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Scientific Note

Report of the establishment of *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) in Central America

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Abstract. Over the last decade, high-end fruits such as berries, have faced the impact of *Drosophila suzukii* (Matsumura, 1931) (Diptera: Drosophilidae) across Asia, Europe, and the Americas. Yet, none of the countries from Central America has officially reported this pest. We carried out surveys in the blackberry fields of Costa Rica. We placed a proprietary mixture of liquid attractants into red-cup traps with lateral holes, as it has been widely demonstrated as best to capture *D. suzukii*. Subsequently, we collected both adults and larvae for analysis. The adults were identified using key morphological features of *D. suzukii*, while the larvae were used for molecular analysis of the samples. The results revealed the first record of establishment of *D. suzukii* infesting blackberry in Costa Rica, Central America. These discoveries reveal the spread of the pest in Latin America and the importance of intensifying the surveillance process in other countries in this region.

Keywords: Costa Rica, SEM, monitoring traps, blackberry.

Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae), commonly named the spotted wing *Drosophila* (SWD), is currently one of the most devastating pests in fruit production around the world (Garcia et al. 2022). Part of the success of this species is the serrated ovipositor, contrary to closely related species, that allows this pest to lay its eggs into thin epicarps of ripening intact fruits (Crava et al. 2020). This ovipositor has allowed this species to find a new ecological niche apart from other species of the same genus, which normally feed on yeasts from rotting fruits (Garcia 2020). This aspect has facilitated SWD to become a serious threat to agriculture worldwide, especially to berries with thin epicarp, which are important economic fruits such as blackberries, blueberries, raspberries, and strawberries (Atallah et al. 2014; Crava et al. 2020; Ioriatti et al. 2020).

Although SWD had its origin in Asia, it has quickly spread through the world in the last 15 years, probably facilitated via international trade, with a presence now across its native Asia, Europe, and more recently North and South America, Africa and Oceania (Garcia et al. 2022). The simultaneous invasion of Spain, Italy, and the United States occurred in 2008, which quickly led to the invasion of closer territories (Cini et al. 2014; Rota-Stabelli et al. 2013). By 2011, the presence of SWD had been reported in several states in the United States and Canada, and by the same year, it was already detected in Mexico (Andreazza et al. 2017; Garcia et al. 2022). In South America, by 2012 and in 2013 the pest was detected in Brazil (Deprá et al. 2014), followed by Uruguay in 2014 (González et al. 2015), Argentina in 2015 (de la Vega et al. 2020) and in Chile in 2017 (Garcia et al. 2022). Interestingly, *D. suzukii* had been reported in Costa Rica and Ecuador back in the late 90s, but the lack of specimens in entomological collections raises rational doubts about the probable misidentification of the samples and the invasion and/or establishment of the pest in these countries back then (Hauser 2011).

Most of these records of the presence of SWD have been within the same climatological conditions and consequently, host range. However, particularly in Latin America, the relative proximity of suitable non-host crop species sharing the same geographical space as preferred hosts is a driving force that might help the establishment of the pest (Castro-Sosa et al. 2017). This is also facilitated by the climatic conditions, as many highlands of neotropical countries offer optimal minimum and maximum temperatures to complete the SWD life cycle all year round, without the barrier that winter is in temperate countries. Interestingly, most prediction models do not rank neotropical countries as highly suitable environments for SWD establishment (Santos et al. 2017; Reyes & Lira-Noriega 2020).

Due to rumors about dipteran larvae attacking blackberries growing in Costa Rica during 2021-2022, the objective was to verify whether *D. suzukii* occurs in these areas through selective capture and to identify key morphological characteristics and molecular analyses. We carried out surveys in a blackberry crop located in El Cedral de León Cortés, San José, Costa Rica (9°42'41" N 84°00'33" W). The crop corresponded to a commercial 0.2 ha plot of the tropical highland blackberry (*Rubus adenotrichos* Schtdl. var. Enana). Evaluations were conducted from November 2021 until January 2023, collecting multiple samples along the development of the crop. Our surveys covered all stages of fruit development. To collect SWD adults, six red-black stripe cup traps with hemispherical dome-shaped lids with 4 mm holes across the red and black surface were distributed at equidistant sites of the blackberry field. The reason for using this trap design was based on the results obtained in Mexico in a comparison of several different designs to increase SWD captures and decrease bycatches (Lasa et al. 2017). Similarly, each trap was baited with the product P258-L (ChemTica Internacional, Heredia), which consists of a proprietary mixture of liquid components that attract SWD and lower bycatches. The traps were monitored weekly for 4 weeks and the insects collected within the traps were transferred to clean plastic vials with ethanol (75%). Pre-selection of individuals as candidate SWD specimens was based on the observation of the most striking morphological characteristic of males SWD which corresponds to the presence of characteristic black apical spots at each wing. Female adults on the other hand were pre-selected from samples by looking at any sign of protruding, enlarged ovipositor of flies with similar characteristics to the males (red eyes, 2-3 mm body size). The specimens were processed and observed by scanning electron microscopy (SEM) using a Hitachi TM-3000 instrument at 5000 kV to identify distinctive features related to female and male adults.

