SHORT COMMUNICATION

AFRICAN
ENTOMOLOGY

First observation of *Aedes albopictus* (Skuse, 1894) (Diptera: Culicidae) in Tshuapa province (Boende), Democratic Republic of the Congo

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In May–June 2021, we collected *Aedes albopictus* (Skuse, 1894) adults near the central hospital in Boende, the capital of Tshuapa province in the Democratic Republic of the Congo (DRC). We identified the mosquitoes using morphological and DNA-based techniques. This is the first report of this species in the DRC outside of Kinshasa and Kongo Central. Given the central location of Boende in the Congo Basin, our finding indicates that the vector might also have spread to other cities which are located on the Congo River and its major tributaries. We highlight the need to investigate the distribution of *Ae. albopictus* and to update disease risk maps for Central Africa, as it is an important vector for human arbovirus transmission.

Aedes albopictus (Skuse, 1894) (Diptera: Culicidae) is considered to be the most invasive mosquito species worldwide (Kraemer et al. 2015). The species originated in Southeast Asia, but successfully expanded to other parts of the world during the second half of the 20th century (Lwande et al. 2020). This range expansion was facilitated by global commerce (e.g. trafficking of used tires) and the mosquito's adaptation to various environments (e.g. ability to breed in different types of container) in both tropical and temperate regions (Eritja et al. 2017; Hawley et al. 2016; Kraemer et al. 2019). This expansion has been linked to the emergence of *Aedes*-borne diseases, such as chikungunya (CHIK), Zika, yellow fever and dengue fever in new locations (Borgherini et al. 2007; Leta et al. 2018; Ryan et al. 2018).

Although the first observation of *Ae. albopictus* in Africa was reported four decades ago (Nigeria) (Kraemer et al. 2019), its presence in the Democratic Republic of the Congo (DRC) was only described for the first time in 2016 (Bobanga et al. 2018). Three years later, we linked a major CHIK outbreak to its recent colonization in that area (the provinces of Kinshasa and Kongo Central during 2019-2020) (Selhorst et al. 2020; Wat'senga Tezzo et al. 2021; Weggheleire et al. 2021). Indeed, *Ae. albopictus* was the main *Aedes* species around the houses of human CHIK cases in Kinshasa (> 80% of the adults and larvae), Kasangulu (> 99% of the adults) and Matadi (> 95% of the adults and larvae). *Aedes aegypti* (Linnaeus, 1762), a primary vector of arboviruses indigenous to Africa, was the only other *Aedes* species detected during this outbreak. Furthermore, we could link the CHIK outbreak to an amino acid substitution in the viral envelope gene E1 (E1-A226V), which made the virus strain more suitable for dissemination by *Ae. Albopictus*, compared to strains that lack the substitution (Selhorst et al. 2020). These previous results indicate that *Ae. albopictus* now has well-established populations in south-western DRC, highlighting the potential for future outbreaks of *Aedes*-borne diseases and the urgent need for additional surveillance and implementation of control methods in the entire country (Mbanzulu et al. 2017; Wat'senga Tezzo et al. 2021).

Hypotheses that could explain why the invasion of *Ae. albopictus* in the DRC increases the risk of arbovirus outbreaks are:

1. Native *Aedes* species were never present in high numbers before the arrival of *Ae. albopictus*;
2. Native *Aedes* species are less suitable vectors for arbovirus transmission; and
3. *Ae. albopictus* occupies a different niche to that of native *Aedes* species, thereby increasing the overall *Aedes* biting rate (and R_0 in human populations).

While hypotheses I and II are less likely, as past outbreaks were linked to high density *Ae. aegypti* populations in the DRC (Mbanzulu et al. 2020), hypothesis III is plausible, as *Ae. albopictus* is found more frequently in periurban areas surrounded by vegetation, while *Ae. aegypti* is most prevalent in neighbourhoods located downtown with higher buildings (Bahun et al. 2020; Kamgang et al. 2018; Leisnham 2009). Here, we provide data on the presence of *Ae. albopictus* in Boende, to encourage scientists to investigate the ecology of *Ae. albopictus* on the Congo River (and tributaries) and to warn policymakers of a potential increase in the number and size of *Aedes*-borne viral outbreaks in the area.

