

Article

Neosilba batesi Curran (Diptera: Lonchaeidae): Identification, Distribution, and Its Relationship with Avocado Fruits

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Abstract

In this study, the association between *Neosilba batesi* (Diptera: Lonchaeidae) and avocado fruits (*Persea americana* L.) was investigated. Fruits showing signs of rot and infested with Diptera larvae were collected from commercial orchards in the states of Michoacán and Jalisco, Mexico. *N. batesi* was identified in association with fruits from both trees and the ground at all sampling sites. Furthermore, a phylogenetic analysis based on the mitochondrial cytochrome c oxidase subunit I (*COI*) gene supported the morphological identification, showing >99% identity with records from Veracruz, and revealed distinct genetic lineages within the *Neosilba* genus. In a study within one Michoacán orchard, infested tree-borne fruits averaged 5.40 cm in length and 3.90 cm in width, with a mean of 9.61 larvae emerging per fruit. Females were observed to lay eggs in openings between the pedicel and the fruit, never piercing the exocarp. In contrast, on fallen fruit, they utilized existing wounds with exposed pulp. Infested avocados exhibit characteristic spots indicating the presence of internal larvae and generally detach from the tree. Larvae can feed on avocados in various stages of decomposition and may either emerge through wounds or pupate within the fruit. These findings support the opportunistic and saprophagous behavior associated with this fly species.

Keywords: lance fly; *Persea americana*; opportunistic species; saprophagous; *COI* gene sequence; phylogenetic analysis



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1. Introduction

The family Lonchaeidae (Diptera: Tephritoidea), commonly known as lance flies, comprises a group of acalyptrate dipterans characterized by stout bodies measuring approximately 3–6 mm in length. Members of this family are typically metallic green, blue, or black in coloration, and have abundant fine setae and black halteres [1,2]. This family has a broad global distribution and currently includes 611 described species, classified into ten genera [3].

Lonchaeids exhibit a wide variety of behaviors. Most species consume decaying plant material during their larval stage; however, some develop on healthy plant tissue [4–7] or act as secondary invaders of fruits associated with other insects (usually Tephritidae) [8,9]. Furthermore, a few species exhibit predatory behavior toward other arthropods at specific developmental stages [10–12].

In the Neotropical region, the genera *Dasiops* Rondani, 1856, and *Neosilba* Wadill&Weems, 1978 contain the highest number of described species, some of which can be considered pests of fruit and vegetables from different plant families [13–17].

Neosilba batesi Curran (Diptera: Lonchaeidae) has been recorded in Colombia, Peru, Central America, Mexico, and the United States [3,8,18,19]. This species is polyphagous, typically saprophagous, and opportunistic on fruits previously infested by *Anastrepha* spp. (Diptera: Tephritidae) [8,20–22]. It takes advantage of wounds caused by prior oviposition or exit holes made by larvae emerging from the fruit. However, *N. batesi* can occasionally act as a primary invader, as it is sometimes the only species that emerges from fruits collected in the field [23,24].

Recently, *N. batesi* has been reported in association with avocado (*Persea americana*) fruits in some regions of Mexico [25,26]. However, these reports lack rigorous species identification and a systematic assessment of its phytosanitary status. Additionally, avocado producers in Michoacán and Jalisco have reported premature fruit drop potentially linked to this species.

Despite these circumstances, no formal study has yet confirmed the identity of *N. batesi* or evaluated its ecological or phytosanitary significance in avocado crops. This is relevant because Mexico is one of the world's leading producers and exporters of avocado fruit [27], with Michoacán and Jalisco accounting for nearly 87% of national production [28]. Therefore, the objectives of this work are to: 1. confirm the taxonomic and molecular identity of *N. batesi*; 2. provide photographic documentation to support its recognition; and 3. generate data on its geographic distribution and relationship with avocado in Mexico.

2. Materials and Methods

2.1. Sampling and Taxonomic Identification of Insects

From November 2022 to January 2023, inspections were conducted in avocado plantations in the municipalities of Tingüindín, Ario de los Rosales, Los Reyes, and Tancítaro (Michoacán state), and Quitupan and Tuxpan (Jalisco state) (Table 1). At each site, we collected fallen Hass fruits infested with Diptera larvae, as well as tree-hanging fruits exhibiting spots or symptoms of rot. A total of 104 and 43 fruits of each type, respectively, were collected.

Fruits from each location were stored separately and transported to the Laboratory of Agrobiology at Universidad Michoacana de San Nicolás de Hidalgo in Uruapan, Michoacán, Mexico. All fruit samples from each site were placed in plastic containers (30 × 20 × 15 cm), covered with organza cloth, and held until adult emergence.

Adult specimens were preserved in 70% ethanol, then sexed, counted, and identified according to [8] and [23]. Photographs of newly emerged larvae from the fruits, pupae, adults, and male genitalia were taken with a Canon EOS Rebel T7 (Tokyo, Japan) camera fitted with a 4× microscope objective. Focus stacking was performed in Helicon Focus 8.2.2 Pro., and final image editing was performed in GIMP 2.10.34.

Table 1. Accession numbers of sequences used in the phylogeny study, with corresponding organism name, submission date, country, and reference.

