







RESEARCH ARTICLE

Geographical distribution of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and genetic diversity of invading population of *Ae. albopictus* in the Republic of the Congo

[version 1; referees: awaiting peer review]

Basile Kamgang ¹, Theodel A. Wilson-Bahun ^{1,2}, Helen Irving³,
Michael O. Kusimo ¹, Arsene Lengua², Charles S. Wondji ^{1,3}

¹Medical Entomology, Centre for Research in Infectious Diseases, Yaounde, P.O. Box 13591, Cameroon

²Faculty of Science and Technology, Marien Ngouabi University, Brazzaville, Congo

³Liverpool School of Tropical Medicine, Liverpool, UK

v1 First published: 25 Jun 2018, 3:79 (doi: [10.12688/wellcomeopenres.14659.1](https://doi.org/10.12688/wellcomeopenres.14659.1))
Latest published: 25 Jun 2018, 3:79 (doi: [10.12688/wellcomeopenres.14659.1](https://doi.org/10.12688/wellcomeopenres.14659.1))

Abstract

Background: The arbovirus vector, *Aedes albopictus*, originating from Asia, has recently invaded African countries, including the Republic of the Congo, where it was associated with a chikungunya outbreak. Up until now, little was known about its distribution in relation to the native *Aedes aegypti* and how the invasion will modify the epidemiology of arboviral diseases. Here, we assessed the current distribution of *Ae. albopictus* and *Ae. aegypti* in the Republic of the Congo and explored the genetic diversity of the invading species, *Ae. albopictus*.

Methods: Immature stages of *Aedes* were collected in nine locations in the Republic of the Congo in 2017 following a north-south transect and reared to adult stage. Adults were morphologically identified, counted and grouped according to species and location. Genetic diversity of *Ae. albopictus* was assessed by analyzing the cytochrome oxidase I (*COI*) gene.

Results: *Ae. albopictus* and *Ae. aegypti* were found together across the country in all the locations investigated. The invasive species is predominant over the native species in all locations except Brazzaville, suggesting that *Ae. albopictus* is displacing *Ae. aegypti* across Congo. When comparing the species distributions across the two largest cities, Brazzaville and Pointe Noire, *Ae. albopictus* was more prevalent than *Ae. aegypti* in the suburbs whereas the opposite situation was reported in the city centre. Mitochondrial DNA analysis revealed very low genetic diversity of *Ae. albopictus* with only three haplotypes recorded across the country supporting the recent introduction of this species in the Republic of the Congo. Phylogenetic tree analysis revealed that *Ae. albopictus* from Congo originated from other tropical Asian countries such as China, likely as a result of increasing trade links.

Conclusion: These findings are important for the implementation of vector control strategies and can serve as a foundation for further research on these vectors in the country.

Open Peer Review

Referee Status: AWAITING PEER

REVIEW

Discuss this article

Comments (0)

Keywords

Aedes albopictus, *Aedes aegypti*, ecological distribution, arbovirus vectors, genetic diversity, Republic of Congo

Corresponding author: Basile Kamgang (kamgang_d@yahoo.fr)

Author roles: **Kamgang B:** Conceptualization, Data Curation, Formal Analysis, Funding Acquisition, Investigation, Methodology, Project Administration, Resources, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing;

Wilson-Bahun TA: Formal Analysis, Investigation, Methodology, Visualization, Writing – Review & Editing; **Irving H:** Data Curation, Formal Analysis, Writing – Review & Editing; **Kusimo MO:** Writing – Review & Editing; **Lenga A:** Supervision, Writing – Review & Editing; **Wondji CS:** Conceptualization, Funding Acquisition, Project Administration, Supervision, Writing – Original Draft Preparation, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

How to cite this article: Kamgang B, Wilson-Bahun TA, Irving H *et al.* **Geographical distribution of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and genetic diversity of invading population of *Ae. albopictus* in the Republic of the Congo [version 1; referees: awaiting peer review]** Wellcome Open Research 2018, 3:79 (doi: [10.12688/wellcomeopenres.14659.1](https://doi.org/10.12688/wellcomeopenres.14659.1))

Copyright: © 2018 Kamgang B *et al.* This is an open access article distributed under the terms of the [Creative Commons Attribution Licence](https://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Grant information: This work was supported by a Wellcome Trust Training Fellowship in Public Health and Tropical Medicine (204862) awarded to Basile Kamgang. The funders had no role in study design, data collection or analysis, decision to publish or preparation of the manuscript. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

First published: 25 Jun 2018, 3:79 (doi: [10.12688/wellcomeopenres.14659.1](https://doi.org/10.12688/wellcomeopenres.14659.1))

Introduction

Arthropod-borne viral diseases such as dengue, zika and chikungunya have emerged or re-emerged in several countries of the world during the past decades^{1–4}.

These viruses are transmitted to vertebrates, including humans, by the bites of infected mosquitoes that share the same ecological niche as the host organism. Indeed, two distinct ecological cycles, enzootic and urban epidemic cycles, have been well documented^{5,6}. The enzootic cycle occurs in the sylvan environment, involving non-human primates and wild mosquitoes, while urban epidemic cycle occurs in urban environments, implicating human beings and urban mosquitoes such as *Aedes aegypti* Linnaeus 1762 and *Ae. albopictus* (Skuse) 1894. Other potential modes of zika virus transmission to humans have been evoked notably via sexual intercourse or via blood donor⁵. Both epidemic vectors, *Ae. aegypti* and *Ae. albopictus*, are found in sub-Saharan Africa, where *Ae. aegypti* is native. Two subspecies of *Ae. aegypti*, *Ae. aegypti formosus* and *Ae. aegypti aegypti*, were formally identified by Mattingly in 1957⁷. *Ae. aegypti formosus*, is a dark colored mosquito confined to African forests while *Ae. aegypti aegypti* is light-colored with white abdominal scales and is found in human-dominated habitats primarily outside Africa. Generally, *Ae. aegypti* collected in central Africa match *Ae. aegypti formosus*⁸.