During the weekly visits to check captures of adults in traps we also checked for fallen fruits. Hence, whenever possible, we collected fruits that fell to the ground during the field season for use in molecular analyses. For each collection at least 25 fallen fruits with living dipteran larvae inside were preserved in a collection vial and brought live to the laboratory, and were labeled as isolates 1, 2, and 3. In the laboratory, 50 milligrams of larval tissue were weighed and placed in 0.5 mL of TRIzol™ reagent. The samples were homogenized for three minutes using a Retsch® macerator, followed by vortexing and incubation at room temperature (RT, approx. 21°C) for five minutes. Subsequently, 0.2 volumes of chloroform (100 µL) were added, manually shaken for 15 seconds, and incubated again at RT for three minutes. The next step involved centrifugation at 17,200 x g / 4°C for 15 minutes, followed by recovery of the supernatant, which was mixed with an equal volume of 100% isopropanol. After manual shaking for 15 seconds, the mixture was incubated for 10 minutes at RT. The samples were centrifuged again (17,200 x g / 4°C for 10 minutes), the pellet was recovered, resuspended in 500 µL of 75% ethanol stored at -20°C, and centrifuged for five minutes at 17,200 x g / 4°C. The supernatant was discarded, and the pellet was air-dried for three minutes at room temperature and then resuspended in 25 µL of water for PCR. DNA concentration and purity were quantified using a Thermo Scientific® nanoDrop Lite, with concentrations around 80 ng/µL and absorbance ratios (260/280nm) near 1.8±0.2. The primers for detection were those reported by Freda & Braverman (2013) for cytochrome oxidase subunit I: COI_s 5'-TTTCTACAAATCATAAAGATATTGG-3' and COI_as 5'-TAAACTTCAGGGTGTCCAAAAAATCA-3'.

PCR reactions with 20 µL were performed using Platinum™ SuperFi II DNA Polymerase (Invitrogen, USA) adding 0.5 µM of primers, and 100 ng of genomic DNA. The thermal profile included an initial denaturation at 98°C for 30 seconds, followed by 30 cycles of 98°C for 10 seconds, 60°C for 10 seconds, and 72°C for 20 seconds; then, a final extension of 5 minutes at 72°C. PCR products were visualized on a 2% agarose gel dissolved in TAE buffer and stained with BrEt. The molecular marker used was 1Kb MassRuler™ DNA Ladder Mix from Thermo Scientific® to confirm bands near 650 bp. The amplicons were purified using the Thermo Scientific GeneJET PCR Purification Kit. Sequencing was performed using the Sanger method.

A phylogenetic reconstruction of the samples and their matches with National Center for Biotechnology Information (NCBI) database accessions was conducted using a BLAST (basic local alignment search). Multiple sequence alignment was performed using the MUSCLE tool from EMBL-EBI (Edgar 2014), and analysis was carried out with MEGA v11.0 software (Tamura et al. 2021) to calculate genetic distances based on the number of base pair substitutions between sequences, eliminating positions with missing data according to the Tamura-Nei model (Tamura & Nei 1993). The phylogenetic tree was generated through the Maximum Likelihood method with a 500-repetition bootstrap. An estimate of evolutionary divergence between sequences was performed following the Tajima and Nei model (Tajima & Nei 1984) and evolutionary analysis was conducted using MEGA v11.0 software (Tamura et al. 2021).

Weekly captures for the period evaluated consistently showed positive captures of adults attracted to the traps set in the blackberry crops of Costa Rica. Assessment and sorting of captures per trap showed that on average 87% of captured insects were *D. suzukii* based on morphological identification (Fig. 1). Furthermore, the sex ratio in captured flies tended to be female-biased which is also a feature that has been reported for SWD trapping (Whitener et al. 2022).

