

1 **Short title: Insecticide susceptibility of *Aedes* spp. in Kinshasa**

2

3 **Insecticide susceptibility of *Aedes (Stegomyia) aegypti* (Linnaeus, 1762) and *Aedes (Stegomyia)***

4 ***albopictus* (Skuse, 1894) in Kinshasa, Democratic Republic of the Congo**

5

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21 **Abstract**

22 *Aedes aegypti* and *Aedes albopictus* are arbovirus vectors of public health concern. Although the
23 Democratic Republic of the Congo (DRC) faces a long-standing risk of *Aedes*-borne viruses, data on
24 insecticide resistance of *Aedes* populations are absent. To address this gap, we investigated insecticide
25 susceptibility of *Ae. aegypti* and *Ae. albopictus* in areas with a high risk of arbovirus transmission. We
26 also investigated the frequency of knock-down resistance (*kdr*) mutations in *Ae. aegypti*. Immature
27 stages of *Ae. aegypti* and *Ae. albopictus* were collected from two sites in Kinshasa (Lingwala and Cité
28 Verte) between April and July 2017 and reared to the adult stage. Wild-caught adult *Ae. aegypti* were
29 collected in 2016 in another site (Ngaliema). Female *Ae. aegypti* (from Lingwala) and *Ae. albopictus*
30 (from Cité Verte) were used in WHO tube insecticide susceptibility tests. The F1534C, V1016I and
31 V410L *kdr* mutations were genotyped in *Ae. aegypti* from Lingwala and Ngaliema. We observed *Ae.*
32 *aegypti* to be susceptible to bendiocarb, propoxur and malathion, suspected resistant to permethrin,
33 and resistant to deltamethrin and DDT. *Aedes albopictus* was susceptible to bendiocarb, propoxur,
34 malathion and permethrin, suspected resistant to deltamethrin and resistant to DDT. While F1534C
35 and V1016I were not detected, a few *Ae. aegypti* from Lingwala were heterozygous for the mutation
36 V410L. This study reports for the first time the insecticide resistance status of *Aedes spp.* and the
37 detection of the *kdr* mutation V410L in *Ae. aegypti* in DRC. Given the resistance profile, carbamates
38 and potentially malathion are recommended insecticide options against *Ae. aegypti* in Kinshasa. It will
39 be important to develop *Aedes* control strategies based on the resistance patterns of *Aedes* in Kinshasa.

40 **Key words:** *Aedes* mosquitoes, pyrethroids, resistance, *kdr* mutations

41

42 **Introduction**

43 *Aedes aegypti* and *Aedes albopictus* are important arbovirus vectors with an increasing distribution
44 range [1]. Whereas *Ae. aegypti* originated in sub-Saharan Africa, *Ae. albopictus* originated in
45 southeast Asia and was first observed in Africa in 1989 (South-Africa) [2, 3, 4]. Currently, *Ae. aegypti*
46 is distributed primarily in tropical and subtropical regions of the world while *Ae. albopictus* is found
47 on all continents except Antarctica [1, 5]. Both *Ae. aegypti* and *Ae. albopictus* can transmit viruses that
48 are pathogenic to humans including dengue, chikungunya, Zika and yellow fever viruses [5].

49

50 The public health threat posed by *Aedes*-borne viruses is increasing due to the rapid spread of their
51 mosquito vectors in new regions [1, 5, 6]. Dengue fever (DF) was first reported in the Americas in the
52 seventeenth century following the introduction of *Ae. aegypti* [7]. Currently, dengue virus (DENV,
53 *Flaviviridae, Flavivirus*) is the most important arbovirus worldwide: endemic in more than 125
54 countries, dengue affects an estimated 390 million people annually [8]. Chikungunya (CHIKV,
55 *Togaviridae, Alphavirus*) and Zika (ZIKV, *Flaviviridae, Flavivirus*) viruses both emerged in East
56 Africa in the middle of the twentieth century and then spread worldwide [9, 10]. Although both
57 diseases are seldom deadly, they can cause a high disease burden in the affected communities [9 - 11].
58 Yellow fever (YF) originated in Africa and emerged in the Americas in the seventeenth century.
59 Despite the existence of an effective vaccine, YF remains a public health concern in Africa and South
60 America. It was estimated that 97,400 YF cases (28,000-251,700) and 4,800 (1,000-13,800) associated
61 deaths occurred worldwide in 2017 [12]. The 2015-2016 YF outbreak in Angola and the Democratic
62 Republic of the Congo (DRC) highlighted once again how threatening this disease can be [13].

