Development Times of the Wild-Type *Aedes albopictus* (Diptera: Culicidae) Reared in Semi-controlled Residential Conditions in Yucatan, Mexico[1]

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J. Entomol. Sci. 60(2): 258–268 (April 2025)
DOI: 10.18474/JES24-28

Abstract Eggs of *Aedes albopictus* (Skuse) (Diptera: Culicidae) were collected in a forested area near Tixkokob, Yucatan, Mexico. The time of juvenile development was monitored under semicontrolled conditions in a home environment in Merida, Yucatan, Mexico. We also amplified and sequenced a fragment of the cytochrome c oxidase subunit 1 mitochondrial gene from the field collected *A. albopictus* to evaluate its genetic similarities with populations from other regions. Over 7 mo, we monitored ovitraps and collected the eggs that were deposited. We transported and counted the eggs in the insectary then transported them to a home where we induced them to hatch and reared them to adulthood. We recorded the development of each immature stage and the daily temperature and relative humidity. We collected a total of 6,891 eggs; 25.67% of the larvae progressed to the pupal stage, and 17.44% reaching adulthood. Among the emerging adults, *A. albopictus* was the most abundant, accounting for 81.95%. The remaining adults were *Aedes epactius* Dyar & Knab, *Aedes cozumelensis* Diaz Nájera, *Aedes scapularis* (Rondani), and *Aedes podographicus* Ingår. The average development time from the first instar larval stage to adulthood of *A. albopictus* was 8.9 days at 29.67 ± 0.84°C and 71.66 ± 3% relative humidity. The *A. albopictus* specimens were most genetically similar to *A. albopictus* from the Republic of the Congo, India, Brazil, and China, indicating the wide global dispersion of this mosquito. These findings provide basic information on the effect of the temperature and humidity in a typical house in Merida on the *A. albopictus* life cycle.

Key Words Asian tiger mosquito, eggs, immature instar, life history, ovitrap

Aedes albopictus (Skuse) (Diptera: Culicidae), commonly referred to as the Asian tiger mosquito, is an invasive mosquito in the Americas and is considered a secondary vector of dengue and Zika viruses in this region (Garcia-Rejon et al.

[1]Received 4 March 2024; accepted for publication 9 April 2024.
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2021). *Aedes albopictus* has been found to harbor RNA from dengue and Zika viruses in Mexico (Correa-Morales et al. 2019, Huerta et al. 2017, Ibañez-Bernal et al. 1997, Sanchez-Rodríguez et al. 2014). Eastern equine encephalitis, Cache Valley, and West Nile viruses have been found in *A. albopictus* in the United States (Gerhardt et al. 2001; Gomes et al. 2003; Holick et al. 2002; Mitchell et al. 1992, 1998; Niebylski et al. 1994, Savage et al. 1993, Stenn et al. 2019), and yellow fever virus has been found in these mosquitos in Brazil (Alencar et al. 2021).

Researchers have widely documented the occurrence and distribution of *A. albopictus* in Mexico (Ortega-Morales et al. 2022), where it has now been reported in 21 Mexican states. Despite the fact that this mosquito has been implicated as an arbovirus vector (Garcia-Rejon et al. 2021), few studies have focused on its biology in Mexico (Casas-Martínez et al. 2020). An understanding of the regional biology of this potential vector is essential to facilitating studies to evaluate the arboviral infection rate and insecticide resistance factors. Therefore, it is crucial to have captive colonies of the local phenotype that are kept under culture conditions that resemble the environment found in nature (Garcia-Rejon et al. 2018, Talavera-Aguilar et al. 2021).

Aedes albopictus develops and survives best in water of 20–27°C, and adults actively search for hosts and oviposit at 18–30°C (Cui et al. 2021, Delatte et al. 2009, Ezeakacha and Yee 2019, Westbrook et al. 2010). In colder climates, *A. albopictus* can survive the winter months in a diapausal egg state (Reinhold et al. 2018). The Yucatan is generally warm, and temperatures seldom fall below 15°C; thus, *A. albopictus* remains active throughout the year (Contreras-Perera et al. 2019, Ortega-Morales et al. 2018). Effective estimation of its vectorial capacity necessitates an understanding of its life history, including the biological cycles of regional phenotypes and local populations. Along with available vertebrate hosts, suitable containers, and harborage, development time is a significant factor in the survival of mosquito populations (Garcia-Rejon et al. 2018). Slower development increases the likelihood of larvae being eaten or parasitized or of larval habitats being eliminated (Service 1993). The main objective of the present study was to estimate the development time of *A. albopictus* and determine its genetic similarity to populations from other regions.

