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# Scelionidae (Hymenoptera) parasitizing eggs of *Bagrada hilaris* (Hemiptera, Pentatomidae) in Mexico

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## Abstract

The painted bug or bagrada bug, *Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), is a key pest of crops in the family Brassicaceae. In this work, three species of Scelionidae (Hymenoptera) are reported for the first time as parasitoids of painted bug eggs in Mexico, at Saltillo, state of Coahuila: *Gryon myrmecophilum* (Ashmead), *Telenomus podisi* Ashmead and *Trissolcus basalis* (Wollaston). This is also the first report of a species of the widespread genus *Telenomus* as an egg parasitoid of *B. hilaris* outside of India. Total percent parasitism, high resolution images, and CO1 sequences are provided for each species. In the future, research in Mexico should be carried out on parasitoid species presented in this work to determine their potential as biological control agents and the feasibility of augmentative, classical or inoculative biocontrol strategies for integrated pest management.

## Keywords

Heteroptera, stink bug, biological control, parasitoid

## Introduction

*Bagrada hilaris* (Burmeister) (Hemiptera: Pentatomidae), known in Mexico with the common names of bagrada bug or painted bug, is a key pest of cole crops (family Brassicaceae) originally distributed in Africa and Asia (Howard 1906; Ahuja et al. 2008; Kavita et al.

2014). This pest first invaded California, USA, in 2008 (Palumbo et al. 2016) and in 2014 was detected in Saltillo, southeastern Coahuila, Mexico, causing economic damage in broccoli (*Brassica oleracea* L. var. italica), cabbage (*B. oleracea* var. capitata), and cauliflower (*B. oleracea* var. capitata) (Sánchez-Peña 2014; Torres-Acosta and Sánchez-Peña 2016).

The family Scelionidae is a cosmopolitan group of parasitoids that attacks the eggs of a variety of arthropods, including Hemiptera. In the Old World, several authors have reported parasitoids of this family attacking painted bug eggs. In India, *Gryon karnalense* (Chacko and Katiyar) and *Telenomus samueli* Mani were reported from the eggs of *B. hilaris* (as *Bagrada cruciferarum* Kirkaldy) (Chacko and Katiyar 1961; Mani and Sharma 1982). In Pakistan, sentinel eggs of *B. hilaris* placed in the field for four days and subsequently incubated in the laboratory yielded three species of hymenopteran parasitoids: *Trissolcus hyalinipennis* Rajmohana & Narendran, *Gryon gonikopalense* Sharma and a species of *Ooencyrtus* Ashmead (Encyrtidae) (Mahmood et al. 2015; Sforza et al. 2019). In the USA, Ganjisaffar et al. (2018) reported *Trissolcus basalis* and *Tr. hyalinipennis* parasitizing painted bug eggs in California. The objective of this work is to determine the presence of parasitoids of painted bug through sentinel eggs in northwestern Mexico and facilitate future work in this line of research.

## Materials and methods

### Field site

The work was carried out in the experimental fields of the Universidad Autónoma Agraria Antonio Narro (**UAAAN**) in Saltillo, state of Coahuila, México (25°21'15.80"N, 101°2'17.98"W, 1746 meters above sea level. The specific irrigated field (0.07 hectares) was planted with an assortment of Brassicaceae cultivars in equal numbers of the following plants: Arugula (*Eruca vesicaria* L. ssp. sativa), broccoli, cabbage, cauliflower, kohlrabi (*Brassica napobrassica* Miller. 1768), mustard (*Sinapis alba* L. 1753), radish (*Raphanus sativus* L. 1753) and turnip (*Brassica rapa* L. 1753 subsp. *rapa*).

### Detection of parasitoids of painted bug through sentinel eggs.

Eggs were obtained by rearing field-collected mating pairs of painted bugs in the laboratory. Eight mating pairs were placed in Petri dishes at a temperature of 26–28 °C with diffuse overhead daylight. After 12 hours in the laboratory, mating pairs in the Petri dishes produced an average of 270 eggs (range of 85–580). The mating pairs were removed and the eggs (on the same uncovered Petri dish bottom they were laid on) were placed on the soil surface at a distance of approximately 5 cm from a broccoli stem. If it was necessary to handle the eggs, a soft number 2 brush (Pinceles Rex, Mexico City) was used.

