Acta Tropica 157 (2016) 30–41

Contents lists available at ScienceDirect

Acta Tropica

journal homepage: www.elsevier.com/locate/actatropica

Population genetics and ecological niche of invasive *Aedes albopictus* in Mexico

Angélica Pech-May[a,b], David A. Moo-Llanes[a], María Belem Puerto-Avila[a], Mauricio Casas[a], Rogelio Danis-Lozano[a], Gustavo Ponce[c], Ezequiel Tun-Ku[a], José Francisco Pinto-Castillo[a], Alejandro Villegas[d], Clemente R. Ibáñez-Piñon[e], Cassandra González[d], Janine M. Ramsey[a,*]

[a] *Instituto Nacional de Salud Pública/Centro Regional de Investigación en Salud Pública, Tapachula, Chiapas, Mexico*
[b] *Instituto Nacional de Medicina Tropical, Ministerio de Salud de la Nación, CONICET, Puerto Iguazú, Misiones, Argentina*
[c] *Universidad Autónoma de Nuevo León, Facultad de Ciencias Biológicas, San Nicolás de los Garza, Nuevo León, Mexico*
[d] *Servicios de Salud del Estado de Morelos, Cuernavaca, Morelos, Mexico*
[e] *Servicios de Salud del Estado de Oaxaca, Oaxaca, Oaxaca, Mexico*

ARTICLE INFO

Article history:
Received 28 August 2015
Received in revised form 7 January 2016
Accepted 20 January 2016
Available online 24 January 2016

Keywords:
Aedes albopictus
Population genetics
Ecological niche
Mexico
Climate change
Invasive species

ABSTRACT

The Asian tiger mosquito *Aedes albopictus* (Skuse), is one of the most invasive mosquito species worldwide. In Mexico it is now recorded in 12 states and represents a serious public health problem, given the recent introduction of Chikungunya on the southern border. The aim of this study was to analyze the population genetics of *A. albopictus* from all major recorded foci, and model its ecological niche. Niche similarity with that from its autochthonous distribution in Asia and other invaded countries were analyzed and its potential future expansion and potential human exposure in climate change scenarios measured. We analyzed 125 sequences of a 317 bp fragment of the cyt *b* gene from seven *A. albopictus* populations across Mexico. The samples belong to 25 haplotypes with moderate population structuring (Fst = 0.081, $p < 0.02$) and population expansion. The most prevalent haplotype, found in all principal sites, was shared with the USA, Brazil, France, Madagascar, and Reunion Island. The ecological niche model using Mexican occurrence records covers 79.7% of the country, and has an 83% overlap with the Asian niche projected to Mexico. Both Neotropical and Nearctic regions are included in the Mexican niche model. Currently in Mexico, 38.6 million inhabitants are exposed to *A. albopictus*, which is expected to increase to 45.6 million by 2070. Genetic evidence supports collection information that *A. albopictus* was introduced to Mexico principally by land from the USA and Central and South America. Prevalent haplotypes from Mexico are shared with most invasive regions across the world, just as there was high niche similarity with both natural and invaded regions. The important overlap with the Asian niche model suggests a high potential for the species to disperse to sylvatic regions in Mexico.

1. Introduction

Aedes albopictus (Skuse) (Diptera: Culicidae), better known as the "tiger mosquito", originated in Asia and is now distributed throughout tropical and temperate areas of all continents (Benedict et al., 2007). Its distribution has dramatically shifted as a result of introduction of the species from the Orient to the New World, Europe and Africa. One of the main dispersal mechanisms is via international trade and ground transport of used tires and lucky bamboo (*Dracena sanderiana*) (Hawley et al., 1987; Craven et al., 1988; Fontenille and Toto, 2001; Scholte et al., 2008; Medlock et al., 2012). The species invasion of the American continent probably initiated in North America, first collected in the USA in 1985 (Sprenger and Wuithiranyagool, 1986). It has subsequently spread throughout the eastern and central US, as it has to South America (Brazil) (Forattini, 1986). Much later, in 1998, the species was identified in the Misiones province of northern Argentina, on the border with Brazil (Rossi et al., 1999).

A. albopictus was first collected in Mexico in the northern state of Coahuila in 1993, along the non-coastal region of the Mexico-USA border (Ibáñez-Bernal and Martínez-Campos, 1994). By a year later, it was also collected from the northeast Gulf of Mexico coast

* Corresponding author.
E-mail addresses: jramsey@insp.mx, janineramseyw@gmail.com (J.M. Ramsey).

http://dx.doi.org/10.1016/j.actatropica.2016.01.021
0001-706X/

area, in and around Monterrey, Nuevo León (Ibáñez-Bernal and Martinez-Campos, 1994; Rodríguez-Tovar and Ortega-Martínez, 1994; Ibáñez-Bernal et al., 1997; Orta-Pesina et al., 2001). The species then dispersed progressively to other northern border states covering the northern width of Mexico from the Pacific coast, over the Neovolcanic Belt, and to the Gulf of Mexico in the span of a decade (Rodríguez-Tovar and Ortega-Martínez, 1994; Orta-Pesina et al., 2001, 2005; Reyes-Villanueva et al., 2013).

In 2002, specimens were collected close to the southern Mexico-Guatemala border although not in central Mexico, as would have been expected had there been natural north-south dispersal (Casas-Martínez and Torres-Estrada, 2003). The species was periodically reported after that year from other southern states by primary healthcare personnel, and finally in 2010 from the Neovolcanic high plains south of Mexico City, in Morelos (Villegas-Trejo et al., 2010). In 2012, a report confirmed the species from the north-east region of the Yucatan Peninsula, in Cancun (Salomón-Grajales et al., 2012). Currently, *A. albopictus* is recorded from 12 states in Mexico (Chiapas, Coahuila, Hidalgo, Morelos, Nuevo León, Oaxaca, Puebla, Querétaro, Quintana Roo, San Luis Potosí, Tamaulipas, and Veracruz) (CONABIO, 2013).

A wide range of amphibians, reptiles, birds, and mammals are natural hosts of *A. albopictus* in conserved, and anthropic environments, suggesting that the species can be a bridge vector of zoonotic pathogens for humans (Delatte et al., 2010). In human domestic habitats, it feeds and rests outdoors and on vegetation (Marquetti et al., 2000; Delatte et al., 2010), which implies that *A. albopictus* densities are usually highest in rural and suburban areas (Hawley, 1988). It is a confirmed secondary vector of dengue virus in the American continent (Serufo et al., 1993; Ibáñez-Bernal et al., 1997), even though vector competence and its specific role in maintenance and transmission of the virus is still being studied (Lourenco-de Oliveira et al., 2003; Lambrechts et al., 2010). Most notoriously, the species is a known vector of alphavirus such as Chikungunya, (recently introduced into southern Mexico in 2014), Eastern equine encephalitis virus and Venezuelan equine encephalitis virus; of Flavivirus such as Saint Louis encephalitis and West Nile virus, and Bunyavirus such as La Crosse encephalitis virus (Francy et al., 1990; Gratz 2004; Paupy et al., 2009; Paupy et al., 2010).

Information about the genetic structure and the molecular basis of the genomic background of specific vectors provides evidence of population diversity, an important parameter to evaluate control interventions (Ayres et al., 2002). Molecular genetic markers have been increasingly applied to assess genetic divergence or similarities among geographically isolated populations (Avise and Ball, 1990). Many authors have used one or several mtDNA markers to analyze the probable origin of this invasive species (Usmani-Brown et al., 2009; Porretta et al., 2012; Zhong et al., 2013). Phylogenetic data reported by Mousson et al. (2005), using cyt *b*, COI and ND5 genes, suggest that *A. albopictus* is part of a paraphyletic lineage with two major clades, an Asian lineage (Cambodia, Vietnam and Thailand) and another from South America (Brazil). A recent study has provided evidence for *A. albopictus* populations in Los Angeles (California) which had greater identity with Asian populations, as compared with those from the eastern and Southeastern USA (Zhong et al., 2013). Some studies comparing autochthonous populations (Asian region- Thailand, Cambodia, Vietnam, and Madagascar) versus invasive non-autochthonous populations (USA, Brazil, and Italy) indicate that population structure may be due to global mobility and gene flow (Usmani-Brown et al., 2009; Kamgang et al., 2011; Raharimalala et al., 2012). However, none of these studies have explored population structure or diversity and the speciesí or populationís geographic distribution.

Given the species' important invasive capacity, several studies have now attempted to develop ecological niche or environmental models in order to understand potential natural species distributions, to analyze disease transmission risk components, and to quantify dispersal potential (Benedict et al., 2007; Medley, 2010; Jacob et al., 2011; Ogden et al., 2014; Campbell et al., 2015). In a study focusing on Asian populations, Porretta et al. (2012) provide evidence that ancestral populations of *A. albopictus* did not experience a fragmentation phase (around the glacial period), but have undergone population growth in Asia as interconnected populations. In contrast, Medley (2010) has argued that the *A. albopictus* niche adapts along with its invasive capacity, although there is no attempt to adjust for realized niche restrictions, such as founder effects in domestic habitats (i.e., sympatric with *Aedes aegypti*). A recent study on the projected dispersal in climate change scenarios using niche models, suggests a doubling of the area with US populations to the northeast by the end of the century (Rochlin et al., 2013), and using climate indicators, another study predicted a general northward range expansion for the US (Ogden et al., 2014).

Global dispersal of *A. albopictus* has occurred via maritime and commercial air routes between continents (Reiter and Sprenger, 1987; Romi, 2001; Gratz, 2004). Similar to its delayed appearance on the west coast of the US, it is noteworthy that *A. albopictus* was not collected in Mexico prior to the 1990s, in either coastal areas or major maritime ports, and that a decade occurred between the first collection of *A. albopictus* along the US border in the northeast Gulf of Mexico region, and that from the southern border, nearest to the Pacific coast, where there is no major mercantile shipping. The question hence arises regarding what dispersal mechanisms and routes have been and may be most important for this invasive species within and to Mexico, and what is the potential for the species to invade sylvatic habitats in either Nearctic or Neotropical regions of the country. It is expected to disperse uniquely among anthropic habitats as it adapts to artificial containers, but it may not be restricted to these habitats if the predicted niche of the populations is similar to the original speciesí niche (Ali and Nayar, 1997). Global travel and sizeable international and intra-national population migrations represent unique challenges for zoonotic and vector-borne diseases. National and global surveillance systems will need timely and complete information in the future not only for vector susceptibility to population control strategies (WHO, 2012), but more specifically for disease transmission models. Vector-borne disease control programs need to understand a vector's ecological tolerance both on a macro-environmental (ecological niche), as well as a local landscape level (rural-urban gradient). The present study analyzes the current population genetics of *A. albopictus* from all major recorded foci and biogeographic regions and the ecological niche of this invasive species in Mexico. Possible future expansion and potential human exposure of *A. albopictus* has been projected in two future climate change scenarios.

