

SCIENTIFIC NOTE

FIRST RECORD OF *CULICOIDES OCCIDENTALIS* IN SINALOA, MEXICO

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ABSTRACT. *Culicoides* species adults were collected in light traps located on the coast of Elota, Sinaloa, Mexico, in February 2022. All specimens were females, and it was determined based on their morphology that they belonged to the *Culicoides variipennis* complex. The identification of the species was carried out by means of a comparative analysis of genetic sequences of the cytochrome *c* oxidase subunit I gene, which resulted in *C. occidentalis*, this being its first report in Sinaloa and its third nationwide.

KEY WORDS Blue tong virus, *Culicoides occidentalis*, *Culicoides variipennis* complex, Sinaloa

The *Culicoides variipennis* are classified within the subgenus *Monoculicoides*, with 6 distinct species currently recognized within the group (*C. albertensis* Wirth and Jones, *C. australis* Wirth and Jones, *C. sonorensis* Wirth and Jones, *C. occidentalis* Wirth and Jones, *C. variipennis* Coquillett, and *C. mullensis* Shults and Borkent (Shults 2021). Morphological characterization of adults, exclusively *C. occidentalis*, represents a challenge for researchers because of their similarities with female *C. sonorensis* and male *C. variipennis*; however, the heterogeneity of developmental habitats and historical geographic distributions of the species may separate them ecologically and thus be important factors in directing efforts to identify the taxa (Holbrook et al. 2000).

Historical records of the *C. variipennis* complex indicate that they are primarily Nearctic species with distributions limited to North America (Wirth and Jones 1957, Holbrook et al. 2000, Huerta et al. 2012). In the USA, *C. sonorensis* has been listed as a competent vector for bluetongue virus (BTV) transmission, being primarily associated with livestock production, particularly cattle (McGregor et al. 2022). The BTV infection rates in field-collected populations of *C. variipennis* and *C. occidentalis* from different US sites were less than 3%, indicating that they are species with low probability of transmitting the virus to the animal host (Tabachnick 1996); however, studies, such as in vitro infections, that test for vector competence between the species are lacking (McGregor et al. 2022).

In Mexico the complex is distributed in 13 states, with *C. sonorensis* being the most widely reported (Table 1; Wirth and Jones 1957; Borkent and Grogan 2009; Holbrook et al. 2000; Huerta et al. 2012, 2020). *Culicoides occidentalis* has been recorded in northwestern Mexico in the state of Baja California, Baja California Sur, as well as in central Mexico in Puebla (Table 1; Wirth and Jones 1957, Alcocer et al. 2001, Huerta et al. 2012). At present, these species have not been related to the BTV transmission, although the virus is widely established in the national territory, affecting sheep and cattle (Suzan et al. 1983, Vilchis et al. 1986, Rojas-Anaya et al. 2023).

Culicoides adults were collected with light traps located on the coast of Elota, Sinaloa (trap 1: 23° 54' 18" N, 106° 57' 29" W, elevation 5 miles above sea level [masl]) trap 2: 23° 54' 20" N, 106° 57' 26" W, elevation 3 masl; trap 3: 23° 54' 32.7" N, 106° 57' 38" W, elevation 3 masl), at twilight hours (5:30 to 7:30 p.m.) in February 2022. The bags collected from the traps holding the specimens were placed in 200-ml plastic vessels containing 100 ml of 70% ethanol and transferred to the entomological collection of the Faculty of Biology, Autonomous University of Sinaloa, for the corresponding studies.

Forty-seven *Culicoides* adults were isolated from the collection bags (from light traps 1, 2, and 3, 14, 20, and 13 specimens were collected, respectively). Specimens were identified by morphology according to the dichotomous keys proposed by Holbrook et al. (2000). The total number of *Culicoides* collected corresponded to females characterized by a wide third palpal segment and a clearly pronounced sensory pit (Fig. 1A, 1B). Since these characteristics are similar in *C. sonorensis* and *C. occidentalis* (Holbrook et al. 2000), and both species have been recorded from states near and adjacent to Sinaloa (Table 1; Wirth and Jones 1957, Holbrook et al. 2000, Borkent and Grogan 2009), morphology in this case is unreliable for species identification. In this regard the specimens were processed by molecular techniques for taxonomic discrimination.

