# Important *Aedes* Spp. Density Levels in Kinshasa, Democratic Republic of Congo

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**Research**

**Keywords:** Kinshasa, Central Africa, Aedes, survey, chikungunya, Democratic Republic of Congo

**Posted Date:** October 28th, 2020

**DOI:** https://doi.org/10.21203/rs.3.rs-37427/v2

**Version of Record:** A version of this preprint was published at Parasites & Vectors on February 1st, 2021. See the published version at https://doi.org/10.1186/s13071-021-04588-7.

# Abstract

BACKGROUND: Dengue, yellow fever, chikungunya and Zika are among the most important emerging infectious vector-borne diseases worldwide. Besides sporadic dengue cases, yellow fever and chikungunya outbreaks have been increasingly reported in Democratic Republic of Congo (DRC) in the last decade. The main vectors of these arboviruses, *Aedes aegypti* and *Aedes albopictus*, were reported in DRC, but there is a lack of detailed information on their presence and spread hampering transmission risk assessments in the region.

METHODS: In 2018, two cross-sectional surveys were realized in Kinshasa province (DRC), one in the rainy (January/February) and one in the dry season (July). Four hundred houses were visited in each of the four selected communes (N'Djili, Mont Ngafula, Lingwala and Kalamu). Breedings sites were recorded, larvae and pupae collected and reared to obtain adults for genus and species identification. A subset of specimens was DNA-barcoded for validation of the morphological species identification.

RESULTS: The most rural commune (Mont Ngafula) had the highest density levels, with a Breteau Index of 82.2 and 19.5/100 houses in rainy and dry season, respectively. The Breteau Index in the other communes Kalamu, Lingwala and N'Djili elevated to 21.5 (4.7), 36.7 (9.8) and 41.7 (7.5) in the rainy (and dry) season. The House index was on average 27.5% and 7.6%; and the Container Index 15.0% and 10.0% in rainy and dry season, respectively. The vast majority of *Aedes* positive containers was found outside the houses (adjusted OR 27.4 (95%CI 14.9-50.1)). The main breeding sites were used tires, water storage containers and trash. *Anopheles* larvae were also found in *Aedes* breeding sites, especially during the rainy season.

CONCLUSIONS: These results show that Kinshasa is highly infested with *Aedes* spp. which indicates a high potential for arbovirus transmission in the area. During the dry season, the most productive containers (for *Aedes* pupae production) are containers used for water storage, whereas in the rainy season this is trash and tires. The present study also evidences that *Aedes* breeding sites are mainly located outdoors. Based on the results of this study, a contextualized *Aedes* control strategy can be designed for Kinshasa.


# Background

Arboviruses are causing a variety of diseases, such as dengue, yellow fever, chikungunya and Zika, which are among the most important emerging infectious diseases worldwide [1–3]. The distribution of these diseases and their transmitting vector are fairly well known in Latin-America and South-East Asia [4–6], but information in Sub-Saharan Africa remains scarce and scattered [7–9]. Of the latter, most information is coming from seroprevalence studies for dengue, showing that there is or has been virus circulation, demonstrated by a 12.5% IgG positivity in Cameroon, 36% in Burkina Faso, 45% in Nigeria [7] and 50.6% in Tanzania [10]. Chikungunya, instead, is mainly reported during outbreaks, such as the one in 2004 in Kenya [11], in 2013 in Tanzania [12], in 2018 in Mozambique [13], and in 2011 [14] and 2019 [15] in

Brazzaville (Republic of the Congo), and in 2000 [16], 2012 [17] and 2019 [18] in Kinshasa, capital of the Democratic Republic of Congo (DRC). In Kenya it has been shown that a chikungunya outbreak infected 67% of the population [19]. In Kinshasa, several alpha-, flavi- and bunyaviruses were found in mosquito samples (*Aedes* and *Culex*) in 2014 [20].There are reports that the dengue virus is circulating in DRC, but up to now there was no outbreak detected. The dengue infections found were in the majority of cases not clinically diagnosed, but detections upon analysis of stored samples. In 2012, the antigen dengue test was positive among three suspected chikungunya cases in Kinshasa [21]; in 2013-14 dried blood spots taken during a Demographic Health Survey were in 0.6% positive for dengue [22]; between 2002 and 2013, in 3.5% of stored samples of yellow fever suspect cases in the Bas Congo region, the dengue virus was identified [23]. More recently, in 2015-16 in an acute fever study in Mont Ngafula (suburban area of Kinshasa) dengue was the identified pathogen in 8.1% of the acute fever cases and chikungunya in 0.9% [24], however previous dengue infection was evidenced in 30.2% of the 342 study participants. In the neighboring country, Angola, a dengue outbreak with an estimated attack rate of 10% has been reported in 2013 [25]. Zika has been rarely detected in this African region [26], but several yellow fever outbreaks, the last one in 2016, were described [27].

Within the sub-Saharan African region, the information on the presence and distribution of the *Aedes* mosquito is even more difficult to find than on the above pathogens. This lack of entomological data leads to the use of suitability maps, based on mathematical models, to estimate arbovirus transmission risk [28], however, real *Aedes* spp. density levels would give a more reliable estimate [29]. Both *Aedes aegypti* and *Aedes albopictus* are found in the region, where *Ae. aegypti* is native, whereas *Ae. albopictus* is native of South-East Asia [30]. The latter, nowadays considered omnipresent in Central Africa [8], was reported for the first time in Central Africa in early 2000 [31] and in Kinshasa (DRC) in 2018 [32]. Both species can be found in human-domesticated environments [30], such as Kinshasa, a mega-city with a high population density and movement, where density levels remain unknown.

In this study, we evaluate the *Aedes* spp. density levels, together with the characteristics of the preferred breeding sites, in order to have an evidence basis for guidance of *Aedes* control efforts and for transmission estimation in Kinshasa, the capital of DRC.

# Methods

*Settings*

The study took place in Kinshasa, capital city of Democratic Republic of Congo (DRC), located in the Central-African region. Kinshasa lies at 279 m above sea level and is characterized by a tropical climate with a rainy season between October and May, and a dry season from June to September. The average temperature varies between 18°C and 32°C and the average monthly rainfall varies between 2 and 222 mm, in dry and rainy season respectively. Kinshasa is expanded over 9965 km² and has an estimated population of almost 12 million people. The city is administratively subdivided in 24 communes, which are grouped in four districts: Tshangu in the East, Lukunga in the North, Mont Amba in the South-East and

Funa in the Center-West. In this study, four communes were selected in different geographical zones of the city having a different ecology, urbanization, water supply systems and history of arbovirus outbreaks (figure 1).

