

THE PRESENCE OF *Aedes hendersoni* IN MEXICO

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ABSTRACT. The mosquito species commonly named Henderson American pointy mosquito, *Aedes hendersoni*, was found during a routine mosquito vector surveillance from 2020 to 2023 in diverse collection sites with conserved forested vegetation at the Eco-Parks Rincón de la Silla and El Salto in Nuevo León, northeastern Mexico. Collected specimens were labeled, mounted, and identified using taxonomic keys. Further confirmation of *Ae. hendersoni* was performed by deoxyribonucleic acid (DNA) barcoding and cytochrome oxidase-I (*COI*) sequences served to confirm the identity of *Ae. hendersoni* by comparison with other sequences of species with *Triseriatus* group and *Protomacleaya* subgenus of *Aedes*. Voucher specimens were curated and are available in 2 Mexican collection repositories and *COI* sequences were deposited in the NCBI GenBank with accession number MG242473.1. Hence, the presence of *Ae. hendersoni* in México is documented for the first time. Now, geographic occurrence of this species is from Canada throughout United States to northeastern México. With this new record, there are the 4 mosquito species in the *Triseriatus* group [*Aedes*: (*Protomacleaya*)] and 252 species in México.

KEY WORDS *Aedes hendersoni*, DNA barcoding, México, *Triseriatus* group

INTRODUCTION

The Henderson American pointy mosquito, *Aedes* (*Protomacleaya*) *hendersoni* Cockerell, also known as one of the eastern tree-hole mosquitoes, was originally described by the American entomologist Theodore Dru Alison Cockerell (1866–1948) as a variety of the other species also commonly named eastern tree-hole mosquito, *Ae. triseriatus* (Say), from specimens collected in the vicinity of Douglas, Wyoming, USA (Cockerell 1918); subsequently, *Ae. hendersoni* was synonymized under the name *Ae. triseriatus* by Dyar (1922, 1928)

and until 1960, *Ae. hendersoni* was considered as either synonym or subspecies of *Ae. triseriatus* (Hedeen 1963). In 1960, *Ae. hendersoni* was elevated from synonymy to the rank of specific valid name by Breland (1960), based on the observation of external characters of reared females, larvae, and chromosome complement comparison. Further differences between *Ae. hendersoni* and *Ae. triseriatus* females were later observed on the tarsal claws (Harmston 1969) and host selection; although *Ae. hendersoni* fed mainly on tree squirrels and raccoons, *Ae. triseriatus* fed predominantly on chipmunks and deer (Nasci 1982). A complete description of all life stages, except eggs of *Ae. hendersoni* and *Ae. triseriatus*, and a revision of their taxonomy, distribution, and bionomics was provided by Zavortink (1972), while the eggs of *Ae. hendersoni* were described and compared with the *Ae. triseriatus* eggs by Linley and Craig (1993). *Aedes hendersoni* was later placed into the genus *Ochlerotatus sensu auctorum*, based on phylogenetic analyses of all life stages on the Aedini tribe (Reinert et al. 2004, 2006, 2008, 2009), and, subsequently, re-stored in the genus *Aedes* by Wilkerson et al. (2015). *Aedes hendersoni* belongs to the *Triseriatus* group (*Aedes*: *Protomacleaya*), which also includes the species *Ae. brelandi* Zavortink, *Ae. triseriatus*, and *Ae. zoosophus* Dyar and Knab (Zavortink 1972, Taylor 1990).

Species in the *Triseriatus* group are distributed predominantly in the Nearctic region, *Ae. triseriatus* and *Ae. hendersoni* occupy vast geographical ranges, and much of North America is inhabited by one or both species. *Aedes hendersoni* occurs abundantly in the eastern United States and small portions of southern Canada, where it was first reported in the provinces of British Columbia (Zavortink 1972), Ontario and Quebec; and, Saskatchewan (Trimble 1972). In the United States, *Aedes hendersoni* extends much further west than *Ae. triseriatus*, reaching the

