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Authors: Yadouleton, Angès, Hounkanrin, Gildas, Tchiboza, Carine, Bialonski, Alexandra, Schmidt-Chanasit, Jonas, et al.

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Short Communication

First Detection of the Invasive Mosquito Vector *Aedes albopictus* (Diptera: Culicidae) in Benin, West Africa, 2021

Anges Yadouleton,¹ Gildas Hounkanrin,¹ Carine Tchiboza,¹ Alexandra Bialonski,²
Jonas Schmidt-Chanasit,^{2,3} and Hanna Jöst^{2,4,✉}

¹Laboratoire des Fièvres Hémorragiques Virales du Bénin, 01BP 918 Cotonou, Bénin, ²Bernhard Nocht Institute for Tropical Medicine, WHO Collaborating Centre for Arbovirus and Hemorrhagic Fever Reference and Research, 20359 Hamburg, Germany, ³Faculty of Mathematics, Informatics and Natural Sciences, Universität Hamburg, 20359 Hamburg, Germany, and ⁴Corresponding author, e-mail: hanna.joest@gmx.de

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Abstract

Aedes albopictus (Skuse) is native to Southeast Asia and has colonized tropical and temperate regions worldwide in the last three to four decades. In Africa, data on its distribution is incomplete. Most studies having focused on the abundance, competition to other species, and phylogenetics of this vector are from the central African region. Here, we report the first detection of *Ae. albopictus* in Benin, West Africa. A total of 13 specimens were collected during the study period in 2021. The phylogenetic analysis of a cytochrome oxidase c subunit I gene fragment revealed a close relationship to populations from tropical India. Because of its close geographical proximity to areas where it has been found, it is assumed that the species was introduced several years before and is currently widely distributed in Benin. Additional studies are needed to explore its distribution, expansion range, and competitive effects on native species.

Key words: *Aedes albopictus*, Africa, Benin

Aedes albopictus, (Skuse) formerly also described as *Stegomyia albopicta*, is one of the most invasive mosquito species and a threat to public health as it is an efficient vector of arboviruses such as dengue virus (DENV), chikungunya virus (CHIKV), West Nile virus (WNV), Zika virus (ZIKV), and Japanese encephalitis virus (JEV) (Medlock et al. 2012). The species originates in Southeast Asia and has invaded five continents during the past 30–40 yrs (Benedict et al. 2007). The ecological plasticity of the species enables its adaptation to various environments and its rapid global spread was driven mainly by human movement and the transport of used tires. A major component of its vectorial capacity is the preference for urban and suburban surroundings, with larval breeding sites ranging from natural sites to artificial containers (Ngoagouni et al. 2015). In addition, the species shows an opportunistic feeding behavior on a wide range of hosts, with a marked preference for humans (Delatte et al. 2010). In Africa, *Ae. albopictus* was first detected in 1989 in South Africa and later in Nigeria, Cameroon, Equatorial Guinea, and Gabon. The displacement of *Ae. aegypti* (Linnaeus, Diptera: Culicidae) by *Ae. albopictus* has been observed worldwide in several places including Florida, the Bermuda Islands, Australia, Reunion

Island, and Cameroon (O'Meara et al. 1995, Bagny et al. 2009, Kaplan et al. 2010, Lounibos and Kramer 2016, Tedjou et al. 2018). In Central Africa, it tends to displace the indigenous *Ae. aegypti* in human-domesticated environment (Tedjou et al. 2018). The predominance of *Ae. albopictus* over *Ae. aegypti* in sympatric areas could result from the higher mating competitiveness (Bargielowski et al. 2013). In Benin, the main *Aedes* species in urban environments is *Ae. aegypti* (Padonou et al. 2020). Several outbreaks of DENV, ZIKV, and CHIKV were observed in the central African region (Bamou et al. 2021) and it was assumed that the establishment of *Ae. albopictus* has led to an epidemiological modification of arboviral diseases (Tedjou et al. 2018). Several phylogeographical studies have been undertaken in order to ascertain the origins of invasive populations. Studies using mitochondrial markers (mtDNA) suggested that the *Ae. albopictus* populations from temperate regions in the United States, Japan, and Italy form a genetic cluster that is distinct from populations in tropical Asia. In Sub-Saharan Africa studies on the genetic diversity were conducted in 2011 in Cameroon (Kamgang et al. 2011), 2012 in the Central African Republic (Kamgang et al. 2013), 2016 on São Tomé island (Reis et al. 2017), and 2017 in



Fig. 1. Map of Southern Benin. Mosquito sampling sites marked with a dark gray star are positive and light gray are negative for *Aedes albopictus*; maps created with d-maps.com.