2. Material and methods

2.1. Study area

Mexico has a highly diverse bio-geographical territory spanning both Nearctic and Neotropical regions, located between the coordinates 14°32′N, 32°43′N and 118°22′W, 86°42′W. The region was divided into 1, 732, 435 pixels at a resolution of 30 arc-s (0.008333° = 1 km) for latitude and longitude. The current Mexican population from the 2010 census is 112, 336, 537 inhabitants, divided into rural population (communities with < 10,000 inhabitants) (38, 491, 814) and urban population (73, 844, 723) (INEGI; www.censo2010.org.mx/; last accessed July 2014). Mexico has two principal mountain ranges along Pacific and Gulf of Mexico coasts: the Sierra Madre Occidental and the Sierra Madre Oriental, with the high plains of the Neovolcanic Belt in between.

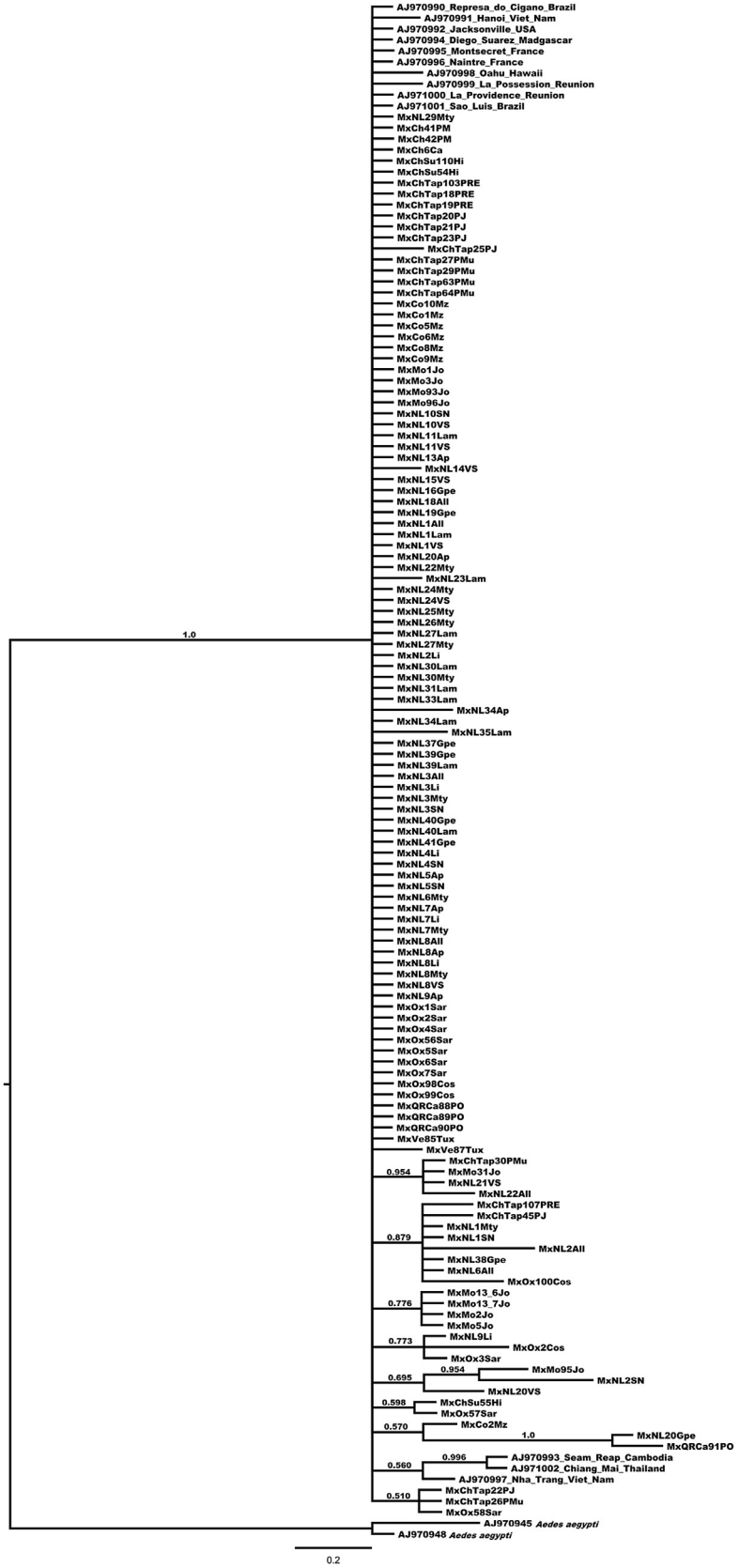

Fig. 1. Bayesian Inference (BI) topology tree for 317 nucleotides of cytochrome *b* (cyt *b*), of *A. albopictus* from Mexico inferred using the GTR + G model. Numbers on each branch (above branch) represent posterior probabilities obtained in the BI. *Aedes aegypti* was used as outgroup. The scale bar represents the expected number of nucleotide substitutions per site.

Table 1
New localities of collected for the distribution of *Aedes albopictus* in Mexico.

State	County	Locality	Sampling site	Date	Sample analyzed		Latitude	Longitude
					Female	Male		
Chiapas	Cacahoatán	Cacahoatán	Cemetery	Sep/2012	X	–	14.99110	92.17040
	Tapachula	Puerto Madero	Cemetery	Sep/2012	X	–	14.72300	92.42760
	Suchiate	Ciudad Hidalgo	Cemetery	Aug/2012	X	–	14.68590	92.14900
	Tapachula	Tapachula	Jardin Cemetery	Sep/2012	X	X	14.89500	92.24660
	Tapachula	Tapachula	Municipal Cemetery	Sep/2012	X	X	14.90000	92.2660
	Tapachula	Ejido Raymundo Enriquez	Cemetery	Sep/2012	X	X	14.86140	92.32460
Coahuila	Múzquiz	Cd. Melchor Múzquiz	Municipal Cemetery	Oct/2012	X	–	27.89172	101.52885
Morelos	Jojutla	Jojutla	Bonanza Suburb	Aug/2012	X	–	18.60590	99.20870
Nuevo León	Allende	Allende	Bicentenary Park	Sep/2012	X	–	25.28908	100.01468
	Apodaca	Apodaca	Cantizalez Suburb	May/2012	X	–	25.78385	100.19213
	Guadalupe	Guadalupe	Rincon de la Sierra suburb	Sep/2012	X	–	25.63899	100.19894
	Lampazos de Naranjo	Lampazos de Naranjo	Centro Suburb	May/2012	X	–	27.02116	100.49905
	Linares	Linares	Nueva Victoria Suburb	Sep/2012	X	–	24.86123	99.57878
	Monterrey	Monterrey	Fundidora Park	Sep/2012	X	–	25.67675	100.27972
	Salinas Victoria	San Nicolas de los Garza	Autonomous University of Nuevo León	Sep/2012	X	–	25.72981	100.30791
	Villa de Santiago	Villa de Santiago	Municipal Cemetery	Sep/2012	X	–	25.39729	100.12009
Oaxaca	Acatlán de Perez Figueroa	Cosolapa Sarmiento	Municipal Cemetery	Sep/2012	X	X	18.36070	96.40580
	San Juan Guichicovi	Paso Real de Sarabia	Municipal Cemetery	Sep/2012	X	X	17.04000	95.01290
Quintana Roo	Benito Juárez	Cancun	Los Olivos Cemetery	Sep/2012	X	–	21.14100	86.87970
Veracruz	Tuxpan	Tuxpan de Rodríguez Cano	Zapote Gordo Suburb	Nov/2009	X	–	20.57310	97.22510

Collection records for all 32 Mexican states reported prior to 2014 for *A. albopictus* were included in this study, with collection registries from the primary healthcare system (PHS) or from academic groups. *A. albopictus* specimens were collected from seven states in 2012: Chiapas, Coahuila, Morelos, Nuevo Léon, Oaxaca, Veracruz and Quintana Roo (Table 1). No specific permissions were required for mosquito collections and the field studies did not involve endangered or protected species. The collections were obtained using entomological nets and manual aspirators from 08:00–12:00 h, ovitraps, or from breeding sites. The specimens (eggs and larvae) were preserved in perforated plastic tubes and stored in sealed containers with desiccant to prevent fungal growth or were placed in breeding cups and maintained until emergence. The adults were individually examined to confirm that they were *A. albopictus* using taxonomic keys (Darsie 1986; Savage and Smith, 1995). Specimens were preserved individually in 80% ethanol and stored at −20 °C for molecular analyses.

2.2. DNA extraction, amplification, and sequencing

Total genomic DNA was extracted from specimens using a modified plasmid extraction protocol (Sambrook et al., 1989; Pech-May et al., 2013). DNA was re-suspended in 40 μl of bi-distilled water and stored at −20 °C. The mitochondrial cytochrome *b* (cyt *b*) was amplified using the oligonucleotides cyt *b* L14841 (5′-AAAAAGCTTCCATCCAACATCTCAGCATGATGAAA-3′) and cyt *b* H15149 (5′AAACTGCAGCCCCTCAGAATGATATTTGTCCTCA-3′), which amplify a fragment of approximately 380 bp (Kocher et al., 1989). PCR was performed in a 25 μl reaction volume containing 5 μl 5x Green Go Taq® reaction buffer, 3.75 mM $MgCl_2$, 0.4 mM dNTPs, 0.2 units of Go Taq® DNA polymerase (PROMEGA, Madison, Wi USA), 10 pmol/μl of each oligonucleotide and 50 ng template DNA. The amplification protocol was 5 min at 95 °C, followed by 35 cycles of 30 s at 97 °C, 45 s at 45.7 °C, and 60 s at 72 °C; followed by a 72 °C extension for 5 min, and 4 °C indefinitely. PCR products of cyt *b* were separated by electrophoresis on 1.5% agarose gels stained with 0.5 μg/ml ethidium bromide, and visualized under UV light. PCR products were purified using the QIAquick™ PCR purification kit (QIAGEN, Valencia, CA) and sequencing was carried out on a Prisma 310 ABI (High-Throughput Genomics Center, University of Washington, Department of Genome Sciences).

