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SCIENTIFIC NOTE

MOSQUITO FAUNA IN THE MANGROVES OF YUCATAN, MEXICO, AND IDENTIFICATION OF ALPHAVIRUS RNA

JULIAN E. GARCIA-REJON,[1] JULIO C. TZUC-DZUL,[1] KAREN Y. LOPEZ-CARRILLO,[1] NOHEMI CIGARROA-TOLEDO,[2] ROSA C. CETINA-TREJO,[1] WILBERT A. CHI-CHIM,[1] LOURDES G. TALAVERA-AGUILAR,[1] LAURA I. LOPEZ-APODACA[1] AND CARLOS M. BAAK-BAAK[1,3]

ABSTRACT. The surveillance of arboviruses in mangrove mosquitoes is a neglected topic in Mexico. The Yucatan State is part of a peninsula and, therefore, is rich in mangroves along its coast. The purpose of the study was to identify alphavirus in the mosquito fauna of mangroves. Mosquitoes were captured in mangrove settings in seven communities in Yucatan between June 2019 and August 2021. From 1900 to 2200 h and from 0500 to 0800 h, mosquitoes were captured with a backpack-mounted aspirator. In total, 3,167 female mosquitoes of five genera and nine species were captured. *Aedes taeniorhynchus* and *Anopheles crucians* were the most abundant mosquitoes collected. Mosquitoes were sorted into 210 pools and tested by reverse transcription-polymerase chain reaction for alphavirus ribonucleic acid (RNA). Alphavirus RNA was found in *Ae. taeniorhynchus*, *An. pseudopunctipennis*, and *An. crucians* collected in the Celestun Mangrove. The community is part of the Ria Celestun Biosphere Reserve, and the presence arbovirus-infected mosquitoes could pose a health risk to residents and visitors alike in the area.

KEY WORDS *Aedes, Anopheles*, coastal areas, arbovirus, Mexico

The surveillance of arboviruses in mangrove mosquitoes is a neglected issue in Mexico. However, there is a background of alphaviruses and orthobunyaviruses in mosquitoes linked to coastal areas (Scherer et al. 1971; Farfan-Ale et al. 2009, 2010; Adams et al. 2012). Experimental studies have determined that *Anopheles freeborni* Atken, *An. gambiae* s.s., *An. quadrimaculatus* Say, and *An. stephensi* Liston are competent vectors of the Mayaro virus (Brustolin et al. 2018). In the mangroves of Colombia, mosquitoes naturally infected with alphavirus and flavivirus RNA were identified (Hoyos-López et al. 2016).

Approximately 70% of the Yucatan Coast is covered with mangroves (Rodríguez-Zúñiga et al. 2013). The mangroves are rich in mosquitoes, mammals, and birds, which are key components in the transmission of arboviruses (Farfan-Ale et al. 2004, Nagelkerken et al. 2008, Bond et al. 2014, Baak-Baak et al. 2016, Manrique-Saide et al. 2016). Disturbances in the mangroves (e.g., agricultural, livestock, aquaculture, and tourism) affect the ecological interactions of native species (Nagelkerken et al. 2008, Rodríguez-Zúñiga et al. 2013), and the emergence of pathogens can occur. In a previous study, wild birds were caught in the Yucatan mangroves, and the green jay, *Cyanocorax yncas* (Boddaert), was positive for the St. Louis encephalitis virus (Farfan-Ale et al. 2004). Therefore, it is critical to figure out whether the mosquito fauna of mangroves are potential vectors and pose a health concern to people who live in or visit these areas. The study aimed to determine the mosquito fauna in mangrove habitats as well as to identify arboviruses of the genus *Alphavirus*.

Yucatan State is located in Mexico's Yucatan Peninsula and is bordered by the states of Quintana Roo and Campeche. The Yucatecan Coast is located in the northern part of the state, where a dry and semi-dry climate predominates. Overall, rainfall is highest from June to October (typically $>$100 mm/month), with sporadic rainfall occurring during the remainder of the year (Baak-Baak et al. 2016). The study was carried out in the mangroves of seven communities located along the coast of the Yucatan State (Fig. 1).

