears the spiders do not feed on the adult ants. Larval myrmecophagy is a possibility, but doubtful. The biology of this myrmecophilic spider may be similar to that of the linyphiid *Masoncus pogonophilus* Cushing that inhabits the nest chambers of the harvester ant, *Pogonomyrmex badius*

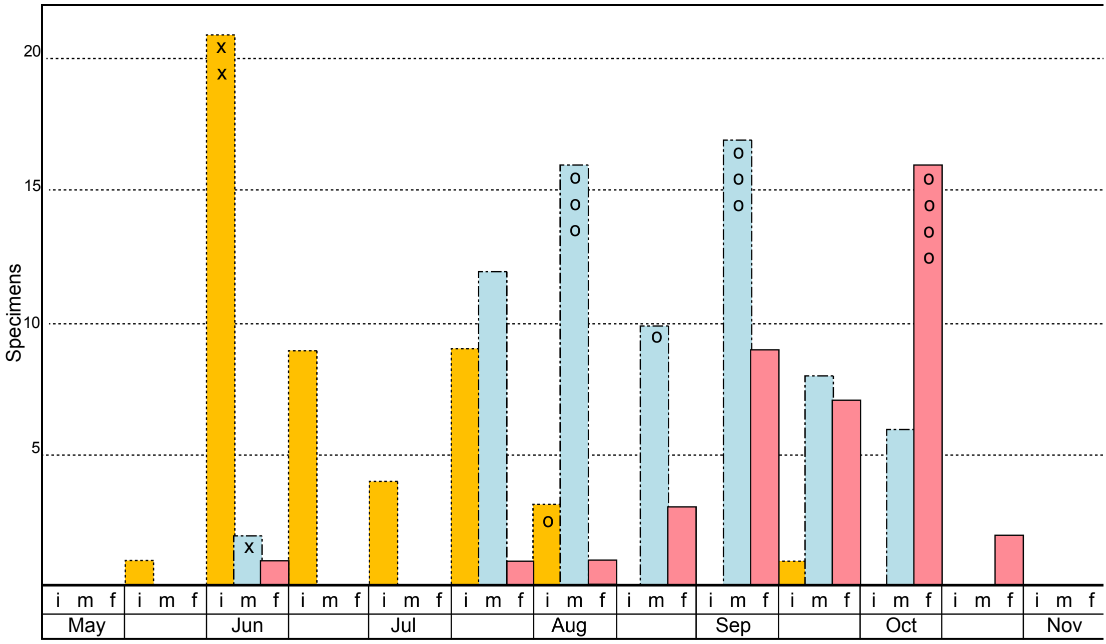


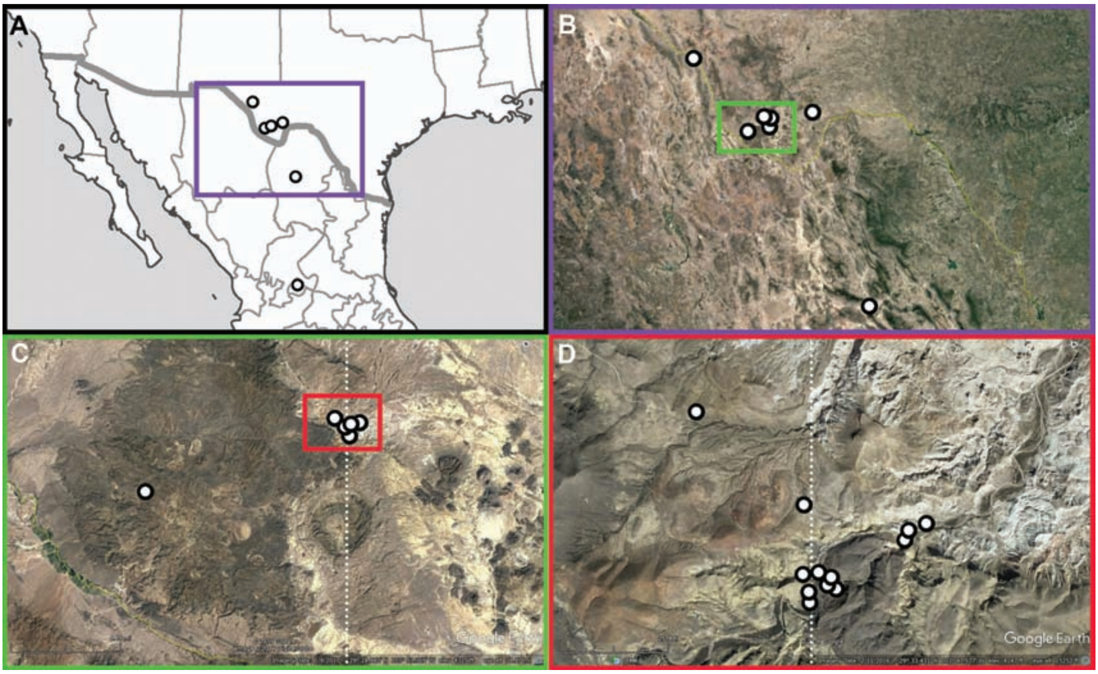
FIG. 12. Phenology of *Myrmecicultor chihuahuensis*, new species, including all collection records. All but 12 (labelled "o") of the 159 specimens collected are from the Dalquest Desert Research Station (DDRS) and, except for those excavated from an ant nest ("x"), were collected in pitfall traps. Juvenile specimens (i) are indicated in orange, males (m) in blue, and females (f) in red. Intermonth records are from trap runs that spanned two months, where each month contained at least 20% of the trap days. For example, results of a trap from 14 September to 9 October are recorded as intermonth, whereas one from 7 October to 5 November is recorded as October.

(Latreille), where it feeds on other small nest symbionts such as Collembola (Cushing, 1995, 1998).

The DDRS collection data suggest that the spiders come to the surface after a rain. Since precipitation records began in 2007, 131 spiders were collected shortly after rain. In contrast, only two juvenile spiders were collected—one in July of 2009 and one in June of 2011—without a close rain event being recorded. We suspect the spiders come to the surface synchronously with alate reproductive ants, which also are common on the surface after rain. Do the spiders come to the surface on their own, or do the worker ants bring them to the surface? The answer is unknown.

PHENOLOGY

As this spider is associated with the harvester ants, *P. rugosus*, *N. albisetosis*, and *N. cockerelli*, it was collected when the ants were most active (fig. 12), i.e., May to November. Of the 159 spiders collected, 48 were juveniles, 71 males, and 40 females. The immatures first appeared, followed by males, then females. May was the earliest record of a single juvenile, and one late season immature was collected in September. 91% of juveniles were collected in June and July. The majority, 99%,



MAP 1. Known localities of *Myrmecicultor chihuahuensis*, new species, plotted on a political map of North America (**A**) and three topographic maps (**B**–**D**) at increasing magnifications, as indicated by the rectangular boxes, with **D** showing the most densely sampled sites at the DDRS. The vertical line in **C** and **D** is the Presidio/Brewster County border.

of the males appeared between mid-July and October, with one male appearing in June. Of the females, 85% were collected during September and October, with the earliest in June and two in November. These data are based on trap samples that could be sorted. Some trap samples were lost due to heavy rainfall, which diluted the propylene glycol and washed sediment into the traps. Males mature about a month before the females (fig. 12). Certainly, overlap occurs, but immatures appear first, then males, followed by the females. Based on this information, the males and females do not overwinter. The juveniles probably overwinter and start their development before the ants become active in the spring. If so, they are feeding on something in the ant nest.

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