Species/Voucher Code	Estate, Country	Accession GenBank	Reference
<i>Neosilba batesi</i> Mx-CIQA-Mich01	Michoacan, Mexico	PV243309	Nucleotide sequences generated by this work
<i>Neosilba batesi</i> Mx-CIQA-Mich02	Michoacan, Mexico	PV243310	
<i>Neosilba batesi</i> Mx-CIQA-Mich03	Michoacan, Mexico	PV243311	
<i>Neosilba batesi</i> INECOL_24/21	Veracruz, Mexico	PV504763	[29]
<i>Neosilba batesi</i> INECOL_24/22	Veracruz, Mexico	PV504764	[29]
<i>Neosilba batesi</i> INECOL_24/30	Veracruz, Mexico	PV504765	[29]
<i>Neosilba batesi</i> CSCA 17X541	FL, USA	MW283302.1	unpublished
<i>Neosilba zadolicha</i> YSUW02121210	Brazil	KR262649.1	[30]
<i>Neosilba pendula</i> IV18-A	Brazil	OQ160349.1	[31]
<i>Neosilba glaberrima</i> INECOL_24/43	Veracruz, Mexico	PV504766	[29]
<i>Neosilba glaberrima</i> INECOL_24/52	Veracruz, Mexico	PV504767	[29]
<i>Neosilba recurva</i> INECOL_24/32	Veracruz, Mexico	PV522074	[29]
<i>Neosilba recurva</i> INECOL_24/39	Veracruz, Mexico	PV522075	[29]
<i>Neosilba recurva</i> INECOL_24/40	Veracruz, Mexico	PV522076	[29]
<i>Neosilba recurva</i> INECOL_24/46	Veracruz, Mexico	PV522077	[29]
<i>Neosilba recurva</i> INECOL_24/51	Veracruz, Mexico	PV522078	[29]
<i>Neosilba</i> sp. INECOL_24/31	Veracruz, Mexico	PV504768	[29]
<i>Neosilba</i> sp. INECOL_24/41	Veracruz, Mexico	PV504769	[29]
<i>Neosilba</i> sp. INECOL_24/42	Veracruz, Mexico	PV504770	[29]
<i>Neosilba</i> sp. INECOL_24/42	Veracruz, Mexico	PV504771	[29]
<i>Neosilba</i> sp. INECOL_24/44	Veracruz, Mexico	PV504772	[29]
<i>Neosilba flavitarsis</i> INECOL24/1M	Veracruz, Mexico	PQ834830	[29]
<i>Neosilba flavitarsis</i> INECOL24/2F	Veracruz, Mexico	PQ834831	[29]
<i>Neosilba flavitarsis</i> INECOL24/3M	Veracruz, Mexico	PQ834832	[29]
<i>Silba adipata</i> 8041568-LCO	Turkey	MK450115	unpublished
<i>Silba adipata</i> INECOL_24/10	Veracruz, Mexico	PV504773	[29]
<i>Silba adipata</i> INECOL_24/11	Veracruz, Mexico	PV504774	[29]
<i>Silba adipata</i> TJMM10	Morelos, Mexico	OM949837	unpublished
<i>Silba fumosa</i> ZFMK-TIS-2574287	Germany	OP831869	[32]
<i>Lonchaea cristula</i> LCR5-1	Colombia	MZ189773	[33]
<i>Lonchaea iona</i> ZFMK-TIS-2575260	Germany	OP831877.1	[32]

2.2. DNA Extraction and Sequencing

Genomic DNA was individually extracted from three morphologically identified *Neosilba batesi* specimens (two males and one female) using the DNeasy Blood&Tissue Kit (QIAGEN, Hilden, Germany), following the manufacturer's protocol for insect tissues, without modifications.

A 508 bp fragment of the 5' region of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the Platinum™ Taq DNA Polymerase High Fidelity Kit (Thermo Fisher Scientific, Waltham, MA, USA). The primers used were COI-FW: 5'-TTATAATTGGDGGWTTTGGWAATTG-3' [34] and HC02198: 5'-TAAACTTCAGGGTGA CCAAAAAATCA-3' [35]. PCR conditions consisted of an initial denaturation at 94 °C for 1 min, followed by 35 cycles of 94 °C for 15 s, 55 °C for 30 s, and 68 °C for 1 min, with a final extension at 72 °C for 5 min.

PCR products were visualized on 1% agarose gels stained with ethidium bromide and purified using the Wizard® SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA). Amplicons were sequenced using the Sanger method on an Applied Biosystems™ 3130/3130xl Genetic Analyzer.

Sequence chromatograms were visualized and edited manually using BioEdit v7.7.1 [36]. Multiple sequence alignment was performed using ClustalW [37], with subsequent manual adjustments to ensure positional homology. Sequence identity was confirmed via BLAST against the NCBI GenBank database (available online: <https://blast.ncbi.nlm.nih.gov/Blast.cgi>, accessed on 22 April 2025).

Phylogenetic analysis was conducted using MEGA11 [38]. The best-fit nucleotide substitution model was determined using a hierarchical likelihood ratio test (hLRT) and comparison of Bayesian Information Criterion (BIC) scores across 31 candidate models. The General Time Reversible model with a discrete Gamma distribution (GTR + G) was identified as the best fit (BIC = 4194.459). Phylogenetic reconstruction was performed using the Maximum Likelihood (ML) method. Initial heuristic trees were generated via Neighbor-Joining (NJ) and BioNJ algorithms based on pairwise distances, with the final topology corresponding to the highest log-likelihood value.