Mosquitoes were captured from June to November 2019, January and February 2020, and August 2021, using a backpack-mounted aspirator (Prokopack Aspirator®, Gainesville, FL). The hours captured were from 1900 to 2200 h and 0500 to 0800 h. Two entomologists sat comfortably, and mosquitoes that flew by or landed on the bodies of the entomologists were captured. Mosquitoes were transported alive to the Laboratory of Arbovirologia at the Universidad Autónoma de Yucatán. Stereomicroscopes and taxonomic keys were used to identify species (Clark-Gil and Darsie 1983, Velasquez 2014). Female mosquitoes were sorted into pools of $\leq$15 and stored at –80°C until required.

Pools of female adults were placed into Eppendorf tubes containing 300 µl of Liebovitz's L15 medium

[1] Laboratorio de Arbovirología, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán, México, C.P. 97225, México.
[2] Laboratorio de Biología Celular, Centro de Investigaciones Regionales Dr. Hideyo Noguchi, Universidad Autónoma de Yucatán, México, C.P. 97000, México.
[3] To whom correspondence should be addressed.

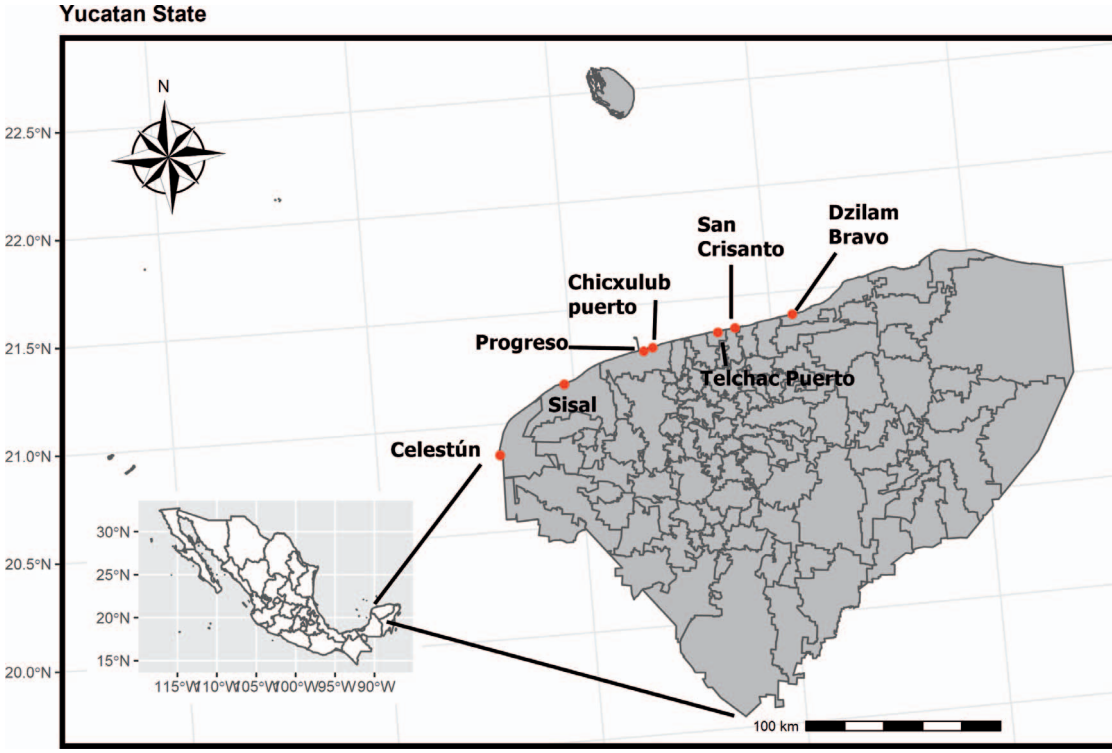

Fig. 1. Study site.

