



## RESEARCH ARTICLE

**REVISED** **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 3; peer review: 3 approved]**

Basile Kamgang <sup>1</sup>, Theodel A. Wilson-Bahun <sup>1,2</sup>, Helen Irving<sup>3</sup>, Michael O. Kusimo <sup>1</sup>, Arsene Lenga<sup>2</sup>, Charles S. Wondji <sup>1,3</sup>

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

<sup>2</sup>Faculty of Science and Technology, Marien Ngouabi University, Brazzaville, Congo

<sup>3</sup>Liverpool School of Tropical Medicine, Liverpool, UK

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### 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

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1. **Yao Lucien Konan** , National Institute of Public Hygiene, Abidjan, Cote d'Ivoire
2. **Sébastien Boyer** , Institut Pasteur of Cambodia, Phnom Penh, Cambodia
3. **Julien B. Z. Zahouli**, Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland

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.

### Keywords

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

Any reports and responses or comments on the article can be found at the end of the article.

**Corresponding author:** Basile Kamgang ([kamgang\\_d@yahoo.fr](mailto: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

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**REVISED Amendments from Version 2**

The manuscript has been slightly modified in accord with the observation of reviewer 3. 'zika' has been replaced by 'Zika' across the manuscript. The last sentence of the conclusion was deleted.

See referee reports

## 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<sup>1–4</sup>.

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<sup>5,6</sup>. 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 donor<sup>5</sup>. 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 1957<sup>7</sup>. *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*<sup>8</sup>.

While *Ae. albopictus* is a native of South East Asia, it has now invaded all the five continents during the past 30–40 years<sup>9,10</sup>. This rapid global spread has been caused mainly by sales and distribution of used tires across the world<sup>11</sup> coupled with the ecological plasticity of the species, enabling its adaptation to various environments<sup>9</sup>. *Ae. albopictus* was reported for the first time in Central Africa in early 2000<sup>12</sup> and is currently present in almost all central African countries<sup>13</sup>, where it tends to supplant the indigenous species *Ae. aegypti* in human-domesticated environment<sup>14,15</sup>. 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*<sup>16,17</sup>. 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<sup>14,15</sup>. 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<sup>15,18</sup>.

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<sup>19,20</sup>. 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 chikungunya<sup>2</sup>, and a large chikungunya outbreak in 2011 in the Republic of the Congo with more than 11, 000 cases<sup>21</sup>. 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<sup>22,23</sup>, where Zika was detected in this species<sup>24</sup>. In Congo, both *Ae. aegypti* and *Ae. albopictus* were found to be positive for chikungunya virus<sup>25</sup>, 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-groups<sup>26</sup>. 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 areas<sup>15</sup>, 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 (Pointe Noire only) corresponding to the rainy season 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* larva or pupa) 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 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 described<sup>27</sup>. 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'-TTTCAACAAA TCATAAAGATATTGG-3' and albCOIR 5'-TAAACTTCTGGA TGACCAAAAAATCA-3'<sup>28</sup>. Polymerase chain reaction (PCR) amplification was performed using a Gene Touch thermal cycler (Bulldog Bio, Portsmouth, USA), as described previously<sup>28</sup>. 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 BioEdit<sup>29</sup>. 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 ( $\pi$ ) were computed with DnaSP 5.10.01 (<http://en.bio-soft.net/dna/dnasp.html>)<sup>30</sup>. The statistical tests of Tajima<sup>31</sup>, and Fu and Li<sup>32</sup> 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<sup>28,33,34</sup>. The same COI region was sequenced at these various regions and the maximum likelihood phylogenetic tree was constructed using MEGA 7.0<sup>35</sup>. Genealogical relationships between haplotype in this current study was assessed using TCS version 1.21<sup>36</sup> and tcsBU (<http://cibio.up.pt/software/tcsBU/>)<sup>37</sup> 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.

