Telenomus alecto (Crawford) (Hymenoptera: Scelionidae), parasitoid of *Diatraea magnifactella* Dyar (Lepidoptera: Crambidae) from Jalisco, Mexico: a study based on morphological and molecular evidence

María de Lourdes Ramírez-Ahuja[1], Mayra A. Gómez-Govea[1], Gerardo J. Trujillo-Rodríguez[1], Enrique Garza-González[2], Iram P. Rodriguez-Sanchez[1], and Elijah J. Talamas[3]

Abstract

The parasitoid wasp, *Telenomus alecto* (Crawford) (Hymenoptera: Scelionidae), is reported for the first time parasitizing *Diatraea magnifactella* Dyar (Lepidoptera: Crambidae) in Jalisco, Mexico. The occurrence of *Te. alecto* was discovered in May 2017 during a survey of egg parasitoids of *D. magnifactella*. The field survey was conducted on *Saccharum officinarum* L. (Poaceae) in Etzatlan, Jalisco, Mexico. In total, 656 eggs were collected, of which 401 were parasitized. The identity of the parasitoids was determined on the basis of morphological evidence and we here provide the barcoding region (COI).

Key Words: Telenominae; egg parasitoids; stem borer; *Saccharum officinarum*

Resumen

El parasitoide *Telenomus alecto* (Crawford) (Hymenoptera: Scelionidae) es reportado por primera vez parasitando *Diatraea magnifactella* Dyar (Lepidoptera: Crambidae) en Jalisco, México. *Telenomus alecto* fue descubierto en mayo de 2017 durante un estudio sobre los parasitoides de huevo de *D. magnifactella*. El estudio de campo se realizó en Etzatlan, Jalisco, México sobre *Saccharum officinarum* L. (Poaceae). En total, se recolectaron 656 huevos, de los cuales 401 estuvieron parasitados. La identidad de los parasitoides se determinó por morfología y mediante la región del código de barras (COI).

Palabras Clave: Telenominae; parasitoides de huevos; barrenador del tallo; *Saccharum officinarum*

Sugar cane (*Saccharum officinarum* L.; Poaceae) is an important crop in Mexico due to its economic and social relevance, and it generates more than 450,000 jobs directly (SIAP 2020). During 2018, production was 55.9 million tons, and the main producing states were Veracruz (37.5%) and Jalisco (13.1%), which together contributed 28.3 million tons (SIAP 2020).

The 2 principal groups of pests that attack sugar cane are the sugarcane borers, *Diatraea grandiosella* Dyar, *Diatraea saccharalis* (F.), *Diatraea considerata* Heinrich, *Diatraea magnifactella* Dyar, *Eoreuma loftini* (Dyar) (all Lepidoptera: Crambidae), and spittlebugs, *Aeneolamia* spp. (Hemiptera: Cercopidae) (Hernández 1994). However, the most important pests are sugarcane borers because losses up to 50% have been reported (Rodríguez-del-Bosque et al. 1989; Hernández 1994; Rodríguez-del-Bosque & Vejar-Cota 2008; Vejar-Cota et al. 2008). These losses depend on the combined effects of the *Diatraea* species composition, the region, the environment, and management factors (Rodríguez-del-Bosque et al. 1989).

To minimize the impact of *Diatraea* pest species, integrated pest management has been carried out, which includes cultural control, legal control, plant resistance, biological control, and chemical control. However, the latter is not recommended due to the price of insecticides, resistance problems, and the resultant environmental damage (Meagher 1996; Rodríguez-del-Bosque & Vejar-Cota 2008).

Diatraea species have several natural enemies, of which parasitoids are considered the most important. For example, the egg parasitoids *Trichogramma atopovirila* Oatman & Platner and *Trichogramma pretiosum* Riley (both Hymenoptera: Trichogrammatidae) are released massively in some states of Mexico (Arredondo-Bernal 2020). The species *Telenomus alecto* (Crawford) and *Telenomus* sp. (Hymenoptera: Scelionidae) also have been reported in Mexico and South America as parasitoids of *Diatraea rufescens* Box and *D. saccharalis* (both Lepidoptera: Crambidae) (Bin & Johnson 1982). During 2017, parasitoids of the genus *Telenomus* Haliday were detected in eggs of *D. magnifactella* in sugar cane in the municipality of Etzatlan in Jalisco. The objective of

