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1 **FINE-SCALE POPULATION GENETIC STRUCTURE OF DENGUE MOSQUITO**
2 **VECTOR,** *Aedes aegypti* **AND ITS ASSOCIATION TO LOCAL DENGUE**
3 **INCIDENCE**

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17 **ABSTRACT**

18 Dengue fever is an important arthropod-borne disease which is transmitted by the mosquito
19 vector, *Aedes aegypti*. Vector control programs rely heavily on targeting the mosquito
20 vector in order to stop the disease transmission cycle. Hence, the present study conducted a
21 fine-scale population genetics of *Ae. aegypti* in a highly urbanized and dengue endemic

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22 region in the Philippines. Furthermore, the study also explored the correlation of population
23 genetic indices to the local dengue incidence of the region. The genetic diversity and
24 population structure of *Ae. aegypti* populations were analyzed by genotyping 11
25 microsatellite loci from 526 adult mosquitoes sampled in 21 study areas in Metropolitan
26 Manila. Five genetic indices and its dengue incidence were then correlated using Pearson's
27 correlation. Results showed low genetic differentiation among mosquito populations
28 indicating high gene flow activity in the region. However, the study also revealed a
29 considerable number of inferred genetic clusters (K=5). The constructed UPGMA
30 dendrogram exhibited close proximity of genetically-similar *Ae. aegypti* mosquito
31 populations that extends in long distances suggesting passive dispersal ability of the
32 mosquito vector. Moreover, a positive and significant correlation was observed between
33 dengue incidence and inbreeding coefficient ($F$is) ($r = 0.52$, $p = 0.02$). Overall, the study
34 showed that population genetic structuring can occur in a fine-scale area which consisted
35 notable clustering and extending patterns of genetically-similar mosquito populations. This
36 infers the potential migration ability of *Ae. aegypti* in different locations of the region
37 where specific vector control zones could be carried out to disrupt its dispersal ability. Also,
38 this is the first study that attempted to correlate genetic indices to dengue incidence that
39 could serve as a supplementary index in identifying high dengue risk areas in the future.

40 **AUTHOR SUMMARY**

41 Dengue disease puts billions of people worldwide at risk. To mitigate this risk, population
42 genetic studies of its vector, *Aedes aegypti*, are being conducted. The information
43 established from these studies can be utilized to reduce mosquito population and thereby,
44 reduce the opportunity for dengue transmission. In this study, we used microsatellite
45 markers to determine genetic structure and diversity followed by correlation analyses
46 between genetic indices and dengue incidence. Results show a low genetic differentiation
47 among mosquito populations in Metro Manila; it also indicates population genetic
48 structuring in a fine-scale area. This suggest a pattern of migration activity of *Ae. aegpyti*
49 which can be used to mitigate dengue transmission. Moreover, the study also explored in

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50 correlating genetic indices and local dengue incidence where it demonstrated significant
51 correlation with the inbreeding coefficient (*F*is). Further investigation is needed on how
52 these genetic indices may be utilized in predicting and identifying high dengue risk areas in
53 endemic areas.

54 **INTRODUCTION**

55 Dengue disease is the most prevalent mosquito-borne viral infection in tropical and
56 subtropical countries [1] with approximately 2.5 billion people worldwide at risk of
57 contracting the disease [2]. Dengue virus is transmitted primarily to humans by the
58 principal mosquito vector, *Aedes aegypti*. This mosquito species is considered to be the
59 most efficient vector of arboviruses because of its highly adaptive nature to the urban
60 environment [3]. Although a dengue vaccine is available [4], the World Health
61 Organization [2] still recommends disease prevention and control towards the mosquito
62 vector.

63 Molecular genotyping of the mosquito vector using microsatellites has provided
64 useful insights towards the improvement of mosquito vector control strategies [5,6].For
65 instance, revealing the gene flow pattern among *Ae. aegypti* populations can be interpreted
66 as the mosquito vector's dispersal pattern [7,8]. Microsatellites is widely used as the
67 standard molecular marker of choice in population genetic studies of *Ae. aegpyti* [9]. Due to
68 the marker's high polymorphism, co-dominance, and broad genome distribution [10], it has
69 been deemed suitable for differentiating both macro- and micro-geographic scale mosquito
70 populations [11,12,13].

71 Despite the many population genetic studies of *Ae. aegypti* using microsatellites
72 worldwide, only a handful of studies have investigated the vector's genetic structure in a
73 fine-scale area [7,11,12,13,14,15,16].These fine-scale studies are defined as having
74 sampling points within city boundaries, or villages with geographic distances of less than
75 50 km. For instance, spatial genetic differentiation across *Ae. aegypti* populations was
76 evident in spatial scales within city boundaries [7,11,12,16], among villages [13,14] and

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77 along a street [15]. It has been claimed that genetic divergence in small spatial scales is
78 common in Southeast Asia [11] and could also be attributed to the type of breeding sites
79 [15]. Furthermore, multiple inferred clusters of genetic mosquito populations (K= 3–9)
80 were also detected within these fine-scale areas [11,14,16]. It was suggested that a single
81 house or groups of closely situated houses may act as assembling units in forming these
82 genetic clusters [11].

83 The information and patterns identified by the population genetic approach can be
84 factored as part of the strategy in reducing the mosquito population, thereby, decreasing the
85 opportunity of dengue transmission. Early fine-scale population genetic studies of *Ae.*
86 *aegypti* [7,8,11,15] had only demonstrated the degree or magnitude of genetic structuring
87 while recent studies [12,16] concentrated on hypothesis testing such as the role
88 urbanization in genetic divergence [14]. Although the results provided relative insights to
89 our understanding of the mosquito vector, it lacks in demonstrating the clustering or
90 distribution of genetically-similar mosquito populations which can reveal notable patterns
91 for it application in vector control. For instance, in Yuunan Province of China, *Ae. aegypti*
92 populations from border areas of the region are genetically-similar among each other and
93 distinct from its two main cities indicating different invasion and colonization conduits [17].
94 The findings was presented simply in a dendrogram which can used in creating a sound
95 basis in disrupting possible invasion of this mosquito vector from neighboring countries. In
96 previous fine-scale population genetic studies, such graphical presentation was not
97 illustrated but only described or portrayed in tabular records of pairwise genetic differences
98 (e.g. F_{ST}, Nei's genetic distance). Illustrating how geographical locations are genetically-
99 similar or distinct may demonstrate the migration activity of *Ae. aegypti* and the extent of
100 its spatial distribution patterns in fine-scale areas.

