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First report of the cherry borer *Grapholita packardi* (Zeller) (Lepidoptera: Tortricidae) attacking hawthorn fruits (*Crataegus mexicana*) in Veracruz, Mexico

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Primer reporte del barrenador de cereza *Grapholita packardi* (Zeller) (Lepidoptera: Tortricidae) atacando frutos de tejocote (*Crataegus mexicana*) en Veracruz, México

RESUMEN. El tejocote *Crataegus mexicana* (Moc. Sessé) es un fruto de México y su consumo es parte de las tradiciones culinarias mexicanas. Por lo tanto, realizamos un estudio sobre la identificación y prevalencia de un tortrícido (Lepidoptera: Tortricidae) en frutos de *C. mexicana* en dos zonas donde se encuentra este árbol frutal en forma silvestre. Del total de los frutos recolectados, los visiblemente dañados fueron el 10% el primer año y el año siguiente el 30%. Los tortrícidos adultos fueron monitoreados y capturados con trampas de feromonas. Se identificaron mediante la quetotaxia en la larva y la extracción de genitalia en los adultos, así como por la amplificación y secuenciación del gen 5.8 S rDNA. La presencia de la especie *Grapholita packardi* (Zeller) se determinó en frutos de tejocote. Dado que no hay secuencias reportadas del gen 5.8 S rDNA en GenBank para esta especie, este es el primer estudio en proporcionar la amplificación y secuenciación de un marcador molecular para esta especie. Es importante realizar un seguimiento de la evolución y distribución de *G. packardi* y los daños que causa. En este trabajo argumentamos que esta especie debería ser considerada como una plaga potencial en México.

PALABRAS CLAVE. 5.8 S rDNA. Identificación. Tortrícidos.

ABSTRACT. Mexican hawthorn *Crataegus mexicana* (Moc. Sessé) is a Mexican fruit consumed as part of the Mexican culinary traditions. We conducted a study to identify and describe the incidence of a tortricid (Lepidoptera: Tortricidae) in *C. mexicana* from two areas where the fruit tree is present on its wild form. From the total of the collected fruits, 10% was visibly damaged during the first year, increasing to 30% the following year. Adult tortricids were monitored and captured using pheromone traps. Identification was carried out using larvae chaetotaxy in the larva and analysis of genitalia in the adults, as well as the amplification and sequencing of the gene 5.8 S rDNA. The presence of the species *Grapholita packardi* (Zeller) was determined in the fruits. Since there are no sequences of the gene 5.8 S rDNA reported in the GenBank database for this species, this is the first study in providing the amplification and sequence of this molecular marker for this insect. It is important to follow-up the evolution and distribution of *G. packardi* and the damages it causes. In this work, we suggest that this species should be considered as a potential pest in Mexico.

KEYWORDS. 5.8 S rDNA. Identification. Tortricids.

The Mexican tejocote *Crataegus mexicana* (Moc. Sessé) (Rosales: Rosaceae) is commonly found in the states of San Luis Potosi, Jalisco, Veracruz, Puebla and Chiapas, although its distribution also includes Central America and Ecuador (CONABIO, 2015). In Mexico, tejocote is consumed as part of the culinary traditions during the Christmas festivities called "posadas". More than 940 hectares of tejocote are cultivated in Mexico, with an estimated production of 8,877 tons *per* year (SIAP, 2016).

