Notas

New host association and first molecular determination of *Peltophorus polymitus* (Coleoptera: Curculionidae) in Mexico

Nueva asociación de hospedero y primera determinación molecular de *Peltophorus polymitus* (Coleoptera: Curculionidae) en México

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Abstract: *Peltophorus polymitus* Boheman (Coleoptera: Curculionidae) is a coleopteran species that feeds on various agave species cultivated in Mexico; however, it has not been formally considered as a pest to date. This beetle is widely distributed throughout Mexico and is associated with several agave species, including *Agave angustifolia* Haw., *Agave cupreata* Trel. & A. Berger, *Agave durangensis* Gentry, *Agave kerchovei* Lem., *Agave palmeri* Engelm., and *Agave tequilana* Weber. This study reports the first documented occurrence of *P. polymitus* feeding on *Agave inaequidens* Koch “maguey alto” in Michoacán, Mexico. Photographic evidence of the plant damage caused by this insect is presented.

Additionally, this is the first report of *P. polymitus* mitochondrial COI gene sequencing, along with a molecular phylogenetic analysis based on COI sequences of American Zygopini species available in GenBank. A total of 166 adult specimens and 172 larvae were recorded in a wild maguey alto plantation in Michoacán. This agave species is used by rural communities for food, medical purposes, and mezcal production.

Keywords: *Agave inaequidens*, Damage illustration, Molecular identification, Spotted agave weevil.

Resumen: *Peltophorus polymitus* Boheman (Coleoptera: Curculionidae) es un escarabajo que se alimenta de varias especies de agaves cultivadas en México, sin que a la fecha haya sido considerado formalmente como una plaga. Este insecto se distribuye ampliamente en todo México y se asocia con varias especies de agave, incluyendo *Agave angustifolia* Haw., *Agave cupreata* Trel. & A. Berger, *Agave durangensis* Gentry, *Agave kerchovei* Lem., *Agave palmeri* Engelm. y *Agave tequilana* Weber. Este trabajo registra el primer reporte de *P. polymitus* alimentándose de plantas de *Agave inaequidens* Koch “maguey alto” en Michoacán, México. Se presentan imágenes de los daños que estos insectos provocan a las plantas. Además, este es el primer reporte de la secuenciación del gen COI mitocondrial de *P. polymitus*, junto con un análisis filogenético molecular basado en secuencias COI de especies americanas de Zygopini disponibles en GenBank. La investigación registró 166 adultos y 172 larvas que se encontraron en una plantación silvestre de maguey alto en Michoacán. Esta especie de agave es utilizada por comunidades rurales con fines alimentarios, medicinales y para la producción de mezcal.

Palabras clave: *Agave inaequidens*, Escarabajo pinto del agave, Identificación molecular, Ilustración de daños.

