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Duponchelia fovealis (Lepidoptera: Crambidae) and its Parasitoid in Cultivated Berries in Guanajuato, Mexico[1]

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The production of berries in Mexico predominantly focuses on the cultivation of blueberries (*Vaccinium* spp.), strawberries (*Fragaria* x *ananassa* Duch), raspberries (*Rubus idaeus* L.), and blackberries (*Rubus ulmifolius* Schott) (Servicio de Información Agroalimentaria y Pesquera [SIAP] 2022, https://nube.siap.gob.mx/gobmx_publicaciones_siap/). The cultivation of these crops has experienced steady growth over the past 10 years, with annual increases ranging from 4.7% in blackberries to as much as 28% in blueberries (SIAP 2022). However, the increased production of these crops comes with an elevated risk of the emergence of previously unreported pests.

One such example is *Duponchelia fovealis* Zeller, commonly known as the European pepper moth. In Mexico, this exotic pest was first reported in 2019 as a harmful agent affecting strawberry crops in the states of Michoacán, Guanajuato, Jalisco, and Aguascalientes (Soria 2019, Periódico Rural AGRO21. 10: 5, https://issuu.com/agro21/docs/ruralagro21_ed_10; Cruz-Esteban and Rojas 2021, Southwest Entomol. 46: 533–536; Rosales-Escareño et al. 2021, Resúmenes LVIII Congreso Nacional de Entomología 91; Jaraleño-Teniente et al. 2022, III Congreso Nacional de Entomología Aplicada 73–76). *Duponchelia fovealis* feeds on at least 73 host plant species, including aquatic and ornamental plants like poinsettia, lisianthus, and begonia, as well as economically important crops such as strawberries, blackberries, chili pepper, tomatoes, maize, and peanuts (Bonsignore and Vacante 2010, Protez. Colt. 3: 40–43; Stocks and Hodges 2011, UF/IFAS Extension, EENY 508:1–10, a series of the Entomology and Nematology Department, https://edis.ifas.ufl.edu/publication/IN910#TOP).

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This moth is considered a significant pest in strawberry cultivation for countries like Italy (Bonsignore and Vacante 2010), France, Turkey (Efil et al. 2014, Zool. Stud. 2: 328–334), and Brazil (Zawadneak et al. 2016, Idesia 34: 91–95). In strawberry crops, the larvae primarily feed on the lower parts of the plant, near the roots (Stocks and Hodges 2011). Some observable damage symptoms include the presence of excrement and silk around the leaves or at the plant's crown (Bethke and Vander-Mey 2013, Univ. California Coop. Ext.). Additionally, the leaves may display crescent-shaped or rounded perforations. In advanced larval stages, they can bore into the plant's crown, disrupting nutrient circulation (Stocks and Hodges 2011). Fruit infestation can be identified by small cavities approximately 1 to 2 mm deep and the presence of larval excrement (Efil et al. 2014). Similar symptoms have been reported by some strawberry growers in Guanajuato.

Accurate pest identification is essential for implementing appropriate management measures. Furthermore, describing the developmental stages of the pest is necessary to identify critical stages in its life cycle, facilitating the implementation of effective control tactics. The search for natural enemies is relevant, as these organisms can play a crucial role in regulating the pest population. Therefore, the objective of this study was to identify the lepidopteran pest affecting strawberry and blueberry crops in the state of Guanajuato, and to describe their developmental stages, as well as determining the natural enemies associated with this pest.

