New and common haplotypes shape genetic diversity in Asian tiger mosquito populations from Costa Rica and Panamá

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Abstract

The Asian tiger mosquito *Aedes albopictus* (Skuse) (Diptera: Culicidae) is a vector of several human pathogens. *Ae. albopictus* is also an invasive species that, over recent years, has expanded its range out of its native Asia. *Ae. albopictus* was suspected to be present in Central America since the 1990s, and its presence was confirmed by most Central American nations by 2010. Recently, this species has been regularly found, yet in low numbers, in limited areas of Panamá and Costa Rica (CR). Here, we report that short sequences (~558bp) of the mitochondrial COI and ND5 genes of *Ae. albopictus*, had no haplotype diversity (HD). Instead, there was a common haplotype for each gene in both CR and Panamá. By contrast, a long COI sequence (~1390bp) revealed that HD (± S.D.) was relatively high in CR (0.72 ± 0.04) when compared with Panamá (0.33 ± 0.13), below the global estimate for reported samples (0.89 ± 0.01). The long COI sequence allowed us to identify 7 (5 new) haplotypes in CR and 2 (1 new) in Panamá. A haplotype network for the long COI gene sequence showed that samples from CR and Panamá belong to a single large group. The long COI gene sequences suggest that haplotypes in Panamá and CR, although similar to each other, had a significant geographic differentiation (K_{st}=1.33, P<0.001). Thus, most of our results suggest a recent range expansion in CR and Panamá.

Key-Words: Mitochodrial COI, ND5, *Aedes albopictus*, invasive species, dengue vectors

45 The Asian tiger mosquito**,** *Aedes albopictus*, is an invasive insect species
46 that has been expanding globally in the last 150 years (Lounibos 2002, Benedict
47 et al. 2007, Bonizzoni et al. 2013). Its successful expansion is mainly due to its
48 desiccation tolerant eggs and adaptation to small aquatic habitats (Lambrechts
49 et al. 2010). These characteristics allowed *Ae. albopictus* to inhabit artificial
50 water containers that promote its close interaction with humans (Bonizzoni et al.
51 2013).Moreover, *Ae. albopictus* aggressive biting behavior (Ponlawat and
52 Harrington 2005) and vectorial competence, allow its females to transmit a wide
53 array of arboviruses (Benedict et al. 2007, Paupy et al. 2009), most notoriously
54 dengue virus (Lambrechts et al. 2010) and Chikungunya virus (Paupy et al.
55 2009). Moreover, for dengue virus, mosquitoes can get infected vertically, i.e.,
56 without involving vertebrate hosts (Martins et al. 2012).
57 Several studies have suggested the geographical origin of *Ae. albopictus* to
58 be in Southeast Asia (Hawley et al. 1987, Khambhampati et al. 1991, Rai 1991,
59 Porretta et al. 2012) from where it likely invaded, mainly by means of maritime
60 trade, most of East Asia before the end of the 19th century (Lounibos 2002).
61 Nevertheless, *Ae. albopictus* gained notoriety in the 1980s, after becoming
62 established in Harris County, Texas, USA, where it became a dominant vector
63 species in the Houston area (Sprenger and Wuithiranyagool 1986). Subsequent
64 molecular genetics studies, and additional ecological evidence, suggested
65 Japan as a likely place for the origin of this infestation (Hawley et al. 1987,
66 Khambhampati et al. 1991, Rai 1991, Lounibos 2002, Bonizzoni et al. 2013).

The detection of the Asian tiger mosquito in USA was not a mere description of a range expansion, it highlighted how the expansion and establishment of this species, like many other invasive species, has been driven by the intensification of global commodity trade (Bonizzoni et al. 2013), first by its detection at seaports (Eads 1972) and its subsequent detection and establishment at the final destination of trade commodities (Reiter and Darsie 1984, Sprenger and Wuithiranyagool 1986). For example, *Ae. albopictus* was already present in Albania in the late 1970s, a time when Albania was the main European commercial partner of China, a country within the native range of *Ae. albopictus* (Adhami and Reiter 1998). Similarly, in the mid1980s the species was detected in São Paulo, Brazil (Forattini 1986), the economic heart of South America. Currently, *Ae. albopictus* has spread over Europe, Oceania, and reports of its presence/establishment all over Africa are becoming increasingly common,with extensive documentation of trade playing a major role on *Ae. albopictus* expansion (Bonizzoni et al. 2013).