The SEM micrographs revealed anatomical features reported for SWD, such as the serrated ovipositor in females (Varón-González et al. 2020) and the presence of sexual combs on the first and second tarsi of the front legs (Kopp 2011) (Fig.1).

The phylogenetic analysis confirmed that the collected individuals correspond to the species *D. suzukii* (Fig. 2), and their divergence links them to multiple previously described accessions of this species (Tab. 1).

The trapping, anatomical, and molecular evidence from the collected individuals indicates that the pest *D. suzukii* is established blackberry crops in Costa Rica, posing a potential threat to the local production of this fruit.

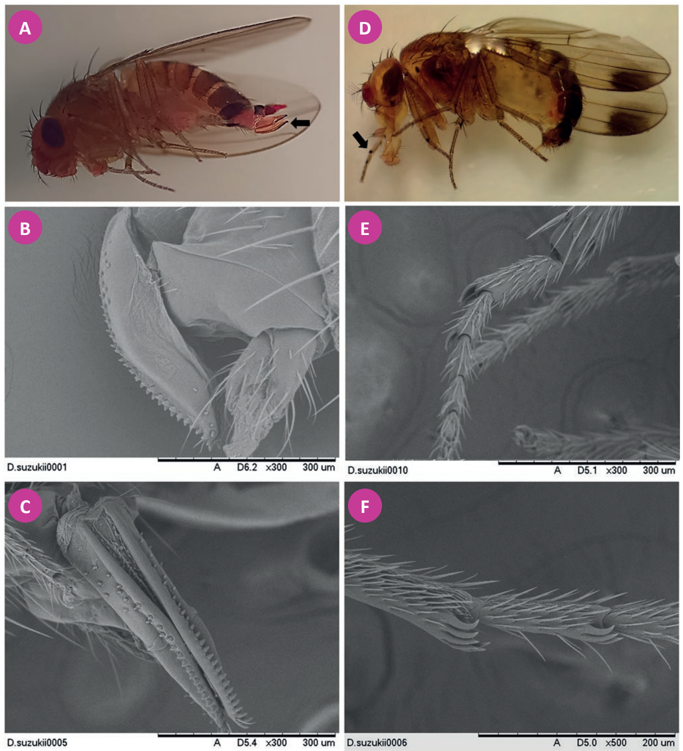

Figure 1. Key anatomical characteristics observed by light and electron microscope from field-collected samples: A, B, C) serrated ovipositor in females. C, D, E) presence of sexual combs on the first and second tarsi of the front legs in males. Arrows indicates highlighted details by electron microscopy.

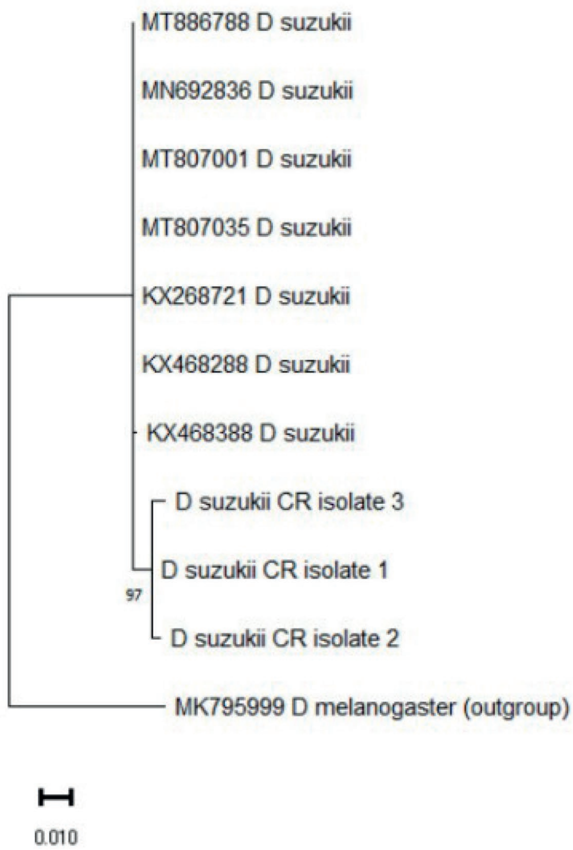

Figure 2. Evolutionary analysis by maximum likelihood method showing the cluster of samples processed from our collections closer to other *D. suzukii* accessions than to *D. melanogaster* used here as an outgroup.