63

64 In the absence of effective vaccines or specific drugs against most *Aedes*-borne viruses, vector control
65 remains indispensable to control and prevent disease outbreaks [14]. Vector control is often based on
66 source reduction (e.g. removing water containers that serve as larval habitats) and the use of
67 insecticides against adult and immature mosquitoes. The insecticides generally used belong to four
68 main families: organochlorines, organophosphates, carbamates and pyrethroids [15]. Pyrethroids are
69 most commonly used against adult *Aedes* because of their broad arthropod toxicity but low toxicity to

70 mammals [15, 16]. Since *Aedes* are increasingly reported as resistant to pyrethroids and other
71 insecticides in many parts of the world, it is essential to monitor the susceptibility of *Aedes* to these
72 insecticides in areas threatened by *Aedes*-borne viruses [14, 17]. Moreover, the determination of the
73 underlying resistance mechanisms is of interest since it can guide the choice of new insecticides by
74 providing a deeper understanding of cross resistance and selection pressures on populations.
75 Insensitivity of insecticide target sites due to mutations and increased insecticide detoxification are the
76 main mechanisms associated with resistance in *Aedes* [17]. Mutations in the voltage gated sodium
77 channel gene causing knock-down resistance (*kdr*) are important target site mutations associated with
78 pyrethroid resistance and are common in *Ae. aegypti* [17]. More than 10 mutations have been recorded
79 globally, of which the F1534C, V1016I, and V410L mutations have been reported so far in *Ae. aegypti*
80 in Africa [17- 21].

81
82 Much of DRC is at high risk of *Aedes*-borne virus transmission [5, 22-25], with YF and chikungunya
83 outbreaks occurring regularly [24 - 28]. Although DF outbreaks have not been reported,
84 seroprevalence studies indicate that DENV strains are circulating in the human population [29 - 31].
85 Both *Ae. aegypti* and *Ae. albopictus* are present in DRC and large areas of the country are suitable for
86 their establishment [5, 32]. In addition, a previous study reported that CHIKV was detected in *Aedes*
87 populations around Kinshasa [33]. Also, *Ae. aegypti* and *Ae. albopictus* were vectors of the 2019
88 chikungunya outbreak in DRC's capital Kinshasa and the major port city of Matadi, respectively [24].
89 Despite the long-standing risk of *Aedes*-borne diseases, insecticide resistance status of *Aedes*
90 populations have not previously been reported from DRC [17]. To address this gap, we investigated
91 the susceptibility of *Ae. aegypti* and *Ae. albopictus* in high risk areas of arbovirus transmission to the
92 following insecticides: dichlorodiphenyltrichloroethane (DDT), deltamethrin, permethrin, bendiocarb,
93 propoxur and malathion. We also investigated the frequency of three *kdr* mutations (F1543C, V1016I,
94 V410L) in *Ae. aegypti*.