[1]Universidad Autonoma de Nuevo Leon, Facultad de Ciencias Biológicas, Laboratorio de Fisiología Molecular y Estructural, San Nicolas de los Garza, Nuevo Leon 66450, Mexico; E-mail: lulu.ahuja@hotmail.com (M. L. R. A.), mayragee@gmail.com (M. A. G. G.), entogerry36@gmail.com (G. J. T. R.), iramrodriguez@gmail.com (I. P. R. S.)
[2]Agrobionsa, Agrobiológicos del Noreste, Calle Rio Mocorito 575 pte, Guadalupe, Culiacán Rosales, Sinaloa, 80220, Mexico; E-mail: egarzag9@hotmail.com (E. G. G.)
[3]Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida 32608, USA; E-mail: elijah.talamas@fdacs.gov (E. J. T.)
*Corresponding author; E-mail: elijah.talamas@fdacs.gov

this work was to determine the species of *Telenomus* using both morphological and molecular data, and the percentage of parasitism of the eggs of *D. magnifactella*.

Materials and Methods

Parasitoids were obtained from field-collected *D. magnifactella* eggs on the leaves of *S. officinarum* in Etzatlan, Jalisco, Mexico (20.970833°N, 104.225000°W), during Sep and Oct 2018 (Table 1). Leaves with eggs of *D. magnifactella* were cut and taken to the laboratory (RH 70%, 25 ± 2 °C). Each egg mass was placed into a 3 cm plastic box (Reyma®, Tijuana, Mexico). Observation of the egg mass continued until the emergence of either *D. magnifactella* larvae or parasitoid adults. Parasitoids that emerged were placed in 96% ethanol for morphological and molecular analyses. The number of eggs, emerged larvae, parasitism, and sex ratio of the parasitoids were recorded.

SPECIES DETERMINATION

We used the key of Bin and Johnson (1982) to identify the species of *Telenomus* and the keys of Pinto (1998) for *Trichogramma*. For *Telenomus* and *Trichogramma* species, examination of the male genitalia often is necessary for species-level identification. To prepare the slides of the male genitalia we followed the protocol of Polaszek and Kimani (1990), which consists of permanent preparations in Canada balsam. Terminology follows Johnson (1984) and Polaszek and Kimani (1990).

COI BARCODING

Genomic DNA was isolated nondestructively from whole specimens using the Qiagen DNeasy kit (Qiagen, Hilden, Germany) as described by Giantsis et al. (2016). Polymerase chain reaction was carried out to amplify the COI barcoding region using the LCO1490 and HCO2198 primers (Folmer et al., 1994). The polymerase chain reaction was performed in a 30 µL reaction volume: 2 µL of DNA, 3 µL of 10x Qiagen polymerase chain reaction buffer containing 15 mM $MgCl_2$, 0.9 µL of each primer, 0.6 µL of dNTPs (25 mM each), and 0.2 µL of 5 U per µL Taq DNA Polymerase (Qiagen, Hilden, Germany). The polymerase chain reaction conditions were as follows: 94 °C for 3 min, followed by 40 cycles of 94 °C for 30 s, 52 °C for 1 min, 72 °C for 1 min with a final extension 72 °C for 10 min. All polymerase chain reaction products were electrophoresed through agarose gel (1%), and then were sequenced in both directions at the Florida Department of Agriculture and Consumer Services, Division of Plant Industry in Gainesville, Florida, USA). Voucher specimens are deposited at Colección de Insectos Benéficos Entomófagos, Facultad de Ciencias Biológicas, Universidad Autonoma de Nuevo Leon, San Nicolas de los Garza, Nuevo Leon, Mexico, and Florida State Collection of Arthropods, Gainesville, Florida, USA. COI barcodes generated during this study were deposited in GenBank with accession numbers MZ614063 (FSCA 00091166); MZ614064 (FSCA 00091167).

Results

A total of 21 egg masses with 656 eggs were collected across all sites surveyed in Etzatlan, Jalisco, Mexico, during 2018. *Telenomus alecto* and *Tr. atopovirilia* were recovered from 401 parasitized eggs (Table 1). A percentage of parasitism of 53% of *Te. alecto* was observed on the eggs of *D. magnifactella*, whereas 7% parasitism was observed on *Tr. atopovirilia*.