Among the pests of economic importance affecting fruit trees cultivation worldwide, species of the genus *Grapholita* are one of the most reported. In Mexico, there are 57 pests reported under epidemiological phytosanitary surveillance in the 31 states and in Mexico city (SENASICA, 2012). *Grapholita packardi* (Zeller) (Lepidoptera: Tortricidae), known as cherry moth, is one of the major pests in fruit trees from the Ericaceae and Rosaceae families (EPPO, 2014; Gilligan & Epstein, 2014). In 1926, it was first reported in apple, rose and hawthorn plants in the North American region (Heinrich, 1926). From 1914 to 1960 it was considered the major pest in *Prunus avium* L. (Hoerner & List, 1952; Oatman & Ehlers, 1962). Finally, in 2014, EPPO mentioned *G packardi* as the main host in cherry (*P. avium*) and as secondary hosts in plum (*Prunus domestica* L.), apple (*Malus domestica* Borkh), pear (*Pyrus communis* L.) and blueberry (*Vaccinium macrocarpon* Aiton). Moreover, it has been occasionally found in quince (*Cydonia oblonga* Miller) and peach (*Prunus persica* (L.) Stokes). Larvae make galleries in the interior of the fruits, with aesthetic damage that reduces the final fruit quality causing economic losses (Gilligan & Epstein, 2014; SAGARPA, 2014). The objective of the present research was to identify the tortricid *G. packardi* in fruits of *C. mexicana* in Mexico as well as its prevalence, to aid the implementation of prevention and control strategies in order to avoid its dispersion.

Study area

This study was conducted in the state of Veracruz, in municipalities of Jalancingo (19° 46' 52.5" N; 97° 17' 03.5" W; 1,996 m.a.s.l.), and Las Vigas de Ramirez (19° 38' 06.7" N; 97° 07' 39.3" W and 19° 38' 32.1" N; 97° 07' 40.4" W; 2,420 m.a.s.l.).

Fruit sampling

The monitoring was carried out weekly on mature hawthorn fruits and the visible damage caused by larvae was identified. Monitoring of adult specimens was performed during October and November 2014, 2015 and 2016 as well as during May, June and July 2015. and 2016 with pheromone traps (TRECE) coated with Pherecon® pheromone. A total of 120 traps were positioned as follows: 60 in Jalacingo and 60 in Las Vigas de Ramirez. Traps were placed in creole apple, plum and wild hawthorn trees presenting healthy fruits and foliage in a quadrant of 200 m^2 *per* trap in family homegardens.

Taxonomic identification of adults and larvae

Adults and larvae in fruits samples were processed at the Laboratory of High Technology Xalapa, S.C. of the Veracruzana University, for the preparation of samples and identification according to morphological characteristics. Identification was carried out using taxonomic keys, setal maps (MacKay, 1959; Weisman, 1986; Stehr, 1987), and extraction of genitalia, using a stereomicroscope (Carl Zeiss, Stemi 1000) and a compound microscope (Carl Zeiss bright field, Axiostar).

Molecular analysis

DNA extraction was performed using a modified version of the method proposed by Yu et al. (2011). Briefly, tissue was extracted from the abdominal larval segments 4 to 8 and PCR performed according to the protocol (SAGARPA-CNRF, 2014). The TW81 (GTTTCCGTAGGTGAACCTGC) and AB28 (ATATGCTTAAGTTCAGCGGGT) molecular markers were used. The 5.8S rDNA amplified gene fragment was purified with Wizard® SV Gel kit and PCR Clean-Up System (Promega, WI, USA), and sequenced in the Institute of Biotechnology of the UNAM (sequencer Applied Bio systems). BLAST sequence homology search was performed within GenBank database NCBI (National Center for Biotechnology Information) on the nucleotide sequences obtained.

Out of the 210 adult specimens, 95 were intercepted on hawthorn trees and 115 on plum and apple trees. However, in the hawthorn fruits, 40% of visual damage observed was caused by larvae of *Conotrachelus crataegi* Walsh (Coleptera: Curculionidae) as Muñiz-Merino et al. (2012) have previously reported as a usual plague in hawthorn in México. Only 10% was due to *G. packardi* larvae in 2015. However, in 2016, the damage by *G. packardi* increased to 30%.

