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The Mosquitoes of Querétaro, Mexico: Distribution, Ecology, and Discovery of *Shannoniana huasteca* n. sp. (Diptera: Culicidae)

Aldo I. Ortega-Morales [1], Luis M. Hernández-Triana [2,*] and Quetzaly K. Siller-Rodríguez [3,*]

[1] Departamento de Parasitología, Universidad Autónoma Agraria Antonio Narro, Unidad Laguna, Torreón 27054, Coahuila, Mexico; agrortega@hotmail.com
[2] Animal and Plant Health Agency, Virology Department, Vector Borne Diseases Research Group, Surrey KT153NB, UK
[3] Facultad de Ciencias Biológicas, Universidad Juárez del Estado de Durango, Gómez Palacio 35010, Durango, Mexico
* Correspondence: luis.hernandez-triana@apha.gov.uk (L.M.H.-T.); qksr@hotmail.com (Q.K.S.-R.)

Abstract: In order to document the diversity and distribution of mosquitoes inhabiting the Querétaro State of México, collection trips were conducted in all physiographic regions and sub-regions of the state (Sierra Madre Oriental, Central Plateau, and Neo-Volcanic Axis). In addition, mosquito specimens collected in Querétaro and deposited in the Collection of Arthropods of Medical Importance (CAIM) were re-examined. A total of 2718 specimens (570 larvae, 384 larval exuviae, 537 pupal exuviae, 30 pupae, 807 females, 368 males, and 22 male genitalia) were analyzed. In total, 2 subfamilies, namely Anophelinae and Culicinae, 5 tribes, 12 genera, 20 subgenera, and 50 species were found. Of these, 3 tribes, 8 genera, 11 subgenera, and 33 species are new records for the mosquito fauna of Querétaro. Two undescribed species were found, and one of them, *Shannoniana huasteca* Ortega n. sp., is described here using morphology and Cytochrome oxidase subunit 1 (*COI*) DNA barcoding. Taxonomic notes, new distribution limits, comments about the medical importance of species, and a key to identify adult females of *Shannoniana* species are provided.

Keywords: mosquitoes; Querétaro; new records; *Shannoniana huasteca*; new species

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1. Introduction

Mexico is divided into 32 political states of which only nine have been systematically studied in terms of the taxonomy, ecology, and distribution of mosquito species: Tlaxcala (26 spp.) [1], Quintana Roo (88 spp.) [2–9], Veracruz (141 spp.) [10–12], Tamaulipas (82 spp.) [13], Hidalgo (57 spp.) [14,15], Nuevo León (67 spp.) [16–18], Tabasco (107 spp.) [19–22], Mexico City (28 spp.) [23,24], and Mexico State (51 spp.) [25]. In Querétaro state, 17 mosquito species had been previously reported. However, most records are based on collections made in urban and sub-urban regions, not on collections from conserved forest and jungle regions, primarily in the north of the state. In this study, all physiographical regions of Querétaro (Sierra Madre Oriental, Central Plateau, and Neo-Volcanic Axis) were sampled with special emphasis on conserved forest regions and other sylvan regions of the state during the dry and rainy seasons.

A current checklist of the mosquito species that inhabit Querétaro state is provided in this study. Moreover, biological notes and medical importance are provided for the newly reported species and for species that reach their distributional limits within the state. Two undescribed species were found during the field collections, one of which is described here: *Shannoniana huasteca* Ortega n. sp. using morphological characters of adult stages and the analysis of DNA-barcodes. The second species, belonging to the genus *Culiseta*, subgenus *Culiseta*, is left undescribed until more material becomes available. Querétaro is the tenth state of Mexico to have the list of mosquito species updated. At present, 50 species

are currently known (Table 1). Specimens collected and examined during this study were deposited in the Culicidae Collection of the Parasitology Department of the Autonomous Agrarian University Antonio Narro, Laguna unit, Torreón, Coahuila, Mexico.

Table 1. Checklist of the mosquito species that occur in Querétaro state. VM-P: [26]. V: [27]. D-NV: [28]. IBMC: [29]. STEA: [30]. WEEA [31] OMEA [a]: [32]. OMEA [b]: [33]. F.R.: First Record. NSR: New State Record (in bold). NS: New Species (in bold). [1] Reported as *Aedes atropalpus* (Coquillett). [2] Reported as *Culex peus* Speiser.

Taxa	F.R.	Taxa	F.R.
Anopheles (*Anopheles*)		26. ***Cx. coronator*** **Dyar and Knab**	**NSR**
1. ***An. apicimacula*** **Dyar and Knab**	**NSR**	27. ***Cx. declarator*** **Dyar and Knab**	**NSR**
2. ***An. eiseni*** **Coquillett**	**NSR**	28. ***Cx. erythrothorax*** **Dyar**	**NSR**
3. ***An. franciscanus*** **McCracken**	**NSR**	29. *Cx. quinquefasciatus* Say	D-NV
4. *An. pseudopunctipennis* Theobald	VM-P	30. ***Cx. restuans*** **Theobald**	**NSR**
5. *An. punctipennis* (Say)	VM-P	31. ***Cx. salinarius*** **Coquillett**	**NSR**
Anopheles (*Nyssorhynchus*)		32. *Cx. stigmatosoma* [2] Dyar	D-NV
6. *An. albimanus* Wiedemann	IBMC	33. ***Cx. tarsalis*** **Coquillett**	**NSR**
Aedes (*Aedimorphus*)		34. ***Cx. thriambus*** **Dyar**	**NSR**
7. *Ae. vexans* (Meigen)	V	***Culex*** **(*Melanoconion*)**	
Aedes (*Georgecriagius*)		35. ***Cx. erraticus*** **(Dyar and Knab)**	**NSR**
8. *Ae. epactius* [1] Dyar and Knab	D-NV	35. ***Cx. peccator*** **Dyar and Knab**	**NSR**
Aedes (*Howardina*)		***Culex*** **(*Neoculex*)**	
9. *Ae. allotecnon* Kumm, Komp, and Ruiz	V	36. ***Cx. apicalis*** **Adams**	**NSR**
10. ***Ae. quadrivittatus*** **(Coquillett)**	**NSR**	37. ***Cx. arizonensis*** **Bohart**	**NSR**
Aedes (*Ochlerotatus*)		***Culex*** **(*Phenacomyia*)**	
11. ***Ae. angustivittatus*** **Dyar and Knab**	**NSR**	38. ***Cx. lactator*** **Dyar and Knab**	**NSR**
12. ***Ae. euplocamus*** **Dyar and Knab**	**NSR**	***Lutzia*** **(*Lutzia*)**	
13. *Ae. scapularis* (Rondani)	D-NV	40. ***Lt. bigoti*** **(Bellardi)**	**NSR**
14. *Ae. shannoni* Vargas and Downs	V	***Culiseta*** **(*Culiseta*)**	
15. *Ae. trivittatus* (Coquillett)	V	41. ***Cs. inornata*** **(Williston)**	**NSR**
Aedes (*Protomacleaya*)		42. ***Cs. particeps*** **(Adams)**	**NSR**
16. ***Ae. amabilis*** **Schick**	**NSR**	43. ***Cs.*** **n. sp.**	**NS**
17. *Ae. brelandi* Zavortink	STEA	***Limatus***	
18. *Ae. podographicus* Dyar and Knab	OMEA [a]	44. ***Li. durhamii*** **Theobald**	**NSR**
19. ***Ae. schicki*** **Zavortink**	**NSR**	***Sabethes*** **(*Sabethoides*)**	
20. *Ae. triseriatus* (Say)	STEA	45. ***Sa. chloropterus*** **(von Humboldt)**	**NSR**
Aedes (*Stegomyia*)		***Shannoniana***	
21. *Ae. aegypti* (Linneaus)	IBMC	46. ***Sh. huasteca*** **n. sp.**	**NS**
22. *Ae. albopictus* (Skuse)	OMEA [b]	***Wyeomyia*** **(*Triamyia*)**	
Haemagogus (*Haemagogus*)		47. ***Wy. aporonoma*** **Dyar and Knab**	**NSR**
23. *Hg. equinus* Theonald	WEEA	***Wyeomyia*** **(*Wyeomyia*)**	
Psorophora **(*Grabhamia*)**		48. ***Wy. adelpha/guatemala***	**NSR**
24. ***Ps. signipennis*** **(Coquillett)**	**NSR**	49. ***Wy. mitchellii*** **(Theobald)**	**NSR**
Culex **(*Anoedioporpa*)**		***Toxorhynchites*** **(*Lynchiella*)**	
25. ***Cx. restrictor*** **Dyar and Knab**	**NSR**	50. ***Tx. moctezuma*** **(Dyar and Knab)**	**NSR**
Culex (*Culex*)			

2. Materials and Methods

2.1. Study Area

Querétaro state is located in the north-central part of Mexico, between 21°40′12″ and 20°00′54″ north latitude and the meridians 99°02′35″ and 100°35′48″ west longitude. The state has an area of 11,699 km^2. It is bordered to the north by the state of San Luis Potosí; to the west by the state of Guanajuato; to the east by the state of Hidalgo; to the southeast by the state of Mexico; and to the southwest by the state of Michoacán. The state is divided into three physiographic regions and four subregions (Figure 1): Sierra Madre Oriental (Carso Huasteco); Central Plateau (Mountains and Plains of Northern Guanajuato); and

Neo-Volcanic Axis (Plains and Mountains of Querétaro and Hidalgo, Thousand Peaks, and Lakes and Volcanoes of Anáhuac) [34]. A description of the regions and subregions of Querétaro and a list of the municipalities sampled are given in Table 2.

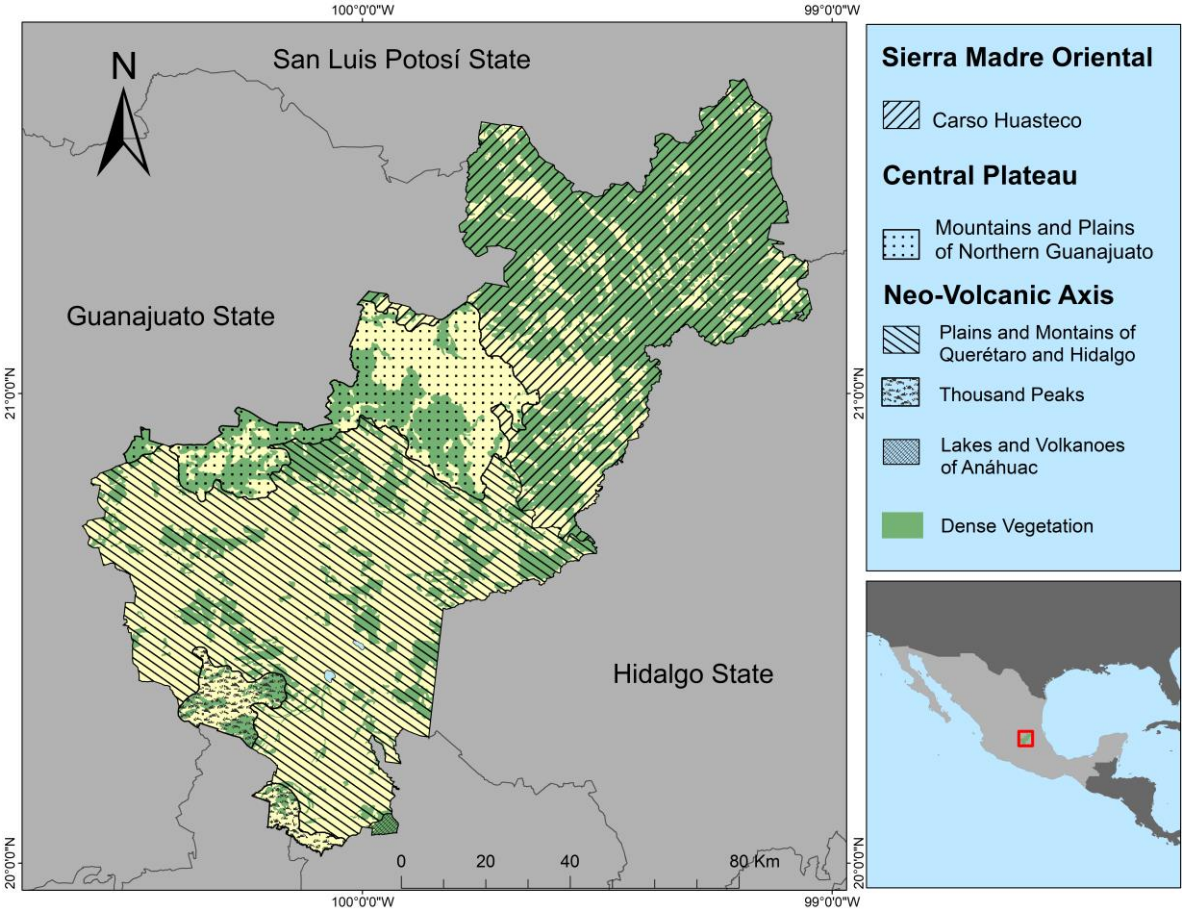

Figure 1. Physiography of Querétaro state. Red bow shows the study area.