During Sep 2018, 297 eggs of *D. magnifactella* were collected, of which 102 eggs were parasitized by *Te. alecto* (Fig. 1). Eggs parasitized by *Tr. atopovirilia* were collected in Oct. Only 3 egg masses were observed where both *Te. alecto* and *Tr. atopovirilia* emerged. In all other egg masses only *Te. alecto* emerged, and of the 359 eggs that were collected in Oct, 252 were parasitized by *Te. alecto* (see Table 1).

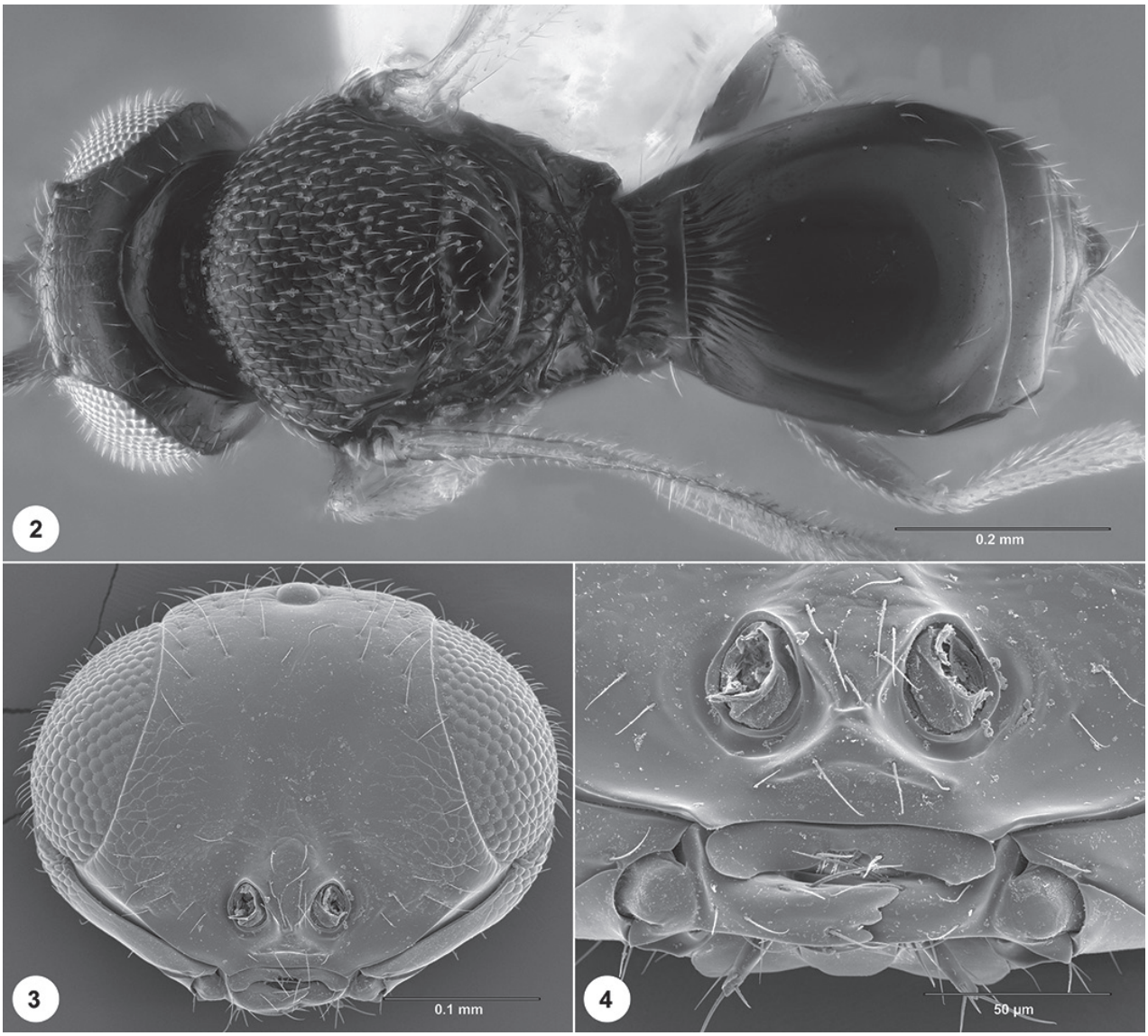
Table 1. Parasitized *Diatraea magnifactella* eggs, field parasitism and sex ratio of *Telenomus alecto* and *Trichogramma atopovirilia* collected from Etzatlan, Jalisco, Mexico.