**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)
<b>All</b>	<b>1 (0.0)</b>	<b>556 (43.9)</b>	<b>3 (100)</b>	<b>65 (36.9)</b>	<b>5 (60.0)</b>	<b>10 (10.0)</b>	<b>640 (42.9)</b>

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



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% 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 ( $H_d=0.24$ ) 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, Ouessou 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

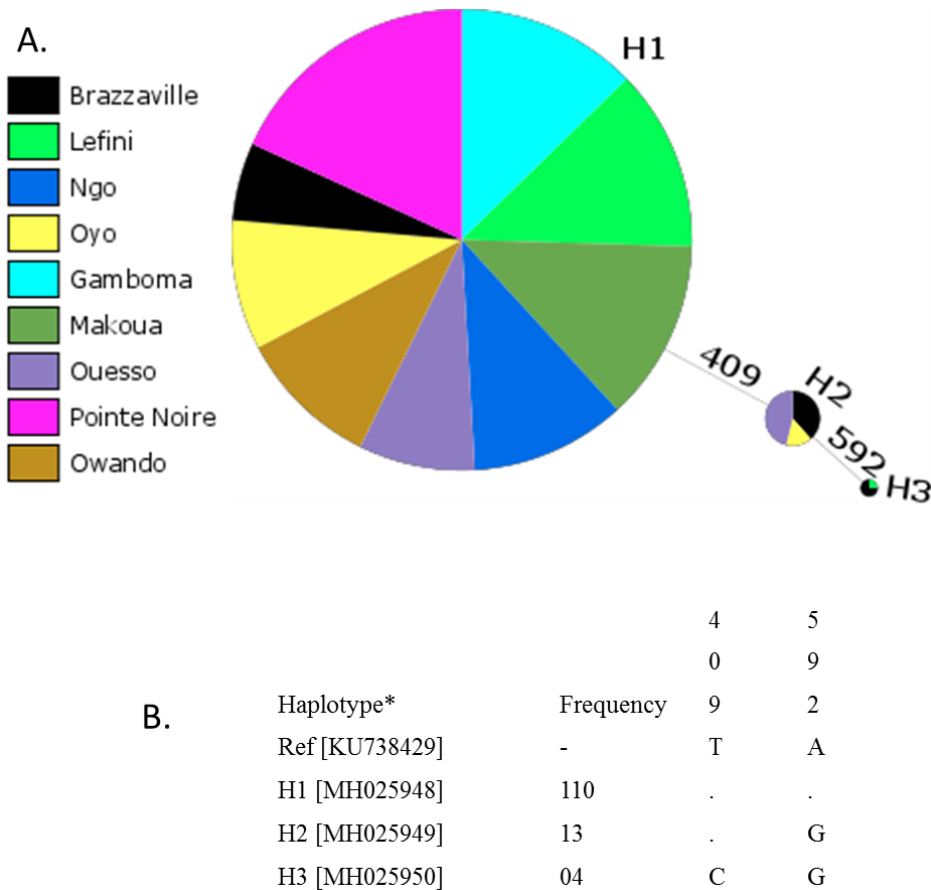
generated and analysed based on 445 nucleotides previously published in GenBank showed that the Republic of the Congo's haplotypes were closely related to the sequences from China, Singapore, Papua New Guinea and Christmas Island (Figure 3).

#### Discussion

This study 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 Brazzaville<sup>25</sup>, 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<sup>14,15,18</sup> 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 environments<sup>9</sup>, as well as its mating competitiveness in areas of sympatry with *Ae. aegypti*<sup>17,38</sup>. The prevalence of each species can vary according to the season in sympatric areas, as shown previously<sup>15,39</sup>, but was specifically linked to the duration of the dry season<sup>40</sup>. 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*<sup>41</sup>. In both major cities where samples were analysed according

**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
<b>All</b>	<b>1852 (27.72%)</b>	<b>4828 (72.28%)</b>	<b>&lt;0.001</b>

