Article

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Urban Green Spaces and Vector-Borne Disease Risk in Africa: Case of the Sibang Forested Park in Libreville (Gabon, Central Africa)

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Abstract: In Africa, vector-borne diseases (VBDs) are still a major public health issue especially in cities that gather an increasing human population. Market gardening practices, for example, can favor the transmission of urban malaria, while insufficient water supply and waste management favor the circulation of arboviroses related to *Aedes* mosquitoes. Urban planning is a major challenge to mitigate vector risks. As a planning strategy, greening is a concept that is increasingly considered as a major element impacting the well-being of inhabitants, but also for the restoration of biodiversity in cities. Nevertheless, the impact of urban green spaces on vector risk remains poorly investigated, as they may serve as refuge for vectors. This is why the diversity of mosquitoes in terms of species and larval habitat, through larval prospections in environmental water collections and human landing catches, is studied here at an intra-urban interface area between a forest and an urban ecosystem in order to assess the vector risk generated by preserving a forest patch in the heart of Libreville, capital of Gabon, central Africa. Out of 104 water containers explored, 94 (90.4%) were artificial, mainly comprising gutters, used tires, and plastic bottles, while 10 (9.6%) were natural, comprising a puddle, streams, and tree holes. The majority of the water collections recovered (73.1%) were found outside of the forested area, natural and artificial ones considered together. A total of 770 mosquitoes belonging to 14 species were collected from water collections. The mosquito community was largely dominated by anthropophilic species like *Aedes albopictus* (33.5%), *Culex quinquefasciatus* (30.4%), and *Lutzia tigripes* (16.5%). The Shannon index of diversity showed that mosquitoes were almost twice less diversified inside the forest (0.7) than outside (1.3). However, both communities were quite similar in terms of common species and relative abundance (Morisita-Horn index = 0.7). Regarding Human landing catches, *Aedes albopictus* (86.1%) was the most aggressive species, putting people at risk of *Aedes*-borne viruses. This study uncovered the importance of considering urban forested ecosystems as potential drivers of disease emergence and spread in urban areas, as they might locally boost urban mosquito densities due to poor environmental practices, mainly maintained by humans through poor environmental practices. In Gabon, this study should contribute to guide targeted vector control strategies, especially regarding the implementation of policies for a better environmental management and vector surveillance in urbanized areas.

Keywords: Vector-borne diseases; urbanization; urban forest; *Aedes; Anopheles; Culex;* Gabon; central Africa

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1. Introduction

Vector-borne diseases (VBDs) account for a significant proportion of human diseases worldwide[1]. On a global scale, Africa is one of the most affected continents, where malaria, arboviral diseases such as yellow fever (YF), chikungunya (CHK), dengue (DEN) or Zika (ZIK), and neglected tropical diseases such as lymphatic filariasis are the cause of several major health crises [1–5]. Among the affected regions, urban localities are concentrating an important part of the VBDs' burden [6]. In central Africa, urban areas are commonly associated with significant prevalence of malaria [7–11] and arboviral diseases [12–15].

In central Africa, as in other sub-Saharan African countries, urbanization is poorly controlled. Insufficient water supply and poor waste management have led to a proliferation of mosquito breeding habitats that put populations at risk for VBDs [16,17]. This makes the issue of urban planning and environmental management of African cities an important challenge to be addressed in order to mitigate the risks associated with VBDs.

Greening cities (i.e., promoting and developing green spaces such as parks, agriculture areas, or ecological corridors within cities) is a concept that has been promoted in discourses because of their potential benefits to the environmental quality as well as human well-being [18]. In addition, urban green spaces are also recognized as sustainable solutions to mitigate climate warming, especially at microclimatic scale by regulating the ambient temperature in cities through the freshening of the air provided by the shade of trees [18]. Alongside its beneficial impact over the quality of life in cities, the development of urban green spaces is also valued for its positive impact on carbon sequestration and biodiversity conservation [19]. However, in spite of these benefits, the potential downside of urban green spaces regarding public health issues, including the vector risk related to VBDs, is not yet well understood. In temperate zones some investigations have shown that urban woodland vegetation cover appears to facilitate dispersal and create movement corridors for *Aedes* mosquito females in search of egg-laying sites, whereas grasslands with few tall grasses seems to limit them [20]. In tropical America, the presence of high vegetation is likely to define a microclimate locally impacting the relative humidity of the air, leading to a positive association with the presence of *Aedes* mosquitoes [21]. Similarly, urban forests have shown substantial changes in mosquito communities, including the two most important arbovirus vectors, *Ae. albopictus* and *Ae. aegypti*. These species, especially *Ae. albopictus*, may use the forest as a refuge and act as "bridge vectors" of arboviruses between forest and anthropogenic settings [22]. However, in Africa, there is a need to elucidate how urban green spaces shape mosquito communities and impact the risk related to VBDs since their potential as refuges or incubator ecosystems for mosquito vectors has received little attention.

In Gabon, 85% of the national population lives in urban areas [23], and these are the places of origin of most of the people affected by VBDs [12,24–26]. With a dense forest covering more than 80% of the national territory, placing the country at the upper end of per capita forest area rates in Africa [27], the question of the impact of urban green spaces on the modulation of the epidemiological patterns of VBDs is of high priority to address. In the present study, we explored the diversity and the larval microhabitat typology of mosquitoes at an intra-urban interface between a frequently visited forested reserve and built-up surroundings in order to evaluate the VBDs' entomological risk related to that ecosystem within the city of Libreville, capital of Gabon, central Africa.

2. Materials and Methods

2.1. Study area

This study was carried out from July 14th to August 20th 2020, during the long dry season (which last from June to September), in the Sibang arboretum (0°24'58''N and 9°29'23''E) and its inhabited surroundings (Figure 1). The study was conducted during the dry season as we suspected that this wooded area may serve as a refuge for mosquitoes during that period of low rainfall, and there was a need to investigate the mosquito communities as well as the vector risk associated to that ecosystem. The Sibang arboretum is a forest park covering 160,000m^2 and located in an urbanized area of the eastern part of Libreville crossed by the Adoung river. Cordier (2000) reported a diversity of 137 plant species and at least forty species of birds, and a poorly quantified diversity of vertebrate animals including reptiles and small mammals such as squirrels. Despite its protected status, the Sibang arboretum is subject to an important anthropogenic pressure, which is strongly marked by the presence of diverse traces of human frequentation and the accumulation of rubbish deposited in certain locations up to several tens of meters inside the arboretum (Figure 2).

