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# Taxonomic Identity of Lepidopteran Insects Associated with Mangrove Mortality in the "La Encrucijada" Biosphere Reserve, Chiapas, Mexico

**Niño-Domínguez, Alicia[1]; Martin-Gómez, Martín F.[2]; Zamorano-Vázquez, Luis A.[2]; Macías-Sámano, Jorge E.[3]; Escalona-Domenech, Raisa Y.[1*]; Aguirre-Cadena, Juan F.[1]; Magallanes-Cedeño, Ricardo[1]**

[1] Universidad Autónoma de Chiapas, Facultad de Ciencias Agrícolas, Campus IV. Huehuetán, Chiapas, C. P. 30660. México.
[2] Comisión Nacional de Áreas Naturales Protegidas, CONANP La Encrucijada. Acapetahua, Chiapas, C. P. 30580. México.
[3] Synergy Semiochemicals Corp. Canada
* Correspondence: raisa.escalona@unach.mx

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## ABSTRACT

**Objective**: To provide the responsible authority, CONANP Encrucijada, with the taxonomic identity of the folivorous larvae found on mangrove species and associated with their mortality.

**Design/Methodology/Approach**: Through field surveys in affected areas, larval specimens were collected for confinement, rearing, and observation until their metamorphosis into adults. External morphological characters were then reviewed, focusing on wing vein patterns, dorsoventral coloration, the presence of light and dark bands, and circular markings such as ocelli, to determine taxonomic identity using keys and image comparisons.

**Results**: The taxonomic identity of the collected larvae corresponded to two families within the order Lepidoptera: Nymphalidae and Hyblaeidae, and the species *Junonia evarete* (Cramer) and *Hyblaea puera* (Cramer), respectively.

**Study Limitations/Implications**: The presence of both species, each capable of consuming large amounts of foliar biomass while exploiting the same host, implies more severe damage to leaf tissue. This necessitates ongoing evaluation and monitoring to understand their impact on the optimal development and recovery of *Avicennia germinans*.

**Findings/Conclusions**: The results represent the first report of the distribution of these two Lepidoptera species in the La Encrucijada Biosphere Reserve. A comprehensive study on the impact of folivory on *Avicennia germinans* and its relationship with environmental degradation is urgently needed.

**Keywords**: folivory, mangrove mortality, taxonomic, coastal wetlands

## INTRODUCTION

Mangrove ecosystems develop in estuaries, lagoons, and river mouths along the coastlines of tropical and subtropical regions worldwide (Yáñez-Arancibia & Lara-Domínguez, 1999). These are areas of high ecological and socioeconomic value due to their rich biodiversity and productivity, driven by their ability to retain sediments and organic matter through the aerial root systems of mangrove species such as *Rhizophora mangle*. This facilitates the