The sentinel egg tests were conducted monthly in the field from 25 November 2017 – 20 December 2018. The eggs were exposed 7–8 days in the field, and subsequently incubated at 24–28 °C and a relative humidity of 60% in the laboratory until the emergence of parasitoids. The wasps that emerged were placed in 96% ethanol until their subsequent identification.

## DNA analysis

Specimens were softened in 70% ethanol for two hours, then DNA was extracted using a DNeasy Blood and Tissue Kit (Qiagen). DNA extracts were quantified using a NanoDrop 2000 spectrophotometer (Thermo Scientific). At least 20 ng of genomic DNA was used per PCR. The 5'-CO1 barcode region was PCR-amplified using the primers LCO1490 and HCO2198 (Folmer et al. 1994). PCRs were performed at 25 µl volumes using HiFi HotStart DNA Polymerase (Kapa Biosystems). PCR thermocycle conditions were: 1) initial denaturing at 95 °C for 2:00 minutes followed by 32 cycles of steps 2–4, 2) 98 °C for 30 seconds, 3) 50 °C for 30 seconds, 4) 72 °C for 40 seconds, and 5) final extension at 72 °C for 7:00 minutes. PCR products were verified by gel electrophoresis and cleaned for sequencing with QIAquick Gel Extraction Kits (Qiagen). Purified PCR products were Sanger sequenced in both directions using BigDye Terminator v3.1 (Applied Biosystems) chemistry on a SeqStudio Genetic Analyzer (Applied Biosystems). Sequence reads were trimmed and sequence contigs were assembled in Sequencher 5.4.6 (Gene Codes Corporation). CO1 barcodes generated during this study were deposited in GenBank. Accession numbers for these sequences are presented in Table 1.

## Morphological identification

Specimens of *G. myrmecophilum* and *Te. podisi* were identified to species using the keys of Masner (1980) and Johnson (1984), respectively. Specimens of *Tr. basalis* were identified using Talamas et al. (2015) and the description by Ganjisaffar et al. (2018) of morphological variation present in individuals that emerge from *B. hilaris* eggs.

Stacks of photographs were taken with a Macropod imaging system and rendered using HeliconFocus. Specimen collection data and host associations are deposited in the Hymenoptera Online Database (htttp://hol.osu.edu). Voucher specimens for all scelionid species are deposited at the Florida State Collection of Arthropods (**FSCA**), Gainesville, Florida, and the Entomology collection, Universidad Autónoma Agraria Antonio Narro, Saltillo, Mexico.

**Table 1.** Accession numbers for specimens of Scelionidae used for DNA sequencing.

| Species | Locality | Collection Unit Identifier | GenBank Accession Number |
|---|---|---|---|
| *Trissolcus basalis* | Saltillo, Mexico | FSCA 00090267 | MK720829 |
| *Telenomus podisi* | Saltillo, Mexico | FSCA 00090266 | MK720830 |
| *Gryon myrmecophilum* | Saltillo, Mexico | FSCA 00090442 | MK720831 |
| *Gryon myrmecophilum* | Saltillo, Mexico | FSCA 00090443 | MK720832 |
| *Gryon myrmecophilum* | Rutgers, NJ, USA | FSCA 00090445 | MK937524 |

## Results

Scelionid wasps were detected only in November 2017 and June, July and August 2018. In a subsequent paper we will discuss in detail the phenology of the parasitoid complex on painted bug eggs at this location in Mexico.

### ***Trissolcus basalis*** **(Wollaston)**

Figs 1–3

As reported by Ganjisaffar et al. (2018), specimens of *Tr. basalis* that emerge from the eggs of *B. hilaris* have reduced episternal foveae and fainter striation on T2 relative to specimens that emerge from larger stink bug eggs (Figs 2–3).

BLAST comparison of the CO1 sequence from specimen FSCA 00090267 resulted in a 100% identity to a *Tr. basalis* sequence in Genbank from the USA (MK188338.1), providing confirmation of the morphological identification.

On the collection date of 25 November 2017, a total of 29 *Tr. basalis* were collected (this date was the only time *Tr. basalis* emerged from sentinel eggs) and the percentage of parasitism was 12.4% (n= 234 eggs).