To root the phylogenetic tree, sequences of *Silba adipata*, *Silba fumosa*, *Lonchaea iona*, and *Lonchaea cristula* (All Diptera: Lonchaeidae) were included as outgroup taxa. These species belong to the subfamily *Lonchaeinae* and were selected to ensure taxonomic relevance and provide intergeneric divergence for robust phylogenetic inference.

2.3. Observation and Description of Damages in Avocado Fruits

The study was carried out in a 5 ha Hass avocado orchard in Los Reyes, Michoacán (19°35'17.20" N, 102°23'4.82" W; 1592 m a.s.l.). From January to April 2024, we conducted four sampling events, selecting tree-hanging fruits of various sizes that exhibited rot symptoms or necrotic epidermal spots.

Each fruit was measured for length and width, then placed individually in a plastic cup filled with vermiculite to serve as a pupation substrate. Cups were covered with organza cloth and checked daily to record and count emerged adults, which were preserved in 70% ethanol.

At every sampling site, we performed visual observations of fly behavior and photographed infested fruits to document larval presence.

3. Results

3.1. Identification and Phylogenetic Analyses

Flies of the family Lonchaeidae that emerged from avocados collected from the ground (n = 131) and trees (n = 85) in nine orchards were identified as *N. batesi* Curran (Figure 1), based on their chaetotaxy and the morphology of the male genitalia. Females were assigned to *N. batesi* because there are no taxonomic keys available for females of the genus *Neosilba*, and no males of other species were detected.

The larvae are musciform in shape and translucent white, with a slender and cylindrical body tapering toward the anterior end. They possess a pair of short, dark, and sclerotized posterior spiracles, characteristic of Lonchaeidae (Figure 1A). The puparia are oval, slightly broader at the front, reddish-brown in color, and exhibit remnants of the posterior spiracles on the caudal part (Figure 1B).

Adults (Figure 1C) are glossy and bluish black, measuring approximately 3–4 mm in length, with reddish eyes, black legs, a setulose lunule, plumose arista, and calypters bearing a group of long black setae, typical of the genus. In males, the epandrium is elongated, and the parameres are bilobed, with the medial lobe being slightly narrower (Figure 1D).

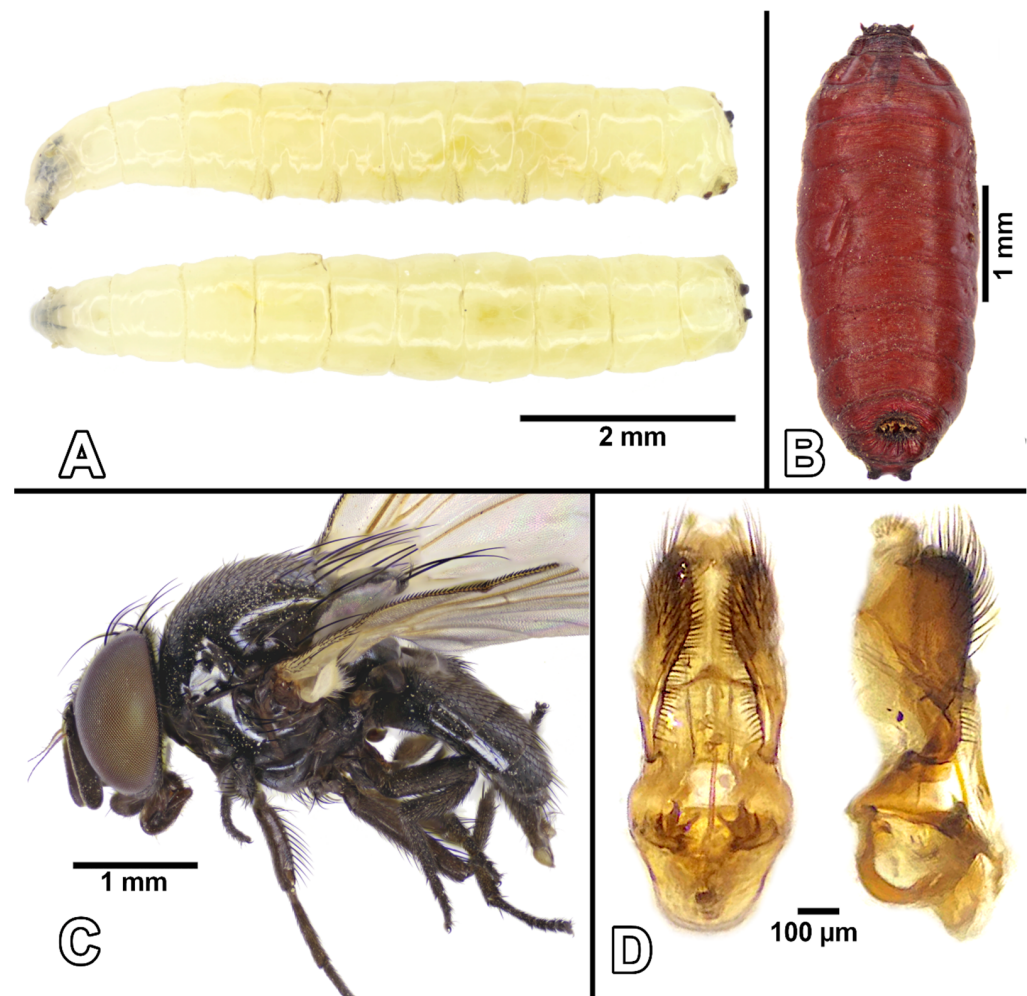


Figure 1. *Neosilba batesi*. (A) Lateral and dorsal view of larva; (B) puparium in ventral position; (C) female in lateral view; (D) male genitalia.