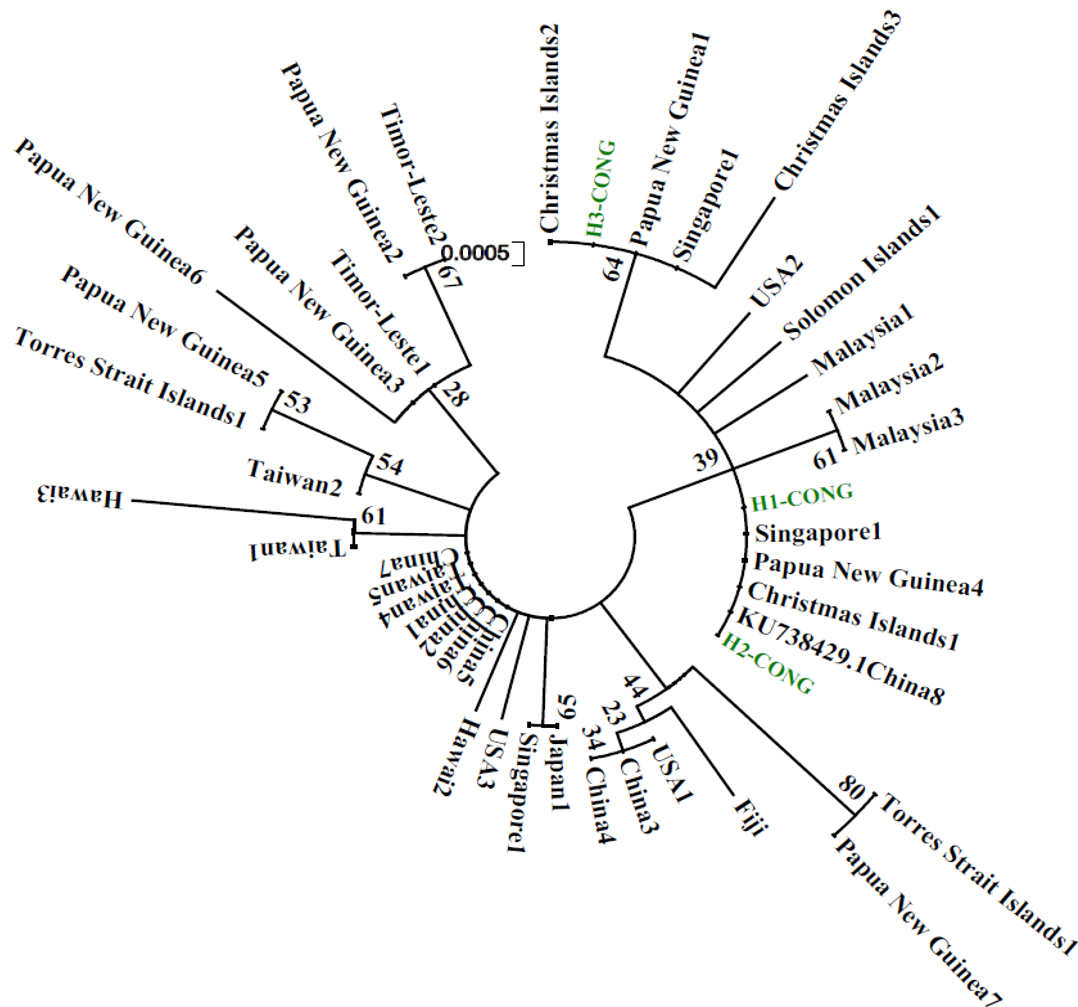


**Figure 2. Genetic diversity of the *COI* gene across Congolese populations of *Ae. albopictus*.** (A) Haplotype network showing the genealogic relationships between three haplotypes detected across Congo. The pie chart represents the proportion of each haplotype per site. (B) *COI* haplotypes found 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	$\pi$ (k)	D	D*	Fs	F*
Brazzaville	14	H1, H2, H3	2	0.692	0.0014 (0.890)	1.127 <sup>ns</sup>	0.935 <sup>ns</sup>	0.612 <sup>ns</sup>	1.021 <sup>ns</sup>
Lefini	15	H1, H3	2	0.133	0.0004 (0.266)	-1.490 <sup>ns</sup>	-1.873 <sup>ns</sup>	0.235 <sup>ns</sup>	-1.844 <sup>ns</sup>
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 <sup>ns</sup>	0.752 <sup>ns</sup>	0.297 <sup>ns</sup>	0.533 <sup>ns</sup>
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 <sup>ns</sup>	0.701 <sup>ns</sup>	1.253 <sup>ns</sup>	0.906 <sup>ns</sup>
Pointe Noire	20	H1	0	0.000	0.0000 (0.000)	NC	NC	NC	NC
<b>Total</b>	<b>127</b>	<b>3</b>	<b>2</b>	<b>0.246</b>	<b>0.0005 (0.2952)</b>	<b>-0.294<sup>ns</sup></b>	<b>0.662<sup>ns</sup></b>	<b>-0.0238<sup>ns</sup></b>	<b>0.417<sup>ns</sup></b>

N, number of sequences; S, number of polymorphic sites; H, haplotype; Hd, haplotype diversity;  $\pi$ , 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.