### ***Telenomus podisi*** **(Ashmead)**

Figs 4–6

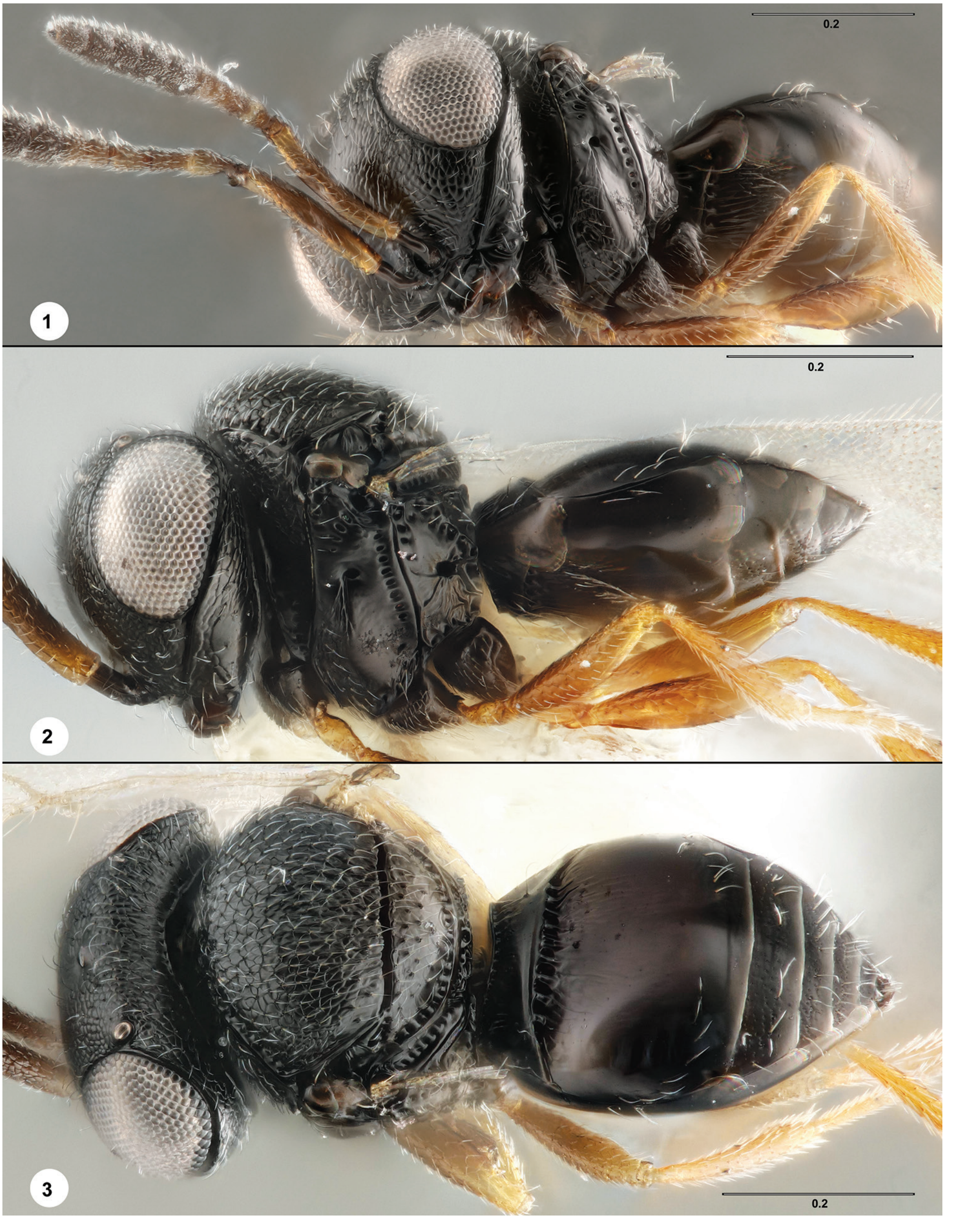
The small size of *B. hilaris* eggs does not influence the diagnostic morphology of *Te. podisi* and no relevant differences were found between the specimens in this study and *Te. podisi* reared from other stink bug eggs. BLAST comparison of the CO1 sequence from specimen FSCA 00090266 resulted in 98.9% sequence identity with *Te. podisi* sequence KR870961.1 from Genbank.