*(Insert here Figure 1)*

N'Djili is a peri-urban commune in the east of the city, pertaining to the Tshangu district, where many informal economic activities, specifically vehicle repair shops, are located. Urban infrastructure, such as waste water infrastructure and garbage collection, is deficient. 97% of the houses have a water supply system in their compound, but an important proportion of them has problems with quality and amount of water availability. The population density of this area is estimated at 39 000 persons/km².

Kalamu II is a commune in the center of town, belonging to the Funa district, and is a residential place with as main economic activity technical service provision. It has an estimated population density of 47 000 persons/km².

Mont Ngafula I is situated in the south of the city, bordering Mont Amba district, and is an example of a semi-urban area with an estimated population density of 730 persons/km². It is geographically characterized by the presence of hills (and accompanying erosions) and small valleys. The main economic activity is agriculture and the selling of agriculture products to Kinshasa city. The place is characterized by unplanned urbanization with a typical deficient water supply system – with in some areas supply frequency as low as two times/week – and a deficient waste water disposal.

Lingwala is a commune in the center of the town, pertaining to the Lukunga district, with a lot of informal markets. Population density is estimated at 33 000 persons/km².

*Study design and data collection*

Two cross-sectional surveys were done in the four selected communes, one in the rainy season (18 January – 16 February, 2018) and one in the dry season (2 – 27 July, 2018). In each of the four selected communes, one neighborhood has been randomly chosen (all neighborhoods per commune listed, followed by random number selection procedure) as study site. In each study site, 400 houses were randomly selected to be surveyed. The sample size was calculated to detect, with a power of 80%, 10% of the houses being positive for *Aedes* spp. mosquitoes with a precision of 3% and allowing for a 5% alfa-error. Each day, 80 houses were inspected, using a systematic sampling approach: on a landmark (roundabout or main road) random points were identified for each team as their starting point to enter the (smaller) avenues. With a sampling interval of three houses, starting on the right side of the avenue, each of the 4 teams inspected the selected houses up to reaching 20 houses/day. When the avenue came to an end and the sampling size of 20 was not yet reached, the team turned back entering the houses on the other side of the street up to reaching the daily sample size. In each selected house, the entire house was inspected inside and outside. If there was more than one house per compound, a random house was chosen to inspect, but the entire outside part of the compound was inspected. The next day, the next

avenue (going left from the one of the previous day) was sampled. By this procedure, an extensive part of the neighborhood was covered with the survey. When one commune was finalized, the four entomological teams went to another commune and followed the same methodology. All communes were covered in four weeks' time. Each entomological survey team consisted of three persons, pre-trained by the entomology department of the 'Institut National de Recherche Biomédicale' (INRB), one entomologist of the INRB (supervisor) and one community health worker.

In each compound, all water holding containers were inspected and if immature stages (larvae or pupae) of mosquitoes were observed, they were collected in plastic bottles (one bottle per breeding site) and transported to the laboratory at INRB for genus identification (*Anopheles, Aedes, Culex*). The place, category and positivity/negativity of each container was reported. For larvae, only positivity and negativity was recorded; for pupae, the number of pupae was counted per positive breeding site. Both surveys were done in a similar way, but starting points of avenue and house selection differed and possibly a same house was visited in both surveys, but this was based on randomness and was not aimed at. Both surveys were largely realized by the same field team members.

*Species identification: morphology and DNA-based*

Each day, a random sample of 50 *Aedes* genus larvae/pupae were reared to adults in the insectarium to allow species identification using morphological keys [33,34]. F0 adults were stored at -20°C for DNA barcoding to validate the morphological identification of *Aedes aegypti* and *Aedes albopictus* and confirm the presence of the identified species in Kinshasa. Therefor five specimens of each species were randomly selected per study site. DNA barcoding is a technique based on the amplification of a standard barcode - the partial mitochondrial cytochrome c oxidase subunit I gene for animals. Sanger sequencing of the 658 bp COI standard barcode was performed using the LCO1490 and HCO2198 universal primers [35,36]. Amplifications were carried out in a 20 µl reaction mixture containing 2 µl of DNA template, 2 µl of 10X buffer, 1.5 mM MgCl2, 0.2 mM dNTP, 0.4 µM of each primer, and 0.03 units/µl of PlatinumTM Taq DNA Polymerase (InvitrogenTM). PCR products and negative controls were checked on a 1.5% agarose gel, using a UV transilluminator and the MidoriGreenTM Direct (NIPPON Genetics Europe) method. Positive amplicons were purified using the ExoSAP-ITTM protocol and sequenced in both directions on an ABI 3230xl capillary DNA sequencer using BigDye Terminator v3.1 chemistry (ThermoFisher Scientific). Subsequently, the generated sequences were compared to a library of reference sequences. A specimen was identified by analyzing its percentage sequence similarity with these reference sequences under the assumption that genetic diversity is lower within than between species. A rooted Neighbour-Joining tree was constructed including a sub-selection of the *Ae. albopictus* and *Ae. aegypti* barcodes available from online repositories, together with the newly generated haplotypes (full details of the protocol can be found in Additional File 2).

*Data analysis*

Data were entered in an Access database and 5% of the data were manually checked to evaluate inconsistencies. Data were cleaned and types of recipients regrouped into categories, adapted from

guidelines used in dengue-endemic regions [37]: big deposits used for water storage (> 15 L); small water deposits used for daily kitchen and cleaning activities (< 15 L); artificial containers, mainly trash that has no specific use or goal; natural sites, such a tree holes and bamboo; artificials that are used in the households and cannot be destroyed (for example animal drinking pots); tires; water evacuation systems/ponds. Data were analyzed using IBM SPSS Statistics, version 25. We calculated per round and per commune House Index (number of houses positive for at least one container with immature stages of *Aedes* spp. per 100 inspected houses), Breteau Index (number of containers positive for immature stages of *Aedes* spp. per 100 inspected houses), Container Index (number of containers positive for immature stages of *Aedes spp.* per 100 inspected containers), and Pupal Index (number of *Aedes* spp. pupae per 100 inspected houses). The relative contribution to pupal productivity was calculated and defined as the total number of pupae of *Aedes* spp. per category of breeding site divided by the total number of pupae of *Aedes* spp. collected per commune and per survey round. A descriptive analysis was done. In order to evaluate the factors determining *Aedes* spp. immature stage positivity, a logistic regression model was made and associated variables were identified based on a backwards conditional model, taking into account the clustering at household-level by inserting the household identification variable as a random factor in the model.

