

1 Short title: *Aedes albopictus* distribution in the Democratic Republic of the Congo

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3 **Geographic distribution and future expansion of *Aedes albopictus* in the Democratic  
4 Republic of the Congo**

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17

18 **Abstract**

19 *Aedes albopictus* with an Asian origin has been reported from central African countries. The  
20 establishment of this mosquito species poses a serious threat as the vector of various  
21 infectious diseases. Since information about *Ae. albopictus* in Democratic Republic of the  
22 Congo (DRC) is scarce, we investigated the current distribution of this mosquito species.  
23 Based on the factors affecting the distribution, we predicted future distribution. We conducted  
24 entomological surveys in Kinshasa and three neighboring cities from May 2017 to September  
25 2019. The survey was extended to seven inland cities. A total of 19 environmental variables  
26 were examined using the maximum entropy method to identify areas suitable for *Ae.*  
27 *albopictus* to establish a population. We found *Ae. albopictus* at 21 of 23 sites in Kinshasa  
28 and three neighboring cities. For the first time *Ae. albopictus* was also found from three of  
29 seven inland cities, while it was not found in four cities located in the eastern and  
30 southeastern parts of DRC. A maximum entropy model revealed that the occurrence of *Ae.*  
31 *albopictus* was positively associated with maximum temperature of the warmest month, and  
32 negatively associated with wider mean diurnal temperature range and enhanced vegetation  
33 index. The model predicted that most parts of DRC are suitable for the establishment of the  
34 mosquito. The unsuitable areas were the eastern and southeastern highlands, which have low  
35 temperatures and long dry seasons. We confirmed that *Ae. albopictus* is well established in  
36 Kinshasa and its neighboring cities. The expansion of *Ae. albopictus* to the inland is ongoing,  
37 and in the future the mosquito may establish in most parts of DRC.

38

39 **Key-words:** *Aedes* mosquito, maximum entropy model, MaxEnt, environmental variables.

40

41

42 **Introduction**

43 *Aedes albopictus* is an invasive mosquito and vector of human disease such arboviruses such  
44 as dengue and chikungunya arboviruses [1-5]. Originating from Asia [6, 7], *Ae. albopictus*  
45 has expanded its distribution globally [3]. In central Africa, this mosquito was first reported  
46 from Cameroon in 2000 [8], and subsequently was found in several other countries [9-13].  
47 Following the mosquito invasion into central Africa, numerous dengue and chikungunya  
48 outbreaks have occurred [12, 14-21].

49

50 *Aedes aegypti* is considered to be the main vector of dengue and chikungunya (CHIKV)  
51 viruses; however, *Ae. albopictus* was largely responsible for the dengue and chikungunya  
52 outbreaks in Gabon in 2007 and 2010 [14, 17, 21]. Furthermore, *Ae. albopictus* is able to  
53 transmit the chikungunya virus variant possessing the E1-226V mutation more efficiently  
54 than *Ae. aegypti* [22, 23]. This mutation was first identified during the chikungunya outbreak  
55 in the African Indian Ocean islands in 2005 [24], and was later isolated in central Africa [18,  
56 19, 25].

57

58 In DRC, 50,000 suspected cases were reported during the first chikungunya outbreaks in  
59 Kinshasa from 1999 to 2000 [16]. Chikungunya outbreaks also occurred in Kinshasa in 2012  
60 and 2019 and in the adjacent Kongo Central Province in 2019 [25, 26]. In addition, the  
61 number of dengue virus infections has also increased in recent years [ 26-29]. Although an  
62 apparent outbreak did not occur, an entomological study caught several *Aedes* mosquitoes  
63 infected with CHIKV in Kinshasa in 2014 [30]. Moreover, a study confirmed involvement of  
64 *Ae. albopictus* for transmitting CHIKV with the E1-A226V mutation in two cities, Matadi and  
65 Kasangulu, of Kongo Central Province during the 2019 chikungunya outbreak [25].

66 Curative treatments and vaccines are not available for dengue and chikungunya [31, 32], and  
67 thus vector control is a valuable available tool for reducing infections [ 33]. As such,  
68 understanding the current distribution of *Ae. albopictus* in DRC is an essential step for the  
69 control. Global level distribution models based on environmental variables indicate that  
70 almost the entire area of DRC is suitable for *A. albopictus* establishment [3, 34, 35]. These  
71 models were constructed without entomological data from DRC, and thus the provided  
72 information was too coarse to apply to local vector control. In the present study, we described  
73 the current distribution of *Ae. albopictus* in DRC based on locally available data. In particular,  
74 we provided detailed information for Kinshasa and the neighboring areas where chikungunya  
75 outbreaks recently occurred. We also revealed important environmental variables related to  
76 the distribution, and attempted to determine if the present distribution is static.

77

## 78 **Materials and methods**

### 79 **Study areas**

80 DRC is the largest country in Sub-Saharan Africa with an area of roughly 2,4 million km<sup>2</sup>,  
81 and possesses a diversity of landscapes and climates. The country is divided into six  
82 geographic regions (western, northern, far-northern, central, eastern, and southeastern) based  
83 on landscape and climate (Fig 1). The landscape of the western region is composed of the  
84 coastal plain, with hills and plateaus in the south. The vegetation type is mainly savannah,  
85 with a tropical humid climate and a three 3-month dry season. This region includes Kinshasa  
86 and Kongo Central province, where chikungunya and dengue outbreaks have occurred. The  
87 Congo Basin and equatorial forests largely occupy the northern region. This region has an  
88 equatorial climate without a dry season. The far-northern region is characterized with  
89 savannahs, and has a tropical humid climate with a three month dry season. Equatorial forests

90 occupy the northern part of the central region, whereas the southern part is mainly plateau  
91 with savannahs and steppes. The central region has a dry tropical climate with a three month  
92 dry season. High hills and mountains dominate the eastern region, and lush vegetation forms  
93 the mountain forests. The region has a temperate mountain climate without a distinct dry  
94 season. The southeastern region is dominated by high plateaus with savannahs. The region  
95 has a dry tropical climate with a six-month dry season.

96

97 **Fig 1. Distribution of *Ae. albopictus* in DRC.** Red dots depict the presence of *Ae.*  
98 *albopictus*, and green dots depict absence at the city level. Mosquitoes were sampled at  
99 several sites within Matadi, Kisantu, Kasangulu, Kinshasa, and Mbandaka, and *Ae. albopictus*  
100 was found at one site at least. Each geographic region is made up of multiple provinces,  
101 represented by boundaries.

102

103 We conducted entomological surveys at 32 sites within 11 cities across four different  
104 geographic regions except the eastern and far-northern regions, from May 2017 to September  
105 2019 (Table 1). First, we focused on the western region in which *Ae. albopictus* has been  
106 recorded [13, 25]. The survey in the western region included 14 sites within Kinshasa and  
107 nine sites in the three cities, Kasangulu, Kisantu, and Matadi, in Kongo Central Province.  
108 Since human-mediated dispersal of *Ae. albopictus* was an immediate concern, the survey also  
109 included nine sites along the major transportation routes (Congo River and national roads) in  
110 the other three regions (Fig 1). These sites were three sites within Mbandaka in the western  
111 part of the northern region; Tshikapa, Mbuji-Mayi, and Kalima in the central region and  
112 Lubumbashi, Kilwa, and Kashobwe in the southeastern region.