Materials and Methods

Study area and study design. We monitored mosquito eggs between January and July 2020. We conducted the research near the municipality of Tixkokob, Yucatan, Mexico, on a 12,000-m^2 plot of medium deciduous forest (20°57′54′′N, 89°20′56′′W). The rainy season extends from May to October, with a mean rainfall of 1,000 mm and a 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. Trees and shrubs typical of the region, such as *Alvaradoa amorphoides* Liebmann, *Bursera simaruba* L., *Ehretia tinifolia* L., *Havardia albicans* (Kunth) Britton & Rose, *Leucaena leucocephala* (Lambert) de Wit, *Mimosa bahamensis* Bentham, *Piscidia piscipula* (L.) Sargent, and *Bromelia karatas* L., covered the area. We placed 15 ovitraps at the base of the aforementioned plants and checked them once per week. The standard ovitrap was a 1-L cup painted black (Service 1993) filled with 700 ml of tap water. Filter paper was used as an oviposition substrate

and was placed directly onto the internal walls of the ovitraps. We collected eggs and placed them in plastic containers, labeled the containers with the date and sample identification number, and transported the containers to the Laboratorio de Arbovirología at the Universidad Autónoma de Yucatán.

Egg hatching and development time. The duration of the development cycle of mosquitoes was measured in a home in Merida City, Yucatan. A digital thermometer (KAMYSEN®, Mexico City, Mexico) recorded the temperature and humidity daily. Eggs collected on filter paper were induced to hatch, and the resulting larvae were reared until they emerged as adults. Larvae were placed in plastic trays 57 × 40 × 30 cm and filled with 5 L of tap water and 1 g of goldfish food (Biopro®, Monterrey, Nuevo Leon, Mexico). Food supplement was added every 2 d. We observed the development of each immature stage, the first to fourth larval instars and the pupal stage, on a daily basis. Emergent pupae were separated into date-labeled containers with approximately 500 ml of water. We examined the containers daily and noted the number of emerging adults (Garcia-Rejon et al. 2018). A portion of the larvae were killed by immersing them in hot water at 60°C and then mounted on microscope slides with Euparal (BioQuip®, Galveston, TX). Stereomicroscopes and taxonomic keys were used to identify species (Berlin 1969, Clark-Gil and Darsie 1983, Rueda 2004).

DNA extraction and molecular analysis of *A. albopictus*. Legs of a female *A. albopictus* were removed, deposited in a 1.5-ml Eppendorf tube, and homogenized in 70 μl of Pat Romans. We centrifuged the homogenate at 10,000 × *g* for 10 min and collected the supernatant. DNA was obtained by using the salt technique (Black and DuTeau 1997).

For molecular identification, a polymerase chain reaction (PCR) protocol was employed to amplify a fragment of the mitochondrial cytochrome oxidase subunit 1 (COI) DNA gene with primers LCO1490 (5′GGTCAACAAATCATAAAGATATTGG-3′) and HCO2198 (5′TAAACTTC AGGGTGACCAAAAAATCA-3′) (Folmer et al. 1994). The primer pair amplifies a ca. 710-base pair fragment of COI. PCRs were performed in a total volume of 25 μl containing 4 μl of genomic DNA (~30 ng/μl), 5.2 μl of 5× buffer, 2.08 μl of $MgCl_2$ (25 mM), 0.4 μl of *Taq* polymerase (GoTaq, Promega, Madison, WI), 0.52 μl of deoxynucleoside triphosphates, 1 μL of each primer (10 mM), and 10.8 μl of molecular grade water. The thermocycling conditions were initial denaturation at 95°C for 1 min followed by 35 cycles of 95°C for 1 min, 46°C for 1 min, and 72°C for 1 min. Hybridization was performed at 72°C for 1 min, and the final extension was performed at 72°C for 5 min. We visualized amplicons on 2% agarose gels with 0.5 μg/ml ethidium bromide using the Gel Doc XR+ Gel Documentation System (Bio-Rad, Hercules, CA). The PCR products were purified with the Zymoclean DNA recovery kit (Zymo Research, Irvine, CA) and sequenced with a 3500xL DNA sequencer (Applied Biosystems, Foster City, CA).