101 Surveillance of the immature or adult stages of *Ae. aegypti* has led to the conception
102 of vector indices (e.g. Container, House, Breteau, Pupal or Adult indices) which can be
103 utilized as a potential predictor of local dengue epidemiology [18]. However, frequent or
104 consistent sampling of immature or adult stages of the mosquito vector has proven to be

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105 laborious and cost intensive [19]. Innovative and alternative avenues are now being
106 explored in determining the population size of *Ae. aegypti* such as the development of non-
107 powered passive adult traps [20] and utilizing container-inhabiting mosquito simulation
108 approach [21,22]. One avenue that has not been explored is the application of genetic
109 indices that characterizes the mosquito vector population. For example, population genetics
110 can estimate the effective population size (*Ne*) of the mosquito vector which is related to its
111 consensus size [23]. With several population genetic studies that have reported the
112 estimated population sizes of local *Ae. aegypti* populations [9,13,24], no study had
113 associated *Ne* or other genetic indices to the local dengue incidence. It was demonstrated
114 that sampling 25-30 individuals per local area is suitable for microsatellite-based
115 population genetic studies [25] where genetic analyses can determine mosquito population
116 size by conducting specific sampling episodes as compared to frequent or regular mosquito
117 collection surveillance.

118 Therefore, the present study has two objectives. First, it determines the population
119 genetic structure of *Ae. aegypti* within a fine-scale area and, second, it explores the
120 correlation of population genetic indices to the local dengue incidence. The results can
121 provide a basis of creating new and innovative approaches in controlling this mosquito
122 vector in highly urbanized or endemic areas in the Philippines.

123 **METHODS**

124 *Study area and Mosquito sampling*

125 Metropolitan Manila, a highly urbanized area, is the National Capital Region (NCR)
126 of the Philippines with an area of 636 km^2. It is located at the eastern shore of Manila Bay
127 in Southwestern Luzon (14°50′ N Latitude, 121°E Longitude), Philippines, Southeast Asia.
128 It is composed of 16 cities and 1 municipality with a total population of 12,877,253 [26].
129 This area is the most urbanized region in the Philippines being the center of the national
130 government, economy, education and culture, country's leading business center, largest
131 manufacturing location and principal port for importation and exportation [27].

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132 In this study, Metropolitan Manila was divided into 21 study areas (Figure 1 and
133 Table S1). Initially, study areas are delineated per city which represents at least one per city.
134 However, some cities comprised of either a large or small land sized area, thus designating
135 more study areas. For example, the largest city, Quezon City, in the region was divided into
136 5 study areas based on its district boundaries. In order to standardize the land size covered
137 by each study area, we merged two small neighboring cities, San Juan and Mandaluyong.
138 The map layer of the administrative city boundaries of Metropolitan Manila was
139 obtained from the Philippine Geographic Information System (GIS) Data Clearinghouse
140 for further analysis [28].

141 Households on each study area were selected based on voluntary informed consent
142 in collecting adult *Ae. aegypti* mosquitoes inside their premises. The number of households
143 per study area ranged from 2 – 14 with an average of 6 households and a total of 134
144 households. The maximum distance among households within study areas ranged from 1.39
145 km to 6.17 km. Since the households are widely dispersed within each study area, we
146 calculated the geographical midpoint (http://www.geomidpoint.com/) to assign a single
147 georeferenced location for each study area in subsequent genetic analysis. The distance
148 among study areas (midpoints) ranged from 2.85 – 39.66 km.

149 Collection of *Ae. aegypti* mosquitoes was done by installing a commercially
150 available mosquito UV Light Trap (Jocanima ©) in each household's outdoor premises for
151 3-5 days. Collected adult mosquito individuals were sorted, then identified accordingly
152 based on the pictorial keys of Rueda et al. [29] and preserved in 99% ethanol. Majority of
153 population genetic studies in *Ae. aegypti* have either used only larval or reared larval to
154 adult samples. For this reason, this could lead to a potential bias in estimating population
155 genetic parameters due to the sampling of full sibling mosquito larvae [30]. This was
156 evidence in the population genetic structure of amphibian larval samples that led to
157 inaccurate estimate of differentiation among populations when compared to adult samples

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158 [31,32]. As such, this study targets adult *Ae. aegypti* samples than conventional egg, or
159 larval samples to prevent collecting mosquito full siblings. From a collection period of May
160 2014 until January 2015, a total of 526 adult *Ae. aegypti* were collected and ranging from
161 12 to 42 individuals per study area (Table S1).

162 *DNA Extraction and microsatellite genotyping*

163 The total genomic DNA of individual mosquito sample was extracted using the
164 QIAGEN Blood and Tissue DNEasy Kit following the modified protocol of Crane [33].
165 We identified 11 microsatellites from Slotman et al. [34] and Chambers et al. [10] for
166 genotyping and grouped them accordingly into four sets for multiplex PCR (Table S2).
167 Each set consisted fluorescent labeled forward primers with different annealing
168 temperatures during the amplification process. Generally, each set composed of 1.2 µL of
169 10X buffer (TAKARA), 0.8 µL of 25 mM $MgCl_2$, 1.6 µL of 10 mM of each dNTPs, 0.6 µL
170 of 10 µM forward and reverse primers and 0.08 µL of 5.0U/ µL of Taq DNA polymerase
171 (TAKARA), 1.5 µl of 10% Dimethyl sulfoxide (DMSO) and 1 µl of template DNA
172 consisting a final volume of 10 µl. Thermocycle conditions are as follows: initial
173 denaturation step of 94 □C for 5 minutes, denaturation step of 94 □C for 30 seconds,
174 annealing step with temperature and duration (in seconds) of each primer set as indicated in
175 Table S2, extension step of 72 □C for 30 seconds following 35 cycles and a final
176 incubation step of 72 □C for 5 minutes. PCR amplicons were checked by electrophoresis in
177 3% agarose gels stained with Midori Green (Nippon Genetics) and visualized under UV
178 light using the Chemidoc XRS Chemiluminescent Gel Documentation Cabinet (BIO-RAD).
179 Prior to fragment size analyses, multiplex PCR products were diluted in 1/15 water and
180 then pooled together. 1ul of each diluted pool were added with 0.5 µl of GS 500 Liz
181 Internal Size StandardTM (Applied Biosystems, USA) and HD formamide for a total
182 volume of 20 µl. Fragment analysis of the amplified products were done using ABI 3500
183 Genetic Analyzer (Life Technologies) while genotyping is done using GeneMapper
184 (Applied Biosystems). Microsatellite data were checked for error and the presence of null
185 alleles with MICROCHECKER [35].