95

96 **Materials and methods**

97 **Study sites**

98 Immature stages of *Ae. aegypti* and *Ae. albopictus* were collected from two sites in Kinshasa (DRC)
99 between April and July 2017, Lingwala (S 04° 19'33'', E 015°18'20'') and Cité Verte (S 04°25'52'', E
100 015°15'35'') (Fig. 1). An additional sample of dead adult *Ae. aegypti* collected in February 2016 in
101 Ngaliema (S 04° 21'7''/E 015° 14'33'') during a previous study was also used for *kdr* genotyping only
102 (Fig. 1) [32]. All these sites have experienced arbovirus outbreaks in the past. Autochthonous cases of
103 YF were reported during the 2016 outbreak in Lingwala and in Selembao, in which the Cité Verte site
104 is located. Probable chikungunya cases were observed in Cité Verte and Ngaliema during the poorly
105 documented chikungunya outbreak in 2012 [27, 30, 31, 34]. Furthermore, antibodies against CHIKV
106 and DENV were reported in approximately 30% of febrile patients from Selembao and Ngaliema,
107 tested in 2005-2006 [29].

108

109 **Fig 1. *Aedes* sampling sites in Kinshasa, DRC.**

110

111 **Mosquito collections, rearing and identification**

112 Tires filled with rain water were used as oviposition traps in sites in Lingwala (n=6) and Cité Verte
113 (n=10). The tires were checked every five days throughout the study duration for the presence of
114 mosquito larvae, which were collected using a pipette or ladle and brought to the insectary at the
115 Tropical Medicine Department of Kinshasa University. Larvae were fed with fish food and reared to
116 the adult stage under ambient conditions, with temperature and humidity within the target ranges of
117 28±2 °C and 80±5%, respectively, throughout the study duration. Adult mosquitoes obtained from
118 immature stages were identified to species using morphological keys according to Huang [35] and
119 kept alive. In the earlier sampling in Ngaliema, adult mosquitoes were collected using electric
120 Prokopack aspirators (John W. Hock, Gainesville, USA) from 3:30 to 6:30 pm [32]. The dead
121 mosquitoes were later identified to species as above.

122

123 **Adult bioassays**

124 To assess the insecticide susceptibility of *Ae. aegypti* and *Ae. albopictus*, we used World Health
125 Organisation (WHO) insecticide susceptibility tube tests performed on (non-blood-fed) F₀ adult female
126 *Ae. aegypti* (from Lingwala) and *Ae. albopictus* (from Cité Verte) [36]. For each test, 3-4 replicates of
127 20-25 females fed *ad libitum* on a 10% sugar solution were exposed for one hour to insecticide-
128 impregnated papers treated with deltamethrin 0.05% (pyrethroid), permethrin 0.75% (pyrethroid),
129 bendiocarb 0.1% (carbamate), propoxur 0.1% (carbamate), malathion 5% (organophosphate), or DDT
130 4% (organochlorine). Although these insecticide concentrations were initially recommended for
131 *Anopheles*, they are commonly used to screen *Aedes* for insecticide resistance in Africa, though it
132 should be noted that the doses for permethrin and malathion are three and approximately six-times
133 higher, respectively, than the recommended *Aedes* doses [17]. Insecticide-impregnated papers were
134 obtained from the Vector Control Research Unit at the Universiti Sains Malaysia, Penang, Malaysia.
135 Controls were also run by exposing 2 replicates of 20-25 adult female mosquitoes to untreated papers.
136 After 60 minutes of insecticide exposure, mosquitoes were transferred into holding tubes and supplied
137 with a 10% sugar solution. Percent mortality was recorded 24 hours after exposure, and the 95%
138 binomial confidence intervals were calculated using SPSS 21.0 (IBM Corp. Armonk, NY, USA).
139 Mortalities were corrected when necessary using Abbott's formula (if control mortality was between 5
140 and 20%) [36]. We used the following WHO criteria to score population-level
141 resistance/susceptibility: Mortality rates <90% were considered resistant, > 97% were considered
142 susceptible and mortality rates between 90 and 97% were suspected resistant [36]. *Ae. aegypti*
143 specimens that survived after pyrethroid exposures were killed by freezing, stored at 0°C and later sent
144 (with *Ae. aegypti* from Ngaliema) to the Centers for Disease Control and Prevention (CDC, USA) for
145 *kdr* genotyping.