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Fig. 1. Map showing current geographic distribution of *Aedes hendersoni*. In the inset of Nuevo León, México, the blue star depicts the collection sites of *Ae. hendersoni* at “Cascadas de Guadalupe” and the green star depicts that at “El Salto”. The Figure was produced with Geographic Information Systems (GIS) using the open-source software QGIS v. 3.22.13 LTR (Available online: <https://qgis.org/es/site/>, accessed on April 28, 2025) and vector layers in Shape format of the Basic Geostatistical Framework 2020 of the National Institute of Statistics, Geography and Informatics (Spanish acronym INEGI), México.

southeastern region of British Columbia and eastern Washington and Oregon in the north as well as the northern regions of Nevada and Utah, eastern Colorado and New Mexico, and mid-northern Texas to the south, although it is absent in peninsular Florida, and without records in the extreme southern Texas (Darsie and Ward 2005, Fitzgerald and Livdahl 2019) (Fig. 1).

MATERIALS AND METHODS

Study area

Routine vector mosquito surveillance from 2020 to 2023 was conducted in diverse collections sites with conserved forested vegetation at the Eco-Parks Cascadas de Guadalupe de Guadalupe municipality (25°38'07.0"N 100°11'59.0"W, 600 m.) and El Salto



Fig. 2. Panel A: A Shannon trap containing humans deployed at the Eco-Park Cascadas de Guadalupe in Nuevo León, México. Here, *Ae. hendersoni* was collected. Panel B: General view of El Salto, Zaragoza, Nuevo León, México. In both places, *Ae. hendersoni* females were collected.

of Zaragoza municipality (23°56'31.4"N 99°45'45.3"W, 1,616 m.) in Nuevo León, northeastern Mexico.

Samples collection and curation

Adult mosquitoes were collected monthly at dusk and night hours (17:00–22:00 h), and occasionally at morning hours (08:00–10:00 h), using three Centers for Disease Control and Prevention (CDC) miniature light traps (John W. Hook Company, Gainesville, FL, USA) with a 0.95 watts incandescent light baited with 3–5 drops of octanol on 1–2 cotton balls per trap. Two Shannon traps containing humans (Fig. 2) were also deployed 100 m apart from CDC light trap collection sites. In addition, host-seeking mosquitoes were caught using mechanical aspirators. Collected specimens were killed in situ using chloroform vapors, labeled, and transported to local labs. Voucher specimens were curated and deposited in 2 Mexican collections repositories: The Agronomy School of the Autonomous University of Nuevo León (FA-UANL), located in Monterrey, Nuevo León and the Antonio Narro Autonomous Agrarian University-Laguna Unit (UAAAN-UL), located in Torreón, Coahuila. Specimens were morphology identified using the keys of Schick (1970), Zavortink (1972), and Darsie and Ward (2005).

DNA barcoding

Genomic DNA from two legs per specimen was isolated employing the modified hotshot technique (Montero-Pau et al. 2008). The cytochrome oxidase 1 (*COI*) barcode gene was amplified with the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAAA TCA-3') (Folmer et al. 1994). Polymerase chain reaction (PCR) reactions were performed using the MyTaq™ Mix (BIO-25041, Meridian Bioscience, Cincinnati, OH) with PCR conditions as described elsewhere (Ortega-Morales et al. 2024). The PCR products were electrophoresed in 1% agarose gel, and then fragments, with the expected amplicon length, were sequenced bidirectionally by Eurofins (Eurofins, Genomics, Louisville, KY) using the traditional Sanger sequencing protocol (Sanger et al. 1977). Phylogenetic analysis of the *COI* sequences was determined with Molecular Evolutionary Genetics Analysis (MEGA 12) software (Tamura et al. 2021) using the neighbor-joining method with 500 replications bootstrap (Saitou and Mei 1987).