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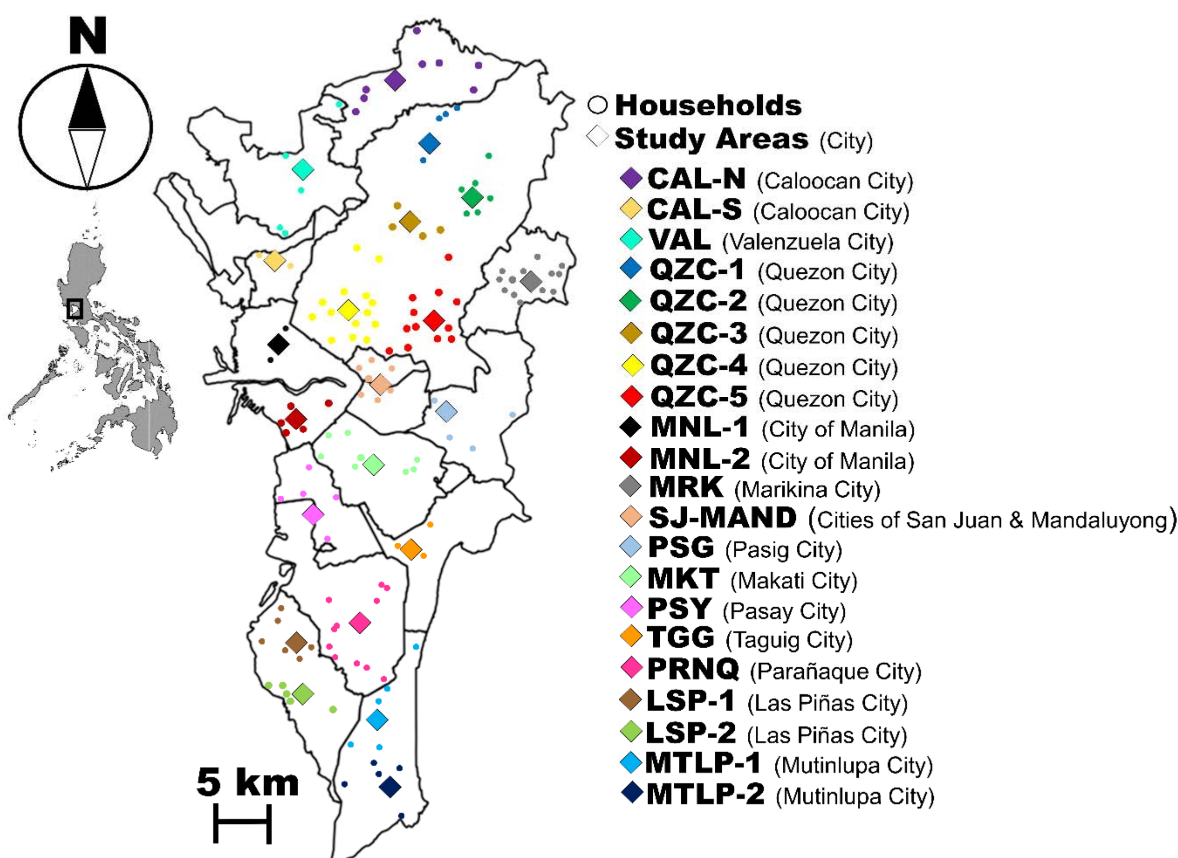

186

187 **Figure 1. Geographic midpoints of *Ae. aegypti* study areas (◊) with its corresponding**
188 **household sites (○) in Metropolitan Manila. Details of each study area can be seen in**
189 **Supplementary Table S1.**

190 The exact Hardy-Weinberg equilibrium (HWE) test and estimations of the Linkage
191 disequilibrium (LD) among all pairs of loci were conducted using GENEPOP v4.2.1
192 [36,37]. Significance levels for multiple testing were corrected using the Bonferroni
193 procedure. The number of alleles, allelic richness and private alleles were calculated using
194 HPRARE [38,39]. Observed heterozygosity (*Ho*), expected heterozygosity (*He*), inbreeding
195 coefficients (Fis) were calculated using the Genetic Analysis in Excel (GenAlEx) version

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196 6.3 [40]. To assess the magnitude of genetic differentiation among sites, pairwise F_{ST}
197 values were calculated using Arlequin v3.5.1.3 [41] with 10,000 permutations. Pairwise
198 gene flow estimates (*Nm*) among sites were manually calculated using the formula of
199 Slatkin and Barton [42] from the calculated pairwise F_{ST}. A dendrogram was constructed
200 based on the pairwise F_{ST} using the unweighted pair group with arithmetic mean (UPGMA)
201 in *fastcluster* package [43] and the optimal number of clusters were determined using the
202 cindex index in the *NbClust* package [44] from the R program [45].

203 *Genetic Structure*

204 The number of genetic clusters (K) was inferred using the Bayesian approach in the
205 software STRUCTURE v2.3.2 [46]. The admixture model was utilized where its alpha
206 value was allowed to vary, and independent allele frequencies was set at lambda equals to
207 one. Twenty (20) independent runs were performed for each value of K (1 – 15) with a
208 burn-in phase of 200,000 iterations followed by 600,000 replications. Structure Harvester
209 v0.6.93 [47] was used to determine the most likely number of clusters by calculating ΔK
210 [48]. Moreover, the software program CLUMPP v1.1.2 [49] was used to summarize the
211 results from STRUCTURE and visualized using the program DISTRUCT v1.1 [50].