In the New World, *Ae. albopictus* spread to Mexico by the early 1990s (Rai 1991, Lounibos 2002) was suspected in most Central American countries by the late 1990s (Eritja et al. 2005), with all countries confirming its presence by 2010 (Bonizzoni et al. 2013).

In Panamá, *Ae. albopictus* was first detected in 2002, in the “24 de diciembre” neighborhood of Panamá city (ICGES 2003). According to dengue entomological surveys from Panamá’s Ministry of Health, *Ae. albopictus* has

been mainly found in urban settings (Espino et al. 2011, Díaz 2012). Nevertheless, from 2002 *Ae. albopictus* has been monotonically increasing its abundance, having a house index close to 0.5 % in 2013 (Díaz 2012). In Costa Rica (CR), *Ae. albopictus* larvae were first recorded during 2007 in coconut shells at Siquirres, in the Atlantic basin of CR (Marín et al. 2009). Incipient *Ae. albopictus* populations, i.e., persistent but low densities per trapping effort, only have been observed in rural settings in the Atlantic basin of CR (Marín et al. 2009, Calderón Arguedas et al. 2012, Marín Rodríguez et al. 2013). *Ae. albopictus* has not been detected in the Central Valley, and is rare across urban and rural settings in the Pacific basin of CR (Morice Trejos et al. 2010).

The ecology of *Ae. albopictus* in CR and Panamá suggests that populations in these two nations, although not established in the sense of widespread infestations like the ones observed in Harris County, TX, USA in the mid 1980s (Sprenger and Wuithiranyagool 1986) and everywhere else the tiger mosquito is now established (Bonizzoni et al. 2013), are incipient, given their persistence, yet in low abundance, at specific locations. It is unclear whether this reflects the relative recent invasion of these territories by *Ae. albopictus*. This hypothesis can be tested with tools from molecular genetics, where a recent invasion would be more likely associated with low genetic diversity (Avise 1994). From a broader ecological perspective, it is expected that Panamanian *Ae. albopictus* populations potentially have a greater genetic diversity given the fundamental role the Panamá Canal plays in global commerce, with ships containing goods

111 from all over the world regularly crossing it (Llacer 2005), and needing to spend
112 at least three days within Panamanian territory. This situation poses a potentially
113 higher propagule pressure (Lounibos 2002) in Panamá than CR, i.e., the
114 recruitment of new individuals from abroad which can contribute unique genetic
115 material is more likely to occur in Panamá, thus leading to the expectation of
116 more introgressions, and perhaps haplotype diversity, in Panamá than CR,
117 considering that both countries have similar strategies for dengue mosquito
118 control.

119 Mitochondrial DNA genes are ideal genetic markers to test hypothesis about
120 ancestry and demographic changes in populations (Avise 1994), due to their
121 lack of recombination, uniparental inheritance, high mutation and nucleotide
122 substitution rates, and the well-defined effective population size of ¼ nuclear
123 genes (Avise 1994, Birungi and Munstermann 2002, Usmani-Brown et al. 2009).
124 Moreover, mitochondrial genes have been frequently used in studies seeking
125 inferences about the genetic relationships of *Ae. albopictus* (Birungi and
126 Munstermann 2002, Mousson et al. 2005, Usmani-Brown et al. 2009, Delatte et
127 al. 2011, Kamgang et al. 2011, Porretta et al. 2012, Raharimalala et al. 2012,
128 Navarro et al. 2013, Zhong et al. 2013) therefore making easy the comparison
129 with samples from several places in the globe. Here, we thus report: (i)
130 haplotypes for the ND5 and COI Mitochondrial genes present in incipient *Ae.*
131 *albopictus* populations of CR and Panamá,(ii) analyze mitochondrial COI and
132 ND5 sequences of samples from Costa Rica and Panamá, to explore genetic

differences between mosquitoes from these neighboring countries and propose a possible geographical origin of *Ae. albopictus* populations in CR and Panamá, nations outside the original native range of this invasive insect. In our analysis we also considered two non-independent sequences of the COI gene, a short (558 bp) and a long (1390 bp), where the short sequence is embedded within the longer sequence, to increase the precision of genetic structure estimates in *Ae. albopictus* from Panamá and CR.