While *Ae. albopictus* is a native of South East Asia, it has now invaded all the five continents during the past 30–40 years^{9,10}. This rapid global spread was caused mainly by sales and distribution of used tires across the world¹¹ coupled with the ecological plasticity of the species, enabling its adaptation to various environments⁹. *Ae. albopictus* was reported for the first time in Central Africa in early 2000¹² and is currently present in almost all central African countries¹³, where it tends to supplant the indigenous species *Ae. aegypti* in human-domesticated environment^{14,15}. The predominance of *Ae. albopictus* over *Ae. aegypti* in sympatric areas has been shown to result from the higher mating competitiveness of *Ae. albopictus* over *Ae. aegypti*^{16,17}. Previous studies in Central Africa showed that both *Ae. aegypti* and *Ae. albopictus* can be found together in the same location and often share the same larval habitats^{14,15}. In this region, the immature stages of both species develop in stagnant water found mainly in peri-domestic containers such as used tires and discarded tanks. However, in the sympatric area, *Ae. albopictus* prefers containers surrounded by vegetation whereas *Ae. aegypti* prefers containers located in neighbourhoods with high building densities^{15,18}.

Dengue, zika and chikungunya were for a long-time considered to be rare in Central Africa, because only sporadic epidemics were reported in the rural environment, with isolation of the viruses in wild mosquitoes and humans^{19,20}. In the past decades, several outbreaks have been reported in this part of the world, notably a concurrent dengue/chikungunya outbreak in Gabon in 2007, with more than 20, 000 cases of chikungunya², and a large chikungunya outbreak in 2011 in the Republic of the Congo with more than 11, 000 cases²¹. This suggests an epidemiological modification of arboviral diseases in the region. During these outbreaks, *Ae. albopictus* was established as the major vector

particularly in Gabon^{22,23}, where zika was detected in this species²⁴. In Congo, both *Ae. aegypti* and *Ae. albopictus* were found to be positive for chikungunya virus²⁵, implicating both species in virus transmission. This investigation was the first to confirm the presence of *Ae. albopictus* in the Republic of the Congo. Since then, no study has been undertaken to compare the geographical distribution and prevalence of *Ae. aegypti* and *Ae. albopictus* in the Republic of the Congo as well as the genetic diversity of the invading species. Indeed, previous studies in Central Africa based on polymorphisms to the cytochrome oxidase subunit 1 (*COI*) gene indicated that *Ae. albopictus* populations in Cameroon are related to tropical rather than temperate or subtropical out-groups²⁶. However, the Central African Republic population segregated into two lineages: the first encompassed specimens from tropical areas including all the haplotypes from Cameroon and the second lineage encompassed temperate and subtropical areas¹⁵, suggesting multiple sources of *Ae. albopictus*.

To improve entomological surveillance and the control of these arbovirus vectors in the Republic of the Congo, we present here the current nation-wide geographical distribution and prevalence of *Ae. aegypti* and *Ae. albopictus* in this country, and establish the genetic diversity of the invading population of *Ae. albopictus* using the *COI* gene.

Methods

Sampling sites

Mosquitoes were collected in May and November 2017 in nine locations in the Republic of the Congo across the north-south transect (Table 1 and Figure 1). The Republic of the Congo is located in Central Africa, straddling the equator. Two main types of vegetation are found. The forest in the north, covering 60% of the national territory, and the savannah, which occupies the remaining parts of the country. There are three types of climate. The equatorial climate is found in the north of the country, characterized by high humidity and rainfall greater than 1,700 mm per year, with an average temperature between 24°C and 26°C. The humid tropical climate in the southwest, where annual average precipitation varies from 1,200 mm to 1,700 mm, with an average monthly temperature between 21°C and 27°C. The subequatorial climate, experienced at the plateau and basin regions, has an average annual rainfall of about 1,600 mm. Because the spread of *Aedes* mosquitoes mainly relies on human activities, sampling was focused on human-domesticated environments spread along the main communication networks, and trade routes throughout the country.

Mosquito collection, rearing and identification

In Brazzaville and Pointe Noire, the two most populated cities of the Republic of the Congo, the difference between downtown and suburban was examined during the investigation. In the other locations, however, samples were collected randomly throughout each city and pooled together. In each selected location, all containers with water were inspected and positive containers (with at least one *Aedes* larvae or pupae) were recorded. Immature stages of *Aedes* were collected, transported to the insectaries, pooled according to the location and reared to adult stage for morphological identification. G0 adults were stored

Table 1. Sampling sites in the Republic of the Congo.

Location	Geographical coordinates	Altitude, m	Climate
Brazzaville	S 4°19'38" E 15°09'12"	278	Subequatorial climate
Lefini	S 2°54'58" E 15°37'56"	314	Subequatorial climate
Ngo	S 2°29'14" E 15°45'00"	636	Subequatorial climate
Gamboma	S 1°52'27" E 15°52'25"	378	Subequatorial climate
Oyo	S 1°09'14" E 15°58'21"	297	Subequatorial climate
Owando	S 0°29'42" E 15°54'41"	275	Subequatorial climate
Makoua	S 0°00'23" E 19°37'33"	350	Equatorial climate
Ouesso	N 1°36'35" E 16°02'58"	339	Equatorial climate
Pointe Noire	N 4°48'19" E 11°53'23"	14	Tropical climate