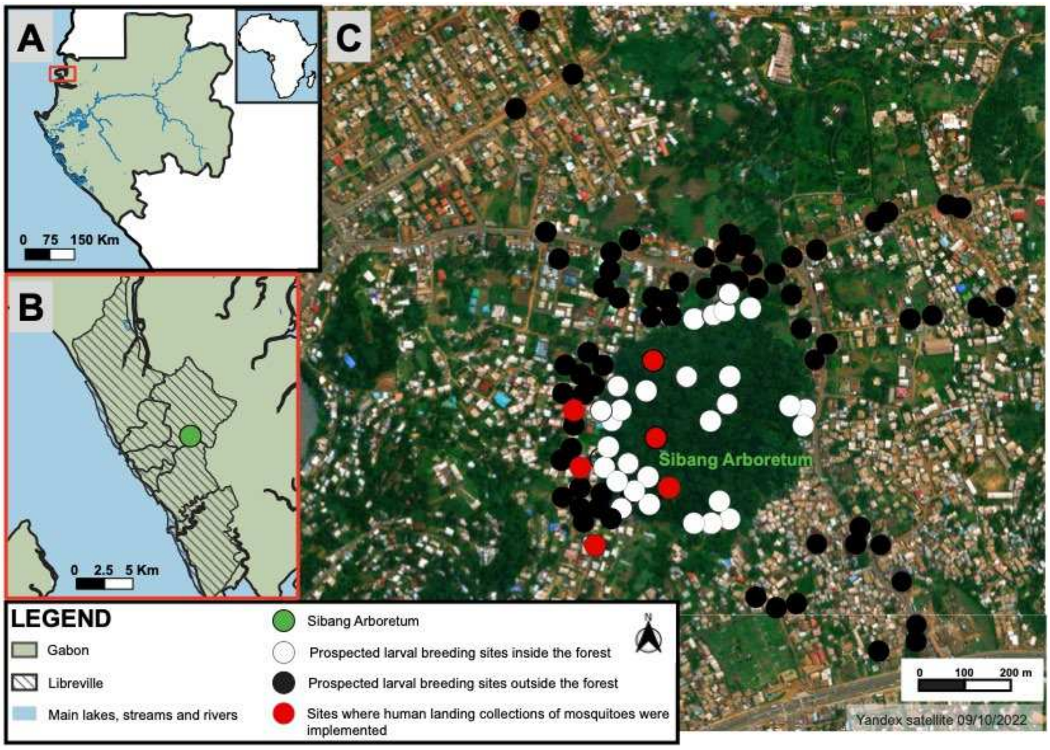

Figure 1. Study area and sampling sites. A: Location of Gabon within Africa; B: Location of the Sibang arboretum within Libreville; C: Sampling sites, with black spots representing prospected larval breeding sites and red spots representing sites where human landing collections were implemented.

Figure 2. Photography of a section of the Sibang arboretum border heavily marked by waste dumping from surrounding households.

2.2. Larval sampling

Larval prospections were conducted during 13 non-consecutive days outside and inside the forest block. Outside the forest, larval prospections were conducted over a radius of 800m around the forest block. Mosquito breeding sites were prospected as exhaustively as possible depending on field accessibility and permission from residents to visit their properties. Over the collection period, the sampling effort for larval prospections lasted of 15 hours and 63 hours inside and outside the forest respectively.

Water containers were explored in sites visited to collect immature stages of mosquitoes (Fig 1C). Larvae and pupae were collected using a dipper or a pipette when required and transferred into vials labeled according to the container type, the site location, and the date. At the entomological laboratory of the Research Institute for Tropical Ecology (IRET), in Libreville, larvae and pupae were placed into labeled trays covered with a mosquito net and maintained at room temperature until the emergence of adults. Upon emergence, adult mosquitoes were successively stored at -20°C for 30 minutes to be euthanized, then morphologically identified to species (or genus) using a binocular microscope (Leica Microsystems ©). Identification was aided by “custom” taxonomic keys based on updates of the Edwards’ identification keys for Ethiopian mosquitoes [29], and the Huang’s key for the subgenus *Stegomyia* of *Aedes* mosquitoes from the Afrotropical region [30]. Species were named according to the online list of valid species (http://mosquito-taxonomic-inventory.info).

2.3. Human landing collection

Adult female mosquitoes looking for blood meal were collected using diurnal human landing collection technique (HLC). The study benefited from ethical approval (permit No. 016/2019/PR/SG/CNE) from the National Ethic Committee. Three volunteers, posted at three fixed capture sites, collected adult females during two consecutive sampling sessions. The first sampling session was conducted inside the forest, and the second one was conducted outside the forest. During each session, the capture sites were distanced by at least 50m from each other (Figure 1C). Each sampling session consisted in 3 consecutive days of diurnal captures, from 10:00 am to 2:00 pm (4h per day), representing a sampling effort of 12h. Mosquitoes were captured with a mouth aspirator upon landing on the volunteer’s bare legs and then transferred into a plastic jar covered with a net to prevent mosquito escape. Once the captures were completed, the mosquitoes were transported to the IRET’s entomological laboratory for identification. After the euthanasia process, mosquitoes were then morphologically identified and counted as described above.

2.4. Data analysis

All statistical analyses were done using the R software v3.6.1 (https://www.r-project.org/) and spatial analyses were performed using Quantum GIS version 3.10.7 (https://www.qgis.org/). Species richness was determined as the number of mosquito species recovered. The species diversity according to the number of species and their respective abundance was assessed using the Shannon-Weaver index (H) [31] using the *“diversity”* function of the *vegan* package. To investigate the similarity in terms of species composition and density between mosquito communities from inside and outside the forest, the Morisita-Horn similarity index (C) [32] was calculated using the “*vegdist*” function of the *vegan* package. Because “*vegdist*” is an analysis of dissimilarity (C'), $C = 1 - C'$ was used for this study. C ranges from 0 (0% of similarity between both compartments) to 1 (100% of identity between both compartments).

Environmental variables were collected to characterize the larval habitats exploited by mosquitoes of the Sibang arboretum and its surroundings. These variables included the physical description of the substrate, the type (artificial *vs* natural), and the spatial location of the larval habitat (inside the forest *vs* outside the forest). We used a Multiple Correspondence Analysis (MCA) to assess the similarity level of larval habitats according to their species composition and environmental characteristics. It allowed to assess the mosquito species' degree of specificity related to the type and the location of larval habitats. The MCA was also used to discriminate the most relevant biotic and environmental variables associated with this larval habitat segregation.