The genus *Peltophorus* Schoenherr, 1845 (Curculionidae: Conoderinae, Zygopini) includes the species *Peltophorus adustus* (Fall, 1906), *Peltophorus jordani* Heller, 1895, and *Peltophorus polymitus* Boheman, 1845, which are found in the southwestern United States, Mexico, and Honduras (Anzaldo, 2017). *Peltophorus* is characterized by the following features: the posterior margin of the mesoventrite is expanded to accommodate the rostrum at rest; large eyes dominate the head; the pygidium is exposed in dorsal view; the subapical pronotal constriction is sulcate; and the second funicular segment is subequal to or shorter than the first (Romo & Morrone, 2012; Anzaldo, 2017). All three species are present in Mexico (González-Hernández et al., 2015; Muñiz-Vélez et al., 2015; Figueroa-Castro et al., 2016; Zaragoza-Caballero et al., 2019), but only *P. adustus* and *P. polymitus* are associated with several economically important agave species (González-Hernández et al., 2015; Figueroa-Castro et al., 2016). *Peltophorus polymitus*, known as the spotted agave weevil, can be distinguished from *P. adustus* by its coloration pattern of the pronotum and propleura, as well as the shape of the pronotum. In *P. polymitus*, the pronotum and propleura are white with irregular black spots, and the lateral edges of the pronotum converge apically (Fig. 1a, b). In contrast, the pronotum of *P. adustus* is black with whitish lateral areas, while the propleura is white, and the lateral edges of the pronotum appear approximately parallel (Sleeper, 1963). Unlike *P. adustus*, which is limited to the state of Guerrero and associated with *Agave angustifolia* Haw. and *Agave cupreata* Trel. & Berger (Figueroa-Castro et al., 2016), *P. polymitus* is recorded in 16 states of the Mexican Republic (Chiapas, Durango, Estado de México, Guerrero, Hidalgo, Jalisco, México, Michoacán, Nuevo León, Oaxaca, Puebla, Querétaro, San Luis Potosí, Sonora, Tamaulipas, and Tlaxcala; Salazar-Rivera et al., 2024), associated with *Agave palmeri* Engelm. (Sleeper, 1963), *Agave durangensis* Gentry (Reyes-Muñoz et al., 2020), *Agave kerchovei* Lem. (Brena-Bustamante, 2012), *Agave tequilana* Weber, *A. angustifolia,* and *A. cupreata* (González-Hernández et al., 2015; Salazar-Rivera et al., 2024). The damage caused by this insect to the leaves (pencas) and/or floral scapes (quiotes) of various agave species is detailed in the studies of Brena-Bustamante (2012), González-Hernández et al. (2015), and Reyes-Muñoz et al. (2020). To date, there are no reports indicating that *Agave inaequidens* Koch, or “maguey alto", serves as a host plant for the spotted agave weevil. Maguey alto is a wild agave species distributed in the Trans-Mexican Volcanic Belt, particularly in the mountain chain stretching through central Mexico and a large part of the state of Michoacán, at elevations of 1700 to 2600 meters above sea level (Torres-García, 2015). For the rural communities of Michoacán, maguey alto has significant cultural and economic value, as it is used for food, living fences, fodder, fiber, medicine, and mezcal production. The primary form of reproduction for this agave is through seeds produced in the pods that develop in the quiote; however, it has also been documented that under cultivation conditions, it can reproduce asexually through offshoots, or "hijuelos" particularly those that sprout in response to physical damage (Torres-García, 2015).

During recent collection efforts in the Área Voluntaria para la Conservación “El Tocuz” (19°29′10.8” N, 101°21′30.1” W and 2338 m), Acuitzio del Canje, Michoacán, on June 25, 2022; August 25, and September 2, 2023, we recovered 166 adults and 172 larvae of *P. polymitus* feeding in a wild plantation of maguey alto (Fig. 1a-h). To document this finding, the adults found on the leaves and floral scapes were killed and preserved in 70 % alcohol, fixed with pins, and identified using Sleeper (1963) and Romo & Morrone (2012) keys. The larvae, which were extracted from pieces of infested quiotes, were killed in acid alcohol (70 % ethanol + glacial acetic acid, 9:1) and preserved in 70% ethanol. All voucher specimens were deposited at the Colección de Plagas Agrícolas from Instituto de Investigaciones Agropecuarias y Forestales, Universidad Michoacana de San Nicolás de Hidalgo (at Tarímbaro, Michoacán, México).