The number of breeding sites with at least one immature stage of *Anopheles spp.* was enumerated and its proportional importance calculated for each season and respective commune.

# Results

The survey in the rainy and dry season allowed to sample a total of 1 678 and 1 598 houses, respectively. In the rainy season, 5 079 water-holding containers, which were potential breeding sites, were inspected against 1 657 in the dry season. The average number of containers per household was different across communes ($p < 0.001$): for example in the rainy season, an average of 1.4 (Standard deviation SD 1.3) in Kalamu, 2.0 (SD 1.7) in Lingwala, 2.9 (SD 2.3) in Mont Ngafula and 5.3 (SD 2.6) in N'Djili. In rainy and dry season, 65.9% and 78.3% of the containers, respectively, were observed outside the sampled houses, i.e. in the open space around the house within the compound, ($p < 0.001$). In the Additional File 1 (table 1), the distribution of type of containers per location, commune and season is detailed.

*Aedes* density levels were higher in the rainy than in the dry season ($p < 0.001$, see table 2), with a Breteau Index (BI) of 45.35 versus 10.39/100 houses, a Container Index (CI) of 14.9% versus 10.02 % and a House Index (HI) of 27.53% versus 7.63%, respectively (Table 1). Mont Ngafula, a rural sub-urban area in the Southern edge of Kinshasa had the highest density levels amongst all visited communes with a BI of 82.21 and 19.50/100 houses in the rainy and dry season respectively, which was about four times higher than Kalamu, a commune that lies within the heart of the center of town. The number of *Aedes* pupae present per 100 houses reached in the rainy season 246 pupae/100 houses in Mont Ngafula, 126 in N'Djili, 90 in Lingwala, and 50 in Kalamu (Table 1). In the rainy season 99.3% of the positive breeding sites were outdoors against 96.4% in the dry season. A wide variety of containers is used by the *Aedes* mosquito to breed: big water deposits, small water deposits, artificials type trash, natural sites, non-

destroyable artificial containers, tires, water evacuation/ponds (Figure 2). Tires were taken as a separate group, as they are frequently present and it is difficult to know if they are just put aside for re-use/temporary storage or to be destroyed.

When analyzing the pupal productivity of breeding sites, we observed a statistically significant difference between rainy and dry season (aOR 3.73, 95% CI (2.21-6.31); p < 0.001). In the dry season, we observed that big water deposits were producing 20.3% of the pupae against 5.5% in the rainy season, indicating seasonal variability in breeding preference of the vector (Figure 3). In the rainy season 64.3% of all inspected containers were small water deposits, but they were only responsible for 46.4% of the pupae production, whereas tires, representing only 11.1% of the inspected containers, were responsible for 35.0% of the pupae production. The breeding containers used for water storage (big and small ones together) contributed relatively more to the pupal productivity in the dry season compared to the rainy season. Furthermore, we observed that productivity of artificial containers (mainly trash) was different across communities ($p < 0.001$) and season ($p < 0.001$) (Figure 4).

Positivity for *Aedes* breeding was higher in the rainy than in the dry season with an adjusted OR of 1.98 (95% CI 1.6 -2.4), and about 27 times (aOR 27.4 (95% CI 14.9 – 50.1)) more outdoors compared to indoors ($p < 0.001$). Mont Ngafula and Lingwala were statistically significantly more infested than N'Djili ($p < 0.001$, see table 2). The water container types most associated with *Aedes* infestation were tires (aOR 4.6 (95% CI 3.5-6.1)) and trash (aOR 1.9 (95% CI 1.4-2.5)) in comparison to big water containers (Table 2).

Based on the morphological identification of F0 adults, *Ae. aegypti* and *Ae. albopictus* were found in both seasons. The morphological identifications were validated by comparing the generated sequences of a subset of specimens against the Identification System of BOLD, with Species Level Barcode Records. The obtained similarity percentages ranged from 99.69 to 100%. The five and 14 haplotypes of *Ae. albopictus* and *Ae. aegypti*, respectively, are clustering only with conspecific sequences from specimens collected worldwide, supported with maximum bootstrap support (Figure 1 of Additional File 2). The generated sequences were deposited in GenBank with following accession numbers: MT345349-MT345426.

9.46% and 9.06% of the total number of containers positive for *Aedes* spp. immature stages, contained also immature stages of other genera, such as *Culex* and *Anopheles*, in the rainy and dry season respectively. This was in 99.3% of the cases observed in outdoor recipients and specifically in big water deposits in the rainy season and in trash in the dry season. Of them, in the rainy season, a total of 32 *Aedes* breeding sites were positive for *Anopheles* against only two in the dry season. *Anopheles* were found in big and small water deposits, trash and tires (Figure 6). In the rainy season *Anopheles* were observed in all communes whereas in the dry season *Anopheles* larvae were only found in small water deposits in Mont Ngafula, the most rural commune of the four study sites (Figure 7).

# Discussion

In both surveys and in all communes, the larval indices (HI, CI, and BI) were higher than the arbovirus transmission threshold values (BI of 5 set out by the World Health Organization) [38,39]. The Breteau

Index was on average 45 per 100 houses in the rainy season, and in comparison to a House Index of on average 27%, it is clear that one house can have different *Aedes* spp. positive breeding sites. In case an arbovirus is introduced in Kinshasa, the high larval and pupal *Aedes* densities suggest that transmission can rapidly occur and cause a major outbreak, such as the one caused by chikungunya in 2019 [18].