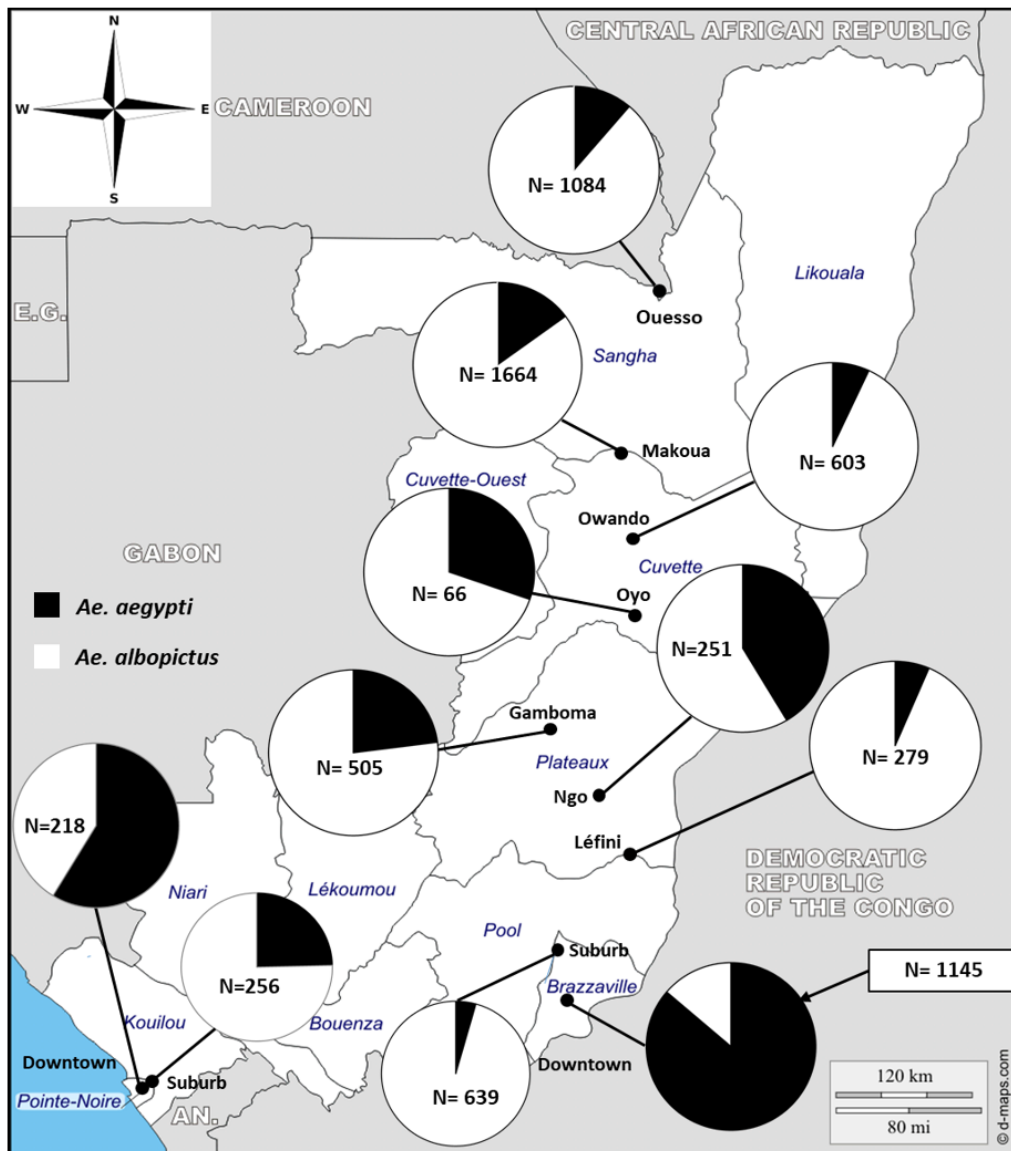


Figure 1. Geographic distribution of *Ae. aegypti* and *Ae. albopictus* across the Republic of the Congo.

at -20°C for molecular and genetic analyses. The comparisons between the prevalence of *Ae. aegypti* and *Ae. albopictus* in each location, across the country were performed using multiple chi-square test.

Mitochondrial DNA analysis for *Ae. albopictus*

Genomic DNA was extracted from 20 whole *Ae. albopictus* per location (nine locations) using the Livak protocol as previously described²⁷. DNA extracts from each location were used as templates to amplify 700-bp fragment of COI gene. The sequences of primers used are: albCOIF 5'-TTTCAACAAAT-CATAAAGATATTGG-3' and albCOIR 5'-TAAACTTCTGGA TGACCAAAAATCA-3'²⁸. Polymerase chain reaction (PCR) amplification was performed using a Gene Touch thermal cycler (Bulldog Bio, Portsmouth, USA), as described previously²⁸. PCR products were detected by agarose gel electrophoresis in Tris-Acid-EDTA buffer (TAE). The gel was prepared with Midori green, staining dye, and visualized with the aid of UV light. PCR products from each location with very good amplification were purified using the Exo-SAP protocol and sent to the Centre for Genomic Research (Liverpool, UK) for sequencing.

Sequence data analysis

Sequences were manually corrected using BioEdit software version 7.2.1 (<http://en.bio-soft.net/format/BioEdit.html>) and aligned using ClustalW, which is present in BioEdit²⁹. Sequences were numbered based on the reference sequences downloaded in GenBank KU738429.1. The number of haplotypes (h), the number of polymorphism sites (S), haplotype diversity (Hd) and nucleotide diversity (π) were computed with DnaSP 5.10.01 (<http://en.bio-soft.net/dna/dnasp.html>)³⁰. The statistical tests of Tajima³¹, and Fu and Li³² were also estimated with DnaSP in order to establish non-neutral evolution and deviation from mutation-drift equilibrium. The different haplotypes detected

were compared to previous sequences published in GenBank (Supplementary Table 1) that originated from China, Papua New Guinea, USA, Singapore, Taiwan, Malaysia, Hawaii, Christmas Islands, Japan, Solomon Islands, Timor Leste and Torres Strait Islands^{28,33,34}. The same COI region was sequenced at these various regions and the maximum likelihood phylogenetic tree was constructed using MEGA 7.0³⁵. Genealogical relationships between haplotype in this current study was assessed using TCS version 1.21³⁶ and tcsBU (<http://cibio.up.pt/software/tcsBU/>)³⁷ software.

Results

Containers inspected and prevalence of *Ae. aegypti* and *Ae. albopictus*

A total of 640 containers with water were investigated across the Republic of the Congo (Table 2). Among them, 42.9% were positive for immature stages of *Aedes*. Containers were classified into three main groups: domestic (flower pot and water storage tanks), peridomestic (used tires, discarded tanks and car wrecks) and natural (axil of plants). Used tires were the most prevalent habitat and most productive containers in all the locations, ranging from 18.1% in Pointe Noire to 100% in Lefini (Table 2). The presence of *Aedes* in other containers was very limited.