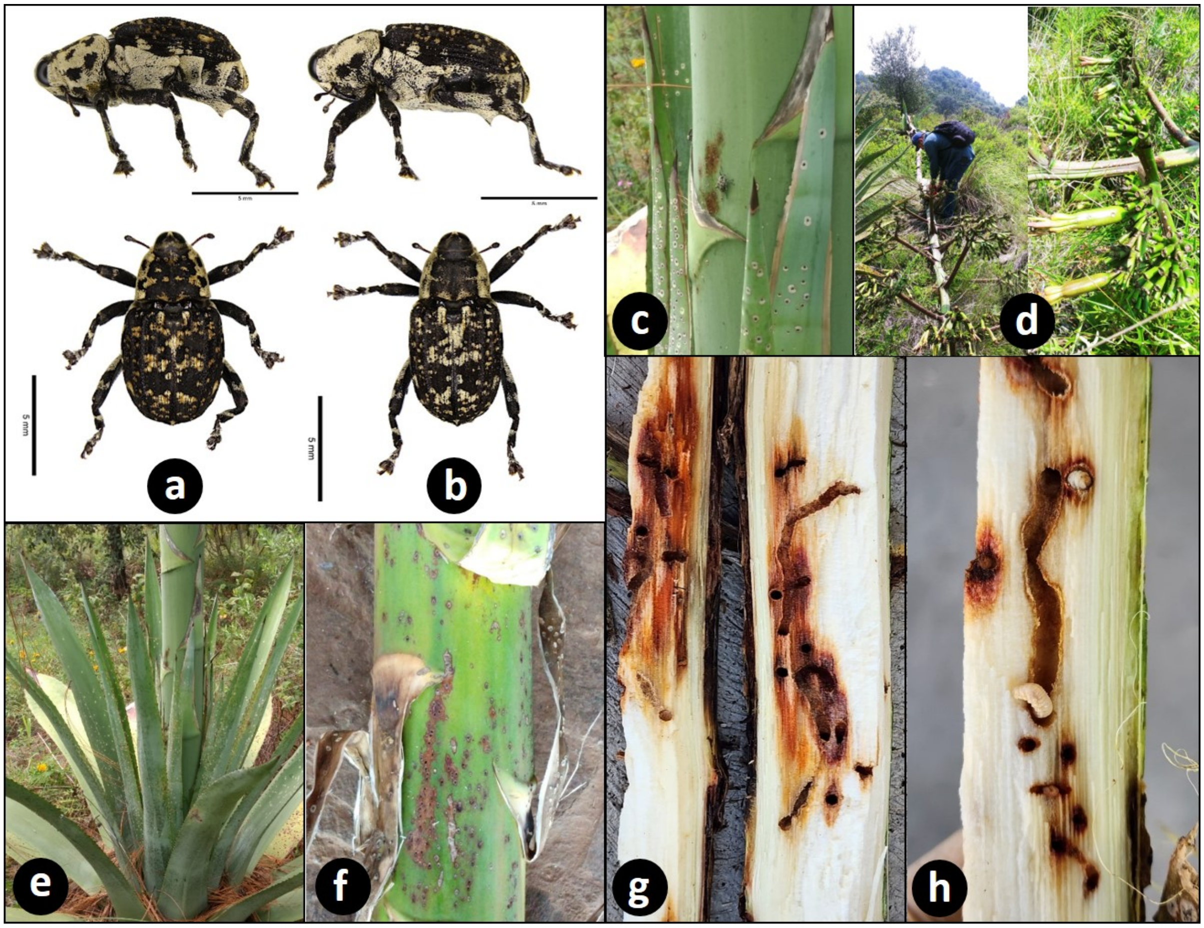

Figure 1
***Peltophorus polymitus*.**
Lateral and dorsal views of female (a) and male (b). c-f. Feeding damage to leaves and quiotes of *Agaveinaequidens* by adults. d. Branches of the quiote with damaged floral pods. g-h. Larvae feeding inside *Agaveinaequidens* quiotes.