2.3. Genetic and phylogenetic analyses

The cyt *b* sequences of *A. albopictus* were manually aligned and edited using the software BioEdit v.7.0.9 (www.mbio.nscu.edu/bioedit/bioedit.html; Hall, 2004) and Mega v.6 (www.megasoftware.net/mega.php; Tamura et al., 2013). All sequences of Mexican haplotypes were deposited in GenBank (Accession Number: KT900921-KT900945). Genetic variability was measured using the number of mutations (η), number of segregating sites (S), number of unique sites (Su), mean number of pairwise differences (k), number of haplotypes (Nh), haplotype diversity (h), nucleotide diversity (π), and nucleotide polymorphism index (θ). In addition, Tajima's D (Tajima, 1989) and D and F indices (Fu and Li, 1993) were estimated to test for neutrality considering segregating sites using DnaSP v.5.10 software (www.ub.edu/dnasp/; Rozas and Librado, 2009).

Observed pairwise nucleotide differences between haplotypes were compared to the expected frequency distribution assuming an expansion model, to test for the effect of population growth (Mismatch distribution) (Roger and Harpending, 1992; Harpending, 1994). Harpending's raggedness index R (Harpending, 1994) and the sum of squares deviations (Ssd) were calculated to validate population expansion, using 10,000 replicates with the Arlequin v.3.5 software (www.cmpg.unive.ch/software/arlequin35/; Excoffier and Lischer, 2010). This statistic quantifies the smoothness of the observed pairwise difference distribution and a non-significant result with unimodal distribution indicates an expanding population whereas populations that have multimodal distribution of pairwise differences indicate demographic equilibrium and a subsequent bottleneck (Roger and Harpending,1992; Harpending, 1994).

Analysis of molecular variance (AMOVA) was used to estimate genetic differentiation (Fst) (Excoffier et al., 1992). Linear geographic distances between communities were estimated with ArcMap® 10. The Kimura 2-parameter (K2P) genetic distance between populations was estimated using the Mega v.6 software (Kimura 1980; Tamura et al., 2013) with 10,000 random

permutations. Thirteen sequences reported by Mousson et al. (2005) in GenBank (Accession Number: AJ970990, AJ970991, AJ970992, AJ970993, AJ970994, AJ970995, AJ970996, AJ970997, AJ970998, AJ970999, AJ971000, AJ971001, AJ971002) were used for phylogenetic analysis (Supplementary S1). The best-fit model of evolution was estimated using the Akaike Information Criterion (AIC) (Akaike, 1974) as implemented in JModeltest v.0.1.1 software (www.darwin.uvigo.es/our-software/; Posada, 2008). Intra-specific phylogeny was analyzed using MrBayes: Bayesian Inference of Phylogeny 3.2 (www.mrbayes.sourceforge.net/download.php; Ronquist et al., 2012) and four Markov Chain Monte Carlo chains were run for 10,000,000 generations (sampled every 1,000 generations) to allow adequate time for convergence ($\leq$0.008). The first 25% of sampled trees were considered as burn in. The tree was visualized with FigTree V1.4. (http://tree.bio.ed.ac.uk/software/figtree/). Sequences of *A. aegypti* were used as outgroup for MrBayes analysis (Accession Numbers: AJ970945 and AJ970948). The relationship between haplotypes was evaluated by constructing a minimum haplotype network using the median-joining method implemented in Network v.4.6 software (http://www.fluxus-engineering.com; Bandel et al., 1999), assuming epsilon of 0 and a transition/transversion of 1:2.

2.4. Ecological niche models

A database was constructed from *A. albopictus* collections reported in published literature, reports from PHS vector program personnel, and authors' unpublished collections for Mexico (N = 207; Supplementary S2). Thirteen environmental layers were used to construct the ecological niche models (ENM) (Moo-Llanes et al., 2013). Nine bioclimatic (bio1, bio4, bio5, bio6, bio7, bio12, bio13, bio14, bio15) data layers were obtained from the Worldclim-Global Climate Data (www.worldclim.org; last accessed June 2014). These bioclimatic variables were selected from 19 by choosing the more meaningfull variables hypothesized to limit speciesí distributions at coarse-grain scale (1 km^2), after analysis of multicolinearity in a correlation matrix with relatively low inter-correlation (r < 0.75) (Moo-Llanes et al., 2013). Additionally, four topographic layers (aspect, elevation, slope, topographic index) obtained from the Hydro 1k data set were used (http://eros.usgs.gov/products/elevation/gtopo30/gtopo30.html; last accessed June 2014). ENM were constructed using the Genetic Algorithm for Rule-set Prediction (GARP) (www.nhm.ku.edu/desktopgarp/; Stockwell and Peters, 1999), since GARP is the preferred model for datasets which may have heterogeneous occurrence records across a broad geographic range. The software randomly divides occurrence points into calibration data for model building (75%) and evolution data for model testing (25%). One hundred replicate models were developed for each species and a soft omission threshold of 20% of the distribution was used (Anderson et al., 2003). Models were evaluated using the partial ROC (software download http://kuscholarworks.ku.edu/dspace/handle/1808/10059), the area under the observed line of model performance is related to the area under the line of random expectations, and a ratio is calculated. Bootstrap manipulations (1000 total), in which 50% of evaluation data are resampled with replacement and AUC ratios recalculated, are used to test the hypothesis that model performance is better than random expectation. The null hypothesis of performance is no better than random expectation when 95% of bootstrap-replicate AUC ratios are 1 (Escobar et al., 2013).

Environmental parameters for future climate change models were derived from Max Planck Institute Earth System Model at base resolution (MPI-ESM-LR). The ECHAM6 is an atmospheric general circulation model, and as such focuses on the coupling between diabatic processes and large-scale circulations, both of which are ultimately driven by radiative forcing. ECHAM6 has an improved representation of radiative transfer in the short wave (or solar) part of the spectrum, a completely new description of aerosol effects, an improved representation of surface albedo, including the treatment of melt pongs on sea ice, and a greatly improved representation on the middle atmosphere as part of the standard model (Stevens et al., 2013). Representative Concentration Pathway (RCP) for the 4.5 emission scenario, representing a median estimated greenhouse gas emission was calculated to assess variation among possible future climates (IPCC, 2013). The ENM for *A. albopictus* in Mexico was projected for 2050 and 2070 using climate models along with topographic variables, and the change in geographic distribution range calculated for all scenarios using the proportion of occupied pixels. The mean and range of elevation change for ENM were also measured using the spatial analyst tools from ESRI ArcMap® 10.0. The total population growth rate for Mexico, projected to increase by 30% for both 2050 and 2070 (i.e., no additional population growth expected in the latter), was generated using projections for fertility, mortality and international migration. Population projections corresponding to ENM at different time periods were calculated using spatial analyst tools (Moo-Llanes et al., 2013).

In addition to the ENM developed for Mexico, databases were constructed for four other regions in order to analyze potential niche prediction across continents: Asia (includes Cambodia, China, Indonesia, Japan, Malaysia, Thailand and Vietnam; N = 1,867), Brazil (N = 29), Italy (N = 53), and USA (N = 5,487) (Supplementary S2). The ENM model for Asia (occupied 3, 894, 689 pixels) is considered the natural area for the species, while those from Brazil (occupied 1, 934, 852 pixels), Italy (occupied 327, 579 pixels), and the USA (occupied 3, 843, 917 pixels) are considered invaded regions. The ENM for each region was re-projected to each of the other four regions, and overlap dimensions calculated from and to the Mexican ENM. We used a background test to identify niche similarity of the Mexican model vs Asia, Mexico vs Brazil, Mexico vs Italy, and Mexico vs United States. The parameters to measure identity were the random test percentage (75%), replicated run type (bootstrap), maximum iterations (500), and the threshold rule (minimum training presence), using ENMtools *ver.*13.2 (Warren et al., 2008; http://enmtools.com/). We calculated the *"Hellinger's distance"* and *"Schoener's distance"* for all pairwise combinations. The empirical measure of niche similarity between populations was compared to a null distribution to test whether they are significantly different from similarity generated from niche models constructed with data points extracted randomly from the distribution range of the compared species. The hypothesis of similarity niche is rejected when the empirically observed value of distance (Hellinger's and Schoener's) is significantly lower than the values expected from the pseudoreplicate datasets (Warren et al., 2008).

3. Results

3.1. Genetic diversity and structure

A total of 125 *A. albopictus* specimens were sequenced from the seven collection states (Supplementary S1). The length of the sequences after alignment was 317 bp, which had 290 unique sites (91.48%), and 27 polymorphic sites (8.51%) and were A-T rich (72.8%). A total of 25 haplotypes were identified with a range of 2–13 haplotypes per population (Table 2). The overall haplotype diversity, nucleotide diversity, and nucleotide polymorphism index were Hd = 0.492 ± 0.05, π = 0.003 ± 0.006, and θ = 0.016 ± 0.003, respectively. Neutrality tests Tajima's D (−2.315), and (Fu & Li, 1993) F(−2.462) were significant (p < 0.05), consistent with population expansion (Table 2). The states of Veracruz and Morelos have the highest haplotype diversity (Hd = 1 ± 0.50 and Hd = 0.733 ± 0.101, respectively), while Quintana Roo had the

Table 2
Genetic variability indices for *Aedes albopictus*. *N*: number of sequences; *h* number of mutations; *S*: number of segregating sites; *Su*: number of unique sites; *k*: mean number of pairwise differences; *Nh*: number of haplotypes; *h*: haplotype diversity; π: nucleotide diversity; θ: nucleotide polymorphism index. SD: standard deviation. Fu & Li's *D* and *F* indices (Fu & Li, 1993) and Tajima's *D* (Tajima, 1989). $^{*}p < 0.05$; $^{**}p < 0.02$.