(Invitrogen, Carlsbad, CA) and mechanically homogenized using sterile pestles. Homogenates were centrifuged at 10,000 × g for 10 min, and supernatants were collected. Total ribonucleic acid (RNA) was extracted from an aliquot (200 µl) of each supernatant, using a Rneasy kit [First-Strand cDNA Synthesis], Catalog A3500 (Qiagen, Valencia, CA). Reverse transcription of RNA to c deoxyribonucleic acid (cDNA) was carried out using a reverse transcription-polymerase chain reaction (RT-PCR) (Promega, Madison, WI). The alphavirus detection protocol was previously described (Eshoo et al. 2007). The RT-PCR procedure was used to amplify a fragment of the alphaviruses' NSP1 gene (~98 nt). Briefly, 2.5 µl of cDNA was added to a tube of PCR Mix containing 1 X of Flexi Buffer, 4 mM of $MgCl_2$, 0.2 mM dNTPs, 0.25 mM, 1 U of Taq polymerase (Invitrogen) and adjusted to 25 µl with molecular grade water. The chikungunya virus (CHIKV) was used as a positive control, and Molecular Biology Grade Water was used as a negative control. The amplification conditions were as follows: an initial denaturation at 94 °C for 3 min, followed by 29 cycles, each consisting of 30 sec, and at 50 °C for 45 sec, hybridization at 72 °C for 10 min, and then a final extension for 5 min at 72 °C. Amplicons were visualized on 2% agarose gels with 0.5 µg/ml ethidium bromide using a Doc™ XR+ Gel Documentation System. Positive amplicons of alphaviruses were tested for chikungunya virus (CHIKV) and Venezuelan equine encephalitis virus (VEEV). The methodology can be in Cigarroa-Toledo et al. (2016).

In total, 3,167 female mosquitoes of five genera and nine species were captured. *Aedes taeniorhynchus* Wiedemann (n = 2,103) and *An. crucians* Wiedemann s.s. (n = 966) were the most abundant mosquitoes and were present in most of the sampling sites (Table 1). The highest percentages of mosquito species were captured in the Celestun (54.91%) and Sisal (24.85%) mangroves (Table 1).

Alphavirus RNA was found in pools of *Ae. taeniorhynchus* ($n = 3$), *An. crucians* s.s. ($n = 1$), and *An. pseudopunctipennis* Theobald ($n = 1$) captured in the Celestun mangrove. Alphavirus-positive mosquitoes were captured in June, October, and November 2019, respectively (Supplemental Figure 1). Positive amplicons of alphaviruses were negative for CHIKV and VEEV.

We found nine species of mosquitoes associated with mangroves, which represented 16.67% of the known species in Yucatan (Baak-Baak et al. 2016, Manrique-Saide et al. 2016). Species richness is minor when compared to extensive work conducted on the Pacific coast of Mexico that included larval sampling in Chiapas, Oaxaca, Guerrero, Michoacan, Colima, Jalisco, Nayarit, and Sinaloa (Bond et al. 2014). *Anopheles pseudopunctipennis* and *An. albimanus* Wiedemann were the most abundant of the 74 mosquito species described in this study. Compared

Table 1. Female mosquitoes captured in the mangroves of the Yucatan coast, Mexico between June 2019 and August 2021.

Mosquito species	Community[1]	No. females
Aedes taeniorhynchus	Celestun	1,707
Anopheles pseudopunctipennis	Celestun	18
Ae. sollicitans	Celestun	6
Psorophora cyanescens	Celestun	4
An. crucians	Celestun	3
Ps. ferox	Celestun	1
An. crucians	Sisal	751
An. pseudopunctipennis	Sisal	19
An. albimanus	Sisal	16
Ae. taeniorhynchus	Sisal	1
An. crucians	Telchac Puerto	204
An. pseudopunctipennis	Telchac Puerto	14
Culex iolambdis	Telchac Puerto	11
Coquillettidia venezuelensis	Telchac Puerto	5
Ae. taeniorhynchus	Telchac Puerto	2
Ae. taeniorhynchus	Dzilam Bravo	186
An. crucians	Dzilam Bravo	1
Ae. taeniorhynchus	San Crisanto	142
An. crucians s.s.	San Crisanto	7
An. pseudopunctipennis	San Crisanto	4
Ae. taeniorhynchus	Progreso	63
Ae. taeniorhynchus	Chicxulub Puerto	2
Total		3,167

