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1 **Detection of a novel insect specific flavivirus across ecologically diverse populations of *Aedes***

2 ***aegypti* on the Caribbean Island of Saint Lucia**

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15 **ABSTRACT**

16 Outbreaks of mosquito-borne arboviral diseases including dengue virus (DENV), Zika virus (ZIKV), yellow

17 fever virus (YFV) and chikungunya virus (CHIKV) have recently occurred in the Caribbean. The

18 geographical range of the principle vectors responsible for transmission, *Aedes (Ae.) aegypti* and *Ae.*

19 *albopictus* is increasing and greater mosquito surveillance is needed in the Caribbean given international

20 tourism is so prominent. The island of Saint Lucia has seen outbreaks of DENV and CHIKV in the past

21 five years but vector surveillance has been limited with the last studies dating back to the late 1970s.

22 Natural disasters have changed the landscape of Saint Lucia and the island has gone through significant

23 urbanisation. In this study, we conducted an entomological survey of *Ae. aegypti and Ae. albopictus*

24 distribution across the island and analysed environmental parameters associated with the presence of

25 these species. Although we collected *Ae. aegypti* across a range of sites across the island, no *Ae.*

26 *albopictus* were collected despite traps being placed in diverse ecological settings. The number of *Ae.*

27 *aegypti* collected was significantly associated with higher elevation and semi-urban settings yielded

28 female mosquito counts per trap-day that were 5-fold lower than urban settings. Screening for

29 arboviruses revealed a high prevalence of a novel insect-specific flavivirus closely related to cell fusing

30 agent virus (CFAV). We discuss the implications that natural disasters, water storage and lack of

31 mosquito surveillance have on arboviral outbreaks in Saint Lucia and implications for insect only

32 flaviviruses on surveillance and detection of pathogenic flaviviruses.

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INTRODUCTION

Medically important arboviruses that cause human morbidity and mortality are predominantly transmitted by mosquitoes. There are more than 600 known arboviruses and related zoonotic viruses with more than 80 known to be human pathogens. Outbreaks of dengue virus (DENV), Zika virus (ZIKV), yellow fever virus (YFV) and chikungunya virus (CHIKV) are increasing [1] and there is potential for zoonotic viruses to spill-over into human populations. Arboviral disease transmission mostly occurs in tropical countries of Southeast Asia and South America and has a significant impact on developing countries [2]. Annual DENV infections are estimated at 100–390 million per year [3] and dengue is 're-emerging' mostly due to the expansion of the geographical range of the principal mosquito vector, *Aedes (Ae.) aegypti,* through globalization and climate change [2,4]. ZIKV is a flavivirus related to dengue virus (DENV) and historically thought to be transmitted by *Ae. aegypti.* Local transmission of ZIKV in the Americas was first reported in early 2014 and 22 countries and territories have now been identified to have autochthonous transmission [5]. YFV is also transmitted by *Ae. aegypti* and can result in large urban outbreaks and rapid spread to distant locations [6]. Yellow fever is now endemic in Central American countries and in several Caribbean Islands [7]. CHIKV is an alphavirus transmitted by *Ae. albopictus* (and to a lesser extent by *Ae. aegypti*) and has spread globally with outbreaks in the mid 2000s in the Indian Ocean and India and even in Europe in 2007 [8]. Transmission of CHIKV has also been seen recently in the Americas and this rapid geographical expansion (in a similar way to DENV) is likely due to the expanding habitat of the mosquito vectors [4].

Outbreaks of arboviral diseases including DENV [9], YFV [7], CHIKV [10] and ZIKV [11] have recently occurred in the Caribbean. The possibility of additional recent arbovirus transmission in the Caribbean must be considered given some infections result in nearly indistinguishable clinical symptoms. For example, Mayaro virus (MAYV) is an alphavirus closely related to CHIKV and has resulted in sporadic outbreaks in South America [12]. MAYV transmission is restricted to South and Central America where it is thought that non-human primates act as reservoir hosts and *Haemogogus* mosquitoes (eg. *H. janthinomys*) found in sylvatic jungle environments are responsible for human cases. Although human cases are strongly correlated with exposure to forest environments, urban transmission of MAYV must be considered given the association of cases and major cities infested with *Ae. aegypti* [13]. As the Caribbean is a destination for many international tourists, surveillance is needed for individual Caribbean islands to determine the risk of facilitating the spread of arboviral diseases. In particular, arboviruses transmitted by *Ae. aegypti* are considered important given that prevention predominantly relies on mosquito vector control. *Ae. aegypti* was first identified in the Caribbean Islands in 1864 [14-16] as has remained present despite the Pan American Health Organization (PAHO) mosquito control campaign in the 1940s-1960s that was launched to eliminate urban yellow fever. *Ae. aegypti* was successfully eradicated in many countries including Brazil, Mexico and Guatemala [17] but eradication was not achieved in other countries such as the USA, Suriname, Guyana, French Guyana, Venezuela and the Caribbean Islands. As the eradication campaign

75   deteriorated in the early 1970s and 1980s, many countries became re-infested with Ae. *aegypti* [18,19] and

76   the geographical expansion of *Ae. aegypti* with urbanization resulted in the introduction of DENV to many

77   countries [20,21]. With the exception of YFV, there are no currently available treatments or vaccines for

78   arboviral diseases transmitted by *Ae. aegypti* and *Ae. albopictus*. Disease control is currently limited to

79   traditional vector control strategies that rely on insecticides or destruction of larval breeding sites. In

80   most DENV-endemic countries, ultra-low volume space spraying is recommended only during dengue

81   outbreaks. However, widespread insecticide resistance has developed in *Ae. aegypti*, including high

82   pyrethroid resistance rates in South America [22] and further north in the Caribbean [23].

83

84   The volcanic island of Saint Lucia is located midway down the Eastern Caribbean Chain between

85   Martinique and Saint Vincent and north of Barbados (**Figure 1**). The first cases of dengue in Saint Lucia

86   were recorded in the 1980s and following Hurricane Thomas in 2011 another outbreak occurred [24].

87   CHIKV was first introduced to Saint Lucia in 2014 [25,26] but despite these outbreaks of major mosquito-

88   borne arboviruses, vector surveillance has been limited and the last documented studies were carried out

89   in 1976 [14-16]. The landscape of Saint Lucia in many areas has changed over the past 40 years due to

90   natural disasters and urbanisation, which has likely changed the distribution of arbovirus vectors. As the

91   density and habitats of Ae. *aegypti* have expanded both in urban and rural areas of many tropical

92   countries, we conducted an initial survey of *Ae. aegypti* and *Ae. albopictus* distribution and analysed any

93   environmental parameters that were associated with the presence of these species. Female mosquitoes

94   were screened for medically important arboviruses and other flaviviruses to investigate whether there was

95   any evidence of infection.

96

97   **METHODS**

98   **Study Sites and mosquito collection**

99   Mosquito collections were carried out on the island of Saint Lucia (Latitude 14.0167˚N, Longitude

100   60.9833˚W) in July 2015. Saint Lucia has a population of ~166,000 people and is 27 miles long and 14

101   miles wide with forest covering 77% of the island. The tropical climate includes a dry season (December

102   to June) and a wet season (July to November). Biogents (BG) Sentinel 2.0 mosquito traps baited with BG

103   lure® were used at various sites across the island (supplementary figure 1) during the beginning of the

104   wet season. Site selection was undertaken based on geographical and environmental variation in urban

105   and semi-urban areas across the island and factors based on island topology including forested areas,

106   brackish water bodies, fresh water bodies and mangrove habitats in communities with previously high

107   mosquito numbers recorded gathered from local knowledge. In some locations traps were placed inside

108   houses. Four permanent traps were connected to power supplies at Canaries Wellness Centre, Soufriere

109   Hospital, Etangs Wellness Centre and the River Doree Anglican Primary School (Figure 1) and traps were

110   run for a period of 22-24 days with mosquitoes collected at 24-hour intervals. Four temporary traps

111   powered by Power King®12 volt batteries were deployed at various locations across the island

112   (supplementary figure 1) to collect mosquitoes over a 24-hour period. Lascar easy log USB data logger

113   2's were placed in permanent traps to record humidity and temperature at hourly intervals. Garmin GPS

114   coordinators were used to determine co-ordinates of both permanent and temporary traps. Trapped

115   mosquitoes were collected, killed on ice for morphological identification to identify individuals belonging to

116   the *Aedes* genus. Larval dipping was undertaken at Soufriere Town, Choiseul Village, Marisule and Gros

117   Islet to sample immature stages (larvae/pupae) from domestic containers (e.g. tanks and drums,

118   discarded containers and tires). Immature stages were reared and allowed to emerge in mosquito cages.

119   Individual mosquitoes that were identified by morphology to be *Ae. aegypti* were placed in RNAlater and

120   stored at -20°C to preserve RNA for molecular analysis.