The COI sequences obtained from two males and one female, each comprising 470 base pairs of the partial mitochondrial COI gene, were identical to one another (GenBank accession numbers: PV243309, PV243310, PV243311; Table 1). BLAST analysis (99% query coverage) showed the highest similarity to *Neosilba zadolicha* (97.05%, accession KR262649.1), followed by *Neosilba pendula* (95.92%, accession OQ160349.1). Sequence identity with *N. batesi* (specimen CSCA_17X541, accession MW283302.1) from Florida was lower, at 93.86%. This relatively low similarity contrasts with the morphological identification of the specimens as *N. batesi*, suggesting potential taxonomic incongruence. However, COI sequences from *N. batesi* specimens from Veracruz, Mexico (PV504763, PV504764, PV504765), recently reported by [29] and included in this study, showed >99% identity with our sequences.

The phylogenetic analysis (Figure 2) supports the taxonomic assignment of the newly generated sequences to *N. batesi*, placing them within a well-supported clade corresponding to the genus *Neosilba*. However, these sequences formed a distinct genetic lineage, clearly separated from both the *N. batesi* specimen from Florida and the clade containing *N. zadolicha* and *N. pendula*. Notably, limitations in the available sequence data were evident, as each of the reference taxa was represented by a single sequence in GenBank, thereby restricting the depth of phylogenetic comparisons and the robustness of taxonomic resolution.

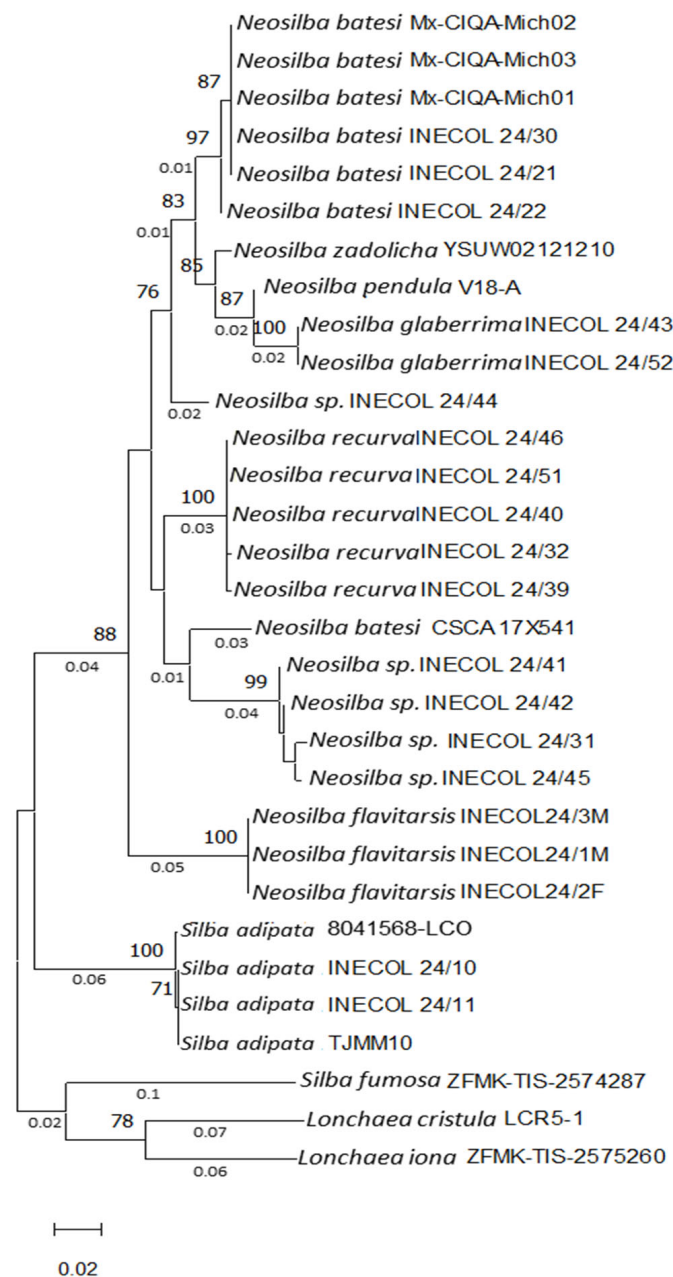


Figure 2. Phylogenetic tree inferred using the Maximum Likelihood method based on the General Time Reversible model [39]. The tree with the highest log likelihood (−1774.16) is shown. Bootstrap support values (percentages) are indicated above the branches. Initial trees for the heuristic search were generated automatically using Neighbor-Joining and BioNJ algorithms based on a pairwise distance matrix estimated via the Maximum Composite Likelihood (MCL) approach, followed by selection of the topology with the highest log likelihood. A discrete Gamma distribution (+G, parameter = 0.3340) was used to model rate variation among sites (five categories). The tree is drawn to scale, with branch lengths representing the number of substitutions per site. The analysis included 31 nucleotide sequences, with the codon positions 1st, 2nd, 3rd, and noncoding. A total of 441 positions were retained in the final dataset. Evolutionary analyses were conducted in MEGA11 [38].

