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Natural egg parasitism by Scelionidae on a Phycitine cactus moth in Mexico

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Species of *Opuntia* Mill. (Cactaceae) (prickly pear) cacti are of great ecological, economic, and cultural importance in North America. In Mexico, *Opuntia* pads (nopales) are fundamental items of the diet; fruits (tunas) also are consumed widely. The introduction of the South American cactus moth (*Cactoblastis cactorum* [Berg]; Lepidoptera: Pyralidae: Phycitini) to control invasive *Opuntia* in Australia is heralded as a textbook case of successful biological control. However, following the accidental invasion of *C. cactorum* to the southeastern US, this moth poses a grave risk to the native *Opuntia* of North America and the species that depend on them (Morrison et al. 2021).

There is keen interest in the natural enemies of *C. cactorum* in southern South America and the potential discovery of worthy biological control agents to be deployed in North America (Folgarait et al. 2018). However, it is essential to first understand the trophic interactions between *C. cactorum* and its natural enemy complex native to North America, as well as the natural enemies of several species of native Phycitini cactus moths closely related to *Cactoblastis* (Heinrich 1956; Neunzig 1997; Simonsen & Brown 2009); among these, the genus *Melitara* Walker (Lepidoptera: Pyralidae). The taxonomic history of *Melitara* is complicated; we follow Neunzig (1997) and his synonymization of *Olycella* with *Melitara*. The following *Melitara* species might occur in Mexico: *Melitara nephelepasa* (Dyar) (south-central plateau); *Melitara junctolineella* Hulst (eastern coast to interior); and the following in northern Mexico: *Melitara apicigramella* Blanchard & Knudson (type locality: Big Bend, Texas, USA); *Melitara dentata* (Grote); *Melitara doddalis* Dyar (type localities in Colorado and New Mexico, USA, respectively, and known from Texas, USA); and *Melitara texana* Neunzig (type locality: Carrizo Springs, south Texas, USA) (all Lepidoptera: Pyralidae). In the study area of northeastern and central Mexico, we therefore anticipate the presence of *M. junctolineella*. Larvae of *Melitara* usually occur at low densities as solitary borers in cactus pads, but in outbreak yr they may be secondary agricultural pests on *Opuntia*. Larvae are referred to as "gusano cebra" or "zebra worm."

The present study stems from a larger body of work investigating parasitoids of several cactus-feeding phycitine moths in Mexico. In particular, we seek to identify potentially specialist parasitoids that offer insights into their natural history and life cycles associated with host specialization in the context of the invasion of *C. cactorum* in North America. Early work by Mann (1969) documented 36 species of generalist and specialist parasitoids across 23 North American cactus-feeding insects in different orders. Pemberton and Cordo (2001) reviewed reports of larval and pupal parasitoids (Braconidae, Chalcididae, Ichneumonidae, and Tachinidae) of *Cactoblastis* spp. in South America. In Florida, surveys of parasitoids on invasive *C. cactorum* included 2 chalcid species from pupae (Pemberton & Cordo 2001) and 3 species of *Trichogramma* (Hymenoptera: Trichogrammatidae) egg parasitoids (Paraiso et al. 2011). Through these studies, we gain better insights on host-specificity to inform the selection of the parasitoid taxa that could be considered for biological control of *Cactoblastis*.