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Table 1. Geographic distribution of *Culicoides variipennis* complex in Mexico.

Species	Locality	Record
<i>Culicoides sonorensis</i>	Mexico State	Wirth and Jones 1957
	Mexico City	
	Cocula, Guerrero	
	Sabinas Hidalgo, Nuevo Leon	
	Matamoros, Puebla	
	Agua Caliente, Sonora	
<i>C. variipennis</i>	San Lorenzo, Coahuila	Huerta et al. 2012
	42 km south of Caballos, Durango	
	Tepic, Nayarit	
	El Salto Falls, San Luis Potosi	
	Otumba, Coyotepec, Mexico State	
<i>C. occidentalis</i>	San Jacinto, Mexico City	Huerta et al. 2020
	Oaxaca, Oaxaca	
	Ensenada, Baja California	
	Lake Tecuitlepa, Puebla	
	Loreto, Baja California Sur	
Playa Ceuta, Elota, Sinaloa	New record	

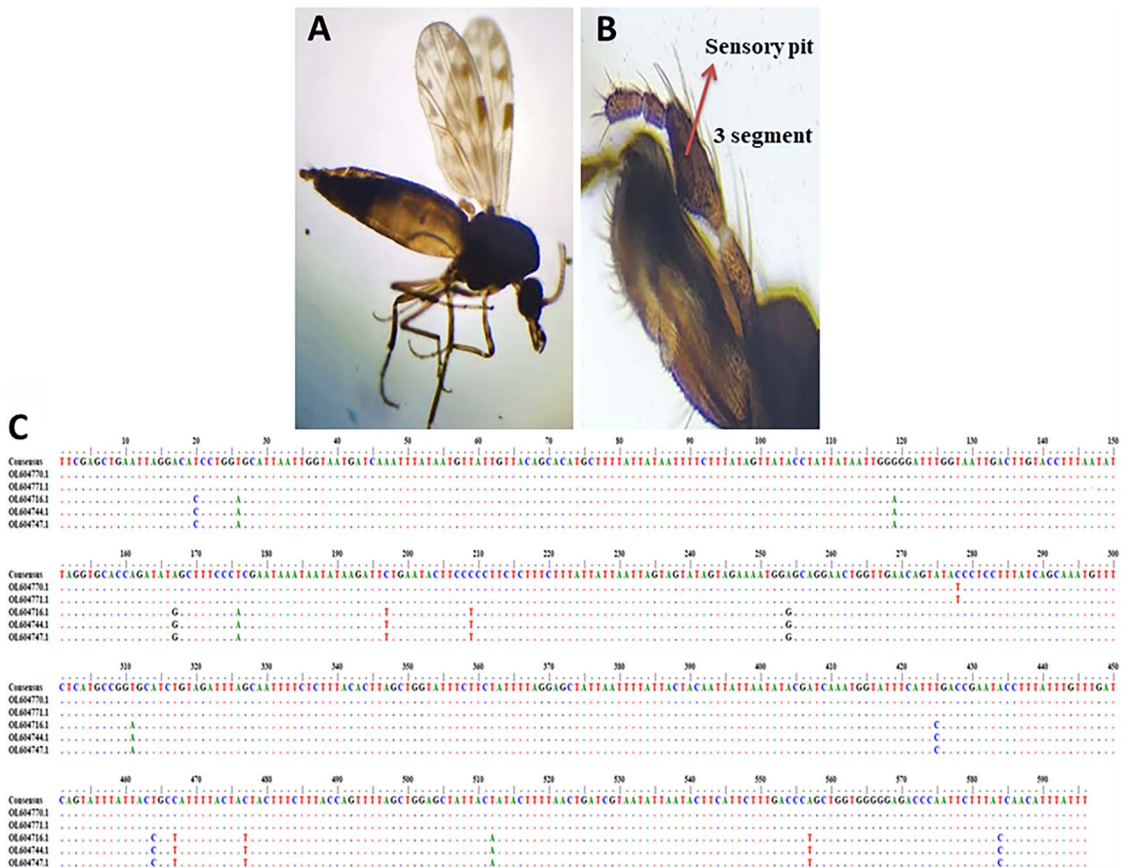


Fig. 1. Morphological and molecular characterization of *Culicoides occidentalis* collected in Sinaloa. (A) Lateral view and (B) palp of a female. (C) Molecular alignment. Consensus: *C. occidentalis* collected in Sinaloa. Sequences available in GenBank: OL604770.1 and OL604771.1, *C. occidentalis*; OL604716.1 and OL60471.1, *C. sonorensis*; OL604744.1 and OL604747.1, *C. variipennis*. Sequence similarity: consensus–*C. occidentalis*, >99%; consensus–*C. sonorensis*, 97.32%; consensus–*C. variipennis*, 97.32%.

The molecular analysis was carried out with mitochondrial DNA because it has been reported to be reliable for the identification of *C. occidentalis* from the rest of the species of the *C. variipennis* complex (Shults 2021, Shults et al. 2022). Fifteen specimens were placed in 1.5-ml plastic tubes and macerated with a sterile pistil. Subsequently, total DNA extraction from adults was performed with the Wizard SV Genomic DNA Purification System kit (Promega, Madison, WI) according to the manufacturer's instructions. A partial sequence of the mitochondrial cytochrome *c* oxidase subunit I (COI) gene was amplified by polymerase chain reaction (PCR) as a molecular marker. The GoTaq Green Master Mix kit (Promega) was used under the manufacturer's conditions, as well as the oligonucleotides LCO sense (5'-ggcaacaatcaataagatattgg-3') and