Table 2. Description of the physiography of Querétaro state and list of municipalities sampled.

Region (Subregion)	Municipalities Sampled	Description of Subregions
Sierra Madre Oriental (Carso Huasteco)	Arroyo Seco, Jalpan de Serra, Landa de Matamoros, Peñamiller, Pinal de Amoles, San Joaquín,	Located in the south of the Sierra Madre Oriental, extends from San Luis Potosí and Guanajuato states to Querétaro, Hidalgo, Veracruz, and Puebla states. This subregion includes cloud and tropical forests, with oak and pine predominate.
Central Plateau (Mountains and Plains of Northern Guanajuato)	Cadereyta de Montes	This region is characterized by being an elevated region made up of wide plains interrupted by scattered mountain ranges, covered for the most part by Cenozoic volcanic rocks. Its average altitude is from 1700 to 2300 m above sea level.
Neo-Volcanic Axis (Plains and Mountains of Querétaro and Hidalgo)	Amealco de Bonfil, Cadereyta de Montes, Colón, Corregidora, El Marqués, Ezequiel Montes, Huimilpan, Pedro Escobedo, San Juan del Río, Santiago de Querétaro, Tequisquiapan, Tolimán	This subregion is an area of rough terrain where rounded hills predominate, some reaching elevations of 2400 m; the weather is dry and semi-warm, with warm temperatures during the summer, and cold during the winter. Includes grasslands and shrublands.

Table 2. *Cont.*

Region (Subregion)	Municipalities Sampled	Description of Subregions
Neo-Volcanic Axis (Thousand Peaks)	Huimilpan	This small subregion has mountainous relief that is made up of volcanic mountains, staggered lava plateaus, basaltic hills and the wide valley of the Lerma River. The climate is temperate and warm sub-humid, the rains occurring between June and October.
Neo-Volcanic Axis (Lakes and Volcanoes of Anáhuac)	Amealco de Bonfil	This subregion has a relief with hills and mountains with elevations above 3000 m; the climate is temperate humid, cool in summer. There are extensive regions of pine and oak forest, as well shrublands and grasslands.

2.2. Mosquito Collection

Immature stages and adult mosquitoes were collected in specific locations in the four physiographic regions of Querétaro (Table 2). The collections were conducted in both the dry and rainy seasons from 2012 to 2021. Immature stages were collected from all bodies of water found in the study area. Larvae and pupae were placed alive in cups with water from the aquatic habitat and transported to the Laboratorio de Biología Molecular of the Parasitology Department of the Universidad Autónoma Agraria Antonio Narro unidad laguna (LBM-UAAAN-UL). A portion of fourth-instar larvae from each collection was mounted on microscope slides using Euparal, whereas the rest of the live larvae were placed in individual emergence tubes to obtain adults with associated larval and pupal exuviae. Male genitalia was dissected to assist identification when required. Adults were collected in the field using CDC light traps, Shannon traps, and/or biting/landing on humans, and they were killed using triethylamine vapors and later mounted on insect pins. Mosquitoes mounted on insect pins were identified using a stereomicroscope Zeiss Discovery V8, while immature stages and exuviae were identified using a microscope Zeiss Primostar. The morphological terminology proposed by Harbach and Knight [35] for mosquito taxonomy was followed in this study.

2.3. Review of Entomological Collections

The Collection of Arthropods of Medical Importance (CAIM) deposited in the Diagnostic and Epidemiologic Reference Institute (InDRE) was reviewed for additional records of mosquitoes of Querétaro. The species found in the CAIM collection that were not collected by us are *Anopheles eiseni* Coquillett, *An. albimanus* Wiedemann, *Haemagogus equinus* Theobald, and *Culex salinarius* Coquillett. The traditional classification of Culicidae [36] was followed in large part, except that we consider only two subfamilies, incorporating *Toxorhynchites* from the tribe Toxorhynchitini into Culicinae, and we followed [37] the arrangement of Aedini taxa that was incorporated into the online classification of the Walter Reed Biosystematics Unit (WRBU) [38]. Generic and subgeneric abbreviations of Culicidae names also followed the WRBU [38].

2.4. DNA Extraction and COI Amplification

For DNA extraction, a modified Hotshot technique [39–41] was employed. Two legs were placed directly into 50 µL of alkaline lysis buffer in micro vials, which were then sonicated in a water bath for 20 min. Micro vials were subsequently incubated in a thermocycler for 30 min at 94 °C and cooled for 5 min. at 4 °C, after which 50 µL of the neutralizing buffer was added to each vial. PCR amplification of the full-length *COI* barcode region [42,43] was performed using Folmer primers (LCO1490 and HCO2198) and a Qiagen PCR system with the following reaction mix with a final volume of 50 µL: 2 µL of the DNA template, 25 µL of H_2O, 5 µL of NH_4, 5 µL of dNTPs (2 mM/µL), 2.5 µL of $MgCl_2$ (25 mM/µL), 0.1 µL of Bioline Taq Polymerase (Bioline Reagents Ltd., London, UK), 5 µL of each primer (each at 10 pmol/µL), and 0.38 µL of bovine serum albumin

(20 mg/mL) [39–41]. The thermal profile consisted of the following: An initial denaturation step at 94 °C for 1 min., 5 cycles of preamplification of 94 °C for 1 min., 45 °C for 1.5 min., 72 °C for 1.5 min., followed by 35 cycles of amplification of 94 °C for 1 min., 57 °C for 1.5 min., and 72 °C for 1 min., followed by a final elongation step of 72 °C for 5 min. All PCR products were visualized with a 1.5% agarose gel, and samples showing bands of the correct size were bidirectionally sequenced using the ABI PRISM® BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) at the Sequencing Unit, APHA.

2.5. Sequence Analysis

DNA sequences generated in both directions were edited manually using BioEdit sequence alignment Editor version 7.0.5.3 [44], and a consensus sequence was generated using ClustalW [45]. Full details for each specimen and sequence information can be found in the Barcode of Life Database (BOLD) within the "Human Pathogens and Zoonoses Initiative", Working Group 1.4. The Digital Object Identifier (DOI) for the publicly available project in BOLD is dx.doi.org/requested. Accession numbers for the sequences of *Shannoniana huasteca* n. sp. were obtained from NCBI (accession numbers requested). For certain species, we used public *COI* barcode sequences publicly available in BOLD: *Shannoniana fluviatilis* (Grench Guiana) (FGMOS1099-16, FGMOS816-16), *Sh. shcedocyclia* (French Guiana) (FGMOS817-16, FGMOS946-16, FGMOS947-16, FGMOS1126-16, FGMOS1134-16). We also compared published sequences of *Sh. moralesi* (Mexico) (MOSQV056-18, MQCCHP015-16, MQCHP016-16, MQCHP017-16, MQCHP018-16, MQCHP019-16, XNSLC054-18, XNSLC055-18), and *Trichoprosopon digitatum* (Mexico) (MQCHP064-16, MQCHP080-16, MQCHP082-16). The dataset was analyzed in MEGA v.6 [41]. The Maximum Likelihood (ML) analysis was performed using the Kimura 2-Parameter distance metric to determine their distribution pattern, and the tree was rooted to *Tr. digitatum*. The tree robustness was measured by the bootstrap approach using 1000 pseudoreplicates [46].

2.6. Nomenclatural Acts

The electronic edition of this article conforms to the requirements of the amended International Code of the Zoological Nomenclature (ICZN), and hence the new name contained herein is available under that Code from the electronic edition of this article. This published work and the nomenclatural acts it contains have been registered in ZooBank, the online registration system for the ICZN. The ZooBank LSIDs (Life Science Identifiers) can be resolved and the associated information viewed through any standard web browser by appending the LSID to the prefix http://zoobank.org/ (access date on 15 January 2023). The LSID for this publication is 4DD7EB32-56DD-41B6-AACC-877947FE26D4. The electronic edition of this work was published in a journal with an ISSN and has been archived and is available from the MDPI digital repositories.

3. Results

3.1. Mosquito Identification

A total of 2718 specimens from 203 collections were studied. Among the specimens were 570 fourth-instar larvae, 384 larval exuviae, 537 pupal exuviae, 30 pupae, 807 adult females, 368 adult males, and 22 dissected male genitalia. The mosquito fauna of Querétaro state consists of 50 species representing the subfamilies Anophelinae and Culicinae, 5 tribes of the subfamily Culicinae, 12 genera, and 20 subgenera (Table 1). Three tribes (Culisetini, Sabethini, and Toxorhynchitini), eight genera (*Psorophora*, *Lutzia*, *Culiseta*, *Limatus*, *Sabethes*, *Shannoniana*, *Wyeomyia*, and *Toxorhynchites*), 11 subgenera (*Grabhamia*, *Anoedioporpa*, *Melanoconion*, *Neoculex*, *Phenacomyia*, *Lutzia*, *Culiseta*, *Sabethoides*, *Triamyia*, *Wyeomyia*, and *Lynchiella*), and 33 species (*Anopheles apicimacula*, *An. eiseni*, *An. franciscanus*, *Aedes quadrivittatus*, *Ae. angustivittatus*, *Ae. euplocamus*, *Ae. amabilis*, *Ae. schicki*, *Psorophora signipennis*, *Culex restrictor*, *Cx. coronator*, *Cx. declarator*, *Cx. erythrothorax*, *Cx. restuans*, *Cx. salinarius*, *Cx. tarsalis*, *Cx. thriambus*, *Cx. erraticus*, *Cx. peccator*, *Cx. apicalis*, *Cx. arizonensis*, *Cx. lactator*,

Lutzia bigoti, *Culiseta inornata*, *Cs. particeps*, *Cs.* n. sp., *Limatus durhamii*, *Sabethes chloropterus*, *Shannoniana huasteca*, *Wyeomyia aporonoma*, *Wy. adelpha/guatemala*, *Wy. mitchellii*, and *Toxorhynchites moctezuma*) are new records for the mosquito fauna of Querétaro. Finally, two new species were discovered, one of which (*Sh. huasteca*) is described herein. The species accumulation curve of 46 of the 50 mosquito species collected is shown in Figure 2.