We performed a k-means analysis based on Ward's method using the *fpc* package [33] coupled with the Calinski Harabasz index (CH index) [34] to determine the minimal parsimonious number of ecological clusters of species. Based on a Principal Component Analysis (PCA) using the *FactoMineR* package [35], we performed an Ascendant Hierarchical Clustering (AHC) to determine and visualize species' clusters likely to occur together within the same micro-ecological niche.

In order to assess the aggressiveness of bloodmeal-seeking female mosquitoes according to the sampling location (inside *vs* outside the forest), we used the Wilcoxon's test based on count data from HLC. An analysis of variance (ANOVA) was performed to determine the differences in the number of captured specimens per person between species within each sampling location.

3. Results

3.1. Typology and positivity of larval habitats inside and outside the forest

A total of 104 water collections were explored in the Sibang district (Figure 1). Out of them, 29 (27.9%) were recovered inside the forest area (5 natural and 24 artificial), and 75 (72.1%) outside the forest area (3 natural and 72 artificial) (Table 1). However, regarding the sampling effort, the number of water collections recovered per hours was 1.9 inside the forest, whereas it was 1.1 outside the forest. Regarding the type of water collection, 10 of them (9.6%) were natural, whereas 94 (90.4%) were artificial (Figure 3A). The natural water collections inspected were tree holes (50%), streams (40%) and a puddle (10%). Artificial water collections included plastic containers (34%), puddles (19.1%) (formed from piped water leakage), gutters (18.1%), worn tires (14.9%), and other kind of detritus each accounting for less than 15%, including a worn freezer, a tin can, a glass jar, a wash basin or various metallic containers (Table 1). Regarding the positivity of water collections (presence of at least one mosquito larva or pupa in water collections), we found that the quasi-totality (inside the forest) or the totality of them (outside the forest) were positive, accounting for 96.4% (n = 27) and 100% (n = 76) respectively, natural and artificial types considered together (Figure 3B). We found that inside the forest, natural and artificial containers were positive at 100% (n = 8) and 95% (n = 19) respectively. However, outside the forest, all the natural (100%, n = 2) and artificial (100%, n = 74) containers were found positive.

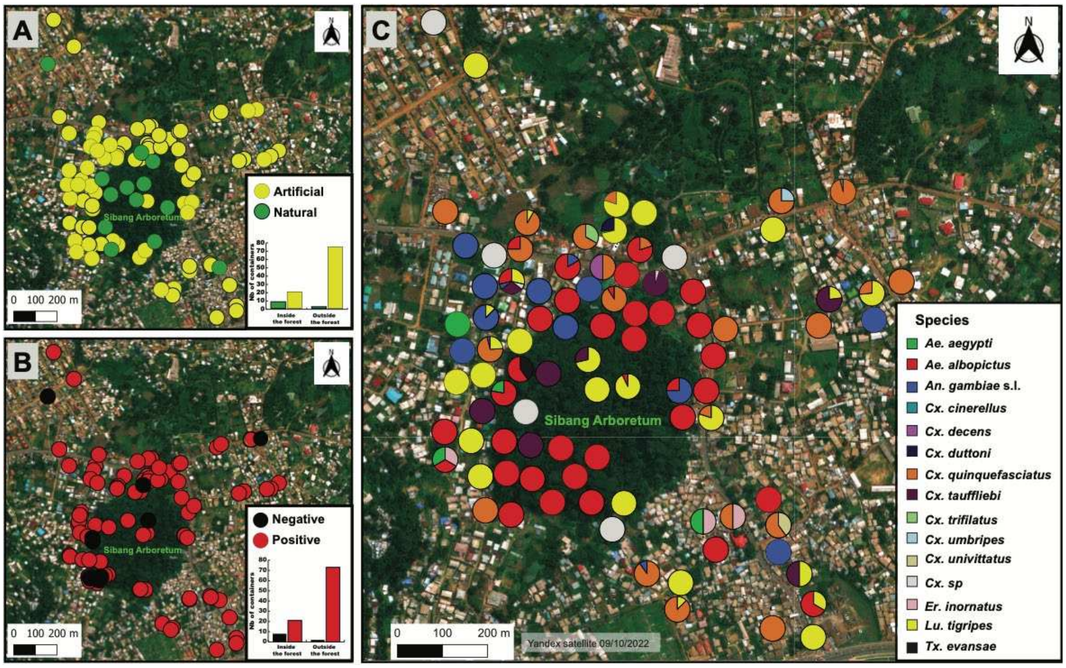

Figure 3. Spatial distribution of larval habitats and relative abundance of mosquito species in each larval habitat. A: The yellow circles correspond to artificial larval habitats and the green ones to natural habitats. B: Red circles represent positive habitats (where there was at least one larva or pupa), and black circles represent negative habitats. C: Pie chart showing the graphical representation of species relative abundance.

Table 1. Water containers explored in the study area This is example 1 of an equation:.

Habitat nature	Inside the forest (n=28)	Outside the forest (N=76)	Overall (N=104)
Artificial			
Glass jars	1 (5.0%)	1 (1.4%)	2 (2.1%)
Gutters	1 (5.0%)	16 (21.6%)	17 (18.1%)
Metallic container	0 (0.0%)	6 (8.1%)	6 (6.4%)
Plastic container	15 (75.0%)	17 (23.0%)	32 (34.0%)
Puddles	0 (0.0%)	18 (24.3%)	18 (19.1%)
Tires	0 (0.0%)	14 (18.9%)	14 (14.9%)
Wash basin	1 (5.0%)	1 (1.4%)	2 (2.1%)
Discarded freezer	0 (0.0%)	1 (1.4%)	1 (1.1%)
Discarded toilet bowl	2 (10.0%)	0 (0.0%)	2 (2.1%)
Subtotal	**20 (100%)**	**74 (100%)**	**94 (100%)**
Natural			
Puddles	1 (12.5%)	0 (0.0%)	1 (10.0%)
Tree holes	5 (62.5%)	0 (0.0%)	5 (50.0%)
Streams	2 (25.0%)	2 (100%)	4 (40.0%)
Subtotal	**8 (100%)**	**2 (100%)**	**10 (100%)**

n: number of water collections.