113

114 **Table 1. Sampling sites, methods and occurrence of *Ae. albopictus*.**

Region, province / city	Site	Date	Latitude	Longitude	Method <sup>a</sup>	Occurrence
<b>Central region</b>						
Kasai/Tshikapa	Tshikapa	2019/8	S 06.417°	E 20.802°	Asp <sup>a</sup>	Present
Kasai Or / Mbuji-Mayi	Bupole	2019/6	S 06.134°	E 23.633°	BGS	Present
Maniema / Kalima	Kalima	2019/7	S 03.073°	E 26.041°	Asp	Absent
<b>Northwestern region</b>						
Equateur / Mbandaka	Mbandaka	2019/7	N 00.048°	E 18.260°	Asp	Absent
Equateur / Mbandaka	Mambenga	2017/5	N 00.061°	E 18.266°	BGS	Present
Equateur / Mbandaka	Bombwanza	2018/5	N 00.048°	E 18.284°	BGS	Present
<b>Southeastern region</b>						
Haut-Katanga / Kilwa	Kilwa	2017/8, 2018/10	S 09.277°	E 28.336°	BGS / Asp	Absent
Haut-Katanga / Kashobwe	Kashobwe	2017/8, 2018/10	S 09.676°	E 28.614°	BGS / Asp	Absent
Haut-Katanga / Lubumbashi	Bel air	2017/8, 2018/10	S 11.662°	E 27.502°	BGS / Asp	Absent
<b>Western region</b>						
Kinshasa / Kinshasa	Lingwala	2019/9	S 04.328°	E 15.302°	Asp	Present
Kinshasa / Kinshasa	Barumbu	2019/8	S 04.311°	E 15.326°	Asp	Absent
Kinshasa / Kinshasa	Tshangu	2019/7	S 04.419°	E 15.427°	Asp	Present
Kinshasa / Kinshasa	UPC	2019/4	S 04.332°	E 15.297°	Asp	Present
Kinshasa / Kinshasa	Echangeur	2019/4	S 04.375°	E 15.343°	Asp	Present
Kinshasa / Kinshasa	Uckin	2019/4	S 04.352°	E 15.241°	Asp	Present
Kinshasa / Kinshasa	Don bosco	2019/4	S 04.366°	E 15.207°	Asp	Present
Kinshasa / Kinshasa	Bu	2019/4	S 04.299°	E 15.924°	BGS	Present
Kinshasa / Kinshasa	Malweka	2019/2	S 04.376°	E 15.220°	Asp	Present
Kinshasa / Kinshasa	Mitendi	2019/2	S 04.468°	E 15.235°	BGS	Present
Kinshasa / Kinshasa	Mbenseke	2019/2	S 04.502°	E 15.226°	BGS	Present
Kinshasa / Kinshasa	Masanga Mbila	2018/12	S 04.443°	E 15.279°	BGS	Present
Kinshasa / Kinshasa	Lingwala II	2018/12	S 04.326°	E 15.305°	BGS	Absent
Kinshasa / Kinshasa	Ngamanzo	2018/9	S 04.173°	E 15.539°	BGS / Asp	Present
Kongo-Central / Kasangulu	Kasangulu	2019/9	S 04.587°	E 15.169°	Asp	Present
Kongo-Central / Kasangulu	Manoka	2019/4	S 04.588°	E 15.173°	Asp	Present
Kongo-Central / Kinsatu	Jardin botanique	2019/9	S 05.132°	E 15.077°	Asp	Present
Kongo-Central / Kinsatu	Kisantu	2019/8	S 05.126°	E 15.070°	Asp	Present
Kongo-Central / Matadi	Kalankala	2019/9	S 05.825°	E 13.460°	Asp	Present
Kongo-Central / Matadi	Soyo	2019/9	S 05.841°	E 13.456°	Asp	Present
Kongo-Central / Matadi	Toulouse	2019/9	S 05.842°	E 13.448°	Asp	Present
Kongo-Central / Matadi	Soyo II	2019/3	S 05.842°	E 13.457°	Asp	Present
Kongo-Central / Matadi	Mvuzi	2019/3	S 05.825°	E 13.460°	Asp	Present

<sup>a</sup> Asp: aspirator, BGS: BG sentinel trap.

116 **Mosquito sampling**

117 Within each site, sampling was focused on places around dwellings which are ecologically  
118 suitable for adults of *Ae. albopictus*, and places where residents reportedly experience  
119 frequent day-time mosquito bites. *Aedes* mosquitoes were collected with electric aspirators  
120 (Prokopack Aspirator, John W. Hock, Gainesville, USA) and/or BG sentinel traps (Biogents  
121 Inc, Regensburg, Germany) from 3:00 pm to 6:00 pm for three to seven consecutive days at  
122 each site. Sampled mosquitoes were identified morphologically to species according to  
123 Huang's identification keys [36]. When at least one *Ae. albopictus* was collected, the site was  
124 considered as a positive site. A distribution map was constructed using the Quantum  
125 Geographic Information System software version 3.4.13 (QGIS Development Team, 2020)  
126 (Fig 1).

127

128 **Environmental variables**

129 We reviewed literature related to modelling *Ae. albopictus* distribution using the maximum  
130 entropy software, MaxEnt [37]. This software is often used for modeling species distribution,  
131 and effectively handles a small number of collection sites [38-42]. Based on the review, we  
132 selected 18 environmental variables which had a permutation importance (PI) of at least 5%  
133 (Table 2) [34, 43-55]. PI indicates the importance of each variable in a MaxEnt model [56].  
134 Among the 18 variables, 15 climatic variables were obtained from the WorldClim database  
135 (<http://www.worldclim.com/version2>) [57]. This climate database provides average\_historical  
136 climate data from 1970 to 2000 with a spatial resolution of 1 km x 1 km. Digital elevation  
137 model (DEM) data was obtained from SRTM imagery/USGS with a resolution of 30.9 m (or  
138 1-arc second) (<https://www2.jpl.nasa.gov/srtm/>). The datasets of two vegetation variables,  
139 Enhanced Vegetation Index (EVI) and Normalized Differentiation Vegetation Index (NDVI),

140 were downloaded from Modis Vegetation Index/USGS with a resolution of 1km x 1 km  
141 (<https://modis.gsfc.nasa.gov/data/dataproducts/mod13.php>). Dry season length was included in  
142 addition to the variables obtained by the literature review [58].

