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First hostplant and last instar of *Hydropionea fenestralis* Barnes & McDunnough, 1914 in the Perote Valley, Mexico (Lepidoptera: Crambidae)

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Abstract

We identified *Hydropionea fenestralis* Barnes & McDunnough, 1914 feeding on seeds of fruits of *Agave salmiana* var. *ferox* (K. Koch) Gentry and *A. salmiana* subsp. *salmiana* (Asparagaceae), based on DNA barcode and adult-stage morphology. This is the first host plant record, the first record last instar, and also constitutes the first Lepidoptera seed borer associated with *Agave* species in Mexico. The *Agave* variety and subspecies are used for the production of traditional fermented beverages called *pulque*, the economic and cultural base of the region. The mean percentage of fruit damage ranges from 13.9±19.9% to 33.2±2% in *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana,* respectably. Molecular evidence confirms the distribution of *H. fenestralis* in central Mexico in the states of Veracruz and Puebla, expanding the known geographic range of the species. This record seems to be an unusual host plant for the current phylogenetic position of *H. fenestralis* within the Glaphyriinae subfamily, whose larvae feed preferably Brassicales plants.

Keywords: Lepidoptera, Crambidae, COI DNA barcode, seed feeder larvae, level of damage, Asparagales, *Hydropionea,* distribution, Mexico.

Primera planta nutricia y estado inmaduro de *Hydropionea fenestralis* Barnes & McDunnough, 1914 en el Valle de Perote, México (Lepidoptera: Crambidae)

Resumen

Identificamos *Hydropionea fenestralis* Barnes & McDunnough, 1914 alimentándose de semillas de frutas de *Agave salmiana* var. *ferox* (K. Koch) Gentry y *A. salmiana* subsp. *salmiana* (Asparagaceae), basándonos en el código de barras de ADN y la morfología en etapa adulta. Este es el primer registro de planta hospedera, último instar, y también constituye el primer Lepidóptera barrenador de semillas asociado con especies de *Agave* en México. La subespecie y variedad de *Agave* se utilizan para la producción de bebidas fermentadas tradicionales llamadas pulque, que son la base económica y cultural de la región. El porcentaje promedio de daño en las frutas varía desde 13.9±19.9% hasta 33.2±2% en *A. salmiana* var. *ferox* y *A. salmiana* subsp. *salmiana*, respectivamente. La evidencia molecular confirma la distribución de *H. fenestralis* en el centro de México, en los estados de Veracruz y Puebla, ampliando el rango geográfico conocido de la especie. Este registro parece ser una planta hospedera inusual para la posición filogenética actual de *H. fenestralis* dentro de la subfamilia Glaphyriinae, cuyas larvas se alimentan preferentemente de plantas Brassicales.

Palabras clave: Lepidoptera, Crambidae, código de barras de ADN, barrenador de semillas, nivel de daño, Asparagales, *Hydropionea,* distribución, México.

Introduction

The superfamily Pyraloidea Latreille, 1809 is one of the most hyperdiverse Lepidoptera groups with over 16,000 described species (Nieukerken et al. 2011; Léger, 2020) and includes two families: Pyralidae Latreille, 1809 and Crambidae Latreille, 1810 (Munroe & Solis, 1999; Regier, 2012). The Pyraloidea has a worldwide geographical distribution with high diversity in the tropics and includes numerous important pests of crops, forests, stored foodstuffs, and ornamental plants (Solis, 2007). It is notable for the ecologically diverse moths it contains, which exhibit various adaptations to explore different sources, including detritivorous, coprophagous, parasitic, and aquatic habitats (Regier, 2012).

Hydropionea Hampson, 1917 genus belongs to the Crambidae family and recently, the genus was transferred from the subfamily Spilomelinae to Glaphyriinae based on morphological evidence (Mally et al. 2019). The *Hydropionea* genus comprises six valid species, and one undescribed species with distribution from North America and Central, Caribbean, and Sud America (Munroe et al. 1995; Janzen & Hallwachs, 2009; Nuss et al. 2003-2022; Scholtens & Solis, 2015; Landry et al. 2020; GBIF, 2024). The subfamily Glaphyriinae *sensu lato* comprises the 'mustard oil clade' whose larvae preferentially feed on Brassicales plants (Regier et al. 2012; Léger et al. 2021). Mustard oils are essential volatiles that use specialized insects to find their host (Hopkins et al. 2009).