146

147 **DNA extraction and *kdr* mutation detection**

148 Real-time PCR was used to identify the F1534C, V1016I, and V410L *kdr* mutations. To estimate the
149 allele frequencies, 28 *Ae. aegypti* female survivors to pyrethroid exposures from Lingwala and 47 wild
150 caught female *Ae. aegypti* from Ngaliema were analyzed. DNA was extracted from individual

151 mosquitoes using the Quanta Biosciences Extracta™ Kit. Each mosquito was placed in a sterile 0.2
152 mL tubes with 25 µL extraction buffer, followed by an incubation at 95°C for 30 min in a C1000 Bio-
153 Rad CFX 96 Touch™ Real-Time System thermocycler. At the end of the incubation, 25 µL of
154 stabilization buffer was added. DNA was quantified using a NanoDrop™ 2000/2000c
155 spectrophotometer (ThermoFisher Scientific). PCR reactions were performed in a Bio-Rad C1000
156 CFX96 Real-Time System thermocycler. Genotypes were determined by analyzing the melting curves
157 of the PCR products.

158
159 The F1534C mutation was detected following the methodology described by Yanola et al. [37] using a
160 final reaction volume of 20 µL comprised of 7.15 µL of ddH₂O, 9 µL of iQ™ SYBR1Green
161 Supermix (Bio-Rad), 0.6 µL of each of the F1534-f forward primer, [5'-GCG GGC TCT ACT TTG
162 TGT TCT TCA TCA TAT T-3'] and CP-r common reverse primer, [5'-TCT GCT CGT TGA AGT
163 TGT CGA T-3']; 0.65 µL of the C1534-f forward primer, [5'-GCG GGC AGG GCG GCG GGG GCG
164 GGG CCT CTA CTT TGT GTT CTT CAT CAT GTG-3'] primer, and 2 µL of DNA template. The
165 cycling conditions were as follows: an initial denaturation at 95°C for 3 min followed by 37 cycles of:
166 95°C for 10 s, 57°C for 10 s, and 72°C for 30 s; and a final extension at 95°C for 10 s. The melting
167 curves were determined by a denaturation gradient from 65°C to 95°C with an increase of 0.2°C every
168 10 s.

169
170 The V1016I mutation was amplified following the methodology described by Saavedra-Rodriguez *et*
171 *al.* [38], using a final reaction volume of 20 µL, containing 8.866 µL of ddH₂O, 8 µL of iQ™
172 SYBR1 Green Supermix (Bio-Rad), 0.4 µL of each of the Iso1016f forward primer, [5'-GCG GGC
173 ACA AAT TGT TTC CCA CCC GCA CTG A-3']; and Iso1011r reverse primer ; [5'-GGA TGA
174 ACC SAA ATT GGA CAA AAG C-3']; 0.34 µL of Val1016f forward primer [5'-GCG GGC AGG
175 GCG GCG GGG GCG GGG CCA CAA ATT GTT TCC CAC CCG CAC CGG-3'] and 1 µL of DNA
176 template. The cycling conditions were as follows: an initial denaturation at 95°C for 3 min followed by
177 35 cycles of: 95°C for 10 s, 60°C for 10 s, and 72°C for 30 s; and a final extension at 95°C for 10 s.

178 The melting curves were determined by a denaturation gradient from 65°C to 95°C with an increase of
179 0.2°C every 10 s.