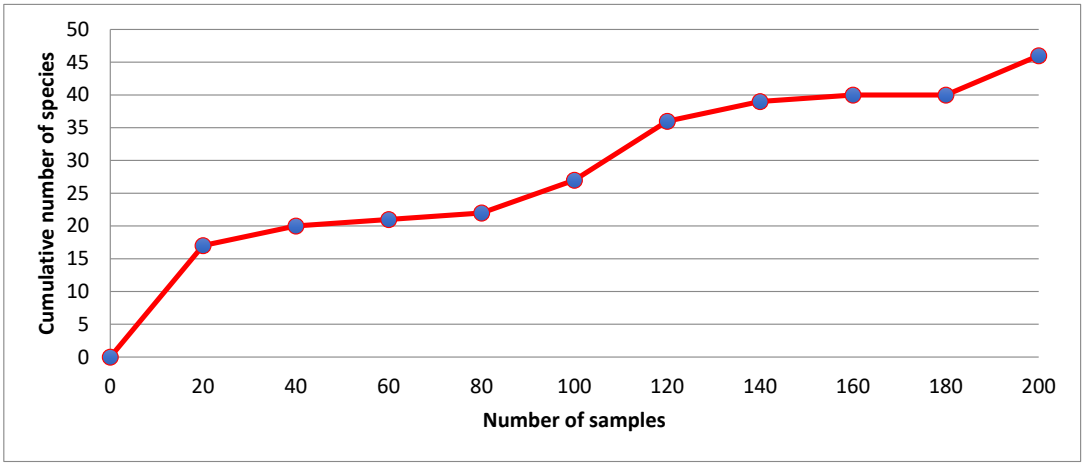

Figure 2. Species accumulation curve for 46 of the 50 mosquito species (203 collections) collected in Querétaro during 2012–2021.

3.2. Biological and Ecological Notes for New State Records

Biological and ecological notes for each species group are reported here. Specific notes including the collection site, date of collection, larval habitat, aquatic parameters, and associated species are shown in Table 3.

3.2.1. Genus *Anopheles*

Both the subgenera *Anopheles* and *Nyssorhynchus* had been previously reported in Querétaro. However, three species within the subgenus *Anopheles* are first recorded in the state: *Anopheles apicimacula*, *An. eiseni*, and *An. franciscanus*. One female of *An. apicimacula* was collected, while immature stages of *An. franciscanus* were collected from swamps, ponds, and stream margins in several locations. One record of *An. eiseni* was obtained from the CAIM collection; this species was not found during our collection trips.

3.2.2. Genus *Aedes*

Of the 16 species of *Aedes* known from Querétaro, five are reported for the first time in the state: *Aedes quadrivittatus*, *Ae. angustivittatus*, *Ae. euplocamus*, *Ae. amabilis*, and *Ae. schicki*. *Aedes quadrivittatus* was one of the most common species within the genus in the forested and conserved regions of the northern part of state; females of this species were collected approaching humans, while immature stages were collected from water in bromeliad axils. Adult females of *Ae. angustivittatus* were collected approaching humans at a single location while immature stages of *Ae. euplocamus* were collected from aquatic habitats at ground level in two sites. *Aedes amabilis* was very common in the conserved regions of the state, and this species frequently approached humans. Only one larvae of *Ae. schicki* was collected from a tree hole, making it the rarest species within the genus in Querétaro.

3.2.3. Genus *Psorophora*

In Mexico, the genus *Psorophora* is a common group of mosquitoes during the rainy season; however, this is the first record of this genus, subgenus *Grabhamia*, and *Ps. signipennis* in Querétaro. Immature stages of this species were collected from ponds during the rainy season. *Psorophora signipennis* is the only species within the genus *Psorophora* known for the state.

Table 3. Collection records of the newly reported mosquito species found in Querétaro, Mexico. The position (geographic coordinates in scale of degrees, minutes, and seconds); elevation (meters above sea level); type of aquatic habitat of the immature stages or environmental condition where adults were collected; environmental parameters of the aquatic habitat (pH, temperature, and dissolved salts in scale of parts per million); and associated species for each collection.

Taxa	Collection Date	Collection Site			Habitat	Aquatic Parameters			Associated Species
		Latitude N	Longitude W	Elev.		pH	Temp	Salts	
An. apicimacula	17 July 2015	20°4′41.2″	100°6′2.0″	1949	Resting on peri-domiciliary	—-	—-	—-	——
An. franciscanus	14 November 2012	20°8′53.3″	99°58′38.5″	2329	Stream margin with clear water, green algae and total shade	6.40	14.5	66	*Ae. epactius*, *Cx. apicalis*
	14 November 2012	20°9′10.7″	100°1′9.6″	2463	Pond with colored water, brown algae and partial shade	6.55	17.2	100	*Cx. tarsalis*
	15 November 2012	20°6′41.9″	100°6′46.9″	2556	Swamp with colored water, green algae and absent shade	7.48	15.3	90	——
	16 November 2012	20°44′38.5″	99°56′19.7″	2058	Spring with clear water, floating vegetation and total shade	7.18	14.1	720	——
	16 November 2012	20°53′30.1″	99°41′44.1″	1539	Pond with clear water, green algae and absent shade	7.19	25.8	701	——
	17 November 2012	21°1′52″	99°36′2.3″	562	Pond with clear water, green algae and partial shade	7.22	24.8	277	*An. pseudopunctipennis*, *Cx. coronator*, *Cx. restuans*
	17 July 2015	20°24′41.2″	100°6′2″	1949	Pond with clear water, aquatic vegetation, green brown and partial shade	8	21	535	*Cx. stigmatosoma*
Ae. quadrivittatus	27 September 2012	21°13′7″	99°5′42″	368	Bromeliad axil with colored water and abundant leaf at bottom, total shade	6.60	26.4	22	*Ae. aegypti*, *Wy. mitchellii*
	27 September 2012	21°13′7″	99°5′42″	368	Bromeliad axil with colored water and abundant leaf at bottom	6.99	24.7	12	*Ae. allotecnon*, *Wy. mitchellii*
	27 September 2012	21°16′14.4″	99°3′20.1″	897	Human biting/landing at day with total shade	—-	—-	—-	*Ae. allotecnon*, *Ae. podographicus*, *Sa. chloropterus*, *Sh. huasteca*, *Wy. mitchellii*
	19 November 2012	21°34′18.8″	99°14′33.3″	—-	Human biting/landing at day with total shade	—-	—-	—-	*Ae. trivittatus*, *Ae. amabilis*
	20 November 2012	21°34′53.6″	99°14′6.8″	686	Human biting/landing at day with total shade	—-	—-	—-	*Ae. trivittatus*, *Ae. amabilis*, *Ae. brelandi*, *Sa. chloropterus*, *Wy. mitchellii*
	20 November 2012	21°34′18.8″	99°14′33.3″	766	Human biting/landing at day with total shade	—-	—-	—-	*Ae. triseriatus*, *Ae. amabilis*
	24 September 2018	21°15′29.3″	99°411.1″	1010	Human biting/landing at day with total shade	—-	—-	—-	*Ae. allotecnon*, *Sh. huasteca*, *Wy. mitchellii*
Ae. angustivittatus	28 September 2012	21°30′59″	99°17′13.3″	1033	Human biting/landing at day with total shade	—-	—-	—-	*Ae. trivittatus*, *Ae. allotecnon*, *Ae. amabilis*, *Ae. brelandi*
Ae. euplocamus	28 September 2012	21°18′48″	99°16′21.7″	965	Rain gutter with colored water, leaves at bottom and total shade	9.70	27.6	58	*Ae. epactius*, *Ae. shannoni*
	20 November 2012	21°34′27.7″	99°12′39.8″	715	Rock hole with clear water, leaves at bottom and total shade	7.63	24	162	*Ae. epactius*, *Cx. coronator*

Table 3. *Cont.*

Taxa	Collection Date	Collection Site			Habitat	Aquatic Parameters			Associated Species
		Latitude N	Longitude W	Elev.		pH	Temp	Salts	
Ae. amabilis	28 September 2012	21°30′32.7″	99°17′22.2″	965	Human biting/landing at day with total shade	—-	—-	—-	——
	28 September 2012	21°30′59″	99°17′13.3″	1033	Human biting/landing at day with total shade	—-	—-	—-	*Ae. angustivittatus*, *Ae. trivittatus*, *Ae. allotecnon*, *Ae. brelandi*
	19 November 2012	21°30′59″	99°17′13.3″	686	Shannon trap baited with human at day				*Ae. trivittatus*, *Ae. triseriatus*
	19 November 2012	21°34′18.8″	99°14′33.3″	—-	Human biting/landing at day with total shade	—-	—-	—-	*Ae. quadrivittatus*, *Ae. trivittatus*
	20 November 2012	21°34′53.6″	99°14′6.8″	686	Human biting/landing at day with total shade	—-	—-	—-	*Ae. quadrivittatus*, *Ae. trivittatus*, *Ae. brelandi*, *Sa. chloropterus*, *Wy. mitchellii*
	20 November 2012	21°34′53.6″	99°14′6.8″	686	Human biting/landing at day with total shade	—-	—-	—-	*Ae. quadrivittatus*, *Ae. triseriatus*
	24 September 2018	21°15′29.3″	99°411.1″	1010	Tree hole with colored water and leaves at bottom	6.73	23	180	——
Ae. schicki	15 November 2012	20°55′51.6″	99°33′16.3″	2381	Tree hole with colored water with abundant leaves at bottom	7.25	12.8	243	——
Ps. signipennis	6 July 2012	20°26′55.9″	100°15′52.1″	2184	Pond with clear water and partial shade	7.3	24	102	——
	6 July 2012	20°26′45.4″	100°16′4.5″	2187	Pond with clear water and partial shade	7.57	25	101	*Ae. trivittatus*
	6 July 2012	20°26′45.4″	100°16′4.5″	2187	Pond with colored water, emerging vegetation and partial shade	7.57	24.7	213	*Ae. trivittatus*
Cx. restrictor	12 September 2012	21°13′13.7″	99°5′42″	368	Tree hole with colored water, leaves at bottom with total shade	8.26	24.6	1132	——
	27 September 2012	21°13′11.2″	99°5′56.9″	140	Discarded tire with clear water, total shade	7.55	22.7	42	*Cx. thriambus*, *Tx. moctezuma*
Cx. coronator	2 April 2012	21°32′8.6″	99°40′59.9″	960	Stream margin with clear water and total shade	8.64	27.9	792	——
	3 April 2012	21°26′45.7″	99°37′58.1″	567	Pond with clear water, aquatic vegetation and partial shade	7.8	26.3	510	*An. punctipennis*, *Cx. restuans*
	3 April 2012	21°23′29.3″	99°34′57.9″	534	Pond with clear water, emerging vegetation and total shade	7.9	30	206	——
	3 April 2012	21°12′35.3″	99°31′59.5″	1095	Irrigation gutter with colored water and total shade	6.8	25.3	482	*Cx. tarsalis*
	4 April 2012	21°02′23.6″	99°46′06.6″	1292	Pond with clear water and partial shade	7.7	34.2	1429	——
	17 November 2012	21°1′52″	99°36′2.3″	562	Pond with clear water, green algae and partial shade	7.22	24.8	277	*An. franciscanus*, *An. pseudopunctipennis*, *Cx. restuans*
	20 November 2012	21°34′27.7″	99°12′39.8″	715	Rock hole with clear water, leaves at bottom and total shade	7.63	24	162	*Ae. epactius*, *Ae. euplocamus*
Cx. declarator	2 April 2012	21°33′45.8″	99°42′27.9″	—-	Artificial container with clear water and partial shade	8.2	31.3	328	*Ae. epactius*
Cx. erythrothorax	2 April 2012	21°26′45.7″	99°38′1.7″	575	Human biting/landing at night	—-	—-	—-	——
	3 April 2012	21°26′44.6″	99°38′6.0″	548	Resting intra-domiciliary	—-	—-	—-	*Ae. aegypti*, *Cx. quinquefasciatus*
	8 July 2012	20°32′13.4″	99°52′59.8″	1898	Human biting/landing at night	—-	—-	—-	*Cx. tarsalis*
	8 July 2012	20°32′13.4″	99°52′59.8″	1898	Resting in vegetation at night	—-	—-	—-	*Cx. quinquefasciatus*, *Cx. stigmatosoma*
	14 November 2012	20°8′19.1″	99°57′8.3″	2403	Resting on caves at day, total shade	—-	—-	—-	——
	15 November 2012	20°2′31.7″	100°4′27.4″	2393	Irrigation gutter with clear water, aquatic vegetation and partial shade	7.02	15.9	170	*Cx. stigmatosoma*, *Cx. tarsalis*, *Cs. inornata*

