Aedes albopictus infection by the native bacterium *Elizabethkingia anophelis* in Southern Mexico

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Abstract

Objective. To evaluate the presence of *Elizabethkingia anophelis* infection in *Aedes albopictus* wild populations of Southern Mexico. **Materials and methods.** Eight sites were selected to collect *Aedes albopictus* in the Soconusco region, Chiapas, females were analyzed to amplify the Gyrase B gene by PCR, the minimum infection rate of *E. anophelis* was calculated and its species was determined by sequencing and phylogeny. **Results.** The presence of *E. anophelis* was only observed in Huehuetán with a minimum infection rate of 37.8%. **Conclusion.** A local strain of *E. anophelis* was detected for the first time in *Ae. albopictus* from Chiapas and this bacterium could be considered a candidate for study as a probable control agent or as a vehicle for transgenesis.

Keywords: *bacterium*; *Aedes*; Mexico; *Elizabethkingia*

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Resumen

Objetivo. Evaluar la presencia de *Elizabethkingia anophelis* en poblaciones silvestres de *Aedes albopictus* en el sur de México. **Material y métodos.** Se seleccionaron ocho sitios de colecta de *Aedes albopictus* silvestre en la región del Soconusco, Chiapas; se determinó la tasa mínima de infección de *E. anophelis* mediante la amplificación por PCR del gen Gyrase B y se determinó su especie por secuenciación y filogenia. **Resultados.** La presencia de *E. anophelis* se observó únicamente en Huehuetán con una tasa mínima de infección de 37.8%. **Conclusión.** Una cepa local de *E. anophelis* se detectó por primera vez en *Ae. albopictus* de Chiapas y esta bacteria podría considerarse un candidato para estudio como probable agente de control o como vehículo para transgénesis.

Palabras clave: bacteria; *Aedes*; México; *Elizabethkingia*

Elizabethkingia anophelis is a gram-negative bacillus distributed in natural environments and found in the microbiota of *Aedes* and *Anopheles* mosquitoes that confers protection against different pathogens.[1,2] Although *E. anophelis* is present in different organs of *Aedes* sp. mosquitoes, its role as a bacterium that induces protection against dengue and Chikungunya has not been described.[3] For this reason, it is relevant to continue

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the study of this bacterium to propose new candidates for probable control agents. In this study, we report the field infection of native *E. anophelis* in *Aedes albopictus* from the Soconusco region, Chiapas, Mexico.

Materials and methods

Females of *Ae. albopictus* were collected in eight localities in the central part of the Soconusco, Chiapas (15°19′ N, 92°44′ W) (figure 1) during the rainy season of 2017. Subsequently, they were stored in groups of five individuals, and preserved at -20 °C. Genomic DNA was extracted using DNAzol kit and analyzed by PCR to determine the presence of *E. anophelis*, through amplification of a 699 bp of the Gyrase B gene (Gyr B).[4] The integrity of DNA was analyzed using specific primers to amplify the actin gene of *Ae. albopictus*.[5] To confirm the presence of *E. anophelis*, PCR products from the Gyr B gene were purified using the QIAquick PCR purification kit (QIAGEN) for sequencing. The electropherograms were visualized and edited in the software Chromas. Each sequence was compared with available sequences in the GenBank database using the BLASTn (http://blast.ncbi.nlm.nih.gov/Blast.cgi). We performed global alignment for the Gyr B gene using the Clustal W algorithm in MEGA version X. A Maximum Likelihood (ML) reconstruction was performed with 1 000 bootstraps, using the Tamura 3-parameters (T92) + I model with a BIC value of 5 403.10. All alignment sites with less than 95% site coverage were eliminated from the analysis. The obtained sequences were deposited in GenBank under the following accessions numbers OM417227, OL711949.