180

181 The V410L mutation was detected following the methodology described by Saavedra *et al.* [39] using
182 a final reaction volume of 20 µL comprised of 8.7 µL of ddH₂O, 9.9 µL of iQTM SYBR1Green
183 Supermix (Bio-Rad), 0.1 µL of each L410fw, [5'-GCG GGC ATC TTC TTG GGT TCG TTC TAC
184 CAT T-3'] and V410fw, [5'-GCG GGC AGG GCG GCG GGG GCG GGG CCA TCT TCT TGG
185 GTT CGT TCT ACC GTG-3'] primers; 0.2 µL of a common reverse primer 410rev [5'-TTC TTC
186 CTC GGC GGC CTC TT-3'] and 2 µL of DNA template. The cycling conditions were as follows: an
187 initial denaturation at 95°C for 3 min followed by 40 cycles of: 95°C for 10 s, 60°C for 10 s, and 72°C
188 for 30 s; and a final extension at 95°C for 10 s. The melting curves were determined by a denaturation
189 gradient from 65°C to 95°C with an increase of 0.2°C every 10 s.

190

191 **Results**

192 **Relative abundance of *Aedes* species by collection site**

193 A total of 4,802 *Aedes* were obtained from immature stages collected throughout the study belonging
194 to two species: *Ae. aegypti* (2,558 specimens) and *Ae. albopictus* (2,244 specimens). A total of 2,544
195 (1,576 females) *Ae. aegypti* and 171 (93 females) *Ae. albopictus* were obtained from Lingwala and 14
196 (4 females) *Ae. aegypti* and 2,073 (1,320 females) *Ae. albopictus* were obtained from Cité Verte. In
197 total 1,851 female *Aedes* were used in bioassays (including controls and 2 tests discarded because of
198 high mortality in the control), out of which 1,097 were female *Ae. aegypti* from Lingwala and 754
199 were female *Ae. albopictus* from Cité Verte.

200

201 **Adult bioassays**

202 *Aedes aegypti* were fully susceptible to bendiocarb and malathion (100% mortality) and also
203 susceptible to propoxur (98% mortality). Suspected resistance was detected to permethrin (97%
204 mortality), and resistance was detected to deltamethrin and to DDT with mortality rates of 73% and

205 25%, respectively (Table 1). *Aedes albopictus* was fully susceptible to permethrin, bendiocarb,
 206 propoxur and malathion (100% mortality). Suspected resistance to deltamethrin (92% mortality) and
 207 resistance to DDT (36% mortality) were detected (Table 1).

208

209 **Table 1: Mortality rates of adult female *Ae. aegypti* (Lingwala) and *Ae. albopictus* (Cit e Verte) 24**
 210 **hours after exposure to insecticides in WHO bioassays.**

Insecticide class	Insecticide type	<i>Aedes</i> species	Number tested (mosquito dead)	% Mortality (95% CI)	Status ^a
Organochlorine	DDT 4%	<i>Ae. aegypti</i>	63 (16)	25 (16 - 37)	R
		<i>Ae. albopictus</i>	84 (30)	36 (26 - 46)	R
Pyrethroid	Deltamethrin 0.05%	<i>Ae. aegypti</i>	94 (69)	73 (64 - 82)	R
		<i>Ae. albopictus</i>	80 (74)	92 (86-98)	SR
	Permethrin 0.75%	<i>Ae. aegypti</i>	97 (94)	97 (93 - 100)	SR
		<i>Ae. albopictus</i>	80 (80)	100 (95-100)	S
Carbamate	Bendiocarb 0.1%	<i>Ae. aegypti</i>	93 (93)	100 (96-100)	S
		<i>Ae. albopictus</i>	80 (80)	100 (95-100)	S
	Propoxur 0.1%	<i>Ae. aegypti</i>	98 (96)	98 (95 - 100)	S
		<i>Ae. albopictus</i>	80 (80)	100 (95-100)	S
Organophosphate	Malathion 5%	<i>Ae. aegypti</i>	94 (94)	100 (96-100)	S
		<i>Ae. albopictus</i>	100 (100)	100 (96-100)	S

^a R: Resistant; S: Susceptible; SR: Suspected Resistant.