For the *Hydropionea* genus, the only known host plant and immature stage are for an undescribed species from Costa Rica that feed on *Capparis uniflora* (Brassicales: Capparaceae) (Janzen & Hallwachs, 2009), which fits with the Glaphyriinae in the clade of Brasicales (Mally et al. 2019; Léger et al. 2021). However, for the remaining six species of *Hydropionea*, the host plants and immature stages are unknown.

During an investigation on the viability of seeds of *Agave* species, in September 2020, the first author found Microlepidoptera larvae feeding on seeds inside fruits of the culture of *A. salmiana* var. *ferox* (K. Koch) Gentry, and *A. salmiana* subsp. *salmiana* (Asparagales: Asparagaceae) in Perote Valley, Veracruz and in the state of Puebla, Mexico. The subspecies and the variety of *Agave* are planted in the boundary of the culture of beans and corn and are used to delimit property and to produce "pulque" (a fermented beverage) which is an economic incentive and cultural activity in the region (Delgado-Lemuset et al. 2014).

The objective of the present work is to identify larvae feeding on seeds of *Agave* fruits, using morphological and DNA barcoding approaches, and estimate seed levels of damage in *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana*.

Materials and Methods

Collecting Area, Rearing and Morphological Study

The collecting area is within the Perote Valley, the central western area of the state of Veracruz mainly, and in the El Carmen Basin in the state of Puebla, Mexico located between parallels 19º 22' and 19º 39' North latitude and the meridian 97º 06' and 97º 26' West longitude, 2,800 masl (Yañez-Garrido & Nava-Bringas, 2017). The *Agave* subspecies is locally known as Tepezorra and the variety as *Agave* Manso or Verde. The scientific accepted names are *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana* (World Flora Online, 2024). We randomly collected *Agave* fruits of both variety and subspecies of *Agave salmiana* from September to November 2020. We inspected some fruits for seed borer larvae for ADN analysis. Four larvae were stored in 90% alcohol for DNA barcode. We also found last instar larvae or pupae inside fruits, which were placed in plastic containers in laboratory conditions until adult emergence. After emergence, we mounted the adults and let them dry for two weeks. We prepared genitalia by boiling abdomens for 15 minutes in 10% KOH solution in a test tube with 5 ml of water (Laguerre, 2014). Images were captured with a Nikon D7500 camera with an AF-S

Nikkor 85 mm macro lens and genitalia with a Leica DM3000 microscope (Leica, Wetzlar, Germany). Identification was based on the original description and illustrations provided by Barnes & McDunnough (1914) and Powell & Opler (2009). Specimens will be deposited in the Colección Lepidopterológica (Heterocera) del Museo de Zoología de ECOSUR/Chetumal (ECO-CH-LN).

DNA Extraction and Analysis

DNA was extracted from the dorsoventral tissue of the larva (2 mm) using the DNeasy Blood & Tissue Kit (QIAGEN, Düsseldorf, Germany), following the manual instructions. Amplified PCR products were submitted to Eurofins Genomics (USA) to forward and reverse Sanger sequencing, targeting the 658 bp barcode region of the mitochondrial cytochrome oxidase subunit I (COI) using the primers LepF and LepR (Hajibabaei et al. 2006). The forward and reverse sequences obtained were aligned, and the consensus sequence was assembled using Bioedit 7.2.5. software (Hall, 1999). Species identification was performed by comparing the consensus sequences against BOLD system (http://www.boldsystems.org/) and using the Blastn tool on the NCBI database (www.ncbi.nlm.nih.gov). Neighbor-Joining (Saitou & Nei, 1987) tree were calculated with MEGA version 11.0.13 (Tamura et al. 2021) using Kimura 2-parameters (Kimura, 1980) distance model to display divergences comparing our sequences with available sequences from NCBI and BOLD system databases. Bootstrap values (Felsenstein 1985) were used to estimate branch support.

Evaluation of *Agave* Seed Damage

To assess the level of damage by the seed borer we collected 10 closed fruits with eight repetitions of both *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana*. We compared the mean percentage with an ANOVA with normalized data with arcsine transformation and at 0.05 confidence interval.