3.2. Distribution of *Neosilba batesi* in the Study Area

From November 2022 to January 2023, specimens of *N. batesi* emerged from the nine sites sampled in the states of Michoacan and Jalisco, Mexico. These sites ranged in elevation from 1301 to 2206 m a.s.l. (Table 2, Figure 3).

Table 2. Sites with *Neosilba batesi* on avocado trees *Persea americana* c.v. Hass and number of emerged adults per orchard of confined soil and tree fruit.

Site	Coordinates/ Height (m a.s.l.)	Date of Fruit Collection	(Number of Fruits)/Emerged Adults	
			Fallen Fruits	Tree Fruits
Tingüindín, Mich.	19°43′46″ N 102°27′7″ W/1882	22 December 2022	(13)/5♂♂11♀♀	(3)/1♂♂3♀♀
Ario de los Rosales, Mich.	19°11′10″ N 101°39′52″ W, 2206	10 January 2023	(8)/4♂♂7♀♀	(6)/4♂♂7♀♀
Los Reyes ¹ , Mich.	19°35′17.20″ N 102°23′4.82″ W/1592	10 November 2022	(19)/10♂♂16♀♀	(4)/2♂♂9♀♀
Los Reyes ² , Mich.	19°34′34″ N 102°19′30″ W, 2141	14 December 2022	(8)/6♂♂10♀♀	(5)/3♂♂5♀♀
Los Reyes ³ , Mich.	19°39′50″ N 102°24′51″ W, 1620	14 December 2022	(10)/8♂♂6♀♀	(5)/5♂♂7♀♀
Los Reyes ⁴ , Mich	19°38′50″ N 102°23′39″ W, 1780	14 December 2022	(6)/4♂♂7♀♀	(4)/6♂♂8♀♀
Tancítaro, Mich.	19°23′28″N 102°25′18″W, 2020	14 January 2023	(14)/4♂♂6♀♀	(6)/3♂♂5♀♀
Tuxpan, Jal.	19°32′5″ N 103°27′50″ W, 1301	28 December 2022	(11)/6♂♂8♀♀	(4)/3♂♂5♀♀
Quitupan, Jal.	19°48′2″ N 102°48′32″ W, 2040	29 December 2022	(15)/5♂♂9♀♀	(6)/4♂♂6♀♀

1,2,3,4 Avocado orchards indicated on the map of the study area (Figure 3).

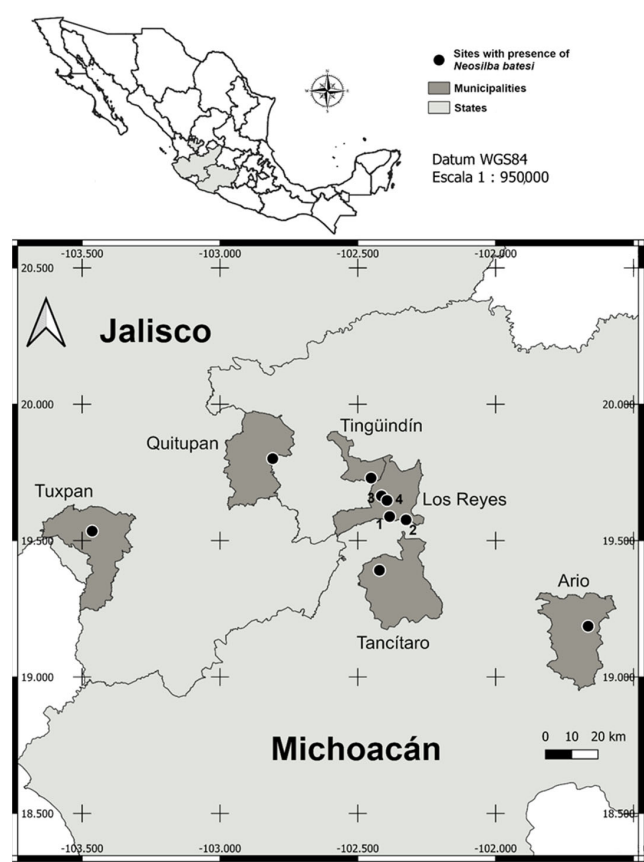


Figure 3. Avocado orchards with the presence of *Neosilba batesi* (numbers 1, 2, 3, and 4 indicate the orchards mentioned in Table 1).

During fruit collection and transport, several larvae emerged and most reached the pupal stage; however, pupal mortality was high. Only adults of each sex were counted. Females consistently outnumbered males, though the female-to-male ratio rarely exceeded 2:1. In addition, specimens of *Drosophila melanogaster* and other *Drosophila* spp. emerged from fallen fruits.

3.3. *Neosilba batesi* and Its Association with Avocado Fruits

In the avocado plantation in Los Reyes, Michoacán, 157 fruits were collected from trees across four inspections in 2024. Fruits infested by *N. batesi* larvae measured, on average, 5.40 cm in length and 3.90 cm in width. The average number of larvae that emerged per fruit was 9.61. Although most larvae successfully reached the pupal stage, mortality in

this stage was high. The maximum dimensions recorded for infested fruits were 7.95 cm in length and 5.05 cm in width (Table 3).