development of numerous aquatic and terrestrial species, many of which are utilized by local communities for economic activities such as aquaculture, agriculture, and the extraction of tannins, wood for construction, and fuel (López *et al.*, 2020). It is estimated that mangrove ecosystems can produce from 8.5 t $ha^{-1}$ $yr^{-1}$ (Félix-Pico *et al.*, 2006) to 24 t $ha^{-1}$ $yr^{-1}$ (Sol-Sánchez *et al.*, 2022) of biomass, a figure considered high compared to forest (Návar-Cháidez & Jurado-Ybarra, 2009) and agricultural (Martínez-Romero & Leyva-Galán, 2014) systems. Loss of mangrove areas leads to a reduction in productivity, which is reflected in decreased fisheries output. For example, it is estimated that the loss of one hectare of mangrove may result in an annual loss of approximately 800 kg of shrimp (Sánchez-Hernández, 2002; Sol-Sánchez *et al.*, 2022). Various factors undermine the ecological stability of mangroves, including storms, hurricanes, and cyclones (Velázquez-Salazar *et al.*, 2021), which occur cyclically each year, causing significant loss of forest cover. However, mangroves also provide natural protection for the coastline and show high resilience to these meteorological events, which is positively related to the organic matter content in affected sites (Amaral *et al.*, 2023; Agraz *et al.*, 2015). At the local scale, biotic factors can also cause mortality of large mangrove areas. Reports frequently document the death of mangrove trees following severe defoliation caused by Lepidoptera larvae or stem boring by coleopterans and their symbiotic phytopathogens (Ortíz-Reyes *et al.*, 2018; Perdomo *et al.*, 2018). In the coastal regions of Brazil, atypical foliar herbivory events have been reported. Mehlig and de Menezes (2005) documented severe herbivory in *Avicennia germinans* (Cramer) caused by *Hyblaea puera* (Cramer) (Hyblaeidae) in the Ajuruteua Peninsula in 1998. In 2016, 66% of mangroves (approximately 20,300 ha) dominated by *A. schaueriana* (Stapf & Leechman) were defoliated by the same insect in Paraná (Ditzel-Faraco *et al.*, 2019). In Mexico, *Anacamptodes* sp. (McDunnough) (Geometridae) was studied in the municipality of Cárdenas, Tabasco, due to its folivory on *A. germinans* (Cramer), the dominant mangrove species (99.6%). The biomass consumed by this lepidopteran exceeded 57% of a 50-ha sample within a total affected area of 3,841 ha in 2011 (Sol-Sánchez *et al.*, 2015). More recently, in the same municipality at Laguna Mecoacán, Vázquez-Vázquez *et al.* (2024) reported intense herbivory on *R. mangle* (L.) and *Laguncularia racemosa* (L.-C.F. Gaertn) caused by the lepidopteran *Hylesia colimatifex* (Dyar) (Saturniidae). While folivory by these Lepidoptera species often occurs as part of the natural energy flow dynamics, epidemic or atypical outbreaks have been linked to progressive mangrove degradation, hydroperiod variation, hydrological flow changes, and meteorological events (Saur *et al.*, 1999; Elster *et al.*, 1999). In the mangroves of the La Encrucijada Biosphere Reserve (RBLE), Chiapas, technical personnel from the managing agency CONANP reported, following monitoring conducted between September and November 2024, a total of 187 ha of mangroves affected by Lepidoptera larval folivory. This represents 0.3% of the RBLE's total area (144,868 ha). The wetlands in this region are among the most productive, with the tallest mangrove trees on the Pacific coast of the Americas, reaching heights of up to 35 meters. Their productivity supports and shelters endemic fauna such as the bird *Campylorhynchus chiapensis* (Salvin & Godman), as well as key mammal species like the jaguar (*Panthera onca* L.), spider monkey (*Ateles geoffroyi* vellerosus Griss), and white-tailed deer (*Odocoileus virginianus* Zimm.), along with numerous commercially important fish species such as mojarra (*Cichlasoma* sp. Swainson), mullet (*Mugil cephalus* L.), and gar (*Lepisosteus tropicus* T. N. Gill) (CONANP, 1999). Therefore, it is crucial to conduct basic scientific studies to identify the biotic factors compromising the ecological stability of the RBLE. The folivory caused by Lepidoptera larvae on mangrove trees is an atypical event, and the populations of these causal agents Lepidoptera larvae had not previously been studied in the RBLE. As such, their taxonomic identity was unknown. The aim of this work was to provide the authorities in charge of the reserve (CONANP) with the formal taxonomic identification of the Lepidoptera larvae responsible for mangrove folivory, as a first step toward guiding ecological studies, population control, and informed management strategies to mitigate their impact on mangrove ecosystem health.

## MATERIALS AND METHODS

### Sampling site

Biological material collections for the identification of the causal damage agent were conducted at the sites Embarcadero Las Garzas (15° 12' 10.5" N; −92° 48' 54.2" W) and Santa Isabel (15° 15.37' 23" N; −92° 54.48' 52.0" W) (Figure 1B and 1A. Geographical location map), where the predominant vegetation is mangrove forest composed of the species *Rhizophora mangle* (L.), *Laguncularia racemosa* (L.-C.F. Gaertn), *Conocarpus erectus* (L.), and *Avicennia germinans* (L.).