121

122   **RNA extraction and PCR analysis of adult *Ae. aegypti***

123   *Ae. aegypti* adult female mosquitoes were pooled according to trap location and date of collection (1-3

124   females/pool) and RNA was extracted using Qiagen 96 RNeasy Kits according to manufacturer's

125   instructions and a Qiagen Tissue Lyser II (Hilden, Germany) with 3mm stainless steel beads to

126   homogenise mosquitoes. RNA was eluted in 45 μl of RNase-free water and stored at -70˚C. A Qiagen

127   QuantiTect Kit was first used to remove any genomic DNA co-purified during the RNA extraction protocol

128   and then reverse transcription was performed to generate cDNA from all RNA extracts using

129   manufacturer's instructions. Confirmation of species identification was undertaken using an Internal

130   transcribed spacer 1 (ITS1) real time PCR assay that discriminates between *Ae. aegypti* and *Ae.*

131   *albopictus* [27]. Arbovirus screening included the major arboviruses of public health importance, suspected

132   or having the potential of being transmitted by *Ae. aegypti* / *Ae. albopictus* in the Caribbean: DENV,

133   CHIKV, ZIKV, YFV and MAYV (table 1). In addition, Pan-Flavivirus PCR screening was undertaken that

134   allows simultaneous detection of numerous flaviviruses using a conserved region of the NS5 gene [28].

135   PCR reactions for all assays except ZIKV were prepared using 5 μl of Qiagen SYBR Green Master mix, a

136   final concentration of 1 μM of each primer, 1 μl of PCR grade water and 2 μl template cDNA, to a final

137   reaction volume of 10 μl. Prepared reactions were run on a Roche LightCycler® 96 System and PCR

138   cycling conditions are described in table 1. Amplification was followed by a dissociation curve (95˚C for

139   10 seconds, 65˚C for 60 seconds and 97˚C for 1 second) to ensure the correct target sequence was

140   being amplified. ZIKV screening was undertaken using a Taqman probe based assay [29]. PCR results

141   were analysed using the LightCycler® 96 software (Roche Diagnostics). Synthetic long oligonucleotide

142   standards of the amplified PCR product were generated in the absence of biological virus cDNA positive

143   controls and each assay included negative (no template) controls.

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147 **Sanger sequencing and phylogenetic analysis**

148 Pan-flavi PCR products were submitted to Source BioScience (Source BioScience Plc, Nottingham, UK)

149 for PCR reaction clean-up, followed by Sanger sequencing to generate both forward and reverse reads.

150 Sequencing analysis was carried out in MEGA7 [30] as follows. Both chromatograms (forward and reverse

151 traces) from each sample was manually checked, edited, and trimmed as required, followed by alignment

152 by ClustalW and checking to produce consensus sequences. Consensus sequences were used to

153 perform nucleotide BLAST (NCBI) database queries. Maximum Likelihood phylogenetic trees were

154 constructed from Sanger sequences as follows. The evolutionary history was inferred by using the

155 Maximum Likelihood method based on the Tamura-Nei model [31]. The tree with the highest log likelihood

156 in each case is shown. The percentage of trees in which the associated taxa clustered together is shown

157 next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying

158 Neighbor-Joining and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum

159 Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value.

160 The trees are drawn to scale, with branch lengths measured in the number of substitutions per site.

161 Codon positions included were 1st+2nd+3rd+Noncoding. All positions containing gaps and missing data

162 were eliminated. The phylogeny test was by Bootstrap method with 1000 replications. Evolutionary

163 analyses were conducted in MEGA7 [30].

164 **Statistical analysis**

165 Count data analysis was conducted using a generalized linear model because the response variable

166 (mosquito counts) had a non-normal error distribution. Models were run using Stata MP (version 14, Stata

167 Corp, College Station, TX, USA). Both Poisson and negative binomial link functions were used in

168 analysis, with the superior model identified from visual inspection of fits (**Supplementary figure 2**). A

169 univariate analysis included elevation, humidity and temperature as continuous explanatory variables,

170 and urbanisation level (urban or semi-urban) as a factor. Incident Rate Ratios (and corresponding 95%

171 confidence intervals) were calculated.

172

173 **Results**

174 A total of 3,701 adult mosquitoes were collected across the island of Saint Lucia over a four-week period

175 using BG sentinel 2 traps (**Table 2**). *Culex* was the dominant genus, comprising 78.7% of the total

176 mosquitoes collected and the remaining 21.3% were morphologically identified as species within the

177 *Aedes* genus. No *Ae. albopictus* females were collected in any of the locations despite traps being

178 placed in diverse ecological settings. *Ae. aegypti* adults were collected in 26/46 trap locations with the

179 largest number of females being collected at Soufriere Hospital (n=196) and Canaries Wellness Centre

180 (n=93) where permanent traps were running for the duration of the collection period. The average

181 number of female *Ae. aegypti* collected over a 24-hour period across all trap locations was 3.09. A

182 particularly high number of *Ae. aegypti* were collected during a 24-hour period from Dugard *(47 females*

183   and 10 males comprising 47.5% of the total collection) (**Figure 2**, **supplementary figure 1**) using a trap

184   placed indoors in a semi-urban area. In contrast, low numbers of *Ae. aegypti* were collected using the

185   permanent trap at River Dorree Anglican Primary School with *Ae. aegypti* compromising 0.5% (n=5) of

186   the collection and an average of 0.13 female mosquitoes per 24 hours of trapping.

187   A generalized linear model (GLM) was used to analyse the associations between the counts of female

188   *Ae. aegypti* (combining counts from both the temporary traps and permanent traps) and four independent

189   variables: peak daily temperature, peak daily humidity, trap elevation and ecological zone (semi-urban or

190   urban). Plotting count frequencies against alternative, competing models assuming either a Poisson or a

191   negative binomial distribution clearly demonstrated the superiority of a negative binomial model in fitting

192   the data distribution (**supplementary figure 2**). Exponentiation of the coefficients resulting from a GLM

193   (negative binomial family) produced the incidence rate ratio (IRR) associated with the independent

194   variables. Here, IRR can be interpreted as the ratio of counts per trap-day associated with the tested

195   variable. These are described along with 95% confidence intervals in **Table 2**. No significant association

196   was found with temperature. Because previous studies have shown a non-monotonic association

197   between *Ae. aegypti* and temperature (i.e. *Ae. aegypti* thrive at a non-trivial optimal temperature) [32] we

198   subsequently attempted to fit a more complex (quadratic) function between these variables but this did

199   not improve model fit (data not shown). Higher counts were significantly associated with higher elevation

200   although the effect size was small; and semi-urban settings yielded female mosquito counts per trap-day

201   that were 5-fold lower than urban settings. We tested for interactions between all covariates but none

202   were found to be significant.

203

204   A sub-sample of adult female *Ae. aegypti* mosquitoes collected from BG traps were screened for

205   arboviruses (**Table 3**). No evidence was seen for infection of the major medically important arboviruses

206   that have historically been transmitted by *Ae. aegypti*. However, the presence of a novel flavivirus closely

207   related to cell fusing agent virus (CFAV) was detected (**Figure 3**) in 17.8% (8/45) individuals screened

208   from Soufriere Hospital, 20% (3/15) of individuals screened from Etangs Wellness Centre, 33% (1/3)

209   screened from Micoud Village, 50% (1/2) individuals from Micoud Highway and 50% (2/4) individuals from

210   Piaye. We also detected this novel flavivirus in adult females that have been reared from larval collection

211   (n=10).   Phylogenetic analysis reveals this flavivirus is an insect specific flavivirus (ISF) clustering with

212   other ISFs and separate from pathogenic flavivirus such as DENV, ZIKV and YFV (**Figure 4).**

213

214   **Discussion**

215   Entomological indices including the abundance of adult mosquitoes are often used to assess the risk of

216   disease transmission and this, in turn, influences vector control strategies. The lack of surveillance

217   studies, to our knowledge, for major vectors of arboviruses of public health importance on the island of

218   Saint Lucia needed to be addressed, given the recent outbreaks of arboviruses such as DENV, CHIKV

219   and ZIKV in the Caribbean and surrounding regions. The principle vector of these arboviral diseases, *Ae.*

220 *aegypti,* is highly invasive and is now present in much of the Americas including the US [5]. In this study,

221 we collected adult mosquitoes to determine the geographical distribution of *Ae. aegypti* across the island

222 of Saint Lucia and to determine any correlation with environmental variables. BG sentinel 2 traps were

223 selected as these traps were redesigned to provide increased durability in field conditions and were

224 recently shown to be effective at trapping *Aedes* species [33]. The durability of traps was particularly

225 important for the four permanent traps that were used for approximately 24 days. Adult collections

226 indicate that *Ae. aegypti* is present throughout the Island of Saint Lucia and population densities are

227 significantly higher in urban areas compared to semi-urban or rural settings. We also demonstrated that

228 adult counts were positively correlated to elevation.