*Aedes aegypti* originated from Africa and is the main vector of arboviruses outside Africa. Yet, the vector competence, unknown for Kinshasa, of this species in Africa seems to be highly variable depending on the vector population, the virus isolate and the ecological context [28]. The presence of *Aedes albopictus*, which is an exotic species for Africa, might change the epidemiology for a number of arboviruses in Africa. Considered as a secondary vector for dengue virus, it can drive transmission of the chikungunya virus, especially the one of the ECSA lineage with the A226A, as shown in Kinshasa in the recent outbreak [40].

Having done surveys, following standardized procedures, in four different communes of Kinshasa during the rainy and dry season is the major strength of this study. The entomological team was trained beforehand and was largely the same for both surveys. A weakness is that the study took place over only one year time and only once per season. As inspection of breeding sites depends on the rigor and professionality of the team doing the fieldwork, quality control was established, namely a fixed supervisor was available in the field site during the survey and there was regular extra control from the international integrant of the study-team. Due to operational issues we were not in a position to identify all larvae to species level, which was another weakness, hence we could not calculate the relative importance of *Ae. aegypti* and *Ae. albopictus*, neither which species has predilection for which container type.

In a place like Kinshasa, where dengue is rarely reported [21–24] and chikungunya and yellow fever cause sporadic outbreaks [16,18,41], we didn't expect to find such high *Aedes* densities. The observed densities are comparable to the ones of other African settings: south-eastern Tanzania has a HI of 4.9 – 6.6, CI of 14.6-18.9 [42]; Burkina Faso a HI of 70, CI of 35 and BI of 10 [43]; north-west Ethiopia a HI of 25.5, CI of 32.9 and BI of 48.4 [44]; Mozambique a CI of 22 [45]; and Angola a HI of 4.3 – 27.9, CI of 2.1-9.3 and BI of 5.8-42.2 [46]. However, the densities were much lower than the one observed in Kenya during a dengue outbreak in 2013-14, where BI reached a value of 270/100 houses [47].

In contrast to findings in Latin-America [48], in Kinshasa *Aedes* breeding sites were mainly found outdoors, a characteristic also seen in other African countries [49]. The prevalence of *Aedes* breeding sites outdoors, together with the behavior in this context (to stay during daytime in the backyard or in the open place in front of the house), suggests a close human-mosquito contact, favoring the development of the *Aedes* spp. cycle, by blood feeding during daytime [50]. The low presence of *Aedes* immature stages inside the homes can also be due to rapid use and cleaning of the few containers found there. These results indicate that for controlling *Aedes* in Kinshasa, management strategies need to target outdoor spaces for breeding sites destruction or reduction.

Used car tires, water storage containers and artificial breeding sites (type trash) were the main containers chosen by *Aedes* mosquitoes for the oviposition coinciding with other studies conducted in the African continent [43–47,49,51]. The water storage containers were also found to be the most productive for *Aedes* pupae, which is a stage in the mosquito cycle which does not need nutrients and which is just before the adult stage of the mosquito [52]. These containers used to store water are always filled (partially or fully) with water due to a deficient water supply system, not depending on rain, which makes them a preferred breeding site, especially in the dry season, even despite being constantly subject to anthropogenic action. In the rainy season, in all study sites, breeding sites are favored by rain and containers typically filled with rain water are the most productive ones for *Aedes* pupae. A nice example are the tires, while they only represent 11% of the potential breeding sites, in the rainy season, about 35% of all pupae are found in them. Temperature, humidity and reduced light inside tires create a suitable environment for *Aedes* mosquito breeding and when tires are stored or discarded for long duration, it makes them a prolific breeding site [53–55]. In these conditions, eggs can be attached to the tires for a long time, playing their role in the preservation of the *Aedes* mosquito population throughout the dry season [56]. Small containers filled with water for kitchen/cleaning purposes were found in large numbers in all study sites and were also highly productive, especially in the rainy season.

In this study, *Aedes* species, which transmits among others chikungunya, dengue, Zika and yellow fever, were dominant in the inspected potential breeding sites, in and around the houses. Also other mosquito genera were found, such as *Culex* and *Anopheles*. *Culex* is common in urban settings using similar breeding sites as the urban *Aedes* species. It is of note that *Anopheles* species was found together with *Aedes* in the same breeding sites [57]. *Anopheles* usually prefers other types of breeding sites, such as ponds with static fresh water and are not particularly attracted to small containers [58]. The presence of *Anopheles* in urban settings is primarily associated with urban agriculture, as in Mont Ngafula [59], though we found *Anopheles* in all four communes in the rainy season, also in the center of Kinshasa. The observation of *Anopheles* larvae in man-made containers suggest that also *Anopheles* species adapt to those kind of containers, which is important in the light of transmission risk of urban malaria in Kinshasa.

# Conclusion

*Aedes* spp. seem to be well established in all four study communes of Kinshasa and are especially abundant in the sub-urban area of Mont Ngafula. This study – the first in its kind in Kinshasa – evidences that an *Aedes* control strategy needs to target outdoor containers, specifically containers for water storage in the dry season and tires in the rainy season. Additional insights in the ecology of the adult *Aedes* mosquito and its insecticide susceptibility will support the design of a comprehensive *Aedes* control strategy to be implemented to prevent a next outbreak of arboviral infections in Kinshasa.

# List Of Abbreviations

DRC Democratic Republic of Congo

INRB Institut National de Recherche Biomédicale

BI Breteau Index

CI Container Index

HI House Index

SD Standard Deviation

OR Odds Ratio

aOR adjusted Odds Ratio

CI Confidence Interval

ITM Institute of Tropical Medicine, Antwerp

# Declarations

- Ethics approval and consent to participate

The study protocol was approved by the 'Comité d'éthique de l'Université de Kinshasa' (authorization number: ESP/CE/032/2018). Before starting the survey in each commune, the study was presented to the 'Médecin Chef de Zone' and the local mayor, in order to have their approval for realizing the study in their area of responsibility. An informed consent was asked to the head of the households of the sampled houses and an oral approval was obtained. Different quality control measures were put in place: in each commune an entomological expert supervised the work of the field teams, the project-leader verified at the end of each day a subset of the data collection forms on completeness and an external entomological expert (Cuban expert) did ad hoc supervisions of the field work and of the laboratory activities.

