ESTRICTOR IN IOHEMI CIGARROA-LOS M. BAAK-BAAK<sup>1,4</sup> neluding competent and id July 2022, mosquito nitoring, we present the

## SCIENTIFIC NOTE

## NEW STATE RECORD FOR *CULEX REJECTOR* AND *CX. RESTRICTOR* IN YUCATAN, MEXICO

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ABSTRACT. Several known mosquito species occur in the Mexican state of Yucatan, including competent and suspected vectors responsible for transmitting zoonotic arboviruses. Between February and July 2022, mosquito collections were carried out in several forested areas in Yucatan. As part of the ongoing monitoring, we present the first reports of *Culex (Microculex) rejector* and *Cx. (Anoedioporpa) restrictor*. Another 14 species were identified during the monitoring: *Aedes albopictus, Ae. bimaculatus, Ae. tormentor, Ae. cozumelensis, Anopheles albimanus, Cx. coronator* s.l., *Cx. erraticus, Cx. lactator, Cx. salinarius, Coquillettidia venezuelensis, Limatus durhamii, Psorophora ciliata, Toxorhynchites theobaldi,* and *Wyeomyia mitchellii.* Currently, the mosquito fauna in Yucatan consists of 65 species. The subgenera *Microculex* and *Anoedioporpa* had not been documented in Yucatan State prior to the current investigation.

KEY WORDS Anoedioporpa, first record, Microculex, mosquito fauna, mosquito update

*Culex daumastocampa* Dyar and Knab and *Cx. rejector* Dyar and Knab, of the subgenus *Microculex*, are reported to occur in Mexico (Ortega-Morales et al. 2018). *Culex imitator* Theobald was first reported in Mexico by Knight and Stone (1977), and subsequent studies reported its occurrence in the Mexican states of Hidalgo, San Luis Potosi, and Tamaulipas (Ortega-Morales et al. 2015, 2018). Following specimen reexamination by the same author, the record for *Cx. imitator* was corrected to *Cx. rejector*. Currently, *Cx. imitator* was removed from the list of mosquitoes in Mexico (Ortega-Morales et al. 2018).

Within the subgenus Anoedioporpa, Cx. conservator (Dyar and Knab) and Cx. restrictor (Dyar and Knab) are recorded to occur in Mexico (Adeniran et al. 2021). In early, now outdated studies, Stone and colleagues (1959) placed *Tinolestes*, Micraedes, and Anoedioporpa in the subgenus Aedinus Bourroul (1904), based on the shared characteristic of males possessing short palps. However, this trait developed independently in several unrelated mosquito species (Belkin 1968); therefore, Anoedioporpa was promoted to subgeneric rank. According to Berlin and

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Belkin (1980), Anoedioporpa can be recognized by a combination of characteristics. Adults have narrow decumbent scales on the vertex; short palpus in both sexes (except in Cx. restrictor); a general absence of acrostichal bristles on the mesonotum (except in Cx. corrigani Dyar and Knab and Cx. restrictor); a generally yellowish pleural integument; and dark tarsi. In male genitalia, a large densely setose area is observed on the inner surface of the sidepiece below the proximal division of the subapical lobe. The siphons of 4th instars sport 4 pairs of subventral hairs, 2 pairs of subdorsal hairs, an apical hook (2-S) with a small submedian branch, dorsal saddle hairs (2,3-X), and a long, single, and ventral brush usually with 5 pairs of hairs. *Culex restrictor* was originally described in Oaxaca, Mexico (Berlin and Belkin 1980), and considered a member of Microculex (Stone et al. 1959, Knight and Stone 1977), but reclassified by Berlin and Belkin (1980) after noting characteristics of both adult and immature mosquitoes resembled those of Anoedioporpa. At present, 12 species are currently included in the Conservator and Restrictor Groups of the subgenus Anoedioporpa. The Restrictor Group is monotypic and contains only Cx. restrictor (Berlin and Belkin 1980) due to highly particular traits not shared with other Anoedioporpa species (Berlin and Belkin 1980). Adult mosquitoes of the Restrictor Group present acrostichal bristles extending from the anterior promontory to the prescutellar area; and a bristled lower mesepimeron. The male genital lobe is distally divided; has specialized setae with 4 apical leaves; and cereal sclerite of proctiger digitiform. Fourth instars have a ventral brush (4-X) with 6 pairs of hairs; and a sclerotized plate at the base of the 2nd hair on the abdominal segment VIII (Berlin and Belkin 1980). The present study's objective was to monitor the

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mosquito fauna found in Yucatan's forested areas and update the state occurrence list, if necessary.