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<sup>15,18,40</sup>. 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*<sup>42,43</sup>. 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 tyres are the main productive for both *Ae. aegypti* and *Ae. albopictus*<sup>13,15</sup>. 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 arboviruses<sup>44</sup>. 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<sup>13,23,25</sup>. During previous studies in central Africa, *Ae. albopictus*, was found to be infected by Zika virus in natural conditions<sup>24</sup>. It was also demonstrated that *Ae. albopictus* from Bangui in Central African Republic is able to transmit enzootic chikungunya virus strains<sup>45</sup>.

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<sup>15,26,46</sup>. 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 previously<sup>47</sup>. 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<sup>21,25</sup>. 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<sup>15,26,46</sup>. 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 emergence or re-emergence of several arboviruses such as dengue, Zika and yellow fever viruses.

## 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>.

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## 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.](#)

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## Version 2

Reviewer Report 31 August 2018

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**Julien B. Z. Zahouli**

Swiss Tropical and Public Health Institute (Swiss TPH), Basel, Switzerland

### 1) General comments

Kamgang *et al.* conducted this study with the overall aim of assessing the geographical distribution of *Aedes aegypti* and *Aedes albopictus* and the genetic diversity of *Ae. albopictus* in the Republic of Congo. Specifically, the authors sampled immature *Aedes* mosquitos among their usual breeding sites in 9 sites across several eco-regions, reared immature to adult stage for species morphological identification, and analyze the diversity of *Ae. albopictus* using CO1 gene method.

This study merits a careful and particular attention. While it is not novel to describe the *Aedes* mosquito species in Africa, this study is the one focusing on the invading Asian tiger *Ae. albopictus* which has been newly introduced into the African continent by the means of transportation and trade. The authors identified three *Aedes* species (*Aedes aegypti*, *Aedes albopictus* and *Aedes simpsoni*) that may have an important impact on public health as they may carry and transmit pathogens. The study shows some clear results, which include the variation of among mosquito species abundance of *Aedes aegypti* and *Aedes albopictus* and genetic diversity of *Ae. albopictus* species as a function of location. The outcomes were represented as texts, figures and tables. The outcomes of this work could be explored for better understanding of the distribution of *Aedes aegypti* and *Aedes albopictus* species that can influence the transmission of arboviruses to humans and may contribute the control arboviral diseases in the Republic of Congo.

Despite its strength, there are a number of weaknesses and gaps to the study that need to be addressed. The study is inherently limited to represent the species and specimens of non-*Aedes* mosquitoes and some features of the containers. The authors did not characterize the larval habits by looking at some ecological features of the containers. Some important characteristics such as the exposure of containers to sunlight, the presence or absence of microbial food inputs, air temperature, rainfall, temperature of water, turbidity of water, color of containers and some biological interactions (predation, competition, sympatry...) involving predators, competitors and other associations among mosquito larvae were not reported. The report of these factors may

probably help to better understand the difference in ecological distribution and patterns between *Aedes aegypti* and *Aedes albopictus* species. The authors talked about the displacement of *Aedes aegypti* due to the presence of *Aedes albopictus* in the abstract without clearly indicating the strength of the displacement and presenting data showing this important ecological phenomenon. The objective of this study is not clear to me. The hypothesis of this study was not provided. The authors should provide the objective and hypothesis of their works.

The writing throughout the manuscript is good to me. However, the writing needs to be improved in some places. In addition, as the authors did not sample the ecological characteristics of sampling areas and containers, they discussed their findings on the basis of the findings of other studies – this seems to consider them as speculations – the authors should focus on the own data, discuss them on the basis of their own data sampled and drawn inferences after comparing the data with previous studies.

Ultimately, above all, more importantly are the paper's weaknesses that should be addressed. I have provided more detailed and specific comments below.