Date	*Diatraea magnifactella* eggs	Emerged parasitoids	*Telenomus alecto*	*Trichogramma atopovirilia*	% parasitism
06-IX-2018	33	0	0		0
06-IX-2018	37	23	15♀, 8 ♂		62.16
06-IX-2018	30	0	0		0
06-IX-2018	26	0	0		0
06-IX-2018	32	18	10♀, 8 ♂		56.25
06-IX-2018	20	0	0		0
06-IX-2018	16	0	0		0
06-IX-2018	25	0	0		0
06-IX-2018	38	29	18♀, 11♂		76.31
06-IX-2018	22	19	12♀, 7♂		86.36
06-IX-2018	18	13	9♀, 4 v		72.22
11-X-2018	27	11	8♀, 3♂		40.74
11-X-2018	56	55	46♀, 9♂		98.21
11-X-2018	29	17	11♀, 6♂		58.62
11-X-2018	30	30	18♀,12♂		100
11-X-2018	31	23	16♀, 7♂		74.19
11-X-2018	32	24	23♀, 1♂		75
11-X-2018	45	42	35♀, 4♂	2♀, 1♂	93.33
11-X-2018	35	29	25♀, 4♂		82.85
11-X-2018	36	33	15♀, 5 v	11♀, 2♂	91.66
11-X-2018	38	35	3♀, 1♂	28♀, 3♂	92.10
TOTAL	656	401	354	47	61.12%

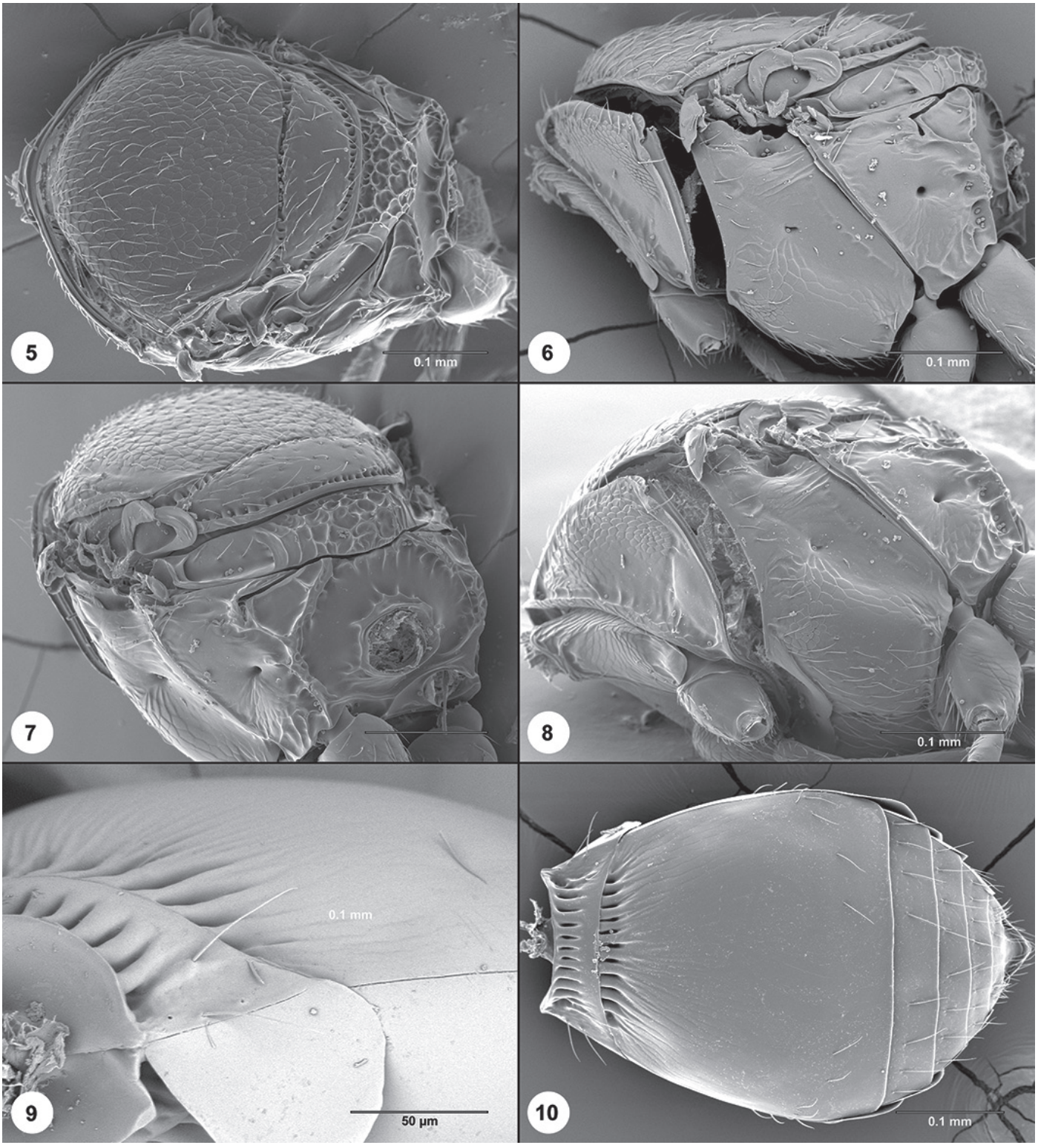
Fig. 1. *Telenomus alecto*, female (FSCA 00091145), lateral habitus.