Occurrence Records

The distribution maps were made in ArcMap 10.8 software using the field collection and data obtained from the Global Biodiversity Information Facility (GBIF 2022; www.gbif.org, accessed on 16 March 2023; https://doi.org/10.15468/dl.j6fz2q.

Results

We obtained adults from larvae seed borers raised on fruits of *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana* used for pulque production (Figure 1A-D). Larvae were recorded from September to October and adults' emergence began on 19 September through November 2020. Fruits showed a characteristic small hole for the entrance/emergence of the larvae or adult (Figure 1E). As far as we could observe one larva caused damage to all seeds in the *Agave* fruits as reached the adult stage (Figure 1F). One adult emerges from a single fruit of *Agave*. The mean percentage of fruit damage was statistically higher in *A. salmiana* var. *ferox* compared with *A. salmiana* subsp. *salmiana* with 33.2±20.6 and 13.9±19.9%, respectably.

The seed borer was identified as *H. fenestralis,* based on DNA barcode and adult morphology. We generated three consensus sequences of 658 bp available at GenBank under accession numbers OQ092765-OQ092767, which match with one available sequence JF847159 in GenBank and the BOLD system with *H. fenestralis* assigned to the Barcode Index Number (BIN) BOLD: ADH9941. (Figure 2).Intraspecific *p*-distance ranging from 0.2% to 0.6% between specimens of central Mexico and Arizona, U.S. (state of the type locality).

The collecting area of *H. fenestralis* represents a new state record from Mexico in the states of Veracruz and Puebla (Figure 3) in a plantation of *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana* at 2,800 altitudes. The region is semi-arid and predominates xerophytic shrubland vegetation.