143

144 **Table 2. Important environmental variables for *Aedes albopictus* distribution.**

Code	Variable	PI (%)	References
Bio1	Annual mean temperature		[34, 47, 54]
Bio2	Mean diurnal temperature range	55.6	[45, 49]
Bio4	Temperature seasonality		[43, 49]
Bio5	Maximum temperature of warmest month	30.8	[43, 45, 47]
Bio6	Minimum temperature of coldest month		[43, 47]
Bio7	Temperature annual range		[45]
Bio10	Mean temperature of warmest quarter		[48, 50, 52, 53]
Bio11	Mean temperature of coldest quarter		[46, 48, 50, 52, 53, 55]
Bio12	Annual precipitation		[47, 55]
Bio13	Precipitation of wettest month		[43, 45, 47, 51]
Bio14	Precipitation of driest month		[44, 47, 49]
Bio15	Precipitation seasonality		[43]
Bio16	Precipitation of wettest quarter		[46]
Bio17	Precipitation of driest quarter		[46, 50, 55]
Bio18	Precipitation of warmest quarter		[49, 54]
DEM	Digital elevation model		[54]
NVDI	Normalized Difference Vegetation Index		[34]
EVI	Enhanced vegetation index	13.6	[55]
	Dry season length		[58]

Permutation importance values are given for variables selected in the final model.

145

146 **Modeling**

147 We selected environmental variables that were significantly different between positive and  
148 negative sites. A relationship of mosquito occurrence with each variable was examined using  
149 the Wilcoxon-Mann-Whitney test (GraphPad Prism version 8.4.2, GraphPad Software, San  
150 Diego, California USA). When numbers of sample size were insufficient ( $n < 4$ ) for the  
151 statistical test, we identified variables which had an extreme median value at negative sites  
152 versus positive sites. We first examined if a negative site median value was within the range  
153 of positive site values in the corresponding geographic region. When the median value was  
154 outside the range, we also compared it to the range of values from all positive sites including  
155 ones from the other regions. When the value was still outside the range, the variable was  
156 considered for modeling.

157 Between the selected variables, we examined the Pearson correlation coefficients [44]. When  
158 the coefficients were above 80%, we retained them based on their apparent importance in past  
159 studies (Table 2) [34, 43-55]. Dry season length was excluded from the analyses because of  
160 the absence of a raster file. Then, we ran a full model including all selected variables with the  
161 default settings of MaxEnt. Based on the results from the full model, we constructed a  
162 reduced model including variables that had a PI above 5%. Since our sample size was small,  
163 we modified the settings in MaxEnt using ten replications, linear feature, and cumulative  
164 output format. The PI from the latter model was used to identify the most important variables.  
165 Response curves were also used to determine how the model changes with a permutation of  
166 each variable. The area under the curve (AUC) was used to assess model accuracy. When an  
167 AUC value was above 0.75, the model was acceptable. With the outputs from the optimal  
168 model, we constructed a predicted geographical distribution map of *Ae. albopictus* in DRC  
169 using the QGIS software.

170 **Results**

171 We collected a total of 2,841 *Aedes* mosquitoes. Of which, 2,331 (82%) were *Ae. albopictus*,  
172 and 510 (18%) were *Ae. aegypti*. The former species was found at 25 of 32 sites within 7 of  
173 11 cities (Table 1, Fig 1). Within Kinshasa, *Ae. albopictus* was collected at 12 of 14 sites  
174 (Table 1). In Kongo Central Province, *Ae. albopictus* was collected at all nine sites. This  
175 species was collected at two of the three sites within one city in the western part of the  
176 northern region. In the central region, we found *Ae. albopictus* in the two cities in the  
177 southern part, Tshikapa and Mbuji-Mayi, but we did not find it in the city in the northeastern  
178 part, Kalima. We did not find *Ae. albopictus* in the three cities, Kilwa, Kashobwe and  
179 Lubumbashi, in the southeastern region (Table 1).

180

181 A total of 19 environmental variables were selected based on a literature review (Table 2).  
182 Wilcoxon-Mann-Whitney tests revealed that the precipitation of the warmest quarter was  
183 significantly greater at the positive sites compared with the negative sites; however, the  
184 differences were not statistically significant for the other variables (Fig 2). The medians of all  
185 environmental variables at the two negative sites in the western region were within the ranges  
186 of values at the positive sites of the same region (Fig 3). In the northern region, the medians  
187 from the negative sites were within the range of values from the positive sites except for the  
188 NVDI (Fig 3R). However, the median of NDVI was within the range of the values from the  
189 positive sites when all regions were considered. The medians of nine variables at the negative  
190 site in the central region were out of the ranges of the two positive sites. When all regions  
191 were considered, the medians were within the range of the positive sites. However, the  
192 maximum temperature of the warmest month at the negative site in the central region was  
193 lower than the range of all positive site values including ones from the other regions. The

194 same negative site of the central region had higher EVI and NDIV than the ranges of all  
195 positive sites. The medians of ten variables at the three negative sites in the southeastern  
196 region were outside the ranges of values at the positive sites. The negative sites had lower  
197 annual mean temperatures, a wider mean diurnal temperature range, lower minimum  
198 temperatures of the coldest month, lower mean temperatures of the coldest quarter, a wider  
199 temperature annual range, greater precipitation seasonality, lower precipitation of the driest  
200 quarter, lower precipitation of the warmest quarter, higher elevation, and longer dry season  
201 length than any of the positive sites. Lubumbashi is located in the southernmost and at the  
202 highest elevation among the sites in the southeastern region, and these environmental  
203 variables of the city were more extreme than the other sites.

204

205 **Fig 2. Comparisons of each environmental variable between the positive and negative**  
206 ***Ae. albopictus* collection sites.** Each panel shows the first quartile, the median, the third  
207 quartile, the minimum and the maximum values in positive (*Ae. albopictus* was found) and  
208 negative (the species was not found) sites by box plots. A: Annual mean temperature (°C); B:  
209 mean diurnal temperature range (°C); C: temperature seasonality (%); D: maximum  
210 temperature of warmest month (°C); E: minimum temperature of the coldest month (°C); F:  
211 mean temperature of the coldest quarter (°C); G: temperature annual range (°C); H: mean  
212 temperature of the warmest quarter (°C); I: annual precipitation (mm); J: precipitation of the  
213 wettest month (mm); K: precipitation of the driest month (mm); L: precipitation seasonality  
214 (%); M: precipitation of the wettest quarter (mm); N: precipitation of the driest quarter (mm);  
215 O: precipitation of the warmest quarter (mm); P: digital elevation model (m); Q: enhanced  
216 vegetation index; R: normalized difference vegetation index; S: dry season length (month).

217 An asterisk indicates that the difference was statistically significant ( $p < 0.05$ ) with  
218 Wilcoxon-Mann-Whitney tests.