- Consent for publication

NA

- Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

- Competing interests

The authors declare that they have no competing interests

- Funding

We acknowledge the financial support of the Belgian cooperation (Directorate-General for Development Co-operation) for the institutional collaboration between INRB, DRC and the Institute of Tropical Medicine (ITM), Belgium. ITM's outbreak research team is supported by the Department of Economy, Science and Innovation of the Flemish government, Belgium. The Barcoding Facility for Organisms and Tissues of Policy Concern (BopCo - http://bopco.myspecies.info/) is financed by the Belgian Science Policy Office (Belspo) as Belgian federal in-kind contribution to the European Research Infrastructure Consortium "LifeWatch". The funding bodies did not have any role in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.

- Authors' contributions
    - WTF, MMC and VV designed the study; MEZ and VW supervised survey activities and the laboratory work; FS organized practically the fieldwork; BMZ, IG and MTR realized the fieldwork; MMC, BMZ, IG and VW did laboratory work; SN did the DNA barcoding; VV and VW did the data analysis; BJA, VW, MMC interpreted the results. All authors did a part of the manuscript writing. All authors read and approved the final manuscript.

- Acknowledgements

We would like to express our sincere gratitude to all the people of the four included communes who collaborated on this study and to Isaac Pankwa for the data-entry at INRB, Kinshasa.

- Authors' information (optional)

NA

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# Tables

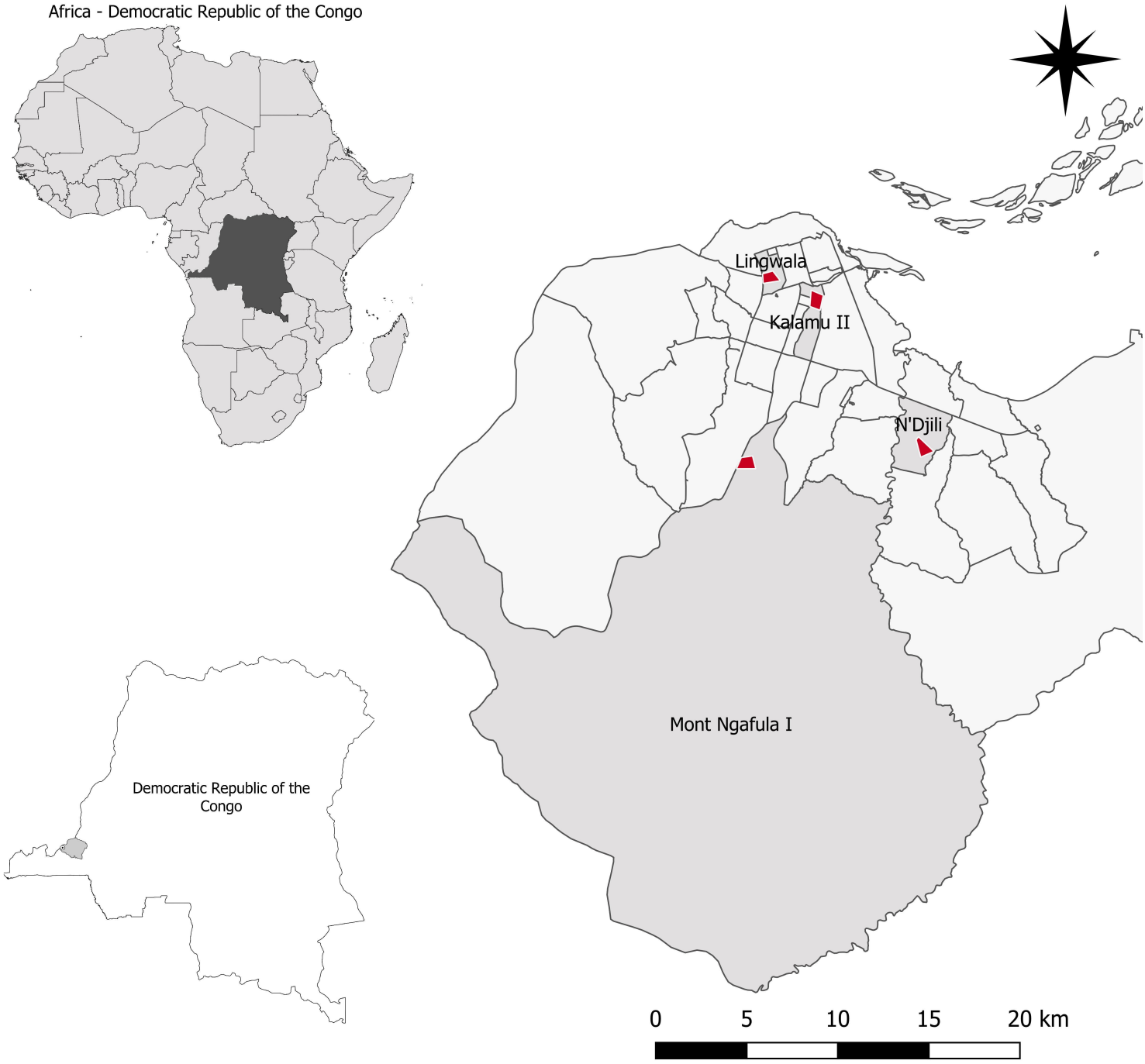
**Table 1. *Aedes spp.* entomological indices of the four study sites, in the rainy and dry season, Kinshasa, 2018.**

| | | TOTAL | LINGWALA | NDJILI | MONT NGAFULA | KALAMU |
|---|---|---|---|---|---|---|
| **Nr of containers inspected** | Rainy/dry season | 5079/1657 | 821/180 | 2550/665 | 1164/634 | 544/178 |
| Container Index (%) | Rainy season | 14.98 | 17.90 | 7.84 | 28.18 | 15.81 |
| | Dry season | 10.02 | 21.67 | 4.51 | 12.30 | 10.67 |
| **Nr of houses inspected** | Rainy/dry season | 1678/1598 | 400/399 | 479/399 | 399/400 | 400/400 |
| Breteau Index (/100H) | Rainy season | 45.35 | 36.75 | 41.75 | 82.21 | 21.50 |
| | Dry season | 10.39 | 9.77 | 7.52 | 19.50 | 4.75 |
| House Index (%) | Rainy season | 27.53 | 22.25 | 27.97 | 44.86 | 15.00 |
| | Dry season | 7.63 | 7.02 | 6.52 | 13.25 | 3.75 |
| Pupae/100 houses | Rainy season | 128.00 | 90.00 | 126.00 | 246.00 | 50.00 |
| | Dry season | 15.00 | 13.00 | 9.00 | 20.00 | 19.00 |