3.2. Mosquito species composition and diversity

After larval rearing at the insectary, a total of 770 adult mosquitoes emerged and were morphologically identified. These mosquitoes belonged to 14 species grouped into 6 genera, including Aedes (2 species), Culex (8 species and 1 undetermined), Anopheles (1 species), Eretmapodites (1 species), Lutzia (1 species), and Toxorhynchites (1 species) (Table 2). Overall, the species assemblage was largely dominated by Ae. albopictus (33.5%), Cx. quinquefasciatus (30.4%), and Lu. tigripes (16.5%), whereas Cx. cinerellus, Cx. decens, Cx. duttoni, Cx. trifilatus, Cx. umbripes, Cx. univittatus, Er. inornatus, and Tx. evansae were relatively less abundant, each one representing less than 1% of the total assemblage (Table 2). Inside of the forest, there was a predominance of Ae. albopictus (77%), Cx.quinquefasciatus (13.8%), and Lu. tigripes (6.9%) in artificial habitats, whereas in natural ones, only Cx. taufliebi (50%) and Lu. tigripes (22.7%) were the most predominant species (Table 2). Concerning larval habitats explored outside the forest, Cx. quinquefasciatus (35.1%), Ae albopictus (29.7%), and Lu. tigripes (17.1%) were the most predominant species in artificial containers. However, in natural ones, Cx. taufliebi (67.8%) and Lu. tigripes (28.6%) were the most predominant species recovered. However, we noticed that Ae. albopictus and An. gambiae s. l. were absent from all natural habitats (Table 2). According to the Shannon-Weaver index, mosquitoes appeared almost twice less diverse inside the forest (H = 0.7) than outside the forest (H = 1.3). However, both compartments showed an overall community similarity level valued at C = 0.7 (i.e. 70% of similarity, which is associated with the amount of shared species between both compartments and their relative abundance within the respective communities).

Table 2. Assemblage of mosquitoes according to the type and the spatial location of breeding sites.

Species	**Inside forest**			**Outside forest**			**Overall**
	Artificial	Natural	Sub-total	Artificial	Natural	Sub-total	
Ae. aegypti	0 (0.0%)	0 (0.0%)	0 (0.0%)	27 (4.3%)	0 (0.0%)	27 (4.1%)	27 (3.5%)
Ae. albopictus	67 (77.0%)	3 (13.6%)	70 (64.3%)	188 (29.7%)	0 (0.0%)	188 (28.5%)	258 (33.5%)
An. gambiae s. l.	0 (0.0%)	1 (4.6%)	1 (0.9%)	41 (6.5%)	0 (0.0%)	41 (6.2%)	42 (5.4%)
Cx. cinerellus	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)	1 (0.1%)	1 (0.1%)
Cx. decens	2 (2.3%)	0 (0.0%)	2 (1.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.3%)
Cx. duttoni	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (0.9%)	0 (0.0%)	6 (0.9%)	6 (0.8%)
Cx. quinquefasciatus	12 (13.8%)	0 (0.0%)	12 (11.0%)	222 (35.1%)	0 (0.0%)	222 (33.6%)	234 (30.4%)
Cx. taufliebi	0 (0.0%)	11 (50.0%)	11 (10.1%)	17 (2.7%)	19 (67.8%)	36 (5.4%)	47 (6.1%)
Cx. trifilatus	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (0.5%)	0 (0.0%)	3 (0.5%)	3 (0.4%)
Cx. umbripes	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.1%)	0 (0.0%)	1 (0.1%)	1 (0.1%)
Cx. univittatus	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (0.6%)	0 (0.0%)	4 (0.6%)	4 (0.5%)
Culex sp.	0 (0.0%)	0 (0.0%)	0 (0.0%)	12 (1.9%)	1 (3.6%)	13 (2.0%)	13 (1.7%)
Er. inornatus	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (0.5%)	0 (0.0 %)	3 (0.5%)	3 (0.4%)
Lu. tigripes	6 (6.9%)	5 (22.7%)	11 (10.1%)	108 (17.1%)	8 (28.6%)	116 (17.5%)	127 (16.5%)
Tx. evansae	0 (0.0%)	2 (9.1%)	2 (1.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.3%)
Overall	87 (100%)	22 (100%)	109 (100%)	633 (100%)	28 (100%)	661 (100%)	770 (100%)

3.3. Larval habitat typology, similarity and species culsturing

According to the type of larval habitats, we observed that Ae. albopictus was mainly found in artificial breeding containers. Additionally, this species was associated with higher relative abundances in both compartments compared to the other ones (Table 2). Similarly, Cx. quinquefasciatus was mostly recovered in artificial breeding containers (13.8% and 35.1% inside and outside the forest respectively). However, Lu. tigripes was most relatively abundant in natural (22.7% and 28.6% inside and outside the forest respectively) (Table 2). Regarding Ae. aegypti and An. gambiae s. l., we observed that the first species was exclusively recovered in artificial breeding containers and outside the forest at 100%. The second one was almost exclusively found in artificial breeding sites located outside the forest (6.5%) (Table 2).

Regarding the level of similarity of larval habitats, the MCA revealed that the best-correlated variables associated with the larval habitat distribution were their type (i.e. whether the habitat was natural or artificial) and spatial location (i.e. whether the habitat was located inside or outside the forest), as well as the presence of the species Ae. albopictus, An. gambiae s. l., Ae. aegypti, Lu. tigripes, Cx. quinquefasciatus, Cx. trifilatus, Cx. duttoni, and Er. inornatus (Figure S1). These variables allowed to explain 38.4% of the total variance associated with the species composition of larval habitats. Thus, the analysis showed a clear segregation of larval habitats according to their spatial location, and according to their type (Figure 4A & 4B). In terms of larval habitat specificity for mosquito species, our results revealed that in the Sibang area, mosquito larval habitats are likely to be exploited by species that could be characterized as specialist (i.e. those with a high level of habitat specificity: Ae. aegypti, An. gambiae s. l., Cx. trifilatus, Cx. duttoni and Er. inornatus), opportunistic (i.e. species that are not highly specialized, having a preferential biotope, but which are able to adapt under a certain range of environmental conditions different from that of specialist species: Ae. albopictus and Lu. tigripes) or ubiquitous (i.e. species highly adapted to occupy and proliferate in varied ecological niches, possibly with a wide geographical distribution: Cx. quinquefasciatus) (Figure 4C-4J). Indeed, our observations revealed that among the most relatively abundant species, the breeding sites that were mostly exploited by species were discarded plastic containers or worn tires for Ae. albopictus (opportunistic species), and metallic or concrete containers (i.e. wash basin, see Table 1) for Cx. quinquefasciatus (ubiquitous species) (Figure 5). However, Lu. tigripes, appeared also opportunistic by exploiting in more equivalent proportions discarded tires, puddles, plastic, metallic or concrete containers (Figure 5). Furthermore, regarding species known as the main vectors of public health concern, the breeding sites that were almost exclusively exploited by Ae. aegypti and An. gambiae s. l. were plastic containers and puddles (both natural and artificial) respectively (Figure 5).