Molecular identification of *A. albopictus*. The obtained sequence (forward and reverse) was analyzed and assembled with BioEdit version 7.0.5.3 (Hall 1999). A consensus sequence was generated with MEGA v. 6.06 (Kumar et al. 2018). The sequence obtained from *A. albopictus* was compared with sequences available in BOLD Systems® (https://www.boldsystems.org/) and GenBank with the Basic Local Alignment Search Tool (http://blast.ncbi.nlm.nih.gov/Blast.cgi). The sequences with >99% identity with that of the Asian mosquito ($n = 22$) were

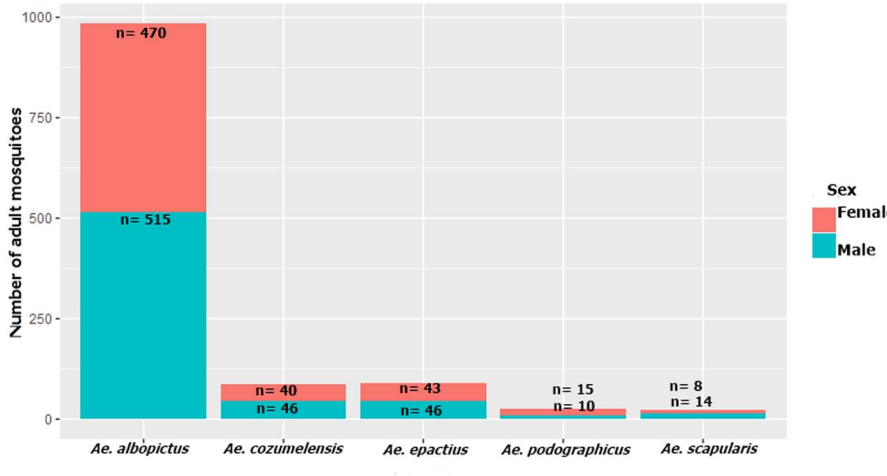

Fig. 1. Number of adult mosquitoes that emerged from eggs.

used to build the tree of genetic relationships (numbers of accesses in the tree), of which 11 belonged to specimens from Mexico. The sequence of this study was named *A. albopictus* 5-Yuc and uploaded to GenBank (GenBank ID: OR690439). The *Anopheles crucians* (GenBank ID OQ272332) sequence was used as an outgroup. Sequence alignment was performed with Clustal W in MEGA 11 with the default parameters (Tamura et al. 2021). The nucleotide substitution model was estimated in MEGA 11 option "find best DNA/protein models (ML)." The evolutionary history was inferred by using the maximum likelihood method and the Tamura three-parameter model (Tamura et al. 2021).

Results

Egg surveillance and length of development time. The mean temperature and accumulated precipitation of the study area during the egg collection were 27.63 ± 2.54°C and 933.5 mm, respectively. In total, 6,891 eggs were collected from ovitraps. Of those, 25.67% (1,769) of the larvae reached the pupal stage, and 17.44% (1,202) reached the adult stage. *Aedes albopictus* was the most abundant species, accounting for 81.95% (985) of the1,202 emerged adults. Adult females and males of *Aedes epactius* Dyar & Knab ($n = 89$), *Aedes cozumelensis* Diaz Nájera ($n = 86$), *Aedes scapularis* Rondani ($n = 22$), and *Aedes podographicus* Igår ($n = 20$) were also identified (Fig. 1). *Aedes albopictus* was identified in all the months of egg collection. However, between January and May, it was the only species collected.

In the home, mosquito development was observed at 29.67 ± 0.84°C and 71.66 ± 3% relative humidity. The average development time of *A. albopictus* from the first larval instar to adulthood was 8.9 ± 0.54 d. *Aedes scapularis* had the shortest life cycle, and *A. cozumelensis* had the longest (Table 1).

Table 1. Mean (±SD) duration of each developmental stage of five mosquito species reared in a residence at 29.67 ± 0.84°C and 71.66 ± 3% relative humidity in Merida, Yucatan, Mexico.

Mosquito species	Average Duration (days) of Each Life Stage					
	Larval 1st Instar	Larval 2nd Instar	Larval 3rd Instar	Larval 4th Instar	Pupae	Adult
Aedes albopictus	1 ± 0.5	1.5 ± 0.5	2 ± 0.5	2 ± 0.5	2.4 ± 1	8.9 ± 0.54
A. epactius	1 ± 0.5	1.5 ± 0.5	2 ± 0.5	2 ± 0.5	2.3 ± 1	8.8 ± 0.51
A. cozumelensis	1.5 ± 0.5	1.5 ± 0.5	3 ± 1	3 ± 1	3 ± 1	12 ± 0.82
A. podographicus	1.5 ± 0.5	1.5 ± 0.5	2 ± 0.5	3 ± 0.5	2.25 ± 1	10.25 ± 0.62
A. scapularis	1 ± 0.5	1 ± 0.5	1 ± 0.5	1.5 ± 0.5	1.5 ± 0.5	6 ± 0.27

