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Artículo científico

How much is known about the genetic diversity of the Asian tiger mosquito? A systematic review

¿Cuánto se conoce acerca de la diversidad genética del mosquito tigre? Una revisión sistemática

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Abstract

Introduction: *Aedes (Stegomyia) albopictus* (Skuse, 1894) is a vector for dengue and chikungunya viruses in the field, along with around 24 additional arboviruses under laboratory conditions. Knowledge of the genetic diversity of insect vectors is critical for the effective control and elimination of vector-borne diseases. **Objective:** We determined the current scenario of the genetic diversity in natural populations of *A. albopictus* through a systematic review. **Methodology:** It was possible to establish the first reports and distribution of *A. albopictus* populations in the world, as well as its genetic diversity, population genetic structure and molecular markers used to determine its genetic diversity. **Results:** *A. albopictus* is distributed worldwide with genetically structured populations and low diversity; however, 89.5% of the genetic diversity known is based on the use of RFLP, allozymes, isozymes, and mtDNA molecular markers that exhibit significant problems according to the literature. After the results were obtained, a critical analysis was carried out and existing shortcomings were detected. **Conclusion:** The current knowledge of genetic diversity of *A. albopictus* is based on genetic markers that exhibit significant problems reported in the literature; therefore, vector control programs targeting *A. albopictus* populations, may be compromised.

Keywords: *Aedes albopictus*; Genetic; Markers; Gene; Flow.

Resumen

Introducción: *Aedes (Stegomyia) albopictus* (Skuse, 1894) es un vector para los virus del dengue y chicunguña en la naturaleza, junto con cerca de 24 arbovirus en condiciones de laboratorio. El conocimiento de la diversidad genética de los insectos vectores es fundamental para el control eficaz y la eliminación de enfermedades transmitidas por estos. **Objetivo:** Aquí se determinó el escenario actual de la diversidad genética en poblaciones naturales de *A. albopictus* a través de una revisión sistemática. **Metodología:** Se pudieron establecer los primeros registros y distribución de las poblaciones de *A. albopictus* en el mundo, así como su diversidad genética, estructura genética poblacional y marcadores moleculares utilizados para determinar su diversidad genética. **Resultados:** *A. albopictus*

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se distribuye en todo el mundo con poblaciones genéticamente estructuradas y baja diversidad; Sin embargo, el 89,5% de la diversidad genética conocida se basa en el uso de RFLP, aloenzimas, isoenzimas y marcadores moleculares mitocondriales que presentan problemas significativos según la literatura. Una vez obtenidos los resultados, se realizó un análisis crítico y se detectaron deficiencias existentes. **Conclusión:** El conocimiento actual de la diversidad genética de *A. albopictus* se basa en marcadores genéticos que presentan problemas significativos reportados en la literatura; Por lo tanto, los programas de control de vectores dirigidos a las poblaciones de *A. albopictus* pueden verse comprometidos.

Palabras clave: *Aedes albopictus*; Marcadores moleculares; Flujo genético.

Introduction

Aedes albopictus, also known as the Asian tiger, is a mosquito from Southeast Asia, the Pacific and Indian Ocean Islands. It has spread and colonized every continent except Antarctica in the past 30–40 years, primarily by trading of tires, and is expected to continue to disperse[1-2]. *A. albopictus* is commonly found in sub-urban, rural, semi-rural and savage environments from tropical, subtropical and temperate regions[2-4]. The Asian tiger mosquito has been linked to the transmission of arboviral and filarial infectious diseases of humans and animals[5-6.] Its high potential to carry a wide range of human pathogens is consequently of wide concern.

A. albopictus presents vector competence for 26 arboviruses from the families Flaviviridae (e.g., Dengue virus, Nile virus, yellow fever, Japanese encephalitis), Bunyaviridae (e.g., Potosí, LaCrosse virus), Togaviridae (e.g., Chikungunya and Ross River virus) and Reoviridae (e.g., Orungo and Nodamura virus)[7-9]. Naturally, *A. albopictus* is able to transmit important diseases such as dengue and chikungunya fever. The Asian tiger mosquito has played a significant role in Chikungunya virus (CHIKV) outbreaks in Central Africa, Asia and Europe[10-13]. In addition to CHIKV, *A. albopictus*, a species that is sympatrically distributed with *Aedes aegypti*, is epidemiologically important in transmitting the dengue viruses (DENV) throughout areas of Southeast Asia, Africa, North America and Europe[14,15].