Table 1. Number of mosquitoes sampled in Benin in 2021

Sampling date	Sampling site	Sampling method	<i>Cx. quinquefasciatus</i>	<i>Ae. aegypti</i>	<i>Ae. albopictus</i>
12 January 2021	Abomey-Calavi	Larvae		16	1
18 May 2021	Abomey-Calavi	HLC		118	1
9 June 2021	Abomey-Calavi	BGS		26	
9 June 2021	Abomey-Calavi	HLC		269	
7 July 2021	Abomey-Calavi	HLC		99	8
1 March 2021	Cotonou	GAT	3	4	
2 March 2021	Cotonou	GAT	6		
2 March 2021	Cotonou	BGS	162		
3 March 2021	Cotonou	BGS	270	9	
3 March 2021	Cotonou	GAT		1	
4 March 2021	Cotonou	BGS	106	4	
5 March 2021	Cotonou	BGS	150	4	
5 March 2021	Cotonou	GAT	3	1	
29 July 2021	Dassa-Zoumè	HLC		101	
30 July 2021	Dassa-Zoumè	HLC		33	
1 August 2021	Dassa-Zoumè	HLC		134	
1 September 2021	Dassa-Zoumè	HLC		100	
2 September 2021	Dassa-Zoumè	HLC		134	1
2 September 2021	Dassa-Zoumè	BGS		32	
4 September 2021	Dassa-Zoumè	HLC		77	
5 September 2021	Dassa-Zoumè	HLC		200	
24 June 2021	Porto Novo	HLC		111	
7 July 2021	Porto Novo	HLC		259	2
25 August 2021	Porto Novo	HLC	5	73	
26 August 2021	Porto Novo	BGS	30	3	
Total			735	1,792	13

HLC, human landing catches; BGS, BG-Sentinel mosquito trap; GAT, Gravid *Aedes* Trap.

the Republic of the Congo (Kamgang et al. 2018). Consistent with the recent arrival of *Ae. albopictus* in the central African region a low mtDNA diversity was observed (3–4 haplotypes). These mtDNA analyses also showed that African *Ae. albopictus* populations are related to tropical, rather than temperate or subtropical outgroups. The finding of several haplotypes using microsatellite data suggests

multiple introductions of *Ae. albopictus* from tropical regions (Kamgang et al. 2011; Tedjou et al. 2018). A previous study from the Central African Republic found that the population comprised two clades, suggesting multiple sources of invasion (Kamgang et al. 2011, 2013). In this study, we report the first detection of *Ae. albopictus* in Benin, West Africa.