219

220 **Fig 3. Medians of each environmental variable at positive sites and negative sites of *Ae. albopictus* in the four regions.** A value for each site is depicted as a dot. The black horizontal  
221 bars indicate the median and vertical bars indicate the range. A: annual mean temperature  
222 ( $^{\circ}\text{C}$ ); B: mean diurnal temperature range ( $^{\circ}\text{C}$ ); C: temperature seasonality (%); D: maximum  
223 temperature of warmest month ( $^{\circ}\text{C}$ ); E: minimum temperature of the coldest month ( $^{\circ}\text{C}$ ); F:  
224 mean temperature of the coldest quarter ( $^{\circ}\text{C}$ ); G: temperature annual range ( $^{\circ}\text{C}$ ); H: mean  
225 temperature of the warmest quarter ( $^{\circ}\text{C}$ ); I: annual precipitation (mm); J: precipitation of  
226 wettest month (mm); K: precipitation of the driest month (mm); L: precipitation seasonality  
227 (%); M: precipitation of the wettest quarter (mm); N: precipitation of the driest quarter (mm);  
228 O: precipitation of the warmest quarter (mm); P: digital elevation model (m); Q: enhanced  
229 vegetation index; R: normalized difference vegetation index; S: dry season length (month).

231

232 Of 12 selected variables, excluding dry season length, five pairs were highly correlated  
233 among eight variables (S1 File). We chose annual mean temperature, mean diurnal  
234 temperature range and the EVI over the others because the past studies showed that they were  
235 more important. As a result, seven variables were included in the full MaxEnt analysis (Table  
236 2). After the model selection, the optimal model contained three variables, maximum  
237 temperature of the warmest month, mean diurnal temperature range, and EVI. Mean diurnal  
238 temperature range was the most important variable, followed by maximum temperature of  
239 warmest month, and EVI (Table 2). The AUC of the optimal model was 0.975. The response  
240 curves revealed that the highest suitable area was predicted with EVI below – 0.017,

241 maximum temperature of the warmest month above 34.3 °C, and mean diurnal temperature  
242 below 6.5°C (Fig 4).

243

244 **Fig 4. Response curves for *Ae. albopictus* suitability in relation to mean diurnal  
245 temperature range (A), maximum temperature of warmest month (B), and enhanced  
246 vegetation index (C).** The curves show how each environmental variable affects the MaxEnt  
247 prediction. The red line is the mean response of the ten MaxEnt replications.

248

249 The model predicted that most of DRC is suitable for *Ae. albopictus* establishment (Fig 5).  
250 The suitability was high in the most parts of the western region; however, it varied between 0  
251 to 75% in the southern area of the region. The suitability was also high in the central region  
252 and the northern region although a noticeable area in the northeastern region had low  
253 suitability. The eastern part of the eastern region and the southern part of the southeastern  
254 region had low suitability. The model successfully predicted all positive sites within the  
255 highly suitable areas and all negative sites within the highly suitable areas in the western, the  
256 northwestern, and the central regions. However, the model predicted two negative sites, Kilwa  
257 and Kashobwe, in the southeastern region to be suitable whereas Lubumbashi was predicted  
258 as being unsuitable area.

259

260 **Fig 5. Suitability map of *Ae. albopictus* in DRC generated by the optimal MaxEnt model.**

261 Dots depict the presence (black) or absence (green) of *Ae. albopictus*. Only 24 out of the 32  
262 dots can be visualized because some sites are overlapped.

263

264 **Discussion**

265 The present study found *Aedes albopictus* in 25 sites in seven cities in DRC. This mosquito  
266 species was newly found in four cities in the western and central regions, but it was absent in  
267 the cities in the southeastern region where many environmental variables showed extreme  
268 values. The MaxEnt model revealed that the occurrence of *Ae. albopictus* was positively  
269 associated with maximum temperature of the warmest month, and negatively with wider  
270 mean diurnal temperature range and enhanced vegetation index. The model predicted that  
271 almost the entire area of DRC is suitable for the establishment of *Ae. albopictus*.

272

273 Within Kinshasa, *Ae. albopictus* was found at 12 of 14 collection sites. This mosquito species  
274 was recorded in Kinshasa for the first time in DRC in 2016 [13]. A recent study reported  
275 within this city a high level of larval infestation of *Ae. albopictus* in artificial containers  
276 together with *Ae. aegypti* [59]. In the adjacent province, *Ae. albopictus* was found at all 9  
277 collection sites within three cities, Kasangulu, Kisantu, and Matadi. The present study  
278 recorded this mosquito species in Kisantu for the first time, while it was recorded in Matadi  
279 and Kasangulu during the 2019 chikungunya outbreak. During the outbreak, *Ae. albopictus*  
280 was more abundant than *Ae. aegypti* in these two cities [25]. The findings from the present  
281 study were sufficient to conclude that *Ae. albopictus* is well established in the western part of  
282 the western region.

283

284 We also confirmed that *Ae. albopictus* has extended its distribution to the inland cities. This  
285 mosquito species was recorded in Mbandaka in the northern region for the first time. We  
286 collected *Ae. albopictus* in the city in 2017 and in the two consecutive years, indicating that  
287 this mosquito quickly spread to the area after its recording in Kinshasa in 2016. This species

288 was likely introduced to Mbandaka from the western region by traffic along the Congo River,  
289 which is the main transportation route to the northern region. In the Philippines a molecular  
290 study showed evidence of *Ae. aegypti* migrations with ships among the islands [60].

291

292 In contrast, we did not find *Ae. albopictus* in Kalima in the upriver region of the Congo River  
293 in the eastern part of the central region. The result is likely due to the distance and the poor  
294 access from the other areas where this species has become established. However, air flight  
295 activity is intense between the area and Kinshasa, and *Ae. albopictus* might be introduced by  
296 air in the future [61]. Either way, the result from one collection site is not enough to confirm  
297 the absence of this mosquito species in the region. On the other hand, *Ae. albopictus* was  
298 found at two cities in the southern part of the central region. The results are likely due to a  
299 larger amount of traffic and a shorter distance between Kinshasa and this area compared with  
300 Kalima. The access is also better through the major roads, and there are frequent flights  
301 between Kinshasa and the area.

302

303 We did not find *Ae. albopictus* at all three cities in the southeastern part of the southeastern  
304 region. The results may be partially due to the distances from the areas where this mosquito  
305 has been established. However, because Lubumbashi is the second largest city in DRC, the  
306 amount of road traffic from the central and western regions is not negligible, and the flight  
307 activities are intense between Kinshasa and Lubumbashi. The intense traffic may introduce  
308 this mosquito species to the area in the near future [62].

309

310 Climate may limit the distribution of *Ae. albopictus* in the southeastern region. The medians  
311 of ten environmental variables at the negative sites in the southeastern region were outside the

312 ranges of the values from the positive sites of the other regions. The results indicate that the  
313 sites in the southeastern region are cooler, and the temperature fluctuates more because of the  
314 inland with high altitudes. Indeed, the MaxEnt model indicated that the climate variables  
315 (maximum temperature of the warmest month and mean diurnal temperature range) are  
316 important for establishment of this mosquito species. On the other hand, the model suggests  
317 that two negative sites, Kilwa and Kashobwe, in the southeastern region are suitable for *Ae.*  
318 *albopictus* establishment. The elevations of these sites are less than 1,000 m, the maximum  
319 temperature of warmest months is 31 to 32 °C and the mean annual temperatures are 23 to  
320 24 °C. Since *Ae. albopictus* could establish in temperate areas with an annual mean  
321 temperature of 11°C and/or 1,350 accumulated degree-days above 11°C per year [63-65], the  
322 temperatures of the two cities are warm enough. These model results suggest that the  
323 distances and traffic from the western region are likely the limiting factors, but this mosquito  
324 species may establish in these two sites in the future.