Worldwide, *Aedes aegypti* is the primary vector for the DENV, a disease that remains a serious public health problem in many tropical and subtropical countries[16]. In the Americas, *A. aegypti* is the only confirmed natural dengue virus vector[17]. Although its geographical distribution is more limited, *A. albopictus* is considered a potential vector in the Americas due to the high level of vector competence of local populations for DENV[18-19]. A meta-analysis of 14 studies on the relative susceptibility of *A. albopictus* and *A. aegypti* for DENV suggests that *A. albopictus* is more susceptible to midgut infections than *A. aegypti*; however, the ability of the virus to disseminate in the latter mosquito is considerable, suggesting a greater potential for transmission in nature[20]. Nevertheless, currently *A. aegypti* is the primary vector for the DENV in the Americas[21-22].

Given the sanitary and epidemiological importance of *A. albopictus*, the understanding of the patterns of genetic structure and gene flow among *A. albopictus* populations is pivotal for the development of rational vector control programs[23]. Population genetics studies of *A. albopictus* have been carried out globally as the species continues to spread and displace *A. aegypti* in some areas[4]. Different genetic markers have been used to study the population genetic structure of *A. albopictus*, such as Isozymes/Allozymes[24-25], Restriction Fragment Length polymorphism (RFLP[26]), Random Amplified Polymorphic DNA (RAPD[27]), Mitochondrial DNA (mtDNA[28-30]) sequence haplotype, ribosomal DNA (rDNA[31]) and Microsatellites[32].

Genetic studies with early populations of *A. albopictus,* using Isozymes/Allozymes, indicated that populations cluster by continent or country of collection[24-25,33-34]. Subsequent researches examined variation at smaller and/or wider geographic scales using molecular markers such as RAPD, mtDNA, rDNA and microsatellite; these genetic studies report varying levels of population differentiation at both local and continental scales[29,35-36].

Population genetic studies provide insights into the basic biology of arthropod disease vectors by estimating dispersal patterns and their potential to spread pathogens[37]. Significant progress has been made in understanding insect diversity and ecology by using protein markers such as isozymes/allozymes[38]. The isozymes, developed in the late 70s, were originally defined as multiple molecular forms of enzymes with identical or similar functions and that are present in the same individual[39-40]. The isozymes may have different

allelic forms known as allozymes[41]. The isozymes application is guided for quantifying heterozygosity, genetic diversity, genetic differentiation and other measures of genetic variation within and among populations. However, one of the problems of the protein markers is the lack of ability to detect polymorphisms between related species, since the proteins are the result of gene expression, which may differ from one tissue to another, from one stage of development to another, or from one environment to another[42].

Protein markers made a significant contribution in the early periods when DNA technologies were not as advanced as it is now. However, with the development of DNA-based marker systems, such as RFLP, RAPD, mtDNA and microsatellites, it was found that a greater level of polymorphism could be obtained by using DNA rather than protein markers in many cases[43]. The RFLP was the first DNA marker used in population studies[44] and is used to detect DNA fragments from different molecular weights (by digestion with the same restriction enzyme) in different organisms, usiny electrophoresis on agarose or polyacrylamide gel[38]. The RFLP has been used for constructing genetic maps, cloning of genes based on maps and for helping to resolve taxonomic and phylogenetic problems[45]. However, the main disadvantage of the RFLP is the requirement of large amounts of high quality DNA to recognize loci single copies, which only detect a fraction of the variability of existing sequences in the genome, which means the information is limited[46].

The RAPD markers method has been reported to be an efficient tool to differentiate geographically and genetically isolated population. The RAPD technique uses the PCR principle for random amplification of DNA sequences. The RAPD-PCR is a dominant type of molecular marker, that is unable tt differentiate heterozygotes from homozygotes[43]. These markers allow the study of a large number of loci and provide a random sampling of DNA, therefore, present high levels of polymorphism compared to RFLP and protein markers[46]. However, they have significant limitations when compared to codominant markers (e.g., microsatellites) and/or haploid (e.g., mtDNA), since, the amplified fragments often do not correspond to DNA bound to a character, but to one repeated, and it does not provide information about the number of copies of genomic DNA containing the amplified sequence[43].

The mtDNA is used for marker analyses largely because of their maternal inheritance, haploid status, and high rate of evolution[47]. The mtDNA is a type of marker used for the recognition of cryptic species, phylogenetic studies and/or genetic structure of populations[48-50]. One of the disadvantages of using mtDNA in population and phylogenetic studies is the presence of nuclear mitochondrial pseudogenes (NUMTs)[51-52]. NUMTs are non-functional copies of mitochondrial sequences that have become incorporated into the nuclear genome[53]. Samples containing mixtures of mtDNA and NUMT sequences are expected to significantly affect the outcome of genealogy- and frequency-based analyses. This is because mtDNA and NUMTs have separate genealogies and thus, evolutionary history[52].