Populations	*N*	*h*	*S*	*Su*	*k*	*Nh*	$h \pm$ SD	$\pi \pm$ SD	$\theta \pm$ SD	Fu & Li		Tajima
										D	*F*	*D*
Chiapas	22	6	6	311	0.866	7	0.541 ± 0.125	0.002 ± 0.0008	0.005 ± 0.002	−0.917	−1.244	−3.889**
Coahuila	7	1	1	316	0.286	2	0.286 ± 0.196	0.0009 ± 0.0006	0.001 ± 0.001	−1.048	−1.101	−1.006
Morelos	10	4	4	313	1.156	4	0.733 ± 0.101	0.003 ± 0.001	0.004 ± 0.002	−1.127	−1.145	−0.701
Nuevo León	66	21	20	297	0.924	13	0.403 ± 0.077	0.002 ± 0.0008	0.013 ± 0.002	−3.904**	−3.998**	−2.387**
Oaxaca	14	8	8	309	1.264	6	0.604 ± 0.150	0.003 ± 0.001	0.007 ± 0.002	−2.165	−2.386	−1.876*
Quintana Roo	4	7	7	310	3.5	2	0.500 ± 0.265	0.011 ± 0.005	0.012 ± 0.004	−0.817	−0.796	−0.817
Veracruz	2	1	1	316	1	2	1 ± 0.50	0.003 ± 0.001	0.003 ± 0.003	X	X	X
Global	125	29	27	290	1.051	25	0.492 ± 0.05	0.003 ± 0.006	0.016 ± 0.003	−1.882	−2.462*	−2.315**

highest nucleotide diversity ($\pi = 0.011 \pm 0.005$) and Nuevo León had the highest nucleotide polymorphism index ($\theta = 0.013 \pm 0.002$). All neutrality tests were significant for the Nuevo León population ($p < 0.05$), whereas Tajimaís *D* was significant only in populations from Chiapas and Oaxaca ($p < 0.05$) (Table 2). The mismatch distribution was unimodal for all populations, consistent with a population expansion model (except for Veracruz, probably due to too few samples). The Raggedness index *R* and the sum of squares deviations (Ssd) were not significant for Chiapas ($R = 0.071$, $p = 0.846$; Ssd = 0.005, $p = 0.721$), Coahuila ($R = 0.265$, $p = 0.606$, Ssd = 0.248, $p = 0.063$), Morelos ($R = 0.115$, $p = 0.594$; Ssd = 0.009, $p = 0.594$), Nuevo León ($R = 0.181$, $p = 0.645$; Ssd = 0.004, $p = 0.605$), while for Oaxaca ($R = 0.121$, $p = 0.997$; Ssd = 0.351, $p = 0.0001$) and Quintana Roo ($R = 0.75$, $p = 0.957$; Ssd = 0.5, $p = 0$), only *R* was not significant. Populations that had significant SSD values were Quintana Roo, which probably had too few samples, and Oaxaca, which had multiples peaks that could indicate a population bottleneck.

There was moderate population structuring (Fst = 0.081, $p < 0.02$), while within-population genetic variation (91.9%) was higher than that among populations (8.2%). Pairwise Fst values among populations were significant ($p < 0.04$) between Morelos and Oaxaca (Fst = 0.163, 361.12 km), Morelos and Nuevo León (Fst = 0.182, 789.77 km), Morelos and Coahuila (Fst = 0.203, 1062.30 km), Morelos and Chiapas (Fst = 0.211, 846.43 km), Morelos and Quintana Roo (Fst = 0.285, 1322.68 km), and Nuevo León-Quintana Roo (Fst = 0.0.287, 1451.07 km). Pairwise genetic distances were highest between populations from Quintana Roo and Oaxaca, and Quintana Roo and Morelos (Table 3).

3.2. Phylogenetic inferences

The Bayesian phylogeny was constructed using the GTR + G model as the most appropriate for the data (-INL = 830.6799, Delta AIC = 0, AIC = 2233.3599) with gamma of 0.402. Analysis provided maximum support (1.0 posterior probability, PP) for the major clade (Fig. 1). The most prevalent haplotype (H1) from the minimum spanning haplotype network included specimens from seven Mexican states (Chiapas, Coahuila, Morelos, Nuevo León, Oaxaca, Quintana Roo, and Veracruz) and is shared with Represa do Cigano and Sao Luis (Brazil), Jacksonville (USA), Diego Suarez (Madagascar), Montserrat and Naintré (France), and La Providence (Réunion) (Fig. 2). The native Asian haplotypes (H2: Hanoi, Vietnam; H3: Seam Reap, Cambodia and Chiang Mai, Thailand; H4: Nha Trang, Vietnam) are separated from H1 by one or two mutations. The most distant haplotypes were H28, a specimen from Nuevo León (MxNL20Gpe) and H29, a specimen from Quintana Roo (MxQRCa91PO) which were five and six mutations from the most prevalent haplotype, respectively. Other haplotypes shared across states were H7 and H9 (Chiapas/Oaxaca), H25 (Nuevo León/Oaxaca), and H11 (Chiapas/Morelos/Nuevo León) (Fig. 2).

3.3. *Ecological niche of* A. albopictus

The ecological niche model constructed for *A. albopictus* from Mexico covers 78.3% of the country, both coasts, although less along the Pacific vs the Gulf coast, and the Neovolcanic high plains. The species is found in both Neotropical and Nearctic biogeographic regions (Fig. 3A). The mean elevation for the ENM was 488 masl, with a range between sea level and 1,920 masl. All models (Asia, Brazil, Italy, Mexico and United States) were statistically significant and better than random expectations, with partial-ROC ratios >1 (Supplementary S3). ENMs modelled individually from Asia, Brazil, and Italy projected distributions in similar proportions to Mexico (63.1%, 50.6%, and 66.3% respectively), but that for the USA was significantly lower (36.1%; $T = 8.297$, df = 2, $p = 0.0142$; Fig. 4 and Table 4). The Mexican ENM for *A. albopictus* predicted greater potential distribution to each of the other regions than each individual ENM, with the exception of Asia: 100% of Brazil, 99.6% for Italy, and 66.9% of the US. The Mexican ENM predicted the same proportional distribution in Asia as the Asian ENM (45.9%) (Fig. 4 and Table 4). The overlap of the Mexican ENM with that of and in each region was greatest for Asia and Brazil. The Asia ENM projected to Mexico had a 67.5% similarity with the Mexican ENM, while that for Brazil was 51.6% similar, Italy 48.1%, and the USA significantly lower (25.2%; $T = 5.126$, df = 2, $p = 0.0360$). Qualitatively, the overlap between the Mexican ENM and the Asian ENM predicts most of the distribution regions in Mexico, within which both Brazilian and US ENMs are included. However, the Italian ENM covered complementary regions to the former, particularly in states within the Nearctic region: Baja California Sur, Chihuahua, Durango, Nuevo León, Tamaulipas, Sinaloa, and Sonora (Fig. 4). All pairwise comparisons of models indicated greater similarity than random in the background test (Mexico vs Asia, Mexico vs Brazil, Mexico vs Italy, and Mexico vs United States).

3.4. A. albopictus *distributions in climate change scenarios*

There was an increase over time in the geographic distribution expected for the Mexican ENM of *A. albopictus*, although a 82.3% increase in 2050 (Fig. 3B) was not sustained and resulted in an overall 75.5% increase from current to 2070 (Fig. 3C). The distribution remained relatively constant through 2050 in the Neotropical region (Chiapas, Chihuahua, Guerrero, Michoacán, Nuevo León, Oaxaca, Peninsula of Yucatán, Puebla, Tabasco, Tamaulipas, Veracruz, San Luis Potosí, and Sinaloa), while greatest overall change to 2070 was in the Nearctic region (Coahuila, Sonora, Baja California Norte, and Baja California Sur). The mean altitude of the ENM increased 73 m (to 561 masl) in 2050, although there was a subsequent decrease to 467 m above sea level in 2070.

Currently, 16, 876, 857 rural inhabitants are exposed to *A. albopictus* in Mexico, lower than that in urban communities (21, 792, 535 inhabitants). There was a 60% and 49% increase in exposed

Table 3
Kimura 2-parameter (K2P) of pairwise genetic distances (below diagonal) and geographical distance in km (above diagonal) between collection sites for *A. albopictus* in Mexico. $p \leq 0.003$.

	Veracruz	Quintana Roo	Oaxaca	Nuevo León	Morelos	Coahuila	Chiapas
Veracruz		1079.26	339.01	640.80	302.22	925.20	819.35
Quintana Roo	0.007		1019.72	1451.07	1322.68	1663.65	899.41
Oaxaca	0.004	0.008		979.42	361.12	1295.07	503.24
Nuevo León	0.003	0.007	0.003		789.77	284.50	1450.95
Morelos	0.004	0.008	0.005	0.004		1062.30	846.43
Coahuila	0.002	0.006	0.002	0.002	0.003		1732.43
Chiapas	0.003	0.007	0.003	0.003	0.004	0.002	

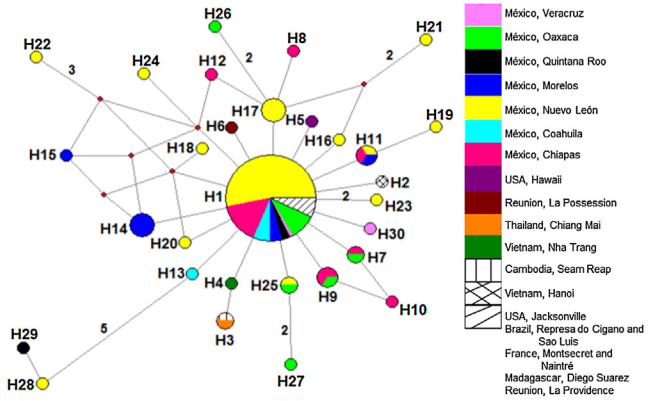

Fig. 2. Minimum haplotype network for *A. albopictus* based on 317 nucleotides of cytochrome *b* (cyt *b*). Haplotype color represents the geographic origin of each sample: The line connecting haplotypes represents one mutational step, whereas numbers along the lines are total number of mutational steps. Circle area is proportional to the frequency of each haplotype.