In total, 6,684 specimens of immature stages of *Aedes* were identified, comprising 72.24% of *Ae. albopictus*, 27.70% of *Ae. aegypti* and 0.06% (four specimens collected in Brazzaville suburb) of *Aedes simpsoni*. *Ae. aegypti* and *Ae. albopictus* were found together in all the locations investigated (Figure 1 and Table 3). However, *Ae. albopictus* was predominant in all the locations except in Brazzaville. When samples from the two major cities, Brazzaville and Pointe Noire, were divided according to the environment (downtown versus suburb), *Ae. albopictus* was found more prevalent in the suburbs (95.62%

Table 2. Containers prospected per location.

Location	Axil of plants	Used tires	Car wrecks	Discarded tanks	Water storages	Flower pots	All
	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Brazzaville downtown	1 (0.0)	59 (49.2)	3 (100)	10 (80.0)	0 (NC)	0 (NC)	73 (54.8)
Brazzaville suburb	0 (NC)	69 (63.8)	0 (NC)	3 (0.0)	0 (NC)	0 (NC)	72 (61.1)
Pointe Noire downtown	0 (NC)	61 (18.1)	0 (NC)	3 (33.3)	0 (NC)	0 (NC)	64 (18.8)
Pointe Noire suburb	0 (NC)	56 (25.0)	0 (NC)	13 (46.2)	4 (50.0)	0 (NC)	73 (30.1)
Lefini	0 (NC)	3 (100)	0 (NC)	0 (NC)	0 (NC)	0 (NC)	3 (100)
Ngo	0 (NC)	47 (38.3)	0 (NC)	0 (NC)	0 (NC)	0 (NC)	47 (38.3)
Gamboma	0 (NC)	58 (37.9)	0 (NC)	2 (50)	1 (100)	0 (NC)	61 (39.3)
Oyo	0 (NC)	43 (48.8)	0 (NC)	0 (NC)	0 (NC)	0 (NC)	43 (48.8)
Owando	0 (NC)	4 (25)	0 (NC)	8 (62.5)	0 (NC)	10 (10.0)	22 (31.8)
Makoua	0 (NC)	59 (54.2)	0 (NC)	10 (30)	0 (NC)	0 (NC)	69 (50.7)
Ouessou	0 (NC)	97 (50.5)	0 (NC)	0 (NC)	0 (NC)	0 (NC)	97 (50.5)
All	1 (0.0)	556 (43.9)	3 (100)	65 (36.9)	5 (60.0)	10 (10.0)	640 (42.9)

N, number of containers found with water; (%), percentage of positive containers; NC, not computed.

Table 3. Prevalence of *Aedes aegypti* and *Aedes albopictus* according to the location.

Location	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>	P-value
Brazzaville downtown	962 (86.28%)	153 (13.72%)	<0.001
Brazzaville suburb	28 (4.38%)	611 (95.62%)	<0.001
Pointe Noire downtown	128 (58.72%)	90 (41.28%)	<0.001
Pointe Noire suburb	63 (24.61%)	193 (75.39%)	<0.001
Lefini	18 (6.45%)	261 (93.55%)	<0.001
Ngo	104 (41.43%)	147 (58.57%)	<0.001
Gamboma	116 (22.97%)	389 (77.03%)	<0.001
Oyo	20 (30.30%)	46 (69.70%)	<0.001
Owando	42 (6.97%)	561 (93.03%)	<0.001
Makoua	249 (14.96%)	1415 (85.04%)	<0.001
Ouessou	122 (11.25%)	962 (88.75%)	<0.001
All	1852 (27.72%)	4828 (72.28%)	<0.001

and 75.39% in Brazzaville and Pointe Noire, respectively) than *Ae. aegypti*, whereas the reverse was true for the downtown areas (Table 3).

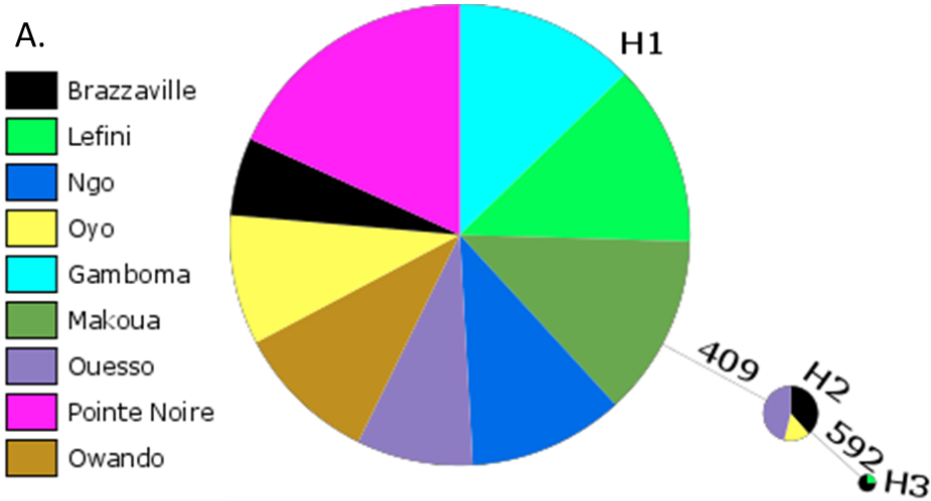
Mitochondrial DNA analysis of *Ae. albopictus*

In total, 127 specimens of *Ae. albopictus* from nine locations across the Republic of the Congo were analysed using the *COI* gene. Sequence analysis, based on 638 nucleotides, revealed a low polymorphism, with only two mutational sites defining three haplotypes namely H1, H2 and H3 (Figure 2B). Consequently, this resulted in low haplotype diversity ($Hd=0.24$) and nucleotide diversity ($\pi=0.00005$) indexes (Table 4). The most frequent haplotype, H1 (86.6%), was detected in all the locations. Supplementary Table 2 shows the haplotype distribution per location. The haplotypes H2 (10.2%) and H3 (3.2%) were found in three (Brazzaville, Ouesso and Oyo) and two (Brazzaville and Lefini) locations, respectively (Table 4 and Figure 2). The dominant haplotype matches perfectly with the *COI* gene sequence deposited in GenBank that originated from China (KU738429.1). A higher genetic diversity was reported in Brazzaville where all the three haplotypes were reported. The haplotype network showed that each haplotype was separated from the others by one mutational step (Figure 2). Overall, Tajima's D ($D=-0.294$) and Fu's F_s ($F_s=-0.024$) statistics were negative, but not statistically significant. Phylogenetic tree generated and analysed based on 445 nucleotides previously published in GenBank showed that the Republic of the Congo's haplotypes were closely related to the sequences from China, Singapore, Papua New Guinea and Christmas Island.