Field observations indicated that, in apparently healthy developing fruits on the tree, females deposit eggs at the junction of the pedicel with the fruit (Figure 4A). Occasionally, several females can be found ovipositing simultaneously on the same fruit, especially when it shows signs of rot. In fallen fruits, oviposition occurs in the exposed pulp at the pedicel attachment site (Figure 4B) or within wounds on the exocarp. Females can also lay their eggs in fruits with previous ovipositions and larvae inside. No direct oviposition through the exocarp was observed.

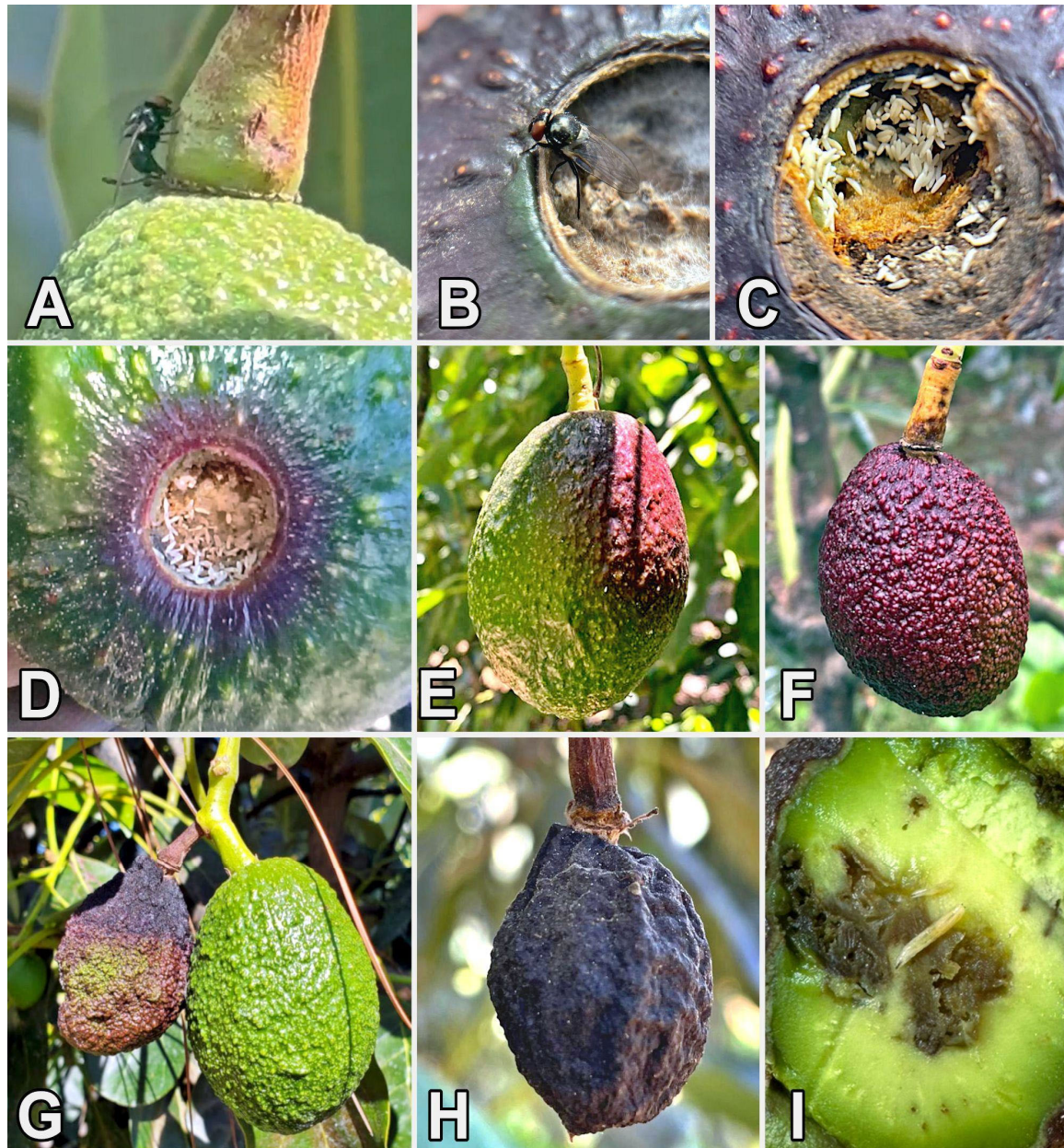


Figure 4. Ovipositions, signs, and symptoms of *Neosilba batesi* larvae in avocado fruits. (A) Female ovipositing on an immature tree fruit. (B) Female ovipositing on a fallen fruit showing signs of rot and white mycelium. (C) Recently laid *N. batesi* eggs at the base of a fallen, rotting fruit. (D) Purple ring at the fruit's base. (E) Infested fruit with a lateral purple spot. (F) Infested fruit is almost entirely covered by rot. (G) Infested and healthy fruits sharing the same peduncle. (H) Mummified fruit attached to the pedicel. (I) Larvae and internal rot within the fruit.

Eggs are whitish, elongated, and may be laid singly or in clusters (Figure 4C). Early infestation is signaled by external symptoms, such as a purple ring at the fruit's base (Figure 4D) or purple to reddish spots that may partially (Figure 4E) or completely cover the surface of the fruit (Figure 4F). Notably, healthy avocados can grow and develop normally even when adjacent to infested ones, including those on the same peduncle (Figure 4G).