Table 4
ENM distribution of *A. albopictus* for four regions or countries, and proportional projection of these ENM to Mexico or of Mexico's ENM to each.

ENM	Proportion of ENM to complete region/country (%)	Proportion of each ENM to Mexico	Proportion of Mexico ENM to each region/country	Proportion of overlap of each region's ENM with the projection of the Mexican ENM to the same region	Overlap of the Mexican ENM to each region's ENM projected to Mexico
Asia	45.7	63.1	45.9	82.99	67.49
Brazil	83.4	50.6	100.0	83.37	51.60
Italy	59.1	66.3	99.6	58.89	48.13
USA	36.1	20.4	66.9	53.87	25.19

rural (27.0×10^6) and urban (32.4×10^6) populations expected in 2050, respectively, while final increases to 2070 were 24% (20.8×10^6) and 14% (24.7×10^6) for rural and urban populations, respectively.

4. Discussion

Although *A. albopictus* has been reported in Mexico for the last two decades, this is the first analysis of its genetic variation and population structure. Mitochondrial genes have been widely used to analyze population genetics and phylogeography of invasive *A. albopictus* in many countries (Urbanelli et al., 2000; Birungi and Munstermann, 2002; Mousson et al., 2005; Usmani-Brown et al., 2009; Kamgang et al., 2011; Porretta et al., 2012; Navarro et al., 2013; Zhong et al., 2013; Futami et al., 2015). Herein, differentiated haplotypes of the cyt *b* gene have been identified from all current collection areas in Mexico, even from where there are few collections (i.e., Quintana Roo and Veracruz). Haplotype diversity was moderate within Mexican states (except Coahuila), similar to that reported by Birungi and Munstermann (2002) using ND5 in Brazil and the USA and Navarro et al. (2013) in Venezuela and Colombia. Futami et al. (2015) using COI also reported similar diversity in populations from Asia, USA, Costa Rica, and Panama. However, nucleotide diversity using the cyt *b* gene in this study was double that estimated using COI and ND5 by Porretta et al. (2012) in Asia, by Futami et al. (2015) using COI in Asia, USA, Costa Rica, and Panama, or using the ND5 gene in Colombia and Venezuela (Navarro et al., 2013). Low genetic variability measured in the present study may be the result of low population size, gene drift, and/or rapid expansion of new haplotypes dispersed due to travel and commerce between urban areas (Birungi and Munstermann, 2002; Mousson et al., 2005; Mirabello and Conn, 2006). Birungi and Munstermann (2002) suggested that insecticide interventions which reduce effective populations, but do not eliminate them, reduce the gene pool and provoke genetic bottlenecks or founder effects, which subsequently accelerate gene drift. These mechanisms may be the case for synanthropic mosquito populations subjected to insecticides for dengue control in Mexico. Generally, following a brief period of low population size, population expansion ensues and results in loss of genetic variation (Nei et al., 1975). Tajima's *D* in this study (<0) supports the conclusion that Mexican populations have been through bottlenecks and population expansions with purifying selection. The unimodal mismatch distributions for all populations also support an expansion model, with the exception of those from Veracruz and Quintana Roo. Specimens from these latter two states were

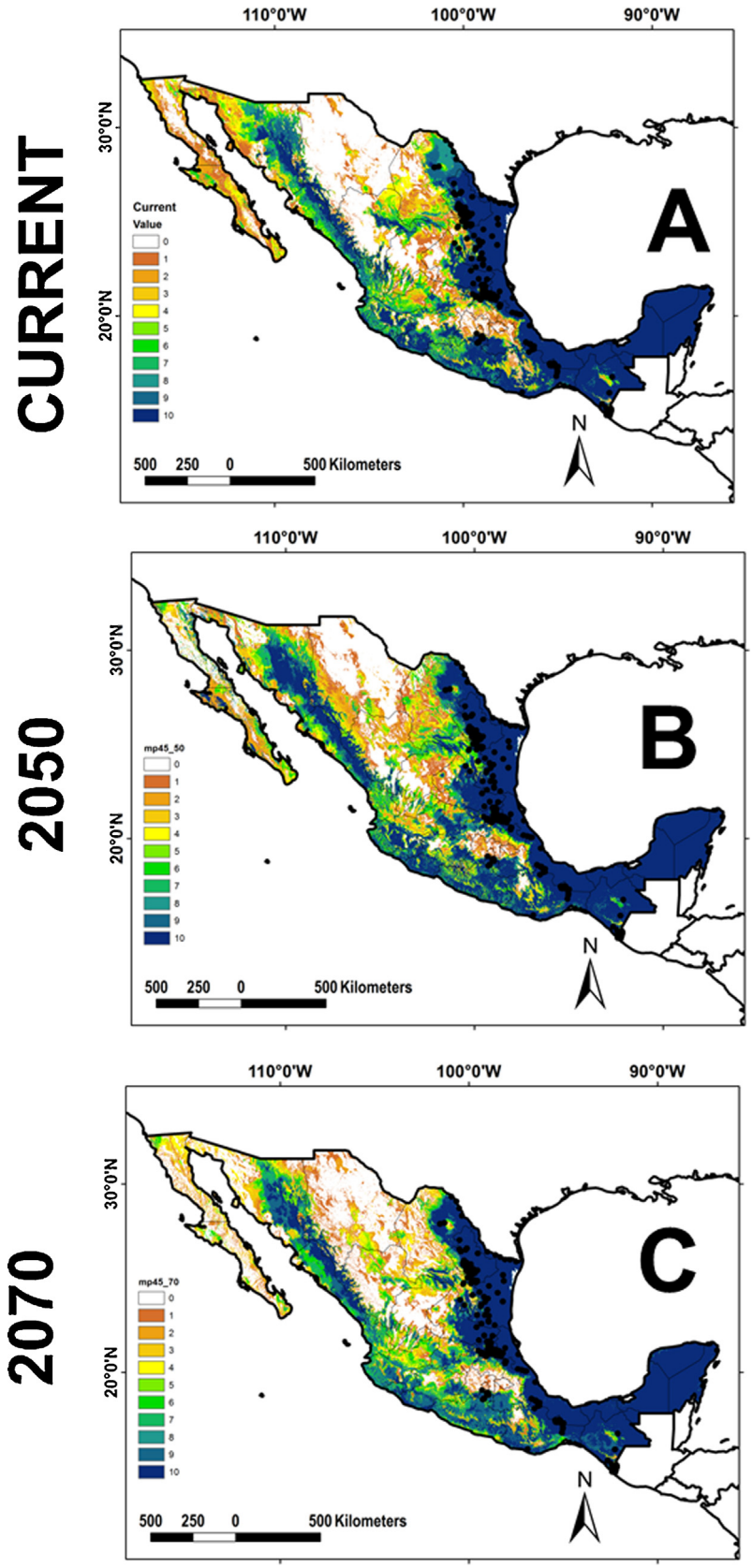

Fig. 3. Ecological niche models for *Aedes albopictus* in current and climate change scenarios. (A) Current ENM; (B) ENM using ECHAM6 model/RCP 4.5 for 2050; (C) ENM using ECHAM6 model/RCP 4.5 for 2070.

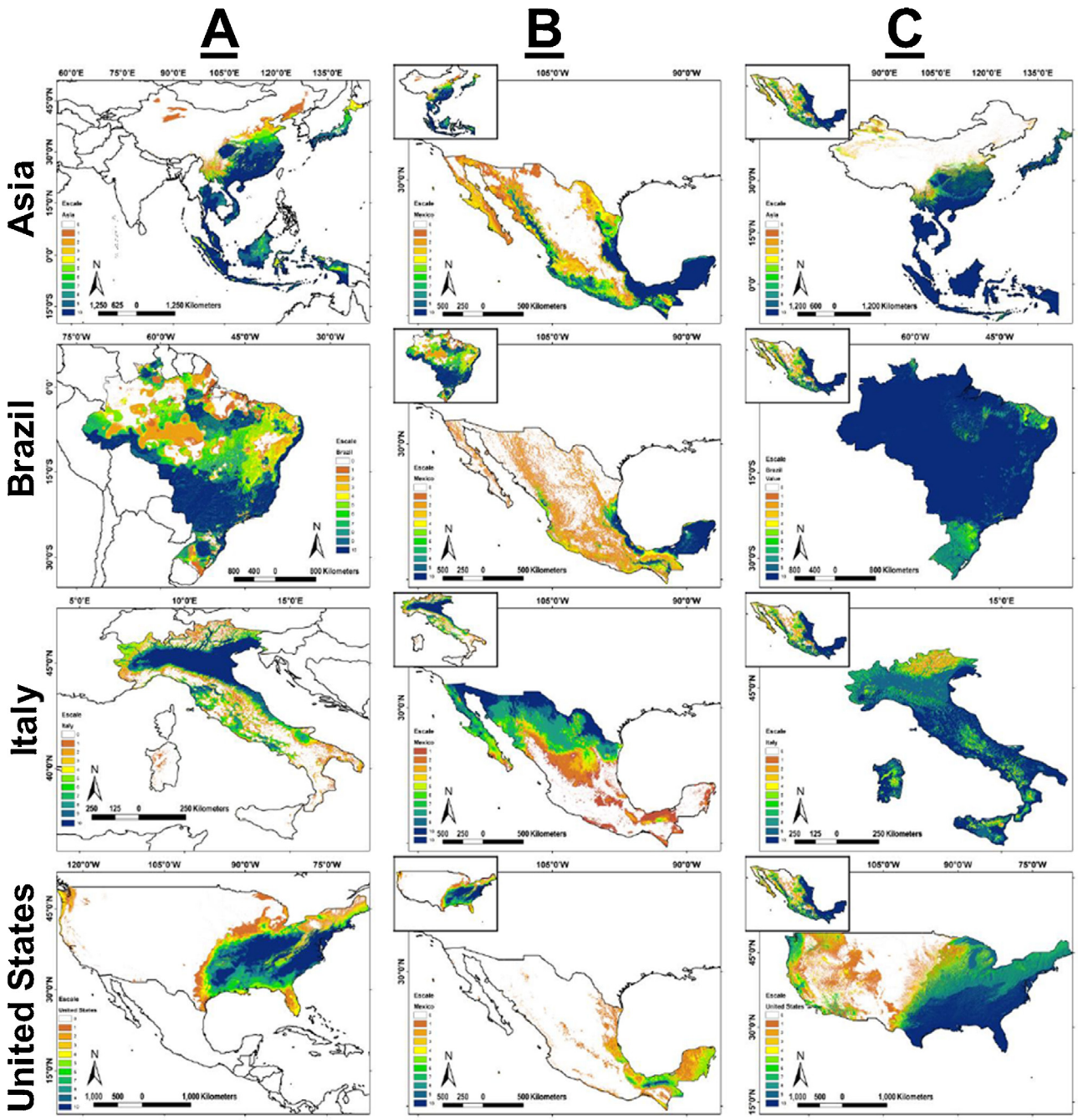

Fig. 4. Ecological niche model for *Aedes albopictus* in and projected to different regions. (A) ENM from Asia, Brazil, Italy, and United States; (B) ENM projections of each region to Mexico; (C) Mexican ENM projected to each region.

too few, while those from Oaxaca had multiples peaks that could indicate population bottlenecks.