211

212 ***kdr* mutations**

213 A total of 75 female *Ae. aegypti* (28 from Lingwala and 47 from Ngaliema) were genotyped for the

214 *kdr* mutations F1534C and V1016I. All mosquitoes tested were wild type at these loci. Concerning

215 mutation V410L, 54 female *Ae. aegypti* (24 from Lingwala and 30 from Ngaliema) were genotyped.
216 Seven mosquitoes were heterozygous for the leucine mutation (all from Lingwala) with a resulting
217 frequency of L410 at 14.5% (CI 95%: 8.7-20.3%) (Table 2).

218

219 **Table 2: Frequency of V410L *kdr* mutation in *Ae. aegypti*.**

Site	n	410			L410 Allele frequency	
		VV	VL	LL	Frequency (%)	95% CI
Lingwala	24	17	7	0	14.5	8.7–20.3
Ngaliema	30	30	0	0	0	–

220

221 Discussion

222 This study determined the susceptibility of *Ae. aegypti* and *Ae. albopictus* from Kinshasa to
223 insecticides. Both *Ae. aegypti* and *Ae. albopictus* showed a high frequency of resistance to DDT and
224 moderate resistance to pyrethroids, although were susceptible to carbamates and organophosphates.
225 These results are consistent with other studies performed in Africa. Results from bioassays in
226 neighbouring Republic of the Congo (RC) and Central African Republic (CAR) showed populations
227 of *Ae. aegypti* and *Ae. albopictus* highly resistant to DDT [40, 41]. Highly-resistant populations to
228 DDT were also recorded elsewhere in Africa including Cameroon and Burkina Faso [42, 43], as well
229 as in Asia and in the Americas [17, 44 - 46]. Some authors have explained the high frequency of DDT
230 resistance in *Aedes* populations as being due to the intense use of this insecticide in the past [40, 41].
231 Indeed, the use of DDT in aerial spraying to control malaria vectors in Kinshasa several decades ago
232 and the intense use in farming could have led to the emergence of resistance in mosquitoes including
233 *Aedes spp.* [47]. Considering the pyrethroids, *Ae. aegypti* was resistant to deltamethrin and suspected
234 resistant to permethrin, while *Ae. albopictus* was suspected resistant to deltamethrin and susceptible to
235 permethrin. Fully susceptible populations of both species to deltamethrin have been recorded in RC,
236 Cameroon, CAR and Nigeria; however, suspected or moderately resistant populations were also
237 recorded by the same studies [40 - 42, 48]. Although, *Ae. aegypti* and *Ae. albopictus* populations have

238 generally shown low levels of resistance to deltamethrin in other parts of Africa, studies in Burkina
239 Faso detected the presence of highly resistant *Ae. aegypti* populations [43, 49, 50]. Also, in Asia and in
240 the Americas, studies have reported the presence of *Aedes* populations highly resistant to deltamethrin
241 [17]. Regarding permethrin, studies in RC and Cameroon detected *Ae. aegypti* and *Ae. albopictus*
242 resistant populations using the insecticide concentration that WHO typically recommends for *Aedes*
243 [41, 42]. However, our study used a permethrin concentration three-fold higher [51]. As such, the
244 permethrin resistance level could have been underestimated. Nevertheless, resistant *Ae. aegypti* and
245 *Ae. albopictus* populations were also recorded in studies in Burkina Faso, Tanzania and Ghana using
246 permethrin concentration similar to our study [52 - 54]. The reduced susceptibility to pyrethroids in
247 both *Ae. aegypti* and *Ae. albopictus* observed in this study is of concern as pyrethroids are broadly
248 recommended in control activities against adult *Aedes* [15, 16]. Moreover, the intense and continuous
249 use of pyrethroids in mosquito nets, home insecticide sprays, and farming in Kinshasa might
250 exacerbate the mosquito resistance [55].