## 2) Specific comments

### Background

Line 3 (It....associated): Please replace “was” with “has been”

Line 4-5 (how ..... diseases): It seems that this paper did not focus on the epidemiology of arboviral diseases, but the ecology of *Aedes* species. Please, it suggested that the authors delete or provide clearer explanation.

### Abstract

#### Background

Line 2 (in 2017): It suggested that the authors clarify the period (month) of the study. The authors should write the date at the start of the sentence or at the end of the sentence, but not at the middle. Please, correct.

#### Results:

The authors stated that *Ae. albopictus* has been newly introduction to Congo and that *Ae. albopictus* has displaced *Ae. aegypti*. I am concerned – please, what was the distribution of *Ae. aegypti* before the introduction of *Ae. albopictus*? Please, how do the authors know that *Ae. Albopictus* is displacing *Ae. aegypti* since I did not see data on such an ecological issue in this manuscript. Please, correct.

Line 4-5 (suggesting .....across Congo): The portion of the sentence seems to be a discussion – may I suggest that the authors displace it into the Conclusion.

Line 9-10 (supporting .....the Congo): The portion of the sentence seems to be a discussion – may I suggest that the authors displace it into the Conclusion.

Line 11-12 (likely .....trade links): The portion of the sentence seems to be a discussion – may I suggest that the authors displace it into the Conclusion.

### Introduction

Please replace “zika” with “Zika” in the whole document (please use a capital letter “Z”

Line (This rapid .....caused mainly...): Please, replace “was” with “has been”

Line (This suggests ..... in the region): This sentence is not clear to me – may the authors clarify? May I suggest that the authors clearly provide the objective and hypothesis of this study?

## Methods

Line (The humid tropical climate.....in the southwest): It seems that words are missing here – maybe “is found”?

Line (In Brazzaville.....downtown and suburban...). How did the authors make the difference between “downtown” and “suburban”? What were the criteria?

Line (with at least one Aedes larvae or pupae) – please, may the authors replace “larvae” with “larva” (singular form!) and “pupae” and “pupa” (singular form)?

Line (....stage for morphological identification.): please, may the authors specify the determination key used for morphological identification and the reference?

Lines (The comparisons between .....chi-square test): Is this a statistical analysis of the data? I am not sure that this corresponds to the title of this paragraph – usually, the statistical analysis of data is separated from the lab works – may the authors write an independent paragraph clearly describing the statistical analysis procedures? It will be interesting if the authors perform geospatial analysis of the data to clearly show the distribution of *Ae. albopictus* and *Ae. aegypti* across sampling areas.

Lines (in each selected location ALL containers, ....were recorded) – I am not sure that it is possible to sample ALL containers in a given site – usually, only readily visible and accessible containers can be sampled – please, it is suggested that the authors reword this sentence?

It appears that the authors did not sample the characteristics of containers and habitats, but only the mosquito immatures – If so, how will they explain the distribution of species? It should be important to collect information on the biotic and abiotic characteristics/variables of the sampling sites and breeding containers.

May the authors explain or justify the importance of using CO1 method to assess the origin of *Ae. albopictus* instead of other technique? What are the advantages of using CO1 method?

## Results

As I said previously, while the authors stated that ALL containers were sampled in such large areas, only 640 containers were sampled? In my experience, it is not possible to sample all containers in such large areas.

Were the breeding sites and land cover not characterized? The characteristics of the breeding containers and land cover are likely to affect species distributions. It is suggested that the authors described the characteristics of *Aedes* breeding sites and land covers surrounding the containers.

It is suggested that the authors add a paragraph focusing on the distribution of both main *Aedes* species, *Ae. aegypti* and *Ae. albopictus*, among the containers samples? May the authors also specify the proportions of containers that both species co-habited?

Table 3: The overall proportions of *Ae. aegypti* (27.72%) and *Ae. albopictus* (72.28%) in the Table 3 does not match with the proportions indicated in the text (In total, 6,684 specimens ..... comprising 72.24%.....27.70%....). Please, explain or correct these differences.

I am concerned: non-*Aedes* mosquitoes were not collected? Usually, *Culex* species, sometimes *Anopheles* species and other predatory larvae of mosquitoes inhabit *Aedes* breeding sites... These species are likely to alter the ecology and distribution of *Ae. aegypti* and *Ae. albopictus*. Please, it is suggested that the authors add other mosquito species if they collected.