Both larval and male genitalia characters were coincident with those reported in this species (MacKay, 1959; Weisman, 1986; Stehr, 1987) (Figs. 1, 2). BLAST analysis indicated 94% of similarity in the 900 bp fragment sequence when compared with *Eucosma siskiyouana* (accession number KC430350.1). The proportion of sequenced gene is greater than 1:1. No similarity to other species of *Grapholita* was obtained. Since no gene sequences for 5.8 s rDNA have been

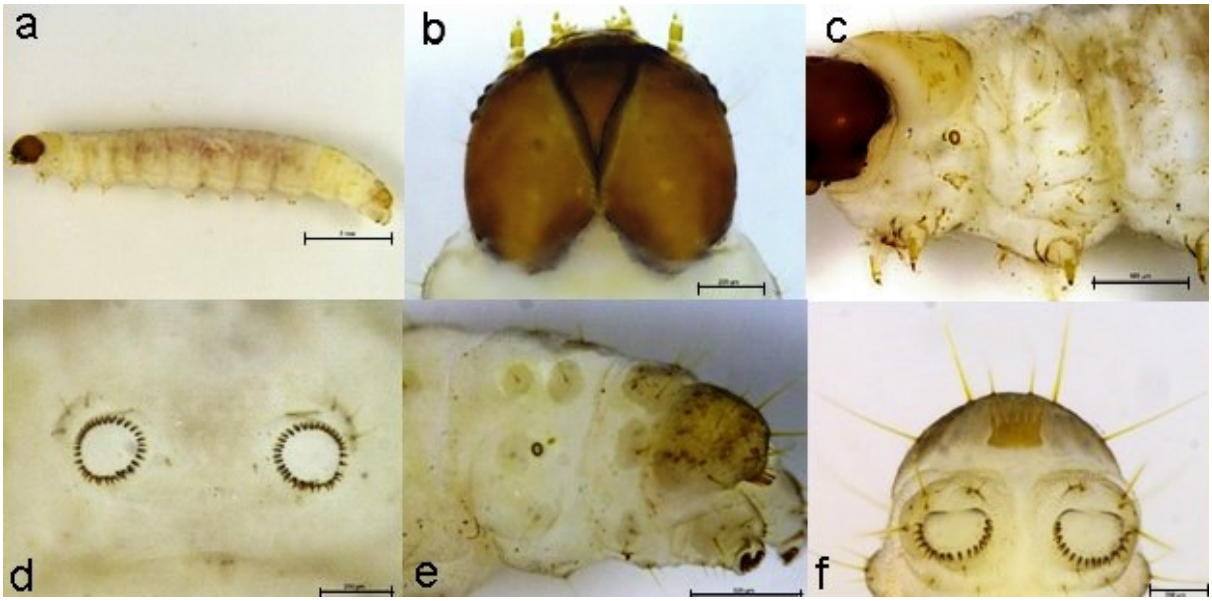

Fig. 1. Larval morphology of *Grapholita packardi*. a. larvae; b. epicranial suture; c. thorax; d. uniordinal crochets; e. terminal segments; f. anal comb. Photographs taken by Entomology Department of the National Reference Center (2017). Bars = 200 μm.