The ribosomal DNA (rDNA) can be found in the mitochondria, chloroplast and nucleus. The rDNA has been analyzed at the structural level in a large number of multicellular eukaryotes, including insects[54]. The rRNA occurs in tandem repetitions and it consists of three highly conserved subunits (18rDNA, 5.8rDNA and 28rDNA),dseparated by two External Transcribed Spacers (ITS1 e ITS2) with high replacement rates[55]. Due to the low rate of substitution present, these sequences are useful in phylogenetic studies on taxa with old divergence time[56]. Nevertheless, it has been found NUMTs in *A.* aegypti derived from the tRNA and rRNA genes throughout the mtDNA genome[53].

Microsatellites are also used as popular markers in insect studies because of the high abundance and highly variable nature of their loci in genome[57]. However, in contrast to most other arthropods (e.g., *Anopheles gambiae* s.[58]), microsatellites appear to be underrepresented within some members of the mosquito subfamily Culicinae (e.g., *Culex pipiens*, *C. pipiens quinquefasciatus*, and *A. aegypti*[59-60]). Nevertheless, in *A. aegypti* for instance, microsatellites are commonly used in population genetics studie[61].

Regarding these marker systems (Isozymes, RFLP, etc.), some details about *A. albopictus* movement, gene flow patterns and genetic structure has been inferred. However, no published article has focused on analyzing the current scenario of the genetic diversity from natural populations of the Asian tiger mosquito. Hence, the objective of this systematic review was to defind the current scenario of the genetic diversity of natural populations of *A. albopictus*. For this purpose, data from the first record and distribution of the vector was compiled and included; besider, discussion as focused on the current knowledge of genetic diversity through different molecular techniques. Finally, some important gaps of knowledge, that needed to be addressed, were identified for further research.

Materials and methods

Throughout May 2014, a systematic review was carried out on articles about: The first records of the vector, Genetic diversity, and distribution of natural populations of *A. albopictus*. Distribution data of the vector was considered from the reviews authored by: Rai[62], Benedict et al.[63], Caminade et al.[2], Medlock et al.[64] and Bonizzoni et al.[1]. The database used for the research of the early records of the vector and the genetic diversity, sere: Web of Knowledge ("all databases", including Biological Abstracts, Biosis, Current Contents Connect, Web of Science, and Zoological Records) by Thomson Reuters and the Google search engine (limited to the first five pages of results). The Google search engine was used to identify reports, conference abstracts, guidelines, etc. Data research was performed including all dates andelimite to sources i: English, Spanish and Portuguese.

keywords used for the research on the early records of the vector was, '*Aedes albopictus*' followed by the phrase 'first record'. Only the first record for country was considered. Regarding the research on genetic diversity, the keyword used was: '*Aedes albopictus*' followed by the terms 'genetic diversity', 'gene flow', 'population structure' 'population genetics', 'mtDNA' and 'nuclear DNA'. From the results of the research, all the titles and abstracts found were read, and from these, only articles related to the search criteriasweretconsidered. After reading the title and abstract, replicas and items that did not meet the inclusion criteria, were removed from the search.

The publications included in the analysis were summarized using a data extraction tool developed from Microsoft Excel 2010. Two data matrices were constructed: one related to the first record-distribution and the other on genetic diversity. The first matrix on the first record-distribution contained data such as: Location (state, city, region, county, district, and street), year, geographic coordinates, distribution, and references. The second one, on the genetic diversity, included data like: Location (state, city, region, county, district, and street), geographic coordinates, genetic diversity (polymorphic diversity / haplotype / gene / nucleotide), molecular technique, genetic structure (*p-value* that indicate genetic structure such as: X^2 test (Isozymes/ Allozyme)/G_{ST} (RAPD)/F_{ST} (mtDNA, Microsatellites)) and references. Maps were designed based om the geographical coordinates of the two matrices and the molecular techniques. The georeferencing data were calculated using Google Earth 7.1.

Results

A total of 65 published articles between 1987 and 2014 were analyzed. From these articles, 63% referreg to the first record of the vector and the other 37% on genetic diversity (**Table 1-2**). The first record of *A. albopictus* outside Asia (place of origin of the vector) was registered in 1979 in Europe (Albania). Since then, the Asian tiger has been dispersed in the continents of Oceania, Africa, Europe and America during the last 36 years (**Figure 1a, Table 1**). In Oceania, the vector is present in 10 of the Torres Strait Islands, since its appearance in Brisbane (Queensland, Australia) in 1988. In Africa, there are records of *A. albopictus* from 1991 in Nigeria (Delta State) and South Africa (Cameroon). However, nowadayt, there are no records of the vector along the African continent. In Europe, *A. albopictus* has been confirmed in 16 countries from the continent after its appearance in Albania (1979), and later in Genova (Italy) in 1990. In America, *A. albopictus* was initially introduced in the middle of the decade of the 1980 in United States (Texas). Consequently, the Asian tiger has been registered in South America and Central America since 1980 until 1990, primarily in Brazil (1986, Rio de Janeiro) and Mexico (1988, Coahuila), and subsequently in the remaining countries (**Table 1**).