The most prevalent and ancestral haplotype present in all Mexican collection sites is identical to that reported from Represa do Cigano and Sao Luis (Brazil), Jacksonville (USA), Diego Suarez (Madagascar), Montsecret and Naintré (France), and La Providence (Réunion). Several authors have reported genetic differences between USA and Brazilian populations using isoenzymes and ND5 (Kambhampati et al., 1991; Birungi and Munstermann, 2002; Lourenco-de Oliveira et al., 2003; Usmani-Brown et al., 2009), although, Navarro et al. (2013) using ND5 reported that the most frequent or ancestral haplotype was present in both USA and Brazilian populations, similar to results from the present study. Effective dispersal (gene flow) is one of the more prevalent evolutionary processes affecting a populationís genetic structure (Hedrick, 2005). Variable genetic differentiation was observed between Mexican populations of *A. albopictus*, although these were consistent with that reported by Kamgang et al. (2011) in Cameroun, and by Zhong et al. (2013) for Italy and Guangzhou, China, or New Jersey, Guangzhou and Japan. Population migration and commerce within and to Mexico are important factors in *A. albopictus* dispersal, since the genetic distances between populations was low, even though higher than that reported from other countries (Usmani-Brown et al., 2009).

Predominant populations of *A. albopictus* in Mexico are curiously patterned along a northeastern-southwest route, along the foothills of the Eastern Sierra Madre across the Isthmus of Tehuantepec, to the southern Pacific coast border with Guatemala. Given

heightened dengue and *A. aegypti* surveillance in the last decade in Mexico, it is tempting to suggest that these collections accurately reflect regions where the species has already dispersed via human migration or commercial transportation routes. The ENM developed from the Mexican collections alone, suggests greater potential distribution in the Yucatan Peninsula and along the Gulf of Mexico coast, although other regions along the Pacific coast and some areas of the high plains also offer appropriate niche. Climate change models do not predict significant changes for these distributions, although changes are expected to be greatest in the Nearctic region, similar to that already described for the USA by Rochlin et al. (2013) and Ogden et al. (2014).

A. albopictus was collected for the first time in Mexico in the northern state of Coahuila in 1992, and three years later from Reynosa, Tamaulipas (Ibáñez-Bernal et al., 1997). It was presumed that secondary invasive populations from the USA had been introduced into Mexico by land. The present study provides evidence that the predominant haplotype in Mexico is from secondary invasive populations, predominant in both the USA and Brazil, and that the same are also found in Europe and more remote Indian Ocean and African regions. Haplotypes from Asia were one or two mutations from the most prevalent haplotype in Mexico, which suggests that *A. albopictus* introduction has not been significant directly from Asia, via Pacific maritime routes. This, however, could change should commerce shift routes or import origins. Longitudinal sampling at key port areas along both Pacific and Gulf coasts should be considered for preventive monitoring to reduce the introduction of new populations, such as that in Australia and New Zealand (Holder et al., 2010).

Early in *A. albopictus* global dispersal, there were attempts to use ENM to predict potential distribution areas across continents and regions, based on the species' natural Asian ENM and using alternate accessible "**M**" data sets (for invaded countries). Even though these models predicted well on macro-scales for some countries (Benedict et al., 2007), they gave rise to the hypothesis that the species' niche "adapted" during its dispersal (Medley, 2010). The present study found no limitation of inter-prediction between Asia and Mexico, although this failed using the same methods for Brazil, USA, and Italy. If we assume that the Asian model, constructed with all current Asian occurrences reflects the sum of all ecological tolerances of the species' natural gene pool, then models developed from occurrences in non-native accessible regions, will be subsets of the former (Nakazawa et al., 2010). These latter are based on invasive populations initiated by few individuals and founder effects, having only a subset of environmental tolerances. If the invasive niche models (i.e., Mexico, Brazil, US, Italy) were constructed from a broad gene pool (such as native populations), or indeed from all current populations and their occurrences (Campbell et al., 2015), the inter-predictability would be high. The Mexican ENM had high predictability to Asia, and vice versa, but this was not the case for any of the other country models, for which the Mexican (and Asian) ENM generally over-predicted potential distributions. The ENM from each country, predict only minor distributions in Mexico, already recorded as part of *A. albopictus* distribution in the country. Curiously, the USA model did not project to the Nearctic region of Mexico, but to Tabasco and Chiapas in the Neotropical region, perhaps a reflection of specimens transported from Central American population migrations. In contrast, the Italian model projected broadly across the northern Nearctic region, from which there have been no collections of the species reported.

Ecological niche models are highly sensitive predictions of the potential distribution of species. They have most recently been used in various efforts as a tool for risk assessment of invasive species and to analyze associations of fitness on population genetics, and congener displacement (Peterson et al. 2001; Peterson, 2003; Nagaraju et al., 2013; Guisan et al., 2014). Despite the fact that GARP was recently evaluated to be the best modelling tool for predicting invasive speciesí distributions (Barbosa et al., 2012), GARP does not have variable analytical tools such as MaxEnt. Although this may be a limitation of the associations analyzed in the present study, they would have affected all models across natural or invaded regions similarly. Despite active discussion and evidence for and against niche conservatism between close or distant congeners, there is no evidence in the literature analyzing niche model differences for specific genotypes within a species, a key variable to study, particularly since invasive species are usually based on reduced founder individuals or populations. In the absence of data regarding proportional representation of specific haplotypes or more robust haplogroup analyses associated with niche variables, model outputs for invasive populations should be interpreted carefully.

A. albopictus populations in Mexico have moderate genetic structuring, but low genetic variation and distance. This is probably the result of local population expansions, while the mix of haplotypes reflects an ample degree of gene flow among even distant collection sites. In Mexico, 38.6 million inhabitants are now exposed to *A. albopictus*, and if current *A. albopictus* populations are sustained, this is expected to increase to 45.6 million by 2070. Introduction of new haplotypes or of greater native diversity from Asia could greatly change this profile and corresponding exposed populations. Since *A. albopictus* is a vector to at least 21 important pathogens, including dengue, Chikungunya, LaCrosse, yellow fever, and West Nile virus (Gratz, 2004), its populations and their infection with arbovirus should be monitored to understand and intervene where possible, such as the current Chikungunya epidemic on Mexico's southern border (Díaz-González et al., 2015).

Acknowledgments

We thank Miguel Muñoz-Reyes, José Luis Aguilar-Rodríguez, Nahum Avendaño-Martínez, Regino Pacheco-García, Heber Juárez-Collado and Rafael Perez-Utrera for their technical support for mosquito field collections. This study received funding from CONACyT-SEMARNAT project # 108158 to JMR.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.actatropica.2016.01.021.

References

Akaike, H., 1974. A new look at the statistical model identification. IEEE Trans. Autom. Control 19, 716–723.

Ali, A., Nayar, J.K., 1997. Invasion spread, and vector potential of *Aedes albopictus* in the USA and its control possibilities. Med. Entomol. Zool. 48, 1–9.

Anderson, R.P., Lew, D., Peterson, A.T., 2003. Evaluating predictive models of species' distributions: criteria for selecting optimal models. Ecol. Model. 162, 211–232, http://dx.doi.org/10.1016/S0304-3800(02)00349-6.

Avise, J.C., Ball, R.M., 1990. Principles of genealogical concordance in species concepts and biological taxonomy. Oxf. Surv. Evol. Biol. 7, 45–67.

Ayres, C.F.J., Romão, T.P.A., Melo-Santos, M.A.V., Furtado, A.F., 2002. Genetic diversity in Brazilian populations of *Aedes albopictus*. Mem. Inst. Oswaldo Cruz 97, 871–875, http://dx.doi.org/10.1590/S0074-02762002000600022.

Bandel, H.J., Forster, P., Röhl, A., 1999. Median-joining networks for inferring intraspecific phylogenies. Mol. Biol. Evol. 16, 37–48.

Benedict, M.Q., Levine, R.S., Hawley, W.A., Lounibos, L.P., 2007. Spread of the tiger: global risk of invasion by the mosquito *Aedes albopictus*. Vector-Borne Zoonotic Dis. 7, 76–85, http://dx.doi.org/10.1089/vbz.2006.0562.

Birungi, J., Munstermann, L.E., 2002. Genetic structure of *Aedes albopictus* (Diptera: Culicidae) populations based on mitochondrial ND5 sequences: evidence for an independent invasion into Brazil and the United States. Ann. Entomol. Soc. Am. 95, 126–132, http://dx.doi.org/10.1603/0013-8746(2002)095.

CONABIO-Comisión Nacional para el Conocimiento and Uso de la Biodiversidad, 2013. CONABIO-Comisión Nacional para el Conocimiento y Uso de la Biodiversidad Información sobre los registros de insectos de la familia Culicidae obtenida del Sistema Nacional de Información sobre Biodiversidad de CONABIO.

Campbell, L.P., Luther, C., Moo-Llanes, D.A., Ramsey, J.M., Danis-Lozano, R., Peterson, A.T., 2015. Climate change influences on global distributions of Dengue and Chikungunya virus vectors. Philos. Trans. R. Soc. B 370, 1665, http://dx.doi.org/10.1098/rstb.2014.0135.