212 *Isolation by Distance and Spatial Autocorrelation*

213 Pairwise geographic distances (km) among study areas and households were
214 calculated using the Vincenty's formulae [51] on Microsoft Excel 2016. To test isolation by
215 distance (IBD), pairwise F_{ST} and geographic distance (km) among study areas were
216 examined using Mantel's test of correlation with 10,000 permutations. Spatial
217 autocorrelation was performed using pairwise Nei's genetic distance among mosquito
218 individuals and geographic distance (km) among households with 10,000 permutations and
219 Bootstrap replications. Results of the permutation were considered significant at the 5%
220 level. In this analysis, a correlogram was produced with 45 distance classes at 1km interval.
221 Both analyses yielded a correlation coefficient of the two data matrices ranging from –1 to

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222 +1, with a test for a significant relationship by random permutation. All analyses were
223 performed using GenAlEx version 6.3 [40].

224 *Correlation Analysis between Genetic indices and Dengue incidence*

225 In order to calculate the dengue incidence of each study area, reported dengue cases
226 per village (*baranggay*) in 2014 were obtained from the National Epidemiology Center of
227 the Department of Health, Philippines while the population census per village were
228 acquired from the Philippine Statistics Authority agency (www.psa.gov.ph). Calculation of
229 dengue incidence was performed by dividing the number of cases to the total population
230 size for a given year multiplied by a factor of 1,000. Pearson's correlation coefficient was
231 calculated based from the computed dengue incidence and the selected population genetic
232 indices namely; Allelic richness, Private Allelic Richness, Observed heterozygosity,
233 Inbreeding coefficient and the effective population size. The correlation analysis was
234 performed using the *stats* package of the R program version 3.3.5 [45].

235 **RESULTS**

236 *Genetic Diversity*

237 We observed a total of 113 alleles across 11 microsatellite loci in 21 study areas
238 from Metropolitan Manila (Table S3). The number of alleles per loci ranged from 3 (F06)
239 to 19 (B07) with an average of 10.25 alleles per loci, suggesting that the chosen
240 microsatellites markers are highly polymorphic (Table S4). Null alleles were present in 4
241 loci (M313, AC4, AG7 and H08) and the null allele frequency ranges from 0.00 – 0.33 in
242 all loci (Table S5). For the 231 tests of HWE of each locus per study area, 91 tests showed
243 statistically significant deviation ($p < 0.05$) where 72 of these significant deviations
244 indicated He > Ho, suggesting heterozygosity deficits (Table S3). The LD test showed a
245 total of 119 of 1155 (10.30%) pairs of loci with significant LD after Bonferroni corrections.

246 Table 2 shows the summary of the genetic diversity per study area. The mean
247 number of different alleles for all study areas ranged from 3.82 (TGG) to 6.36 (QZC-3)

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248 while the mean number of effective alleles for all study areas showed to be 2.74 (QZC-1) to
249 3.51 (LSP-2). On the other hand, the mean allelic richness ranged from 3.24 (QZC-1) to
250 3.85 (MRK) for all study areas while the proportion of private alleles ranged from 0.02
251 (LSP-2) to 0.23 (PSY). Overall, all study areas except for one (MKT) did not conform to
252 Hardy-Weinberg equilibrium expectations (*He* > *Ho*), indicating heterozygosity deficits
253 and the possibility of inbreeding within each study area. The effective median population
254 size (*Ne*) across all study areas was calculated to be from 6.2 to 4,607 with two study areas
255 estimated as an infinite number.

256 **Table 2. Dengue Incidence and Genetic Diversity among 21 *Ae. aegypti* populations**
257 **based on 11 Microsatellites in Metropolitan Manila, Philippines**

Study Areas	Dengue Incidence	Allelic richness	Private allelic richness	Observed Heterozygosity	Inbreeding coefficient (Fis)	Median effective population size (*Ne*)
CAL-N	1.92	3.68	0.08	0.493	0.169	78.1
CAL-S	4.76	3.49	0.11	0.468	0.215	8.5
VAL	5.22	3.49	0.04	0.508	0.143	6.2
QZC-1	0.25	3.24	0.10	0.470	0.111	116.1
QZC-2	3.92	3.45	0.06	0.520	0.047	20.7
QZC-3	3.26	3.44	0.03	0.525	0.071	57.7
QZC-4	3.68	3.63	0.08	0.541	0.077	86.8
QZC-5	4.45	3.37	0.05	0.500	0.117	58.2
MNL-1	1.32	3.53	0.08	0.524	0.090	186.7
MNL-2	3.70	3.55	0.03	0.468	0.132	386.8
MRK	3.64	3.85	0.07	0.511	0.112	4607.1
SJ-MND	4.08	3.29	0.03	0.496	0.191	38.1
PSG	2.81	3.56	0.08	0.524	0.099	31.7
MKT	2.02	3.59	0.06	0.558	0.036	∞
PSY	3.18	3.71	0.23	0.506	0.080	477.3
TGG	3.80	3.27	0.05	0.536	0.071	24.1
PRNQ	8.74	3.78	0.04	0.564	0.076	84.7
LSP-1	8.37	3.65	0.12	0.536	0.104	38.4
LSP-2	8.95	3.44	0.02	0.432	0.296	∞
MTLP-1	8.44	3.79	0.13	0.438	0.240	79.4
MTLP-2	7.51	3.51	0.03	0.449	0.240	94

258 *Genetic differentiation and structure*

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259 The overall F_{ST} was estimated to be 0.016 and pairwise F_{ST} values between study
260 areas ranged from -0.002 – 0.054 (Table S6). With this, significant genetic differentiation
261 was demonstrated in 87 (out of 201, 41.4%) pairwise values. Pairwise gene flow (*Nm*)
262 estimates among study areas ranged from 3.404 – 290.448. 11 pairwise gene flow estimates
263 were not calculated due to the estimated negative and zero F_{ST} values. The dendrogram
264 based on the pairwise F_{ST} values revealed the spatial pattern and distribution of genetically
265 similar study areas (Figure 2). Further analysis showed that the optimal number of cluster
266 groups is 6 where 4 indicated groups of genetically similar study areas. It is shown that
267 highly genetic-similar study areas are proximal to each other. This is exemplified by
268 genetic group 2 where mosquito populations in the south area except for LSP-2 are
269 genetically similar. There are also neighboring study areas that are genetically similar such
270 as in genetic groups 3 (VAL, CAL-S), 1 (QZC-2 and QZC-4) and 4 (PSG-MRK).
271 Furthermore, the pattern of the identified genetic groups extends in long distances as
272 demonstrated in genetic groups 1 and 4.