Table 3. *Cont.*

Taxa	Collection Date	Collection Site			Habitat	Aquatic Parameters			Associated Species
		Latitude N	Longitude W	Elev.		pH	Temp	Salts	
Cx. restuans	3 April 2012	21°26′45.7″	99°37′58.1″	567	Pond with clear water, aquatic vegetation and partial shade	7.8	26.3	510	*An. punctipennis*, *Cx. coronator*
	14 November 2012	20°8′53.3″	99°58′38.5″	2329	Stream margin with clear water and total shade	6.39	17.4	223	*Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cx. arizonensis*, *Cs. particeps*
	20 November 2012	21°33′50.2″	99°13′11.4″	—-	Rock hole with clear water, leaves at bottom and total shade	7.22	17.2	101	——
Cx. tarsalis	3 April 2012	21°18′50.0″	99°30′21.9″	549	Pond with clear water, with floating vegetation and total shade	8.20	30.2	171	*An. punctipennis*, *Cx. stigmatosoma*
	3 April 2012	21°18′50.0″	99°30′21.9″	549	Pond with clear water, with floating vegetation and total shade	8.4	31.7	175	*An. punctipennis*, *Cx. tarsalis*, *Cx. peccator*
	3 April 2012	21°12′35.3″	99°31′59.5″	1095	Irrigation gutter with colored water and total shade	6.8	25.3	482	*Cx. coronator*
	4 April 2012	21°09′32.4″	99°34′37.9″	1785	Spring with clear water and total shade	8.3	13	225	*Cx. stigmatosoma*, *Cx. arizonensis*, *Lt. bigoti*, *Cs. particeps*
	6 July 2012	20°22′34.3″	100°16′28.8″	—-	Rock hole with clear water and partial shade	9.1	20.5	156	*Ae. trivittatus*, *Cs. particeps*
	7 July 2012	20°25′08.9″	100°17′40.7″	2151	Pond with clear water, emerging vegetation and absent shade	7.2	19.5	111	——
	7 July 2012	20°22′22.8″	100°17′0.85″	—-	Artificial container with clear water and partial shade	8.3	19.7	223	*Cx. stigmatosoma*
	7 July 2012	20°22′27.3″	100°16′30.1″	2305	Pond with clear water, emerging vegetation and partial shade	7.4	20.3	90	*Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cx. thriambus*
	7 July 2012	20°11′15.5″	100°8′57″	2639	Flower vase with clear water and absent shade	8.4	23.2	298	——
	7 July 2012	20°23′2.7″	100°0′35.8″	1915	Resting in vegetation at day with partial shade	—-	—-	—-	*Ae. trivittatus*, *Cx. quinquefasciatus*, *Cx. stigmatosoma*
	8 July 2012	20°46′34.7″	100°03′10.4″	1941	Resting in vegetation at day with total shade	—-	—-	—-	*Cx. stigmatosoma*
	8 July 2012	20°54′13.9″	99°55′53.9″	1756	Flower vase with colored water, leaves at bottom and partial shade	7.7	26.4	631	*Cx. stigmatosoma*
	8 July 2012	20°39′46.2″	99°53′29.1″	1996	Pond with clear water, emerging vegetation and absent shade	9.4	25.1	212	*Cx. stigmatosoma*
	8 July 2012	20°32′13.4″	99°52′59.8″	1898	Human biting/landing at night	—-	—-	—-	*Cx. erythrothorax*
	14 November 2012	20°8′53.3″	99°58′38.5″	2329	Rock hole with clear water, leaves at bottom, partial shade	6.59	22.7	279	*Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cs. particeps*
	14 November 2012	20°9′10.7″	100°1′9.6″	2463	Pond with dark water, aquatic vegetation and total shade	7.20	15.3	777	*An. franciscanus*
	15 November 2012	20°2′31.7″	100°4′27.4″	2393	Irrigation gutter with clear water, aquatic vegetation and partial shade	7.02	15.9	170	*Cx. stigmatosoma*
	16 November 2012	20°46′12.6″	99°55′4″	1932	Irrigation gutter with clear water, aquatic vegetation and partial shade	7.11	23.5	29	*Cx. apicalis*

Table 3. *Cont.*

Taxa	Collection Date	Collection Site			Habitat	Aquatic Parameters			Associated Species
		Latitude N	Longitude W	Elev.		pH	Temp	Salts	
Cx. thriambus	7 July 2012	20°9′10.7″	100°1′9.6″	2463	Pond with clear water, emerging vegetation and partial shade	7.4	20.3	90	*Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cx. tarsalis*
	12 September 2012	21°13′7″	99°5′42″	368	Resting on caves with total shade	—-	—-	—-	*Ae. albopictus*
	12 September 2012	21°13′11.2″	99°5′56.9″	140	Discarded tire with clear water, total shade	7.55	22.7	42	*Cx. restrictor*, *Tx. moctezuma*
	29 September 2012	21°8′6.1″	99°37′27.6″	1790	Flower vase with clear water, leaves at bottom, green algae and total shade	7.94	19.9	484	—-
	29 September 2012	21°8′6.1″	99°37′27.6″	1790	Discarded tire with clear water, leaves at bottom and total shade	8.26	19.4	389	*Cx. stigmatosoma*
	16 November 2012	20°43′59.3″	99°56′16.7″	2080	Artificial container with colored water, leaves at bottom and absent shade	7.21	15.2	555	*Cx. quinquefasciatus*
	16 November 2012	20°53′23.7″	99°34′55.1″	2399	Discarded tire with clear water and partial shade	7.29	22.5	177	—-
	16 November 2012	20°56′4.4″	99°33′35.3″	2416	Discarded tire with colored water, leaves at bottom and total shade	7.24	12	780	—-
	16 November 2012	20°56′4.4″	99°33′35.3″	2416	Cattle drinker with clear water, leaves at bottom and partial shade	7.24	13.4	158	*Ae. trivittatus*, *Cs. particeps*
	17 November 2012	20°59′45.9″	99°34′40.3″	1884	Spring with clear water, aquatic vegetation, brown algae and total shade	7.10	15	289	*An. pseudopunctipennis*, *Cx. stigmatosoma*
	17 November 2012	20°59′45.9″	99°34′40.3″	1684	Artificial container with clear water, leaves at bottom and total shade	7.10	16	328	*Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cs. particeps*
	24 September 2018	21°8′38″	99°37′15.1″	2292	Discarded tire with clear water, leaves at bottom and total shade	7.15	20	190	*Cs.* n. sp., *Tx. moctezuma*
	22 September 2021	21°15′29.3″	99°4′11.1″	1010	Tree hole with colored water, leaves at bottom with total shade	7.50	20	1095	*Cx. lactator*
Cx. erraticus	3 April 2012	21°18′50.0″	99°30′21.9″	549	Pond with clear water with floating vegetation and total shade	8.4	31.7	175	*An. punctipennis*, *Cx. tarsalis*, *Cx. peccator*
Cx. peccator	3 April 2012	21°18′50.0″	99°30′21.9″	549	Pond with clear water with floating vegetation and total shade	8.4	31.7	175	*An. punctipennis*, *Cx. tarsalis*, *Cx. erraticus*
	3 April 2012	21°18′50.0″	99°30′21.9″	549	Resting in vegetation at day with total shade	—-	—-	—-	—-

Table 3. *Cont.*

Taxa	Collection Date	Collection Site			Habitat	Aquatic Parameters			Associated Species
		Latitude N	Longitude W	Elev.		pH	Temp	Salts	
Cx. apicalis	14 November 2012	20°8′53.3″	99°58′38.5″	2329	Stream margin with clear water, green algae and total shade	6.40	14.5	66	*An. franciscanus*, *Ae. epactius*
	15 November 2012	20°16′59.9″	100°10′5.7″	2296	Stream margin with clear water, emerging vegetation, green algae and partial shade	7.49	22	65	*An. punctipennis*
	15 November 2012	20°20′22.3″	100°14′53.7″	—-	Pond with colored water, aquatic vegetation and absent shade	7.07	25	38	——
	15 November 2012	20°20′19.9″	100°19′50.4″	2418	Rock hole with dark water, emerging vegetation and partial shade	7.11	23.5	29	Cx. arizonensis
	16 November 2012	20°34′43″	100°19′21.7″	2037	Discarded tire with colored water, leaves at bottom and total shade	7.43	17.11	682	Ae. epactius
	16 November 2012	20°53′23.7″	99°34′55.1″	2399	Discarded tire with clear water and partial shade	7.28	20.4	110	——
Cx. arizonensis	4 April 2012	21°09′33.7″	99°34′35.9″	1813	Waterhole with clear water and total shade	7.56	16.8	269	——
	4 April 2012	21°09′32.4″	99°34′37.9″	1785	Spring with clear water and total shade	8.3	13	225	*Cx. stigmatosoma*, *Cx. tarsalis*, *Lt. bigoti*, *Cs. particeps*
	29 September 2012	21°8′6.1″	99°37′27.6″	1790	Flower vase with clear water, leaves at bottom, green algae and total shade	9.76	19.6	277	*Ae. albopictus*, *Cx. stigmatosoma*, *Cs. particeps*
	14 November 2012	20°8′1.1″	99°56′52.6″	2369	Resting on caves at day, total shade	—-	—-	—-	*Cx. stigmatosoma*, *Cs. particeps*
	14 November 2012	20°8′53.3″	99°58′38.5″	2329	Stream margin with clear water and total shade	6.39	17.4	223	*Cx. quinquefasciatus*, *Cx. restuans*, *Cx. stigmatosoma*, *Cs. particeps*
	15 November 2012	20°20′19.9″	100°19′50.4″	2418	Rock hole with dark water, emerging vegetation and partial shade	7.11	23.5	29	*Cx. apicalis*
Cx. lactator	22 September 2021	21°15′29.3″	99°4′11.1″	1010	Human biting/landing at day	—-	—-	—-	*Ae. allotecnon*, *Sa. chloropterus*, *Sh. huasteca*, *Wy. aporonoma*, *Wy. mitchellii*
	22 September 2021	21°15′29.3″	99°4′11.1″	1010	Tree hole with colored water, leaves at bottom with total shade	7.50	20	1095	*Cx. thriambus*
Lt. bigoti	4 April 2012	21°09′32.4″	99°34′37.9″	1785	Spring with clear water and total shade	8.3	13	225	*Cx. stigmatosoma*, *Cx. tarsalis*, *Cx. arizonensis*, *Cs. particeps*
	28 September 2017	21°16′46.9″	99°8′29.2″	1828	Artificial container with clear water, leaves at bottom and total shade	6	24.6	23	*Ae. albopictus*
Cs. inornata	15 November 2012	20°2′31.7″	100°4′27.4″	2393	Irrigation gutter with clear water, aquatic vegetation and partial shade	7.02	15.9	170	*Cx. erythrothorax*, *Cx. stigmatosoma*, *Cx. tarsalis*