Literaturs on genetic diversity showed that the Asian tiger populations have been studied in all ite distribution arear (**Figure 1b**). A total of 267 vector populations have been studied throughout the world. The largest number of populations studied was founs in the American continent (37%) followed by Europe (21%), Africa (20%), Asia (16%) and Oceania (6%). The 37% in the American continent is distributed into: 56% in North America (United States), 41% in South America (mainly Brazil) and 3% Central America (Dominican Republic, Guatemala and Cayman Islands; **Figure 1b**, **Table 2**).

In general, most of the populations of *A. albopictus* have hat genetic structure studies at regional and global levels. The genetic diversity (*Hd*) of the Asian tiger populations ranged from 0.0 (Central Africa) to 0.83 (China, Singapore, Japan, Italy, United State), rnonetheless, most of the *Hd* studies results were lower than 0.7. Furthermore, the haplotype diversity (π) of the Asian tiger populations ranged from 0.00 to 0.30 (**Table 2**).

The data were obtained from the published literature (**Table 1-2**). The colors indicate vector distribution: Gray (Unknown or no data), Red (Indigenous) and Blue (Current distribution range).

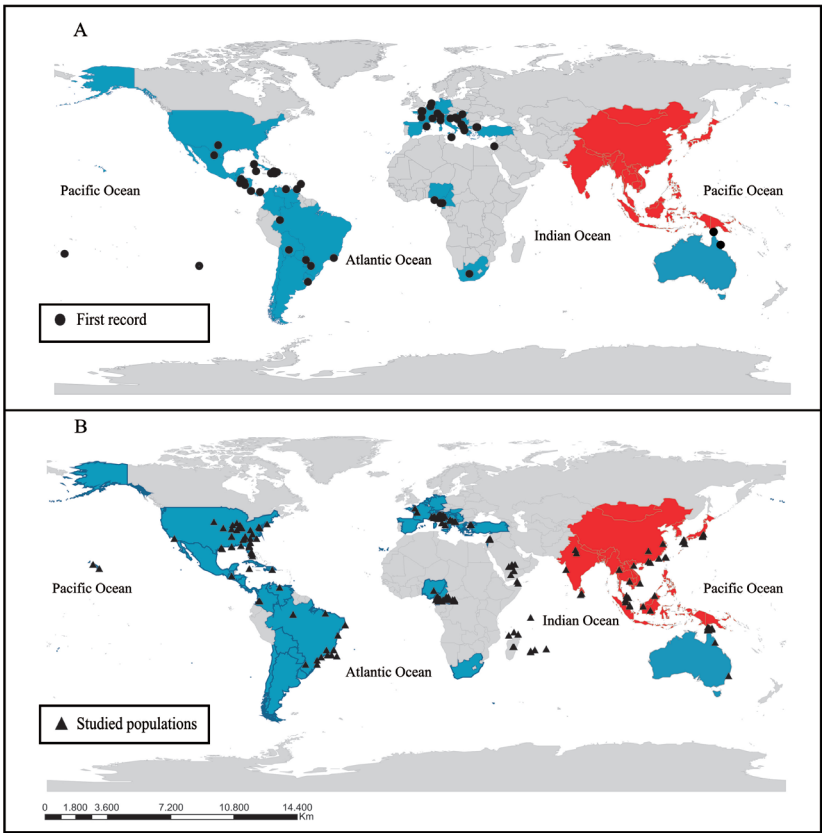

Figure 1. Political maps indicating: **A.** The first record and distribution of *A. albopictus*, and **B.** The *A. albopictus* populations used in genetic diversity studies.

Table 1. First records (in chronological order) of natural populations of *A. albopictus* in the world.