On the monthly collection dates between January-December 2018, a total of 51 *Te. podisi* was collected in the months of June and July, resulting in 9.2 and 9.9% of parasitism respectively (n= 532 eggs).

### ***Gryon myrmecophilum*** **(Ashmead)**

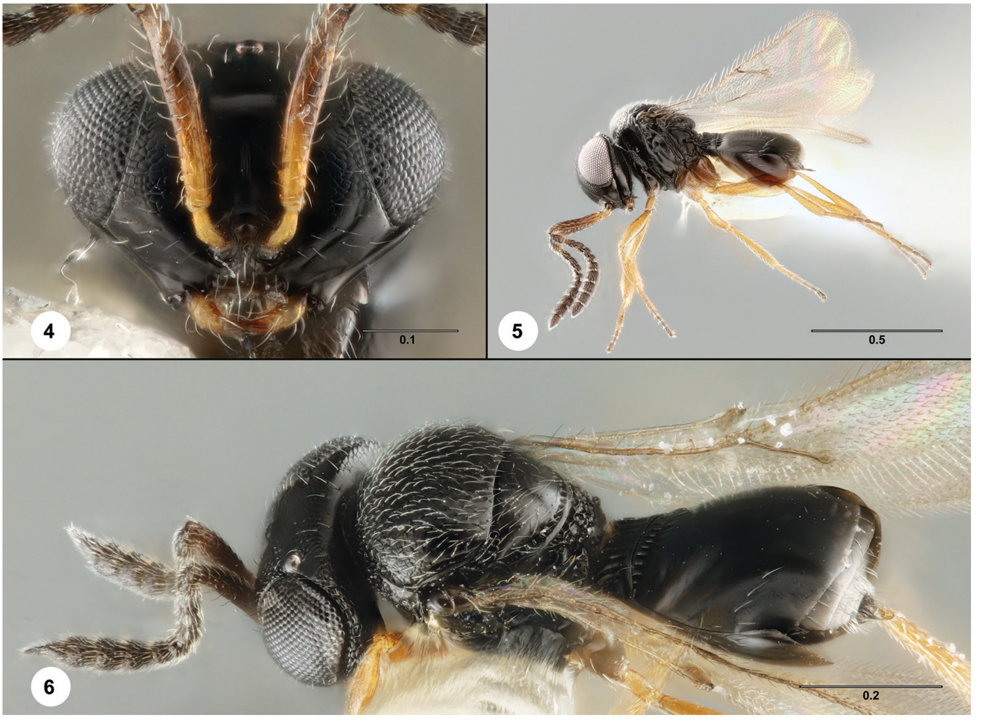
Figs 7–11

Our identification of this species is based on the revision of the genus by Masner (1980) and the specimens in this study were compared to photographs of the holotype specimen made available by Talamas et al. (2017). The systematics of *Gryon* is currently under revision by the second author. Preliminary analysis indicates that *G. myrmecophilum* belongs to a cosmopolitan cluster of similar species, some of which may have intercontinental distributions. A specimen of *G. myrmecophilum* from New




**Figures 1–3.** *Trissolcus basalis* female (FSCA 00090267) **1** head, mesosoma, metasoma, ventrolateral view **2** head, mesosoma, metasoma, lateral view **3** head, mesosoma, metasoma, dorsolateral view. Scale bars: in millimeters.

Jersey (FSCA 00090445) was sequenced to provide a comparison with a specimen closer to the type locality (Washington, DC). Comparison of the sequences from Mexico and New Jersey showed 88% sequence identity, indicating that *G. myrmecophilum*




**Figures 4–6.** *Telenomus podisi* **4** female (FSCA 00033549) head, anterior view **5** female (FSCA 00090266) habitus, lateral view **6** female (FSCA 00033275) head, mesosoma, metasoma, dorsolateral view. Scale bars: in millimeters.