Table 3. *Cont.*

Taxa	Collection Date	Collection Site			Habitat	Aquatic Parameters			Associated Species
		Latitude N	Longitude W	Elev.		pH	Temp	Salts	
Cs. particeps	4 April 2012	21°09′32.4″	99°34′37.9″	1785	Spring with clear water and total shade	8.3	13	225	*Cx. stigmatosoma*, *Cx. tarsalis*, *Cx. arizonensis*, *Lt. bigoti*
	4 April 2012	21°09′32.4″	99°34′37.9″	1811	Resting in vegetation at day with total shade	—-	—-	—-	——
	7 July 2012	20°11′15.5″	100°8′57″	2639	Flower vase with clear water, leaves at bottom and partial shade	9.9	21.9	197	*Cx. stigmatosoma*
	29 September 2012	21°8′6.1″	99°37′27.6″	1790	Flower vase with clear water, leaves at bottom, green algae and total shade	9.76	19.6	277	*Ae. albopictus*, *Cx. stigmatosoma*, *Cx. arizonensis*
	14 November 2012	20°8′1.1″	99°56′52.6″	2369	Resting on caves at day, total shade	—-	—-	—-	*Cx. stigmatosoma*, *Cx. arizonensis*
	14 November 2012	20°8′53.3″	99°58′38.5″	2329	Stream margin with clear water and total shade	6.39	17.4	223	*Cx. quinquefasciatus*, *Cx. restuans*, *Cx. stigmatosoma*, *Cx. arizonensis*
	16 November 2012	20°56′4.4″	99°33′35.3″	2416	Cattle drinker with clear water, leaves at bottom and partial shade	7.24	13.4	158	*Ae. trivittatus*, *Cx. thriambus*
	17 November 2012	20°59′45.9″	99°34′40.3″	1684	Artificial container with clear water, leaves at bottom and total shade	7.10	16	328	*Cx. quinquefasciatus*, *Cx. stigmatosoma*, *Cx. thriambus*
Cs. n. sp.	24 September 2018	21°8′38″	99°37′15.1″	2292	Discarded tire with clear water, leaves at bottom and total shade	7.15	20	190	*Cx. thriambus*, *Tx. moctezuma*
Li. durhamii	28 September 2017	21°16′46.9″	99°8′29.2″	1824	Artificial container with clear water, leaves at bottom and total shade	6.2	25	53	——
Sa. chloropterus	27 September 2012	21°16′14.4″	99°3′20.1″	897	Human biting/landing at day with total shade	—-	—-	—-	*Ae. allotecnon*, *Ae. quadrivittatus*, *Ae. podographicus*, *Sh. huasteca*, *Wy. mitchellii*
	20 November 2012	21°34′53.6″	99°14′6.8″	686	Human biting/landing at day with total shade	—-	—-	—-	*Ae. quadrivittatus*, *Ae. trivittatus*, *Ae. amabilis*, *Ae. brelandi*, *Wy. mitchellii*
	28 September 2017	21°16′46.9″	99°8′29.2″	1828	Human biting landing at day with total shade	—-	—-	—-	*Ae. allotecnon*, *Ae. podographicus*, *Sh. huasteca*, *Wy. adelpha/guatemala*
Sh. huasteca	27 September 2012	21°16′14.4″	99°3′20.1″	897	Human biting/landing at day with total shade	—-	—-	—-	*Ae. allotecnon*, *Ae. quadrivittatus*, *Ae. podographicus*, *Sa. chloropterus*, *Wy. mitchellii*
	28 September 2017	21°16′46.9″	99°8′29.2″	1828	Human biting/landing at day with total shade	—-	—-	—-	*Ae. allotecnon*, *Ae. podographicus*, *Sa. chloropterus*, *Wy. adelpha/guatemala*
	24 September 2018	21°15′29.3″	99°411.1″	1010	Human biting/landing at day with total shade	—-	—-	—-	*Ae. allotecnon*, *Ae. quadrivittatus*, *Wy. mitchellii*
	22 September 2021	21°15′29.3″	99°4′11.1″	1010	Human biting/landing at day	—-	—-	—-	*Ae. allotecnon*, *Cx. lactator*, *Sa. chloropterus*, *Wy. aporonoma*, *Wy. mitchellii*

Table 3. *Cont.*

Taxa	Collection Date	Collection Site			Habitat	Aquatic Parameters			Associated Species
		Latitude N	Longitude W	Elev.		pH	Temp	Salts	
Wy. aporonoma	22 September 2021	21°15′29.3″	99°4′11.1″	1010	Human biting/landing at day	—-	—-	—-	*Ae. allotecnon*, *Cx. lactator*, *Sa. chloropterus*, *Sh. huasteca*, *Wy. mitchellii*
Wy. adelpha/guatemala	28 September 2017	21°16′46.9″	99°8′29.2″	1828	Human biting/landing at day with total shade	—-	—-	—-	*Ae. allotecnon*, *Ae. podographicus*, *Sa. chloropterus*, *Sh. huasteca*,
Wy. mitchellii	27 September 2012	21°13′7″	99°5′42″	368	Bromeliad axil with colored water and abundant leaf at bottom, total shade	6.60	26.4	22	*Ae. quadrivittatus*, *Ae. aegypti*
	27 September 2012	21°13′7″	99°5′42″	368	Bromeliad axil with colored water and abundant leaf at bottom	6.99	24.7	12	*Ae. allotecnon*, *Ae. quadrivittatus*
	27 September 2012	21°16′14.4″	99°3′20.1″	897	Human biting/landing at day with total shade	—-	—-	—-	*Ae. allotecnon*, *Ae. quadrivittatus*, *Ae. podographicus*, *Sa. chloropterus*, *Sh. huasteca*
	18 November 2012	21°33′27.4″	99°9′20.4″	1024	Human biting/landing at day with total shade	—-	—-	—-	*Ae. allotecnon*
	19 November 2012	21°34′39″	99°13′41.9″	806	Human biting/landing at day with total shade	—-	—-	—-	*Ae. trivittatus*, *Ae. podographicus*
	20 November 2012	21°35′7.1″	99°14′15.7″	739	Human biting/landing at day with partial shade	—-	—-	—-	*Ae. triseriatus*
	20 November 2012	21°34′53.6″	99°14′6.8″	686	Human biting/landing at day with total shade	—-	—-	—-	*Ae. quadrivittatus*, *Ae. trivittatus*, *Ae. amabilis*, *Ae. brelandi*, *Sa. chloropterus*
	20 November 2012	21°35′7.1″	99°14′15.7″	739	Bromeliad axil with clear water, leaves at bottom and partial shade	7.61	20	44	——
	20 November 2012	21°34′27.7″	99°12′39.8″	715	Human biting/landing at day with total shade	—-	—-	—-	——
	24 September 2018	21°15′29.3″	99°411.1″	1010	Human biting/landing at day with total shade	—-	—-	—-	*Ae. allotecnon*, *Ae. quadrivittatus*, *Sh. huasteca*
	22 September 2021	21°15′29.3″	99°4′11.1″	1010	Human biting/landing at day	—-	—-	—-	*Ae. allotecnon*, *Cx. lactator*, *Sa. chloropterus*, *Sh. huasteca*, *Wy. aporonoma*
Tx. moctezuma	27 September 2012	21°13′11.2″	99°5′56.9″	140	Discarded tire with clear water, total shade	7.55	22.7	42	*Cx. restrictor*, *Cx. thriambus*
	27 September 2012	21°13′11.2″	99°5′56.9″	140	Discarded tire with clear water, total shade	7.48	22.3	77	——
	24 September 2018	21°8′38″	99°37′15.1″	2292	Discarded tire with clear water, leaves at bottom and total shade	7.15	20	190	*Cx. thriambus*, *Cs.* n. sp.
	24 September 2018	21°15′29.3″	99°4′11.1″	1010	Tree hole with colored water, leaves at bottom and total shade	7.39	23.3	270	*Ae.* (*Protomacleaya*) sp.

3.2.4. Genus *Culex*

The genus *Culex* is the most diverse group of mosquitoes in Querétaro, including fifteen species in the state, of which two had previously been reported. The subgenera *Anoedioporpa*, *Melanoconion*, *Neoculex*, and *Phenacomyia* are recorded for the first time in Querétaro. Immature stages of *Cx. restrictor* were collected from two locations, in a tree hole and a discarded tire; this type of larval habitat is common for this species in Mexico. The new records within the subgenus *Culex* are *Cx. coronator*, *Cx. declarator*, *Cx. erythrothorax*, *Cx. restuans*, *Cx. salinarius*, *Cx. tarsalis*, and *Cx. thriambus*. The Coronator complex includes five recognized species: *Cx. camposi* Dyar, *Cx. coronator*, *Cx. ousqua* Dyar, *Cx. ousquatissimus* Dyar, and *Cx. usquatus* Dyar [47]. Based on male genitalia morphology, *Cx. coronator s.s.* was identified from material collected in Querétaro; this is a common species whose immature stages were collected from a variety of aquatic habitats, mostly natural habitats at ground level. Although *Cx. declarator* is a common species in the central-northern region of Mexico, immature stages of this species were collected from an artificial container in only a single location. *Culex erythrothorax*, *Cx. restuans*, *Cx. tarsalis*, and *Cx. thriambus* are common species that were found frequently and collected from a variety of aquatic habitats such as natural and artificial containers. The record of *Cx. salinarius* was obtained from the CAIM collection. The subgenus *Melanoconion* in Querétaro includes *Cx. erraticus* and *Cx. peccator*; immature stages of both species were collected from natural habitats at ground level in two locations. Within the subgenus *Neoculex*, the species found in Querétaro are *Cx. apicalis* and *Cx. arizonensis*. The subgenus *Phenacomyia* and *Cx. lactator* are recorded for the first time in Querétaro. Adult females of this species were collected approaching humans during the day at a single location.

3.2.5. Genus *Lutzia*

The genus *Lutzia*, subgenus *Lutzia*, and *Lt. bigoti* are recorded for the first time in Querétaro. Immature stages of this species were collected from one spring and an artificial container with clear water, predating larvae of *Ae. albopictus* and *Cx.* spp., respectively.

3.2.6. Genus *Culiseta*

The tribe Culisetini, genus *Culiseta*, and subgenera *Culiseta*, *Culiseta inornata,* and *Cs. particeps* are recorded for the first time in Querétaro. Immature stages of *Cs. inornata* were collected from an irrigation gutter in one location, while immature stages of *Cs. particeps* were collected from a variety of aquatic habitats, such as natural ponds and swamps, artificial containers, and discarded tires. Immature stages of one undescribed species within this genus were discovered in discarded tires. These specimens are in a poor condition; hence, this species will be formally described when more specimens are obtained.