229

230 The trapping of a high number of *Ae. aegypti (*47 females and 10 males) during a 24-hour period from

231 Dugard using a trap placed indoors in a semi-urban area suggests an interesting behavioural observation.

232 The biology and behaviour of *Ae. aegypti* in the Caribbean has not been extensively studied but previous

233 work on a Trinidad strain using human landing catches revealed the periodicity consisted of 90% arriving

234 during daylight and twilight and 10% during the night [34]. This study included both urban and rural sites

235 and a consistently larger number of mosquitoes were collected outside vs. inside houses. Light intensity

236 was also significantly correlated with mosquito landing patterns [34]. The trapping of *Ae. aegypti* inside

237 houses in Saint Lucia could indicate a change in behavior with mosquitoes biting indoors during the night

238 in houses with lights on (an anecdotal observation that occurred during our study). Indoor resting of *Ae.*

239 *aegypti* has recently been documented in Mexico [35] which has implications for control methods. Other

240 studies in the Caribbean have also shown that high temperatures in open environments can result in *Ae.*

241 *aegypti* breeding in underground sites [36] and indoor oviposition has been demonstrated [37,38].

242

243 Confirmation of the presence of *Ae. aegypti* on the island of Saint Lucia is not particularly surprising given

244 this species is widespread throughout the Caribbean and is now widespread in the Americas [4,5]. The

245 association with urban environments in Caribbean Islands is seen with the most common breeding sites

246 being drums/barrels, uncovered tanks and cisterns, brick holes, flower pots, used tyres and utility

247 manholes [39,40]. Saint Lucia now provides the ideal environment for *Ae. aegypti* due to recent changes in

248 the climate. The El Nino period in 2009 - 2010 introduced dry hot periods and provided an environment

249 that was not conducive for mosquito production [40]. Water conservation has become a critical issue for

250 Saint Lucia and the majority of the water supply comes from surface runoff collected in rivers, streams

251 and dams. Rain water is collected and stored haphazardly and inappropriately in various containers such

252 as water tanks, drums, and buckets, creating ideal breeding grounds for this species [1,40]. This study was

253 undertaken during the commencement of the wet season with the average rainfall in June and July 2015

254 being 37.1mm and 175.8mm respectively. This indicates that greater mosquito abundance is likely

255 throughout later stages of the wet season and follow-up studies should be undertaken to determine this.

256   Clearly climatic patterns resulting in unpredictable rainfall will provide ideal breeding grounds (unpolluted

257   water in artificial and natural containers) for *Ae. aegypti* in Saint Lucia [36].

258

259   *Ae. albopictus* was not identified in the mosquitoes collected in our study despite many traps being set in

260   or near forested areas. Although the range of *Ae. albopictus* has expanded to Europe, USA and many

261   South American countries [4], this species has only been found in the Eastern Caribbean [40]. However,

262   recent outbreaks of CHIKV on Saint Lucia and neighbouring Caribbean islands suggest that there might

263   be a possibility that *Ae. albopictus* may also play a role in the spread of the disease. Although not

264   confirmed in most Caribbean islands, the Dominican Republic was the first country to confirm the

265   presence of Ae. *albopictus* [41]. With *Ae. albopictus* present in the US to the north and the Cayman Islands

266   to the south, Saint Lucia is clearly considered at risk for establishment of *Ae. albopictus* [42]. *Ae. albopictus*

267   has also been shown to harbour Eastern equine encephalitis virus (EEEV) [43], highlighting the potential

268   transmission risk of additional arboviruses. The traditional ways of importing *Ae. albopictus* through the

269   trade of tyres is also a possible source of introduction for this species. Although Saint Lucia has signed

270   onto the International Health Regulations (IHR) 2005, to prevent and control the international spread of

271   disease, port surveillance systems are not fully implemented and might not be sufficient to monitor

272   containers present on ships and ensure that they are fumigated before they arrive in port. Saint Lucia is

273   also faced with the problem of tyre disposal where there is no functional shredding equipment, which is of

274   great concern, particularly so because tyres in landfills are in close proximity to urban communities.

275   The detection of a novel flavivirus closely related to CFAV in diverse ecological populations of *Ae. aegypti*

276   across the island of Saint Lucia suggests the potential for undiscovered viruses in the Caribbean. A large

277   study was undertaken in Trinidad screening more than 185,000 mosquitoes representing 46 species and

278   85 different viruses were isolated [44]. The isolation of Mucambo virus (MUCV), a Venezuelan Equine

279   Encephalitis complex subtype IIIA), follows a history of isolating alphaviruses from mosquitoes in Trinidad

280   [45]. A potentially novel strain of CFAV was discovered in *Ae. aegypti* populations from Mexico [46] and

281   CFAV was detected in *Ae. aegypti* populations from Kenya [47]. Interestingly, CFAV infection

282   significantly enhanced replication of DENV (and vice versa) in *Ae. aegypti* Aag2 mosquito cells [48].

283   CFAV has been shown to be vertically transmitted in *Ae. aegypti* lab colonies suggesting the

284   possibility of using CFAVs and closely related ISFs for control of medically important arboviruses [49].

285   The presence of insect-specific viruses in *Ae. aegypti* might be underestimated given a recent study

286   suggested up to 27 insect-specific viruses (23 currently uncharacterized) in populations from Cairns

287   (Australia) and Bangkok (Thailand) [50]. The question remains as to whether insect specific viruses like

288   CFAV have not yet gained the ability to infect vertebrates and therefore become arboviruses or whether

289   they have lost this ability [51]. Phylogenetic studies focussed on the E gene of flaviviruses would suggest

290   CFAV is a basal lineage that diverged prior to the separation of mosquito and tick-borne flaviviruses [52].

291   Our results indicate the presence of a flavivirus but it has been shown that some flavivirus genome-

292 integrated sequences can be transcribed and therefore cautious must be taken to assume the presence

293 of an active flavivirus infection [53]. The impact of arboviral diseases is increasing due to the expanding

294 geographical range of many mosquito species, particularly *Ae. aegypti* and *Ae. albopictus*. As most

295 arboviral diseases occur in sporadic epidemics, vector control options are often limited to the use of

296 insecticides that are becoming less effective due to insecticide resistance. As re-emerging arboviral

297 diseases such as DENV and ZIKV continue to spread geographically, the fight to eradicate or reduce the

298 transmission potential of *Ae. aegypti* is increasing in importance. Outbreaks of arboviral diseases,

299 including DENV, CHIKV and ZIKV, have a history of occurring in small tropical islands. ZIKV emerged for

300 the first time outside of Africa and Asia in Yap State in Micronesia and then a large outbreak in French

301 Polynesia was followed by transmission in other Pacific islands [54]. Small Islands Developing States and

302 territories (SIDS) such as Saint Lucia are particularly vulnerable to arboviral disease outbreaks for several

303 reasons [55]. Natural disasters are more frequent and these change the geographical landscape allowing

304 rapid mosquito proliferation. SIDS often lack safe water supplies and sanitation and local governments

305 have limited resources to undertake vector control and manage outbreaks. An increasing ability for travel

306 between SIDS and continental regions facilitate the spread of arboviruses to previously unexposed

307 populations. For these reasons, surveillance strategies need to be monitored, risk areas need to be

308 mapped out and epidemic trends recorded for predicting future outbreaks. For the Caribbean island of

309 Saint Lucia, further research is needed to determine the diversity of current mosquito species and this

310 should be extended to the neighbouring smaller Caribbean islands.

311

References

312 **References**

313 1 Weaver, S. C. & Reisen, W. K. Present and future arboviral threats. *Antiviral Research* **85**, 328-
314 345, doi:10.1016/j.antiviral.2009.10.008 (2010).
315 2 Guzman, M. G. *et al.* Dengue: a continuing global threat. *Nature reviews. Microbiology* **8**, S7-16,
316 doi:10.1038/nrmicro2460 (2010).
317 3 Bhatt, S. *et al.* The global distribution and burden of dengue. *Nature* **496**, 504-507,
318 doi:10.1038/nature12060 (2013).
319 4 Furuya-Kanamori, L. *et al.* Co-distribution and co-infection of chikungunya and dengue viruses.
320 *BMC Infect Dis* **16**, 84, doi:10.1186/s12879-016-1417-2 (2016).
321 5 Yakob, L. & Walker, T. Zika virus outbreak in the Americas: the need for novel mosquito control
322 methods. *Lancet Glob Health* **4**, e148-149, doi:10.1016/S2214-109X(16)00048-6 (2016).
323 6 Johansson, M. A. *et al.* Assessing the risk of international spread of yellow fever virus: a
324 mathematical analysis of an urban outbreak in Asuncion, 2008. *The American journal of tropical*
325 *medicine and hygiene* **86**, 349-358, doi:10.4269/ajtmh.2012.11-0432 (2012).
326 7 Auguste, A. J. *et al.* Yellow fever virus maintenance in Trinidad and its dispersal throughout the
327 Americas. *Journal of virology* **84**, 9967-9977, doi:10.1128/JVI.00588-10 (2010).
328 8 Staples, J. E., Breiman, R. F. & Powers, A. M. Chikungunya fever: an epidemiological review of a
329 re-emerging infectious disease. *Clinical infectious diseases : an official publication of the*
330 *Infectious Diseases Society of America* **49**, 942-948, doi:10.1086/605496 (2009).
331 9 Sharp, T. M. *et al.* Virus-specific differences in rates of disease during the 2010 Dengue epidemic
332 in Puerto Rico. *PLoS neglected tropical diseases* **7**, e2159, doi:10.1371/journal.pntd.0002159
333 (2013).
334 10 Mowatt, L. & Jackson, S. T. Chikungunya in the Caribbean: An Epidemic in the Making. *Infect Dis*
335 *Ther* **3**, 63-68, doi:10.1007/s40121-014-0043-9 (2014).