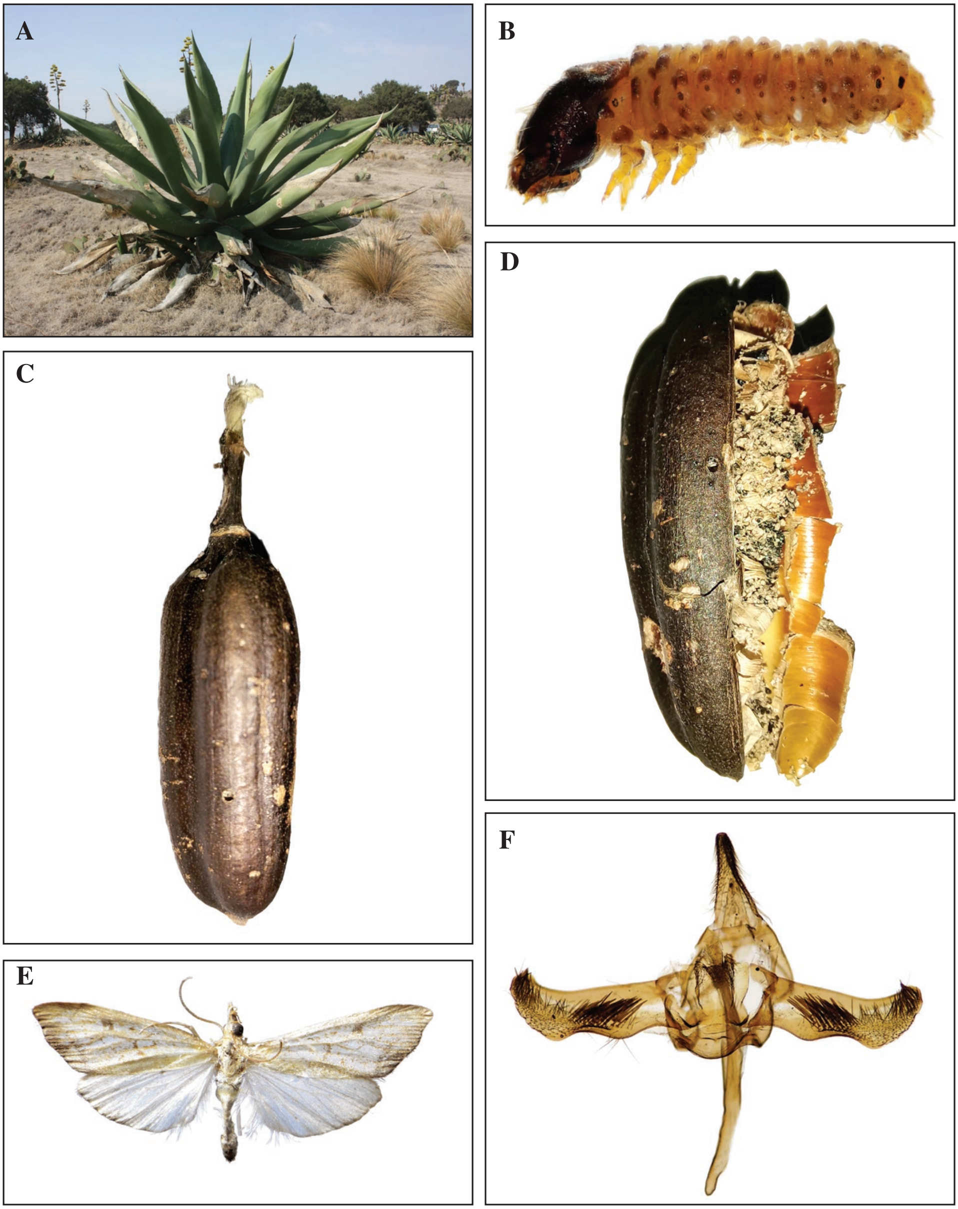

Figure 1. Natural history of *Hydropionea fenestralis* Barnes & McDunnough, 1914 in central Mexico. **A**) Habitat of *H. fenestralis* in *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana* culture surrounded by xerophytic shrubland vegetation. **B**). Last instar of immature state of *H. fenestralis.* **C**) Fruit of *A. salmiana* var. *ferox* with small hole damage (arrow) and **D**) Internal damage of the seed of *A. salmiana* var. *ferox* by the larva of *H. fenestralis*. **E**) Dorsal habitus of *H. fenestralis* reared from larva in mature fruits of *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana*. **F**) *H. fenestralis* male genitalia with aedeagus attached.