325

326 The model suggests that Lubumbashi is not suitable for *Ae. albopictus* survival. This city is  
327 situated at an elevation of about 1,200 m, and the mean annual temperature is 21°C. While the  
328 maximum temperature of the warmest month is 31°C, the minimum temperature of the  
329 coldest month, July, drops to 9 °C. The coldest month occurs in the middle of the six-month  
330 dry season when the monthly rainfall often becomes less than 1 mm. While the lengths of the  
331 dry season are similar among the three cities in the region, the lower temperature and wider  
332 diurnal temperature range may make the climate condition of Lubumbashi less favorable for  
333 *Ae. albopictus*. Even though eggs of this mosquito are tolerant to desiccation [66], egg  
334 survivorship would become less with decreases of temperature and humidity during the dry  
335 season [60, 67]. Furthermore, a greater fluctuation of temperature may make the conditions

336 less favorable for survival [67, 68]. The conditions may become even tougher for *Ae.*  
337 *albopictus* strains originating from tropic regions, which are less tolerant to cooler climate  
338 compared with strains from temperate regions [69, 70].

339

340 A study in Madagascar reported that the distribution of *Ae. albopictus* is largely limited to the  
341 eastern part of the island, with high humidity, a temperature of the coldest months above  
342 12 °C, and dry season shorter than six months in length [58]. The study, however, found *Ae.*  
343 *albopictus* breeding in used tires and captured adults in residential areas in the southwestern  
344 region with an annual precipitation less than 600 mm and an eight-month dry season. The  
345 findings in Madagascar suggest that this mosquito species is able to establish in an area where  
346 suitable man-made habitats are available as long as the temperature is warm enough.

347 Although *Ae. albopictus* distribution in Asia, from which it originated, occurs more in rural  
348 areas with greater vegetation, it also utilizes artificial habitats such as discarded containers in  
349 urban areas [1, 13]. Probably the entry point of a new region is likely an urban area with a  
350 larger amount of traffic. This partially explains the negative association of this species with  
351 the enhanced vegetation index indicated by the MaxEnt model.

352

353 Although our field survey did not cover the far-northern region and the eastern region, the  
354 model suggests that most of the far-northern region and the western part of the eastern region  
355 are also suitable for establishment of this mosquito species. *Ae. albopictus* might have already  
356 reached these regions, or it may reach there in the near future. In contrast, the model suggests  
357 that the eastern part of the eastern region is not suitable for this mosquito species. The area is

358 2,000 m above sea level, and includes mountains above 4,000 m. The harsh climate likely  
359 does not allow *Ae. albopictus* to establish in the area [63-65].

360

361 **Limitation**

362 The number of collection sites was small relative to the size of the country. Including the far-  
363 northern region and the eastern region, a larger number of collection sites could provide a  
364 better picture of the relationships of *Ae. albopictus* with the environmental variables. The  
365 Wilcoxon-Mann-Whitney tests revealed that precipitation of the warmest quarter was greater  
366 at the positive sites than the negative sites. Although this is the only variable statistically  
367 different between them, other variables might become significant with a larger number of  
368 collection sites.

369

370 We collected mosquitoes mainly within urban areas. Mosquitoes are more frequently  
371 introduced to urban areas with human activities, and thus sampling approach was practical to  
372 identify sites in which *Ae. albopictus* was established when considering the large size of the  
373 country. For instance, with fewer negative sites, the Max Ent model might be affected by the  
374 highest EVI value at the single negative site in the central region. As a result, EVI became  
375 one of the three important environmental variables, and it was negatively associated with the  
376 presence of *Ae. albopictus*. This result contradicts the past studies in the other areas [71]. A  
377 more precise picture would be produced with a finer spatial scale which can recognize small  
378 patches of vegetation within an urban area, though it is still challenging with free satellite  
379 data.

380

381 The environmental variables used in the present study were selected based on studies  
382 conducted mostly in temperate areas, because few studies were conducted in Africa.  
383 Appropriate variables for the African situation might be different.

384

### 385 **Conclusion**

386 *Aedes albopictus* has established populations in the major cities of the western region of  
387 DRC. This mosquito species is expanding its geographical distribution toward the inland. The  
388 migration is likely facilitated by the major transportation routes including the Congo River.  
389 The MaxEnt model based on environmental variables suggests that most of the country is  
390 suitable for the establishment of *Ae. albopictus*, except the areas in the eastern and the  
391 southeastern parts of the country. The results from our study suggest that low temperatures  
392 and a long dry season limit the distribution of *Ae. albopictus*. This is the first report to provide  
393 the current and future *Ae. albopictus* distributions in DRC using locally collected mosquito  
394 data.

395

### 396 **Implication**

397 Autochthonous cases of chikungunya and dengue have been reported from the western region  
398 and the southern part of the central region where we found *Ae. albopictus* [72]. Although *Ae.*  
399 *albopictus* was found in the southwestern part of the northern region, autochthonous cases of  
400 the viral diseases have not been reported. The diseases have not been reported from the  
401 northern part of the central region and the southeastern region where we did not find this  
402 mosquito species. Moreover, the diseases have not been reported from the far-northern area  
403 and the eastern region. Our model implies that, following the expansion of mosquito  
404 distribution, chikungunya and dengue may also spread to most parts of the country in the near

405 future. Country-wide entomological surveillance is needed to detect the signs of impending  
406 epidemics.

407

408 **Acknowledgments**

409 We would like to thank Pitshou Mampuya, Herve Michel Vulu and Kelvin Landu for their  
410 helpful contribution in the field activities. Fabien Vulu is grateful to the Program for  
411 Nurturing Global Leaders in Tropical and Emerging Communicable Diseases, Graduate  
412 school of Biomedical Sciences, Nagasaki University, and Japan International Cooperation  
413 Agency (JICA) for their support.