336   11   Lednicky, J. *et al.* Zika Virus Outbreak in Haiti in 2014: Molecular and Clinical Data. *PLoS*
337   *neglected tropical diseases* **10**, e0004687, doi:10.1371/journal.pntd.0004687 (2016).
338   12   Weaver, S. C. & Reisen, W. K. Present and future arboviral threats. *Antiviral Res* **85**, 328-345,
339   doi:10.1016/j.antiviral.2009.10.008 (2010).
340   13   Tesh, R. B. *et al.* Mayaro virus disease: an emerging mosquito-borne zoonosis in tropical South
341   America. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of*
342   *America* **28**, 67-73, doi:10.1086/515070 (1999).
343   14   Belkin, J. N. & Heinmann, S. J. COLLECTION RECORDS OF THE PROJECT MOSQUITOES
344   OF MIDDLE AMERICA PART 6 SOUTHERN LESSER ANTILLES BARBADOS DOMINICA
345   GRENADA ST-LUCIA ST-VINCENT. *Mosquito Systematics* **8**, 237-297 (1976).
346   15   Belkin, J. N. & Heinemann, S. J. COLLECTION RECORDS OF THE PROJECT MOSQUITOES
347   OF MIDDLE AMERICA PART 5 FRENCH WEST-INDIES GUADELOUPE AND MARTINIQUE.
348   *Mosquito Systematics* **8**, 163-193 (1976).
349   16   Belkin, J. N. & Heinemann, S. J. COLLECTION RECORDS OF THE PROJECT MOSQUITOES
350   OF MIDDLE AMERICA PART 4 LEEWARD ISLANDS ANGUILLA ANTIGUA BARBUDA
351   MONTSERRAT NEVIS ST-KITTS. *Mosquito Systematics* **8**, 123-162 (1976).
352   17   Soper, F. L. The 1964 status of Aedes aegypti eradication and yellow fever in the Americas. *The*
353   *American journal of tropical medicine and hygiene* **14**, 887-891 (1965).
354   18   Gubler, D. J. The changing epidemiology of yellow fever and dengue, 1900 to 2003: full circle?
355   *Comparative Immunology Microbiology and Infectious Diseases* **27**, 319-330,
356   doi:10.1016/j.cimid.2004.03.013 (2004).
357   19   Gubler, D. J. The global pandemic of dengue/dengue haemorrhagic fever: Curent status and
358   prospects for the future. *Annals Academy of Medicine Singapore* **27**, 227-234 (1998).
359   20   Gubler, D. J. Human arbovirus infections worldwide. *West Nile Virus: Detection, Surveillance, and*
360   *Control* **951**, 13-24 (2001).
361   21   Gubler, D. J. Resurgent vector-borne diseases as a global health problem. *Emerging Infectious*
362   *Diseases* **4**, 442-450 (1998).
363   22   Lima, E. P. *et al.* Insecticide resistance in Aedes aegypti populations from Ceara, Brazil.
364   *Parasites & vectors* **4**, 5, doi:10.1186/1756-3305-4-5 (2011).
365   23   Marcombe, S. *et al.* Insecticide resistance in the dengue vector Aedes aegypti from Martinique:
366   distribution, mechanisms and relations with environmental factors. *PloS one* **7**, e30989,
367   doi:10.1371/journal.pone.0030989 (2012).
368   24   PAHO, P. A. H. O.   (2011).
369   25   Fischer, M., Staples, J. E., Arboviral Diseases Branch, N. C. f. E. & Zoonotic Infectious Diseases,
370   C. D. C. Notes from the field: chikungunya virus spreads in the Americas - Caribbean and South
371   America, 2013-2014. *MMWR Morb Mortal Wkly Rep* **63**, 500-501 (2014).
372   26   Centers for Disease, C. & Prevention. Update on emerging infections: news from the Centers for
373   Disease Control and Prevention. Notes from the field: chikungunya virus spreads in the
374   Americas-Caribbean and South America, 2013-2014. *Ann Emerg Med* **64**, 552-553,
375   doi:10.1016/j.annemergmed.2014.08.002 (2014).
376   27   Hill, L. A. *et al.* Rapid Identification of Aedes albopictus, Aedes scutellaris, and Aedes aegypti Life
377   Stages Using Real-time Polymerase Chain Reaction Assays. *American Journal of Tropical*
378   *Medicine and Hygiene* **79**, 866-875 (2008).
379   28   Johnson, N. *et al.* Assessment of a novel real-time pan-flavivirus RT-polymerase chain reaction.
380   *Vector Borne Zoonotic Dis* **10**, 665-671, doi:10.1089/vbz.2009.0210 (2010).
381   29   Lanciotti, R. S. *et al.* Genetic and serologic properties of Zika virus associated with an epidemic,
382   Yap State, Micronesia, 2007. *Emerg Infect Dis* **14**, 1232-1239, doi:10.3201/eid1408.080287
383   (2008).
384   30   Kumar, S., Stecher, G. & Tamura, K. MEGA7: Molecular Evolutionary Genetics Analysis Version
385   7.0 for Bigger Datasets. *Molecular biology and evolution* **33**, 1870-1874,
386   doi:10.1093/molbev/msw054 (2016).
387   31   Tamura, K. & Nei, M. Estimation of the number of nucleotide substitutions in the control region of
388   mitochondrial DNA in humans and chimpanzees. *Molecular biology and evolution* **10**, 512-526,
389   doi:10.1093/oxfordjournals.molbev.a040023 (1993).