MORPHOLOGICAL IDENTIFICATION

We based our identification on the following characters presented in the description by Bin and Johnson (1982), and compared our specimens to images of the holotype provided by Talamas et al. (2017): Vertex broadly rounded, coriaceous and setose; without hyperoccipital carina (Fig. 2); occiput with same sculpture as vertex, weakly effaced near the occipital carina; occiput dorsally appearing flat; occipital carina quite distinct, complete and simple; eyes setose (Fig. 3); mandibles with 3 small teeth (Fig. 4). Mesoscutum strongly depressed, coriaceous, setose, setal bases not pustulate; notauli absent; scutellum strongly transverse; smooth, setose (Figs. 5 & 7); acetabular carina simple (Figs. 6 & 8). Tl with 1 pair of sublateral setae, 3 pairs of lateral setae (Figs. 9 & 10). Wings clear, surpassing apex of metasoma; fore wing with basal vein not pigmented, postmarginal vein longer than stigmal (see Fig. 1); hind wing broad, greatest width more than twice width of fringe at that point. Male genitalia: digiti small with 3 digital teeth, lamina volsellaris in form of elongated plate with lateral indications of rods more pigmented; aedeagal lobe short and rounded apically (Fig. 11).



Figs. 2–4. *Telenomus alecto*, female: (2) FSCA 00091145, head, mesosoma, metasoma, dorsal view; (3) FSCA 00091497, head, anterior view; (4) FSCA 00091497, mouthparts, anteroventral view.



Figs. 5–10. *Telenomus alecto*, female: (5) FSCA 00091198, mesosoma, dorsal view; (6) FSCA 00091198, mesosoma, lateral view; (7) FSCA 00091198, mesosoma, posterolateral view; (8) FSCA 00091198, mesosoma, ventrolateral view; (9) FSCA 00091197, T1–T2, anterolateral view; (10) FSCA 00091198, metasoma, dorsal view.

Comments. *Telenomus alecto* examined in this work differs in color from the description by Bin and Johnson (1982). In Mexican specimens the coxae are dark brown to black, trochanters are yellow, and the antenna, femora and tibiae are brown. Coloration of the appendages is known to vary within some species of Telenominae, and in some cases is host related (Ganjisaffar et al. 2020). Our specimens were reared from a different species of *Diatraea* than those in the description by Bin and Johnson (1982), indicating that this phenomenon may occur in *Te. alecto* as well.

Host: *Diatraea magnifactella* Dyar, 1911 (Lepidoptera: Crambidae).

Material examined: Mexico, Municipality of Etzatlan, state Jalisco; 13-VII-2019; Enrique Garza González; 20.970833°N, 104.225000°W; *Saccharum officinarum*; (354 specimens) (CIBE 18-029).