Immature stages of lepidopterans causing damage to strawberries were collected in October 2021 in fields with Albion variety in the municipalities of Jerécuaro (20° 1' 45"N - 100° 28' 5"W) and Tarandacuao (19° 58' 42"N; 100° 31' 13"W), as well as in blueberry fields with Biloxi variety in the municipality of Acámbaro (20°04'53.9"N; 100° 43'21.4"W), Guanajuato. In strawberry plants, the search for immature pest stages was conducted through the examination of the crown, leaves, and fruit of the plant, as well as in the furrows with the presence of organic matter, as the larvae can also feed on decomposing leaves (Stocks and Hodges 2011). In blueberry plants, leaves, and the base of the stem were inspected. The selection of plants for collection was directed towards those displaying signs of defoliation or decline. Any immature stage of the lepidopteran pest was kept in paper bags and transported to the laboratory. The collected biological material was maintained in a bioclimatic chamber (25°C ± 2, 70% relative humidity [RH], and 12L:12D) in the Biological Control Laboratory at the Colegio de Postgraduados, Campus Montecillo, Texcoco, State of México, México. The larvae were fed with a natural diet consisting of fresh Albion strawberry leaves, in anticipation of the emergence of pest adults or their parasitoids. To identify the pest species and its parasitoid, photographs of all insect stages were taken using a Canon® DS126211 camera adapted to a stereoscopic microscope (Zeiss® Discovery V.20). The identification of the emerged moths was performed through the comparison of morphological characteristics of adults and the use of taxonomic keys (Solís 2006, Keys to some frequently intercepted lepidopterous larvae). The description of all developmental stages of the lepidopteran was conducted using individuals maintained under the aforementioned conditions. Taxonomic keys for identifying the parasitoids at the genus level were sourced from Riedel (2017, Spixiana 40: 95–137). Some of the emerged parasitoids were sent to Dr. Enrique Ruiz Cancino, a specialist in the Ichneumonidae family affiliated with the Universidad Autónoma de Tamaulipas, Ciudad Victoria, Tamaulipas, México, for confirmation. Since the morphological identification of the parasitoid was only at the genus level, molecular studies were also conducted. Lithium chloride was used for DNA obtainment and digestion, and phenol-chloroform utilized for extraction (Sambrook and Rusell 2001, Cold Spring Harbor Laboratory, NY 1: 112). DNA was amplified using fragments of the 16S and 28S ribosomal RNA genes, as well as the mitochondrial cytochrome oxidase I (COI) gene, through polymerase chain reaction (PCR). The PCR products were sent to the Unit of DNA Synthesis and Sequencing (USSDNA) at the Universidad Nacional Autónoma de México (UNAM) (Cuernavaca, Morelos, México) for sequencing. Evolutionary history was inferred using the maximum likelihood method and the Tamura-Nei model (Tamura and Nei 1993, Mol. Biol. Evol. 10: 512–526). Initial phylogenetic trees for heuristic search were automatically obtained by applying the Neighbor-Join and BioNJ algorithms to a pairwise distance matrix estimated using the Tamura-Nei model, and then selecting the topology with the highest logarithmic likelihood value. This analysis involved 10 nucleotide sequences. Evolutionary analyses were conducted in MEGA X (Kumar et al. 2018, Mol. Biol. Evol. 35: 1547–1549).

The moth species collected from strawberry and blueberry crops was identified as *Duponchelia fovealis* Zeller (Lepidoptera: Pyraloidea: Crambidae). Regarding the characteristics of the immature stages, the eggs are flat and lenticular, measuring approximately 700 μm in length and 500 μm in width. Freshly laid eggs are cream-colored and turn pinkish-orange before hatching (Fig. 1A). The eggs are laid in groups of 3 to 5. Neonate larvae measure about 1.0 mm in length and are light orange in color. The head is dark brown (Fig. 1B). They go through five instars, with the final instar reaching a length of 20.0 mm. The larva has a dark brown thoracic plate with numerous setae on the prothorax, and the setigerous pinnacles are brown, appearing as spots around the body (Fig. 1C). The body color of the larva varies depending on the type of food. Larvae reared on a meridic diet are cream-colored, while field-collected larvae fed on detritus (leaves from the first defoliation of strawberries) have a dark-grayish color. The pupa is obtect, brown in color, and measures approximately 10.0 mm (Fig. 1D). Adults have a wingspan of approximately 18.0 mm and a length of 12.0 mm. One of the main characteristics is the presence of 2 white transverse lines on the forewings. The white line near the distal edge exhibits a “prolongation” or fovea downward (Fig. 1E), which is a characteristic of the species. Adult males have a longer and upwardly curved abdomen compared to females (Fig. 1F). In the municipality of Jerécuaro, 49 larvae and 3 pupae were collected in strawberry cultivation, while in Tarandacuao, only 72 larvae were collected. In the municipality of Acámbaro, just 2 larvae were found in blueberry cultivation. The presence of larvae in strawberry cultivation was primarily detected in the crown of the plants, with fewer larvae found on leaves in contact with the ground. Damage symptoms caused by larvae in strawberry plants were mainly perforations at the base of the stem and shoots. No larvae were observed in strawberry fruits. The highest infestation was observed in strawberry plots with a high proportion of organic matter in the soil. This organic matter consisted of decaying plant debris from the plant’s first defoliation, which remained between the rows, and more than 90% of the collected *D. fovealis* larvae were found beneath this plant material. There are reports of *D. fovealis* feeding on organic matter or detritus (Stocks and Hodges 2011). The behavior of the larvae, which show a preference for feeding on organic matter rather than the crop, suggests the possibility of exploring new research directions. This could be particularly relevant for developing ecological