RESULTS

Fourteen *Ae. hendersoni* females were caught at Cascadas de Guadalupe using Shannon traps deployed in two collection sites from August to September 2020

and from March to April and July 2021. Only 7 *Ae. hendersoni* females were caught with mechanical aspirators at the same collection sites. However, none *Ae. hendersoni* was caught in the CDC light traps. Other mosquito species were also caught: *Anopheles pseudopunctipennis* Theobald, *Ae. amateuri* Ortega and Zavortink, *Ae. trivittatus* (Coquillett), *Ae. amabilis* Schick, *Ae. podographicus* Dyar and Knab, *Ae. triseriatus*, *Ae. (Protomacleaya)* sp., *Ae. aegypti* (L.), *Ae. albopictus* (Skuse), *Haemagogus equinus* Theobald, *Psorophora ferox* (von Humboldt), *Culex coronator* Dyar and Knab, *Cx. interrogator* Dyar and Knab, and *Cx. nigripalpus* Theobald. Seven and 11 *Ae. hendersoni* females were also caught at El Salto using mechanical aspirators in October 2020 and 2023 along with other species: *Ae. quadrivittatus* (Coquillett), *Ae. trivittatus*, *Ae. podographicus*; and *Ae. quadrivittatus*, *Ae. triseriatus*, *Ae. amabilis*, *Ae. albopictus*, and *Wyeomyia mitchellii* (Theobald), respectively (Table 1).

The *Ae. hendersoni* (GenBank accession number: MG242473.1) sequences (n = 6) of this study were compared with *Ae. hendersoni* sequences (n = 2) downloaded from the GenBank of National Centre for Biotechnology Information (NCBI), and with sequences (n = 20) from specimens belonging to the subgenus *Protomacleaya* collected in Nuevo León such as *Ae. amabilis* (MT999244.1) (n = 7), *Ae. podographicus* (MT552480) (n = 6), and *Ae. triseriatus* (JX259710.1 and MG242523.1) (n = 6), and an outer group *Cx. pipiens* L. (CYTC704) from Greece (n = 1). The PCR amplification, sequencing, Basic Local Alignment Search Tool (BLAST), and phylogenetic analysis of the *COI* gene revealed that the specimens examined belong to *Ae. hendersoni*, clustering and sharing 97.72% identity with *Ae. hendersoni* (MG 242498.1) (Fig. 3). The *COI* sequences of *Ae. hendersoni* have been deposited in the NCBI GenBank with accession number MG242473.1.

DISCUSSION

The adult female of *Ae. hendersoni* are readily recognized among species of the *Triseriatus* group by having tarsal segments 1–5 of all legs entirely dark scaled; bristles of the anterior portion of mesoscutum relatively numerous and well developed; light scaling of fossa usually extensive and extending mesad of anterior dorso-central bristles, the scaling more or less bordered on all sides by conspicuous setae lightly to moderately pigmented (Fig. 4); and postspiracular scale patch absent to small (Zavortink 1972). Further differences between *Ae. hendersoni* and *Ae. triseriatus* females were observed: in *Ae. hendersoni*, some few pale scales on the anterior portion of mesoscutum are always present, and these can form a slightly and narrow complete acrostichal line of pale scales, reaching the prescutellar area, which is present in some specimens observed. Nevertheless, in most of them, this acrostichal line is absent (Fig. 4). Figure 4 shows the presence of narrow and complete dorso-central pale scales. In *Ae. triseriatus*, there is no sign of any pattern or

Table 1. Total number of *Aedes hendersoni* females collected in Nuevo León, México along with other associated species caught in 2020–2023.