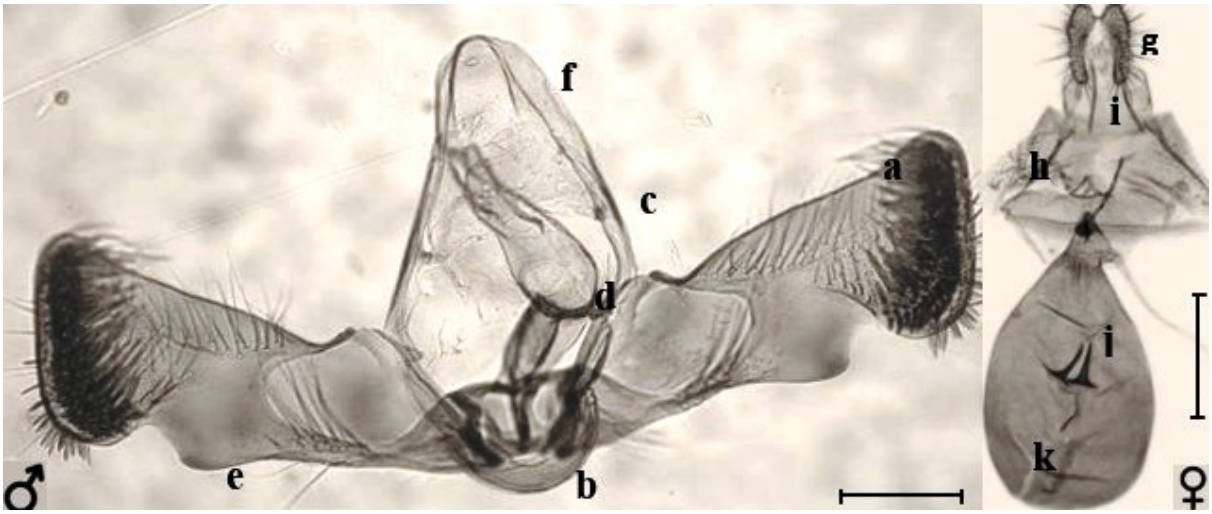

Fig. 2. Male and female genitalia of *Grapholita packardi*. a. valva; b. vinculum; c. tegumen; d. phallus; e. sacculus; f. uncus; g. papillae anales; h. previous apophyses; i. posterior apophyses; j. signum; k. bursae. Photographs taken by San Martín-Romero, E. (2017). Bars = 100 μm.

reported in the GenBank for this species, this is the first study of the amplification and sequencing of *G. packardi*. The nucleotide sequence of *G. packardi* was registered in GenBank under the accession number KX357711.

Tortricidae family includes pests in a wide variety of agricultural, horticultural, and forest crops. Even though it has a cosmopolitan distribution, the family is best represented in temperate, tropical and subtropical regions (Meijerman & Ulenberg, 2000; Valera-Fuentes et al., 2009). In general, members of the subfamily Tortricinae tend to be polyphagous. Dicotyledonous are the most common hosts, yet there are groups of Tortricinae species and genera specialized in gymnosperms. This study found that hawthorn trees are preferred by *G. packardi*, instead of cherry, the primary host in Veracruz, Mexico (Brown et al., 2003; Gilligan & Epstein, 2014).

An important aspect of tortricid dispersion and survival is that larvae employ a wide range of feeding

strategies, from burrowing into root or seeds, or feed on flowers, to feed on leaf litter, even though the latter is unusual (Horak & Brown, 1991; Powell et al., 1998; Cepeda & Cubillos, 2011). It is important to point out that, as this research shows, there was a low incidence of *G. packardi* burrowing into hawthorn fruits.

Patterns of oviposition of tortricids have been studied as ecological indicators of insect-plant interactions (Thomson & Pellmyr, 1991). The great adaptation and plasticity of this family might result in the adoption of new hosts, as in the case of *Cydia pomonella* L. (Tortricidae) found in *Magnolia schiedeana* Schltdl (Salinas-Castro et al., 2014). It is worth mentioning that if *G. packardi* establishes in Mexico, it would affect the production of Rosaceae fruit trees such as cherry, plum, cranberry, pear, apple, peach and hawthorn, which, according to SIAP (2016), represents 1,028,017.36 tons, with a production value of 334,516.82 thousand dollars in 2015. However, this study found, so far, no damage by *G. packardi* in creole fruits of apple and plum.

Therefore, monitoring the spread and damage caused by this potential pest in Mexico would help to prioritize epidemiological studies in order to prevent possible outbreaks in the state of Veracruz and even in the entire country.

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LITERATURE CITED

Brown, J.W., Robinson, G., & Powell, J.A. (2003) Food Plant Database for the Leafrollers of the world (Lepidoptera: Tortricidae). http://www.tortricidae.com/foodplants.asp

Cepeda, D.E., & Cubillos, G.E. (2011) Descripción del último estado larvario y recopilación de registros de hospederos de siete especies de Tortricidos de importancia económica en Chile (Lepidoptera: Tortricidae). *Gayana*, **75(1)**, 39-70.

CONABIO (2015) *Crataegus pubescens* (Kunth) Steud. ROSACEAE. *Nomenclator Botanicus*, **2**, 433-1841.

EPPO (2014) PQR-EPPO database on quarantine pest. http://www.eppo.int

Gilligan, T.M.,& Epstein, M.E. (2014) Tortricids of Agricultural Importance. http://idtools.org/id/leps/tortai/information.html

Heinrich, C. (1926) Revision of the North American moths of the subfamilies Laspeyresiinae and Olethreutinae. *Bulletin of the U.S. National Museum*, **132**, 1-216.