HCO antisense (5'-taaacctcagggtgacaaaataatca-3'; Folmer et al. 1994). The final reaction was 12.5 µl (6.25 µl of PCR mix, 0.5 µl of each oligonucleotide [10 pMol], 2 µl of DNA, and 3.25 nuclease-free water) and amplified with the following conditions: 4 min at 95°C, followed by 34 cycles at 95°C for 40 sec, 55°C for 40 sec, and 72°C for 50 sec, and a final extension of 72°C for 5 min. The amplicon pools of *Culicoides* were sequenced at Macrogen (Seoul, Korea) and compared with *C. occidentalis* sequences previously reported in GenBank (Shults et al. 2022) with the BioEdit program (version 7.2.5) (Hall 2011).

Specimens were collected within a few meters of the temporary salt marsh at Ceuta (range approximately 50–100 m), which is consistent with the developmental habitats of immature stages (Schmidtman et al. 2000). The results of PCR and sequence analysis verified that the species collected at the study sites corresponded to *C. occidentalis*, this by observing a >99% similarity with sequences previously reported in GenBank (accession numbers OL604770.1 and OL604771.1) for this species. This is its first record in Sinaloa. The comparison of sequences with other species of the *C. variipennis* complex historically reported in Mexico was approximately 97% (Fig. 1C).

In Sinaloa the *Culicoides* genus is poorly known, and, according to historical records, 4 species, *C. blantoni*, *C. phlebotomus*, *C. cancer*, and *C. furens*, are distributed (Huerta et al. 2012, Mendez-Andrade and Ibáñez-Bernal 2023). The dipteran *C. occidentalis* is 1 of the 3 species of the *C. variipennis* complex that has been least studied in Mexico, and its record in Sinaloa opens the possibility of understanding its ecology through population comparisons in different geographical areas, as well as elucidating its status as a vector of etiological agents with medical and veterinary importance, which is still uncertain (McGregor et al. 2022). In this study we report for the first time the presence of *C. occidentalis* in the state of Sinaloa, extending its distribution to 3 states of the Mexican Republic.