Materials and Methods

Mosquito Sampling

Mosquitoes were collected in CR by Departmento de Control de Vectores, Ministerio de Salud, CR, at an organic pineapple farm “Finca Corsicana” located in La Virgen de Sarapiquí, Sarapiquí county, Province of Heredia, CR (10° 26’ 03.80” N, 84° 07’ 14.75” W). This farm has had a persistent infestation by *Ae. albopictus*, probably associated with the pesticide-free nature of its agricultural production. For the collection three CDC backpack aspirators (Clark et al. 1994) were operated by personnel of CR’s Ministry of Health. After a total of six hours of operation, we collected 58 adult females on a surface of 1.6 hectares of land cultivated with pineapples, surrounded by patches of tropical rainforest, the native vegetation of the area. The sampling was performed in December 2012.

In Panamá mosquitoes were collected in urban areas from July to September 2012 by personnel from the Departamento de Control de Vectores, Ministerio de Salud, República de Panamá. Mosquitoes were collected in Chepo

155 (9º 9’ 52” N, 79º 5’ 43.37” W), Province of Panamá and Arco Iris (9º 20’ 21.39” N,
156 79º 53’ 26.80” W), Province of Colón, all locations with persistent infestations by
157 *Ae. albopictus* (Espino et al. 2011). Assuming a house index of 0.5% a total of
158 10057 houses were surveyed, expecting to find around 50 houses with *Ae.*
159 *albopictus*.

160 In Panamá, the sampling procedure was performed following the Ministry of
161 Health protocol for dengue entomological surveillance. Briefly, trained crews
162 visited randomly selected households in each location and collected all
163 containers with larval mosquitoes. These containers were then processed by the
164 ICGES Department of Medical Entomology, where all larvae from a positive
165 container were transferred into a 1 L container with 1 g of yeast as food source.
166 Pupae from the surveys and containers were then transferred to emergence
167 containers kept at 25 °C and with an 80-90% relative humidity. For the molecular
168 analysis we considered a single individual per positive house, totaling three from
169 Chepo and 15 from Arco Iris.

170 In both Panamá and Costa Rica, adult mosquitoes were killed by flash
171 freezing the individuals at -5 °C, before an identification based on morphological
172 characters (Rueda 2004). Morphological characters included, the narrow white
173 medial longitudinal stripe on the scutum, but also other major morphological
174 characters; the V-shaped patch of white scales on the mesepimeron, the lack of
175 white scales on the clypeus, the white transverse bands on the anterior
176 abdominal terga and the complete white rings in the last tarsal segment of tarsus

III. For preservation, mosquitoes were kept in ethanol at 99 % shortly after the identification process.

DNA extraction, PCR amplification and sequencing

We analyzed 58 adult females from Costa Rica and 18 adults from Panamá (two females and one male from Chepo, and 15 females from Arco Iris) for molecular analysis. Three legs from each adult were placed in a 1.5-ml PCR reaction tube. Each sample was homogenized in a mixture of extraction solution (20 µl) + tissue preparation solution (5 µl) (REDExtract-N-AmpTM Tissue PCR Kit; SIGMA, St. Louis, MO, USA) for DNA extraction. The solution was heated at 95 ºC for 3 min and neutralized (Kawada et al. 2011).

PCR amplification targeted two mtDNA gene fragments: a 406bp fragment of NADH dehydrogenase subunit 5 (ND5) and a 1390bp fragment of cytochrome-oxydase subunit 1 (COI) excluding primer sequences. One primer set for ND5 and two primer sets for COI were used: for ND5, ND5albof (5'-TCCTTAGAATAAAATCCCGC-3') and ND5albor (5'-GTTTCTGCTTTAGTTCATTCTTC-3') (Birungi and Munstermann 2002); for the upstream COI, albo1454F (5' GGTCAACAAATCATAAAGATATTGG 3') and albo2160R (5' TAAACTTCTGGATGACCAAAAAATCA 3'); for the downstream COI, albo2027F (5' CCCGTATTAGCCGGAGCTAT 3') and albo2886R (5' ATGGGGAAAGAAGGAGTTCG 3') (Zhong et al. 2013). Each 10 µl of master mix contained 1 x PCR buffer, 0.2mM dNTP, 0.2 µM each primer and 0.25 unit of