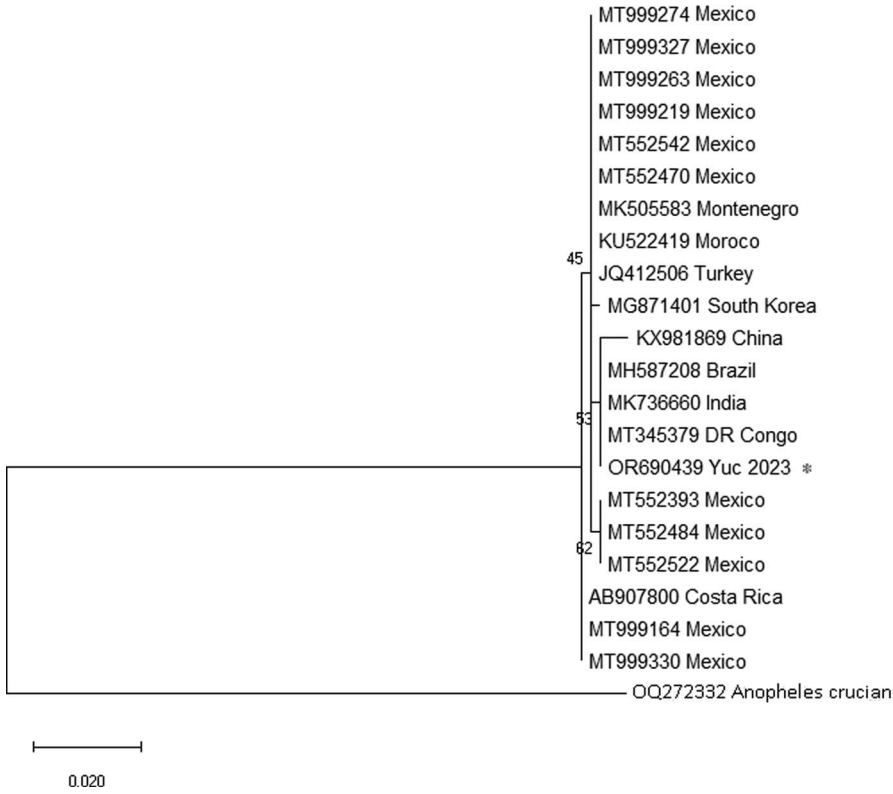

Fig. 2. Phylogeny of *Aedes albopictus* inferred from a fragment of the 600-base pair region of the COI gene. The tree is drawn to scale, with branch lengths indicating the number of substitutions per site. The asterisk indicates the sequence generated in the present study.

Sequence alignments and phylogenetic analysis. A female *A. albopictus* was used to amplify and sequence the fragment of the COI gene. The 600-base pair sequence from the *A. albopictus* in the present study (GenBank ID OR690439) was 100% similar to 22 sequences of *A. albopictus* found in GenBank and the BOLD System. Phylogenetic analysis indicated that specimens collected were most closely related to *A. albopictus* from the Republic of the Congo, India, Brazil, and China (Fig. 2).

Discussion

The egg surveillance of *A. albopictus* included 4 mo of the dry season (January to April) and 3 mo of the rainy season (May to July). In both seasons, *A. albopictus* was the most abundant species in the monitored forest area. This finding suggests that this population can survive at the region's environmental temperature of 27.63 ± 2.54°C. In 2011, *A. albopictus* was first identified on the Yucatan Peninsula, with

larvae found in Cancun, Quintana Roo (Salomón-Grajales et al. 2012). In 2017, both larvae and adults were found on the Yucatan (Ortega-Morales et al. 2018). In 2018, *A. albopictus* was found in the suburbs of Merida, the capital of Yucatan State (Contreras-Perera et al. 2019), and in 2019, larvae were reported in Campeche (Hernández-Rodríguez et al. 2020). Clearly, this exotic mosquito has successfully established itself and survives in Yucatan's forested areas all year. In contrast to these findings, populations of *A. albopictus* in cemeteries in Florida decrease dramatically during the dry season (Juliano et al. 2002).

Mosquitoes are ectotherms; thus, their internal temperatures reflect ambient temperature and environmental conditions (Ezeakacha and Yee 2019, Reinhold et al. 2018). The life history and vector competence of *A. albopictus* have been studied under various temperature regimes. For example, adults kept at low temperatures ($<$20°C) enter quiescence, and temperatures $>$34°C reduce female fecundity and overall survival (Ezeakacha and Yee 2019, Westbrook et al. 2010).

Aedes albopictus larval development takes an average of 35 d at 15°C and 8.8 d at 30°C (Delatte et al. 2009). Larvae grown at 18 and 24°C developed more slowly and into larger adults compared with larvae grown at $\geq$32°C (Delatte et al. 2009, Ezeakacha and Yee 2019, Westbrook et al. 2010). Adult females with the largest body size that were produced at 18°C were six times more likely to be infected with the chikungunya virus than were females reared at 32°C (Westbrook et al. 2010). Temperature also regulates blood digestion and egg production. Short gonotrophic cycles increase the contact of female mosquitoes with vertebrate hosts through more frequent blood feeding. The gonotrophic cycle of the Asian mosquito is 2.9 d at 30°C but 6.7 d at 20°C (Delatte et al. 2009). In Mexico, the gonotrophic cycle of *A. albopictus* was estimated at 3.2–3.7 d at 26.5°C (Casas-Martínez et al. 2020).