251
252 Both *Ae. aegypti* and *Ae. albopictus* were susceptible to bendiocarb, propoxur and malathion. These
253 results are similar to other studies performed in neighbouring countries including RC, CAR and
254 Tanzania in which some *Aedes* populations were susceptible to carbamates and organophosphates [40,
255 41, 56]. In other parts of Africa (e.g. Burkina Faso), carbamate-resistant *Aedes* populations are more
256 common [49, 50]. On the other hand, bioassays conducted with malathion across Africa have shown
257 that both *Ae. aegypti* and *Ae. albopictus* populations were susceptible to this insecticide [34, 40, 48 -
258 50, 53]. This result is in contrast to some *Aedes* populations observed in the Americas and Asia where
259 high malathion resistance has been reported [17, 45, 57]. However, we highlight that our study (and
260 most other studies performed in Africa) used a malathion concentration that was six times higher than
261 what is typically recommended for *Aedes* by the WHO [51]. Our results suggest that carbamates and
262 potentially malathion could be recommended insecticide options against *Ae. aegypti* in Kinshasa.

263
264 To determine the underlying resistance mechanisms in *Ae. aegypti*, we also tested for *kdr* mutations.
265 While the *kdr* mutations F1534C and V1016I were not detected in our study, V410L was detected at a

266 low frequency in the *Ae. aegypti* population from Lingwala. The F1534C mutation was previously
267 reported in *Ae. aegypti* in West Africa [21, 49, 53, 58] but not in three studies conducted in central
268 Africa [19, 40, 41]. The V1016I mutation in *Ae. aegypti* was also reported in Africa in Ghana, Burkina
269 Faso, Cote d'Ivoire, Angola and Cape Verde [18, 19, 21, 49]. The V410L mutation was first detected
270 in *Ae. aegypti* in Brazil [59]. Results from that study [59] revealed that this mutation alone could be
271 responsible for a decrease in *Ae. aegypti* susceptibility to insecticides. Another study in Mexico
272 reported this mutation in samples of *Ae. aegypti* collected in 2002 [39], well before the samples from
273 the Brazilian study. The Mexican study revealed an increase in the frequency of this mutation reaching
274 very high levels by 2016 [39]. In Africa, the V410L mutation was first reported in *Ae. aegypti* in
275 Angola and Cape Verde [19]. This low mutation frequency was surprising given the usual tight
276 linkage observed between the V1016I and V410L *kdr* loci, although occurrence of the 410L mutation
277 alone has occasionally been observed [19, 39]. While mosquito surviving pyrethroid exposures were
278 used for *kdr* genotyping in *Ae. aegypti* from Lingwala, mosquitoes of unknown resistance phenotype
279 were used from Ngaliema. The V410L mutation may be contributing to *Aedes* insecticide resistance in
280 Kinshasa, but given its low frequency, there are likely other resistance mechanisms that are also
281 important. Future research can hopefully explore what those mechanisms are, as well as the
282 frequencies of *kdr* mutations and other mechanisms to mutations that might cause resistance in *Ae.*
283 *albopictus*.

284

285 This study had several limitations. First, the insecticide susceptibility tests were performed on
286 mosquitoes from limited sampling areas. Moreover, higher doses of permethrin and malathion than
287 recommended for *Aedes* were used in the bioassays, so resistance may be underestimated. Also, a
288 limited number of mosquitoes were used to detect *kdr* mutations, so sampling a greater proportion of
289 mosquitoes would give a more accurate estimate of allele frequencies. Despite these limitations, this
290 study is valuable because for the first time, it reports data on the resistance patterns of *Aedes* in
291 Kinshasa, including the detection of the *kdr* mutation V410L in *Ae. aegypti* in the DRC. This
292 information will be useful to guide future insecticide resistance surveillance programmes and to
293 develop control strategies for areas at high risk of *Aedes*-borne arboviruses in the DRC.