Yucatan State (20°50'00"N, 89°00'00"W) is located on Mexico's Yucatan Peninsula, and bordered by the states Quintana Roo and Campeche. The rainy season extends from May to October, with a mean rainfall of 1,000 mm and mean temperature of 27.5°C. The dry season lasts from November to April, with a mean rainfall of 300 mm and a mean temperature of 25.1°C (Baak-Baak et al. 2016). Immature and adult mosquitoes were collected from February to July 2022. Immature mosquitoes from artificial and natural breeding sites were collected by nets, turkey basters, and pipettes, placed into plastic containers that were labeled with collection date, study site, and sample identification number, and transported to the Laboratorio de Arbovirología at Universidad Autónoma de Yucatán. A portion of the larvae were killed in hot water (60°C) and mounted on microscope slides in Euparal (BioQuip®, Galveston, TX). The remaining larvae were individually reared to adults and identified to the species level. Adult mosquitoes were collected using a Mosquito Magnet trap (American Biophysics Corp., North Kingstown, WA) and a mouth aspirator. Stereomicroscopes and taxonomic keys were used to identify species (Dyar and Knab 1906, Berlin 1969, Berlin and Belkin 1980, Clark-Gil and Darsie 1983, Rueda 2004). Voucher specimens were stored in the Arbovirology Laboratory at the Universidad Autónoma de Yucatán, México.

In our study, we identified 16 mosquito species belonging to 8 genera, with *Cx. restrictor* and *Cx. rejector* as new records for Yucatan State. Table 1 shows the collected number of immature and adult mosquitoes per species, as well as the location and type of breeding grounds.

Culex restrictor was confirmed following the description of Berlin and Belkin (1980). Larvae were recognized by the presence of a ventral brush (4-X) with 6 pairs of hairs, a siphon with both subdorsal (2a-S) and subventral hairs (1, la-S) composed of 5 pairs, and hair 2-VIII on a sclerotized plate. The immature mosquito was found in deciduous forest near the community of Ekmul (20°57'54"N, 89°20'56"W), located 27 km east of Yucatan's capital city of Merida. The positive hatchery was located 15 m from a thoroughfare that connects Ekmul and Hubila in the municipality of Tixkokob. The area is populated by trees typical of the region, such as Alvaradoa amorphoides Liebm., Bursera simaruba (L.) Sarg., Ehretia tinifolia L., Havardia albicans (Kunth) Britton and Rose, Leucaena leucocephala (Lam.) deWit, Mimosa bahamensis Benth., Piscidia piscipula (L.) Sarg., and Bromelia karatas L. On June 16 and July 7, 2022, 12 mosquito larvae were collected in a 10-liter-capacity plastic bucket filled with dark-colored water with high organic matter, i.e., fallen leaves and decomposing arthropods.

Culex rejector was confirmed following the description of Dyar and Knab (1906). Larvae were recognized by the presence of setae 6-III-VI of the abdomen with 2 hairs and comb scales with very long spines. Five Cx. rejector larvae were found on July 2, 2022, in the water-filled leaf axils of the bromeliad Aechmea bracteata (Swartz) Grisebach. The Cx. rejector was found in the community of Esmeralda (20°42'03"N, 87°36'34"W) located within the states of Quintana Roo and Yucatan, and 240 km east of Yucatan's capital, Merida. The site is represented by trees from the region, such as *Manilkara sapota* (L.) P. Royem, Brosimum aliscastrum Sw., Piscidia piscipula, Bursera simaruba, Metopium brownei Roxb., and Vitex gaumeri GreenM. The epiphytic and terrestrial forms of Aechmea bracteata bromeliad are abundant.