Discussion

This study has assessed the geographical distribution of *Ae. aegypti* and *Ae. albopictus* in the Republic of the Congo, revealing the co-occurrence of both species across the country. Analyses showed that the invasive species *Ae. albopictus* is the

predominant species in all locations investigated except Brazzaville. The co-occurrence of *Ae. aegypti* and *Ae. albopictus* across the Republic of the Congo suggests that the environmental factors which prevail in the country are favourable for the development of both species. The presence of *Ae. albopictus* in the Republic of the Congo was confirmed in 2011 during the chikungunya outbreak in Brazzaville²⁵, suggesting its recent introduction. Indeed, previous studies in some central African countries such as Cameroon and Central Africa Republic showed that the co-occurrence of *Ae. aegypti* and *Ae. albopictus* is limited to the southern part of the country up to 6°N^{14,15,18} suggesting that climate is a limiting factor for invasion. The predominance of the invading species, *Ae. albopictus*, over the indigenous species, *Ae. aegypti*, has been previously reported in areas where both species are found together in Central Africa. The ecological plasticity of *Ae. albopictus* has been suggested as the main cause of its adaptation to different environments⁹, as well as its mating competitiveness in areas of sympatry with *Ae. aegypti*^{17,38}. The prevalence of each species can vary according to the season in sympatric areas, as shown previously^{15,39}, but was specifically linked to the duration of the dry season⁴⁰. Although, *Ae. aegypti* and *Ae. albopictus* have desiccant-resistant eggs, previous studies showed that *Ae. aegypti* eggs are more tolerant to high temperatures than those of *Ae. albopictus*⁴¹. In both major cities where samples were analysed according to peri-urban and downtown environment, results revealed the predominance of *Ae. aegypti* in downtown areas but less in peri-urban areas. Similar findings to these were reported previously in Central Africa^{15,18,40}. These observations are consistent with former studies indicating the segregation of habitats in sympatric areas according to urban environmental gradients as the main factor responsible for the coexistence of *Ae. aegypti* and *Ae. albopictus*^{42,43}. Used tyres were the most common container found positive for *Aedes* in all the locations. This is in accordance with previous studies in Central Africa showing that used tires are



Haplotype*	Frequency		
		4	5
Ref [KU738429]	-	T	A
H1 [MH025948]	110	.	.
H2 [MH025949]	13	.	G
H3 [MH025950]	04	C	G

Figure 2. Genetic diversity of the COI gene across Congolese populations of *Ae. albopictus*. (A) Haplotype network showing the genealogic relationships between three haplotypes detected across Congo. The pie chart represents the proportion of each haplotype per site. (B) COI haplotypes found across the Republic of the Congo. Only polymorphic positions are shown and are numbered with reference (Ref) to the published *Ae. albopictus* sequences for COI (JF309317; China). Dots represent identity with respect to the reference. The numbers above nucleotides indicate the position where mutations were found. *GenBank accession number shown in brackets.

Table 4. Summary statistics for COI gene polymorphism in *Aedes albopictus* from the Republic of the Congo.

Locality	N	H	S	Hd	π (k)	D	D*	Fs	F*
Brazzaville	14	H1, H2, H3	2	0.692	0.0014 (0.890)	1.127 ^{ns}	0.935 ^{ns}	0.612 ^{ns}	1.021 ^{ns}
Lefini	15	H1, H3	2	0.133	0.0004 (0.266)	-1.490 ^{ns}	-1.873 ^{ns}	0.235 ^{ns}	-1.844 ^{ns}
Ngo	12	H1	0	0.000	0.0000 (0.000)	NC	NC	NC	NC
Gamboma	14	H1	0	0.000	0.0000 (0.000)	NC	NC	NC	NC
Oyo	12	H1, H2	1	0.303	0.0005 (0.303)	-0.195 ^{ns}	0.752 ^{ns}	0.297 ^{ns}	0.533 ^{ns}
Owando	11	H1	0	0.000	0.0000 (0.000)	NC	NC	NC	NC
Makoua	14	H1	0	0.000	0.0000 (0.000)	NC	NC	NC	NC
Ouessou	15	H1, H2	1	0.514	0.0008 (0.5143)	1.376 ^{ns}	0.701 ^{ns}	1.253 ^{ns}	0.906 ^{ns}
Pointe Noire	20	H1	0	0.000	0.0000 (0.000)	NC	NC	NC	NC
Total	127	3	2	0.246	0.0005 (0.2952)	-0.294^{ns}	0.662^{ns}	-0.0238^{ns}	0.417^{ns}

N, number of sequences; S, number of polymorphic sites; H, haplotype; Hd, haplotype diversity; π , nucleotide diversity; k, mean number of nucleotide differences; D, Tajima statistic; D* and F*, Fu and Li statistics; Fs, Fu statistic; NC, not computed; ns, not significant.