Collection date and hour	Type of trap	Total number of <i>Aedes hendersoni</i> caught	Other associated species caught
August 8, 2020 20:00–21:00	Shannon containing humans	One	<i>Anopheles pseudopunctipennis</i> , <i>Ae. amateuri</i> , <i>Ae. trivittatus</i> , <i>Ae. amabilis</i> , <i>Ae. podographicus</i> , <i>Ae. triseriatus</i> , <i>Ae. (Protomacleaya)</i> sp., <i>Ae. aegypti</i> , <i>Ae. albopictus</i> , <i>Psorophora ferox</i> , <i>Culex coronator</i> , <i>Cx. interrogator</i> , <i>Cx. nigripalpus</i>
August 27, 2020 20:00–21:00	Shannon containing humans	Two	<i>An. pseudopunctipennis</i> , <i>Ae. amateuri</i> , <i>Ae. trivittatus</i> , <i>Ae. podographicus</i> , <i>Ae. triseriatus</i> , <i>Ae. (Protomacleaya)</i> sp., <i>Ae. albopictus</i> , <i>Ps. ferox</i> , <i>Cx. coronator</i> , <i>Cx. interrogator</i>
September 22, 2020 20:00–21:00	Shannon containing humans	One	<i>Ae. amateuri</i> , <i>Ae. trivittatus</i> , <i>Cx. coronator</i> , <i>Cx. interrogator</i>
September 23, 2020 18:00–19:00	Shannon containing humans	One	<i>Ae. aegypti</i>
September 24, 2020 19:00–20:00	Shannon containing humans	One	<i>Ae. albopictus</i>
October 2, 2020 12:00–13:00	Manual aspirator (human-seeking)	Seven	<i>Ae. quadrivittatus</i> , <i>Ae. trivittatus</i> , <i>Ae. podographicus</i>
March 22, 2021 18:00–19:00	Shannon containing humans	Six	<i>Ae. triseriatus</i>
April 28, 2021 18:00–19:00	Shannon containing humans	One	<i>Ae. amabilis</i> , <i>Ae. podographicus</i> , <i>Ae. albopictus</i> , <i>Ps. ferox</i>
July 28, 2021 19:00–20:00	Shannon containing humans	One	<i>Ae. trivittatus</i> , <i>Ae. amabilis</i> , <i>Ae. podographicus</i> , <i>Ae. albopictus</i> , <i>Ps. ferox</i> , <i>Hg. equinus</i>
October 5, 2021 15:00–18:30	Manual aspirator (human-seeking)	Eleven	<i>Ae. quadrivittatus</i> , <i>Ae. triseriatus</i> , <i>Ae. amabilis</i> , <i>Ae. albopictus</i> , <i>W. mitchellii</i>
October 27, 2023 21:00–23:00	Manual aspirator (human-seeking)	Five	<i>An. pseudopunctipennis</i> , <i>Ae. trivittatus</i> , <i>Ae. amabilis</i> , <i>Ae. podographicus</i> , <i>Ae. albopictus</i> , <i>Cx. coronator</i>
October 28, 2023 8:00–9:00	Manual aspirator (human-seeking)	Two	<i>Ae. amabilis</i> , <i>Ae. podographicus</i> , <i>Ae. aegypti</i> , <i>Ae. albopictus</i> , <i>Hg. equinus</i>