### Field collections:

Extensive surveys were conducted at two locations where mangrove tree defoliation had been reported. The first site, "Embarcadero Las Garzas" (Figure 1B. Geographical location map), included seven inspection points, and the second site, "Santa Isabel" (Figure 1B. Geographical location map), also included seven inspection points. The purpose was to record the mangrove species exhibiting defoliation and mortality. Additionally, larvae feeding on mangrove leaves were collected and transported alive to the CONANP station in La Encrucijada.

### Larval rearing:

The collected larvae were placed in one-liter jars with leaves from their host plant. The jar lids were perforated to allow air exchange. Leaves were replaced every three days until the larvae pupated. Upon successful emergence, adult Lepidoptera were transferred to waxed paper bags with their wings folded to minimize friction that could damage scale patterns and wing vein structures.

### Adult identification:

Emerging adults were removed from the waxed paper bags and mounted on a stretcher to allow clear observation of the anterior and posterior wing veins. Taxonomic identification was performed using taxonomic keys and comparison with digital images of adults, focusing on wing vein patterns, circular spots, and coloration (Comstock, 1918), as well as other external morphological traits such as antenna type (García-Barrios, 2015).

**Figure 1**. Geographic location map of the sites: A) Santa Isabel B) Las Garzas Piers.

## RESULTS AND DISCUSSION

The defoliation observed during the surveys consistently affected *Avicennia germinans* trees, from which all specimens ranging from early larval stages to the final stage before pupation were collected. During the Lepidoptera larval defoliation alert, some larvae were collected at the end of August 2024 for taxonomic identification. A total of 25 larvae were collected and transported to the CONANP-Encrucijada station. After an average rearing period of ten days, 15 larvae reached the pupal stage, and of these, five successfully emerged as adults within 4 to 7 days. The successfully emerged adults were used for taxonomic identification, resulting in two Lepidoptera species: *Hyblaea puera* (Cramer) (Hyblaeidae) and *Junonia evarete* (Cramer) (Nymphalidae). Larvae of *H. puera* (Cramer) are eruciform in type, with an elongated, cylindrical body lacking urticating hairs and exhibiting a grayish-green coloration. The pupa is shiny reddish in color, smooth in appearance, and slightly curved. The adult (Figure 2. *Hyblaea puera* (Cramer)) has filiform antennae, a head with a developed proboscis, and maxillary palps. The wings span an average of 3.5 cm, with distinctive coloration between dorsal and ventral sides (Figure 2a-c), as well as between forewings and hindwings. Dorsally, the forewings are grayish brown, while the hindwings are black (Figure 2a), featuring irregular transverse orange bands extending from the inner edge of the wing to about three-quarters of the way outward. On the ventral view, a wing coupling system is observed, consisting of a jugum (a lobe on the forewing) and a frenulum (a spine on the anterior edge of the hindwing) (Figure 2e-f).

The vein configuration (Figure 3. Wing vein structure in *H. puera* Cramer) in the forewing shows the first branch of the median vein (M1) fused with the last branch of the radial vein (R5), and the median vein M2 joined with M3, resulting in an open discal cell. Additionally, a third branch of the anal vein is present (Figure 3A). In the hindwing, the median vein M2 is free, and the median vein M3 is joined with the cubital vein Cu1, also resulting in an open discal cell (Figure 3a-B-D). Three anal veins (1A, 2A, and 3A) are present (Figure 3B).