414

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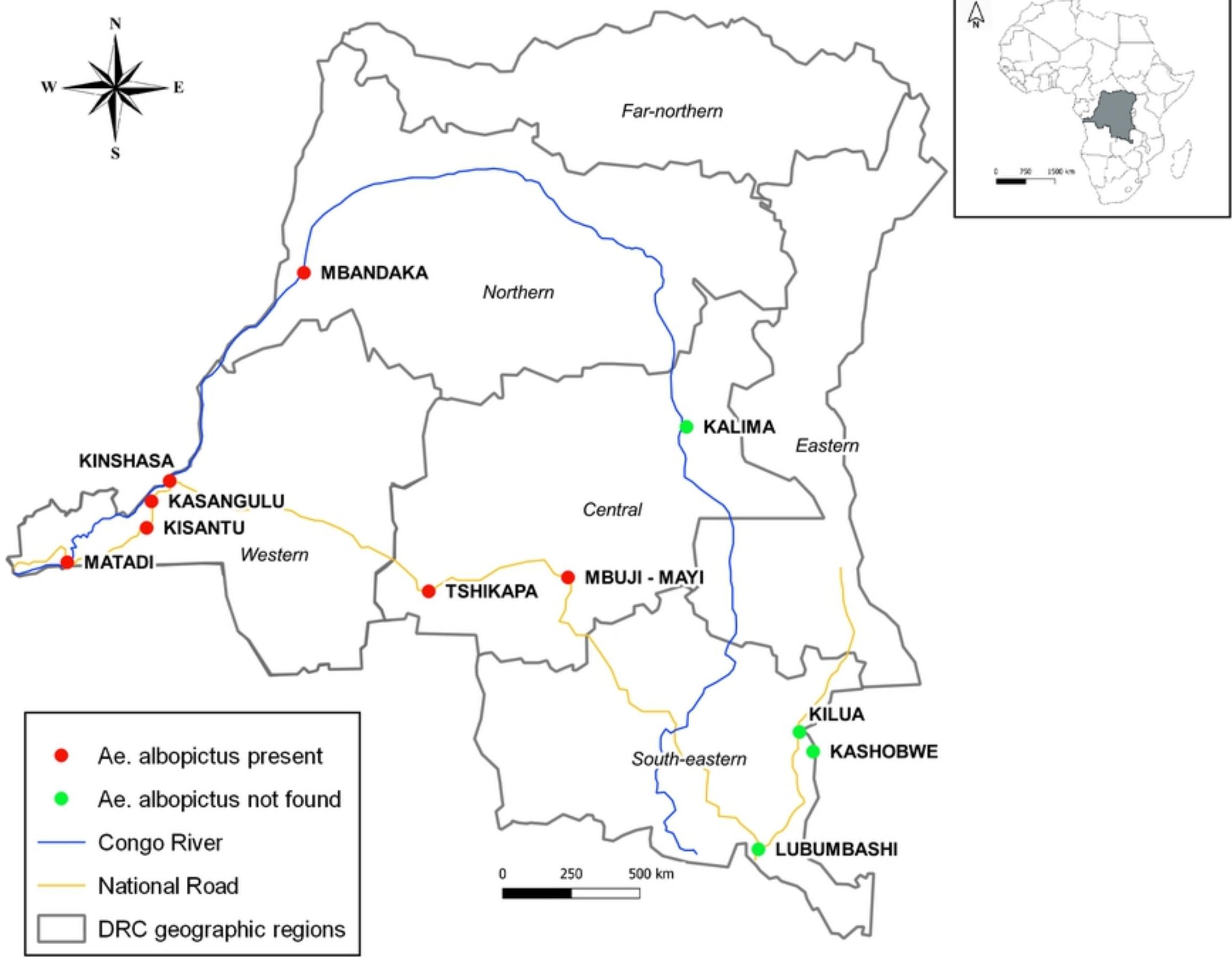
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686 **Supporting file captions**

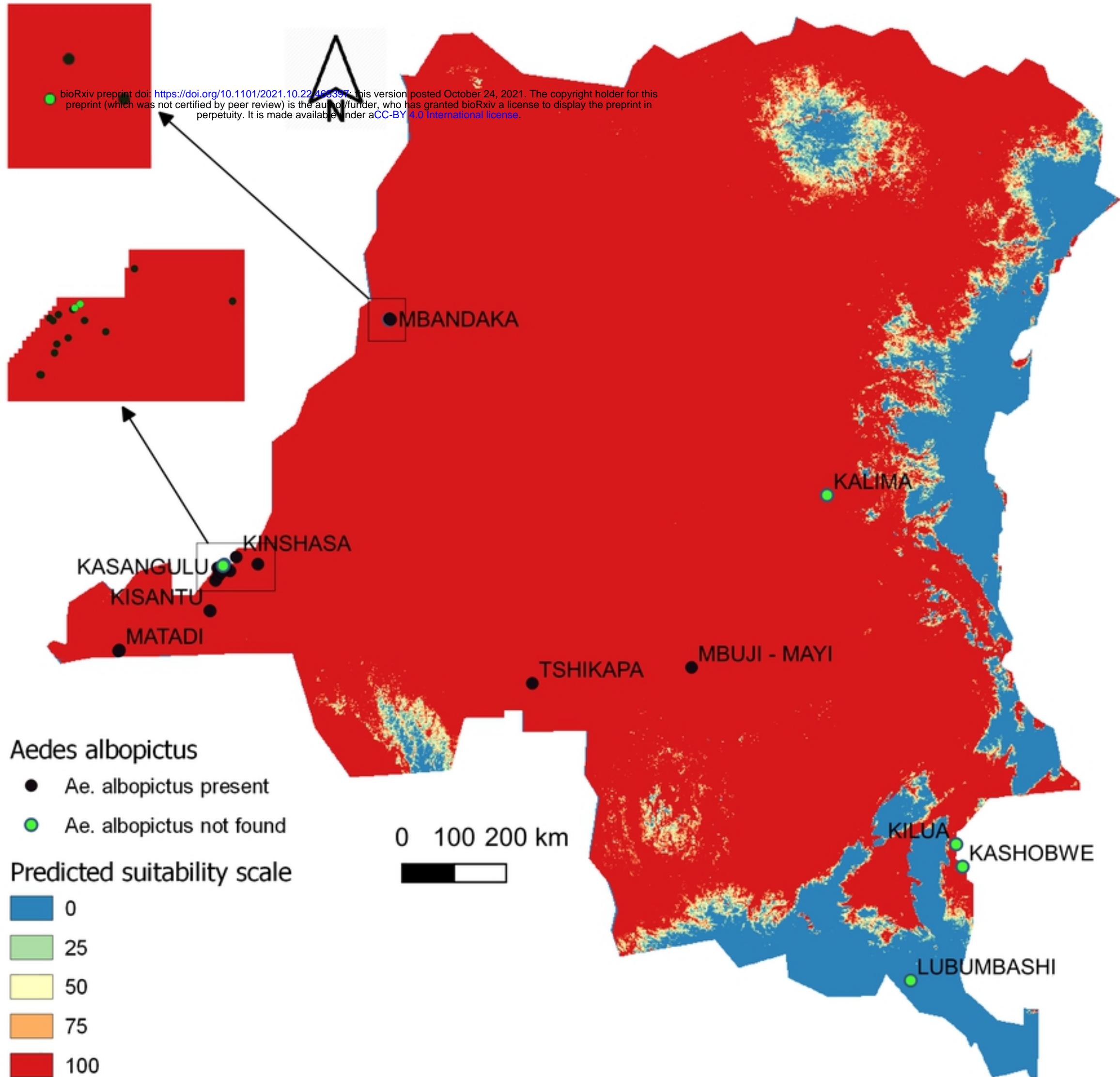
687 **S1 File. Pearson correlations between environmental variables.**