As infestation progresses, internal decay and pedicel rot lead to fruit drop, although some fruits desiccate and mummify while remaining attached (Figure 4H). Dissection of infested fruits reveals larvae of various instars feeding on the mesocarp (Figure 4I).

Larvae consume pulp at different stages of decomposition (Figure 5A,B) and may exit through wounds in the exocarp, either at the pedicel scar or at impact-induced openings. No exit holes produced by larvae through the exocarp were observed. Puparia develop either in the soil or within the fruit (Figure 5C), including mummified avocados that remain on the tree (Figure 4H).

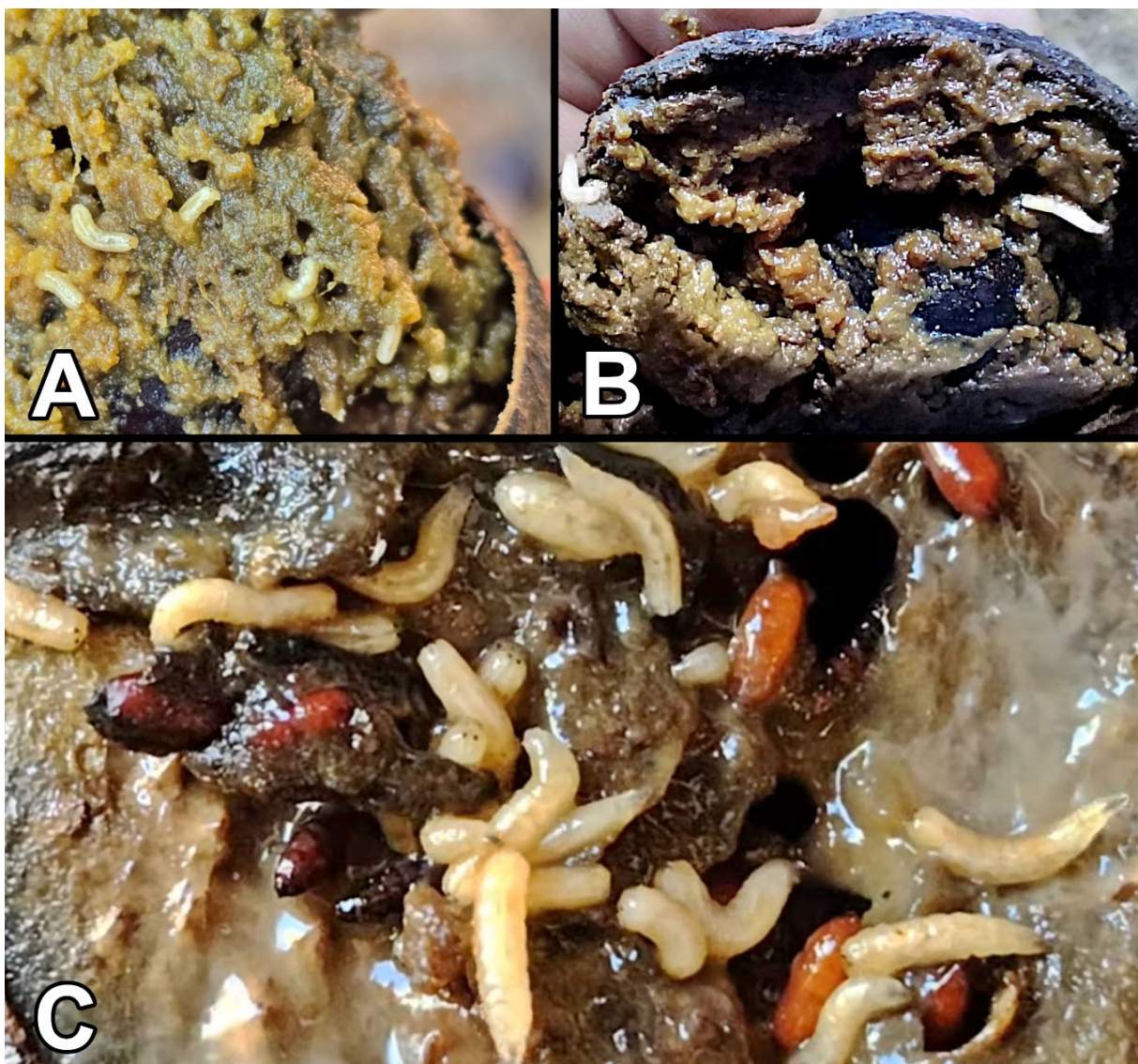


Figure 5. Decomposing avocado fruits with different developmental stages of *N. batesi*. (A,B) Larvae inside fruits in an advanced state of decomposition. (C) Larvae and pupae within a rotting fruit.

Table 3. Size of avocados sampled from trees and number of *Neosilba batesi* larvae and adults obtained across four sampling dates.

Collect Date	Number of Fruits Collected	Average Sizes of Infested Avocado Trees (cm)		Average Number of Emerged Larvae/Fruit	Average Number of Adults
		Long	Width		
27 January 2024	18	7.95	5.05	4.94	1.72
3 February 2024	65	4.57	3.4	5.07	1.06
5 March 2024	44	4.05	3.2	15.09	2.5
18 April 2024	30	5.04	3.93	13.34	3.8

4. Discussion

In this study, *N. batesi* was identified in association with avocados both on the tree and on the ground. In tree fruits, females exhibit opportunistic oviposition behavior by exploiting potential wounds or openings at the junction between the pedicel and the fruit. Conversely, on fallen fruits, they deposit their eggs in areas where the pulp is exposed, even when the fruit is in an advanced state of decomposition.