390   32   Eisen, L. *et al.* The impact of temperature on the bionomics of Aedes (Stegomyia) aegypti, with
391   special reference to the cool geographic range margins. *Journal of medical entomology* **51**, 496-
392   516 (2014).
393   33   Arimoto, H. *et al.* Comparison of Trapping Performance Between the Original BG-Sentinel(R)
394   Trap and BG-Sentinel 2(R) Trap (1). *Journal of the American Mosquito Control Association* **31**,
395   384-387, doi:10.2987/moco-31-04-384-387.1 (2015).
396   34   Chadee, D. D. & Martinez, R. Landing periodicity of Aedes aegypti with implications for dengue
397   transmission in Trinidad, West Indies. *Journal of vector ecology : journal of the Society for Vector
398   Ecology* **25**, 158-163 (2000).
399   35   Dzul-Manzanilla, F. *et al.* Indoor Resting Behavior of Aedes aegypti (Diptera: Culicidae) in
400   Acapulco, Mexico. *J Med Entomol*, doi:10.1093/jme/tjw203 (2016).
401   36   Chadee, D. D. & Martinez, R. Aedes aegypti (L.) in Latin American and Caribbean region: With
402   growing evidence for vector adaptation to climate change? *Acta Trop* **156**, 137-143,
403   doi:10.1016/j.actatropica.2015.12.022 (2016).
404   37   Chadee, D. D. Resting behaviour of Aedes aegypti in Trinidad: with evidence for the re-
405   introduction of indoor residual spraying (IRS) for dengue control. *Parasites & vectors* **6**, 255,
406   doi:10.1186/1756-3305-6-255 (2013).
407   38   Garcia-Rejon, J. *et al.* Dengue virus-infected Aedes aegypti in the home environment. *The
408   American journal of tropical medicine and hygiene* **79**, 940-950 (2008).
409   39   Chadee, D. D., Shivnauth, B., Rawlins, S. C. & Chen, A. A. Climate, mosquito indices and the
410   epidemiology of dengue fever in Trinidad (2002-2004). *Annals of Tropical Medicine and
411   Parasitology* **101**, 69-77, doi:10.1179/136485907x157059 (2007).
412   40   Rawlins, S.   (Pan American Health Organization, 2014).
413   41   PAHO, P. A. H. O. PAHO Advisory:Aedes albopictus in the Carribbean. (1993).
414   42   Benedict, M. Q., Levine, R. S., Hawley, W. A. & Lounibos, L. P. Spread of the tiger: Global risk of
415   invasion by the mosquito Aedes albopictus. *Vector-Borne and Zoonotic Diseases* **7**, 76-85,
416   doi:10.1089/vbz.2006.0562 (2007).
417   43   Mitchell, C. J. *et al.* Isolation of eastern equine encephalitis virus from Aedes albopictus in
418   Florida. *Science* **257**, 526-527 (1992).
419   44   Auguste, A. J. *et al.* Isolation and characterization of sylvatic mosquito-borne viruses in Trinidad:
420   enzootic transmission and a new potential vector of Mucambo virus. *The American journal of
421   tropical medicine and hygiene* **83**, 1262-1265, doi:10.4269/ajtmh.2010.10-0280 (2010).
422   45   Auguste, A. J. *et al.* Isolation and phylogenetic analysis of Mucambo virus (Venezuelan equine
423   encephalitis complex subtype IIIA) in Trinidad. *Virology* **392**, 123-130,
424   doi:10.1016/j.virol.2009.06.038 (2009).
425   46   Espinoza-Gomez, F. *et al.* Detection of sequences from a potentially novel strain of cell fusing
426   agent virus in Mexican Stegomyia (Aedes) aegypti mosquitoes. *Archives of virology* **156**, 1263-
427   1267, doi:10.1007/s00705-011-0967-2 (2011).
428   47   Iwashita, H. *et al.* Mosquito arbovirus survey in selected areas of Kenya: detection of insect-
429   specific virus. *Tropical medicine and health* **46**, 19, doi:10.1186/s41182-018-0095-8 (2018).
430   48   Zhang, G., Asad, S., Khromykh, A. A. & Asgari, S. Cell fusing agent virus and dengue virus
431   mutually interact in Aedes aegypti cell lines. *Scientific reports* **7**, 6935, doi:10.1038/s41598-017-
432   07279-5 (2017).
433   49   Contreras-Gutierrez, M. A., Guzman, H., Thangamani, S., Vasilakis, N. & Tesh, R. B.
434   Experimental Infection with and Maintenance of Cell Fusing Agent Virus (Flavivirus) in Aedes
435   aegypti. *The American journal of tropical medicine and hygiene* **97**, 299-304,
436   doi:10.4269/ajtmh.16-0987 (2017).
437   50   Zakrzewski, M. *et al.* Mapping the virome in wild-caught Aedes aegypti from Cairns and Bangkok.
438   *Scientific reports* **8**, 4690, doi:10.1038/s41598-018-22945-y (2018).
439   51   Calisher, C. H. & Higgs, S. The Discovery of Arthropod-Specific Viruses in Hematophagous
440   Arthropods: An Open Door to Understanding the Mechanisms of Arbovirus and Arthropod
441   Evolution? *Annual review of entomology* **63**, 87-103, doi:10.1146/annurev-ento-020117-043033
442   (2018).
443   52   Crabtree, M. B., Sang, R. C., Stollar, V., Dunster, L. M. & Miller, B. R. Genetic and phenotypic
444   characterization of the newly described insect flavivirus, Kamiti River virus. *Archives of virology*
445   **148**, 1095-1118, doi:10.1007/s00705-003-0019-7 (2003).

446 53 Blitvich, B. J. & Firth, A. E. Insect-specific flaviviruses: a systematic review of their discovery, host
447   range, mode of transmission, superinfection exclusion potential and genomic organization.
448   *Viruses* **7**, 1927-1959, doi:10.3390/v7041927 (2015).
449 54 Musso, D., Cao-Lormeau, V. M. & Gubler, D. J. Zika virus: following the path of dengue and
450   chikungunya? *Lancet* **386**, 243-244, doi:10.1016/S0140-6736(15)61273-9 (2015).
451 55 Cao-Lormeau, V. M. Tropical Islands as New Hubs for Emerging Arboviruses. *Emerg Infect Dis*
452   **22**, 913-915, doi:10.3201/eid2205.150547 (2016).
453 56 Hill, L. A. *et al.* Rapid identification of Aedes albopictus, Aedes scutellaris, and Aedes aegypti life
454   stages using real-time polymerase chain reaction assays. *The American journal of tropical*
455   *medicine and hygiene* **79**, 866-875 (2008).
456 57 Lai, Y. L. *et al.* Cost-effective real-time reverse transcriptase PCR (RT-PCR) to screen for
457   Dengue virus followed by rapid single-tube multiplex RT-PCR for serotyping of the virus. *Journal*
458   *of clinical microbiology* **45**, 935-941, doi:10.1128/JCM.01258-06 (2007).
459 58 Ummul Haninah, A. *et al.* Development and evaluation of a one-step SYBR-Green I-based real-
460   time RT-PCR assay for the detection and quantification of Chikungunya virus in human, monkey
461   and mosquito samples. *Tropical biomedicine* **27**, 611-623 (2010).
462 59 Dash, P. K., Boutonnier, A., Prina, E., Sharma, S. & Reiter, P. Development of a SYBR green I
463   based RT-PCR assay for yellow fever virus: application in assessment of YFV infection in Aedes
464   aegypti. *Virology journal* **9**, 27, doi:10.1186/1743-422X-9-27 (2012).
465 60 Llagonne-Barets, M. *et al.* A case of Mayaro virus infection imported from French Guiana. *J Clin*
466   *Virol* **77**, 66-68, doi:10.1016/j.jcv.2016.02.013 (2016).

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475   **Author contributions statement**

476   All authors contributed to the design of the study. MW and LW performed mosquito collections. CLJ, MW,

477   LW and TW performed molecular analysis of samples. CLJ performed Sanger sequencing and

478   phylogenetic analysis. CLJ, MW, LY and TW undertook data analysis. All authors read and approved final

479   version of the manuscript.

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481   **Competing interests:** The authors declare that they have no competing interests.

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483   **Availability of data and material:** All data generated or analysed during this study are included in this

484   article.

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Tables

Table 1. PCR gene targets and primer sequences for the screening analysis undertaken on *Ae. aegypti* mosquito cDNA.

Target gene	fluorescence detection	Primer sequences (5'-3')	Reference
Ae. aegypti its1	SYBR green	CGCTCGGACGCTCGTAC CTTCGAGCTTCGACGACACA	56
Pan-Flavi NS5	SYBR green	GCMATHTGGTWCATGTGG GTRTCCCAKCCDGCNGTRTC	28
Pan-DENV	SYBR green	TTGAGTAAACYRTGCTGCCTGTAGCTC CTGAAGACATTGGCCCCAC	57
CHIKV E1	SYBR green	ACGCAGTTGAGCGAAGCAC CTGAAGACATTGGCCCCAC	58
YFV 5' NTR/ capsid gene junction	SYBR green	AATCGAGTTGCTAGGCAATAAACAC TCCCTGAGCTTTACGACCAGA	59
MAYV E1	SYBR green	TTCCRAAYCAAGTGGGATTC CACTTTACGTAYGGKGATGG	60
ZIKV	Taqman	AARTACACATACCARAACAAAGTGGT TCCRCTCCCYCTYTGGTCTTC Probe: AGCCTACCTTGACAAGCAGTCAGA CACTCAA (FAM)	29

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526 **Table 2**. Collection site locations and characteristics with total numbers of adult mosquitoes collected

527 from each site.