273 Mantel test between the pairwise genetic (F_{ST}) and geographic distances of all study
274 areas showed very low and non-significant correlation (R^2= 0.01, p=0.172), indicating no
275 isolation by distance. High genetic similarity among adult mosquitoes is limited up to 1 km
276 based on the spatial autocorrelation analysis (Figure S1). This suggests the limited dispersal
277 capability of *Ae. aegypti*.

278 STRUCTURE analysis found that the most likely number of genetically
279 differentiated groups is K = 5 (Figure S2). Figure 3 shows the distribution and proportion
280 of inferred genetic cluster assignment of each mosquito individuals per study area. It is
281 observed that either 4 or all inferred genetic clusters are present in each study area.

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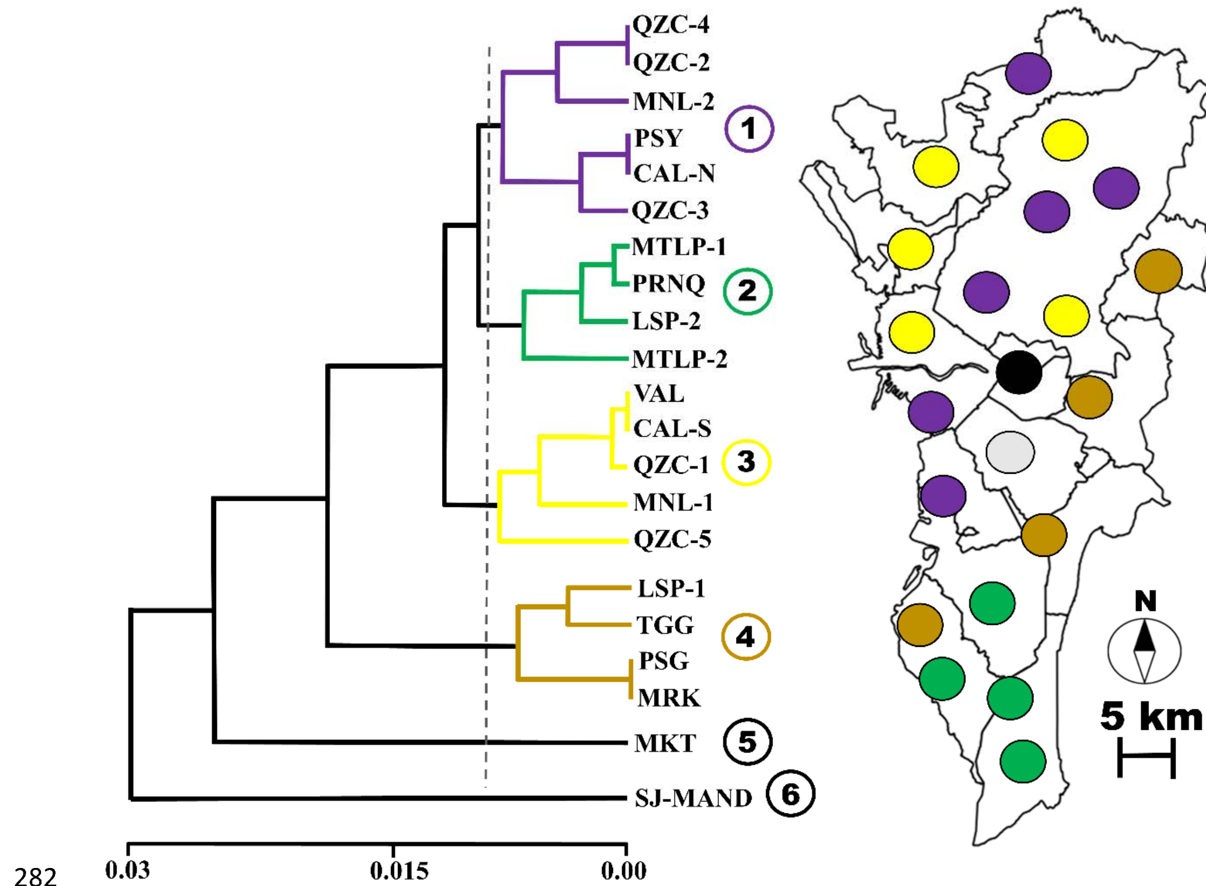

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284 **Figure 2. (a) Dendrogram showing the genetic relatedness of each study area based on**
285 **its pairwise F_{ST} estimates. Colored lines indicate the genetic groups. (b) Map showing**
286 **selected study areas in respect to their genetic group assignment. Colored circles**
287 **indicate the genetic group.**

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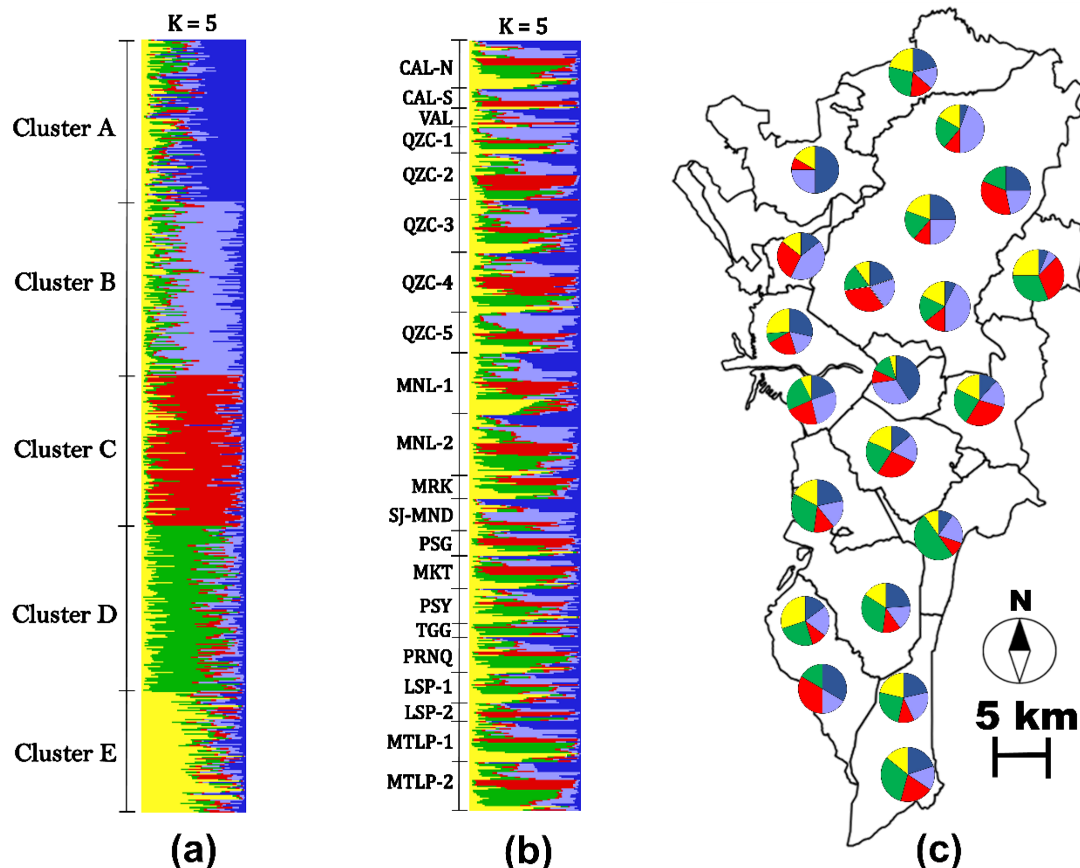