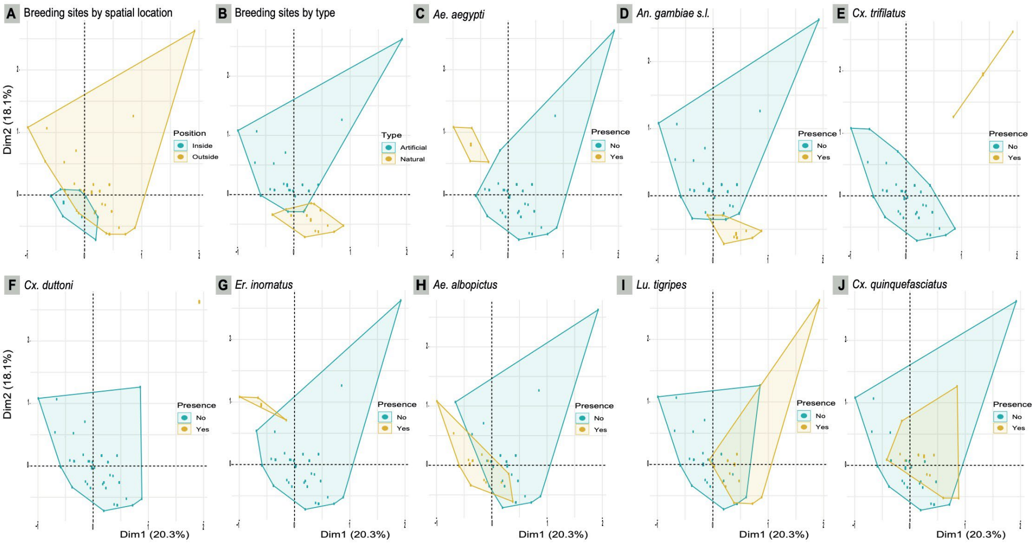

Figure 4. Distribution of larval habitats according to the retained variables processed in the MCA. The MCA explained 38.4% of the total variability of larval habitats in terms of species composition. These variables include spatial location (A) and type (B) of larval habitats. They also comprise the main species recovered (C-J). Intersection areas refer to habitats that tend to be similar in type (natural/artificial), location (inside/outside forest) and specific composition.

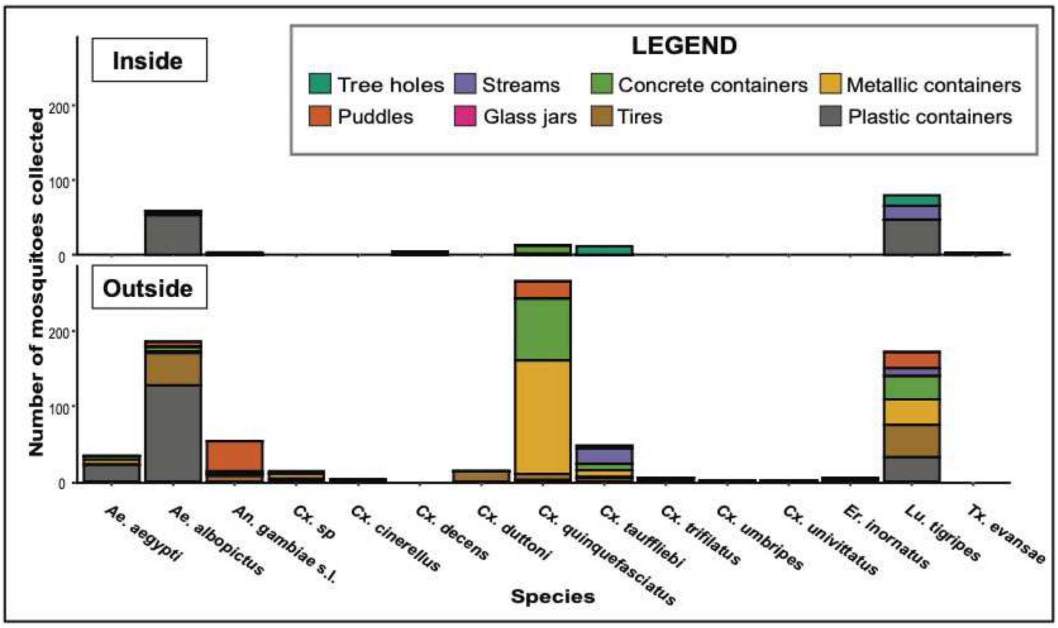

Figure 5. Mosquito species' distribution based on the nature of larval habitats.

Concrete containers include wash basin and gutters.

In order to assess the degree of ecological niche similarity among mosquito species at the larval microhabitat scale, the k-means method based on the PCA (72.8% of the total explained inertia of count data over the first two principal components, see Figure S2) and the CH index revealed that the minimal parsimonious number of ecological clusters of species is four (Figure 6A). These clusters included two that were mono-specific (cluster 2: *Ae. albopictus*; cluster 3: *Cx. quinquefasciatus*) and two that were multi-specific ones (cluster 1: *Ae. aegypti, Tx. evansae, Cx. umbripes, Cx. decens, Cx. trifilatus, Cx. cinerellus, Cx. univittatus, Cx. duttoni, Culex* sp., *Er. inornatus*; cluster 4: *Cx. taufliebi* and *Lu. tigripes*) (Figure 6B). Indeed, our analysis showed that the species that belonged to the same cluster were more likely to share the same type of larval ecological niche in the Sibang area.