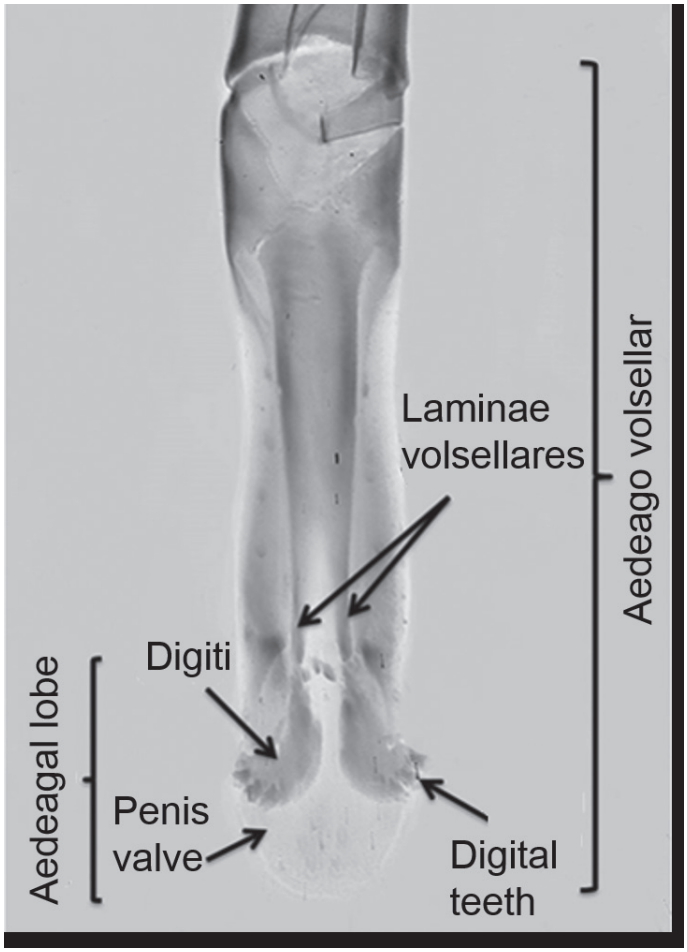


Fig. 11. Genitalia ♂ (CIBE 18-029).

Discussion

Telenomus alecto has been reported as a parasitoid of *D. rufescens* and *D. saccharalis* eggs in Central and South America (Bin & Johnson 1982). *Telenomus alecto* had been reported already in some sugar cane areas as a parasitoid of *Diatraea* spp. in Mexico since 1995 (Rodríguez-del-Bosque & Smith 1997). In 2009, this parasitoid was reported in Sinaloa parasitizing *D. considerata* and *D. grandiosella* (Rodríguez-del-Bosque & Smith 1997; Rodríguez-del-Bosque 2009). In this work, we report *Te. alecto* parasitizing *D. magnifactella* in Jalisco, Mexico, and present the first DNA barcode for this species. Reliable identification of parasitoids is important in biological control programs because they are a sustainable management alternative for controlling pests of agricultural importance, in this case the sugarcane borer. DNA barcoding is a rapid and efficient method of species discovery and identification, but it requires that sequences are attached to reliably identified specimens.

In this work, eggs masses of *D. magnifactella* were collected in different mo and the results showed the egg masses varied in size from 16 to 56 eggs. The eggs collected in Sep were parasitized only by *Te. alecto*. In this mo, no parasitism was observed for *Tr. atopovirilia*, a species that is produced massively and released to control sugarcane borer species in some states of Mexico (Arredondo-Bernal 2020). During Oct, it was observed that both species shared 3 egg masses, but there was a greater emergence of *Te. alecto*. From only 1 egg mass emerged 31 specimens of *Tr. atopovirilia* and 4 *Te. alecto*. This behavior of competing for the same host of *Te. alecto* also has been detected with other *Trichogramma* spp. (Rodriguez del Bosque & Smith 1997) although no further studies have been conducted to determine what kind of interaction would occur between these parasitoids if they both were released massively to control *Diatraea* spp.

It is necessary to study the feasibility of mass production of *Te. alecto*, as well as its percentage of parasitism on *Diatraea* species that occur in Mexico, since it could be an option for biological control within an integrated pest management program.

Acknowledgments

The first author is grateful to the Consejo Nacional de Ciencia y Tecnología (CONACYT, Mexico) for the scholarship granted for her doctoral studies. Elijah Talamas was supported by the Florida Department of Agriculture and Consumer Services, Division of Plant Industry (FDACS-DPI). We also thank Matthew Moore, Cheryl Roberts, and Lynn Combee (FDCAS-DPI) for their assistance with generating COI barcodes, and Jonathan Bremer (FDACS-DPI) for scanning electron microscopy.