688 **S1 Dataset. Environmental variable data from all collection sites.**

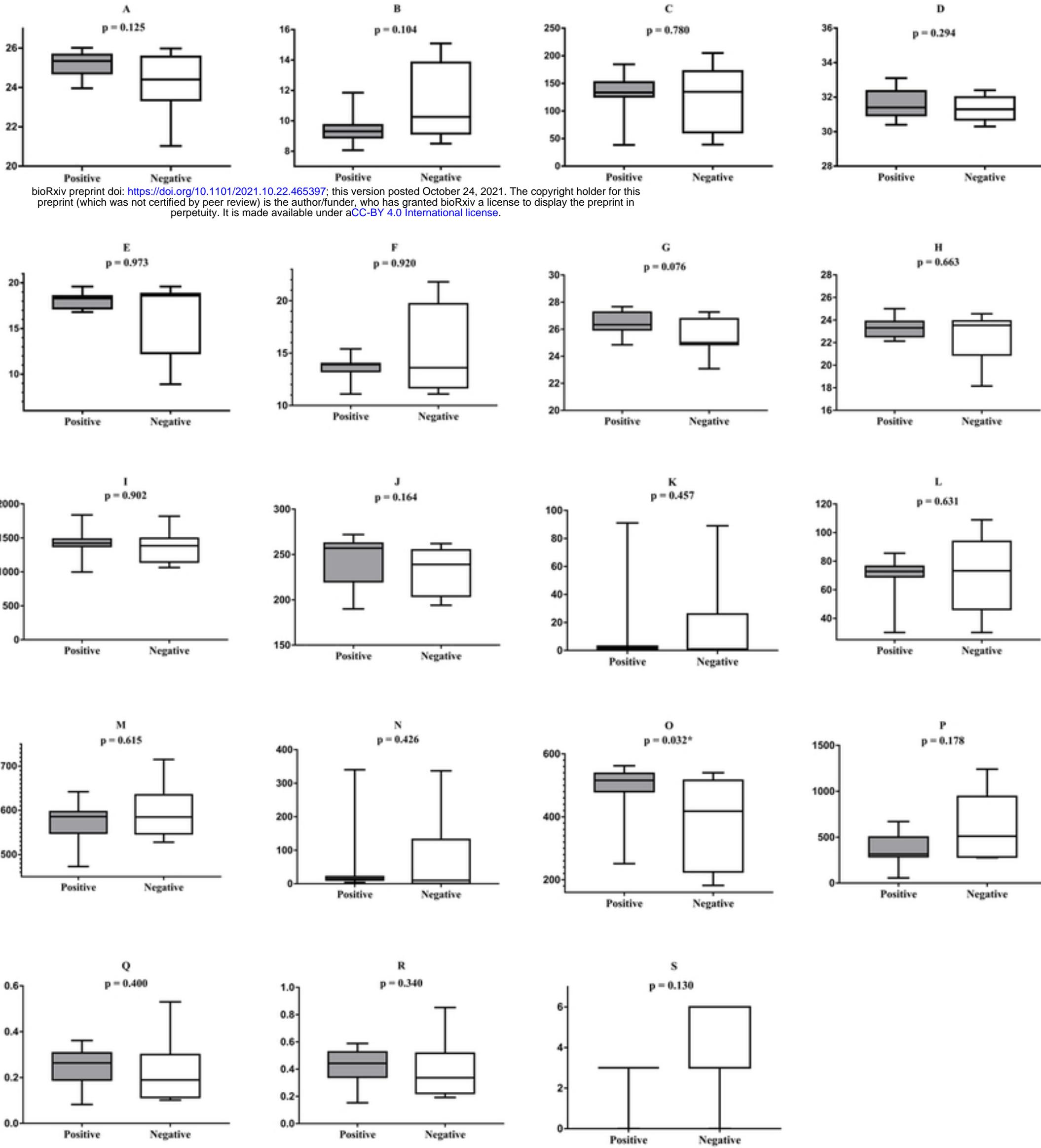
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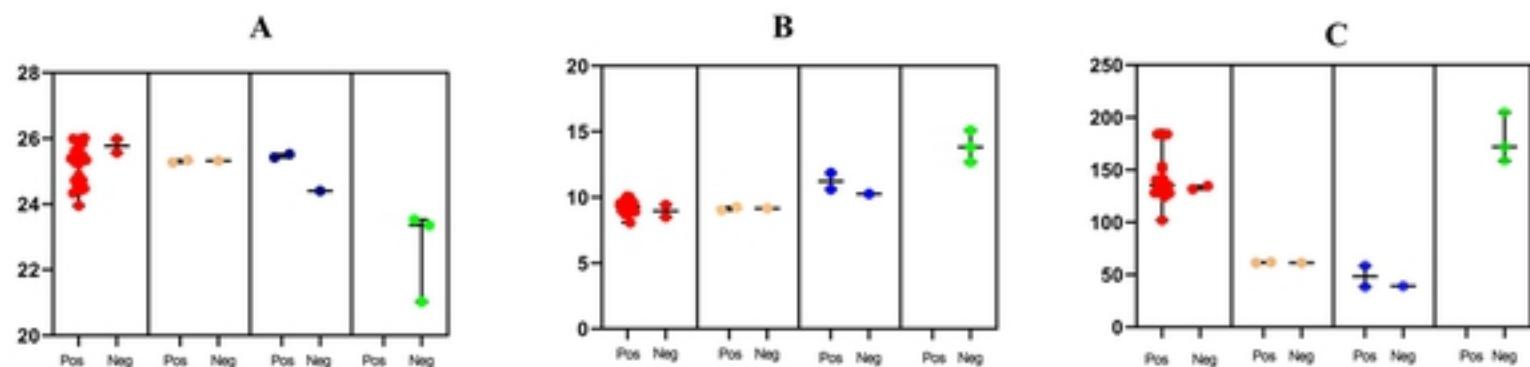
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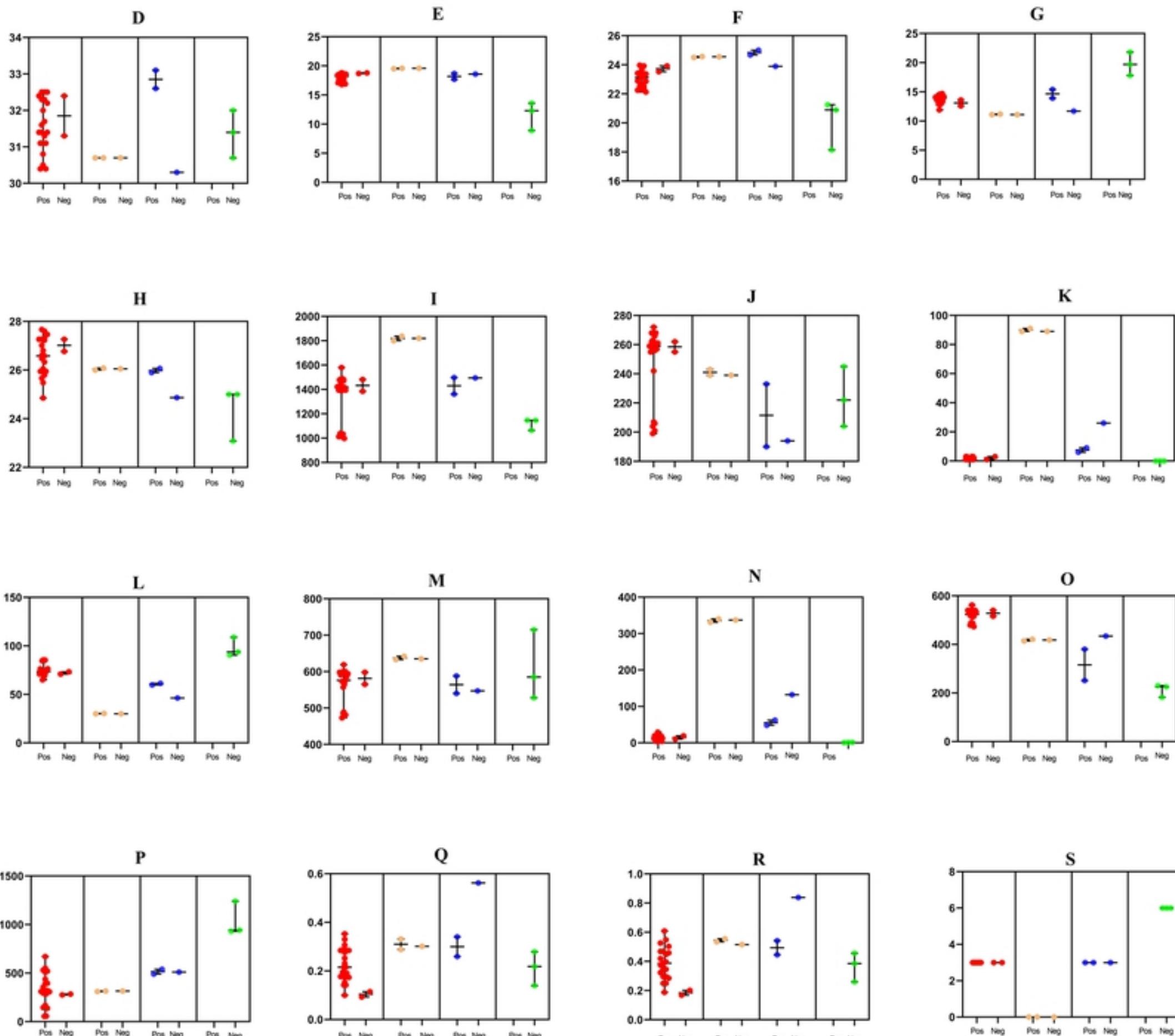