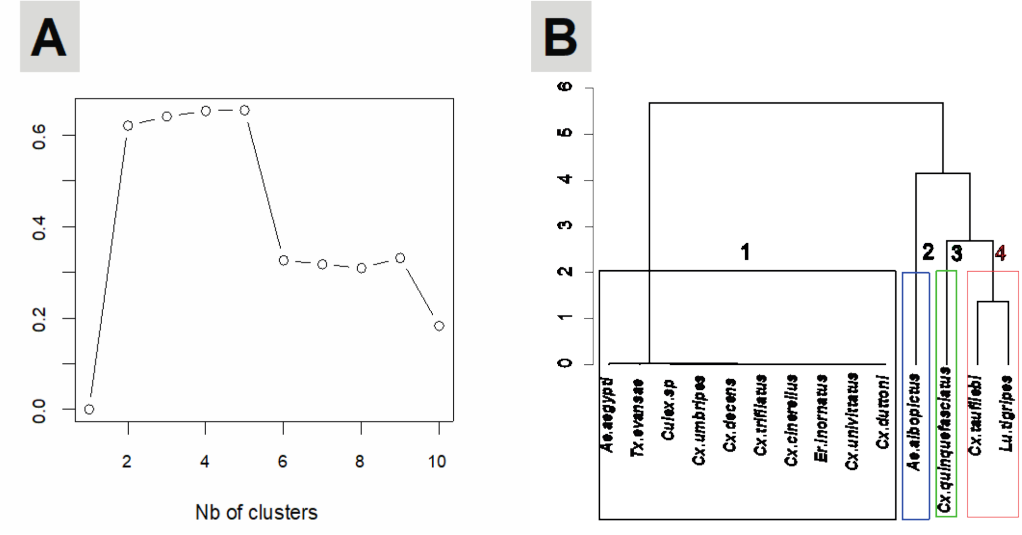

Figure 6. Species clustering based on the CH index and AHC, and analytical synopsis of the niche similarity level across mosquito species based on larval microhabitats. A: Results of Ward k-means analysis combined with the CH Index revealing the parsimonious number of species clusters (n = 4). B: Representation of the species clusters based on the AHC.

3.4. Biting patterns of mosquito species

A total of 874 female mosquitoes distributed into three species were captured and identified. Among them, 755 (86.4%) were captured inside the forest and 119 (13.6%) outside the forest during the time slot surveyed (10:00 am to 2:00 pm). Regarding the species composition of mosquitoes collected according to the sampling, *Ae. albopictus*, with 753 specimens captured (86.1%) was to a large extent the main aggressive species for human, with a peak of aggressiveness of 35.2 bites/person/hour (bph) reached between 11:00 am and 12:00 pm inside the forest (Figure 7A). *Aedes aegypti* and *Er. inornatus* for which 108 (12.4%) and 13 (1.5%) specimens were captured respectively appeared less aggressive with less than 5 bph regardless of the spatial location. Globally, the ANOVA test showed that *Ae. albopictus* was significantly more aggressive than *Ae. aegypti* and *Er. inornatus* (F = 11.8; df = 2; $p < 0.001$). When comparing the aggressiveness inside and outside the forest, *Ae. albopictus* was significantly more aggressive inside the forest than outside (W = 140, $p < 0.001$) (Figure 7B), whereas no significant difference was found between both locations for *Ae. aegypti* (W = 84.5, p = 0.5) (Figure 7B). Analyzing data for each species separately based on the spatial location revealed that. *Er. inornatus* was only captured inside the forest (Figure 7B).

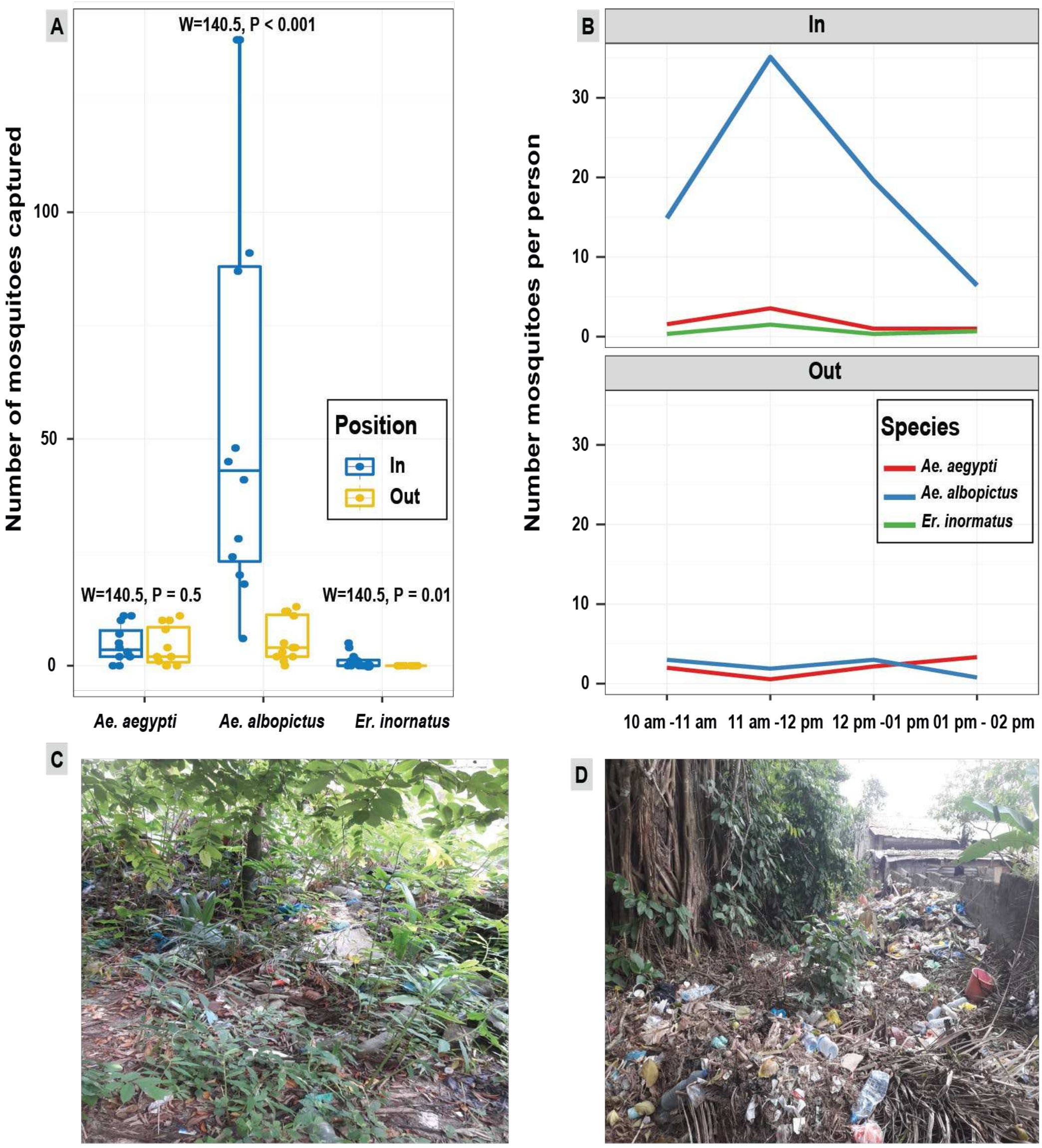

Figure 7. Number and biting rate of human-baited mosquitoes in the Sibang arboretum and its surroundings. A: Mean number of captured mosquito females per person according to the spatial location. B: Number of captured mosquito females per person over time inside and outside of the forest. C and D: A view of wastes accumulating in the forest and its surroundings illustrating the human footprints.