288

289 **Figure 3. Bayesian analysis (K=5) of *Ae aegypti* populations in Metropolitan Manila.**
290 **Bar plots represent the (a) genetic clusters and (b) study areas. Each individual is**
291 **represented by a single horizontal line. Brackets are shown to separate genetic cluster**
292 **or study areas. (c) Spatial map that shows the proportion of all genetic clusters to each**
293 **study area. Colors represent the estimated individual proportion of cluster**
294 **membership.**

295 *Correlation between Population Genetic indices and Dengue incidence*

296 Five genetic indices were used to correlate with dengue incidence. It revealed that
297 allelic richness ($r = 0.30$) and Fis ($r = 0.52$) showed a positive correlation to dengue
298 incidence. Among these indices, it was only Fis that showed statistical significance (Figure
299 4). On the other hand, private allelic richness ($r = -0.14$), observed heterozygosity ($r = -0.26$)
300 and *Ne* ($r = -0.10$) demonstrated a negative correlation with no statistical significance.

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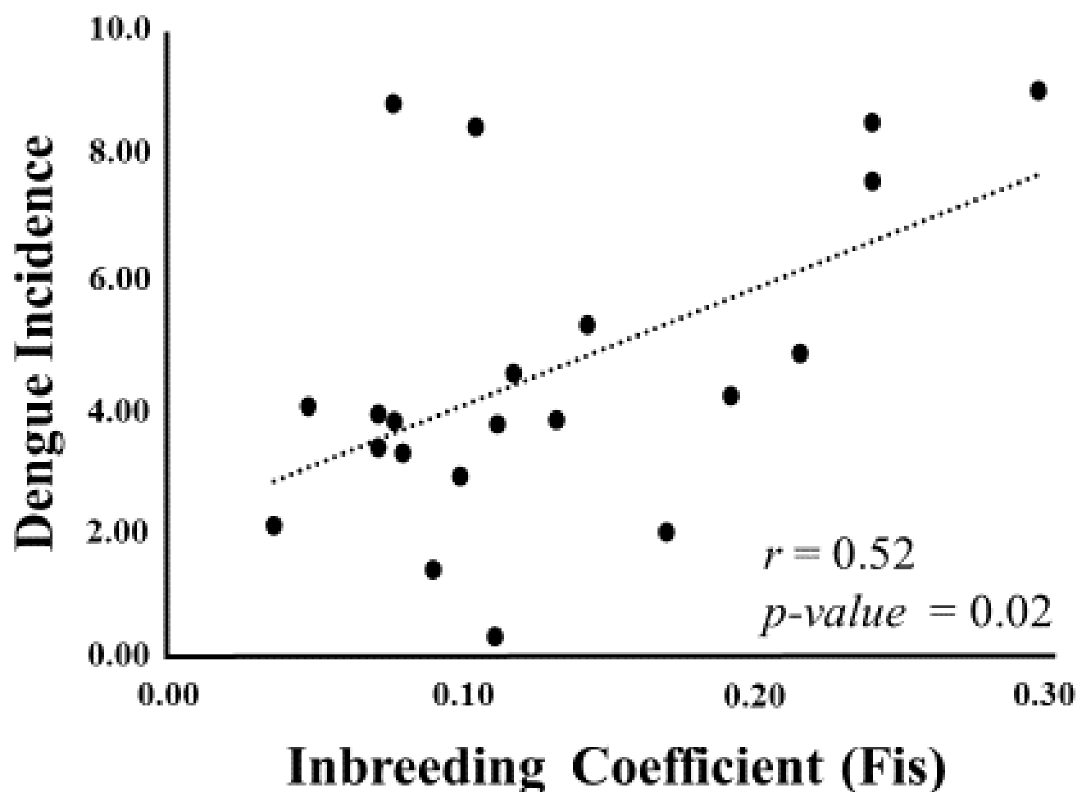

301

302 **Figure 4. Correlation of Dengue Incidence and Inbreeding coefficient (Fis) among 21**
303 **study areas**

304 **DISCUSSION**

305 *Fine-Scale Genetic Structuring and Dispersal*

306 Our study revealed low genetic differentiation among study areas (F_{ST} = 0.006 –
307 0.054) which is similar from previous population genetic studies of *Ae. aegypti* that
308 consisted of a micro-geographic or fine-scale study area. In Sao Paulo, Brazil, for example,
309 the level of genetic differentiation ranged from 0.002 to 0.094 with a maximum distance
310 among collection sites of 30 km [14]. Cities in Southeast Asian countries also showed low
311 levels of genetic differentiation from 0.026 - 0.032 with a spatial scale of 5 - 50 km [11].