In the present study, we identified 16 mosquito species from sylvan habitats, including 2 species never before reported in Yucatan State: Cx. restrictor and Cx. rejector. In the last update of mosquitoes of Yucatan, 52 species were registered (Baak-Baak et al. 2016). In this list, Wyeomyia mitchellii (Theobald) was not included (Díaz-Nájera and Vargas 1973). In recent years, the following 12 species have been identified: Ae. albopictus (Skuse), Ae. bimaculatus (Coq.), Ae. tormentor, Anopheles punctipennis (Say), Coquillettidia venezuelensis (Theobald), Cx. erythrothorax Dyar, Cx. rejector (present study), Cx. restrictor (present study), Cx. taeniopus Dyar and Knab, Cx. trifidus Dyar, Mansonia dyari Belkin, Heinemann, and Page, and Toxorhynchites moctezuma Theobald (Baak-Baak et al. 2016, Bond et al. 2020). With the addition of our findings, the current mosquito fauna of Yucatan consists of 65 species.

Culex rejector was originally described from Veracruz, Mexico, from immature mosquitoes discovered and removed from leaf axils of bromeliads (Dyar and Knab 1906). Further distribution is listed in the countries Belize, Costa Rica, Guatemala, Honduras, and Nicaragua (WRBU 2022). Anoedioporpa mosquitoes are container breeders (Berlin and Belkin 1980). Their immature stages are typically collected in natural, phytotelma breeding sites such as leaf axils, bamboo internodes, and tree holes (Adeniran et al. 2021; Ortega-Morales et al. 2010, 2019b); they have also been found in discarded tires (Ortega-Morales et al. 2015, 2019a, 2019c). Mosquito adults are generally sylvan, though some have been found in trees in suburban areas (Ortega-Morales et al. 2019b, Adeniran et al. 2021).

*Culex restrictor* is currently distributed in the Mexican states of Hidalgo, Jalisco, Mexico, Nuevo Leon, Quintana Roo, San Luis Potosi, Tabasco, Tamaulipas, Veracruz, and Yucatan (Berlin and Belkin 1980; Ortega-Morales et al. 2010, 2015, 2019a, 2019b, 2019c; Adeniran et al. 2021). Previous studies reported *Cx. restrictor* from natural and artificial containers cohabiting with *Ae. albopictus, Ae. aegypti* (L.), *Ae. epactius* Dyar and Knab, *Ae. muelleri* Dyar, *Ae. podographicus* Dyar and Knab,

Table 1. Mosquito species colle	ected in forest	environme	nts in Yucatan State, Mexico, between February and Jul of collection, and collection site with coordinates	tween February and n site with coordin	Table 1. Mosquito species collected in forest environments in Yucatan State, Mexico, between February and July of 2022, with information on mosquito gender, stage, method of collection site with coordinates.	I mosquito gender, stage, method
Subgenus and species	Female	Male	Collecting method <sup>1</sup>	Inmature	Source of collection	Coordinates
Stegomyia Aedes albopictus	9	2	MMT, mouth aspirator	Larvae (41) Pupae (17)	Disposable container, tree hole, bromeliad axil, <sup>2</sup> sink	20°57′54″N, 89°20′56″W; 20°42′03″N, 87°36′34″W
Ochlerotatus Ae. bimaculatus Ae. tormentor	1		Mouth aspirator Mouth aspirator	, , ,		20°42'03″N, 87°36'34″W 20°51'24″N, 90°22'32″W
Howardina Ae. cozumelensis				Larvae (9)	Sink, rock hole	20°57′54″N, 89°20′56″W; 20°42′03″N_87°36′34″W
Nyssorhynchus Anopheles albimanus	3		TMM	[		20°42′03″N, 87°36′34″W
Melanoconion Culex erraticus				Larvae (14)	Lagoon	20°39′38″N, 87°36′48″W
Cutex Cx. coronator s.l. Cx. salinarius				Larvae (40) Larvae (15) Pupae (2)	Sink, rock hole, drum Disposable container	20°42'03″N, 87°36'34″W 20°57'54″N, 89°20'56″W
Phenacomyia Cx. lactator				Larvae (6)	Rock hole	20°42′03″N, 87°36′34″W
Anoeatoporpa Cx. restrictor <sup>3</sup>				Larvae (12) Pupae (3)	Disposable container	20°57′54″N, 89°20′56″W
Microculex Cx. rejector <sup>3</sup>				Larvae (5)	Bromeliad axil <sup>2</sup>	20°42′03″N, 87°36′34″W
khynchotaenta Coquillettidia venezuelensis	2		MMT, mouth aspirator			20°42′03″N, 87°36′34″W
Limatus Limatus durhamii Preverbanc	1		MMT	Larvae (11)	Disposable container	
Esorophora Psorophora ciliata Lunchiolle				Larvae (2)	Tire	20°28′07″N, 89°42′52″W
Lyncnieua Toxorhynchites theobaldi Wveomvia				Larva (1)	Bromeliad axil <sup>2</sup>	20°42′03″N, 87°36′34″W