4. Discussion

4.1. Mosquito communities in the urban forested area of Sibang

This entomological survey aimed to describe the species diversity and the larval microhabitat typology of mosquito communities in an urban forested reserve and in its direct surroundings to evaluate how such a green area could impact the VBDs' entomological risk within the city of Libreville, Gabon. A hundred and four potential breeding sites were surveyed, of which the large majority were found positive with mosquito larvae both inside and outside the forest area. The types of larval habitats recovered, especially for *Aedes*, *Culex* and *Lutzia* mosquitoes which were the most predominant genera, were consistent with what has been reported (i.e. tires, plastic containers, gutters, or tree holes) for these mosquito genera in previous investigations done elsewhere, including in Gabon for *Aedes*, *Culex* and *Lutzia* mosquitoes [36–38], central Africa [39–41], in Asia [42,43], and in south America for *Aedes* and *Culex* mosquitoes [44–46].

Regarding the species composition within larval habitats, we found that overall, among all the species recovered, *Ae. albopictus*, *Cx. quinquefasciatus* and *Lu. tigripes* were the most predominant species. Indeed, as shown earlier, the predominance of *Ae. albopictus* can be explained by the predominance and the availability of artificial water containers which are known as suitable habitats for this species, as previously demonstrated elsewhere [40]. Previous studies demonstrated the natural predatory ability of *Lu. tigripes* over other mosquito species, including mosquitoes of *Aedes* genus [47–50]. Thus, the abundance and distribution of *Lu. tigripes* could follow the dynamics of the other predominant species that it uses as prey. Regarding our results, the relatively high abundance of *Cx. quinquefasciatus* confirms its ubiquity in exploiting various types of larval habitats, especially artificial habitats mostly found in highly populated areas such as urban settings [39].

Although one specimen of *An. gambiae* s. l., the major malaria vector in the world, was recovered in a natural puddle inside the forest, this species was almost only found in artificial larval habitats located outside of the forest which were exclusively open puddles, solely maintained by domestic wastewater runoff, which are among the typical larval habitats known to be created by human activity for this species [51–54]. However, we found three specimens of this species in a discarded tire outside the forest. As this species is known so far to exploit a well-defined range of natural or artificial breeding sites, including but not limited to temporary or permanent ground puddles or pools, water-filled ruts, roadside gutters, or unused dugouts [51], this result confirm the use of unusual microhabitats, including those exploited by *Aedes* mosquitoes larval habitats, as described for *An. stephensi*, an emerging malaria vector in Africa [55].

Although we did not explore particular sites such as epiphytic plants or tree holes at high elevations, or underground animal burrows for potential breeding sites because of access difficulties, our observations indicate that the mosquito diversity within the forested compartment is 2-fold lower than the diversity of mosquitoes outside the forest. In the forested compartment, we did not find any species presumed to be exclusively sylvatic. However, we found some species which have already been observed in a natural sylvatic condition elsewhere in Africa. Indeed, Diallo et al. (2019) reported *An. gambiae s.l., Cx. decens* and *Cx. quinquefasciatus* in forest canopy in Senegal (in a forested area of the Kédougou region), although these three species were described as rare in that forested habitat. Similarly, Pereira dos Santos et al. (2018) reported *Ae. albopictus* in relatively high abundance up to several hundred meters inside an urban forest in Brazil. The absence of exclusively sylvatic species could be due to a lack of suitable conditions for sylvatic species, most of the time characterized by the existence of productive natural breeding sites (e.g. leaf axils, tree holes, rock holes, fruit shells, etc.), or the absence of animal host species on which sylvatic mosquito species depends for their blood meals. However, performing this study in the rainy season could have increased the number of species to detect, including potential forest specialist species. Overall, in spite of their difference in mosquito diversity, both investigated compartments (i.e. inside and outside the forest) showed quite an important similarity during the dry season. This result is mostly due to the high relative abundance of the bulk of species they have in common (i.e. *Ae. albopictus* and *Cx. quinquefasciatus*), thus rendering their communities to be similar.

Our analyzes showed a clear segregation of larval habitats based on their type and their spatial location, that could be explained by the inherent ecological preference of all the species recovered. We could then deduct for the Sibang district a range of species that we could describe as specific, with a limited ecological niche such as *Ae. aegypti,* which was exclusively found in plastic artificial containers outside of the forest, clustering with other species including mainly *Culex* spp. It is also the case for *An. gambiae* s. l., which was almost exclusively found in ground puddles. In the same way, we could also identify opportunistic species, characterized by a higher ecological plasticity during larval development such as *Ae. albopictus* and *Lu. tigripes,* which were found exploiting artificial or natural microhabitats inside and outside of the forest (e.g. discarded tires, plastic, metallic or concrete recipients, puddles, or tree holes). *Aedes albopictus* and *Lu. tigripes* were not clustering together maybe because both species do not have the same microhabitat preference in this particular environmental setting. Finally, we could identify ubiquitous species, characterized by a large ecological niche, especially in man-made microhabitats. This is the case of *Cx. quinquefasciatus* that we recovered mostly across metallic and concrete containers, but also tires and plastic containers, even to a lesser extent.

4.2. The mosquito proliferation drivers in the urban forested area of Sibang

On the field, we observed that the infestation level of artificial water collections was very high, even in the arboretum (the interior of the forest), which is obviously used as a waste dump (see Figure 7 C&D). Moreover, this abundance of human-sourced breeding sites tends to indicate a convergence of breeding site types (mostly associated with waste dumping), available hosts, and consequently mosquito communities, both inside and on the forest's periphery. Indeed, there is a high level of anthropogenic pressure from neighboring households on the arboretum ecosystem, which is characterized by an excessive accumulation of domestic waste inside the forest, and prone to favor the proliferation of major disease vector species, especially those exploiting artificial breeding containers such as *Ae. albopictus,* or *Ae. aegypti* or *Cx. quinquefasciatus*.