Casas-Martínez, M., Torres-Estrada, J.L., 2003. First evidence of the presence of *Aedes albopictus* (Skuse) in southern Chiapas México. Emerg. Infect. Dis. 9, 606–607.

Craven, R.B., Eliason, D.A., Francy, D.B., Reiter, P., Campos, E.G., Jakob, W.L., Smith, G.C., Bozzi, C.J., Moore, C.G., Maupin, G.O., Monath, T.P., 1988. Importation of *Aedes albopictus* and other exotic mosquito species into the United States in used tires from Asia. J. Am. Mosq. Control Assoc. 4, 138–142.

Díaz-González, E.E., Kautz, T.F., Dorantes-Delgado, A., Malo-García, I.R., Laguna-Aguilar, M., Langsjoen, R.M., Chen, R., Auguste, D.I., Sanchez-Casas, R.M., Danis-Lozano, R., Weaver, S.C., Fernandez-Salas, I., 2015. First report of *Aedes aegypti* transmission of Chinkungunya virus in the Americas. Am. J. Trop. Med. Hyg., http://dx.doi.org/10.4269/ajtmh.15-0450.

Darsie Jr., R.F., 1986. A Survey and bibliography of the mosquito fauna of Mexico (Diptera: Culicidae). J. Am. Mosq. Control Assoc. 12, 298–306.

Delatte, H., Desvars, A., Bouétard, A., Bord, S., Gimonneau, G., Vourc'h, G., Fontenille, D., 2010. Blood-feeding behavior of *Aedes albopictus*, vector of Chikungunya on La Réunion. Vector-Borne Zoonotic Dis. 10, 249–258, http://dx.doi.org/10.1089/vdz.2009.0026.

Escobar, L.E., Peterson, A.T., Favi, M., Yung, V., Pons, D., Medina-Vogel, G., 2013. Ecology and geography of transmission of two bat-borne rabies lineages in Chile. PLoS Negl. Trop. Dis. 7, e2577, http://dx.doi.org/10.1371/journal.pntd.0002577.

Excoffier, L., Lischer, H.E.L., 2010. Arlequin suite ver 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Mol. Ecol. Resour. 10, 564–567, http://dx.doi.org/10.11111/j.1755-0998-2010.02847.x.

Excoffier, L., Smouse, P.E., Quattro, J.M., 1992. Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131, 479–491.

Fontenille, D., Toto, J.C., 2001. *Aedes* (*Stegomyia*) *albopictus* (Skuse), a potential new dengue vector in Southern Cameroon. Emerg. Infect. Dis. 7, 1066–1067, 10.3201eid0706.010631.

Forattini, O.P., 1986. Identificacao de *Aedes* (*Stegomyia*) *albopictus* no Brasil. Rev. Saude Publica 20, 244–245.

Francy, D.B., Karabatsos, N., Wesson, D.M., Moore, C.G., Lazuick, J.S., Niebylski, M.L., Tsai, T.F., Craig Jr., C.G., 1990. A new arbovirus from *Aedes albopictus*, an Asian mosquito established in the United States. Science 250, 1738–1740, http://dx.doi.org/10.1126/science.2270489.

Fu, Y.X., Li, W.H., 1993. Statistical tests of neutrality of mutations. Genetics 133, 693–709.

Futami, K., Valderrama, A., Baldi, M., Minakawa, N., Marin-Rodriguez, R., Chaves, L.F., 2015. New and common haplotypes shape genetic diversity in Asian tiger mosquito populations from Costa Rica and Panama. J. Econ. Entomol. 1, 1–8, http://dx.doi.org/10.1093/jee/tou028.

Gratz, N.G., 2004. Critical review of the vector status of *Aedes albopictus*. Med. Vet. Entomol. 18, 215–227, http://dx.doi.org/10.11111/j.0269-283X.2004.00513.x.

Guisan, A., Petitpierre, B., Broennimann, O., Daehler, C., Kueffer, C., 2014. Unifiing niche shifg studies insights from biological invasions. Trends Ecol. Evol. 29, 260–269, http://dx.doi.org/10.1016/j.tree.2014.02.009.

Hall, T., 2004. Bioedit Sequence Alignment Editor. V.7.0.9. Ibis Therapeutics, Carlsbad, CA.

Harpending, H., 1994. Signature of ancient population growth in a low-resolution mitochondrial DNA mismatch distribution. Hum. Biol. 66, 591–600.

Hawley, W.A., 1988. The biology of *Aedes albopictus*. J. Am. Mosq. Control Assoc. 1, 1–39.

Hedrick, P.W., 2005. Genetic of Populations. Jones and Bartlett Publishers, Subdury, Massachusetts.

Holder, P., George, S., Disbury, M., Singe, M., Kean, J.M., McFadden, A., 2010. A biosecurity response to *Aedes albopictus* (Diptera: Culicidae) in Auckland, New Zealand. J. Med. Entomol. 47, 600–609.

IPCC-Intergovernmental Program on Climate Change Climate Change, 2013. The physical science basic. Contribution of working group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press Cambridge.

Ibáñez-Bernal, S., Martinez-Campos, C., 1994. *Aedes albopictus* in Mexico. J. Am. Mosq. Control Assoc. 10, 231–232.

Ibáñez-Bernal, S., Briseño, B., Mutebi, J.P., Argot, E., Rodríguez, G., Martinez-Campos, C., Paz, R., de la Fuente-San, R.P., Tapia-Conyer, R., Flisser, A., 1997. First record in America of *Aedes albopictus* naturally infected with dengue virus during the 1995 outbreak at Reynosa in Mexico. Med. Vet. Entomol. 11, 305–309.

Jacob, B.G., Morris, J.A., Caamano, E.X., Griffith, D.A., Novak, R.J., 2011. Geomapping generalized eigenvalue frequency distributions for predicting prolific *Aedes albopictus* and *Culex quinquefasciatus* habitats based on spatiotemporal field-sampled count data. Acta Trop. 117, 61–68, http://dx.doi.org/10.1016/j.actatropica.2010.10.002.

Kambhampati, S., Black IV, W.C., Rai, K.S., 1991. Geographic origin of the US and Brazilian *Aedes albopictus* inferred from allozyme analysis. Heredity 67, 85–94, http://dx.doi.org/10.1038/hdy.1991.67.

Kamgang, B., Brengues, C., Fontenille, D., Njiokou, F., Simard, F., Paupy, C., 2011. Genetic structure of the tiger mosquito, *Aedes albopictus*, in Cameroon (Central Africa). PLoS One 6, e20257, http://dx.doi.org/10.1371/journal.pone.0020257.

Kimura, M., 1980. A simple method for estimating evolutionary rate of base substitutions through comparative studies of nucleotide sequences. J. Mol. Evol. 16, 111–120.

Kocher, T.D., Thomas, W.K., Meyer, A., Edwards, S.V., Pääbo, S., Villablanca, F.X., Wilson, A.C., 1989. Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. PNAS 86, 6196–6200.

Lambrechts, L., Scott, T.W., Gubler, D.J., 2010. Consequences of the expanding global distribution of *Aedes albopictus* for Dengue virus transmission. PLoS Negl. Trop. Dis. 4, e646, http://dx.doi.org/10.1371/journal.pntd.0000646.

Lourenco-de Oliveira, R., Vazeille, M., De Filippis, A.M., Failloux, A.B., 2003. Large genetic differentiation and low variation in vector competence for dengue and yellow fever viruses of *Aedes albopictus* from Brazil the United States, and the Cayman Islands. Am. J. Trop. Med. Hyg. 69, 105–114.

Marquetti, M.C., Valdes, V., Aguilera, L., 2000. Tipificación de habitats de *Aedes albopictus* en Cuba y su asociación con otras especies de culícidos, 1995–1998. Rev. Cubana Med. Trop. 52, 170–174.

Medley, K.A., 2010. Niche shifts during the global invasion of the Asian tiger mosquito, *Aedes albopictus* Skuse (Culicidae), revealed by reciprocal distribution models. Global Ecol. Biogeogr. 19, 122–133, http://dx.doi.org/10.11111/j. 1466-8238.2009.00497. x.

Medlock, J.M., Hansford, K.M., Schaffner, F., Versteirt, V., Hendrickx, G., Zeller, H., Van Bortel, W., 2012. A review of the invasive mosquitoes in Europe: ecology, public health risks, and control options. Vector-Borne Zoonotic Dis. 12, 435–447, http://dx.doi.org/10.1089/vbz.2011.0814.

Mirabello, L., Conn, J.E., 2006. Molecular population genetics of the malaria vector *Anopheles darlingi* in Central and South America. Heredity 96, 311–321, http://dx.doi.org/10.1038/sj.hdy.6800805.

Moo-Llanes, D.A., Ibarra-Cerdeña, C.N., Rebollar-Téllez, E.A., Ibáñez-Bernal, S., González, C., Ramsey, J.M., 2013. Current and future niche of north and central american sand flies (Diptera: Psychodidae) in climate change scenarios. PLoS Negl. Trop. Dis. 7, e2421, http://dx.doi.org/10.1371/journal.pntd.0002421.

Mousson, L., Dauga, C., Garrigues, T., Schaffner, F., Vazeille, M., Failloux, A.B., 2005. Phylogeography of *Aedes* (*Stegomyia*) *aegypti* (L.) and *Aedes* (*Stegomyia*) *albopictus* (Skuse) (Diptera: Culicidae) based on mitochondrial DNA variations. Genet. Res. 86, 1–11, http://dx.doi.org/10.1017/S0016672305007627.

Nagaraju, S.K., Gudasalamani, R., Barve, N., Ghazoul, J., Narayanagowda, G.K., Ramanan, U.S., 2013. Do ecological niche model predictions reflect the adaptive landscape of species ? A test using *Myristica malabarica* Lam an endemic tree in the Western Ghats, India. PLoS One 8, e82066, http://dx.doi.org/10.1371/journal.pone.0082066.

Nakazawa, Y., Willans, R.A.J., Peterson, A.T., Mead, P.S., Kugeler, K.J., Petersen, J.M., 2010. Ecological niche modeling of *Francisella tularensis* subspecies and clades in the United States. Am. J. Trop. Med. Hyg. 82, 912–918, http://dx.doi.org/10.4269/ajtmh.2010.09-0354.