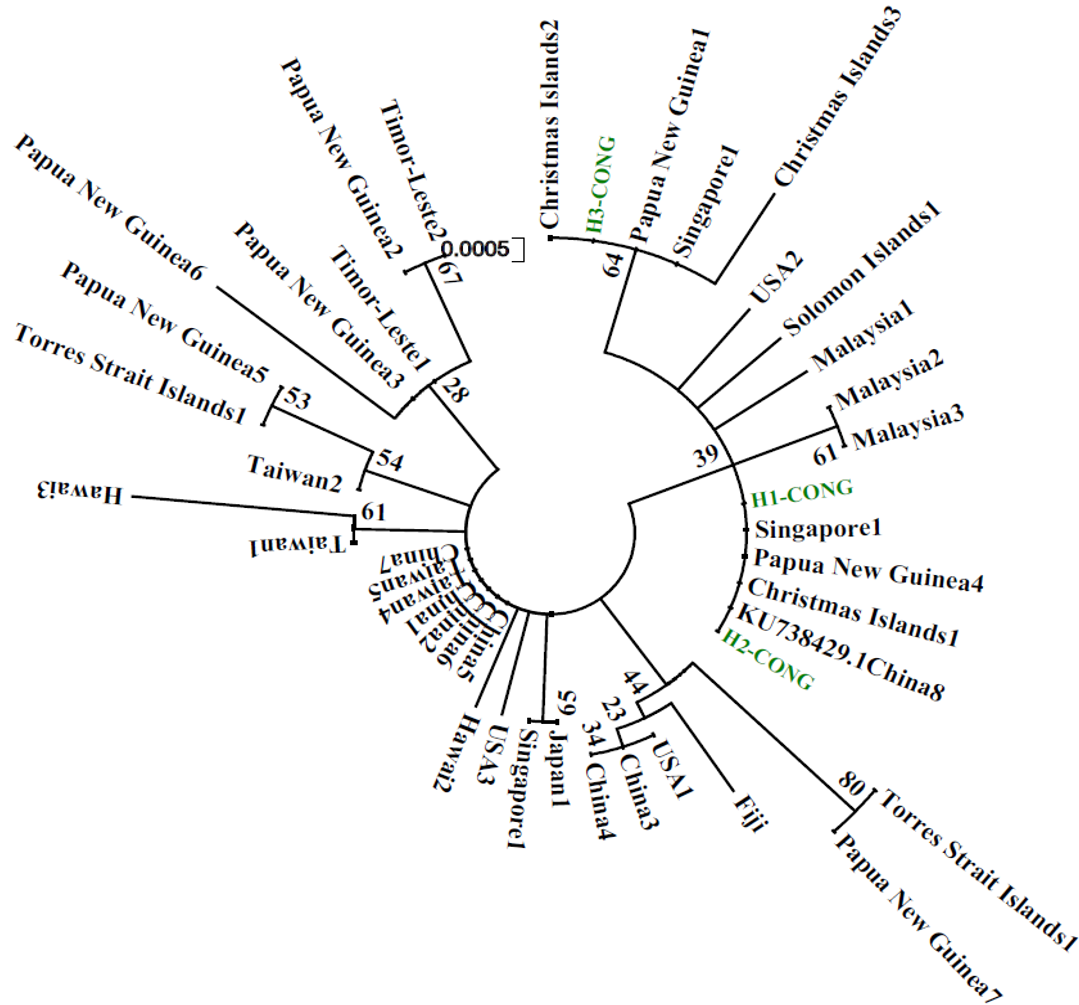


Figure 3. Molecular phylogenetic analysis using the Maximum Likelihood method. The evolutionary history was inferred using the Maximum Likelihood method based on the Tamura 3-parameter model. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. There was a total of 445 positions in the final dataset.

the main productive for both *Ae. aegypti* and *Ae. albopictus*^{13,15}. However, the current study targeted mainly garages and tire shops to increase the chances of discovering immature *Aedes*.

The presence and predominance of *Ae. albopictus* across the Republic of the Congo can increase the risk of mosquito-borne arboviral diseases since *Ae. albopictus* has been found competent to transmit about 22 arboviruses⁴⁴. Notably, the emergence of dengue and chikungunya viruses in the human dominated environment in central Africa coincides with the invasion of *Ae. albopictus* in this area where it was found as the main vector^{13,23,25}. During previous studies in central Africa, *Ae. albopictus*, was found to be infected by zika virus in natural conditions²⁴. It was also demonstrated that *Ae. albopictus* from Bangui in Central African Republic is able to transmit enzootic chikungunya virus strains⁴⁵.

A very low polymorphism of *Ae. albopictus* discovered in the Republic of the Congo in this study is in agreement with the

previous studies using the *COI* gene in areas newly colonised by this species including some Central African countries^{15,26,46}. This low polymorphism is consistent with the recent introduction from a founder *Ae. albopictus* population or could be related to ubiquitous *Wolbachia* infection in populations of this species, as suggested previously⁴⁷. Brazzaville, the capital city of the Republic of the Congo would be probably the main entry point of *Ae. albopictus* in the country, as higher levels of polymorphism (all the three haplotypes recorded) were detected at this location. For instance, *Ae. albopictus* was reported for the first time in the Republic of the Congo in Brazzaville during a chikungunya outbreak which occurred in the country^{21,25}. Phylogenetic analysis showed that the haplotype sequences from the Republic of the Congo are very close to the sequences isolated from populations originating from China, New Papua Guinea, Singapore and Christmas Islands. Primers used in this current study were not the same as those used in the previous study in Cameroon, Central African Republic and Sao Tome island. Therefore, the haplotypes in the current study cannot be compared with those detected in

Central Africa. Nevertheless, these data indicate that the population of *Ae. albopictus* found in Central Africa probably originated from other tropical regions as previously suggested^{15,26,46}. It will be interesting to perform other studies at macro-geographic scale using other markers such as double-digest restriction-site-associated DNA sequencing to assess the genetic structure and the level of the gene flow between these populations.

Conclusion

To our knowledge, this is the first study assessing the distribution of *Ae. aegypti* and *Ae. albopictus* in the Republic of the Congo since *Ae. albopictus* was reported in 2011. Both species were found across the country with *Ae. albopictus* predominating in almost all locations. Low genetic polymorphism of *Ae. albopictus* indicated a recent introduction into the country. The spread of the invading species across the country could change the epidemiology of arboviral diseases in the Republic of the Congo. Thus, it will be important to assess urgently, the vector competence of both *Aedes* species from the Republic of the Congo to prevent several emergence or re-emergence of arboviruses such as dengue, zika and yellow fever viruses. Assessing the susceptibility profile of these species to insecticide will be an important information needed to prepare the country against potential future outbreaks.

Data availability

Sequence for *Aedes albopictus* haplotype H1 cytochrome oxidase subunit I (*COI*) gene, partial cds; mitochondrial,

GenBank accession number MH025948: <http://identifiers.org/ncbigi/GI:1402399558>.

Sequence for *A. albopictus* haplotype H2 cytochrome oxidase subunit I (*COI*) gene, partial cds; mitochondrial, GenBank accession number MH025949: <http://identifiers.org/ncbigi/GI:1402399560>.