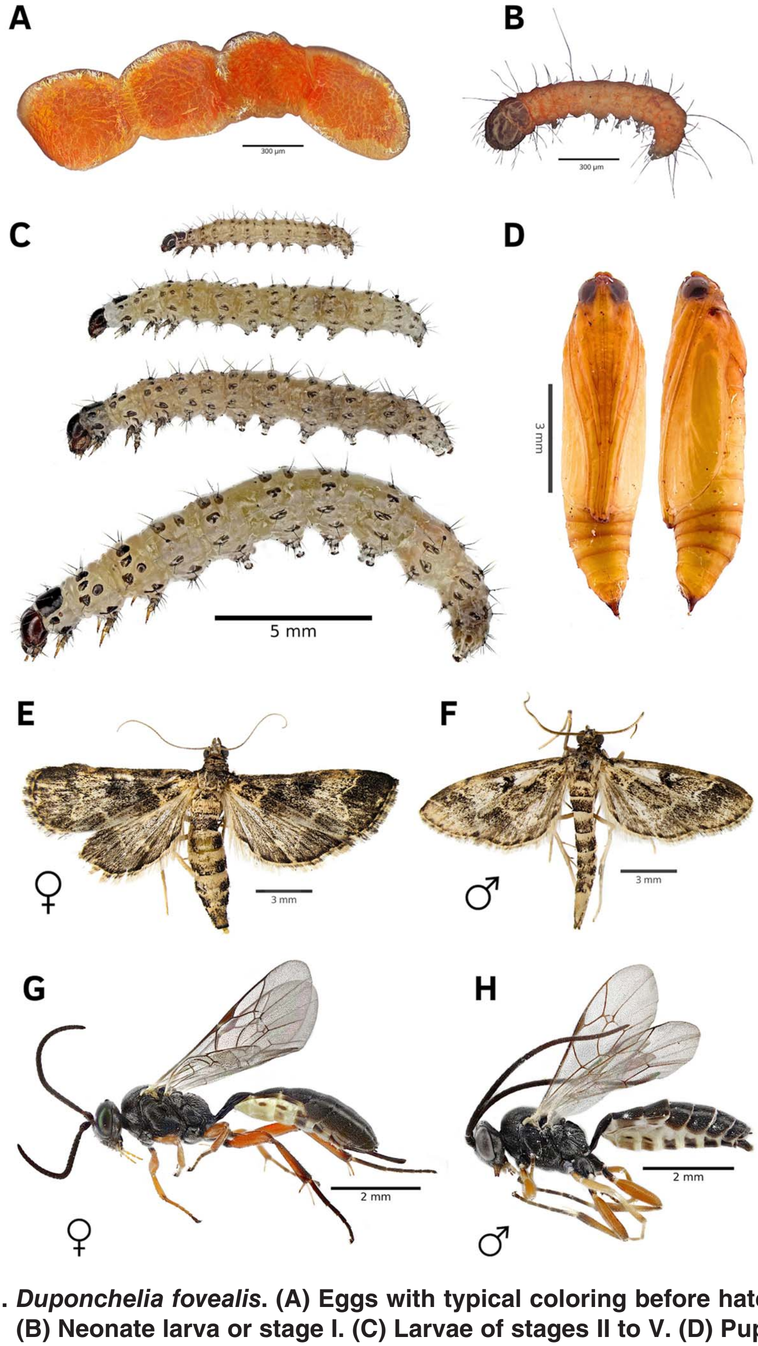

Fig. 1. ***Duponchelia fovealis*. (A) Eggs with typical coloring before hatching. (B) Neonate larva or stage I. (C) Larvae of stages II to V. (D) Pupa. (E) Female. (F) Male. *Campoletis* sp., parasitoids associated with *Duponchelia fovealis.* (G) Female. (H) Male.**