We searched for phycitine "egg sticks" (egg masses) and larvae at 2 sites, Bustamante and García, Nuevo Leon, Mexico, separated by about 60 km. Like *C. cactorum* and *Melitara prodenialis* Walker (Lepidoptera: Pyralidae) from Florida (Stephens et al. 2012), some native phycitines lay their eggs in piles ("egg sticks," Fig. 1A, B) at the tip of spines. Insects were collected from the dominant *Opuntia* species at both sites (*Opuntia streptacantha* Lem.; Cactaceae). At Bustamante (26.54911°N, 100.55973°W), the vegetation is submontane scrub, with barreta (*Helietta parvifolia* [A. Gray ex Hemsl.] Benth.; Rutaceae), guayacán (*Guaiacum angustifolium* Engelm.; Zygophyllaceae), mesquite (*Prosopis glandulosa* Torr.; Fabaceae), and creosote bush (*Larrea tridentata* [DC] Coville; Zygophyllaceae). Sampling was carried out by 3 individuals over a 4-h period spanning 1.5 to 2 ha on 28 Mar 2021. Egg sticks and larvae also were searched for in García, Nuevo Leon, Mexico (25.69878°N, 100.6314°W) over a 10-h period spanning an area of 6 ha during the 2021 dates of 25 Apr, 4 May, and 7 Jun. Sampling activity was carried out by 5 individuals (including the previous 3 individuals in the Bustamante sampling event). The vegetation at García is also submontane scrub, with mesquite, cholla (*Cylindropuntia* sp. [Engelm.] F. M. Knuth; Cactaceae), mescalbean or frijolillo (*Dermatophyllum secundiflorum* [Ortega] Gandhi & Reveal; Fabaceae), and sparse interior live oak (*Quercus fusiformis* Small; Fagaceae). Collected egg sticks were incubated in the laboratory in 30 mL plastic cups (Solo®, Dart Container, Mason, Michigan, USA), and emerging insects were imaged in a Hitachi TM-3000 Scanning Electron Microscope at 15 kV (Hitachi High-Tech Corporation, Fukuoka, Japan).

Collected phycitines from both localities (3 larvae from Bustamante and 3 adults reared from larval populations at García) were sequenced to obtain the Cytochrome Oxidase subunit I barcode for identification. Larvae were processed according to López-Monzón et al. (2019) with DNA denatured at 95 °C for 5 min, followed by 35 cycles of denaturing, annealing, and extension (94 °C, 1 min; 49.8 °C, 1 min; and 72 °C, 1 min, respectively) then extension at 72 °C for 10 min. For reared adults, a single leg from vouchers (TAM0258, GenBank OL960104; TAM0290, Genbank OL960105; and TAM0291, Genbank OL960106) was used.

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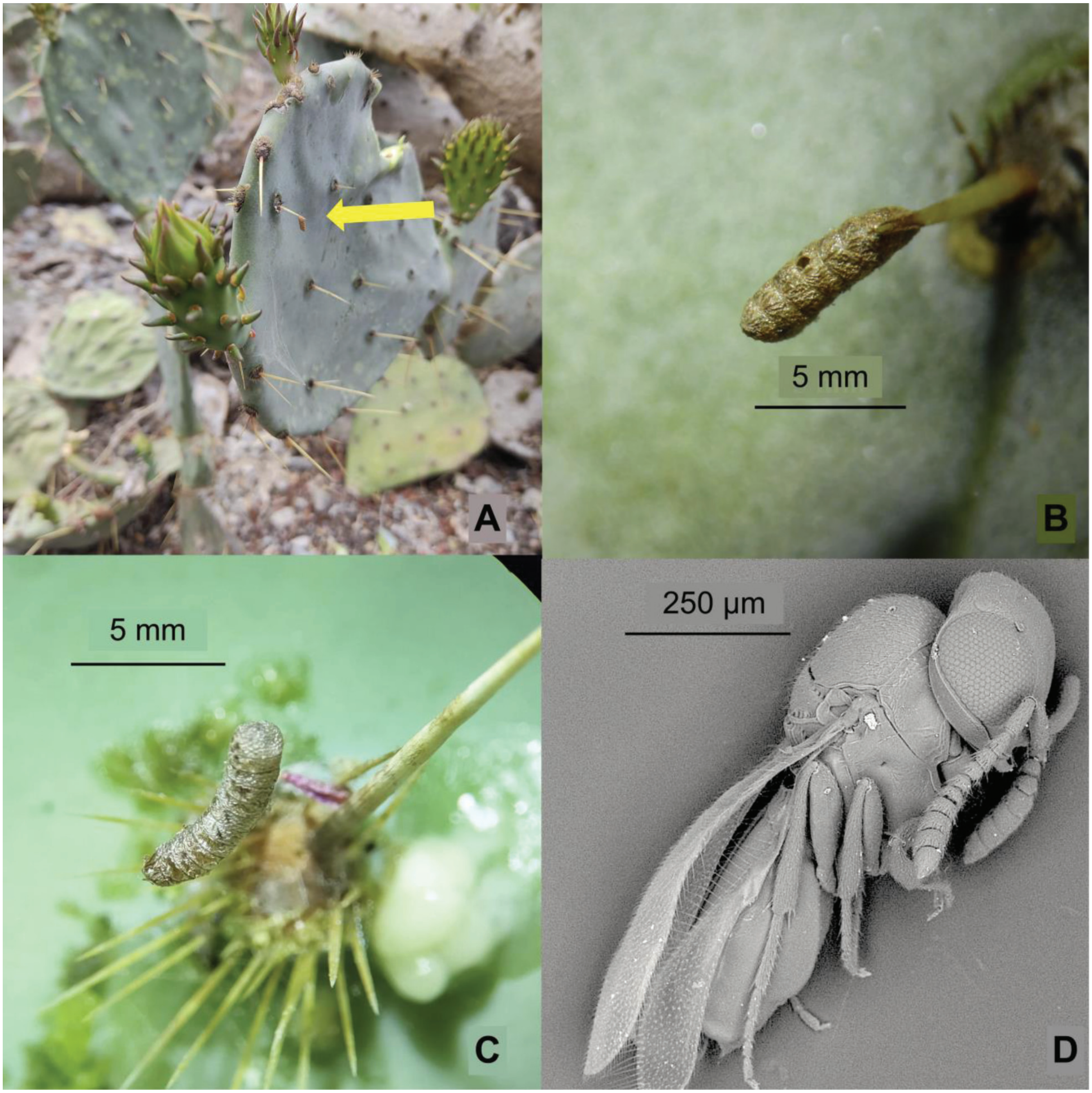