[1] Geographic coordinates of Celestun (20°51′20.02″N, 90°23′32.47″W), Sisal (21°9′33.24″N, 90°2′50.51″W), Telchac Puerto (21°20′17.01″N, 89°16′1.959″W), Chicxulub Puerto (21°17′38.26″N, 89°35′43.079″W), Dzilam Bravo (21°23′29.08″N, 88°53′30.829″W), San Crisanto (21°21′5.61″N, 89°10′50.58″W), and Progreso (21°16′51.74″N, 89°38′28.499″W).

to an earlier investigation, more species were identified in the Celestun mangrove in the current research (Manrique-Saide et al. 2016). In the previous study, in 14 days of sampling, the researchers found *Ae. taeniorhynchus*, *Ae. sollicitans* (Walker), *An. crucians*, *Coquillettidia venezuelensis* Theobald, and *Psorophora confinnis* (Dyar and Knab). *Aedes taeniorhynchus* was the most abundant in mangroves in both studies. Six *Anopheles* species have been reported in Yucatan (Baak-Baak et al. 2016). *Anopheles albimanus*, *An. crucians* s.s., and *An. pseudopunctipennis* were the only three anopheline species captured.

Aedes taeniorhynchus, *An. crucians*, and *An. pseudopunctipennis* were positive for alphavirus RNA, but negative for CHIKV and VEEV. Among the alphaviruses reported in mosquitoes from Mexico, subtype IE of VEEV was identified in *An. pseudopunctipennis*, *Cq. nigricans* Coquillett, *Mansonia titillans* (Walker), *Culex nigripalpus* Theobald, and *Cx. taeniopus* Dyar and Knab (Scherer et al. 1971, Adams et al. 2012). Chikungunya virus has been frequently identified in *Ae. aegypti* (Cigarroa-Toledo et al. 2016), Mayaro virus in humans (Navarrete-Espinosa and Gomez-Dantes 2006), and western equine encephalitis virus in horses (Cobos-Marin et al. 2019).

Five arboviruses of zoonotic origin with the capacity to infect humans have been isolated from *Ae. taeniorhynchus*. In Guatemala in 1969, an epizootic caused by VEEV occurred. The virus was isolated from horses and *Ae. taeniorhynchus* (Sudia et al. 1971). Likewise, in Costa Rica, VEEV was identified in humans, horses, and *Ae. taeniorhynchus* (Martin et al. 1972). In South Carolina, USA, an epidemiological surveillance of the eastern equine encephalitis virus (EEEV) reported *Ae. taeniorhynchus* and *Culiseta melanura* (Coquillett) (Ortiz et al. 2003). In Venezuela, the Itaqui virus was isolated from *Ae. taeniorhynchus*. The Itaqui virus causes headache and fever in humans (Walder et al. 1984). It should be noted that, in the city of Merida, Yucatan, females of *Ae. taeniorhynchus* were tested positive for Cache Valley and Kairi viruses. Both viruses primarily infect livestock and wildlife, and incidentally, humans (Farfan-Ale et al. 2009). In Colombian mangroves, the RNA of the VEEV was found in *Deinocerites atlanticus* Adames, *Ma. titillans*, *Ps. confinnis*, and *Ae. scapularis* (Rondoni). West Nile virus, SLEV, and yellow fever virus were detected in *Ma. titillans* and *Haemagogus* sp. (Hoyos-López et al. 2016). It is worth noting that the alphavirus genome was detected in mosquitoes caught in the Celestun mangroves during the rainy season. In this area, SLEV was discovered in the bird *C. yncas* (Farfan-Ale et al. 2004). To further understand the dynamics of arbovirus transmission in mangrove regions, future research should incorporate surveillance of small mammals, birds, and mosquitoes.

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