To strengthen this study, we also used molecular data to confirm the identity of *P. polymitus* and provide an additional tool for its identification. For this, 10 adults from the same samples were preserved in 96 % alcohol for molecular analysis. The molecular characterization of the spotted agave weevil involved sequencing the mitochondrial gene Cytochrome Oxidase subunit I (COI) from two adults of approximately 6.5 to 8.5 mm in length. We amplified the COI gene using primers designed by Folmer et al. (1994) (LCO-1490f and HCO-2198r). DNA extraction was conducted with a 2 % CTAB solution (Doyle, 1991) and sodium acetate buffer, while purification was performed using the Wizard® Genomic DNA Purification Kit. For DNA quantification, we used spectrophotometry with a NanoDrop 2000 (Thermo Scientific RMA, Wilmington, Delaware, USA). PCR amplification of the product was carried out under specific conditions. The master mix included 7.86 μL of ultra-pure water, 3 μL of 10X buffer, 0.6 μL of dNTPs, 0.18 μL of LCO-HCO primers, 0.18 μL of Taq DNA polymerase, and 3 µL of DNA (20 ng). The cycling program featured an initial denaturation at 94 °C for 3 minutes, followed by 35 cycles of 94 °C for 30 seconds, annealing at 45 °C for 1.5 minutes, and 72 °C for 1 minute for the final extension, utilizing a C1000 Touch thermal cycler (Bio-Rad, Foster City, California, USA). Amplified products were visualized by electrophoresis on a 1.5 % agarose gel (SeaKem, Lonza, Greenwood, South Carolina, USA), and the results were documented using an Infinity system 3026/WL/LC/26 MX X-Press (Vilber Lourmat, Deutschland GmbH, Eberhardzell, Germany). For DNA sequencing, the amplified products were cleaned with ExoSAP (Affymetrix, Santa Clara, California, USA) and sequenced using a 3130 DNA 4-capillary Genetic Analyzer (Applied Biosystems, Foster, California, USA). When the sequences were obtained, they were assembled using BioEdit v.7.0.5 software (Hall, 1999) and compared with multiple zygopines sequences through the Basic Local Alignment Search Tool (BLASTN, developed by Altschul et al. [1997] on the National Center for Biotechnology Information [NCBI] platform). This alignment method incorporated gaps (Higgins et al., 2005). The generated sequence was compared with those of American Zygopini species available at NCBI, using the *Copturus aguacatae* Kissinger (Lechriopini) sequence (from Mexico) to test the monophyly of the group and as an external conoderine (Table I). A phylogenetic analysis was performed with MEGA v. 11 software, using the neighbor-joining tree method (with 1000 bootstrap replicates). Phylogenetic distances were calculated using the Jukes-Cantor method (1969) and were measured in the number of base substitutions per site. All ambiguous positions were removed from each sequence pair (pairwise deletion option).

ID CODE	Database	Locality/Country	Molecular analysis species	Identity (%)	Deposito
PV790451	GenBank	Michoacán, MEXICO	*Peltophorus polymitus* 1	100	IIAF-UMSNH
PV790451	GenBank	Michoacán, MEXICO	*Peltophorus polymitus* 2	100	IIAF-UMSNH
MH404110.1	GenBank	USA	*Peltphorus* sp.	99	Life Sciences, Natural Histo
OP837063.1	GenBank	BRAZIL	*Hemicolpus abdominalis*	82.36	Biodiversity and Bioestatisti
FJ859935.1	GenBank	USA	*Cylindrocopturus* sp.	81.49	Organismic & Evolutionary
DQ346712.1	GenBank	PERU	*Zygops wiedii*	81	Department of Biology, NY
HQ599843.1	GenBank	Oaxaca, MEXICO	*Copturus aguacatae*	78.04	American Museum of Natur

Table I

Nucleotide sequences from *Peltophorus polymitus* and other zygopine species which were downloaded from the GenBank database

Note: Length sequences in all species were between 600 and 660 base pairs (bp).

This finding constitutes the first record of adults and larvae of *P. polymitus* feeding on leaves and floral scapes of maguey alto, in the state of Michoacán. Notches or punctures in the leaves and floral scapes created by adults, as well as galleries in the floral scapes created by larvae (Fig. 1c-h), are consistent with the damage recorded on *A.*

angustifolia, *A. cupreata*, *A. duranguensis*, *A. kerchovei,* and *A. tequilana* plants (Brena-Bustamante, 2012; González-Hernández et al., 2015; Reyes-Muñoz et al., 2020). Current evidence suggests that while *P. polymitus* does not represent a serious threat to cultivated agave species in Michoacán; its feeding behavior on the quiotes of maguey alto may potentially impact seed production and sexual reproduction in wild populations.