Fig. 1. (A) *Melitara* cf. *junctolineella* egg stick on cactus pad spine, visible towards the center of the pad (arrow); (B) *Melitara* cf. *junctolineella* egg stick with emergence hole of parasitic scelionid wasps; (C) non-parasitized *Melitara* cf. *junctolineella* egg stick with larval emergence holes; (D) adult scelionid wasp that emerged from *Melitara* cf. *junctolineella* egg stick.

DNA extraction was performed using the protocol and material from Nucleospin Tissue 250 kit (Macherey-Nagel, Düren, Germany). Polymerase chain reaction profile and LCO/HCO primers outlined in Wahlberg and Wheat (2008) were used. Polymerase chain reaction products from adults were sent to Eurofins Genomics (Louisville, Kentucky, USA) for sequencing and the subsequent results inspected in Geneious (Kearse et al. 2012).

Larvae and adults were identified as *Melitara* cf. *junctolineella*. Adult vouchers TAM0258, TAM0290, and TAM0291 were assigned to *M. junctolineella* in the Barcode of Life Data System (BOLD) (99.83% pairwise similarity). However, *M. junctolineella* appears to be closely related to *Melitara subumbrella* (Lepidoptera: Pyralidae); the 2 species apparently diagnosed by slight characters of the palpi (Neunzig 1997). Until the taxonomy of these species is studied more closely employing nuclear markers and broad geographic sampling, the identification here should be viewed as tentative. Biogeographically, we note that *M. subumbrella* appears to occur north of 30°N (Mann 1969; Neunzig 1997) and therefore out of the study range.

Two phycitine egg sticks, with 5 and 6 eggs each, were collected from 2 *O. streptocantha* plants at Bustamante (Fig. 1A-C). In contrast, a total of 42 egg sticks were collected at García. Egg stick density thus appeared to be greater at García than in Bustamante (0.84 vs. 0.16 egg sticks found per person per h, respectively). Overall, the collected egg sticks appeared to have fewer eggs than those of *C. cactorum* (63 ± 20, range 17–99) and *M. prodenialis* (23 ± 10.2, range 6–53) (Stephens et al. 2012).