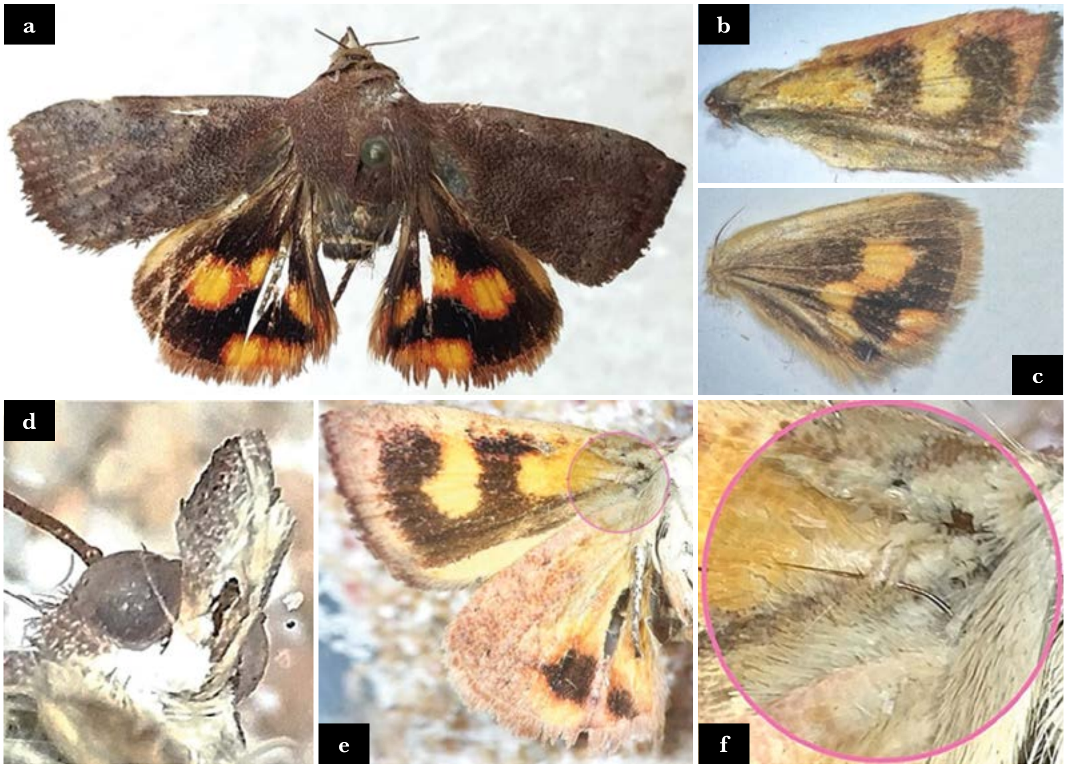

**Figure 2**. Adult *Hyblaea puera* (Cramer): a. Wing spread showing differential coloration between forewing and hindwing. b-c. Ventral view of the forewing and hindwing, respectively. d. Developed proboscis. e-f. Coupling structures: frenulum and jugum.