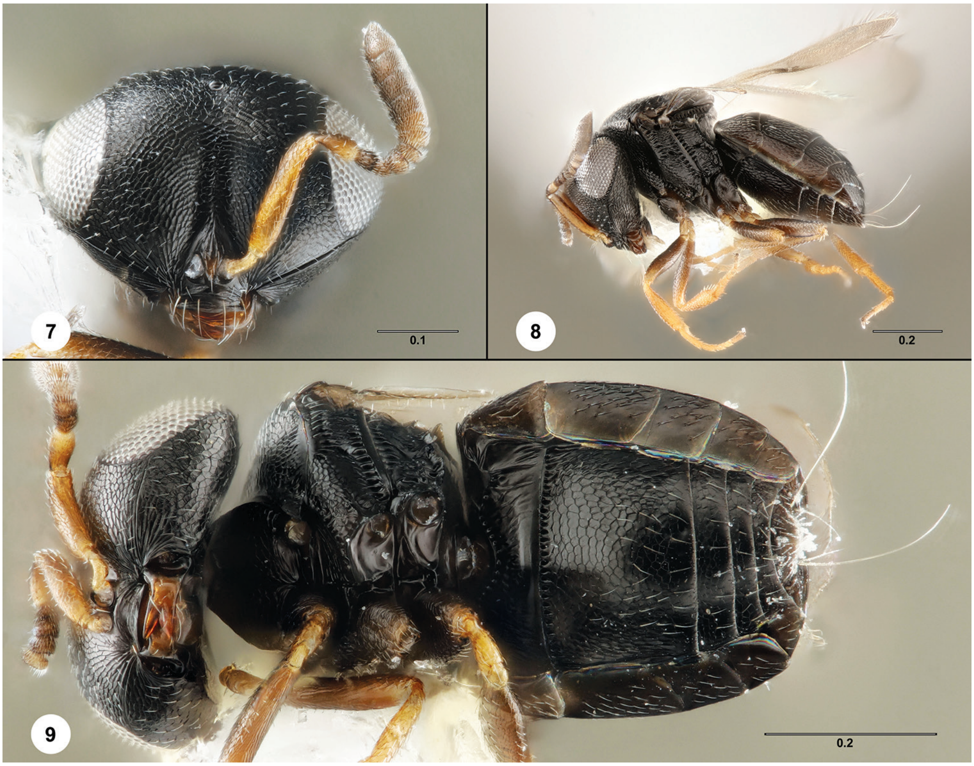
exhibits a high degree of variability in this gene region, or it is possibly a cryptic species complex.

On the monthly collection dates between January–December 2018, a total of 115 *G. myrmecophilum* were collected in the months of June, July and August, resulting in 3.0, 7.6 and 43.2% of parasitism respectively (n=786 eggs).

## Discussion and conclusion

In 2017, only *Tr. basalis* emerged from eggs, in the month of November (29 specimens), for 12.4% of parasitism. This wasp is a near-cosmopolitan parasitoid of stink bug eggs for which one widespread host is the southern green stink bug, *Nezara viridula* (L.) (Powell and Shepard 1982).

During the 2018 monthly sampling dates, scelionid wasps were detected only in June–August. The percentages of egg parasitism by all Scelionidae in the months of June, July and August 2018 correspond to 12.2, 17.4 and 49.6% respectively (total of 166 wasp specimens). Out of the total monthly percent parasitism, *Gryon myrmecophilum* contributed with 24.2, 43.5 and 100% in June, July and August, respectively (115 specimens total); and *Te. podisi* contributed with 75.8 and 56.5%, for the months