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312 The same was observed among villages in Thailand with geographical distances up to 27
313 km showing an average genetic differentiation of 0.037 [13]. Our findings along with
314 previous studies suggest that mosquito populations within fine-scale areas may consist of
315 similar allele frequencies, thus, exhibit continuous and active exchange or sharing of alleles
316 among study areas. This is corroborated with the high gene flow estimates and the lack of a
317 detected signal of isolation by distance observed in the study.
318
319 Our constructed dendrogram showed that genetically similar *Ae. aegypti* mosquito
320 populations are in close proximity with each other. This clustering pattern is highly
321 exemplified in the southern cities which comprises genetic group 2 (MTLP1, MTLP-2,
322 PRNQ, LSP-2) and the eastern part of the region (MRK, PSG and TGG). It demonstrates
323 that the dispersal of *Ae. aegypti* is limited which is also supported by our spatial
324 autocorrelation analysis. We can only infer such limited dispersal could be attributed by
325 landscapes in relation to the accessibility and location of each cities. For example, the
326 southern cities which comprises genetic group 2 (MTLP1, MTLP-2, PRNQ, LSP-2) can
327 only be accessed by one major highway while majority of the total land size of eastern
328 cities (MRK, PSG and TGG) are completely separated by a major highway and a river (e.g.
329 Marikina River). Therefore, access to these southern or eastern cities, may be difficult since
330 a few roads, highways, or bridges connect it from the rest of the region. For this reason, it
331 can potentially limit (but not isolate) the continuous migration and genetic exchange of
332 mosquito populations from other study areas in the entire region. This information provides
333 the potential migration or dispersal activity of *Ae. aegypti* that can be utilized in defining
334 specific vector control zones along landscape corridors (e.g. roads) within certain group of
335 cities.
336
337 What is also notable is that genetically-similar groups can extend in long distances
338 such as observed in the northern to the central (genetic group 1) and the eastern to the
339 southern parts (genetic group 4) of the region. If one overlays the major highways and road
340 networks of Metropolitan Manila, it suggests the “passive” dispersal capability of *Ae.*

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341 *aegypti* by human-mediated transportation. It is believed that mosquito vectors occasionally
342 travel in long distances by taking advantage human-aided transportation routes via land, sea
343 or air [52,53,54] as *Ae. aegypti* eggs, larvae and adults have been found in commercial
344 trucks and ships through tire importation [55,56]. In addition, transportation zones such as
345 airports [57] and docks/ports [54,56] can be littered with larvae and pupae of the mosquito
346 vector, thus acting as the source population. Rapid urbanization (e.g. commercialization)
347 may also intensify this long distance migration or passive dispersal of *Ae. aegypti* by
348 promoting mosquito population admixture over distant areas [15,58].
349
350 Due to the high genetic similarity and migration activity observed among study
351 areas, we expected a low number of inferred genetic clusters (K=2 at the most). However, it
352 revealed a considerable number of genetic clusters (K=5). These findings are consistent
353 with previous population genetic studies with micro-geographic scales [11,14,16] and local
354 studies in the Philippines [54,59]. Furthermore, these studies reported low genetic
355 differentiation but with substantial number of inferred genetic clusters (K = 3-9). The
356 substantial number of genetic clusters in fine-scale areas may be explained by two
357 hypotheses. The first hypothesis could be the result of divergence from a single ancestry
358 and, over time, produced multiple genetic clusters in this area. It is argued that a single or
359 closely-situated houses may act as a clustering unit in forming genetically structured
360 clusters [11,52,60]. This could be due to the limited flight performance of *Ae. aegypti*
361 where it prefers to stay within a small area of about 10 – 500 meters [61] for a stable
362 breeding site and availability of blood hosts [52]. As a result of rapid urbanization, these
363 distinct genetic mosquito groups may have migrated to distant locations through "passive"
364 dispersal, establishing colonies thereafter.
365
366 To some extent, our results support the first hypothesis where it revealed the limited
367 dispersal capability of *Ae. aegypti* in short distances (up to 1 km) based from spatial
368 autocorrelation analysis and, in turn, may have generated the 5 inferred genetically
369 structured groups. Since Metropolitan Manila is considered highly urbanized with

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370 numerous transportation routes, it could have facilitated the passive dispersal of the
371 mosquito vector in distant locations based on the interpretation of high gene flow activity
372 among the study areas. However, limited dispersal cannot only be the single factor that can
373 explain the occurrence of multiple clusters. Without high mutation, the mosquito
374 population cannot diversify within a small spatial scale for a short evolutionary time. Hence,
375 the second hypothesis infers that it could be due to the immigration of mosquito
376 populations from neighboring regions or provinces of Metropolitan Manila, but a larger
377 spatial scale genetic data is needed to test this hypothesis.
378
379 *Correlation of Genetic indices towards Dengue incidence*
380
381 Among the genetic indices, we expected the estimated population size (*Ne*) to show
382 a significant and positive correlation. However, it resulted in a non-significant and negative
383 relationship with dengue incidence. In our study, collecting adult mosquito samples was the
384 ideal choice to perform a better estimation of the effective population size (*Ne*). Previous
385 population genetic studies conducted their sampling using either only larval samples or
386 larval samples reared to adult stages but majority of mosquito larvae do not become adults
387 in the natural setting [62]. Thus, collecting larvae to estimate the effective population size
388 of the adult may be deemed inappropriate.
389
390 One possible reason of detecting no correlation between *Ne* and dengue incidence
391 may be that the calculated *Ne* is either under- or over-estimated. Estimating the precise *Ne*
392 of natural populations can be difficult, especially if there is a large time interval between
393 sampling points or temporal disruptions such as migration or population replacement. It has
394 been demonstrated that lower *Ne* estimates are generated by the presence of temporal
395 disruptions while large intervals in sampling points generate higher *Ne* estimates [23].
396 Nevertheless, the calculated *Ne* estimates of the study is consistent with those calculated by
397 Saarman et al. [23] from mosquito populations worldwide. But further and thorough