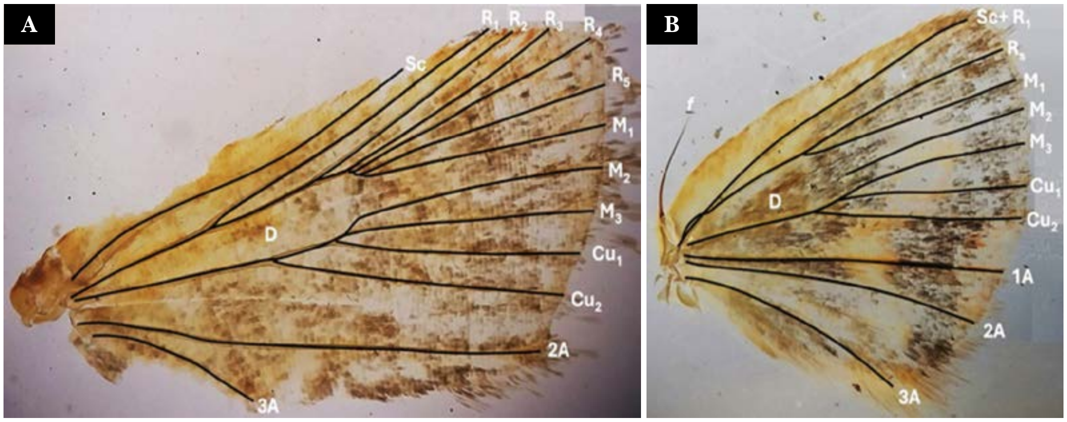

**Figure 3**. Wing vein structure of *H. puera* (Cramer). A. Forewing. B. Hindwing.

In *Junonia evarete*, the eruciform larvae are black with orange spots along the sides; the cylindrical body is adorned with spines. The pupa is grayish with spiny projections on the thorax and attaches to the substrate via a cremaster. The adult (Figure 4. *Junonia evarete* adult) has antennae on the head that are clubbed at the tip, black dorsally and light brown ventrally (Figure 4c-d), and also features well-developed palps and proboscis (Figure 4e). The wingspan measured 4.8 cm. The wings are black (Figure 4a) with distinguishable morphological features such as two short orange bands along the costal margin of each forewing (Figure 4b, upper), while each hindwing displays two transverse bands along the inner margin one wide orange band followed by a narrow light brown band both bordered by black lines (Figure 4b, lower). Additionally, the wings show, on the dorsal side and near the inner margin, circular ocellus-like spots of various sizes (Figure 4a). On the forewings, a small ocellus is located between the median veins M1 and M2, and a larger one between the cubital veins Cu1 and Cu2. On the hindwings, two ocelli appear near the inner margin (Figure 4b), the larger one between veins M1 and M2, and the smaller between Cu1 and Cu2, similar to the forewings (Figure 4a). Each ocellus contains centrally iridescent purple scales (Figure 4e-f). All ocelli are visible in the ventral view of the wings, where a third ocellus (Figure 4c) appears on the hindwings, bordered by a faint black line and located between veins Rs and M1.

The vein configuration (Figure 5. Wing vein structure of *Junonia evarete* Cramer) in the forewing shows the median vein M2 joined with vein M1, and the median vein M3