Country	Year	Reference	Country	Year	References
Albania	1979	Adhami and Reiter[108]	Equatorial Guinea	2001	Toto, et al.[109]
Trinidad and Tobago	1983	Le-Maitre and Chade[110]	Serbia and Montenegro	2001	Mousson, et al.[111]
United States	1985	Sprenger and Wuithiranyagool[112]	Hungary	2001	Scholte and Schaffner[113]
Brazil	1986	Forattini[114]	Panama	2002	ISID[115]
Mexico	1988	Ibañez-Bernal and Martínez-Campos[116]	Switzerland	2003	Flacio, et al.[117]
Australia	1988	Kay, et al.[118]	Nicaragua	2003	Lugo, et al.[119]
Italy	1990	Sabatini, et al.[120]	Uruguay	2003	Rossi and Martínez[121]
Nigeria	1991	Savage, et al.[122]	Israel	2003	Pener, et al.[123]
Southern Africa	1991	Cornel and Hunt[124]	Belgium	2004	Schaffner, et al.[125]
Barbados	1993	Reiter[126]	Spain	2004	Aranda, et al.[127]
Dominican Republic	1993	Peña[128]	Croatia	2004	Klobučar, et al.[129]
Cuba	1995	Broche and Borja[130]	Netherlands	2005	Scholte, et al.[131]
Guatemala	1995	Ogata and Samayoa[132]	Greece	2005	Samanidou-Voyadjoglou, et al.[133]
Honduras	1995	Ogata and Samayoa[132]	Slovenia	2005	Petrić, et al.[134]
El Salvador	1995	Benedict, et al.[63]	Bosnia and Herzegovina	2005	Petrić, et al.[134]
Bolivia	1997	Rai[62]	Germany	2007	Pluskota, et al.[135]
Cayman Islands	1997	Benedict, et al.[63]	Malta	2009	Gatt, et al.[136]
Argentina	1998	Rossi, et al.[137]	Costa Rica	2009	Calderon-Arguerdas, et al.[138]
Colombia	1998	Velez, et al.[139]	Venezuela	2009	Navarro, et al.[140]
Paraguay	1998	Benedict, et al.[63]	Haiti	2010	Marquetti-Fernández, et al.[141]
France	1999	Schaffner and Karch[142]	Turkey	2011	Oter, et al.[143]
Cameroon	2000	Fontenille and Toto[144]	Tonga	2011	Guillaumot, et al.[145]
Chile	2000	MSC[146]	Slovakia	2013	Bocková, et al.[147]

Findings indicate that molecular techniques used in studies on genetic diversity of *A. albopictus* are: RFLP, Allozymes, Isozymes, RAPD, mtDNA (Cytb, COI, ND5), microsatellites and ITS2. However, there were also found studies in which more than one molecular technique was used such as: mtDNA and microsatellite and mtDNA and ITS2 and (**Table 2**). From the total of the population studied, 50.9% have been analyzed using mtDNA, 24.7% allozymes, 7.5% isozymes, 6.4% RFLP, 4.5% mtDNA and microsatellites, 3.0% mtDNA and ITS2, 2.6% RAPD and 0.4% microsatellites (**Figure 2**). On the other hand, the 89.5% of the known genetic diversity is based on the use of RFLP, allozymes, isozymes, and mitochondrial molecular markers, which exhibie problems reported on the literature. Molecular techniques (in inverse chronological order) used to estimate genetic diversity in *A. albopictus* populations are: ITS2 (2013-present), microsatellites (2011-present), mtDNA and RAPD (2002-present), and RFLP, allozymes and isozymes (1988- 2003) (**Table 2**).

Table 2. Genetic diversity worldwide observed (in chronological order) in natural populations of *A. albopictus* using various molecular markers.