4.3. Mosquito aggressiveness in the urban forested area of Sibang

Within the time frame of the human landing captures (10:00 am – 2:00 pm), *Ae. albopictus* was by far the most predominant species collected, with a peak of aggressiveness observed between 11:00 am and 12:00 pm, which was associated with a biting rate of 35.2 bph inside the forest. In previous studies, Kamgang et al. (2012) in Cameroon and Delatte et al. (2010) in La Reunion reported peaks of aggressiveness for *Ae. albopictus* later during daytime between 4:00 pm and 5:30 pm, suggesting that it is quite possible that the aggressiveness of this species in the Sibang area might be much higher than what we have observed.

Among the species recovered, *Ae. albopictus* rises as a worldwide invasive arboviral vector of major public health concern [60], which has already been responsible for past chikungunya outbreaks in Gabon [12,61,62]. To the best of our knowledge, data comparing the biting rate associated with *Ae. albopictus* from inside and outside of a forested compartment, both in anthropized and wild environments, are currently not well documented. However, in the present study, the level of aggressiveness associated with *Ae. albopictus* was more than 2-fold higher than what has been observed in Libreville previously in suburban neighborhoods (15.7 bph) [37], some of which were wooded areas with chikungunya transmission records, but never under forest cover. Furthermore, past investigations conducted in 2009 in the Central African Republic (CAR) which included forested peri-domestic areas among the sampling sites, reported relatively low biting rates reaching a peak of 1.7 bph [63]. This result might be because the period Diallo and colleagues conducted their study corresponded to the early stage of the invasion of *Ae. albopictus* in the CAR, probably associated with low densities of the former. Another reason could be that this forest had not been an area of waste dumping, which seems to be, based on our results, a driver of *Ae. albopictus* proliferation and aggressiveness. To go further, supplementary surveys will be needed to have estimations of the level of biting rate associated with *Ae. albopictus* over a longer period of time during the day in the region. In terms of public health concerns, our results show that in reason of a large population of *Ae. albopictus* sourced from the Sibang urban forest, the exposure of the human populations from this side of Libreville to diseases transmitted by *Ae. albopictus* is to be regarded as a matter of priority for disease outbreak surveillance programmes, especially for those living in the surroundings of the arboretum.

Ae. aegypti was the second most aggressive species, far behind *Ae. albopictus*, especially inside the forest. The low biting rate and the non-significant difference in the captured females of *Ae. aegypti* between the inside and the outside of the forest could be the result of the scarcity of this species in the Sibang district, regardless of the special location (inside vs outside of the forest). In addition, the fact that larval stages of this species were found in relatively low proportion compared to *Ae. albopictus*, suggest that this species could be prone to a population decline resulting from the successful invasion of *Ae. albopictus*, as suggested in central Africa [37,41,64], and elsewhere in the world [65,66].

Among both *Aedes* (*Stegomyia*) species, *Ae. albopictus* is well known for its opportunistic blood-feeding behavior and high vector competence for a number of viruses with pathogenic effects in humans (Pereira-dos-Santos et al., 2020). Studies conducted in the early 2000s in the Sibang arboretum reported a high biodiversity of vertebrate animals, including several species of small mammals, reptiles and birds [28]. Thus, the high density of *Ae. albopictus* would be likely to not only increase the risk of inter-human transfer of *Ae. albopictus*-borne pathogens, but also represent a risk for potential zoonotic pathogens that could be hosted by the vertebrate animals in this forested patch, including birds and rodents, and be transmissible by this vector. Indeed, both animal groups are recognized hosts (or potential hosts) for zoonotic arboviruses, including but not limited to West Nile virus for birds [67].

4.4. Mosquito aggressiveness in the urban forested area of Sibang

This study in the urban forested area of Sibang, which highlighted the important level of aggressiveness of the arbovirus vector *Ae. albopictus*, stressed out the potential risk of arbovirus transmission associated with this urbanized and forested district of Libreville. Such a risk could be exacerbated by a potential boost effect of the forested compartment of the Sibang district, which might constitute a human-maintained incubator ecosystem and resting place for vectors, and which might facilitate and sustain disease spread during epidemic periods.

Our results also highlighted as well the issue of the vegetation of urban cities, which might modulate the burden of vector-borne diseases by being a factor that intensifies (due to a high density of mosquitoes) or dilutes (due to the presence of alternative hosts for mosquitoes to feed on) the transmission of these diseases in such areas. As it has been shown by Araujo et al. (2015) in a Brazilian city that incidence of dengue was higher in heat islands than in forested neighborhoods considered as more fresh. Here we could suggest that urban forests could be considered as “islands of coolness” that might mitigate the spread of arboviruses, for example by slowing down the replication rate of viruses in mosquito vectors that live under the forest cover. Thus, the benefit of developing green spaces (e.g. in temperate poorly forested countries) or managing them (e.g. in tropical and highly forested countries) could be effective in terms of service to biodiversity, climate warming mitigation and the well-being of urban citizens. Therefore, urban forest island such as the Sibang forest should rather be well planned and/or managed to mitigate the risks associated with these urban forested ecosystems regarding environmental-driven diseases in general, and vector-borne diseases in particular.

5. Conclusions

In conclusion, this study conducted in the urban forested area of Sibang, allowed the recording of a bit more than 12% of the number of the currently known mosquitoes species reported in Gabon [37,69–72]. The investigations revealed a variety of larval habitats exploited by the mosquito species recovered from the study area. These habitats were artificial in majority and mainly created by humans. The study uncovered the importance of considering urban forested ecosystems as potential drivers of disease emergence and spread in urban areas, which should be included in vector control strategies. In Gabon, this study should contribute to guide vector control strategies, particularly through the implementation of policies to ensure a good environment management and the surveillance of vectors in urbanized areas.

Supplementary Materials: The following supporting information can be downloaded at: Preprints.org.

Author Contributions: Conceptualization, Judicaël Obame-Nkoghe and Boris Kevin Makanga; Data curation, Judicaël Obame-Nkoghe; Formal analysis, Judicaël Obame-Nkoghe, Boris Kevin Makanga and Prune Komba; Funding acquisition, Judicaël Obame-Nkoghe and Christophe Paupy; Investigation, Judicaël Obame-Nkoghe, Boris Kevin Makanga, Sylvie Brizard Zongo, Aubin Armel Koumba, Rodolphe Akone-Ella, Lynda Nkoghe-Nkoghe, Marc-Flaubert Ngangue-Salamba and Patrick Yangari; Methodology, Judicaël Obame-Nkoghe and Boris Kevin Makanga; Project administration, Judicaël Obame-Nkoghe; Resources, Sophie Aboughe-Angone; Supervision, Christophe Paupy; Visualization, Judicaël Obame-Nkoghe and Boris Kevin Makanga; Writing –

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