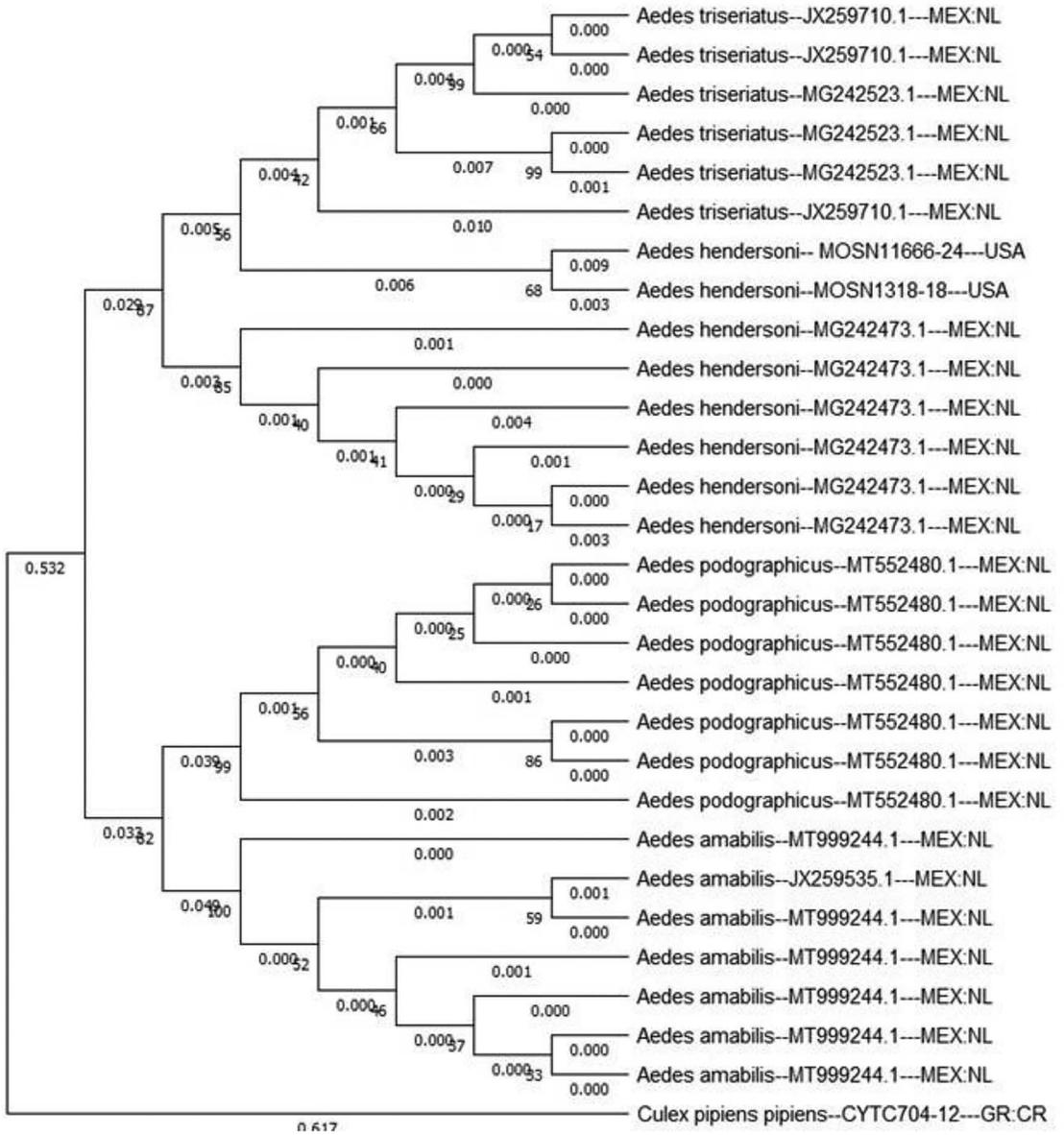


Fig. 3. Phylogenetic tree of *COI* DNA barcode showing the clustering pattern of *Aedes hendersoni* (n = 6) sequences (this study) with those of other *Ae. hendersoni* (n = 2) from USA (downloaded from the GenBank) and closely related species (e.g. *Ae. triseriatus* n = 6).

presence of pale scales on the acrostichal or dorsocentral areas in the observed specimens. These differences were observed and documented by Zavortink (1972) from American “Western” populations of *Ae. hendersoni*. Differences between *Ae. hendersoni* and *Ae. triseriatus* are more noticeable in the larval stage, in *Ae. hendersoni* by having acus of siphon well detached from the base of siphon, anal gills about the same length and much longer than ninth segment, and in general, the light color of body integument (Breland 1960, Grimstad et al. 1974).

Aedes triseriatus is the primary vector of La Crosse (LAC) virus, responsible for encephalitis, especially in young children (Watts et al. 1973, Reno et al. 2000), but *Ae. hendersoni* is incompetent as vector because of its salivary gland barrier to LAC virus (Watts et al. 1975, Grimstad et al. 1985, Paulson and Grimstad 1987, Paulson et al. 1992). Routine vector surveillance of *Ae. triseriatus* and *Ae. hendersoni* is paramount; although the role of *Ae. hendersoni* as an inefficient vector of LAC virus is well understood, that of other closely related