Country[n]	Genetic diversity	Molecular marker							References
	(*Hd*; π)*	A	B	C	D	E	F	G	
India[1]	(0.25-0.37; MD)*		**X**						Gupta and Preet[35]
Central Africa[9]	(0.0-0.5; 0.0000-0.0009)*				**X**	**X**			Kamgang, et al.[151]
France[1]	(0.6; 0.0009-0.002759)*						**X**		Delatte, et al.[32]
Central Africa[6]	(0.00-0.53; 0.0000-0.0005)*				**X**	**X**			Kamgang, et al.[29]
Venezuela[3], Colombia[3]	(0.749; 0.00358)					**X**			Navarro, et al.[100]
Turkey[15]	(MD; MD)				**X**				Oter, et al.[143]
Italy[6]	(MD; MD)				**X**			**X**	Shaikevich and Talbalaghi[31]
China[4], Singapore[1], Japan[1], Italy[1], United State[5]	(0.37-0.83; 0.06-0.30)*				**X**				Zhon, et al.[148]
Lebanon[5]	(MD; MD)			**X**	**X**	**X**			Haddad, et al.[30]
Madagascar[9]	(MD; MD)				**X**	**X**			Raharimalala, et al.[149]
France[3], Mauritius[2], Seychelles[1], Southeastern Africa[1]	(0.0-0.7; 0.00-0.02)				**X**				Delatte, et al.[150]
Cameroon[12]	(0.0-0.64; 0.001)*				**X**	**X**	**X**		Kamgang, et al.[151]
Croatia[8], Serbia and Montenegro[3]	(0.282; 0.000064)				**X**	**X**			Zitko, et al.[68]
Brazil[5]	(0.187; 0.00044)					**X**			Maia, et al.[152]
Italy[1], Cameroon[3], United States[2]	(0.457; MD)*					**X**			Usmani-Brown, et al.[36]
Australia[17]	(MD; MD)				**X**				Ritchie, et al.[153]
Brazil[1],Cambodia[1],France[2],Madagascar[1],Réunion[2], Thailand[1], United States[2], Vietnam[2]	(MD; MD)			**X**	**X**	**X**			Mousson, et al.[111]
Brazil[10], United Kingdom[1], United States[9]	(MD; MD)*	**X**							Lourenço de Oliveira, et al.[34]
Brazil[6]	(0.365; MD)		**X**						Ayres, et al.[27]
Brazil[4], Guatemala[1],Indonesia[1], Italy[1], Japan[1], Madagascar[1], Malaysia[1], Nigeria[1], Dominican Republic[1], United States[6]	(MD; MD)					**X**			Birungi and Munstermann[28]
Italy[18]	(MD; MD)*	**X**							Urbanelli, et al.[33]
Brazil[1], China[1], India[1], Japan[3], Malaysia[1], Mauritius[1],Singapore[1], Sri Lanka[1], Taiwan[1], United State[6]	(MD; MD)	**X**							Kambhampati and Karamjit[26]
Borneo[2], Brazil[4], China[2], India[2], Japan[7], Madagascar[1], Malaysia[3], Sri Lanka[1], United States[20]	(MD; MD)*	**X**							Kambhampati, et al.[25]
United States[6]	(MD; MD)*	**X**							Black, et al.[2])

[n] = Number of cities/towns/sampled regions; ***Hd*** = haplotype diversity; π = nucleotide diversity; ***** = Genetic structuring ($p < 0.05$); **MD** = missing data; **A** = RFLP/Isozymes/Allozymes; **B** = RAPD; **C** = Cytb; **D** = COI; **E** = ND5; **F** = Microsatellites; **G** = ITS2.