## Discussion

Line (This study has assessed....): please, it is suggested deleting “has”.

Line (Analyses showed.....is....Brazzaville): please, may the authors replace “is” with “was” (past simple / preterit) – as the authors are reporting their own data?



Line (The co-occurrence of *Ae. aegypti* .....environmental factors.....both species) – please, may the authors explain which environmental factors that influence the co-occurrence of both *Aedes* species? I did not see a clear link/association/correlation between such environmental factors and *Ae. aegypti* and *Ae. albopictus* distribution. It should be good if the authors sampled and reported the environmental variables (vegetation, land cover, temperature, humidity, rainfall, season, sunlight radiation, shade, nature of water of the containers, type of breeding sites, foods, predators, competitors,) in each specific location and look at the relationships between these variable with the presence/or abundance of the species. May the authors provide such relationship between environmental parameters and the occurrence of *Aedes* species?

Line (The ecological plasticity....): The data did not clearly report the ecological plasticity/elasticity in *Ae. albopictus* which means that the species colonize different habitats (for instance, vegetated/open environment), containers (natural, peridomestic, domestic...), land covers (rural, urban, forests, savannah...).... Please, may I suggest that the authors report such findings in the Results section to support their discussion?

Line (The prevalence.....peri-urban areas) - Here the authors seem to link the difference in the distribution of *Aedes* species between downtown and peri-urban environment to eggs tolerance/desiccation to temperature variations. But, as they did not record the temperature among both environments, it is difficult to understand such an inference... Why not other environmental parameters such as human density, human activities/behaviors, shade/sunlight, the nature of breeding sites or containers?

Line (This is in accordance with .....the main productive for both *Ae. aegypti* and *Ae. albopictus*): It seems that some words are missing in the sentence – it appears to be “...main productive - breeding sites- for..”

Line (However, the .....garages and tire shops...) – may I suggest that the authors in the Methods that the larval collection was performed mainly in the garages and tire shops? It seems that the authors were mixing UK/British spelling and US spelling! As the overall manuscript is written using UK English, they should write “tyre” /UK English) instead of “tire” (US English). Please, correct in the whole document.

Line (...*Ae. albopictus*, was found to be infected....) – please, it suggested deleting coma “,”.

## Conclusion

Line (...SEVERAL emergence or re-emergence of arboviruses....) – may I suggest “..... emergence or re-emergence of SEVERAL arboviruses...”

Line (Assessing the susceptibility.....future outbreaks) – yes, but I do see the link between the current study and insecticide susceptibility issue. Please, it is suggested to delete this sentence.

## Is the work clearly and accurately presented and does it cite the current literature?

Yes

## Is the study design appropriate and is the work technically sound?

Yes

## Are sufficient details of methods and analysis provided to allow replication by others?

Yes

## If applicable, is the statistical analysis and its interpretation appropriate?

Partly

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Author Response 19 Dec 2018

**Basile Kamgang**, Centre for Research in Infectious Diseases, Yaounde, Cameroon

Our study aim is to assess the geographical distribution of *Ae. aegypti* and *Ae. albopictus* in the Republic of Congo as well as the genetic diversity of invading species. In this study, sampling was focused mainly in garages and tire shops due to the previous data reported in Central Africa demonstrating that *Ae. aegypti* and *Ae. albopictus* breed mainly in the used tires. Thus, this strategy was used to increase the chance collected immature stage of Aedes mosquitoes in domesticated environment.

In each city, we sampled in downtown and in suburb basing also to the previous result in Central Africa showing that in the cities in which both species are found, *Ae. aegypti* is most prevalent in down town with higher building density whereas *Ae. albopictus* is rather more abundant in periphery area of the city surrounded by the vegetation (kamgang et al, 2010; Kamgang et al., 2013; Kamgang et al., 2017).

I wanted to precise that the aim was not to characterize the breeding sites of Aedes, this can explain why the data on presence of vegetation, sun light exposure, color of breeding sites, distance between plants and containers,... were not recorded.