<sup>1</sup> MMT, Mosquito Magnet trap. <sup>2</sup> Aechmea bracteata. <sup>3</sup> New record for Yucatan State.

Wyeomyia mitchellii

20°42'03"N, 87°36'34"W

Bromeliad axil<sup>2</sup>

Larvae (20) Larva (1)

Cx. coronator Dyar and Knab, Cx. corniger Theobald, Limatus durhamii Theobald, Tx. moctezuma, and Wy. mitchellii (Berlin and Belkin 1980; Ortega-Morales et al. 2010, 2019a, 2019b, 2019c). In a plastic bucket, we found Cx. restrictor cohabiting with Ae. albopictus. The area of the finding is in new growth in young deciduous forest, although it is also common to find it in oak forest and in suburban areas (Ortega-Morales et al. 2019b, Adeniran et al. 2021). Its distribution range appears to be wide, ranging from the Yucatan peninsula in southeastern Mexico to Tamaulipas in the northeastern part of the country (Berlin and Belkin 1980: Ortega-Morales et al. 2010, 2015, 2019a. 2019b, 2019c; Adeniran et al. 2021). Little is known about the biology of Cx. restrictor and Cx. rejector and their vectorial capacity, although they are not currently incriminated as vectors of pathogens. The findings of the present study confirm the broad distribution pattern of the species.

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## **REFERENCES CITED**

- Adeniran AA, Hernandez-Triana LM, Ortega-Morales AI, Garza-Hernández JA, Cruz-Ramos J, Chan-Chable RJ, Vazquez-Marroquín R, Huerta-Jimenez H, Nikolova NI, Fooks AR, Rodríguez-Perez MA. 2021. Identification of mosquitoes (Diptera: Culicidae) from Mexico State, Mexico using morphology and COI DNA barcoding. *Acta Trop* 213:105730. https://doi.org/10.1016/j. actatropica.2020.105730
- Baak-Baak CM, Cigarroa-Toledo N, Arana-Guardia R, Chi-Chim WA, Chan-Orilla JA, Machain-Williams C, Torres-Chable OM, Ortega-Morales AI, Moo-Llanes DA, Elizondo-Quiroga A, García-Rejon JE. 2016. Mosquito fauna associated with *Aedes aegypti* (Diptera: Culicidae) in Yucatan State of Southeastern Mexico, and checklist with new records. *Fla Entomol* 99:703–709. https://doi.org/10.1653/024.099.0420
- Belkin JN. 1968. Mosquito studies (Diptera, Culicidae). IX. The type specimens of New World mosquitoes in European museums. *Contrib Am Entomol Inst* 3:1–69.
- Berlin O, Belkin J. 1980. Mosquito studies (Diptera, Culicidae) XXXVI. Subgenera Aedinus, Tinolestes and Anoedioporpa of Culex. Contrib Am Entomol Inst 17:1– 104.
- Berlin OG. 1969. Mosquito studies (Diptera, Culicidae). XII. A revision of the Neotropical subgenus *Howardina* of *Aedes. Contrib Am Entomol Inst* 4:1–190.
- Bond JG, Moo-Llanes DA, Ortega-Morales AI, Marina CF, Casas-Martinez M, Danis-Lozano R. 2020. Diversity and potential distribution of culicids of medical importance of the Yucatan Peninsula, Mexico. *Salud Publica Mex* 62:379–387. https://doi.org/10.21149/11208
- Bourroul C. 1904. "Mosquitos do Brasil" [thesis]. Faculdade de Medicina da Bahia, Bahia, Brazil.