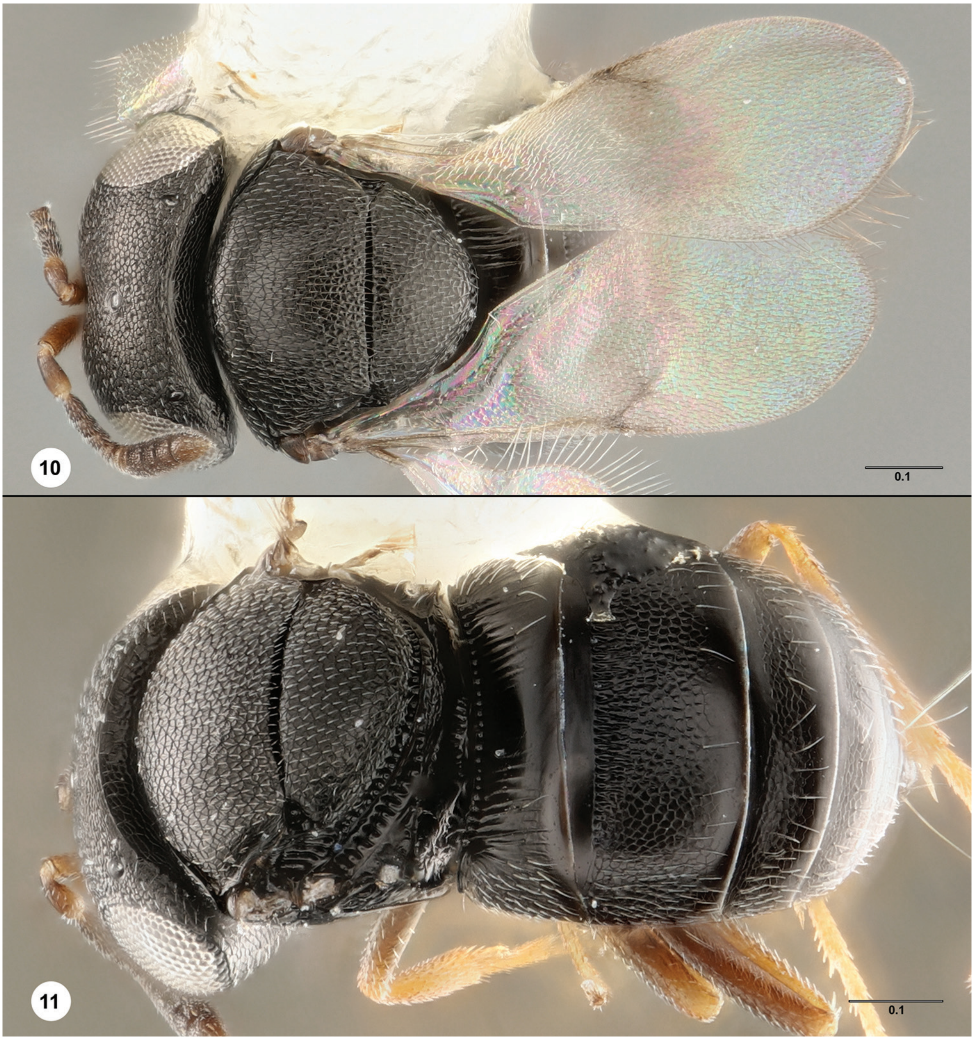



**Figures 7–9.** *Gryon myrmecophilum* **7** female (FSCA 00090446) head, anterior view **8** female (FSCA 00090447) habitus, lateral view **9** female (FSCA 00090446) head, mesosoma, metasoma, ventral view. Scale bars: in millimeters.

of June and July, respectively (51 specimens total). Species of *Telenomus* have been reported as a parasitoids of other stink bugs, including *Euschistus heros* (F.), *Halyomorpha halys* (Stål), *Oebalus insularis* Stål, *Piezodorus guildinii* (Westwood) and *Tibraca limbativentris* (Stål).

In Pakistan, Mahmood et al. (2015) reported that *Tr. hyalinipennis* and *G. gonikopalense* had a combined parasitism rate of 32–38%. This level is similar to the parasitism level obtained in our work. In California, USA, Ganjisaffar et al. (2018) reported that *Tr. basalis* and *Tr. hyalinipennis* parasitized 4.0–20.0% of *B. hilaris* sentinel eggs in January of 2018. We did not observe parasitism by scelionids in January, but it should be noted that our study includes only a small number of sampling dates. Additional sampling is required to describe the phenology of these wasps on *Bagrada* eggs.

To our knowledge, the present work reports the highest percentage of field parasitism of painted bug eggs in the USA and Mexico. It is also the first report of *Te. podisi* parasitizing painted bug eggs. We continue studying the identity, distribution and population fluctuation of beneficial wasps associated with painted bug eggs at selected localities in Mexico. Future research should be carried out on these species, and possibly others that have yet to be detected, to determine their potential




**Figures 10–11.** *Gryon myrmecophilum,* female (FSCA 00090447) **10** head and mesosoma, dorsal view **11** habitus, dorsal view. Scale bars: in millimeters.

as biological control agents. In particular, there is a need for critical comparative analysis of the different modalities of biological control (classical, augmentative or inoculative) that can utilize parasitic wasps in the integrated pest management of the painted bug in Mexico.

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