| Location of collection	GPS coordinates		Elevation (m)	EcoZone Category (trap placement)	Culex spp.		Ae. aegypti		Other Aedes spp.	
	North	West			♀	♂	♀	♂	♀	♂
Canaries Wellness Centre	N 13°54.291	W 061°04.084	4	Urban (outdoor)	162	75	93	16	46	7
Soufriere Hospital	N 13°51.382	W 060°03.546	14	Urban (outdoor)	501	438	196	87	52	11
Etangs Wellness Centre	N 13°50.120	W 061°01.628	289	Semi-Urban (outdoor)	77	25	34	1	14	0
River Doree Anglican Primary School	N 13°45.842	W 061°02.141	67	Semi-Urban (outdoor)	421	173	3	0	5	1
Monzie	N 13°48.605	W 061°01.300	374	Rural (outdoor)	0	0	0	0	0	0
Roblot	N 13°48.011	W 061°01.442	318	Semi-Urban(outdoor)	0	4	2	0	2	0
De Brieul	N 13°47.991	W 061°01.427	308	Semi-Urban(outdoor)	1	0	0	0	2	0
Reunion	N 13°46.353	W 061°02.510	84	Urban(outdoor)	20	6	1	0	1	0
Delcer	N 13°46.948	W 060°58.182	199	Semi-Urban (indoor)	0	2	1	0	0	1
Upper Augier	N 13°44.680	W 060°57.390	33	Semi-Urban (outdoor)	11	18	0	0	4	0
Lower Augier	N 13°43.678	W 060°57.229	25	Urban (indoor)	6	2	0	0	3	0
Desrisseaux	N 13°45.209	W 060°59.553	86	Semi-Urban (outdoor)	0	1	0	0	0	0
Perriot	N 13°46.214	W 060°58.776	162	Rural (indoor)	20	1	0	0	7	0
La Faruge	N 13°44.196	W 060°58.233	17	Semi-Urban (outdoor)	0	0	0	0	0	0
Sauzay	N 13°43.859	W 060°56.983	40	Semi-Urban (outdoor)	19	14	1	0	2	0
Laborie High Way	N 13°44.927	W 060°58.852	44	Semi-Urban (outdoor)	1	0	0	0	0	0
Vieux- Fort Town	N 13°43.510	W 060°56.868	13	Urban (outdoor)	0	0	0	0	0	0
Montete	N 13°43.477	W 060°56.876	14	Urban (outdoor)	30	10	0	0	0	0
Fond Dor	N 13°46.358	W 061°02.393	85	Urban (indoor)	50	97	1	0	0	0
Dennery Highway	N 13°46.525	W 061°02.329	102	Semi-urban(outdoor)	2	1	0	0	0	0
Micoud Village	N 13°49.186	W 060°53.816	12	Urban (outdoor)	7	10	5	0	2	0
Micoud Village	N 13°49.238	W 060°53.921	21	Urban (outdoor)	10	4	9	4	3	0
Micoud Highway	N 13°49.228	W 060°53.873	10	Urban (outdoor)	40	14	4	0	10	1
Micoud Health Centre	N 13°49.178	W 060°53.826	13	Urban (outdoor)	77	47	0	0	3	2
Fond Doux	N 13°49.048	W 061°02.956	347	Forest-fringe (outdoor)	19	27	9	3	2	0
Choiseul Village	N 13°46.474	W 061°02.994	15	Urban (outdoor)	36	27	2	4	0	0
Dugard	N 13°48.547	W 061°01.373	315	Forested (outdoor)	2	1	0	0	0	0
Belle Plain	N 13°49.243	W 061°01.664	466	Forest-fringe (outdoor)	0	0	0	0	0	0
Lamaze	N 13°48.295	W 061°01.104	313	Forested (outdoor)	0	0	0	0	0	0
Montete	N 13°54.663	W 060°53.463	12	Urban (indoor)	10	6	7	0	1	0
Vieux Fort Town	N 13°54.563	W 060°53.633	15	Urban (outdoor)	3	0	0	0	0	0
La Ressource	N 13°54.528	W 060°53.636	10	Rural (outdoor)	70	63	7	0	0	0
Vieux- Fort Town	N 13°46.472	W 061°02.255	108	Urban (outdoor)	1	0	0	1	0	0
Mongouge	N 13°44.981	W 060°56.621	11	Rural (outdoor)	2	0	1	4	0	0
Beanfield	N 13°45.007	W 060°59.701	10	Semi-urban (outdoor)	0	0	0	0	0	0
Dugard	N 13°44.830	W 060°57.863	38	Semi-urban (indoor)	32	29	47	10	2	0
Palmiste	N 13°48.110	W 061°01.772	281	Urban (indoor)	5	1	0	0	0	0
Laborie Town	N 13°51.561	W 061°03.394	54	Urban (indoor)	6	14	6	1	0	0
Sapphaire	N 13°45.497	W 061°01.055	57	Urban (outdoor)	1	0	0	1	0	0
Dennery Village	N 13°46.616	W 061°00.770	138	Semi-urban (outdoor)	12	3	4	1	0	0
Piaye	N 13°48.276	W 061°00.787	292	Semi-urban (outdoor)	11	14	14	5	2	4
Saltibus	N 13°46.251	W 061°01.412	92	Semi-urban (indoor)	11	5	5	2	0	0
Rainforest	N 13°50.345	W 060°58.563	321	Forested (outdoor)	48	0	0	0	0	0
Richfond	N 13°56.086	W 060°55.320	35	Rural (indoor)	23	8	0	0	0	0
Castries City	N 14°00.765	W 060°59.096	16	Urban (indoor)	1	1	1	0	1	0
Ford St Jacques	N 13°49.138	W 061°02.631	338	Semi-Urban (outdoor)	12	15	0	1	0	0

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533 **Table 3.** Incidence Rate Ratios (IRR) and corresponding 95% confidence intervals resulting from

534 univariate generalized linear models with negative binomial link function.

Environmental variable	IRR	Std. Err.	z	P>z	95% Confidence Interval	
elevation	**1.012516**	**0.004744**	**2.65**	**0.008**	**1.00326**	**1.021858**
humidity	1.005635	0.01621	0.35	0.727	0.974361	1.037913
temperature	1.039605	0.089212	0.45	0.651	0.878666	1.230023
semi-urban	**0.188336**	**0.046641**	**-6.74**	**0.000**	**0.115914**	**0.306008**

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564   **Figures**
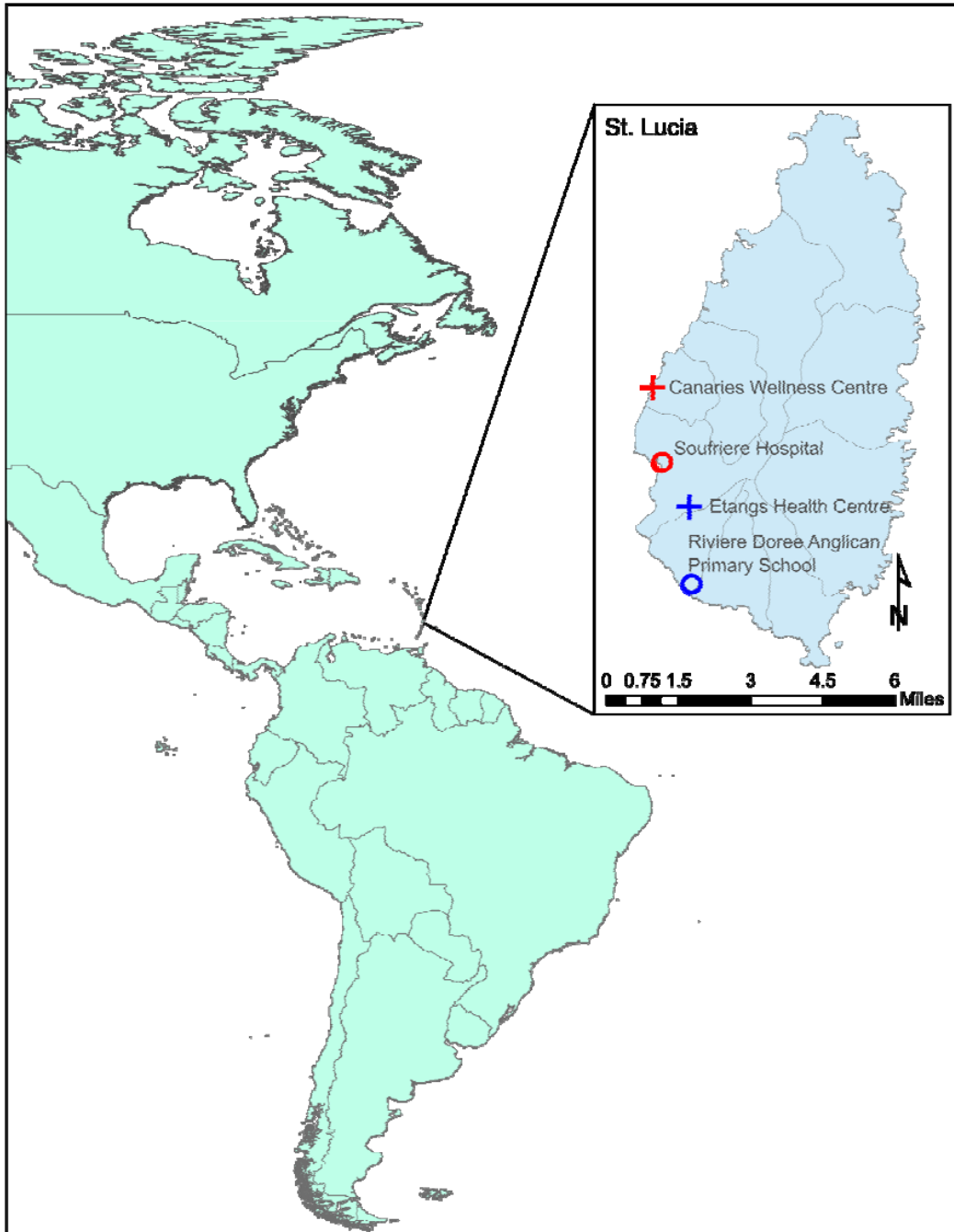
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567   **Figure 1**. Sampling locations of the longer-term mosquito traps on the island of Saint Lucia used

568   throughout the duration of the study (July 2015). Inset: a representative BG Sentinel 2 trap placed in the