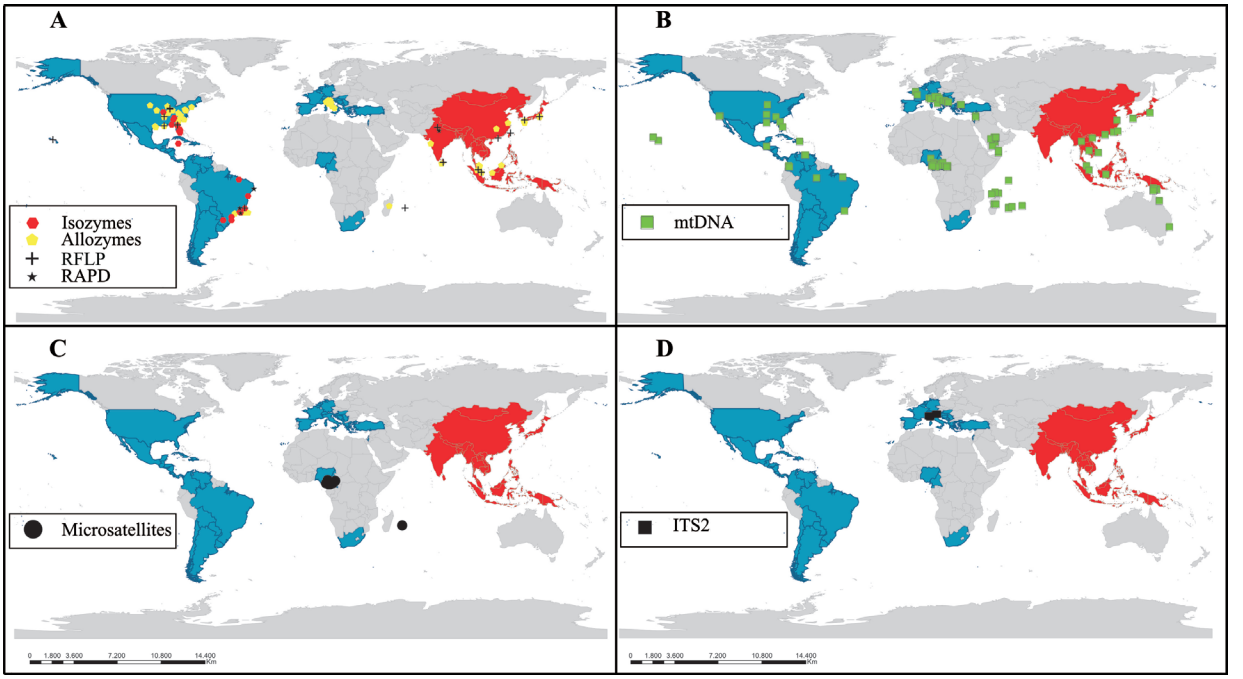

Figure 2. Political maps showing the populations of *A. albopictus* analyzed in genetic diversity studies using the molecular techniques: **A.** Isozymes, Allozyme, RFLP and RAPD; **B.** mtDNA (Cytb, COI and ND5); **C.** Microsatellite; and **D.** ITS2. The colors indicate vector distribution: Gray (Unknown or no data), Red (Indigenous) and Blue (Current distribution range).

Discussion

This study revealed that *A. albopictus* is distributed globally wity structured populations exhibiting low genetic diversit,; most of the genetic diversity known is based on genetic markers that present witw significant problemse. For the last 36 years, the Asian tiger has spread form Asia (place of origin) to Oceania, Africa, Europe and the Americas. However, mathematical models of distribution indicate that *A. albopictus* will continue spreading all over the world due to factors such as transportation means, the environment and climate change[20-65]. Successful dispersion of *A. albopictus* is associated mainly to its ecological plasticity (i.e., the vast array of breeding habitats ranging from tree-holes and cut bamboo to a wide variety of man-made containers), and also, to its passive transport of eggs through the international trade of semi-new tires, plants shipping (*Dracaena spp.*) from Asia, accidental transportation of adults in aircrafts and other means of transportation[1-64]. These situations make *A. albopictus* a highly invasive species, and also link the gene flow among *A. albopictus* populations to the human transportation, as it was globally observed in *A. aegypti* populations[66].

The pattern observed of genetic variation in populations of *A. albopictus* may be attributed to the chemical measures used in vector control programs[67]. Worldwide, extensive and repeated insect control activities have involved source reduction and insecticide application, leading to the reduction and/or eradication of *A. albopictus* populations[68-69]. As a result, reduced levels of genetic variation were observed in the current study. Increased use of insecticides for agricultural pest control, for direct control of *A. albopictus* or for control of sympatric vectors (e.g., other Anophelinae and Culicinae species), has imposed selection pressures on *A. albopictus* populations for increased resistance, as it was observed in *A. albopictus* populations from Asia, Africa, Central America and South America[70-71]. In these resistant populations, genetic polymorphisms could have decreased quickly on any part of the mosquito genome due to the use of insecticides, thereby showing a low genetic diversity.

Low genetic diversity is most likely a result of a decline in population size caused by insecticide use, as it was observed in American *A. aegypti* populations[72-74]. However, some studies have revealed the presence of greater genetic diversity in areas that are frequently treated with insecticides, as shown in *A. aegypti* populations from French Polynesia and Brazil[75-76]. In our findings, most of the genetic diversity of the Asian tiger populations were lower than 0.7. Those results were lower than in other studies on the mtDNA ND4 gene

of *A. aegypti*, a genetic marker widely used in genetic diversity studies in *A. aegypti*.[47,77-78]. For instance, in 36 locations in the Americas, Asia and Africa (*Hd* = 0.80) (79) and five states in Brazil (*Hd* = 0.80)[80] showed higher genetic diversity than the observed in *A. albopictus* populations.

Most Asian tiger populations were genetically structured, a trend also found on *A. aegypti* populations from Asia, Africa, and America[23,61,72]. The genetic structure of *A. albopictus* populations have implications for vector control program, since, studies on selection pressure in *A. aegypti* populations using insecticides such as organophosphates and/or pyrethroids under laboratory conditions, show the fixation of the population resistance phenotype in only a few generations[81-85].

For the development of control programs, it is important to know the dispersal patterns and genetic diversity of the vector[79,86]. Genetic markers are widely used to understand the biology and population dynamics of disease vectors[87]. However, in our study, the 89.5% of the known genetic diversity is based on the use of RFLP, allozymes, isozymes, and mitochondrial molecular markers, which have problems reported in the literature. For instance, the RFLP, allozymes and isozymes markers (developed in the late 70s) are no longer employed in genetic diversity studies, since they present significant limitations when compared to microsatellites and/or mtDNA, due to these show little variation, need sufficient training time and also are poorly reproducible in the laboratory[88,89].