**Table 2. Determinants of *Aedes* spp. positive breeding sites in Kinshasa, 2018.**

| Parameter | Category | Total | Positive N (%) | Multivariate | |
|---|---|---|---|---|---|
| | | | | OR (95% CI) | p-value |
| **Season** | Rainy | 5079 | 761 (15.0) | 1.98 (1.63-2.40) | <0.001 |
| | Dry | 1657 | 166 (10.0) | 1 | |
| **Commune** | Kalamu | 722 | 105 (14.5) | 0.97 (0.74-1.28) | 0.857 |
| | Lingwala | 1001 | 186 (18.6) | 1.53 (1.20-1.96) | 0.001 |
| | Mont Ngafula | 1798 | 406 (22.6) | 2.67 (2.19-3.25) | <0.001 |
| | N’Djili | 3215 | 230 (7.2) | 1 | |
| **Position** | Exterior | 4646 | 916 (19.7) | 27.36 (14.9-50.1) | <0.001 |
| | Interior | 2090 | 11 (0.5) | 1 | |
| **Container type** | Big water deposits | 1080 | 94 (8.7) | 1 | |
| | Small water depositis | 4373 | 395 (9.0) | 0.99 (0.77-1.27) | 0.918 |
| | Artificials (trash) | 533 | 134 (25.1) | 1.89 (1.39-2.55) | <0.001 |
| | Natural | 5 | 0 (0) | 0 | 1 |
| | Artificials not destroyable | 18 | 4 (22.2) | 0.998 (0.32-3.13) | 0.997 |
| | Tires | 710 | 296 (41.7) | 4.60 (3.50-6.06) | <0.001 |
| | Water evacuation | 17 | 4 (23.5) | 2.06 (0.62-6.79) | 0.236 |

# Figures




## Figure 1

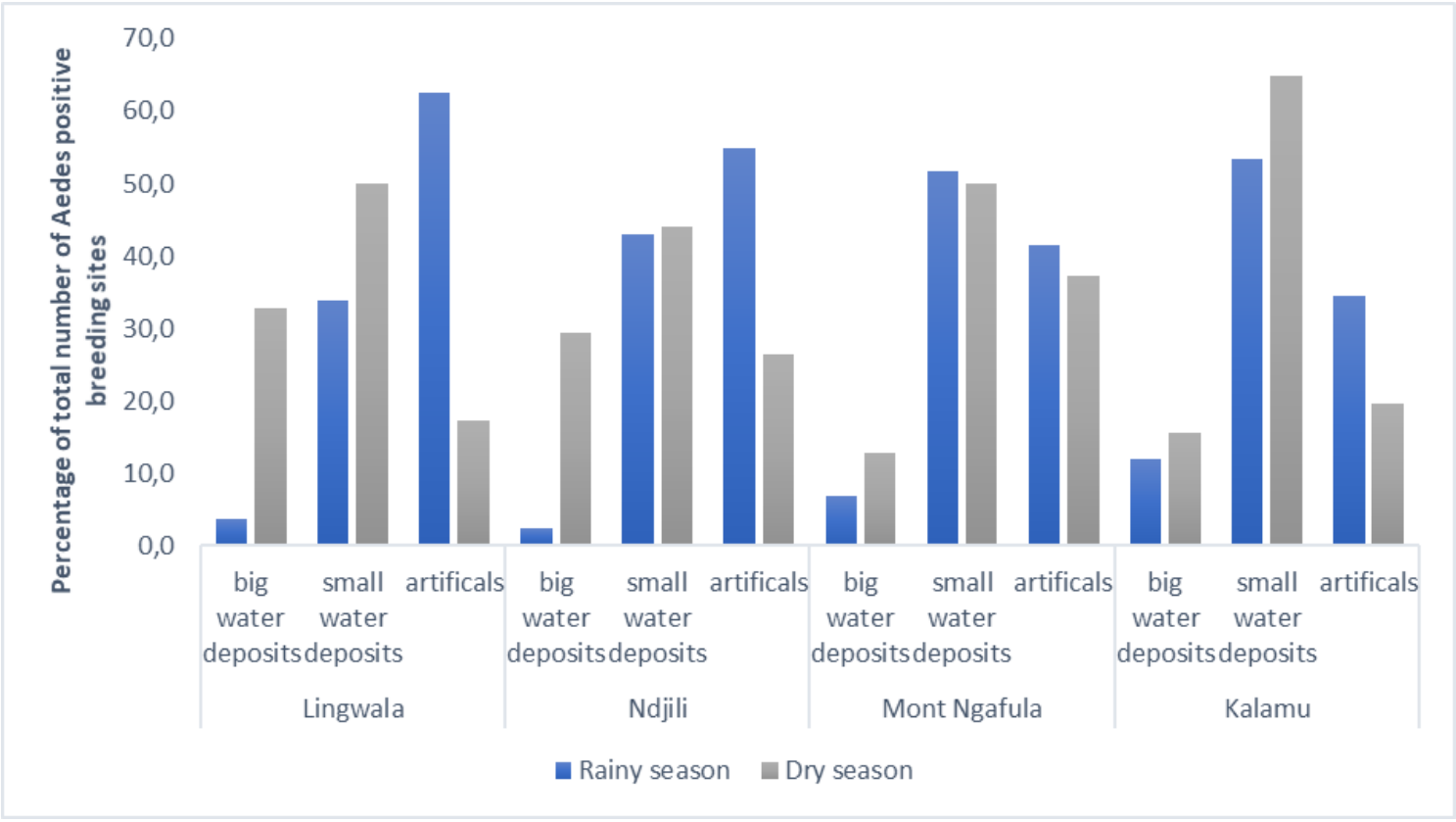
The geographical presentation of the study sites. Legend: The four study communes in Kinshasa (in light grey) with localization of the sampling area (red dots), Kinshasa, 2018.