569   Des Cartier rainforest (a temporary trap location).

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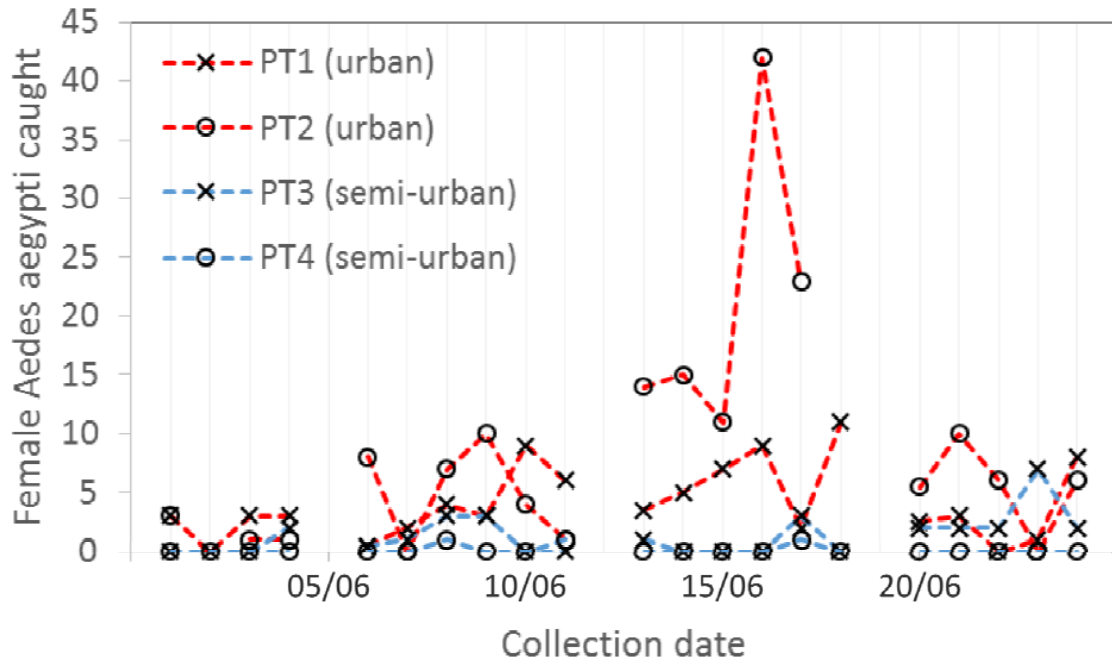
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572 **Figure 2.** Population dynamics of local female *Aedes aegypti* mosquitoes caught from the four longer-
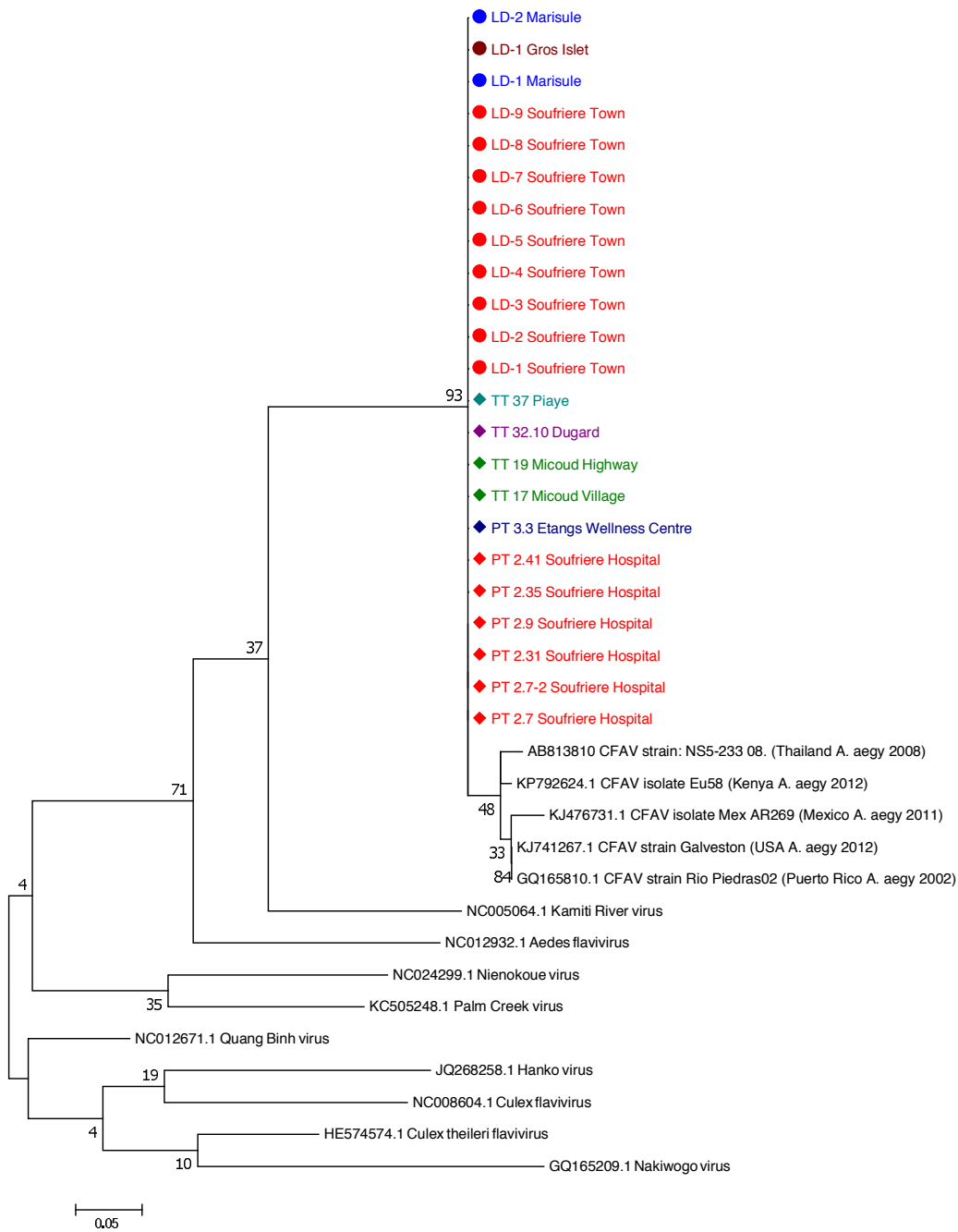
573 term traps positioned in field sites detailed in Figure 1.

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584 **Figure 3**. Maximum Likelihood molecular phylogenetic analysis of Pan-flavi NS5 sequences from field-

585 collected *Ae. aegypti* mosquitoes. The tree with the highest log likelihood (-1077.12) is shown. The tree is

586 drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis

587 involved 37 nucleotide sequences. There were a total of 124 positions in the final dataset.