No parasitoid wasps emerged from egg sticks collected at García. On the other hand, after 11 d, parasitic wasps (Hymenoptera: Scelionidae) emerged from all eggs (100% of parasitism, *n* = 11) in both egg sticks from Bustamante (Fig. 1D). No mating was observed while the wasps were in the plastic dishes. Wasps were preserved in 100% ethanol 24 h after emergence. The taxonomic placement of this unreported species is underway.

As far as we are aware, there are no records of Scelionidae parasitizing native phycitine cactus moths in North America. Although several studies have analyzed different ecological aspects of native cactus moths, including analysis of eggs, no egg parasitoids have been reported (Baker & Stiling 2009; Paraiso et al. 2011; Baker et al. 2012; Stephens et al. 2012). Paraiso et al. (2011) and others have detected several species of egg parasitoids in the genus *Trichogramma* attacking *C. cactorum* in Florida.

These observations are the first to show native populations of scelionid wasps in Mexico parasitizing eggs of *Melitara*, a member of the Phycitini. Because this host is a close relative with similar life history to the invasive cactus moth, *C. cactorum*, it is possible that these parasitoids also may attack the latter in similar ecological situations. In fact, Pemberton and Cordo (2001) indicate a possible existing case of host-switching in cactus moths (from native Phycitine moths to invasive *Cactoblastis* in Florida) for the native chalcidid *Brachymeria pedalis* (Cresson) (Hymenoptera: Chalcididae). Reports indicate that species or morphospecies of wasps in the Scelionidae are not species-specific (as is usually the case of egg parasitoids) but rather are restricted to hosts in the same insect family or subfamily, i.e., *Gryon myrmecophilum* (Ashmead), *Telenomus podisi* Ashmead and *Trissolcus basalis* (Wollaston) (Hymenoptera: Scelionidae) are found on species of Pentatominae (Hemiptera: Pentatomidae) (Felipe-Victoriano et al. 2019); *Telenomus remus* Nixon (Hymenoptera: Scelionidae) is a parasite of larvae of several species of Noctuidae (Lepidoptera) (Kenis et al. 2019). However, the assignment of specialist or generalist sometimes has needed review, such as in the case of *Apanteles* Förster (Lepidoptera: Braconidae) wasps. Whereas initial reviews suggested that some *Apanteles* species may be somewhat generalist (Pemberton & Cordo 2001), further studies have revealed that this genus comprises multiple cryptic species, which are host-specific (Smith et al. 2008; Goñalons et al. 2014). Therefore, further studies are needed to better understand dimensions of cactophagous moths and their parasitoids, especially in North America where they may interact with *C. cactorum*, and possibly provide an important complement to biocontrol by parasitoids introduced from the moth's native range.

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Summary

The detection of native natural enemies that can use *Cactoblastis cactorum* as a host is relevant after its invasion of North America. We detected wasps (Scelionidae) parasitizing eggs of *Melitara* cf. *junctolineella* (a closely related cactus moth in the Phycitini) in Bustamante, Nuevo Leon, northeastern Mexico. This is the first report of Scelionidae parasitizing eggs of phycitine cactus moths. The capability of this wasp to parasitize *C. cactorum* eggs should be investigated.

Key Words: agriculture; desert; invasive species; North America; herbivore; biological control

Sumario

La detección de enemigos naturales nativos que pueden utilizar a *Cactoblastis cactorum* como hospedero, es relevante tras su invasión a Norteamérica. Detectamos avispas (Scelionidae) parasitando huevos de *Melitara* cf. *junctolineella* (una polilla del cactus en Phycitini estrechamente relacionada con *C. cactorum*) en Bustamante, Nuevo Leon, noreste de México. Este es el primer reporte de Scelionidae parasitando huevos de polillas del cactus (Phycitini). Debe investigarse la capacidad de esta avispa para parasitar los huevos de *C. cactorum*.

Palabras Clave: agricultura; desierto; especies invasoras; Norteamérica; herbívoro; control biológico

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