## Figure 2

Illustration of various breeding sites identified/investigated in the study area. Legend: a. big water deposits; b. small water deposits; c. artificial type trash; d. natural sites; e. non destroyable artificial containers; f. tires; g. water evacuation/ponds

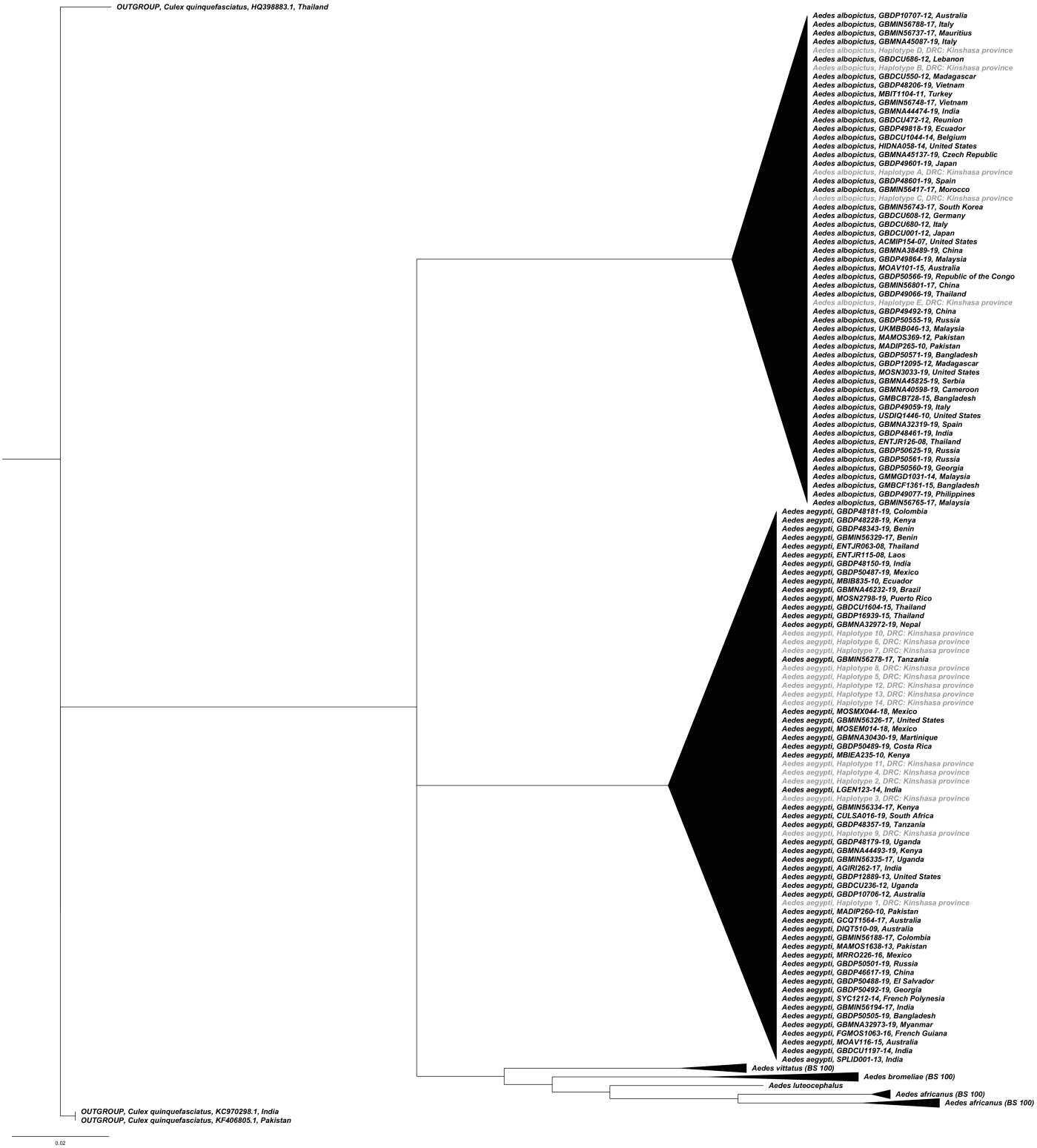

## Figure 3

The productivity of the breeding sites for Aedes spp. immature stages, Kinshasa, 2018