REFERENCES CITED

- Alcocer J, Escobar EG, Lugo A, Lozano LM, Oseguera LA. 2001. Benthos of a seasonally-astatic, saline, soda lake in Mexico. *Hydrobiologica* 466:291–297.
- Borkent A, Grogan WL Jr. 2009. Catalog of the New World biting midges north of Mexico (Diptera: Ceratopogonidae). *Zootaxa* 2273:1–48.
- Folmer O, Black M, Hoeh W, Lutz R, Vrijenhoek R. 1994. DNA primers for amplification of mitochondrial cytochrome C oxidase subunit I from diverse metazoan invertebrates. *Mol Mar Biol Biotechnol* 3:294–299.
- Hall T. 2011. BioEdit: An important software for molecular biology. *GERF Bull Biosci* 2:60–61.
- Holbrook FR, Tabachnick WJ, Schmidtman ET, McKinnon CN, Bobian RJ, Grogan WL. 2000. Sympatry in the *Culicoides variipennis* complex (Diptera: Ceratopogonidae): a taxonomic reassessment. *J Med Entomol* 37:65–76.
- Huerta H, Benítez-Alva JI, Concha-Suarez J, Ibáñez-Piñón CR, Manrique-Saide P. 2020. New records of genus *Culicoides* Latreille from Oaxaca, Mexico (Diptera: Ceratopogonidae). *Acta Zool Mex* 36:1–27.
- Huerta H, Rodríguez-Castrejón AM, Grogan WL Jr, Ibáñez-Bernal S. 2012. New records of biting midges of the genus *Culicoides* Latreille from Mexico (Diptera: Ceratopogonidae). *Insecta Mundi* 211:1–20.
- McGregor BL, Shults PT, McDermott EG. 2022. A review of the vector status of North American *Culicoides* (Diptera: Ceratopogonidae) for bluetongue virus, epizootic hemorrhagic disease virus, and other arboviruses of concern. *Curr Trop Med Rep* 9:130–139.
- Mendez-Andrade A, Ibáñez-Bernal S. 2023. An updated catalogue of biting midges of the genus *Culicoides* Latreille, 1809 (Diptera, Ceratopogonidae) of Mexico and their known distribution by state. *Zookeys* 14:1167:1–47.
- Rojas-Anaya E, Cerón-Téllez F, Yáñez-Garza LA, Gutiérrez-Hernández JL, Sarmiento-Salas RE, Loza-Rubio E. 2023. Detección molecular de un fragmento del virus de lengua azul en borregos de diferentes regiones de México. *Rev Mex Cien Pecu* 14:110–121.
- Schmidtman ET, Bobian RJ, Belden RP. 2000. Soil chemistries define aquatic habitats with immature populations of the *Culicoides variipennis* complex (Diptera: Ceratopogonidae). *J Med Entomol* 37:58–64.
- Shults P, Hopken M, Eyer PA, Blumenfeld A, Mateos M, Cohnstaedt LW, Vargo EL. 2022. Species delimitation and mitonuclear discordance within a species complex of biting midges. *Sci Rep* 12:1730.
- Shults PA. 2021. Study of *Culicoides* biting midges in the subgenus *Monoculicoides*: population genetics, taxonomy, systematics, and control. [Ph.D. dissertation]. Texas A&M University, College Station, TX.
- Suzan VM, Misao O, Romero EA, Yosuke M. 1983. Prevalence of bovine herpesvirus-1, para-influenza-3, bovine rotavirus, bovine viral diarrhoea, bovine adenovirus 7, bovine leukemia virus and bluetongue virus antibodies in cattle in Mexico. *Jpn J Vet Res* 31:125–132.
- Tabachnick WJ. 1996. *Culicoides variipennis* and bluetonguevirus epidemiology in the United States. *Annu Rev Entomol* 41:23–43.
- Vilchis C, Gay J, Batalla D. 1986. Determinación de anticuerpos contra el virus de lengua azul en ovinos por la técnica de inmunodifusión. *Tec Pecu Mex* 51:116–121.
- Wirth WW, Jones RH. 1957. *The North American subspecies of Culicoides variipennis (Diptera, Heleidae)*. Technical Bulletin 1170. Washington, DC: United States Department of Agriculture. 35 p.