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398 investigation should be performed in the future to correctly estimate the *Ne* and its
399 correlation with dengue incidence.
400
401 Notably, the correlation between dengue incidence and the inbreeding coefficient
402 (Fis) revealed a positive and significant correlation (r =0.52, p =0.02). One plausible
403 mechanism may be due to the transovarial or vertical transmission of the dengue virus to its
404 succeeding mosquito offspring. This viral transmission process has been demonstrated in
405 field-collected *Ae. aegypti* mosquitoes until its F2 generation from Quezon City,
406 Metropolitan Manila [63]. Earlier studies also revealed that the transovarial route can
407 sustain the viral infection up to the 15th generation [64]. Therefore, we infer that the
408 inbreeding within the mosquito population may result in producing a substantial number of
409 next generation mosquitoes carrying the dengue virus. This suggests that dengue virus
410 infection is not only maintained by the human-vector cycle but also within mosquito
411 generations. Because of such mechanism, it can possibly lead to an increased disease
412 transmission within local areas. It should be known there are limitations in our
413 interpretation of this correlation. First, no viral detection was done to each individual *Ae.*
414 *aegypti* mosquitoes to ascertain their viral transmission capability. Performing this
415 endeavor could provide the necessary credence that can strongly support such correlation.
416 Secondly, it is unclear whether the dengue virus infection in the human population
417 originated from the exact study area. It has been suggested that dengue infections in the
418 human population especially in urbanized areas can be obtained from other locations such
419 as public spaces, schools or workplaces rather than their place of residence [65,66,67,68].
420
421 Nonetheless, this is the first attempt to directly link mosquito population genetic
422 data to epidemiological data. Although several previous studies signified the application of
423 population genetics towards vector control, they only reported the mosquito gene flow
424 pattern and the association of mosquito genetic structure and its landscape [54,69,70]. Our
425 findings may provide future implications in predicting or identifying high dengue risk areas
426 where the genetic index (e.g. *F*is) can be utilized as a supplementary index with

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427 conventional mosquito-based indices. But further research is needed to ascertain on how
428 these genetic indices can directly influence the local dengue epidemiology.

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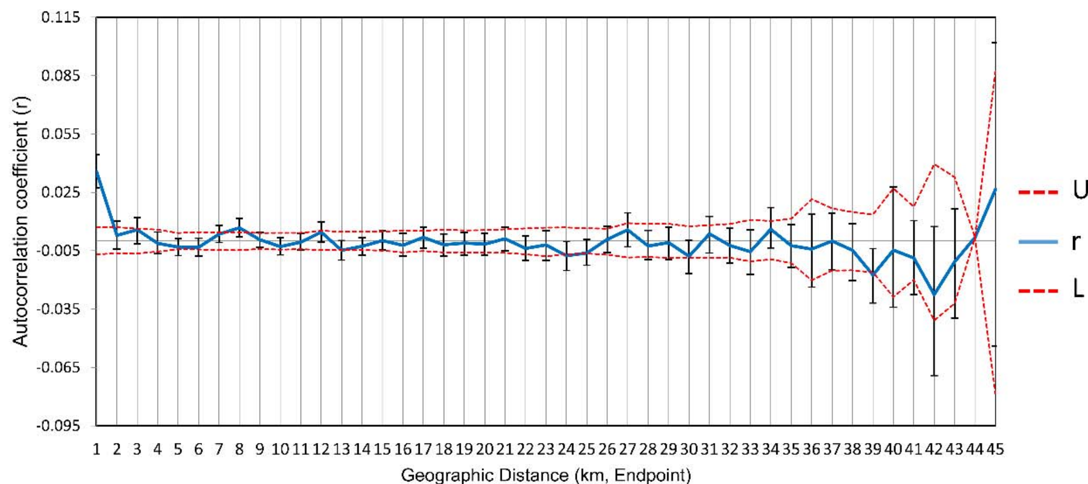
632

633 **SUPPORTING INFORMATION**

634



635

636 **Figure S1.** Correlogram of spatial autocorrelation showing the coefficient (r) up to 45 km
637 with 1km intervals. U and L are upper and lower confidence interval limit respectively.

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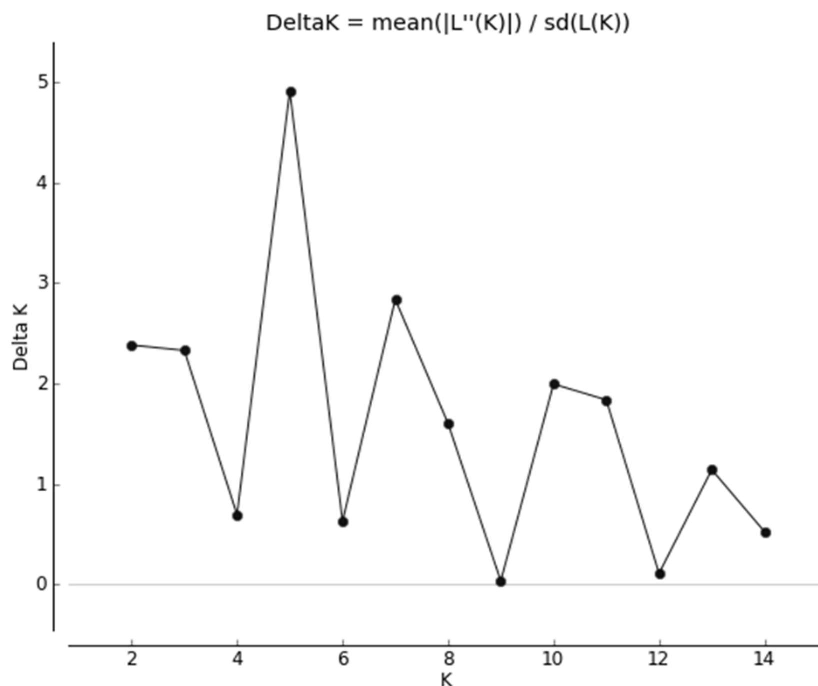

638

639 **Figure S2.** Graph of ΔK against K showing K = 5 as the probable number of genetic
640 clusters

641 **Table S1.** *Ae aegypti* collection sites and population size in Metropolitan Manila,
642 Philippines

643 **Table S2.** List and Characteristics of Microsatellites markers in *Ae. aegypti* used in this
644 study

645 **Table S3.** Analysis of the Genetic Diversity of *Ae aegypti* using 11 microsatellite loci

646 **Table S4.** Null allele frequency estimates per locus per *Ae aegypti* populations

647 **Table S5.** Allele frequencies of the 11 microsatellite loci in *Ae aegypti* populations in
648 Metropolitan Manila

649 **Table S6.** Genetic differentiation using F_{ST} (below diagonal) and Gene flow (*Nm*) (above
650 diagonal) of *Ae aegypti* populations in Metropolitan Manila, Philippines