Sequence for *A. albopictus* haplotype H3 cytochrome oxidase subunit I (*COI*) gene, partial cds; mitochondrial, GenBank accession number MH025950: <http://identifiers.org/ncbigi/GI:1402399562>.

Competing interests

No competing interests were disclosed.

Grant information

This work was supported by a Wellcome Trust Training Fellowship in Public Health and Tropical Medicine (204862) awarded to Basile Kamgang.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Acknowledgments

We thank the people living around all the sampling sites for their cooperation during the field investigations.

Supplementary material

Supplementary Table 1. Outgroup sequences used for phylogenetic analysis.

[Click here to access the data.](#)

Supplementary Table 2. H1, H2 and H3 haplotype distribution per location.

[Click here to access the data.](#)

References

- Hennessey M, Fischer M, Staples JE: **Zika Virus Spreads to New Areas - Region of the Americas, May 2015-January 2016.** *MMWR Morb Mortal Wkly Rep.* 2016; **65**(3): 55–58. [PubMed Abstract](#) | [Publisher Full Text](#)
- Leroy EM, Nkoghe D, Ollomo B, *et al.*: **Concurrent chikungunya and dengue virus infections during simultaneous outbreaks, Gabon, 2007.** *Emerg Infect Dis.* 2009; **15**(4): 591–593. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Bhatt S, Gething PW, Brady OJ, *et al.*: **The global distribution and burden of dengue.** *Nature.* 2013; **496**(7446): 504–507. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Nsoesie EO, Kraemer MU, Golding N, *et al.*: **Global distribution and environmental suitability for chikungunya virus, 1952 to 2015.** *Euro Surveill.* 2016; **21**(20). [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Sharma A, Lal SK: **Zika Virus: Transmission, Detection, Control, and Prevention.** *Front Microbiol.* 2017; **8**: 110. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Thiboutot MM, Kannan S, Kawalekar OU, *et al.*: **Chikungunya: a potentially emerging epidemic?** *PLoS Negl Trop Dis.* 2010; **4**(4): e623. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Mattingsly PF: **Genetical aspects of the *Aedes aegypti* problem. I. Taxonom and bionomics.** *Ann Trop Med Parasitol.* 1957; **51**(4): 392–408. [PubMed Abstract](#) | [Publisher Full Text](#)
- Gloria-Soria A, Ayala D, Bheecarry A, *et al.*: **Global genetic diversity of *Aedes aegypti*.** *Mol Ecol.* 2016; **25**(21): 5377–5395. [PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
- Paupy C, Delatte H, Bagny L, *et al.*: ***Aedes albopictus*, an arbovirus vector: from the darkness to the light.** *Microbes Infect.* 2009; **11**(14–15): 1177–1185. [PubMed Abstract](#) | [Publisher Full Text](#)
- Benedict MQ, Levine RS, Hawley WA, *et al.*: **Spread of the tiger: global risk of**