Table 1. Evolutionary divergence between sequences showing the similarity of isolates from Costa Rica with those accessions of *D. suzukii,* in stark contrast with the isolate of *D. melanogaster* used as an outgroup.

Accesion	1	2	3	4	5	6	7	8	9	10	11
1. *D. suzukii* CR isolate 1		0,002	0,003	0,003	0,003	0,003	0,003	0,003	0,003	0,003	0,014
D. suzukii CR isolate 2	0,003		0,003	0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,014
D. suzukii CR isolate 3	0,005	0,008		0,004	0,004	0,004	0,004	0,004	0,004	0,004	0,014
4. *D. suzukii* GB: KX468288	0,006	0,009	0,011		0,000	0,000	0,000	0,000	0,000	0,001	0,013
5. *D. suzukii* GB: KX268721	0,006	0,009	0,011	0,000		0,000	0,000	0,000	0,000	0,001	0,013
6. *D. suzukii* GB: MT807035	0,006	0,009	0,011	0,000	0,000		0,000	0,000	0,000	0,001	0,013
7. *D. suzukii* GB: MT807001	0,006	0,009	0,011	0,000	0,000	0,000		0,000	0,000	0,001	0,013
8. *D. suzukii* GB: MT886788	0,006	0,009	0,011	0,000	0,000	0,000	0,000		0,000	0,001	0,013
9. *D. suzukii* GB: MN692836	0,006	0,009	0,011	0,000	0,000	0,000	0,000	0,000		0,001	0,013
10. *D. suzukii* GB: KX468388	0,008	0,011	0,012	0,002	0,002	0,002	0,002	0,002	0,002		0,013
11. *D. melanogaster* GB: MK795999	0,099	0,102	0,104	0,092	0,092	0,092	0,092	0,092	0,092	0,093	

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Authors' Contributions

RCC: Conceptualization, Investigation and Writing-original draft; LAM: Investigation and Writing-review and editing; AGH: Conceptualization, Supervision and Writing-review and editing; FGF: Conceptualization, Investigation, Supervision and Writing-original draft.

Conflict of Interest statement

The authors declare no conflict of interest.

References

Andreazza, F.; Bernardi, D.; dos Santos, R. S. S.; Garcia, F. R. M.; Oliveira, E. E.; Botton, M.; Nava, D. E. (2017) *Drosophila suzukii* in Southern Neotropical Region: Current Status and Future Perspectives. *Neotropical Entomology*, 46: 591-605. doi: 10.1007/s13744-017-0554-7

Atallah, J.; Teixeira, L.;, Salazar, R.; Zaragoza, G.; Kopp, A. (2014) The making of a pest: The evolution of a fruit-penetrating ovipositor in *Drosophila suzukii* and related species. *Proceedings of the Royal Society B: Biological Sciences*, 281(1781): 20132840. doi: 10.1098/rspb.2013.2840

Castro-Sosa, R.; Castillo-Peralta, M. R.; Monterroso-Rivas, A. I.; Gomez-Díaz, J. D.; Flores-González, E; Rebollar-Alviter, Á. (2017) Potential Distribution of *Drosophila suzukii* (Diptera: Drosophilidae) in Relation to Alternate Hosts in Mexico. *Florida Entomologist*, 100(4): 787-794. doi: 10.1653/024.100.0403

Cini, A.; Anfora, G.; Escudero-Colomar, L. A.; Grassi, A.; Santosuosso, U.; Seljak, G.; Papini, A. (2014) Tracking the invasion of the alien fruit pest *Drosophila suzukii* in Europe. *Journal of Pest Science*, 87(4): 559-566. doi: 10.1007/s10340-014-0617-z

Crava, C. M.; Zanini, D.; Amati, S.; Sollai, G.; Crnjar, R.; Paoli, M.; Rossi-Stacconi, M. V.; Rota-Stabelli, O.; Tait, G.; Haase, A.; et al. (2020) Structural and transcriptional evidence of mechanotransduction in the *Drosophila suzukii* ovipositor. *Journal of Insect Physiology*, 125: 104088. doi: 10.1016/j.jinsphys.2020.104088

de la Vega, G. J.; Corley, J. C.; Soliani, C. (2020) Genetic assessment of the invasion history of *Drosophila suzukii* in Argentina. *Journal of Pest Science*, 93(1): 63-75. doi: 10.1007/s10340-019-01149-x