Hoerner, J.L., & List, G.M. (1952) Controlling cherry fruitworm in Colorado. *Journal of Economic Entomology*, **45**, 800-805.

Horak, M., & Brown, R.L. (1991) Taxonomy and phylogeny. *Tortricid Pests. Their Biology, Natural Enemies and Control* (ed. Van der Geest, L.P.S., & Evenhuis, H.H.), pp. 23-48. Elsevier, Amsterdam.

Mackay, M.R. (1959) Larvae of the North American Olethreutinae (Lepidoptera). *The Memoirs of the Entomological Society of Canada*, **91(S10)**, 5-338.

Meijerman, L., & Ulenberg, S.A. (2000) Arthropods of economic importance: Family Tortricidae Leafrollers. Eurasian Tortricidae. Zoological Museum University of Amsterdam. http://ip30.eti.uva.nl/bis/tortricidae.php?menuentry=inleiding)

Muñiz-Merino, M., Cibrián-Tovar, J., & Nieto, R.A. (2012) Fuentes de Atracción y preferencia de ovoposición de *Conotrachelus crataegi* Walsh (Coleoptera: Curculionidae) en *Crataegus* spp. (Rosaceae: Maloideae). *Revista Chapingo Serie Horticultura*, **18(1)**, 21-37.

Oatman, E.R., & Ehlers, C.G. (1962) Cherry insects and diseases in Wisconsin. *Wisconsin Agricultural Experiment Station Bulletin,* **555**.

Powell, J. A., Mitter, C., & Farrell, B. (1998) Evolution of larval food preference in Lepidoptera. *Handbook of Zoology. Arthropoda: Insects, Part 35 Lepidoptera, Moths and Butterflies, Vol. IV* (ed. Kristensen, N.P.), pp. 403-422. Walter de Gruyter, Berlin.

SAGARPA (2014) Palomilla de las cerezas *Grapholita packardi* (Zeller). Servicio Nacional de Sanidad e Inocuidad y Calidad Agroalimentaria. *Programa de Vigilancia Epidemiológica Fitosanitaria.* Ficha Técnica No. 50.

SAGARPA-CNRF (2014) Nematodos de Importancia cuarentenaria en Papa para México. Protocolos de Diagnóstico. http://www.senasica.gob.mx/DEFAULT.ASP?id=5951

Salinas-Castro, A., Mazin-Pérez Sandi, M.T., Ramírez-Reyes, T., Luna-Rodríguez, M., & Trigos, A. (2014) An unusual food plant for *Cydia pomonella* (Linnaeus) (Lepidoptera, Tortricidae) in Mexico. *Revista Brasileira de Entomologia*, **58(3)**, 261–264.

SENASICA (2012) Programa de Prevención y Manejo de Riesgos Componente: Sanidades Subcomponente: Vigilancia Epidemiológica Fitosanitaria.

SIAP (2016) Servicio de Información Agroalimentaria y Pesquera. http://www.siap.gob.mx/

Stehr, F.W. (1987) Immature Insects. Kendall/Hunt, Dubuque,

IA Thomson, J.N., & Pellmyr, O. (1991) Evolution of oviposition behavior and host preference in Lepidoptera. *Annual Review of Entomology*, **36**, 65-89.

Varela-Fuentes, S., Brown, J.W., & Silva-Aguirre, G. (2009) Record of *Platynota rostrana* (Walker, 1863) (Lepidoptera: Tortricidae) in citrus from Mexico. *Acta Zoológica Mexicana (n. s.)*, **25(3)**, 651-654.

Weisman, D.M. (1986) *Keys for the identification of some frequently intercepted lepidopterous larvae*. APHIS 81-47, U.S.D.A., Washington, D.C.

Yu H., Chronis, D., Lu., S., & Wang, X. (2011) Chorismate mutase: an alternatively spliced parasitism gene and a diagnostic marker for three important *Globodera* nematode species. *European Journal of Plant Pathology*, **129**, 89-102.