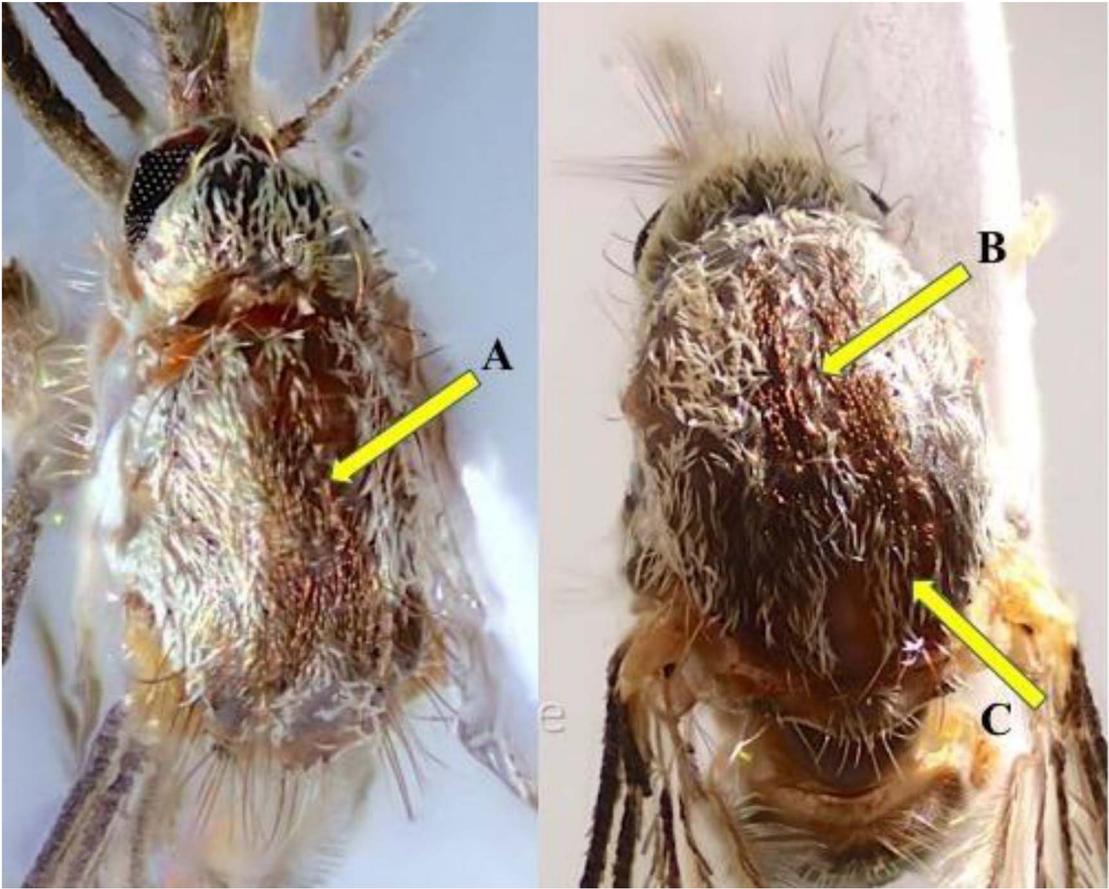


Fig. 4. Different scaling patterns of *Aedes hendersoni* females. Yellow arrow A: Incomplete acrostichal line of pale scales; Arrow B: Slightly and narrow complete acrostichal line of pale scales, reaching the pre-scutellar area; Arrow C: Presence of narrow and complete dorsocentral pale scales.

species as vectors of LAC virus remains to be fully elucidated. The LAC virus has not been reported yet in Mexico because encephalitis, as a vector borne disease, is neglected. Although *Aedes hendersoni* is not recognized as a dangerous or incriminated vector of public health importance, it is associated with being a human nuisance in tourist areas elsewhere.

By adding *Ae. hendersoni* to the mosquito fauna of Mexico, currently there are 253 species, of which, 69 species are of Nuevo León. Voucher specimens are available in 2 Mexican mosquito collection repositories: the Collection of Arthropods with importance in One Health of the FA-UANL, Nuevo León, with reference numbers NL-02-240820, NL-02-270820, NL-03-220920, NL-04-240920, NL-04-230920, 01-220321-CS, 02-220321-CS, 07-220321-CS, 01-230321-CS, 02-230321-CS, 02-280421-CS, 04-260521-CS, 09-240621-CS, 09-280721-CS, 09-250621-CS, 05-250621-CS, 04-270721-CS, 05-270721-CS; and UAAAN-UL of Coahuila, with reference numbers 01271023-RS and 01281023-RS; collection code: Tesis_camada_10.

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