In this study, we produced the first sequence of *P. polymitus* with COI gene, which consists of approximately 656 bp. This sequence was submitted to the GenBank database under accession number PV790451. Molecular phylogenetic analysis revealed a close evolutionary relationship between *P. polymitus* from Mexico and *Peltophorus* sp. from the United States, supported by a high bootstrap value (99/100). This analysis also showed that the zygopine species *Hemicolpus abdominalis* Hustache, *Cylindrocopturus* sp., and *Zygops wiedii* (Germar) are lineages related to *Peltophorus,* which evolved early from a common ancestor (Fig. 2). The presence in Mexico of species belonging to the genera *Hemicolpus*, *Cylindrocopturus,* and *Zygops* (Maes & O´Brien, 1990; Hespenheide, 2018; Palemón-Alberto et al., 2022) suggests that *P. polymitus* and those species probably share the same biogeographic origin.

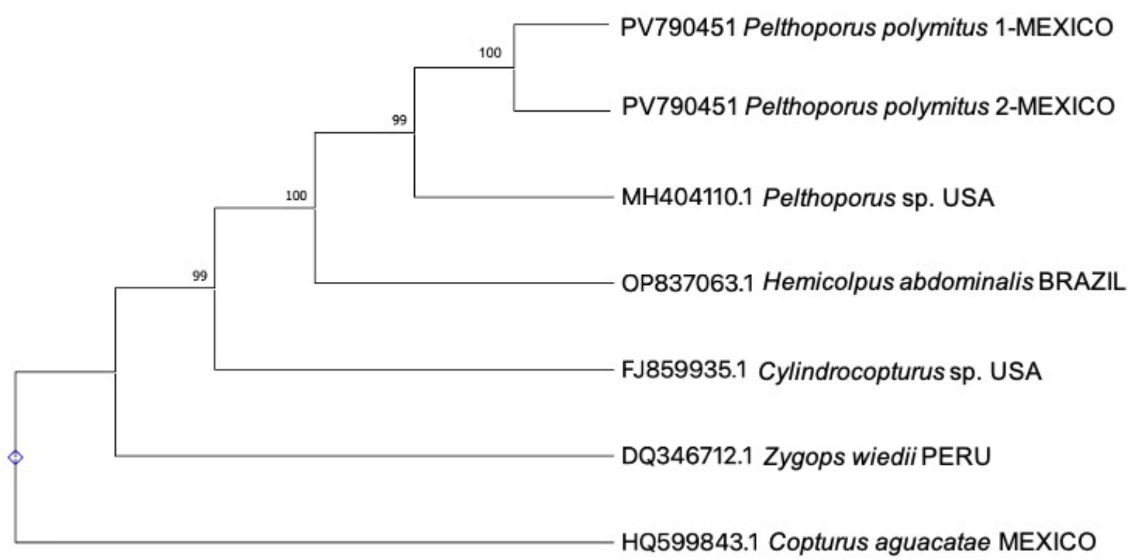

Figure 2.

Phylogenetic tree based on Neighbor-Joining (NJ).

Analysis showing the evolutionary relationships between *Peltophorus polymitus* and other zygopine species. The numbers on the nodes indicate bootstrap support values (based on 1000 replicates). The scale represents genetic distance.

Within the Zygopini tribe there are at least 83 species known from the New World (Anzaldo, 2017), relatively few of them have been sequenced. This is probably the reason why zygopine species have been poorly represented in molecular phylogenies (Anzaldo, 2019). Although it is too early to draw conclusions about Zygopini phylogeny, we conclude that further studies involving sequence data from zygopine species are required to clarify the tribe´s taxonomic status and evolutionary relationships.

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Notes

DATA STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

COMPETING INTERESTS

The authors declare no conflict of interest.

AUTHORS CONTRIBUTIONS

All authors contributed to the conception and design of the study. AUCR, ITM, LJPC, JMCY, and JLLM were responsible for preparing the materials, collecting data, and conducting the analysis. The initial draft of the manuscript was prepared by JIFR, SRO, AMMC, and SPG, followed by revisions and feedback from all authors. All authors read and approved the final manuscript.

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