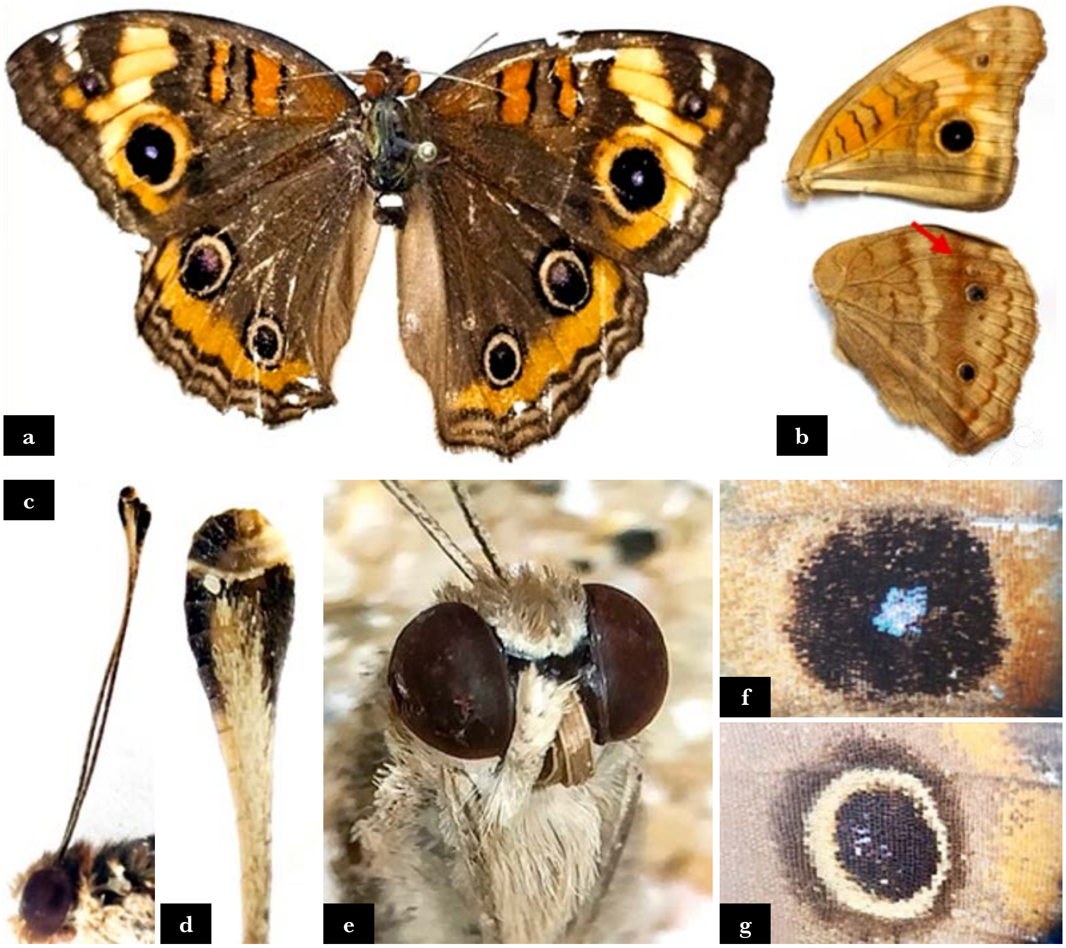

**Figure 4**. Adult *Junonia evarete* (Cramer). a. Wing spread showing the pattern and differential coloration between forewings and hindwings. b. Ventral view of the forewing (upper) and hindwing (lower). C-d. Clubbed antenna. e. Proboscis. f-g. Ocellus-like spots with light blue iridescent scales. Red arrow indicates the third distinguishable ocellus on the hindwing.

joined with the cubital vein Cu1, resulting in an open discal cell (Figure 5A-D). The same configuration occurs in the hindwing, where two anal veins, 2A and 3A, are also present (Figure 5B).

Insects, through herbivory and their various strategies to access resources, fulfill the function of reintegrating nutrients into the ecosystem by consuming biomass from leaves, branches, stems, or roots of living trees in stages of degradation, decline, or weakening. However, an increase in the intensity of herbivory particularly folivory over extended periods prevents the host plant from recovering lost vegetative tissue, thereby reducing its fitness, production of defensive secondary metabolites, and ultimately leading to death (Marquis, 1992; Návar-Cháidez, 2009).

In mangrove ecosystems, folivory occurs at all stages of host development and is mainly carried out by crustaceans of the genus Aratus sp., insects from the order Orthoptera (Families: Tettigoniidae and Acrididae), and Lepidoptera larvae. In this latter group, the intensity of foliar biomass consumption may be enhanced in communities with varying levels of environmental degradation (Gómez-García *et al.*, 2015). Factors such as pH alteration, salinity, and dissolved oxygen in interstitial water affect nutrient availability for mangroves, which in turn compromises their defensive capacity by limiting the production of chemical compounds that reduce leaf palatability to herbivores (Tong *et al.*, 2001; Agraz Hernández *et al.*, 2022). This creates conditions conducive to population outbreaks uncontrolled population explosions sustained as long as the susceptible resource remains available.

The Lepidoptera species *Junonia evarete* (Cramer) is distributed throughout the Neotropical zone of the Americas, ranging from Florida (USA), through the Caribbean, Mexico, Central America, to tropical and subtropical South America. This species is commonly found in secondary vegetation, disturbed environments, and riparian forests (Calhoun, 2010). Although previously unreported in the RBLE, its presence is plausible and suggests that it is part of the local ecosystem. It is worth noting that, in general, the taxonomic identification of species within this genus has not been fully resolved due to incomplete speciation, which results in a high rate of hybridization among species. Recent genomic studies have not included coastal populations from the southeastern Mexican