We estimated the development of *A. albopictus* from the first larval instar to adult as 8.9 d at 29.67°C and estimated the duration of the life cycle in a typical house in Merida. In contrast, Cui et al. (2021) observed that the average larval to pupal development times were 5.2 $\pm$ 0.1 d at 29°C. *Aedes epactius* has a similar development cycle, but *A. podographicus* and *A. cozumelensis* have a longer biological cycle of ca. 10 and 12 d, respectively. In contrast, *A. scapularis* needs only 6 d to complete a cycle from larva to adult. Information on biological cycles is important when mosquitoes are potential vectors of pathogens and it is necessary to evaluate vector competence and the status of resistance to insecticides.

Aedes albopictus has the genetic plasticity to adapt to a wide range of climates, and more studies must be conducted to understand its bionomics and vector capacity at the local level. The results of this work highlight the wide dispersal of this pest. According to the phylogenetic analysis, the genetic sequences of *A. albopictus* from Mexico were grouped into four clades. One clade includes samples from Mexico and Costa Rica. Another clade included only those sequences from Mexico (MT552393, MT552484, and MT552522), whose mosquitoes were collected in a nature reserve in Chiapas that borders Guatemala (Hernández-Triana et al. 2021). The next clade included samples from Mexico, Montenegro, Morocco, Turkey, and South Korea. The sequence of samples in the present work (OR690439) is grouped with *A. albopictus* from the Republic of the Congo (MT345379), India (MK736660), Brazil (MH587208), and China (KX981869). *Aedes albopictus* collected in Yucatan

was not similar to populations from Chiapas (MT999274, MT999219, MT999263, MT999327, MT552542, and MT552470) in southeastern Mexico (Hernández-Triana et al. 2021) probably due to different introductions of *A. albopictus* in this country. The range of *A. albopictus* in Mexico covers areas above sea level, such as the Yucatan Peninsula, up to 2,240 m of elevation in Mexico City, including warm and temperate areas (Contreras-Perera et al. 2019; Dávalos-Becerril et al. 2019; Ortega-Morales et al. 2018, 2022). However, *A. albopictus* also had some genetic similarity to mosquitoes from countries in Asia, the Americas, and Europe (Battaglia et al. 2016, Hernández-Triana et al. 2021, Wat'senga Tezzo et al. 2021). We also collected biological information on mosquitoes in wild habitats. These data could be used for studies on the vector competence of viruses that involve small mammals, birds, and mosquitoes in natural environments. One of the limitations of the study is that the development cycle of these mosquitoes was evaluated in a single home and may not represent all households in the city of Merida.

Acknowledgments

We thank the personnel of the Arbovirology Laboratory at the Dr. Hideyo Noguchi Centro de Investigaciones Regionales of the Universidad Autónoma de Yucatán for their invaluable support during the entire course of the study.

References Cited

Alencar, J., C. Ferreira de Mello, C. Brisola Marcondes, A. Érico Guimarães, H.K. Toma, A. Queiroz Bastos, S. Olsson Freitas Silva and S. Lisboa Machado. 2021. Natural infection and vertical transmission of Zika virus in sylvatic mosquitoes *Aedes albopictus* and *Haemagogus leucocelaenus* from Rio de Janeiro, Brazil. Trop. Med. Infect. Dis. 6(2): 99. https://doi.org/10.3390/tropicalmed6020099.

Battaglia, V., P. Gabrieli, S. Brandini, M.R. Capodiferro, P.A. Javier, X.G. Chen, A. Achilli, O. Semino, L.M. Gomulski, A.R. Malacrida, G. Gasperi, A. Torroni and A. Olivieri. 2016. The worldwide spread of the tiger mosquito as revealed by mitogenome haplogroup diversity. Front. Genet. 7: 208. https://doi.org/10.3389/FGENE.2016.00208.

Berlin, O.G.W. 1969. Mosquito studies (Diptera, Culicidae) XII. A revision of the neotropical subgenus *Howardina* of *Aedes*. Contrib. Am. Entomol. Inst. 4(2): 1–190.

Black, W.C. and N.M. DuTeau. 1997. RAPD-PCR and SSCP analysis for insect population genetic studies, Pp. 524–531. *In* Crampton, J.M. and C. B. Beard (eds.), The Molecular Biology of Insect Disease Vectors. Chapman & Hall, New York, NY.