199 TaKaRaExTaq, and 1μl of template DNA. The temperature profile for ND5
200 consisted of an initial denaturation at 98 ºC for 5 min, followed by 35 cycles at 95
201 ºC for 1 min, 60 ºC for 1 min, 72 ºC for 1 min 30 s, then final extension at 72 ºC
202 for 3 min. The profile for both primer sets of COI consisted of a 94 ºC for 3 min as
203 initial denaturation, followed by 35 cycles of 94 ºC for 30 s, 55 ºC for 30 s, 72 ºC
204 for 1 min, then final extension at 72 ºC for 6 min (Zhong et al. 2013). To confirm
205 amplification, 4 μl of the PCR products were mixed with 2 μl of EZ-Vision
206 (Amresco Inc., USA) and loaded for electrophoresis with 2% agarose gel. The
207 bands were visualized with an UV transilluminator. When the amplification was
208 confirmed, remaining PCR products (approximately 5 μl) were treated with 0.2 μl
209 of ExoSAP-IT (Affymetrix, Inc., CA) for 30 min at 37 ºC followed by 15 min at 80
210 ºC in a thermal cycler.

211 The purified products were sequenced using BigDye Terminator v3.1 Cycle
212 Sequencing Kit (Applied Biosystems, CA). Each reaction mix contained 0.5 μl of
213 Big Dye terminator, 1.8 μl of 5x sequencing buffer, 0.2 μM of forward or reverse
214 primer used at PCR amplification, and 1 μl of purified PCR products. The
215 reaction consisted of an initial denaturation step for 1 min at 96 ºC, followed by
216 25 cycles of 10 s at 96 ºC, 5 s at 50 ºC, and 4 min at 60 ºC. Sequencing reaction
217 products were purified by ethanol precipitation method and dissolved in 10 μl of
218 Hi-Di^{TM}Formamide (Applied Biosystems). The product was denatured at 95 ºC
219 for 2 min and rapidly cooled on ice, and upstream and downstream sequences
220 were analyzed on an ABI 3730 or ABI 3130 automatic sequencer (Applied

Biosystems).We separated the 1390 bp into upstream and downstream sequences, and amplified them separately. Since the amplified regions overlapped, we connected the two streams to build the sequence for each sample.

Data analysis

Processing of ND5 and COI sequences

ND5 and long COI gene sequences obtained from our mosquito samples were manually aligned using MEGA 5.2.1 (Tamura et al. 2011). Newly obtained long COI sequences were trimmed at both ends and rearranged into short sequences (short COI, 558bp) consistent with those presented by Mousson et al. (2005) excluding the primer nucleotides. Haplotypes from long COI (1390 bp) sequences presented by Zhong et al (2013) were considered into subsequent analyses and also trimmed into short COI sequences for haplotype diversity comparison. Published ND5 and short COI haplotype sequences were obtained from the GenBank and ND5 sequences not present in the GenBank were extracted from a report by Navarro et al (2013). COI sequences longer than 500bp were selected for the short COI analysis. Each haplotype was identified by calculating the number of different sites between each sequence pair using MEGA 5.2. This allowed us to identify sequences sharing the same exact haplotype. Haplotype codes for ND5, short and long COI sequences are presented in the online only supplementary Tables S1, S2 and S3, respectively.

243 *Haplotype Networks*

244 Haplotype networks were built with the statistical parsimony algorithm
245 implemented in TCS (Clement et al. 2000). Haplotype networks show haplotype
246 frequencies in each population and their relatedness, which is useful information
247 to infer the plausible geographical origin of a population. It is expected that
248 ancestral populations should have a larger allele diversity than colonizing
249 populations, which are expected to exhibit the loss of rare haplotypes or to
250 present new haplotypes linked to the likely ancestral haplotypes (Clement et al.
251 2000). For the analyses we considered the frequency of haplotype reported in
252 previously published studies used for comparison. Gaps were treated as missing
253 data, and the parsimony threshold probability was set at 0.95%.

254 ***Population genetic analyses with long COI gene sequences***

255 The long COI sequences of CR, Panamá and the 12 populations studied by
256 Zhong et al. (2013)were used to estimate several parameters useful to describe
257 the genetic structure of *Ae. albopictus*.

258 A first group of parameters assessed molecular diversity. These included:
259 the number of polymorphic sites, haplotype diversity (Hd) and nucleotide
260 diversity (π).