Results

A total of 185 specimens of *Ae. albopictus* were collected and grouped in 37 pools of five individuals each, Huixtla (2.7%), Suchiate (13.51%), Tuzantán (2.7%), Huehuetán (27%), Mazatán (5.4%), Cacahoatán (2.7%), Unión Juárez (5.4%) and Tapachula (40.52%) (figure 1). In general, *E. anophelis* infection rate was 37.8 % in *Ae. albopictus*. The presence of *E. anophelis* was only observed in mosquitoes from Huehuetán with an infection in 7/10 pools (70% infection) (figure 2A, lanes 8-10), while the bacterium was not found in the other localities (figure 2A, lanes 3-7). The Gyr B sequences obtained (OM417227; OL711949) exhibited similarities of 100% with the sequence of *E. anophelis* endosymbiont of *Anopheles sinensis* from China (CP023404.1) and *Anopheles gambiae* (CP023402) and Sweden (CP023401) (figure 2B).

Discussion

The presence of *E. anophelis* in a single place, could be associated with the composition of the environmental microbiota where the immature stages live, which is probably different from the other study areas.[6] The ML analysis showed a clustered with a

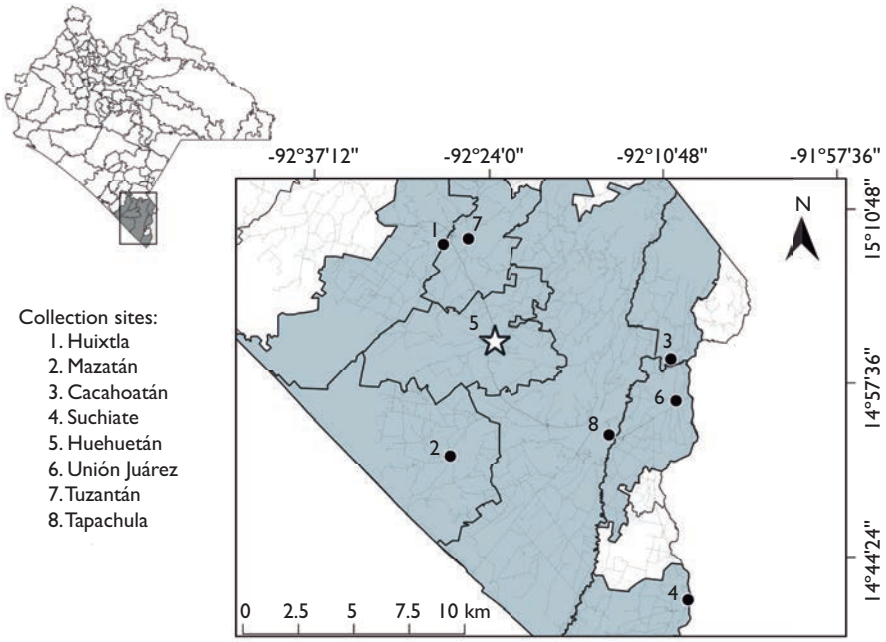

Figure 1. Study area. Collection sites sampled from mosquitoes. May to July, 2017

bootstrap value of 100% with other species of the genus *Elizabethkingia*, and a bootstrap of 83% with the sequences mentioned above. According with the genetic distance the sequences obtained in this study are identical to the sequences of China, Sweden and USA, meanwhile with the rest of the sequences of the same clade the genetic distances ranged from 0.1 to 0.6% (figure 2B).

Conclusion

This study represents the first record of *E. anophelis* in wild populations of *Ae. albopictus* collected in the Soconusco region, Chiapas, Mexico. This evidence may be relevant because *E. anophelis* is an emerging bacterium that inhabits different natural environments and has been described to be present in the salivary glands,