Casas-Martínez, M., R. Tamayo-Domínguez, J.G. Bond-Compeán, J.C. Rojas, M. Weber and A. Ulloa-García. 2020. Oogenic development and gonotrophic cycle of *Aedes aegypti* and *Aedes albopictus* in laboratory. Salud Publica Mex. 62: 372–378. https://doi.org/10.21149/10164.

Clark-Gil, S. and R.F. Darsie. 1983. The mosquitoes of Guatemala, their identification, distribution and bionomics, with keys to adult females and larvae. Mosq. Syst. 15: 151–294.

Contreras-Perera, Y.J., M. Briceño-Mendez, A.E. Flores-Suárez, P. Manrique-Saide, J.A. Palacio-Vargas, H. Huerta-Jimenez and A. Martin-Park. 2019. New record of *Aedes albopictus* in a suburban area of Merida, Yucatan, Mexico. J. Am. Mosq. Control Assoc. 35: 210–213. https://doi.org/10.2987/18-6797.1.

Correa-Morales, F., C. González-Acosta, D. Mejía-Zúñiga, H. Huerta, C. Pérez-Rentería, M. Vazquez-Pichardo, A.I. Ortega-Morales, L.M. Hernández-Triana, V.M. Salazar-Bueyes and M. Moreno-García. 2019. Surveillance for Zika in Mexico: Naturally infected mosquitoes in urban and semi-urban areas. Pathog. Glob. Health 113: 309–314. https://doi.org/10.1080/20477724.2019.1706291.

Cui, G., S. Zhong, T. Zheng, Z. Li, X. Zhang, C. Li, E. Hemming-Schroeder, G. Zhou and Y. Li. 2021. *Aedes albopictus* life table: Environment, food, and age dependence survivorship and reproduction in a tropical area. Parasit. Vectors 14: 568. https://doi.org/10.1186/S13071-021-05081-X.

Dávalos-Becerril, E., F. Correa-Morales, C. González-Acosta, R. Santos-Luna, J. Peralta-Rodríguez, C. Pérez-Rentería, J. Ordoñez-Álvarez, H. Huerta, M. Carmona-Perez, J.A. Díaz-Quiñonez, M.D. Mejía-Guevara, G. Sánchez-Tejeda, P. Kuri-Morales, J.F. González-Roldán and M. Moreno-García. 2019. Urban and semi-urban mosquitoes of Mexico City: A risk for endemic mosquito-borne disease transmission. PLoS One 14: e0212987. https://doi.org/10.1371/JOURNAL.PONE.0212987.

Delatte, H., G. Gimonneau, A. Triboire and D. Fontenille. 2009. Influence of temperature on immature development, survival, longevity, fecundity, and gonotrophic cycles of *Aedes albopictus*, vector of chikungunya and dengue in the Indian Ocean. J. Med. Entomol. 46: 33–41. https://doi.org/10.1603/033.046.0105.

Ezeakacha, N.F. and D.A. Yee. 2019. The role of temperature in affecting carry-over effects and larval competition in the globally invasive mosquito *Aedes albopictus*. Parasit. Vectors 12: 123. https://doi.org/10.1186/s13071-019-3391-1.

Folmer, O, M. Black, W. Hoeh, R. Lutz and R. Vrijenhoek. 1994. DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. Mol. Mar. Biol. Biotechnol. 3: 294–299.

Garcia-Rejon, J.E., J.A. Chan-Orilla, N. Cigarroa-Toledo, W.A. Chi-Chim, O.M. Torres-Chable, G.A. Cruz-Escalona, C. Machain-Williams, J. Mendez-Galvan, J.C. Tzuc-Dzul and C.M. Baak-Baak. 2018. Laboratory evaluation of the Ile1,016 mutation-effect on several life-history parameters of *Aedes aegypti*. Int. J. Mosq. Res. 5: 112–120.

Garcia-Rejon, J.E., J.C. Navarro, N. Cigarroa-Toledo and C.M. Baak-Baak. 2021. An updated review of the invasive *Aedes albopictus* in the Americas; geographical distribution, host feeding patterns, arbovirus infection, and the potential for vertical transmission of dengue virus. Insects 12 (11): 967. https://doi.org/10.3390/INSECTS12110967.

Gerhardt, R.R., K.L. Gottfried, C.S. Apperson, B.S. Davis, P.C. Erwin, A.B. Smith, N.A. Panella, E.E. Powell and R.S. Nasci. 2001. First isolation of La Crosse virus from naturally infected *Aedes albopictus*. Emerg. Infect. Dis. 7: 807–811. https://doi.org/10.3201/eid0705.017506.