Deprá, M.; Poppe, J. L.; Schmitz, H. J.; De Toni, D. C.; Valente, V. L. S. (2014) The first records of the invasive pest *Drosophila suzukii* in the South American continent. *Journal of Pest Science*, 87(3): 379-383. doi: 10.1007/s10340-014-0591-5

Edgar, R. C. (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput, *Nucleic Acids Research*, 32(5): 1792-1797. doi: 10.1093/nar/gkh340

Freda, P. J.; Braverman, J. M. (2013) *Drosophila suzukii*, or Spotted Wing Drosophila, Recorded in Southeastern Pennsylvania, U.S.A.. *Entomological News*, 123(1): 71-75. doi: 10.3157/021.123.0116

Garcia, F. R. M. (2020) Basis for Area-Wide Management of *Drosophila suzukii* in Latin America. In: Garcia, F. R. M. (Ed). *Drosophila suzukii* Management, pp. 93-110. Springer. doi: 10.1007/978-3-030-62692-1_5

Garcia, F. R. M.; Lasa, R.; Funes, C. F.; Buzzetti, K. (2022) *Drosophila suzukii* Management in Latin America: Current Status and Perspectives. *Journal of Economic Entomology*, 115(4): 1008-1023. doi: 10.1093/jee/toac052

González, G.; Mary, A. L.; Goñi, B. (2015) *Drosophila suzukii* (Matsumura) found in Uruguay. *Drosophila Information Service*, 98: 103-107.

Hauser, M. (2011) A historic account of the invasion of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) in the continental United States, with remarks on their identification. *Pest Management Science*, 67(11): 1352-1357. doi: 10.1002/ps.2265

Ioriatti, C.; Anfora, G.; Grassi, A.; Puppato, S.; Rossi Stacconi, M. V. (2020) Current status of the *Drosophila suzukii* control in Europe. *Acta Horticulturae*, 1277: 387-396. doi: 10.17660/ActaHortic.2020.1277.55

Kopp, A. (2011) *Drosophila* sex combs as a model of evolutionary innovations. *Evolution & Development*, 13(6): 504-522. doi: 10.1111/j.1525-142x.2011.00507.x

Lasa, R.; Tadeo, E.; Toledo-Hérnandez, R. A.; Carmona, L.; Lima, I.; Williams, T. (2017) Improved capture of *Drosophila suzukii* by a trap baited with two attractants in the same device. *PLOS ONE*, 12(11): e0188350. doi: 10.1371/journal.pone.0188350

Reyes, J. A.; Lira-Noriega, A. (2020) Current and future global potential distribution of the fruit fly *Drosophila suzukii* (Diptera: Drosophilidae). *The Canadian Entomologist*, 152(4): 587-599. doi: 10.4039/tce.2020.3

Rota-Stabelli, O.; Blaxter, M.; Anfora, G. (2013) *Drosophila suzukii*. *Current Biology*, 23(1): R8-R9. doi: 10.1016/j.cub.2012.11.021

Santos, L. A.; Mendes, M. F.; Krüger, A. P.; Blauth, M. L.; Gottschalk, M. S.; Garcia, F. R. M. (2017) Global potential distribution of *Drosophila suzukii* (Diptera, Drosophilidae). *PLOS ONE*, 12(3): e0174318. doi: 10.1371/journal.pone.0174318

Tajima, F.; Nei, M. (1984) Estimation of evolutionary distance between nucleotide sequences, *Molecular Biology and Evolution*, 1(3): 269-285. doi: 10.1093/oxfordjournals.molbev.a040317

Tamura, K.; Nei, M. (1993) Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Molecular Biology and Evolution*, 10(3): 512-526.doi: 10.1093/oxfordjournals.molbev.a040023

Tamura, K.; Stecher, G.; Kumar, S. (2021) MEGA 11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, 38(7): 3022-3027. doi: 10.1093/molbev/msab120

Varón-González, C.; Fraimout, A.; Delapré, A.; Debat, V.; Cornette, R. (2020) Limited thermal plasticity and geographical divergence in the ovipositor of *Drosophila suzukii*. *Royal Society Open Science*, 7(1): 191577. 10.1098/rsos.191577

Whitener, A. B.; Smytheman, P.; Beers, E. H. (2022) Efficacy and Species Specificity of Baits and Lures for Spotted-Wing Drosophila, *Drosophila suzukii* (Diptera: Drosophilidae). *Journal of Economic Entomology*, 115(4): 1036-1045. doi: 10.1093/jee/toac020