3.2.7. Genus *Limatus*

The tribe Sabethini, genera *Limatus*, *Sabethes*, *Shannoniana*, and *Wyeomyia*; subgenera *Sabethoides*, *Triamyia*, and *Wyeomyia*; species *Li. durhammi*, *Sa. chloropterus*, *Sh. huasteca*, *Wy. apronoma*, *Wy. adelpha/guatemala*, and Wy. mitchellii are recorded for the first time in Querétaro. Immature stages of *Li. durhamii* were collected from an artificial container with clear water in one location with no associated species.

3.2.8. Genus *Sabethes*

In Mexico, the genus *Sabethes* is divided into two subgenera: *Sabethes* and *Sabethoides*. The latter is reported for the first time in Querétaro and is represented by *Sa. chloropterus*. Adult females of this species were collected approaching humans during the day.

3.2.9. Genus *Shannoniana*

In Mexico, three species of the genus *Shannoniana* had been previously recorded: *Sh. fluviatilis* (Theobald), *Sh. moralesi* (Dyar and Knab), and *Sh. schedocyclia* (Dyar and Knab).

In the present study, we discovered a fourth species within the genus. Adult females of *Sh. huasteca* n. sp. were collected approaching humans and males were collected resting in vegetation and approaching humans together with the females. Adults of both sexes are described herein.

3.2.10. Genus *Wyeomyia*

Three species of the genus *Wyeomyia* are reported for the first time in Querétaro: Adult females of *Wy. aporonoma* were collected approaching humans in one location in association with *Ae. allotecnon*, *Cx. lactator*, *Sa. chloropterus*, *Sh. huasteca*, and *Wy. mitchellii*. Since *Wy. guatemala* is possibly a synonymy of *Wy. adelpha* [48], and both species are treated here as a single taxon. Adult females of *Wy. adelpha*/*guatemala* were collected approaching humans during the day in one location, while immature stages of *Wy. mitchelli* were collected from bromeliad axils and adult females were collected approaching humans in several locations of Querétaro.

3.2.11. Genus *Toxorhynchites*

The tribe Toxorhynchitini, genus *Toxothynchites*, and subgenera *Lynchiella* and *Tx. moctezuma* are recorded for the first time in Querétaro. Immature stages of *Tx. moctezuma* were collected from discarded tires and one tree hole, always with clear water and predating on larvae of *Ae*. sp. and *Cx. thriambus* in tropical and conserved regions of the state.

3.3. Molecular Analysis

In total, we analyzed 25 DNA barcodes for five species within the genus *Shannoniana* (four taxa) and *Trichoprosopon* (one taxa) (Table 4). In general, all specimens of the same species clustered together (Figure 3), although there was a deep split in *Sh. fluviatilis* (BOLD:ACZ4319, BOLD:ACZ4320) and *Sh. schedocyclia* (BOLD:ACZ3895, BOLD:ACZ:3896), where two BINs were found in each taxon. All specimens identified as *Sh. huasteca* n. sp. were grouped closely with *Sh. moralesi*, although both groups are well separated with high support bootstrap values (Figure 3). The average genetic divergence was 0.08%; the intra-specific genetic divergence varied from 0.04% in *Sh. huasteca* n. sp., *Sh. moralesi* (0.55%), *Sh. schedocyclia* (1.31%), and *Tr. digitatum* (0.20%). In *Sh. fluviatilis,* the genetic divergence was above 2% (3.96%). Interspecific genetic divergence varied from 4.70% to 13.13%; the pair *Sh. moralesi*/*Sh. fluviatilis* were the more divergent species (13.13%), while the pair *Sh. huasteca* n. sp./*Sh. moralesi* were less divergent (4.70%).

Table 4. Percentage of interspecific (between groups) pairwise K2P genetic divergence of unique DNA barcodes (658 bp), representing five species of Sabethini.

	Sh. fluviatilis	***Sh. shcedocyclia***	***Sh. moralesi***	***Tr. digitatum***
Sh. fluviatilis	—	—	—	—
Sh. schedocyclia	7.43%	—	—	—
Sh. moralesi	13.13%	12.78%	—	—
Tr. digitatum	11.34%	9.93%	12.67%	—
Sh. huasteca n. sp.	12.29%	12.60%	4.70%	12.72%

3.4. Description of New Species

Shannoniana huasteca Ortega n. sp. 4DD7EB32-56DD-41B6-AACC-877947FE26D4. Type specimens: *Holotype*: adult female (A♀) without associated larval and pupal exuviae [CC-UL, 04240918-CN], Camino a Neblinas, Landa de Matamoros, Querétaro, Mexico (21°15′29.3″ N–99°4′11.12″ W) (Figure 4), elevation 1010 m, 24 Sep 2018, 17:00–18:00, human biting at day, tropical cloud forest with oaks and conserved vegetation (Figure 5), col. A.I. Ortega-Morales. *Paratypes*: 10A♀, (same data as *holotype*); [CAIM]. *Allotypes*: 3♂with dissected genitalia, (same data as holotype); [CC-UL] (Table 3).

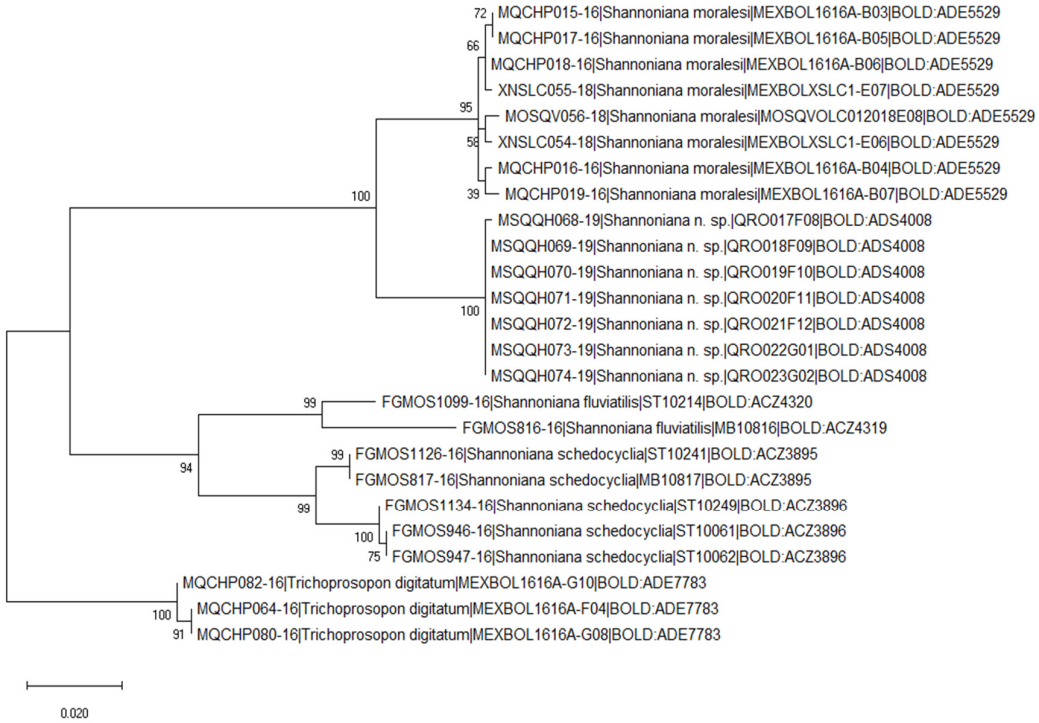

Figure 3. Maximum likelihood tree base on the Kimura 2-parameter of the *COI* DNA barcodes (658 bp) for species of *Shannoniana* (n = 4) and *Trichoprosopon* (n = 1). A divergence of >2% may be indicative of separate operational taxonomic units.

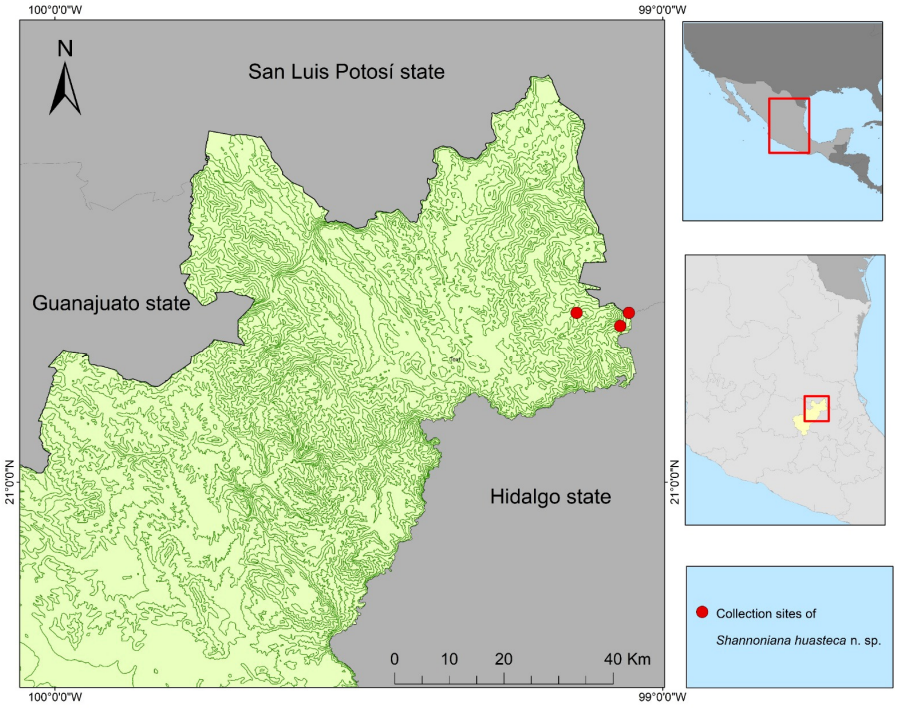

Figure 4. Distribution of *Sh. huasteca*.

Figure 5. Collection site of *Shannoniana huasteca* n. sp., showing the tropical and conserved types of vegetation. The site is near Neblinas road, Landa de Matamoros, Querétaro, Mexico.

Female. Head: Occiput and vertex covered with flat decumbent blue, green and silver scales, with purple and greenish reflections, with a row of erect scales (Figure 6A), interocular setae large, pedicel bare, yellow-brown. Antennae approximately 0.50–0.75 forefemur length. Clypeus bare, dark-brown. Maxillary palpus approximately 0.25 proboscis length, three-segmented, third palpomere longer than the first two, dark-scaled with purplish reflections. Proboscis as long as forefemur, sometimes slightly longer forefemur length of 1.10–1.20, with dark scales with purplish reflections. Thorax: Integument of scutum golden, covered with pale golden-brown decumbent narrow scales without iridescent reflections, acrostichal and dorsocentral setae absent (Figure 6B). Scutellum trilobed, with 7–10 setae on lateral lobes and 5–7 setae on mid lobe, all lobes covered with flat dark-blue scales with purplish reflections. Row of erect dark setae above the paratergite and the wing. Postpronotum covered with flat yellow-golden scales with golden reflections, without setae. Antepronotum lobe with silvery scales, with 4–5 setae. Integument of mesokatepisterum and mesanepimeron dark-golden, mostly covered with large patch of silvery flat scales (Figure 7A), mesanepimeron with 10–12 dark-brown setae. Wing: Approximately 1.30–1.50 mm, scales on veins flat and light-brown (Figure 7B). Halter: Dark-brown with blue scales. All trochanters with patches of silvery scales. All femora dark-scaled, with some iridescent scales bluish-greenish on dorsal line, with a small knee spot of pale scales, fore and midfemora predominantly dark-scaled, with bluish reflections, hindfemur with dorsal dark scaled line and ventral line withe-scaled. Hindtibia dark-scaled, with a complete ring of white-yellow scales apically. Foretarsus covered predominantly with dark scales, mid and hindtarsus with tarsomeres 1–4 dark-scaled, tarsomere 5 with dark scales on dorsal line and white scales on ventral line. Abdomen: All terga covered with dark scales with bluish reflections, apical corners of dark scales on terga extending into 0.50 of sternal segments, sterna covered with white scales.