In May 2021, two of the authors (JM and NL) were bitten by mosquitoes while walking in the garden of a hotel in Boende (Tshuapa province, 0°16′40.45″S, 20°52′39″E). These mosquitoes were identified as *Ae. albopictus* by their characteristic white stripe on the scutum. Six female *Ae. albopictus* were collected during human-landing and confirmed morphologically following Walter Reed's identification keys (Reed 2021). Later that day JM went to the central hospital in Boende (0°16′38.5″S, 20°52′59″E) and was again bitten by the same species. One month later (June 2021), *Ae. albopictus* mosquitoes bit the authors once more when they returned to the same two locations.

These findings suggest that an established population of the mosquito species occurs close to the Tshuapa River in Boende.

The morphological identifications were validated by DNA-barcoding, a technique based on the amplification of the partial mitochondrial cytochrome *c* oxidase subunit I (COI) gene (Hebert et al. 2003). Sanger sequencing of the 658-base pair barcode was realised following laboratory protocols and methods as described in Wat'senga Tezzo et al. (2021). The generated COI sequences (N_{Boende} = 5) were compared against the BOLD Identification System with Species Level Barcode Records. All similarity percentages reached 100%, with the identification of one *Ae. albopictus* haplotype in Boende. In addition, a Neighbour-joining tree was constructed, including available DNA sequences of the six medically important *Aedes* species of the subgenus *Stegomyia* that occur in the Afrotropical region (Kimura 1980; Kumar et al. 2016). All *Ae. albopictus* COI sequences, including the newly generated sequences, group together in a highly supported cluster (100 BS, Figure 1), validating the morphological identifications. The generated sequences were deposited in GenBank® using the following
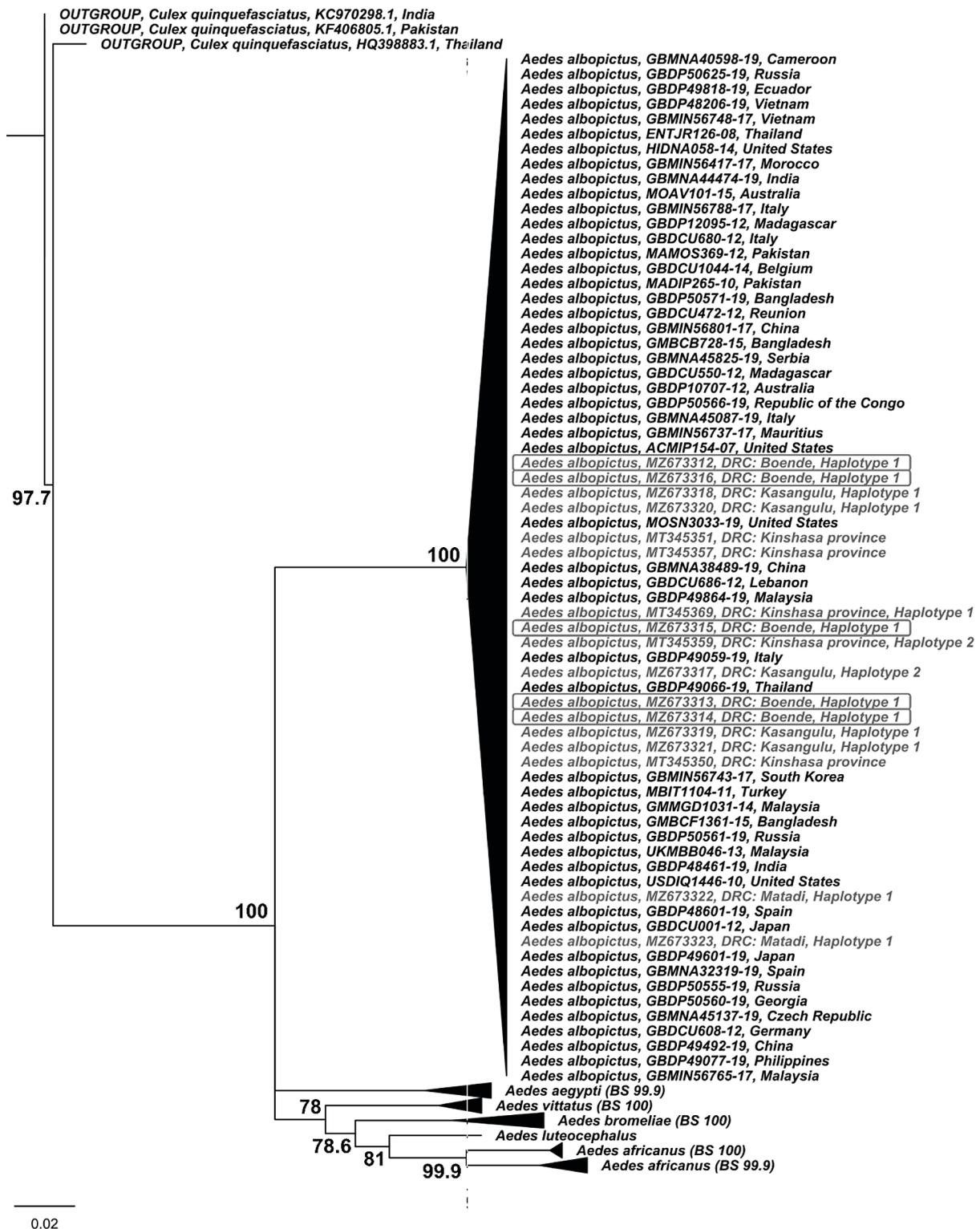