- invasion by the mosquito *Aedes albopictus*. *Vector Borne Zoonotic Dis.* 2007; 7(1): 76–85.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
11. Reiter P, Fontenille D, Paupy C: *Aedes albopictus* as an epidemic vector of chikungunya virus: another emerging problem? *Lancet Infect Dis.* 2006; 6(8): 463–464.
[PubMed Abstract](#) | [Publisher Full Text](#)
 12. Fontenille D, Toto JC: *Aedes (Stegomyia) albopictus* (Skuse), a potential new Dengue vector in southern Cameroon. *Emerg Infect Dis.* 2001; 7(6): 1066–1067.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 13. Ngoagouni C, Kamgang B, Nakouné E, et al.: Invasion of *Aedes albopictus* (Diptera: Culicidae) into central Africa: what consequences for emerging diseases? *Parasit Vectors.* 2015; 8: 191.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 14. Simard F, Nchoutpouen E, Toto JC, et al.: Geographic distribution and breeding site preference of *Aedes albopictus* and *Aedes aegypti* (Diptera: culicidae) in Cameroon, Central Africa. *J Med Entomol.* 2005; 42(5): 726–731.
[PubMed Abstract](#) | [Publisher Full Text](#)
 15. Kamgang B, Ngoagouni C, Manirakiza A, et al.: Temporal patterns of abundance of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) and mitochondrial DNA analysis of *Ae. albopictus* in the Central African Republic. *PLoS Negl Trop Dis.* 2013; 7(12): e2590.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 16. Bargielowski IE, Lounibos LP, Shin D, et al.: Widespread evidence for interspecific mating between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in nature. *Infect Genet Evol.* 2015; 36: 456–461.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 17. Bargielowski IE, Lounibos LP, Carrasquilla MC: Evolution of resistance to satyriation through reproductive character displacement in populations of invasive dengue vectors. *Proc Natl Acad Sci U S A.* 2013; 110(8): 2888–2892.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 18. Kamgang B, Happi JY, Boisier P, et al.: Geographic and ecological distribution of the dengue and chikungunya virus vectors *Aedes aegypti* and *Aedes albopictus* in three major Cameroonian towns. *Med Vet Entomol.* 2010; 24(2): 132–141.
[PubMed Abstract](#) | [Publisher Full Text](#)
 19. Desdoutis M, Kamgang B, Berthet N, et al.: Genetic characterization of Chikungunya virus in the Central African Republic. *Infect Genet Evol.* 2015; 33: 25–31.
[PubMed Abstract](#) | [Publisher Full Text](#)
 20. Berthet N, Nakouné E, Kamgang B, et al.: Molecular characterization of three Zika flaviviruses obtained from sylvatic mosquitoes in the Central African Republic. *Vector Borne Zoonotic Dis.* 2014; 14(12): 862–865.
[PubMed Abstract](#) | [Publisher Full Text](#)
 21. Moyen N, Thiberville SD, Pastorino B, et al.: First reported chikungunya fever outbreak in the republic of Congo, 2011. *PLoS One.* 2014; 9(12): e115938.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 22. Paupy C, Kassa Kassa F, Caron M, et al.: A chikungunya outbreak associated with the vector *Aedes albopictus* in remote villages of Gabon. *Vector Borne Zoonotic Dis.* 2012; 12(2): 167–169.
[PubMed Abstract](#) | [Publisher Full Text](#)
 23. Paupy C, Ollomo B, Kamgang B, et al.: Comparative role of *Aedes albopictus* and *Aedes aegypti* in the emergence of Dengue and Chikungunya in central Africa. *Vector Borne Zoonotic Dis.* 2010; 10(3): 259–266.
[PubMed Abstract](#) | [Publisher Full Text](#)
 24. Grard G, Caron M, Mombo IM, et al.: Zika virus in Gabon (Central Africa)—2007: a new threat from *Aedes albopictus*? *PLoS Negl Trop Dis.* 2014; 8(2): e2681.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 25. Mombouli JV, Bitsindou P, Elion DO, et al.: Chikungunya virus infection, Brazzaville, Republic of Congo, 2011. *Emerg Infect Dis.* 2013; 19(9): 1542–1543.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 26. Kamgang B, Brengues C, Fontenille D, et al.: Genetic structure of the tiger mosquito, *Aedes albopictus*, in Cameroon (Central Africa). *PLoS One.* 2011; 6(5): e20257.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 27. Livak KJ: Organization and mapping of a sequence on the *Drosophila melanogaster* X and Y chromosomes that is transcribed during spermatogenesis. *Genetics.* 1984; 107(4): 611–634.
[PubMed Abstract](#) | [Free Full Text](#)
 28. Maynard AJ, Ambrose L, Cooper RD, et al.: Tiger on the prowl: Invasion history and spatio-temporal genetic structure of the Asian tiger mosquito *Aedes albopictus* (Skuse 1894) in the Indo-Pacific. *PLoS Negl Trop Dis.* 2017; 11(4): e0005546.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 29. Thompson JD, Higgins DG, Gibson TJ: CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 1994; 22(22): 4673–4680.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 30. Librado P, Rozas J: DnaSP v5: a software for comprehensive analysis of DNA polymorphism data. *Bioinformatics.* 2009; 25(11): 1451–1452.
[PubMed Abstract](#) | [Publisher Full Text](#)
 31. Tajima F: Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics.* 1989; 123(3): 585–595.
[PubMed Abstract](#) | [Free Full Text](#)
 32. Fu YX, Li WH: Statistical tests of neutrality of mutations. *Genetics.* 1993; 133(3): 693–709.
[PubMed Abstract](#) | [Free Full Text](#)
 33. Zhong D, Lo E, Hu R, et al.: Genetic analysis of invasive *Aedes albopictus* populations in Los Angeles County, California and its potential public health impact. *PLoS One.* 2013; 8(7): e68586.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 34. Beebe NW, Ambrose L, Hill LA, et al.: Tracing the tiger: population genetics provides valuable insights into the *Aedes (Stegomyia) albopictus* invasion of the Australasian Region. *PLoS Negl Trop Dis.* 2013; 7(8): e2361.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 35. Tamura K, Stecher G, Peterson D, et al.: MEGA6: Molecular Evolutionary Genetics Analysis version 6.0. *Mol Biol Evol.* 2013; 30(12): 2725–2729.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 36. Clement M, Posada D, Crandall KA: TCS: a computer program to estimate gene genealogies. *Mol Ecol.* 2000; 9(10): 1657–1659.
[PubMed Abstract](#) | [Publisher Full Text](#)
 37. Múrias dos Santos A, Cabezas MP, Tavares AI, et al.: tcsBU: a tool to extend TCS network layout and visualization. *Bioinformatics.* 2016; 32(4): 627–628.
[PubMed Abstract](#) | [Publisher Full Text](#)
 38. Bargielowski IE, Lounibos LP, Shin D, et al.: Widespread evidence for interspecific mating between *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in nature. *Infect Genet Evol.* 2015; 36: 456–461.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 39. Reiskind MH, Lounibos LP: Spatial and temporal patterns of abundance of *Aedes aegypti* L. (*Stegomyia aegypti*) and *Aedes albopictus* (Skuse) (*Stegomyia albopictus* (Skuse)) in southern Florida. *Med Vet Entomol.* 2013; 27(4): 421–429.
[PubMed Abstract](#) | [Publisher Full Text](#)
 40. Kamgang B, Yougang AP, Tchoupo M, et al.: Temporal distribution and insecticide resistance profile of two major arbovirus vectors *Aedes aegypti* and *Aedes albopictus* in Yaoundé, the capital city of Cameroon. *Parasit Vectors.* 2017; 10(1): 469.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 41. Juliano SA, O'Meara GF, Morrill JR, et al.: Desiccation and thermal tolerance of eggs and the coexistence of competing mosquitoes. *Oecologia.* 2002; 130(3): 458–469.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 42. Rey JR, Nishimura N, Wagner B, et al.: Habitat segregation of mosquito arbovirus vectors in south Florida. *J Med Entomol.* 2006; 43(6): 1134–1141.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 43. Cox J, Grillet ME, Ramos OM, et al.: Habitat segregation of dengue vectors along an urban environmental gradient. *Am J Trop Med Hyg.* 2007; 76(5): 820–826.
[PubMed Abstract](#)
 44. Gratz NG: Critical review of the vector status of *Aedes albopictus*. *Med Vet Entomol.* 2004; 18(3): 215–227.
[PubMed Abstract](#) | [Publisher Full Text](#)
 45. Ngoagouni C, Kamgang B, Kazanjji M, et al.: Potential of *Aedes aegypti* and *Aedes albopictus* populations in the Central African Republic to transmit enzootic chikungunya virus strains. *Parasit Vectors.* 2017; 10(1): 164.
[PubMed Abstract](#) | [Publisher Full Text](#) | [Free Full Text](#)
 46. Reis S, Cornel AJ, Melo M, et al.: First record of *Aedes albopictus* (Skuse 1894) on São tomé island. *Acta Trop.* 2017; 171: 86–89.
[PubMed Abstract](#) | [Publisher Full Text](#)
 47. Armbruster P, Damsky WE Jr, Giordano R, et al.: Infection of New- and Old-World *Aedes albopictus* (Diptera: Culicidae) by the intracellular parasite *Wolbachia*: implications for host mitochondrial DNA evolution. *J Med Entomol.* 2003; 40(3): 356–360.
[PubMed Abstract](#) | [Publisher Full Text](#)