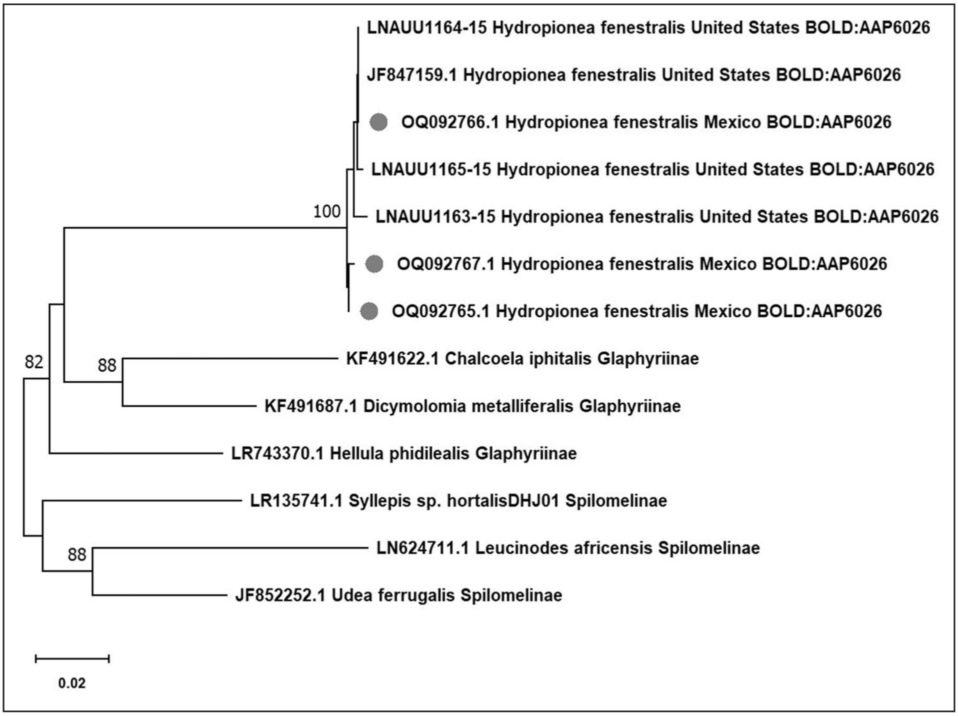

Figure 2. A Neigbor-Joining optimal tree was constructed with Mega 11 using the Kimura 2-parameter base on COI sequences (658 bp) with a gamma distribution using four shape parameters and pairwise deletion option. We used sequences obtained in this study (gray circle) and sequences of the species *Hydropionea fenestralis*, Spilomelinae, and Glaphyriinae subfamilies species from NCBI and BOLD. Bootstrap support values (1000 replications) higher than 70% are shown above the respective branches. *H. fenestralis* sequences include country and Barcode Index Number (BIN) from BOLD system database.

Discussion

We successfully identified the larvae feeding on fruits of *Agave salmiana* variety and subspecies as *Hydropionea fenestralis,* through morphological and DNA barcode analysis. This is the first record of *H. fenestralis* feeding on *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana,* and we also illustrate the last instar of *H. fenestralis* for the first time.

A. salmiana var. *ferox* and *A. salmiana* subsp. *salmiana* are among the main taxa used to produce alcoholic beverages, and the fermenting sap for the beverage called *pulque* is linked to the economy and the culture of the people of the region (Delgado-Lemuset et al. 2014; Trejo et al. 2020). The damage caused by *H. fenestralis* larvae can significantly reduce seed production, as the larvae consume or damage the whole seeds of fruits. The range of fruit damage is from 13.9±19.9% to 33.2±2% for *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana* respectably.

Interaction between *H. fenestralis* with *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana* could lead to detrimental consequences for both populations. The production of pulque involves harvesting *Agave* plants before they reach maturity (Martínez-Salvador et al. 2015), which might leave *H. fenestralis* larvae without fruits for their development. Conversely, a high population of *H. fenestralis* could reduce the availability of viable seeds in the few *Agave* individuals that can reach

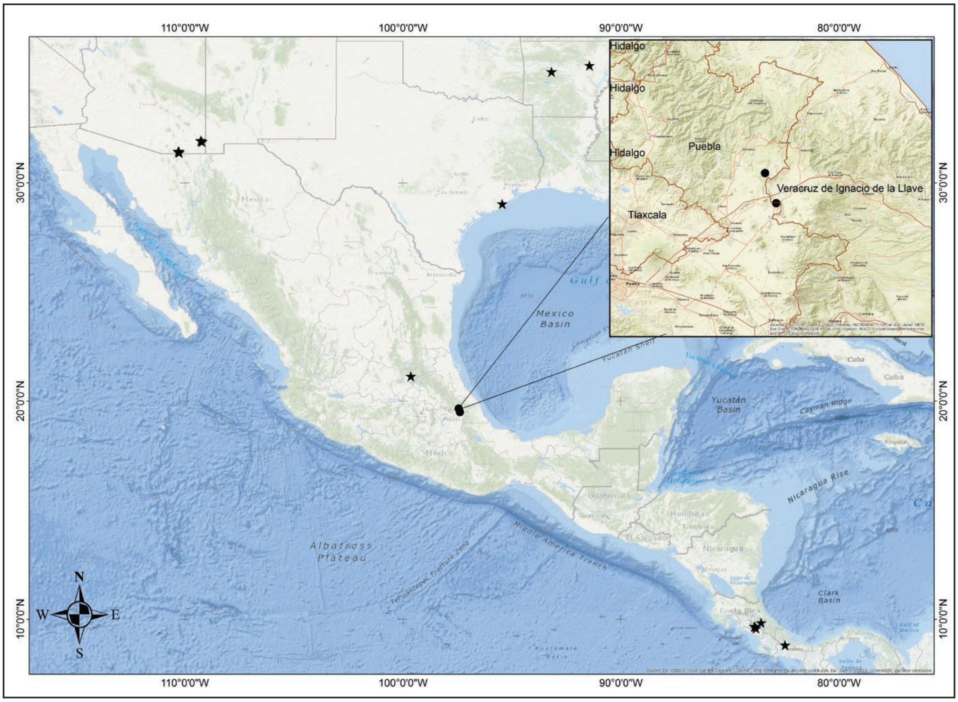

Figure 3. Geographical distribution of *Hydropionea fenestralis*. Previous records (stars), new records (circles).

maturity, thereby further impacting the genetic diversity of cultured variety and subspecies of *Agave* (Rojas et al. 2007; Vasconcelos et al. 2020).