Nevertheless, the main concern is that most of the genetic diversity found in the Asian tiger mosquito populations (51%) is through the use of mtDNA markers. In the last decade, the use of mtDNA has been widely used in population genetics studies for reconstructing historical patterns of population demography, admixture, biogeography and speciation in arthropods, included *A. albopictus*[47,69]. However, integration of mitochondrial sequences in nuclear DNA (referred to as NUMTs) has been discovered in many eukaryotes, including *A. aegypti*[53,90-93]. Thus, PCR amplification using mtDNA marker loci using total genomic DNA can potentially amplify these nuclear copies. These sequences complicate the employment of mtDNA as a molecular marker in genetic studies. In insects, because of the relative small genome size, high copy number of NUMTs sequences may interfere in effective separation of mtDNA from its nuclear paralogs[52,94]. This has been evident among 85 sequenced eukaryotic genomes where the NUMTs sequences were found to have different mitochondrial origin[95]. Thus, population studies using mitochondrial markers derived from these loci can potentially mislead the results.

Another problematic issue of using mtDNA markers has been identified in cases where the host insect harbours maternally inherited microorganisms such as *Wolbachia*. It is a gram-negative endosymbiotic bacterium that causes many developmental defects such as cytoplasmic incompatibility, feminization and sex ratio distortion[96]. As the *Wolbachia* infection sweeps through an insect species, the frequency of mitochondria from infected individuals also increases in the population due to the similar mode of transmission used by *Wolbachia* and the mitochondria. As a result, the spread of the mtDNA from infected individuals reaches high prevalence in these populations, phenomenon commonly referred to as 'genetic hitchhiking'. Thus, inferring evolutionary history of populations solely based on use of mtDNA markers in insect species harboring such maternally inherited microorganisms may be misleading[97]. *Wolbachia* is commonly found in mosquitoes including *A. albopictus*. This species naturally carries two strains of the bacterium *Wolbachia*, wAlbA and wAlbB[98]. *Wolbachia* inherited bacteria are able to invade insect populations using cytoplasmic incompatibility and provide new strategies for controlling mosquito-borne tropical diseases, such as dengue and Chikungunya fever, as shown by Blagrove, et al.[99] and Mousson, et al.[98] in their works.

Currently, there is no presence of NUMTs in *A. albopictus*, therefore, further studies should be done in order to reduce the error caused by NUMTs in the published mtDNA (COI, Cytb, ND5) sequences. Here, we suggest the search for heterozygous sites in the chromatogram and additional termination codons. Common analysis applied on population genetics studies in *A. aegypti* when mtDNA markers are used (see: Gonçalves, et al.[47]; Aguirre-Obando, et al.[72]).

Despite the mtDNA markers have been widely used in vector genetic diversity studies, including *A. albopictus*[29,100], these are not as sensitive to detect genetic variation as microsatellites and/or SNPs (Single Nucleotide Polymorphism) are[61,87]. Microsatellites have been used as genetic markers for a number of arthropod vectors of human diseases, including *A. albopictus*[32]. However, there are a few studies using microsatellites in *A. albopictus* as our findings show. Nevertheless, the use of microsatellites in *A. albopictus* populations has shown they are highly polymorphic. Delatte et

al.[32] using 10 microsatellites (two of them previously used in *A. aegypti*) in *A. albopictus* populations from Reunion Island, in the southwest Indian Ocean, found population genetic structuring. An alternative to increase the number of polymorphic microsatellites in population genetics studies in *A. albopictus* would evaluate the microsatellites described for *A. aegypti* (33 microsatellite loci[60]), as some of them has proved to be highly polymorphic[32].

On the other hand, the SNPs, are the most common way of molecular variation in vertebrates and invertebrates[101-104]. Currently, SNPs have become one of the selectable markers for studies on population genetics, characterization of genes or disease to elucidate the evolutionary processes at the molecular level, since they are easy to detect when compared, for example, with microsatellites[87,105]. In vectors diseases such as *Anopheles gambiae*, *A. funestus* (vectors of malaria in Africa) and *A. aegypti*, SNPs have been highly polymorphic[87,104,106-107]. For *A. aegypti*, Paduan & Ribolla[106] sequenced seven genes of 16 Brazilian populations of this species. These genes revealed the existence of 53 individual SNPs; eight of them are independent and highly polymorphic to be used in genetic diversity studies. Since, our search did not find any work related to the use of SNPs in *A. albopictus*, we suggest to test the polymorphic SNPs described for *A. aegypti* in *A. albopictus*, since other molecular markers developed in *A. aegypti* like microsatellites, have shown highly polymorphic in *A. albopictus*[32]. It can be concluded then, that the current scenario of genetic diversity in *A. albopictus* populations, is based on genetic markers that present significant problems reported in the literature, thus vector control programs, understanding of the vectors transmission, and the spread of genetic traits, such as vector competence and insecticide resistance, may be compromised.

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Conflict of interest

We declared there is no potential conflict of interest.

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