These findings are consistent with previous reports. For example, Ref. [8] documented the first association of *N. batesi* with fallen avocados in the state of Chiapas, Mexico. Later, Ref. [21] classified this species as a saprophagous fly associated with decaying fruits, and Ref. [22] identified *N. batesi* as a fruit-decomposing species in the soil, associated with *Persea americana* cv. Criollo, Fuerte and Hass in Michoacán, Mexico. A related association was also partially reported by [18] in FL, USA, where the species was found in wounded avocados and captured in traps. Conversely, a study on the diversity of Tephritoidea in Colombia [24] reports an association of *N. batesi* with avocado and other fruit species; however, the characteristics of the collected fruits are not specified.

Neosilba batesi is distributed across countries in South, Central, and North America [6], and has been reported to infest fruits of sweet orange (*Citrus sinensis* L.), papaya (*Carica papaya* L.), mango (*Mangifera indica* L.), *Inga* spp., peach palm (*Guilielma gasipaes* Kunth), fig (*Ficus carica* L.), soursop (*Annona muricata* L.), and other wild *Annona* spp. [8,20,23,29].

Several species of this genus, including *N. zadolicha*, *Neosilba certa*, *N. glaberrima*, *N. pendula*, *Neosilba parva*, and other *Neosilba* spp., have been associated with fruits of *Persea americana* in Brazil [40,41]. Among these, only *N. glaberrima* has been reported in Mexico [8,23,29]. No male specimens of *N. glaberrima* were detected in the present study.

The phylogenetic analysis supports the morphological identification, placing the newly obtained sequences from Michoacán within the same clade as other *N. batesi* from Veracruz sequences recently described by [29]. In contrast, *N. batesi*_CSCA 17X541, collected in Florida and submitted to GenBank in 2020 (accession number MW283302.1), clustered in a separate clade. This record lacks an associated publication and supporting taxonomic evidence, leading to the same conclusion reached by [29]: the identification of this isolate requires confirmation. Furthermore, no additional sequence data are currently available for *N. batesi*, *N. zadolicha*, or *N. pendula*. Therefore, further molecular data are needed to achieve a more accurate taxonomic resolution for these species.

During the study conducted in the avocado orchard in Los Reyes, Michoacán, fruits infested with *N. batesi* larvae (from 27 January to 18 April 2024) exhibited characteristics that would prevent their commercialization. These fruits were typically small to medium in size, falling below marketable calibers. Additionally, they appeared visually immature and contained a variable number of larvae. Notably, many pupae failed to reach adulthood, likely due to the unfavorable conditions within the containers where they were confined.

In harvested or fallen fruits, the presence of laid eggs can be detected with the naked eye at the pedicel–fruit attachment point. Additionally, the chorion may still be visible after the eggs have hatched and the larva has emerged. Infestation by *N. batesi* larvae in tree fruits can be visually identified early on, typically by the presence of a purple ring at the base of the fruit (at the pedicel junction), along with distinct purple to reddish discolorations that may extend to cover the entire fruit surface. Most of these fruits eventually fall to the ground, although some remain attached to the pedicel and can easily be detached.

The premature detachment of fruits can be attributed to several factors. Larval feeding activity creates galleries and induces internal decomposition, leading to structural damage that weakens the fruit's attachment to the pedicel. Furthermore, it is probable that microorganisms are involved, enhancing the rotting process and thereby triggering the fruit's natural abscission mechanisms. However, these hypotheses require further confirmation.

Fallen fruits in advanced stages of decomposition usually harbor large numbers of eggs and larvae at different instars. These larvae feed on the fruit pulp, which corroborates their saprophagous feeding habits. Based on our observations, such fallen fruits appear to serve as key reservoirs for future fly generations. Therefore, collecting and removing them from the ground could represent an effective cultural practice to reduce *N. batesi* populations in the field.

We hypothesize that *N. batesi* females prefer to oviposit on fallen, decomposing fruit rather than on apparently healthy fruit still on the tree. However, further research is required to confirm this behavioral pattern. Interestingly, no direct ovipositions through the epidermis of the fruits were observed, and some visually healthy fruits lacking larvae, wounds, or signs of disease continued to develop normally, even in the presence of *N. batesi*-infested fruits around on the tree and in the soil.

This observation suggests that the presence of a pre-existing pathogen or damage between the pedicel and the avocado might influence oviposition behavior on tree fruits, which is consistent with the opportunistic behavior associated with this species.

Traps baited with hydrolyzed protein or torula yeast have been reported as effective tools for capturing *N. batesi* in other crops [19,29,42] and may be useful for monitoring or managing this species in avocado orchards. However, comparative field studies are needed to evaluate their effectiveness under local conditions.

Given the signs of oviposition, the obvious symptoms of infestation, the small dimensions of the fruits that are typically infested and premature fruit drop caused by *N. batesi*, it is unlikely that infested fruits would reach the avocado export chain, which includes multiple strict phytosanitary inspections in both orchards and packing facilities.

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