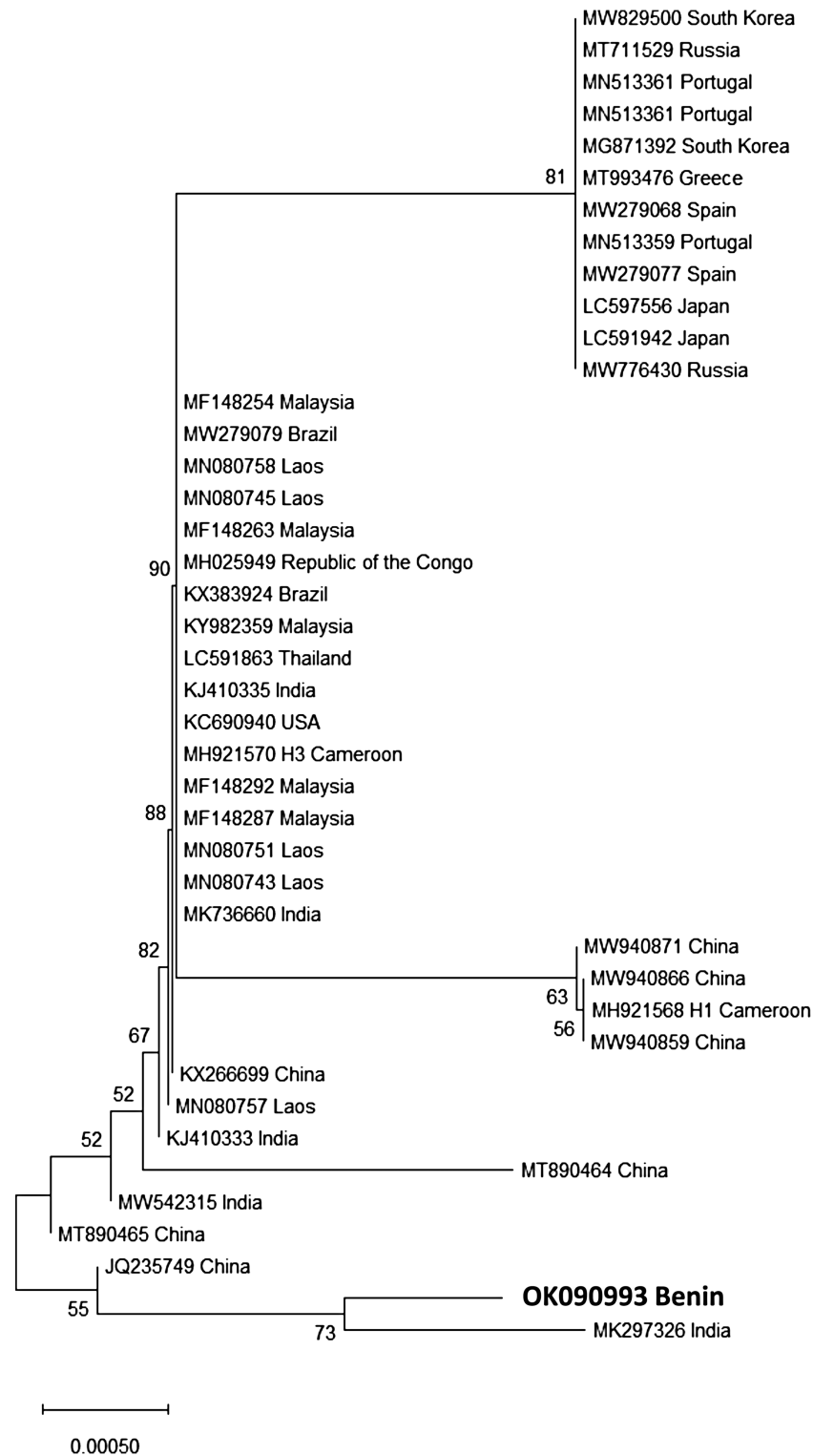


Fig. 2. Phylogenetic tree based on *Aedes albopictus* cytochrome oxidase c subunits 1 sequence fragment (636 nucleotides). The tree was built using the Neighbor-Joining method and the Tamura-Nei nucleotide substitution model. Statistical support for tree nodes was assessed by bootstrap (1,000 iterations). The analysis involved 42 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option).