The proportion of Aedes in the text included four *Ae. simpsoni* collected in Brazzaville. Mitochondrial DNA gene (COI) used here has been extensively used to assess genetic diversity of *Ae. albopictus* even it seem not really suitable in the region recently invaded due to their low polymorphism.

It true that there is no previous data indicated the abundance of *Ae. aegypti* in the Republic of Congo meanwhile taking other the studies implemented in Central African Republic and Cameroon showing that *Ae. albopictus* became dominant species some years after his introduction, we can conclude that the same situation is ongoing in the Republic of Congo. In general, all the hypothesis did in the discussion section are based on the findings in the previous works in the region.

**Competing Interests:** No competing interests were disclosed.

## Version 1

Reviewer Report 07 August 2018

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**Sébastien Boyer** 

Unit of Medical Entomology, Institut Pasteur of Cambodia, Phnom Penh, Cambodia

The study is important and essential in the Public Health domain, especially in the Republic of Congo where data on vectors are missing, and where doing field study is not that easy. Even knowing it is difficult to do field studies, from a scientific point of view, there is no replicate in this experiment. The authors went into the field one time in May 2017 and one time in November 2017. It should not be count as two independent samples of the same sites, but it should be considered as one sample during one season, and one sample during the other season. It is better to have a representation of the geographic distribution in the both season to observe if there is a variation than to have some chi-square tests that are not really useful here. It didn't deserve the study. As I mentioned earlier, I truly think that the data are important here, including the percentage of positive container for each species, but is should be presented per season also, with no replicate.

For representing the data illustrated in the Figure 2, it should be better to represent it by chart by city. Here we didn't see very well what is the percentage of H1, H2 and H3 in Brazzaville for example. It is the same problem for the proportion of H1 and H2 in Ouessou, H1 and H2 in Oyo and H1 and H3 in Lefini. By the way, it is very interesting to observe that these 3 haplotypes are nearby Asia and SouthEast Asia haplotypes.

In total, the study is well realized, and the conclusions are directly dependent of the results. But I suggest 2 only minor changes regarding the presentation of the results, especially for the sampling in May and November.

I also suggest to replace the Figure 2 by a map with the % of each haplotype (circle or histograms) on each city.

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Partly

**If applicable, is the statistical analysis and its interpretation appropriate?**

Not applicable

**Are all the source data underlying the results available to ensure full reproducibility?**

Partly

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Medical Entomology

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Author Response 07 Aug 2018

**Basile Kamgang**, Centre for Research in Infectious Diseases, Yaounde, Cameroon

Thank you so much for your comments. Mosquitoes were sampled in May in 8 locations (Brazzaville, Lefini, Ngo, Owando, Makoua, Ouessou, Oyo and Gamboma, and in November in Pointe Noire only) corresponding to rainy season in each location. Precision was made in the manuscript.

The number of haplotypes detected per city is presented in supplement Table S2.

**Competing Interests:** No competing interests were disclosed.

Reviewer Report 23 July 2018

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**Yao Lucien Konan** 

Vector Control Department, National Institute of Public Hygiene , Abidjan, Cote d'Ivoire

The work is clearly written in a precise way with recent references used.

At the method level, it should be noted the months of mosquito collection were indicated without the seasonal correspondence in the different localities.

In the results, the authors mention productivity of used tires while the larvae and pupae found in the different containers inspected were not counted. They must replace "productive" with "positive".

**Is the work clearly and accurately presented and does it cite the current literature?**

Yes

**Is the study design appropriate and is the work technically sound?**

Yes

**Are sufficient details of methods and analysis provided to allow replication by others?**

Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**

Yes

**Are all the source data underlying the results available to ensure full reproducibility?**

Yes

**Are the conclusions drawn adequately supported by the results?**

Yes

**Competing Interests:** No competing interests were disclosed.

**I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.**

Author Response 23 Jul 2018

**Basile Kamgang**, Centre for Research in Infectious Diseases, Yaounde, Cameroon

I agree with the reviewer that the season was not indicated.

Mosquitoes were collected in May and November 2017 corresponding to the rainy season in nine locations in the Republic of the Congo across the north-south transect.

It is true that the number of pupae and larvae has not been counted, but the number of mosquitoes emerging from each type of larval habitat has been counted.

**Competing Interests:** No competing interests were disclosed.