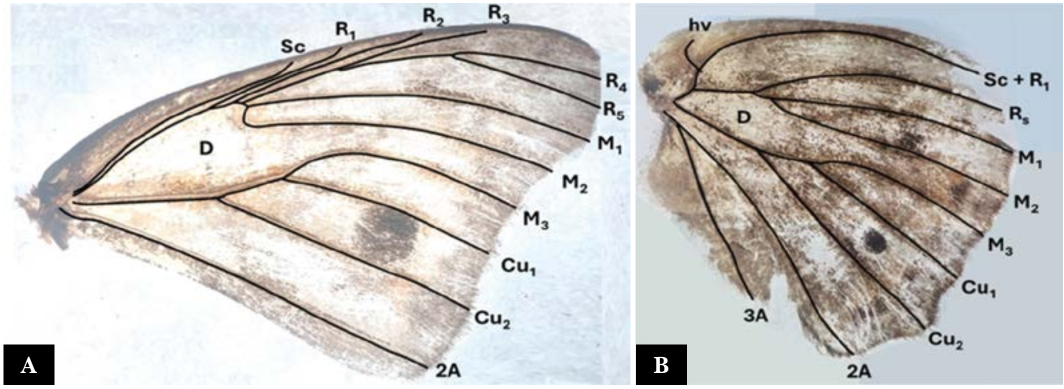

**Figure 5**. Structure of the wing veins of *J. evarete* (Cramer). A. anterior wing. B. hind wing.

Pacific (Coing *et al.*, 2020), highlighting the need for genetic research including these populations to confirm the identity of *J. evarete* (Cramer) in the RBLE. Regarding *Hyblaea puera* (Cramer), this species originates from Southeast Africa. According to FAO (2007), it is distributed across Southern and Eastern Africa, Northern Australia, China, and is also present in the southern U.S. states of Arizona, Texas, and Florida, as well as in the Mexican states of Campeche, Tabasco, and Veracruz, throughout Central America, and down to Paraguay. It inhabits tropical and subtropical forests and is commonly referred to as a major pest in *Tectona grandis* (L.) plantations, where its voracious feeding during the rainy season can reduce commercial wood volume by up to 44% (Cibrián, 2013). *H. puera* (Cramer) larvae exhibit similar behavior when feeding on *Avicennia germinans* (L.), having caused massive mortality of this mangrove species along the coast of Brazil (Mehlig & de Menezes, 2005).

The presence of *H. puera* (Cramer) in the RBLE, as with *J. evarete* (Cramer), has not previously been reported, and it is possible that the species was introduced via teak plantations in the area. Given its status as a potentially introduced species, it is essential to study its biology, ecology, and population dynamics within the region to determine its population status (endemic, epicentric, or epidemic) and to anticipate control strategies to mitigate its impact on *A. germinans* (L.). The presentation of wing vein structures aims to complement the morphological description of each species. Notably, the open discal cell (D cell) observed in both forewings and hindwings may represent a potential identification trait within this taxonomic group, although its diagnostic value remains tentative in the absence of a definitive reference.

## CONCLUSIONS

The identity of the larvae collected and reared for taxonomic identification as adults corresponds to the species *Junonia evarete* (Cramer) and *Hyblaea puera* (Cramer), which are reported for the first time through this study in the coastal zone of the southern Mexican Pacific. Given their potential to consume large amounts of foliar biomass, the co-occurrence of both species in the same site implies a greater impact of folivory on *Avicennia germinans* (L.). Therefore, studies are needed on their biology, ecology, and population dynamics, as well as on the estimation of herbivory impact across different developmental stages of the host. In addition to research focused on the causal agents of folivory, it is recommended to include diagnostic studies to evaluate the degree of environmental degradation that may better explain the causes of atypical (epidemic) herbivory observed in *J. evarete* (Cramer) and *H. puera* (Cramer) populations in the RBLE. This would allow for the establishment of severity criteria and the development of guidelines for their study, control, and informed management, aimed not only at reducing the impact on *A. germinans* (L.) but also at improving the environmental conditions that compromise the health of mangrove ecosystems.

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