- Clark-Gil S, Darsie RF. 1983. The mosquitoes of Guatemala, their identification, distribution and bionomics, with keys to adult females and larvae. *Mosq Syst* 15:151–294.
- Díaz-Nájera A, Vargas L. 1973. Mosquitos mexicanos, distribución geográfica actualizada. *Rev Invest Salud Pública* 33:111–125.
- Dyar HG, Knab F. 1906. The larvae of Culicidae classified as independent organisms. J NY Entomol Soc 14:205– 222.
- Knight KL, Stone A. 1977. Catalog of the mosquitoes of the world (Diptera, Culicidae). 2nd edition. College Park, MD: Entomological Society of America, Thomas Say Foundation.
- Ortega-Morales A, Garza-Hernandez JA, Gonzalez-Alvarez VH, Hernandez-Triana LM, Rodriguez-Perez MA. 2018. First record of *Culex (Microculex) daumastocampa* (Diptera: Culicidae) in Mexico, with notes on *Cx. rejector* and *Cx. imitator. Neotrop Entomol* 47:577–581. https://doi.org/10.1007/S13744-018-0600-0
- Ortega-Morales AI, Mendez-Lopez R, Garza-Hernandez JA, Gonzalez-Alvarez VH, Ruiz-Arrondo I, Huerta-Jiménez H, Rodriguez-Martinez LM, Rodriguez-Perez MA. 2019a. The mosquitoes (Diptera: Culicidae) of Tabasco, Mexico. J Vector Ecol 44:57–67. https://doi. org/10.1111/JVEC.12329
- Ortega Morales AI, Mis-Avila P, Elizondo-Quiroga A, Harbach RE, Siller-Rodríguez QK, Fernandez-Salas I. 2010. The mosquitoes of Quintana Roo State, Mexico (Diptera: Culicidae). *Acta Zool Mex* 26:33–46.
- Ortega-Morales AI, Zavortink TJ, Garza-Hernandez JA, Siller-Rodríguez QK, Fernandez-Salas I. 2019b. The mosquitoes (Diptera: Culicidae) of Nuevo León, Mexico, with descriptions of two new species. *PLoS ONE* 14: e0217694. https://doi.org/10.1371/journal.pone.0217694
- Ortega-Morales AI, Zavortink TJ, Huerta-Jimenez H, Ibañez-Bernal S, Siller-Rodríguez QK. 2019c. The mosquitoes (Diptera: Culicidae) of Hidalgo State, Mexico. Acta Trop 189:94–103. https://doi.org/10.1016/ J.ACTATROPICA.2018.07.003
- Ortega-Morales AI, Zavortink TJ, Huerta-Jimenez H, Sanchez-Ramos FJ, Valdés-Perezgasga MT, Reyes-Villanueva F, Siller-Rodríguez QK, Fernandez-Salas I. 2015. Mosquito records from Mexico: the mosquitoes (Diptera: Culicidae) of Tamaulipas State. *J Med Entomol* 52:171–184. https://doi.org/10.1093/JME/TJU008
- Rueda LM. 2004. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. *Zootaxa* 589:1–60. https://doi.org/10. 11646/zootaxa.589.1.1
- Stone A, Knight KL, Starcke H. 1959. A synoptic catalog of the mosquitoes of the world (Diptera, Culicidae). Washington, DC: Entomological Society of America, Thomas Say Foundation.
- WRBU [Walter Reed Biosystematics Unit]. 2022. Systematic catalog of Culicidae [Internet]. Suitland, MD: Walter Reed Biosystematics Unit [accessed June 27, 2022]. Available from: http://mosquitocatalog.org/.

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