294 **Disclaimer**

295 The views expressed in this manuscript are those of the authors and do not necessarily reflect the
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297

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300

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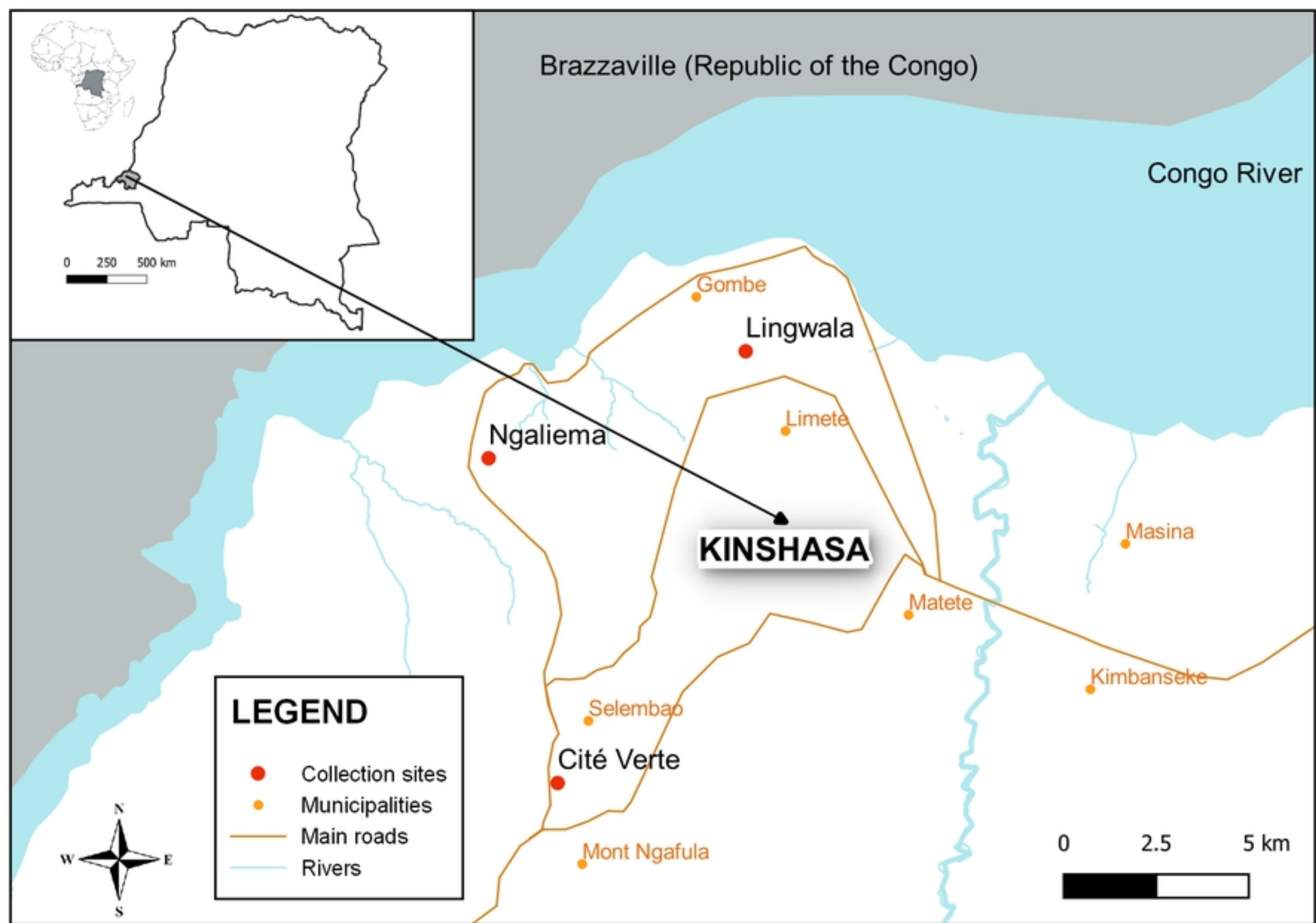
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516 **Supporting file captions**

517 **S1 Appendix. Frequency of *kdr* mutations in *Ae. aegypti*.**

518



Figure