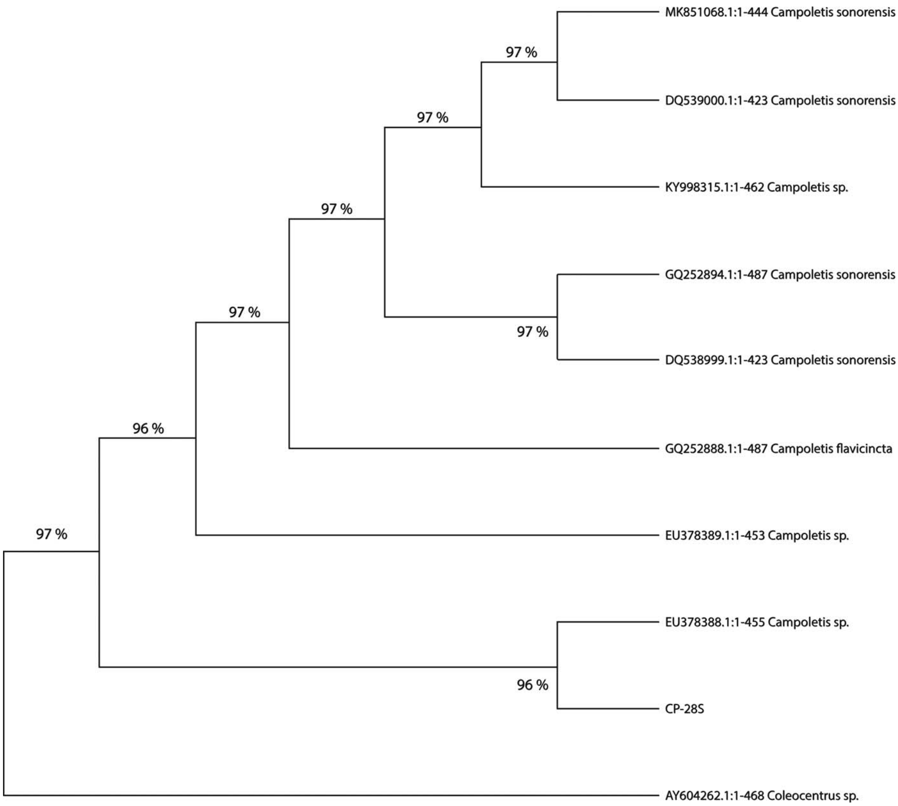

Fig. 2. Dendrogram inferred from maximum likelihood analysis of partial sequences of the 28S gene from the parasitoid emerged from *Duponchelia fovealis* larvae. *Coleocentrus* sp. was used as an outgroup.

pest management tactics, such as the implementation of a "trap crop" consisting of organic matter for monitoring pest population density (Moreau and Isman 2011, Pest Manag. Sci. 67: 408–413).

The parasitism of *D. foveali*s larvae in strawberry plants ranged from 4.32% in the municipality of Jerécuaro to 11.9% in Tarandacuao. Female and male parasitoids emerged from the collected *D. fovealis* larvae (Fig. 1G and H). Species identification of the parasitoid through classical or morphological taxonomy by the specialist was inconclusive at species level. However, it was determined that they belonged to the genus *Campoletis* (Hymenoptera: Ichneumonidae). Morphological structures did not correspond to any of the reported species of the *Campoletis* genus in Mexico. Phylogenetic analysis of the 28S gene identified the analyzed parasitoid (CP-28S) as a member of the *Campoletis* genus. It was identified as another species (EU378388) with a bootstrap value of 96%, distinct from other sequences of the species *Campoletis sonorensis* (Cameron) and *C. flavicincta* (Ashmed), with bootstrap values of 97% (Fig. 2). The genus *Campoletis* is distributed worldwide, and their species are known for being parasitoids of lepidopteran larvae from families such as Noctuidae, Pieridae, and Crambidae (Camargo et al. 2015, Brazil. Braz. J. Biol. 75: 989–998). These species are endoparasites of the first to the third larval stage of various lepidopteran pest species. *Campoletis sonorensis* (Cameron) and *C. flavicincta* (Ashmed) have been recorded in México, often recognized as parasitoids of *Spodoptera frugiperda* (J.E. Smith) (Bahena and Cortez 2015, Casos de Control Biológico en México 2, 181–250), making them potential candidates for biological control programs of that pest (Camargo et al. 2015). Natural control by parasitoids on *D. fovealis* has been documented in Turkey, with parasitism levels of 26.68% by *Campoletis rapax* Gravenhorst (Efil et al. 2014). The parasitoid *Apanteles* sp. (Hymenoptera: Braconidae) was found in Brazil, where this pest was recently reported; however, there was no report of parasitism levels (Zawadneak et al. 2016). Phylogenetic analyses suggest the presence of an unidentified and undescribed species of *Campoletis*. The identification of this parasitoid, which likely exerts a certain level of natural control over *D. fovealis*, opens new perspectives for conducting experiments to evaluate its potential as a biological control agent for this pest.

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