Material and Methods

During a longitudinal study on *Ae. aegypti*, regular mosquito trapings were made at four sites in Benin (Fig. 1) in 2021: Abomey-Calavi (6.418736N, 2.3425287E), Dassa-Zoumè (7.783625°N, 2.185264°E), Porto Novo (6.510439°N, 2.604147°E), and Cotonou (6.364528°N, 2.441564°E) (Table 1). The sampling site at Abomey-Calavi was in the botanical garden of the University. The University is close to Cotonou, the economic center of Benin, with one of the largest ports in West Africa. It features a tropical climate, with alternating rainy (April–July and September–October) and dry seasons. Mosquito larvae were sampled on 12th of January 2021 from ovitraps and were reared to adults in the laboratory (27–28°C, 75–80% RH, ambient light). The sampling sites Porto Novo and Cotonou were densely populated urban neighborhoods with sparse vegetation in the center of the cities. The sampling site at Dassa-Zoumè in central Benin was a suburban site at the periphery of the city with lush vegetation around. For the sampling of adults, human landing catches (HLC), BG-Sentinel mosquito traps (BGS), and passive Gravid *Aedes* Trap (BG-GAT) (Biogents, Regensburg, Germany) were used. HLC carefully followed the WHO guidelines and protocols (WHO Pesticide Evaluation Scheme 2013). As a part of the consent process, the participants in the human trials were fully advised of the nature and objectives of the test and the potential health risks from exposure to mosquito bites. Ethical concerns have been raised and evaluated and to minimize exposure risk HLC participants used malaria prophylaxis. Three volunteers, 2 males and 1 female, all professional entomologists performed the catches. For the tests, volunteers were wearing long trousers and long-sleeved shirts as protection against mosquito bites. One leg of the trousers was rolled up to expose the skin used as the attract area. Volunteers were seated motionless in chairs with the exposed leg extended while observation, aspiration of mosquitoes was made by assistants. HLC were conducted for 2 h per day during dusk and dawn. GATs were set up for 24 h infused with rain water. BGS trappings were carried out for 24 h with BG-Lure (Biogents, Regensburg, Germany) as attractant. Adults were identified morphologically using the key of Becker et al. (2010). Morphologic species determination of one *Ae. albopictus* specimen collected on 12th of January 2021 at Abomey-Calavi was confirmed by DNA sequence analysis. The other *Ae. albopictus* found in May, July, and September 2021 were only identified morphologically. Total DNA was extracted from one mosquito leg using the QiaAMP viral RNA MiniKit (Qiagen, Hilden, Germany). A fragment of the cytochrome oxidase c subunits I (COI) region was amplified using the primers LCO1490 (5'-GGTCAACAAATCATAAAGATATTGG-3') and HCO2198 (5'-TAAACTTCAGGGTGACCAAAAATCA-3') (Folmer et al. 1994). Polymerase chain reaction (PCR) products were subjected to Sanger sequencing. The resulting sequence was submitted for species identification by the basic alignment search tool (BLAST) in the GenBank DNA sequence databases (<https://blast.ncbi.nlm.nih.gov/>). For phylogenetic analysis, the COI sequence from Benin was aligned to published mtDNA sequences using Geneious R9 (Geneious, Auckland, New Zealand). A phylogenetic tree was inferred in MEGA X (Kumar et al. 2018), using the Neighbor-Joining method (Saitou and Nei 1987). The evolutionary distances were computed using the Tajima-Nei method (Tajima and Nei 1984).

Results and Discussion

We record for the first time the occurrence of *Ae. albopictus* in Benin. A total of 13 specimens were found during the sampling period (January to September 2021). Ten *Ae. albopictus* were sampled in a botanical garden in Abomey-Calavi in the south of Benin (Fig. 1).

Two *Ae. albopictus* were found at Porto Novo and one was identified at Dassa-Zoumè in central Benin (Table 1). It is known that the species is preferring suburban or natural habitats, especially when *Ae. aegypti* is present as the dominant species (Ngoagouni et al. 2015). In this study *Ae. albopictus* were collected at suburban sites with lush vegetation and also at densely populated urban neighborhoods with sparse vegetation. At Abomey-Calavi the *Ae. albopictus* larvae was found in the same ovitrap as *Ae. aegypti* larvae which shows the sympatry of the two species. The arrival and establishment of this species was expected due to the geographical proximity to areas where it has been previously recorded. In neighboring Nigeria, the species has been detected in 1991 (Savage et al. 1992) and since then, published data showed that *Ae. albopictus* has become well established in the region. The data also indicated the dominance of *Ae. albopictus* over *Ae. aegypti* in some regions of Nigeria (Adeleke et al. 2015). In Benin, further studies are necessary to estimate the distribution and abundance of *Ae. albopictus* in urban, suburban, rural, and sylvatic biotopes.

The analyzed Beninese specimen clustered with *Ae. albopictus* from the tropical state of Kerala, India (Fig. 2). Because of its high conservation, the COI gene is most widely used for phylogenetic analysis and is the standard for molecular mosquito identification, although disadvantages and limitations of these marker should be taken into account (Lee et al. 2019, Artigas et al. 2021). In accordance with phylogenetic studies from Central Africa, our results suggest that *Ae. albopictus* in Benin is closer related to tropical rather than temperate or subtropical populations. A longitudinal monitoring is necessary to determine the source of invasion and the species' dynamics in space, time, and relation to the other major mosquito vectors. *Ae. albopictus* is a vector for medically important arboviruses and its spread has the potential to affect the epidemiology of emerging or reemerging arboviruses. Therefore, there is the need for further investigations to be prepared to control disease outbreaks.

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