This study is also the first record of a microlepidoptera from the Crambidae family feeding on *Agave* seeds in Mexico (Rodríguez et al. 2019). The *Agave* genus has various associations, both beneficial and detrimental with insects, only Mexico has registered 273 species of insects belonging to 63 families and 7 orders (Rodríguez et al. 2019). Among these species, *Peltophorus adustus* Fall and *Enoclerus zonatus* (Klug) are the only known ones that feed on *Agave* seeds (Figueroa-Castro et al. 2017; Rodriguez et al. 2019).

Our distribution record of *H. fenestralis* extends more than 1200 km from the type locality in Arizona, U.S., and north of Mexico to Puebla y Veracruz states (Barnes & McDunnough, 1914; Scholtens and Solis, 2015). DNA barcode reveals low intraspecific variation with less than 0.07% differences between specimens from Arizona and central Mexico. Additional distribution areas, as documented in the Global Biodiversity Information Facility (GBIF) database (http://www.gbif.org/), included the states of Texas and Arkansas U.S, Queretaro in Mexico, Central America, Costa Rica, and Panama.

The variety and subspecies of *Agave salmiana* grow in wild populations in the states of Durango, Hidalgo, Puebla, Querétaro, and San Luis Potosí and cultivated species extend from Coahuila and Nuevo León, Veracruz to Chiapas (Magallán-Hernández & Hernández-Sandoval, 2000; Reynoso-Santos, 2012). *Hydropionea fenestralis* may occur in the states with wild populations of *A. salmiana* and some cultivation areas for *pulque* production, as in the case of this study. Since *A. salmiana* var. *ferox* and *A. salmiana* subsp. *salmiana* are native to Mexico, *H. fenestralis* it is possible that *H.*

fenestralis might feed on another *Agave* species in the northernmost distribution in Arizona and south in Panama. The distribution of the *Agave* genus spans from the southern U.S. to Colombia, the Caribbean, and Venezuela (García-Mendoza, 2002). However, little is known about the distribution of *H. fenestralis* and further confirmation is needed to establish the specialist association between *H. fenestralis* with *Agave* species.

The only known host plant for the *Hydropionea* genus comes from an undescribed species raised on *C. uniflora*, which is a plant that produces mustard oil, congruent with the phylogenic clade of Glaphyriinae (Janzen & Hallwachs, 2009; Regier et al. 2012; Léger et al. 2021). Interestingly, the genus *Agave,* a monocotyledonous plant of the order Asparagales, does not fit the clade of Brassicales or mustard oil clade (Glaphyriinae sensu lato), where recently the genus *Hydropionea* was transferred (Mally et al. 2019). Glucosinolates are chemical compounds commonly found in Brassicales, that in the presence of myrosinase enzyme result in different hydrolytic products like isothiocyanates (mustard oils), which are essential volatiles in specialized insects that use them as host-finding cues (Hopkins et al. 2009). Although mustard oils are found mainly in the Brassicales clade, other plant species outside of the Brassicales also exhibit mustard oils such as *Drypetes* (Malpighiales: Putranjivaceae), which is the host plant of *Trischistognatha*, a species in the genus within the Glaphyriinae (Soltis & Soltis, 2004; Kala & Khan, 2020; Léger et al. 2021). This led to the suggestion of a host-plant switch from an ancestral Brassicales host plant to *Drypetes* (Léger et al. 2021). However, the occurrence of *H. fenestralis* in *Agave* may suggest a different evolutionary history, as the family Agavaceae and the order Asparagales are not recognized as glucosinolate or isothiocyanate-producing plants (Dahlgren, 1980; Rodman et al. 1998). Further ADN analysis using mitochondrial and nuclear genes will confirm the phylogenetic position of *Hydropionea* genus.

The *Agave* genus is endemic to America, and Mexico has the highest richness with 160 species and 130 endemic species, which represent 76 and 62% of the continental richness (Jimenez-Pérez et al. 2021). Additional investigations are needed targeting fruits of *Agave* species to discover new host plant species and new distribution areas for *H. fenestralis* or related species.

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