Figure 1. Neighbour-joining tree (1000 bootstrap, 75 support threshold), including the six medically important *Aedes* species of the subgenus *Stegomyia* occurring in the Afrotropical region. Available COI sequences for *Aedes albopictus* of the Democratic Republic of the Congo (DRC) are highlighted in grey, including the sequences generated in this study from specimens collected in Boende (in boxes)

accession numbers: MZ673312-MZ673316.

Although Boende is situated approximately 750 km from Kinshasa, in the middle of the rain forest, the discovery of *Ae. albopictus* in the city is not unexpected (Roiz et al. 2018). Boende is located on the left bank of the Tshuapa River (one of the tributaries of the Congo River) and boats arrive daily from large cities downstream, such as Mbandaka and Kinshasa. These boats are usually very crowded and stacked with various goods, including water containers and used tires, making them ideal habitat for *Ae. albopictus* larvae and eggs (Müller et al. 2016). This assumption is further supported by the presence of the same haplotype in Boende, Kinshasa and Kongo Central (Figure 1) (Wat'senga Tezzo et al. 2021). This was also the most common haplotype in the latter study (Haplotype$_{frequency}$ = 0.71). Furthermore, the average precipitation (2109 mm per year) and daily temperatures (ranging from 24 °C to 30 °C year-round) fall within the optimal range for *Ae. albopictus* development (Doty et al. 2017; Ryan et al. 2018). Given that many cities in the DRC have similar climate and transport links to Kinshasa, we expect that established populations of *Ae. albopictus* also exist in other cities located near the Congo River (or its tributaries) throughout the Congo Basin. While *Ae. aegypti* is likely to have been present in these cities for decades (e.g. we captured one *Ae. aegypti* in downtown Boende, data not shown) and is considered to be a primary vector for arboviruses (Mbanzulu et al. 2020), we assume that the recent invasion of *Ae. albopictus* significantly increases the outbreak risk, due to its overall higher vector densities in areas that are less suitable for *Ae. aegypti* (e.g. periurban areas). If this is the case, we predict that the frequency and magnitude of *Aedes*-borne viral outbreaks will increase significantly in the future across the central DRC. This would be consistent with what we have observed in the south-western DRC (Selhorst et al. 2020).

To prevent and control future outbreaks of *Aedes*-borne viruses in the Congo Basin, it will be crucial to monitor both the viruses and their vectors in the region (Roiz et al. 2018). While research in the DRC is often restricted to Kinshasa, for logistical reasons, the eco-epidemiological drivers of disease transmission could be significantly different in other regions of the country (e.g. due to differences in seasonal dynamics, insecticide resistance, landscape elements, human-social behaviours, immunological responses). Further research is clearly needed to investigate the distribution and persistence (established or transient populations) of *Ae. albopictus* in Boende and the rest of the country.

ETHICS STATEMENT

Ethical approval is not required for this type of study.

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