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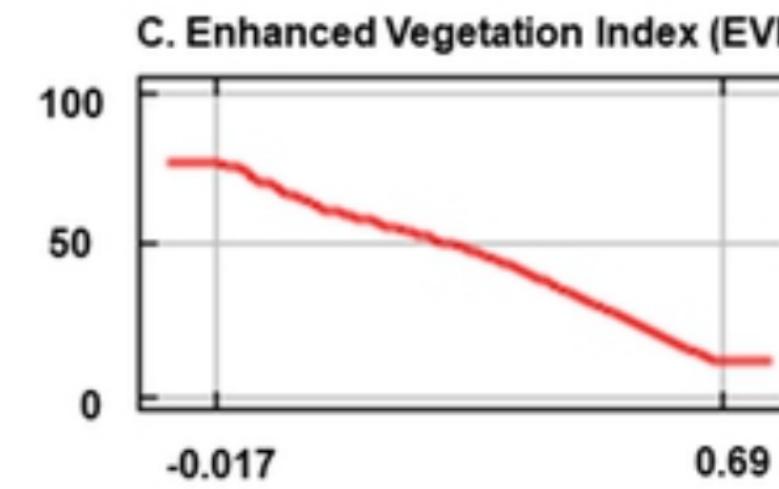
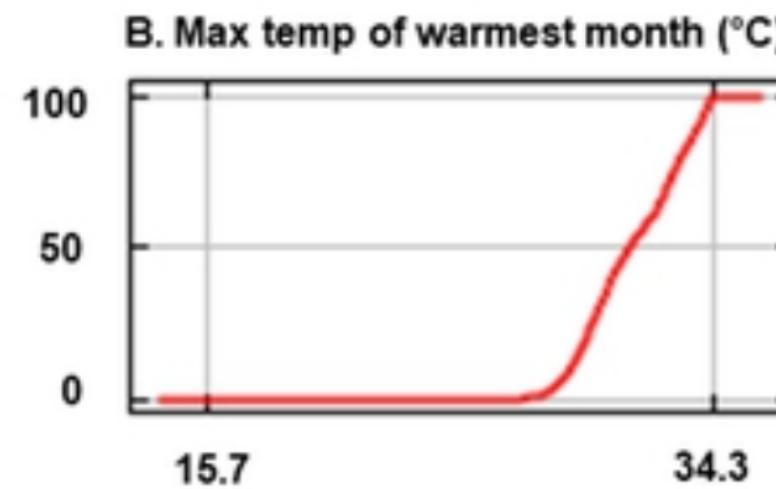
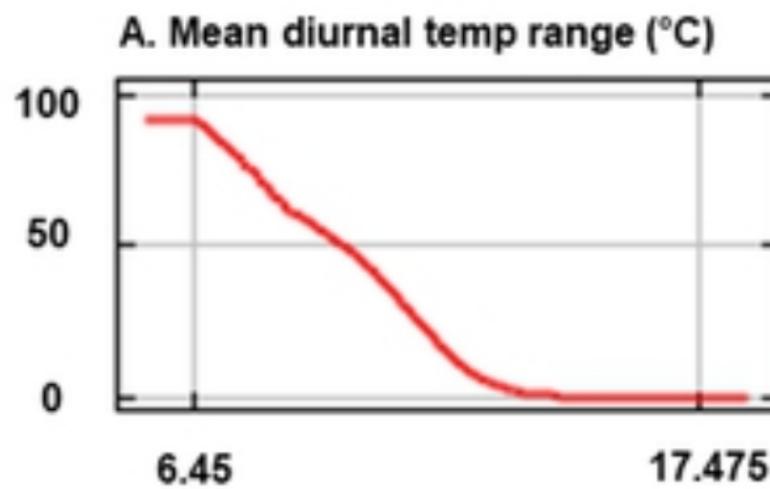


- Western
- Northern
- Central
- South-eastern



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Figure