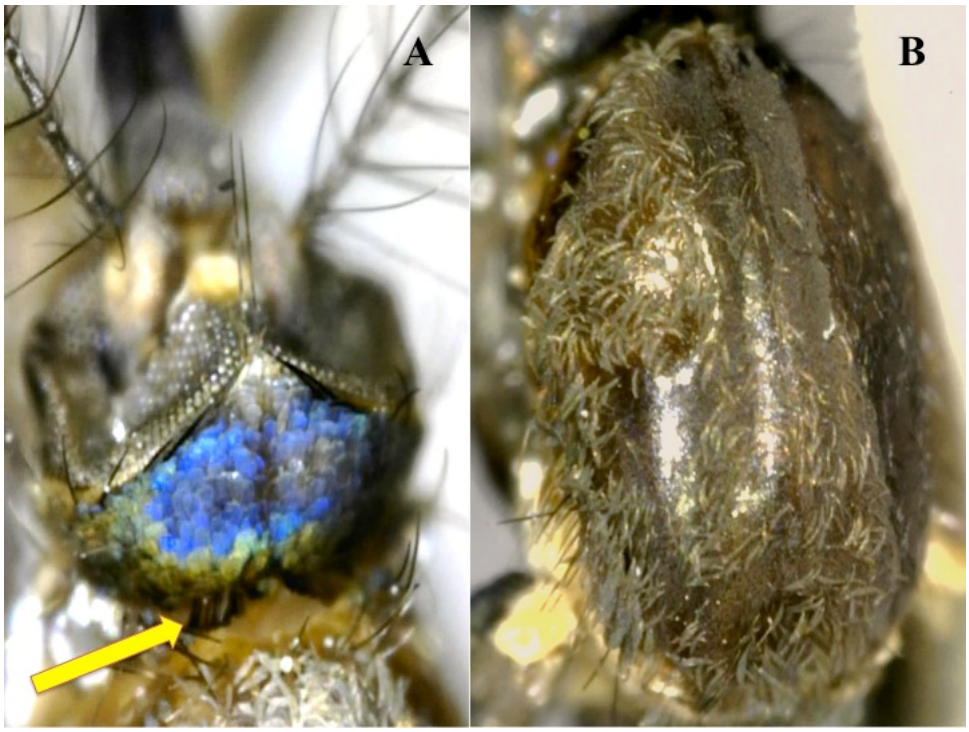

Figure 6. Adult female of *Shannoniana huasteca*, holotype. (**A**) Occiput showing the decumbent green, blue, and silvery scales, arrow showing the row of erect scales; (**B**) scutum covered with pale golden-brown narrow scales.

Figure 7. Adult of *Shannoniana huasteca* n. sp. (**A**) General aspect of lateral view of thorax showing the integument golden, mesokaterpisternum, and mesanepimeron dark-golden (holotype), arrow shows the posprocoxal area; (**B**) wing covered with light-brown scales and dark scales on abdominal terga (holotype); (**C**) general aspect of male genitalia (allotype).

Male. In general, as in females except for sexual characteristics and plumose antennae.

Male genitalia (Figure 7C). Segment IX: Tergum with deep emargination between tergal lobes, the lobes slightly longer than broad, with 6–8 long, curved setae. Gonocoxite: Length approximately three times median width, basal tergomesal lobe well developed, with 5–7 long and strong setae. Gonostylus: Narrow, simple, and long, slightly curved apically. Aedeagus strongly sclerotized, simple and ovate.

Larva. Unknown.

Pupa. Unknown.

Systematics. Females of *Sh. huasteca* n. sp. are distinguished from all three previously described species within the genera *Shannoniana* (*Sh. fluviatilis* (Theobald), *Sh. moralesi* (Dyar and Knab), and *Sh. schedocyclia* (Dyar and Knab)) by having all legs covered with dark scales, except for tarsomere five of hind leg, which has white scales in a ventral line; silvery, decumbent scales on vertex not extending to the ocular line, but interrupted by a patch of dark scales with bluish and greenish reflections; and the absence of a patch of silvery scales on postprocoxal area. The males are readily distinguished by the structure of the male genitalia (Figure 7C), especially the narrow and long gonostyle, slightly curved apically.

Bionomics. Although the type locality was visited on numerous occasions to search for immature stages of *Sh. huasteca* n. sp., these were not found. Immature stages of other species were collected in different aquatic habitats such as containers and phytotelmata (e.g., axils of bracts of *Xanthosoma* spp. and bromeliads). In addition, ovitraps were displayed at different elevations from ground level, but all those collections failed to find immature stages of *Sh. huasteca* n. sp. Adult females were collected approaching humans probing to bite during the day in association with *Aedes allotecnon*, *Ae. quadrivittatus*, *Culex lactator*, *Sabethes chloropterus*, *Wyeomyia aporonoma*, and *Wy. mitchellii*. The medical importance of *Sh. huasteca* n. sp. is unknown, but since females can be persistent biters of humans, the species could be involved in the transmission of pathogens.

Distribution. *Shannoniana huasteca* n. sp. has been collected in the northern region of the state of Querétaro (Neblinas road, location of Landa de Matamoros County). Locations in which the species was collected belong to Huasteco Carso of the Sierra Madre Oriental. *Shannoniana huasteca* n. sp. may occur in the forested regions of the states adjacent to Querétaro such as the southeastern San Luis Potosí state and northwestern Hidalgo state, with both states sharing physiographical conditions belonging to the Carso Huasteco of the Sierra Madre Oriental.

Etymology. This species is named *huasteca* because of the type locality in the Carso Huasteco sub-region. "Huasteco" is a word derived from the huasteco language, which means someone from an Amerindian tribe of the Mayan family that lives in the Mexican states of Tamaulipas, San Luis Potosí, Querétaro, and Veracruz.

3.5. Keys to Species of Adult Female of Shannoniana

ADULT FEMALE (Figure 8) (Modified from Lane and Cerqueira [49] and Clark-Gil and Darsie [48]).

1. Hind tarsi with basal rings of white scales on segments I–IV (Figure 9A) . . . . . *Sh. schedocyclia* Distr.: Bolivia, Brazil, French Guiana, Guatemala, Mexico, Nicaragua, Panama, Venezuela [50] (Distr. Mex.: Chiapas [27,28,51–53], Oaxaca [27,28], Veracruz [10,28,52]).

- Hind tarsi with segments I–IV covered only with dark scales (Figure 9B). 2

2(1) Tarsomere V of hind leg covered completely with dark scales (Figure 9C) . . . . . .Sh. *fluviatilis*

Distr.: Argentina, Bolivia, Brazil, Colombia, Ecuador, French Guiana, Guatemala, Guyana, Mexico, Nicaragua, Panama [50] (Distr. Mex.: Chiapas [28,52,54], Veracruz [10,28,52,55], Oaxaca [28], Quintana Roo [2,7]).

- Tarsomere V of hind leg with white scales (Figure 9D) 3

3(2) Silver scales on occiput extending to the ocular line and reaching the inner corner of the eye, mostly with silver reflections (Figure 10); postprocoxal area with a patch of silvery scales . . . . . *Sh. moralesi*

Distr.: Belize, Guatemala, Mexico, Panama [50] (Distr. Mex.: Chiapas [27,28,51–53,55,56], Veracruz [10,12,27,28,52], Oaxaca [27,28], Tabasco [20]).

Silver scales on occiput are restricted to the occiput and do not extend to the eyes, the rest of scales on ocular line are dark, with purplish and greenish reflections (Figure 6A); postprocoxal area without a patch of silvery scales (Figure 7A). *Sh. huasteca* n. sp. Ortega

Distr.: Mexico (Distr. Mex.: Querétaro).

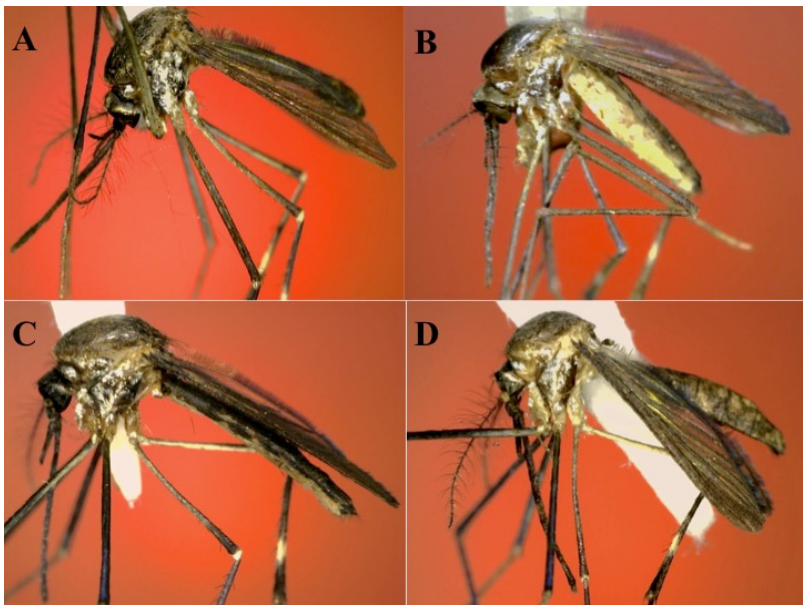

Figure 8. General aspect of adult female of *Shannoniana* spp. (**A**) *Sh. schedocyclia* (Chiapas, Mexico); (**B**) *Sh. fluviatilis* (Quintana Roo, Mexico, MX-QROO-19); (**C**) *Sh. moralesi* (Chiapas, Mexico, 01010818-EU); (**D**) *Sh. huasteca* n. sp. (holotype).

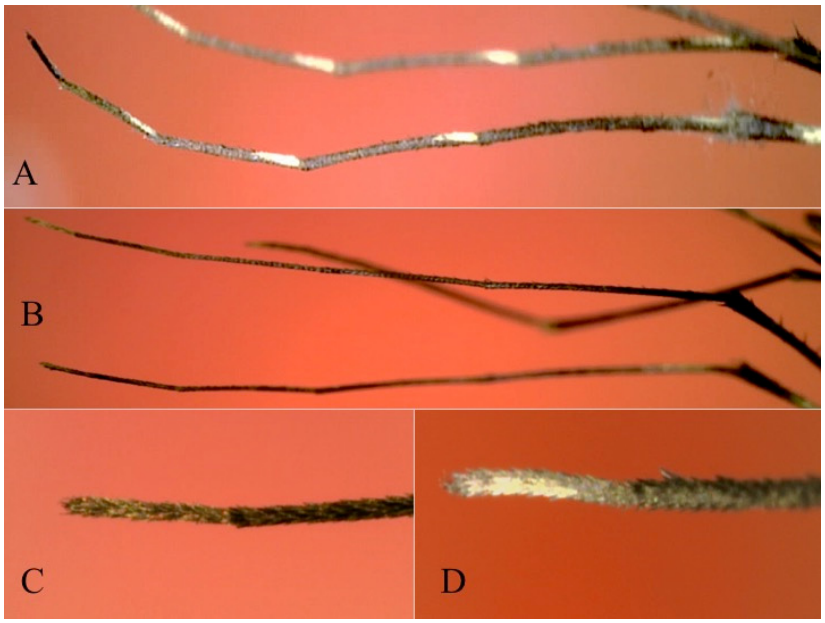

Figure 9. Hind tarsi of adult female of *Shannoniana* spp. (**A**) *Sh. schedocyclia* (Chiapas, Mexico); (**B**,**C**) *Sh. fluviatilis* (Quintana Roo, Mexico, MX-QROO-19); (**D**) *Sh. huasteca* n. sp.