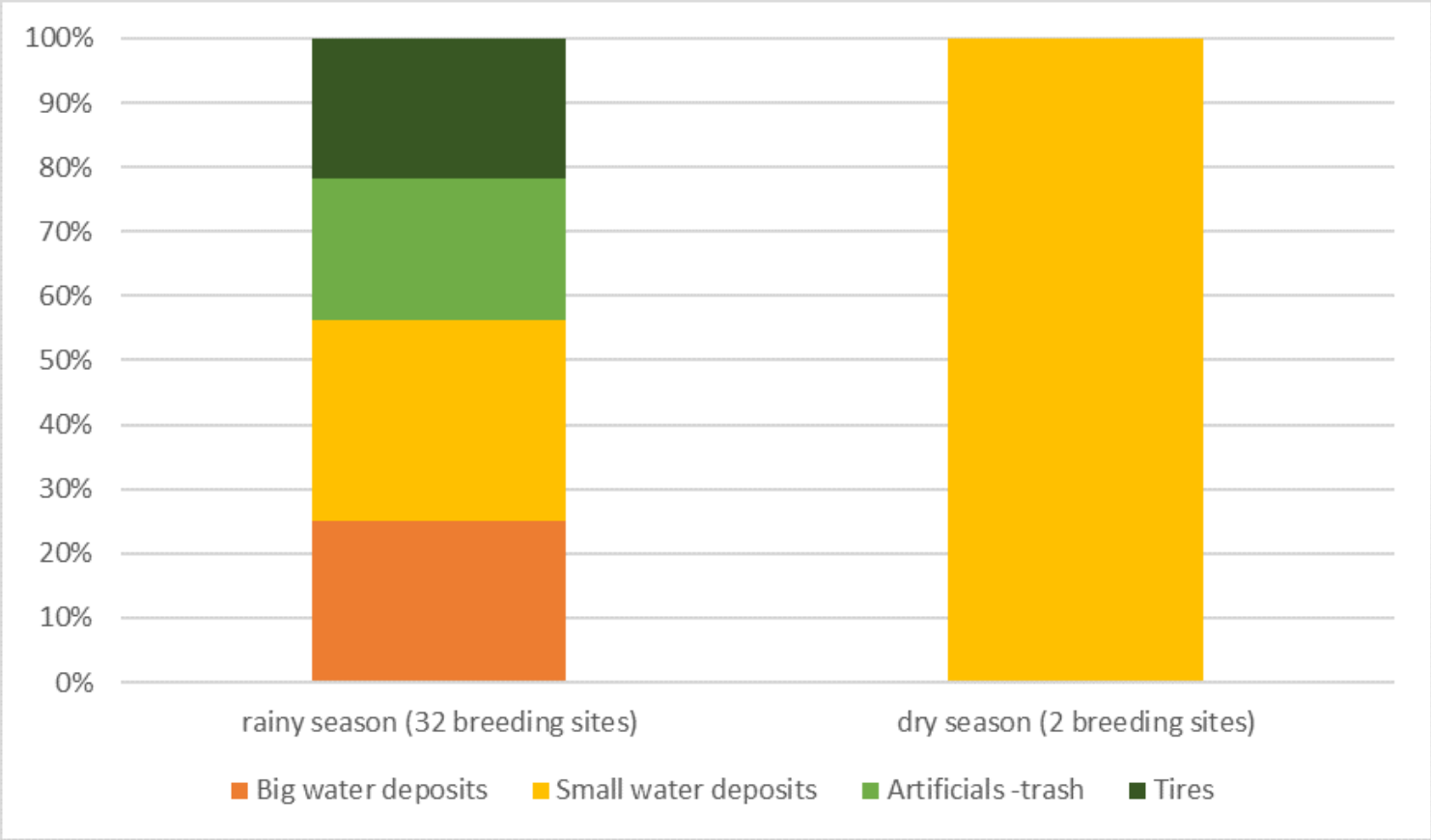

## Figure 4

The geographical and seasonal difference of the most productive Aedes spp. breeding sites, Kinshasa, 2018.




## Figure 5

Neighbor-joining tree including the six medically important Aedes species of the subgenus Stegomyia occurring in the Afrotropical region. Legend: The generated haplotypes of Aedes albopictus and Aedes aegypti of DRC specimens are highlighted in grey.




## Figure 6

Types of breeding sites where Anopheles spp. were encountered in Kinshasa study sites, 2018 (n=32 mixed breeding sites positive for Anopheles).

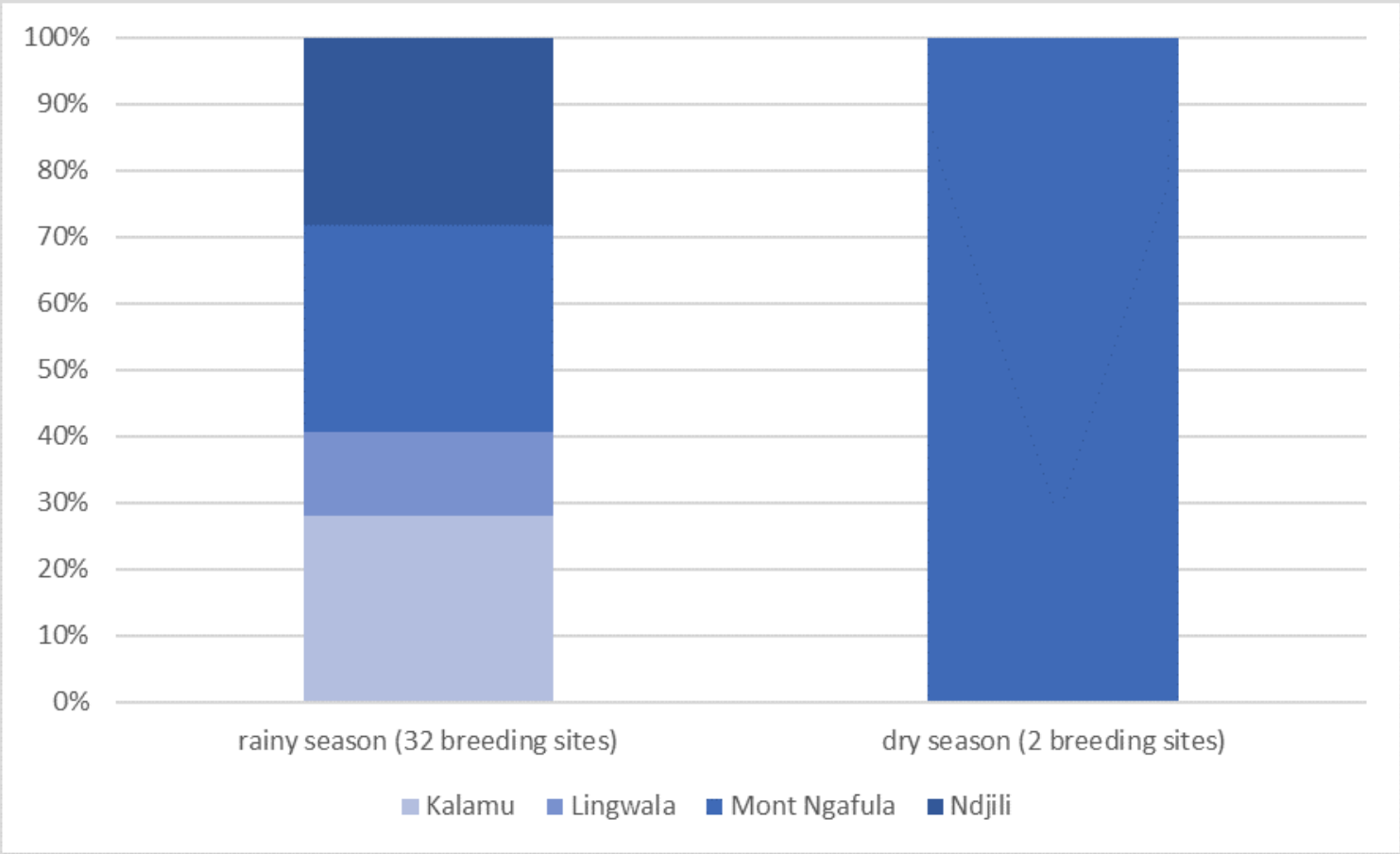



**Figure 7**

Distribution of Anopheles spp. positive breeding sites in the four study communes in the rainy and dry season, Kinshasa, 2018 (n=32 mixed breeding sites positive for Anopheles).

# Supplementary Files

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