Gomes, A.C., N.N. Silva, G.R. Marques and M. Brito. 2003. Host-feeding patterns of potential human disease vectors in the Paraíba Valley region, State of Säo Paulo, Brazil. J. Vector Ecol. 28: 74–78.

Hall, T.A. 1999. BioEdit: A user-friendly biological sequence alignment editor and analysis. Nucleic Acids Symp. Ser. 41: 95–98.

Hernández-Rodríguez, J.L., R. Perez-Pacheco, A. Vásquez-López, M.C. Mejenes-Hernández, C.A. Granados-Echegoyen, I.D.R. Arcos-Cordova, C. Pérez-Rentería, J.I. Benítez-Alva, P. Manrique-Saide and H. Huerta. 2020. Asian tiger mosquito in Yucatan Peninsula: First record of *Aedes* (*Stegomyia*) *albopictus* (Diptera: Culicidae) in Campeche, Mexico. J. Med. Entomol. 57: 2022–2024. https://doi.org/10.1093/JME/TJAA133.

Hernández-Triana, L.M., J.A. Garza-Hernández, A.I. Ortega Morales, S.W.J. Prosser, P.D.N. Hebert, N.I. Nikolova, E. Barrero, E.J. de Luna-Santillana, V.H. González-Alvarez, R. Mendez-López, R.J. Chan-Chable, A.R. Fooks and M.A. Rodríguez-Pérez. 2021. An integrated molecular approach to untangling host-vector-pathogen interactions in mosquitoes (Diptera: Culicidae) from sylvan communities in Mexico. Front. Vet. Sci. 7: 564791. https://doi.org/10.3389/FVETS.2020.564791.

Holick, J., A. Kyle, W. Ferraro, R.R. Delaney and M. Iwaseczko. 2002. Discovery of *Aedes albopictus* infected with West Nile virus in southeastern Pennsylvania. J. Am. Mosq. Control Assoc. 18(2): 131

Huerta, H., J.F. González-Roldán, G. Sánchez-Tejeda, F. Correa-Morales, F.E. Romero-Contreras, R. Cárdenas-Flores, M.L. Rangel-Martínez, J.M. Mata-Rivera, J.J. Siller-Martínez, G.M. Vazquez-Prokopec, P. Manrique-Saide, F. Dzul-Manzanilla, M.

Vázquez-Pichardo, C. Rosales-Jiménez, M.L. Torres-Rodríguez, A. Núñez-León, B. Torres-Longoria, I. López-Martínez, C. Ruíz-Matus, P.A. Kuri-Morales and J.A. Díaz-Quiñónez. 2017. Detection of Zika virus in *Aedes* mosquitoes from Mexico. Trans. R. Soc. Trop. Med. Hyg. 111: 328–331. https://doi.org/10.1093/trstmh/trx056.

Ibañez-Bernal, S., B. Briseno, J.P. Mutebi, E. Argot, G. Rodríguez, C. Martínez-Campos, R. Paz, P. de la Fuente-San Román, R. Tapia-Conyer and A. Flisser. 1997. First record in America of *Aedes albopictus* naturally infected with dengue virus during the 1995 outbreak at Reynosa, Mexico. Med. Vet. Entomol. 11: 305–309. https://doi.org/10.1111/j.1365-2915.1997.tb00413.x.

Juliano, S.A., G.F. O'Meara, J.R. Morrill and M.M. Cutwa. 2002. Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. Oecologia 130: 458–469. https://doi.org/10.1007/S004420100811.

Kumar, S., G. Stecher, M. Li, C. Knyaz and K. Tamura. 2018. MEGA X: Molecular evolutionary genetics analysis across computing platforms. Mol. Biol. Evol. 35: 1547–1549. https://doi.org/10.1093/MOLBEV/MSY096.

Mitchell, C.J., L.D. Haramis, N. Karabatsos, G.C. Smith and V.J. Starwalt. 1998. Isolation of La Crosse, Cache Valley, and Potosi viruses from *Aedes* mosquitoes (Diptera: Culicidae) collected at used-tire sites in Illinois during 1994–1995. J. Med. Entomol. 35: 573–577. https://doi.org/10.1093/jmedent/35.4.573.

Mitchell, C.J., M.L. Niebylski, G.C. Smith, N. Karabatsos, D. Martin, J.P. Mutebi, G.B. Craig Jr. and M.J. Mahler. 1992. Isolation of eastern equine encephalitis virus from *Aedes albopictus* in Florida. Science 257: 526–527. https://doi.org/10.1126/science.1321985.

Niebylski, M.L., H.M. Savage, R.S. Nasci and G.B. Craig Jr. 1994. Blood hosts of *Aedes albopictus* in the United States. J. Am. Mosq. Control Assoc. 10: 447–450.