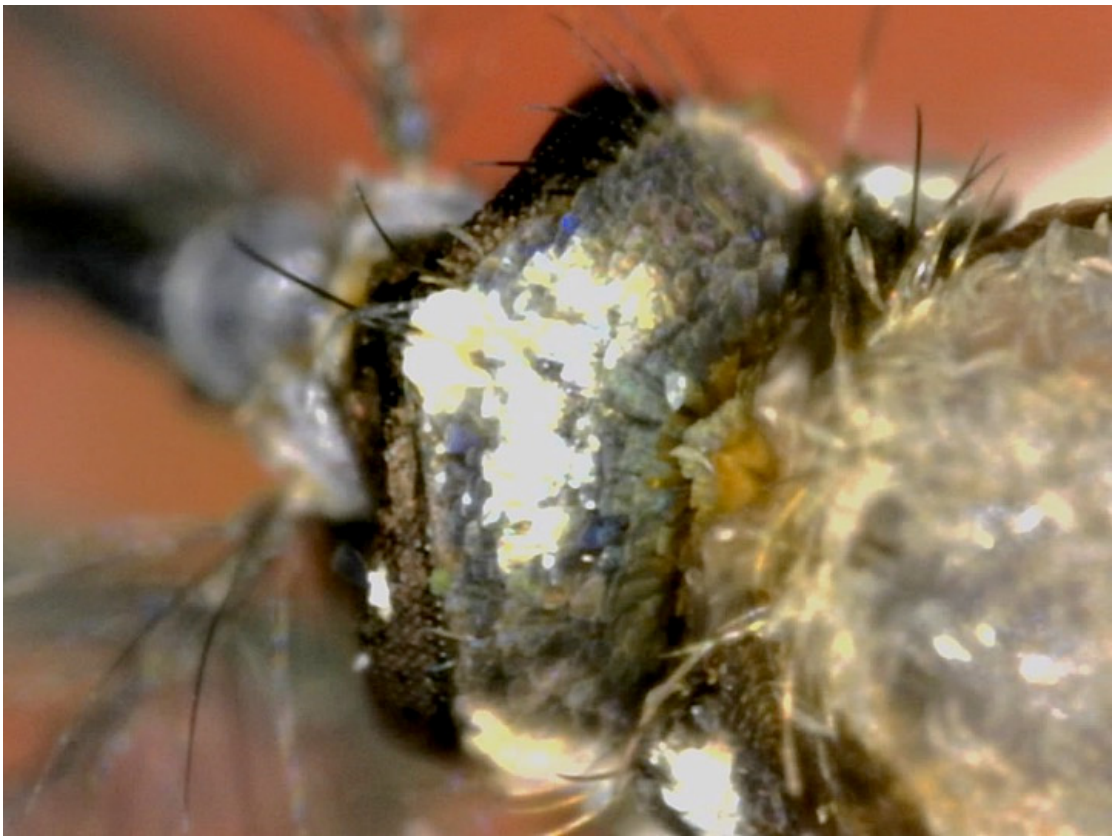

Figure 10. Occuput of *Sh. moralesi* (Chiapas, Mexico, 01010818-EU) showing the decumbent scales mostly with silver reflections.

4. Discussion and Conclusions

4.1. Ecology and Distributional Groups of Species

Based on our collection records and the known distributions of the mosquito species collected in Querétaro state, three groups of species are recognized. The species of each group have similar geographical distributions, reaching their southern or northern distributional limits across the state. The immature stages of groups 1 and 2 develop in phytotelmata such as tree holes and bromeliad axils, while immature stages of group 3 develop in ponds and swamps (Table 3).

4.1.1. Group 1

Species that occur in the Nearctic Region and extend into northern Mexico where they reach their southern limit of distribution in Querétaro include *Aedes brelandi*, *Ae. triseriatus* and the endemic species *Ae. schicki*. Immature stages of those species develop in tree holes filled with rainwater; the presence of species in this group is restricted to the forested and conserved areas of the northern part of the state, extending from the Nearctic Region into the Huasteco Carso of the Sierra Madre Oriental in Querétaro.

4.1.2. Group 2

Species of this group occur in tropical forests in the Neotropical Region and extend into Querétaro where they reach their northern limit. Only one species is reported in this group, *Wyeomyia apronoma*, whose immature stages occur in bamboo internodes, tree holes, and coconut shells. This species has been previously reported in several states of southeastern Mexico, but the distribution is restricted to the north region by the Huasteco Carso of the Sierra Madre Oriental in Querétaro state.

4.1.3. Group 3

Ground pool inhabiting species that extend from the Neotropical Region into Middle Mexico in Querétaro state but no farther north are *Aedes euplocamus*, which has been previously reported in tropical regions of southeastern Mexico where it is a common species during the rainy season, and the Mexican endemic species *Ae. shannoni*, which has been previously reported in the states of Michoacán, Morelos, Querétaro, and Mexico State, but reaches its northernmost distributional limit in Querétaro.

4.2. Species from Adjacent Regions That May Occur in Querétaro

Some species of mosquitoes that have not yet been reported from Querétaro occur in adjacent states and may occur within the state. Included among these are 20 species that have been previously recorded in the state of Hidalgo [14]: *Anopheles aztecus* Hoffmann, *An. crucians* Wiedemann, *An. parapunctipennis* Martini, *An. punctimacula* Dyar and Knab, *An. argyritarsis* Robineau-Desvoidy, *Aedeomyia sqamipennis* Lynch-Arribálzaga, *Aedes muelleri* Dyar, *Psorophora ferox* (von Humboldt), *Culex bidens* Dyar, *Cx. interrogator* Dyar and Knab, *Cx. nigripalpus* Theobald, *Cx. pinarocampa* Dyar and Knab, *Cx. pseudostigmatosoma* Strickman, *Cx. stenolepis* Dyar and Knab, *Cx. rejector* Dyar and Knab, *Cx. territans* Walker, *Cx. corniger* Theobald, *Sabethes gymnothorax* Harbach and Petersen, *Uranotaenia coatzacoalcos* Dyar and Knab, and *Ur. sapphirina* (Osten Sacken); and 12 species that have been previously recorded in the state of México [25]: *Ae. ramirezi* Vargas and Downs, *Ae. guerrero* Berlin, *Ae. lorraineae* Berlin, *Ae. chionotum* Zavortink, *Ae. gabriel* Schick, *Ae. idanus* Schick, *Ae. kompi* Vargas and Downs, *Ae. vargasi* Schick, *Ae. zoosophus* Dyar and Knab, *Haemagogus mesodentatus* Komp and Kumm, *Culiseta incidens* (Thomson), and *Ur. geometrica* Theobald.

4.3. Medical Importance of Mosquitoes of Querétaro

Some of the species reported in Querétaro are of medical and veterinary importance because they are vectors of pathogens causing diseases. In Table 5 the most important public health species that occur in Querétaro are listed.

4.4. Molecular Analysis

The DNA barcode sequences of specimens belong to five Sabethini species of mosquitoes we analyzed in this study grouped together, although a discrepancy in BINs were found in *Sh. fluviatilis* and *Sh. schedocyclia*. This agrees with Talaga [57] in their analysis of the Culicidae DNA barcodes from French Guiana. As we have not been able to examine the voucher specimens from where the DNA barcodes sequences were obtained, we cannot make further comments with regards to the taxonomic status of these two BINs. The specimen we identified as *Sh. huasteca* n. sp. separate with strong support values from those identified as *Sh. moralesi*, which supports our hypothesis that they represent a new species. The latter finding is also supported by the different morphological traits found in the adult female general coloration and the male genitalia.

4.5. Mosquitoes Diversity in Querétaro and Mexico

With the addition of the new mosquito records found in Querétaro reported here, there are currently 50 species known in the state. The state ranks eighth in species richness of the ten Mexican states that have been systematically inventoried for mosquito species. With the addition of *Shannoniana huasteca* n. sp. to the list of mosquito species in Mexico, there are currently 247 known species in the country.

Table 5. Medical importance and pathogens of mosquito vector species collected in Querétaro state, Mexico. Mal: Malaria. DI: *Dirofilaria immitis*. DENV: Dengue virus. ZIKV: Zika virus. CHIKV: Chikungunya virus. YF: Yellow fever virus. SLE: St. Louis encephalitis virus. WNV: West Nile virus. VEEV: Venezuelan equine encephalitis virus. EEEV: Eastern equine encephalitis virus. WEEV: Western equine encephalitis virus. LCV: La Crosse virus.

Taxa	Mal	DI	DENV	ZIKV	CHKV	YF	SLE	WNV	VEEV	EEEV	WEEV	LCV
Anopheles pseudopunctipennis	√											
An. punctipennis	√											
An. albimanus	√											
Aedes vexans		√		√			√	√	√	√	√	
Ae. angustivittatus									√			
Ae. scapularis		√				√	√		√			
Ae. trivittatus		√						√		√	√	√
Ae. triseriatus								√		√		√
Ae. aegypti			√	√	√	√		√	√	√		√
Ae. albopictus		√	√	√	√	√		√	√	√	√	√
Haemagogus equinus						√						
Culex quinquefasciatus					√		√	√	√	√		
Cx. restuans							√	√		√		
Cx. salinarius							√	√		√		
Cx. tarsalis								√	√		√	
Cx. erraticus								√				
Culiseta inornata								√			√	
Sabethes chloropterus						√	√					

Author Contributions: A.I.O.-M. and L.M.H.-T.—study conceptualization; A.I.O.-M. and L.M.H.-T.—methodology; A.I.O.-M.—sampling; A.I.O.-M. and L.M.H.-T.—data curation; A.I.O.-M.—mosquito identification; L.M.H.-T. and Q.K.S.-R.—molecular assays; A.I.O.-M., L.M.H.-T., and Q.K.S.-R.—analyses; A.I.O.-M. and Q.K.S.-R.—figures conceptualization; A.I.O.-M.—writing—original draft preparation; L.M.H.-T. and Q.K.S.-R.—writing—review and editing; A.I.O.-M., L.M.H.-T. and Q.K.S.-R.—visualization and supervision; A.I.O.-M. and L.M.H.-T.—project administration and funding acquisition. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: Not applicable.

Data Availability Statement: All mosquitoes (mounted on pins and/or microscope slides) collected in Querétaro State were deposited in the Culicidae Collection (CC-UL), Parasitology Department, Universidad Autónoma Agraria Antonio Narro unidad laguna (Autonomous Agrarian University Antonio Narro laguna unit), Torreón, Coahuila, Mexico.

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