References Cited

Arredondo-Bernal HC. 2020. Directorio de Laboratorios Reproductores y Comercializadores de Agentes de Control Biológico en México. https://www.gob.mx/senasica/documentos/directorio-de-laboratorios-reproductores-y-comercializadores-de-agentes-de-control-biologico (last accessed 13 May 2022).

Bin F, Johnson NF. 1982. Some new species of *Telenomus* (Hym., Scelionidae) egg-parasitoids of tropical pyralid pest (Lep., Pyralidae). Redia 65: 229–252.

Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Molecular Marine Biology and Biotechnology 3: 294–299.

Ganjisaffar F, Talamas EJ, Bon MC, Perring TC. 2020. First report and integrated analysis of two native *Trissolcus* species utilizing *Bagrada hilaris* eggs in California. Journal of Hymenoptera Research 80: 49–70.

Giantsis IA, Chaskopoulou A, Bon MC. 2016. Mild-vectolysis: a nondestructive DNA extraction method for vouchering sand flies and mosquitoes. Journal of Medical Entomology 53: 692–695.

Hernández M. 1994. Implementación del control biológico de barrenadores de la caña de azúcar en el Ingenio Aarón Sáenz Garza y El Mante, pp. 56–58 *In* Memoria del Curso sobre Control Biológico de Barrenadores de la Caña de Azúcar. Centro Nacional de Referencia de Control Biológico, Tecomán, Colima, México.

Johnson NF. 1984. Systematics of Nearctic *Telenomus*: classification and revisions of the *podisi* and *phymatae* species groups (Hymenoptera: Scelionidae). Bulletin of the Ohio Biological Survey 6: 1–118.

Meagher RL. 1996. MIP en Caña de Azúcar, *In* El Texto Mundial de MIP. University of Minnesota, Minneapolis, Minnesota, USA.

Pinto JD. 1998. Systematics of the North American species of *Trichogramma* Westwood (Hymenoptera: Trichogrammatidae). Memoirs of the Entomological Society of Washington 22: 1–287.

Polaszek A, Kimani SW. 1990. *Telenomus* species (Hymenoptera: Scelionidae) attacking eggs of pyralid pest (Lepidoptera) in Africa: a review and guide to identification. Bulletin of Entomological Research 80: 57–71.

Rodríguez-del-Bosque LA. 2009. Los barrenadores del tallo de la caña de azúcar en México. http://idtools.org/id/leps/lepintercept/RodriguezdelBosque_2009.pdf (last accessed 13 May 2022).

Rodriguez-del-Bosque LA, Smith JW. 1997. Biological control of maize and sugarcane stemborers in Mexico: a review. International Journal of Tropical Insect Science 17: 305–314.

Rodríguez-del-Bosque LA, Vejar-Cota G. 2008. Barrenadores del tallo (Lepidoptera: Crambidae) del maíz y caña de azúcar, pp. 9–22 *In* Arredondo Bernal HC, Rodríguez-del-Bosque LA [Eds.], Casos de Control Biológico en México. Mundi-Prensa, México.

Rodríguez-del-Bosque LA, Smith JW, Browning HW. 1989. Exploration for parasites of sugarcane and corn stalkborers in the huastecas area of Mexico, May 1985. Texas Agricultural Experiment Station Progress Report 4595. College Station, Texas, USA.

SIAP – Servicio de Información Agroalimentaria y Pesquera. 2020. https://www.gob.mx/siap/articulos/la-produccion-de-cana-de-azucar-supera-las-55-millones-de-toneladas-en-2018?idiom=es (last accessed 13 May 2022).

Talamas EJ, Thompson J, Cutler A, Fitzsimmons Schoenberger S, Cuminale A, Jung T, Johnson NF, Valerio AA, Smith AB, Haltermann V, Alvarez E, Schwantes C, Blewer C, Bodenreider C, Salzberg A, Luo P, Meislin D, Buffington ML. 2017. An online photographic catalog of primary types of Platygastroidea (Hymenoptera) in the National Museum of Natural History, Smithsonian Institution. Journal of Hymenoptera Research 56: 187–224.

Vejar-Cota G, Rodríguez-del-Bosque LA, Sahagún D. 2008. Economic and ecological impacts of hand removing dead hearts caused by *Diatraea considerata* (Lepidoptera: Crambidae) on sugarcane in Mexico. Southwestern Entomologist 33: 157–159.