Navarro, J.C., Quintero, L., Zorrilla, A., González, R., 2013. Molecular tracing with mitochondrial ND5 of the invasive mosquito *Aedes* (*Stegomyia*) *albopictus* (Skuse) in Northern South America. JEZS 1, 32–38.

Nei, M., Muruyama, T., Chakraborty, R., 1975. The bottleneck effect and genetic variability in populations. Evolution 29, 1–10.

Ogden, N.H., Milka, R., Caminade, C., Gachon, P., 2014. Recent and projected future climatic suitability of North America for the Asian tiger mosquito *Aedes albopictus*. Parasites Vectors 7, 532, http://dx.doi.org/10.1186/s13071-014-0532-4.

Orta-Pesina, H., Mercado-Hernández, R., Valdez-Rodríguez, M.A., 2001. *Aedes albopictus* in Allende city Nuevo León Mexico. Am. J. Trop. Med. Hyg. 17, 260–261.

Orta-Pesina, H., Mercado-Hernández, R., Elizondo-Leal, J.F., 2005. Distribución de *Aedes albopictus* (Skuse) en Nuevo León México, 2001–2004. Salud Pública Mex. 47, 163–165.

Paupy, C., Delatte, H., Bagny, L., Corbel, V., Fontenille, D., 2009. *Aedes albopictus*, an arbovirus vector: from the darkness to the light. Microbes Infect. 11, 1177–1185, http://dx.doi.org/10.1016/j.micinf.2009.05.005.

Paupy, C., Ollomo, B., Kamgang, B., Moutailler, S., Rousset, D., Demanou, M., Hervé, J.P., Leroy, E., Simard, F., 2010. Comparative role of *Aedes albopictus* and *Aedes aegypti* in the emergence of dengue and chikungunya in Central Africa. Vector-Borne Zoonot Dis. 10, 259–266, http://dx.doi.org/10.1089/vbz.2009.0005.

Pech-May, A., Marina, C.F., Vázquez-Domínguez, E., Berzunza-Cruz, M., Rebollar-Téllez, E.A., Narváez-Zapata, J.A., Moo-Llanes, D.A., Ibáñez-Bernal, S., Ramsey, J.M., Becker, I., 2013. Genetic structure and divergence in populations of *Lutzomyia cruciata*, a phlebotomine sand fly (Diptera: Psychodidae) vector of *Leishmania mexicana* in southeastern Mexico. Infect. Genet. Evol. 16, 254–262, http://dx.doi.org/10.1016/j.meegid.2013.02.004.

Peterson, A.T., Vieglais, D.A., 2001. Predicting species invasions using ecological niche modeling: new approaches from bioinformatics attack a pressing problem. Bioscience 51, 363–371.

Peterson, A.T., 2003. Predicting the geography of species invasions via ecological niche modelling. Q. Rev. Biol. 78, 419–433.

Porretta, D., Mastrantonio, V., Bellini, R., Somboon, P., Urbanelli, S., 2012. Glacial history of a modem invader: phylogeography and species distribution modelling of the Asian Tiger mosquito *Aedes albopictus*. PLoS One 7, e44515, 10.13717journal.pone.00444515.

Posada, D., 2008. jModelTest: phylogenetic model averaging. Mol. Biol. Evol. 25, 1253–1256, http://dx.doi.org/10.1093/molbev/msn083.

Raharimalala, F., Harivelo, L., Ravelonandro, P., Sahondra, L., Zouache, K., Tran-Van, V., Mousson, L., Failloux, A.B., Hellard, E., Moro, C.V., Ralisoa, B.O., Mavingui, P.,

2012. Biogeography of the two major arbovirus mosquito vector, *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae), in Madagascar. Parasites Vectors 5, 56, http://dx.doi.org/10.1186/1756-3305-5-56.
Reiter, P., Sprenger, D., 1987. The used tire trade: a mechanism for the worldwide dispersal of container breeding mosquitos. J. Am. Mosq. Control Assoc. 3, 494–501.
Reyes-Villanueva, F., Garza-Hernández, J.A., García-Munguía, A.M., Howard, A.F.V., Ortega-Morales, A.I., Adeleke, M.A., Rodríguez-Pérez, M.A., 2013. *Aedes albopictus* in northeast Mexico: An update on adult distributions and first report of parasitism by *Ascogregarina taiwanensis*. J. Vector Borne-Dis. 50, 202–205.
Rochlin, I., Ninivaggi, D.V., Hutchinson, M.L., Farajollahi, A., 2013. Climate change and range expansion of the Asian Tiger mosquito (*Aedes albopictus*) in Northeastern USA: implications for public health practitioners. PLoS One 8, e69874, http://dx.doi.org/10.1371/journal.pone.0060874.
Rodríguez-Tovar, M.L., Ortega-Martínez, M.G., 1994. *Aedes albopictus* in Muzquiz City, Coahuila, México. J. Am. Mosq. Control Assoc. 10, 587.
Roger, A.R., Harpending, H., 1992. Population growth makes waves in the distribution of pairwise genetic differences. Mol. Biol. Evol. 9, 559–569, http://dx.doi.org/10.1093/bioinformatics/btp187.
Romi, R., 2001. *Aedes albopictus* in Italy: an underestimated health problem. Ann. Inst. Super Sanita 37, 241–247.
Ronquist, F., Teslenko, M., van der Mark, P., Ayres, D.L., Darling, A., Hohna, S., Larget, B., Lui, L., Suchard, M.A., Huelsenbeck, J.P., 2012. MrBayes 3.2: efficient bayesian phylogenetic inference and model choice across a large model space. Syst. Biol. 61, 539–542, http://dx.doi.org/10.1093/sysbio/sys029.
Rossi, G.C., Nelci, T.P., Krsticevic, F.J., 1999. First record of *Aedes albopictus* (Skuse) from Argentina. J. Am. Mosq. Control Assoc. 15, 422.
Rozas, J., Librado, P., 2009. DnaSP v.5: a software for comprehensive analysis of DNA polymorphism data. Bioinformatics 25, 1451–1452.
Salomón-Grajales, J., Lugo-Moguel, G.V., Tinal-Gordillo, V.R., Cruz-Velázquez, J., Beaty, B.J., Eisen, L., Lozano-Fuentes, S., Moore, C.G., García-Rejón, J.E., 2012. *Aedes albopictus* mosquitoes, Yucatan Peninsula Mexico. Emerg. Infect. Dis. 18, 525–527, 10.320.eid1803.111626.
Sambrook, D.J., Fritsch, E.F., Maniatis, T., 1989. Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory Press, Cold Spring Harbor.
Savage, H.M., Smith, G.C., 1995. *Aedes albopictus* y *Aedes aegypti* en las Américas: implicaciones para la transmisión de arbovirus e identificación de hembras. Bol. Oficina Sanit. Panam. 118, 473–477.
Scholte, E.J., Dijkstra, E., Blok, H., de Vries, A., Takken, W., Hofhuis, A., Koopmans, M., de Boer, A., Reusken, C.B., 2008. Accidental importation of the mosquito *Aedes albopictus* into the Netherlands : a survey of mosquito distribution and the presence of dengue virus. Med. Vet. Entomol. 22, 352–358, http://dx.doi.org/10.11111/j.1365-2915.2008.00763. x.
Serufo, J.C., Oca, H.M., de Tavares, V.A., Souza, A.M., Rosa, R.V., Jamal, M.C., Lemos, J.R., Oliveira, M.A., Noqueira, R.M., Schatzmaryr, H.G., 1993. Isolation of dengue virus type 1 from larvae of *Aedes albopictus* in Campos Altos City State of Minas Gerais Brazil. Mem. Inst. Oswaldo Cruz 88, 503–504.
Sprenger, D., Wuithiranyagool, T., 1986. The discovery and distribution of *Aedes albopictus* in Harris County Texas. J. Am. Mosq. Control Assoc. 2, 217–218.
Stevens, B., Giorgetta, M., Esch, M., Mauritsen, T., Crueger, T., Rast, S., Salzmann, M., Schmidt, H., Bader, J., Block, K., Brokopf, R., Fast, I., Kinne, S., Kornblueh, L., Lohmann, U., Pincus, R., Reichler, T., Rockner, R., 2013. Atmospheric component of the MPI-M earth system model: ECHAM 6. JAMES 5, 1–27, http://dx.doi.org/10.1002/jame.20015.
Stockwell, D., Peters, D., 1999. The GARP modeling system: problems and solutions to automated spatial prediction. Int. J. Geogr. Inf. Sci. 13, 143–158.
Tajima, F., 1989. Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. Genetics 123, 585–595.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis Version 6.0. Mol. Biol. Evol. 30, 2725–2729.
Urbanelli, S., Bellini, R., Carrieri, M., Sallicandro, P., Celli, G., 2000. Population structure of *Aedes albopictus* (Skuse): the mosquito which is colonizing Mediterranean countries. Heredity 84, 331–337, http://dx.doi.org/10.1046/j.1365-2540.2000.00676.x.
Usmani-Brown, S., Cohnstaedt, L., Munstermann, L.E., 2009. Population genetics of *Aedes albopictus* (Diptera: Culicidae) invading populations, using mitochondrial nicotinamide adenine dinucleotide dehydrogenase subunit 5 sequences. Ann. Entomol. Soc. Am. 102, 144–150, http://dx.doi.org/10.1603/008.102.0116.
Villegas-Trejo, A., Manrique-Saide, P., Che-Mendoza, A., Cruz-Canto, W., González-Fernández, M., González-Acosta, C., Dzul-Manzanilla, F., Huerta, H., Arredondo-Jímenez, J.I., 2010. First report of *Aedes albopictus* and other mosquito species in Morelos Mexico. J. Am. Mosq. Control Assoc. 26, 321–323.
WHO-World Health Organization, 2012, Global Strategy for Dengue Prevention and Control, 2012–2020 Geneva, Switzerland.
Zhong, D., Lo, E., Hu, R., Metzger, M.E., Cummings, R., Bonizzoni, M., Fujioka, K.K., Sorvillo, T.E., Kluh, S., Healy, S.P., Fredregill, C., Kramer, V.L., Chen, X., Yan, G., 2013. Genetic analysis of invasive *Aedes albopictus* populations in los Angeles County, California and its potential public health impact. PLoS One 8, e68586, http://dx.doi.org/10.1371/journal.pone.0068586.