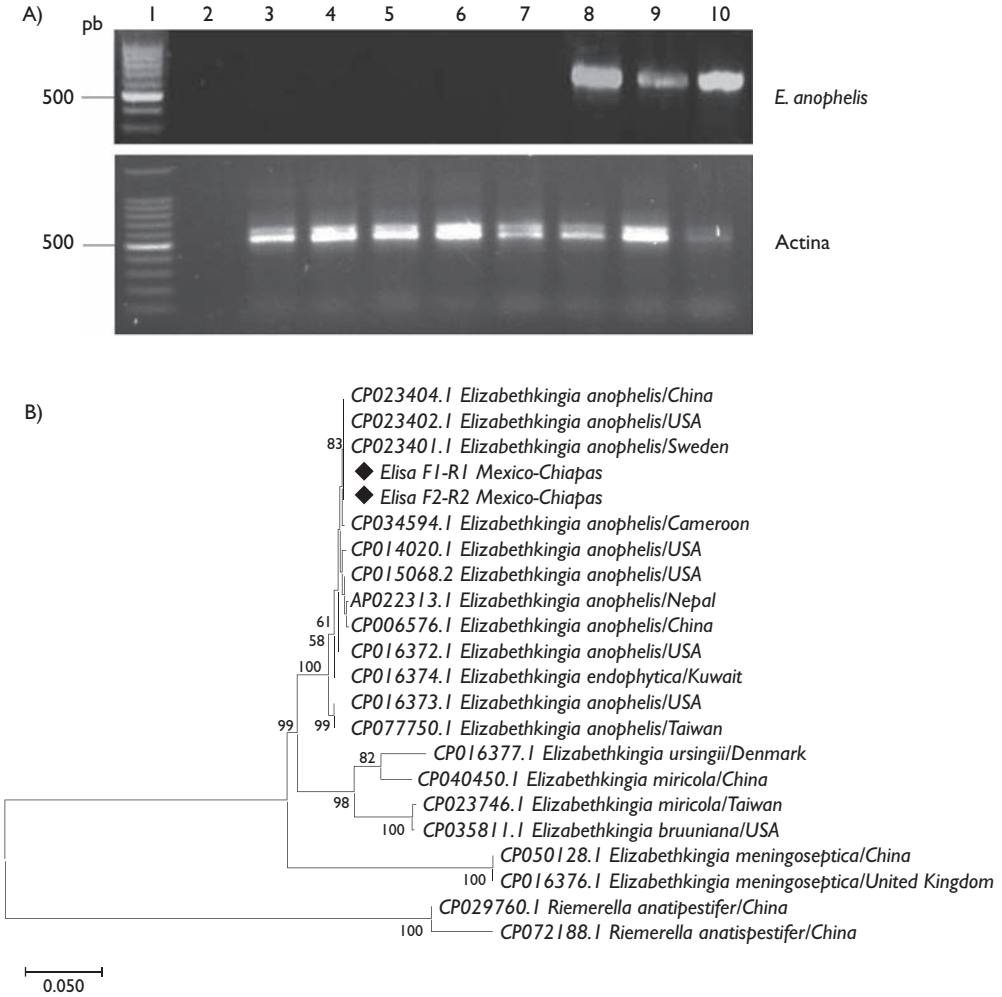

FIGURE 2. *ELIZABETHKINGIA ANOPHELIS* GYRASE B (GYRB) GENE AMPLIFICATION IN MOSQUITOES *AE. ALBOPICTUS* AND PHYLOGENETIC RELATION. PANEL A) IN THE LOWER PANEL THE ACTIN GENE AMPLIFICATION OF THE SAME DNA SAMPLES IS SHOWN AS CONTROL. 1) 100 BP LADDER; 2) NEGATIVE CONTROL; 3) *AE. ALBOPICTUS* FROM TAPACHULA; 5) CACAHOATÁN; 6) HUIXTLA; 7) MAZATÁN; 8), 9), Y 10) *AE. ALBOPICTUS* FROM HUEHUETÁN. IN THE UPPER PANEL, THE AMPLIFICATION OF *E. ANOPHELIS* IS SHOWN ONLY IN THE HUEHUETAN SAMPLES. PANEL B) PHYLOGENETIC ANALYSIS USING MAXIMUM LIKELIHOOD FOR A FRAGMENT OF THE *E. ANOPHELIS* GYRB GENE DETECTED IN HUEHUETÁN, CHIAPAS. THE OBTAINED SEQUENCES ARE MARKED WITH A DIAMOND

midgut, and reproductive organs of mosquitoes, which is transmitted vertically and horizontally, and has blocking effects for viruses. Therefore, the identification of *E. anophelis* begins the possibility of proposing candidates for the study of control agents that could help the expression of antipathogenic molecules to prevent viral replication.

Declaration of conflict of interests. The authors declare that they have no conflict of interests.

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