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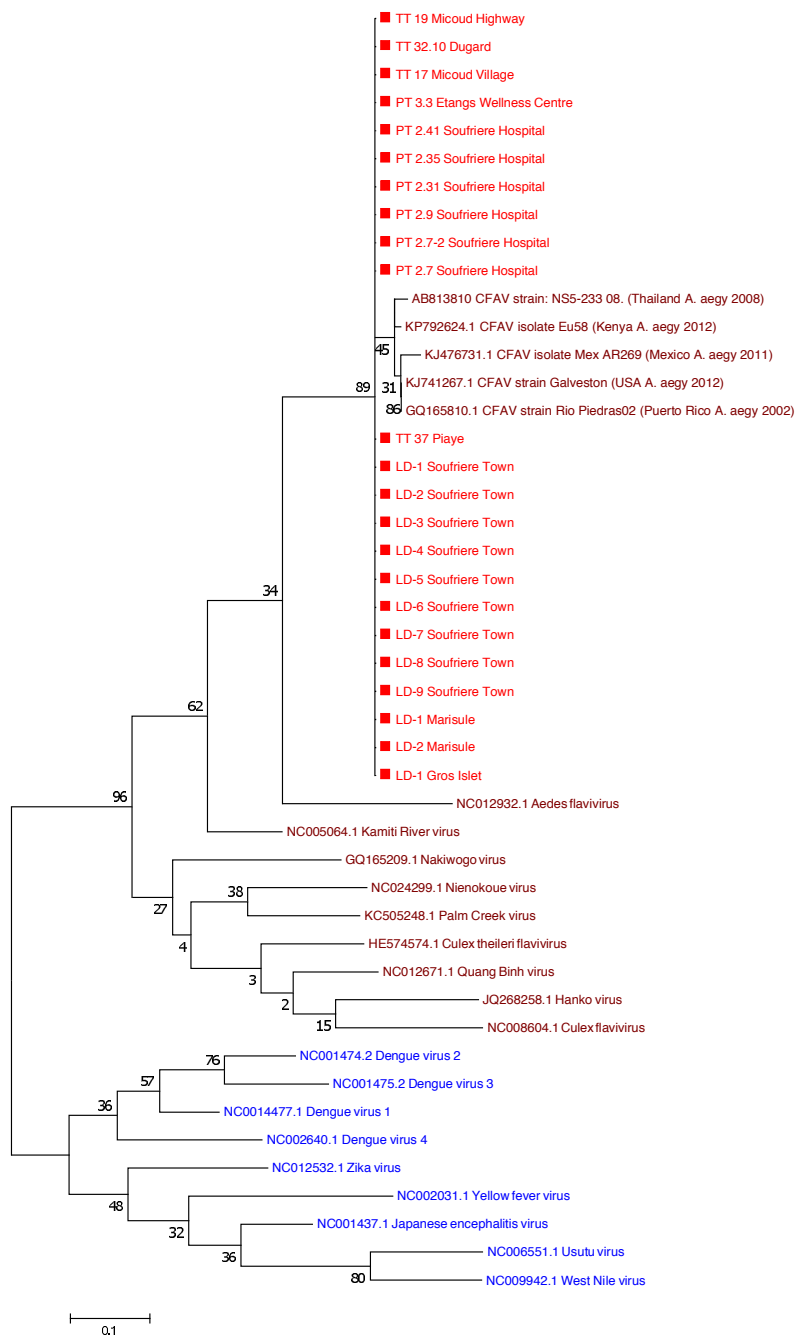
Figure 4. Maximum Likelihood molecular phylogenetic analysis of Pan-flavi NS5 sequences showing the novel flavivirus clustering with insect specific flaviviruses. The tree with the highest log likelihood (-1951.89) is shown. The tree is drawn to scale, with branch lengths measured in the number of substitutions per site. The analysis involved 46 nucleotide sequences. There were a total of 124 positions in the final dataset.
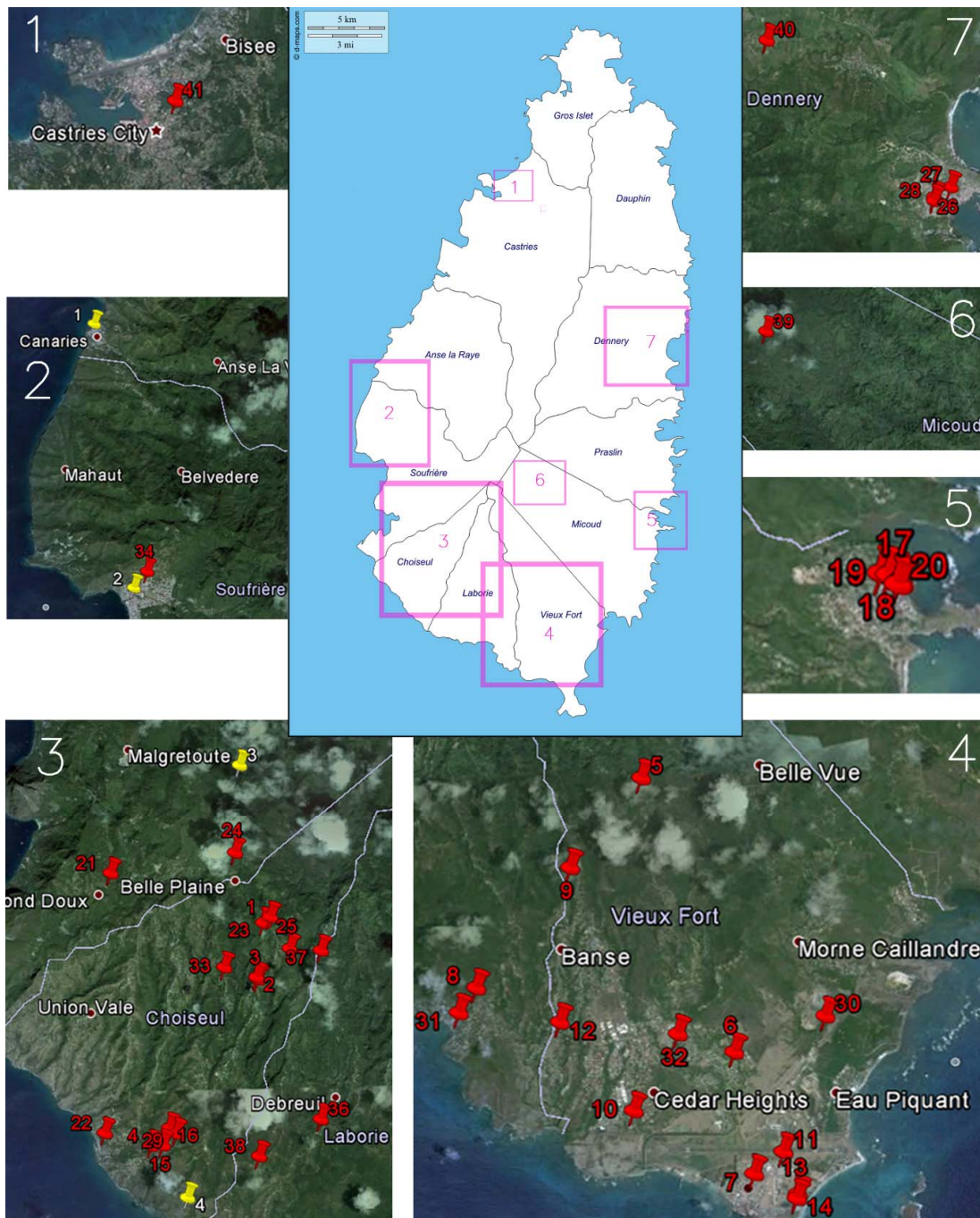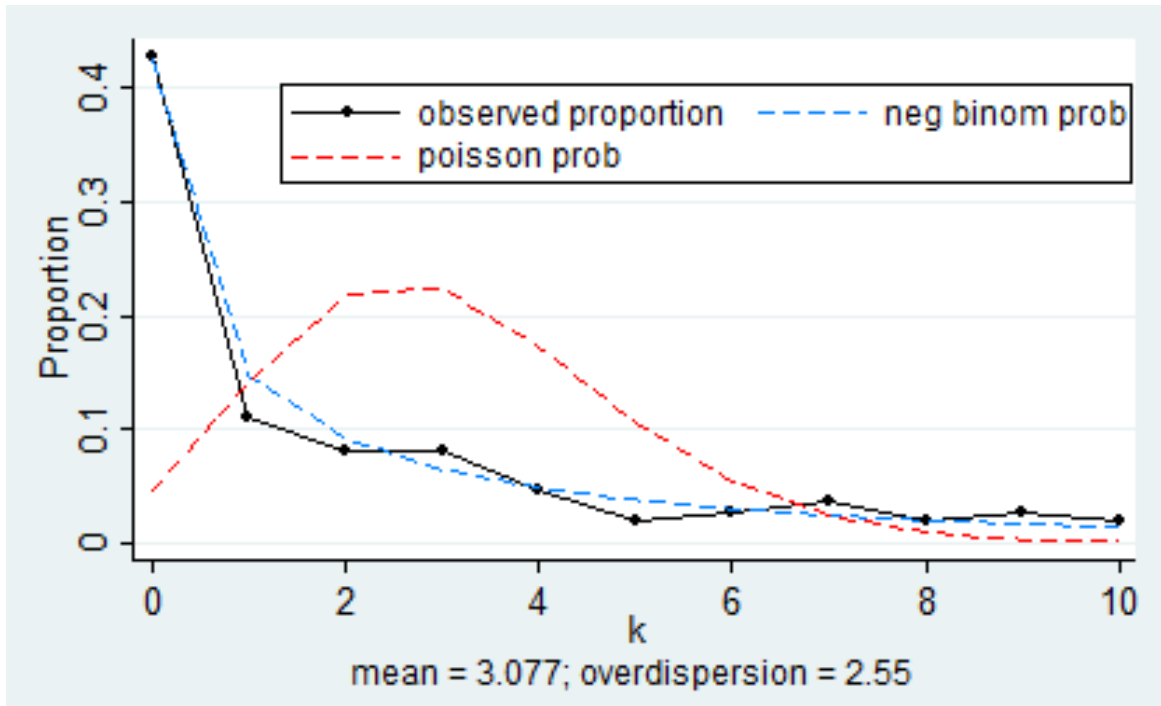
596 **Supplementary information**

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598 **Supplementary Figure 1**: Map of Saint Lucia showing the location of BG Sentinel 2 traps used in the

599 study. GPS coordinates were plotted using Google Earth. Red pins represent locations of temporary

600 traps and yellow pins permanent traps.

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605 **Supplementary Figure 2.** The observed proportions along with the Poisson and negative binomial

606 probabilities for the count type variable (using 'nbvargr' function in Stata). The Poisson probabilities are

607 computed using an estimate of the Poisson mean. The negative binomial probabilities use the same

608 mean and an estimate of the over dispersion parameter.

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