Ortega-Morales, A.I., G. Bond, R. Méndez-López, J.A. Garza-Hernández, L.M. Hernández-Triana and M. Casas-Martínez. 2018. First record of invasive mosquito *Aedes albopictus* in Tabasco and Yucatan, Mexico. J. Am. Mosq. Control Assoc. 34: 120–123. https://doi.org/10.2987/18-6736.1.

Ortega-Morales, A.I., C. Pérez-Rentería, J. Ordóñez-Álvarez, J.A. Salazar, F. Dzul-Manzanilla, F. Correa-Morales and H. Huerta-Jiménez. 2022. Update on the dispersal of *Aedes albopictus* in Mexico: 1988–2021. Front. Trop. Dis. 2. https://doi.org/10.3389/FITD.2021.814205.

Reinhold, J.M., C.R. Lazzari and C. Lahondère. 2018. Effects of the environmental temperature on *Aedes aegypti* and *Aedes albopictus* mosquitoes: A review. Insects 9(4): 158. https://doi.org/10.3390/INSECTS9040158.

Rueda, L.M. 2004. Pictorial keys for the identification of mosquitoes (Diptera: Culicidae) associated with dengue virus transmission. Zootaxa 589(1). https://doi.org/10.11646/zootaxa.589.1.1.

Salomón-Grajales, J., G.V. Lugo-Moguel, V.R. Tinal-Gordillo, J. de La Cruz-Velázquez, B.J. Beaty, L. Eisen, S. Lozano-Fuentes, C.G. Moore and J.E. García-Rejón. 2012. *Aedes albopictus* mosquitoes, Yucatan Peninsula, Mexico. Emerg. Infect. Dis. 18: 525–527. https://doi.org/10.3201/EID1803.111626.

Sanchez-Rodríguez, O.S., R.M. Sanchez-Casas, M. Laguna-Aguilar, M.S. Alvarado-Moreno, E.A. Zarate-Nahon, R. Ramirez-Jimenez, C.E. Medina de la Garza, R. Torres-Zapata, M. Dominguez-Galera, P. Mis-Avila and I. Fernandez-Salas. 2014. Natural transmission of dengue virus by *Aedes albopictus* at Monterrey, northeastern Mexico. Southwest. Entomol. 39: 459–468. https://doi.org/10.3958/059.039.0307.

Savage, H.M., M.L. Niebylski, G.C. Smith, C.J. Mitchell and G.B. Craig Jr. 1993. Host-feeding patterns of *Aedes albopictus* (Diptera: Culicidae) at a temperate North American site. J. Med. Entomol. 30: 27–34. https://doi.org/10.1093/jmedent/30.1.27.

Service, M. 1993. Mosquito Ecology. Field Sampling Methods. Elsevier Science, London, U.K.

Stenn, T., K.J. Peck, P.G. Rocha and N.D. Burkett-Cadena. 2019. Vertebrate hosts of *Aedes aegypti, Aedes albopictus,* and *Culex quinquefasciatus* (Diptera: Culicidae) as

potential vectors of Zika virus in Florida. J. Med. Entomol. 56: 10–17. https://doi.org/10.1093/jme/tjy148.

Talavera-Aguilar, L.G., R.A. Murrieta, S. Kiem, R.C. Cetina-Trejo, C.M. Baak-Baak, G.D. Ebel, B.J. Blitvich and C. Machain-Williams. 2021. Infection, dissemination, and transmission efficiencies of Zika virus in *Aedes aegypti* after serial passage in mosquito or mammalian cell lines or alternating passage in both cell types. Parasit. Vectors 14(1): 261. https://doi.org/10.1186/S13071-021-04726-1.

Tamura, K., G. Stecher and S. Kumar. 2021. MEGA11: Molecular evolutionary genetics analysis, version 11. Mol. Biol. Evol. 38: 3022–3027. https://doi.org/10.1093/MOLBEV/MSAB120.

Wat'senga Tezzo, F., S. Fasine, Z.E. Manzambi, M.D.C. Marquetti, G.B. Mbuka, G. Ilombe, R.M. Takasongo, N. Smitz, J.A. Bisset, W. Van Bortel and V. Vanlerberghe. 2021. High *Aedes* spp. larval indices in Kinshasa, Democratic Republic of Congo. Parasit. Vectors 14(1): 92. https://doi.org/10.1186/S13071-021-04588-7.

Westbrook, C.J., M.H. Reiskind, K.N. Pesko, K.E. Greene and L.P. Lounibos. 2010. Larval environmental temperature and the susceptibility of *Aedes albopictus* Skuse (Diptera: Culicidae) to chikungunya virus. Vector Borne Zoonotic Dis. 10: 241–247. https://doi.org/10.1089/VBZ.2009.0035.