261 A second group of parameters assessed the genetic structure of the studied
262 samples. We studied pairwise geographical subdivision in our samples with the
263 K_{st} statistic, a statistic able to detect geographic differentiation with just ten
264 samples per locality (Hudson et al. 1992). Significance of the K_{st} was tested

265 through Markov Chain Monte Carlo, MCMC (1000 replications).Neutrality tests
266 were conducted via the estimation of Tajima's D, a test for population expansion
267 (Tajima 1989). Briefly, Tajima's D has a null hypothesis of neutral variation when
268 it is not different from 0, and alternative hypotheses of: (i) a recent population
269 bottleneck (or contraction) when it is significantly positive or (ii) a recent
270 population expansion when it is significantly negative (Tajima 1989).
271 All population genetic parameters mentioned in this section were estimated
272 with the software DnaSP5.10 (Librado and Rozas 2009).
273
274 **Results**
275 *Haplotype Diversity*
276 We were able to successfully sequence 57 samples from CR and 16
277 samples from Panamá for the ND5 and COI gene sequences. For ND5 all our 73
278 samples had a unique haplotype NH3 (Table S1). This haplotype had the same
279 sequence of haplotype 3 from a previous study (Birungi and Munstermann 2002),
280 which is globally widespread (Table S1).
281 All samples from CR and Panamá had a unique short COI haplotype, SH03
282 (Table S2). This haplotype had the same sequence of H3 in Mousson et al.
283 (2005), which is globally widespread. In contrast, the long COI sequence (1390
284 bp) revealed 7 haplotypes in CR. Five of them (H67-H71) were new haplotypes,
285 and the remaining two, H17 and H37, had already been described (Table S3). In
286 Panamá, H37 and a new haplotype, H72, were observed, thus totaling 2

haplotypes (Table S3). Accesion codes for the new haplotypes are presented in Table 1. Haplotype and nucleotide diversity for the long COI gene were larger in CR than Panamá, and when compared with the diversity observed in other areas, they were low in Panamá, but relatively high in CR (Table 2).

A total of 18 haplotypes were identified when combining data on short COI haplotypes from Zhong et al. (2013) and our samples from CR and Panamá (SH1-SH18, Table S2). In contrast, the long COI sequences for the same data had a total of 72 haplotypes (Table S3).The number of haplotypes from the long COI sequence was linearly correlated with the number of haplotypes from the short COI sequence, the slope of a linear regression, *b*, not being different from one but with an intercept, *a*, different from zero when there is one haplotype (Fig. 1). This result indicates that, as expected, the number of haplotypes increased linearly with sequence length and proportionally with the number of short sequence haplotypes. Fig. 1 also highlights that for CR, given the large number of mosquito samples, the number of haplotypes was unusually large for the long COI sequence, reflecting that haplotype number has an error expected to be proportional to sample size. The linear regression was able to explain 47% of the variability in the relationship between the number of long and short COI haplotypes (Fig. 1).

Haplotype Networks

The long COI network showed the five newly identified haplotypes from CR were placed near each other and where connected with H17 and H37, which are

309 relatively widespread haplotypes (see online only Table S3). H37 was a
310 haplotype shared with Panamá. A new haplotype found in Panamá, H72, was
311 linked with the most common haplotype, H03 but not with H37. We did not
312 generate haplotype networks for ND5 and the short COI gene sequence given
313 their lack of diversity.

314 *Population Genetic Structure*

315 The pairwise K_{st} tests (Table 3) showed that all population pairs were
316 significantly differentiated (K_s =1.673, K_{st}=0.348, P<0.001).Tajima's D based on
317 the long COI sequences suggest that populations in both Costa Rica (D=1.43,
318 P>0.05) and Panamá (D=0.23, P>0.005) were in genetic equilibrium and neither
319 expanding or contracting, as expected under the neutral mutation hypothesis
320 once a population is established (Tajima 1989)

321 **Discussion**

322 Our phylogeographic analysis revealed some interesting patterns about *Ae.*
323 *albopictus* in Panamá and CR. The first conclusion is that mosquitoes belong to
324 a large group, which based on inferences from ND5 and short COI sequences,
325 represent the most common and widespread haplotypes reported for each of
326 those two gene sequences (Birungi and Munstermann 2002, Mousson et al.
327 2005, Usmani-Brown et al. 2009, Raharimalala et al. 2012). The increased
328 accuracy in the inferences brought by the use of long COI sequences, on the
329 one hand supports that *Ae. albopictus* in CR and Panamá, belongs to a large
330 group of haplotypes. Haplotypes in Panamá and CR were closely related with

each other, one of the eight haplotypes found in our samples being common in the two countries, H37, and also in the two Panamanian sampling locations. The five newly identified haplotypes from CR were placed near each other, and these haplotypes linked groups 2 and 3 from Zhong et al.(2013), which likely emerged as an artifact of sample absence from the Middle USA and Central America. Two haplotypes from CR (H17 and H37) were shared with other countries. H17 was mainly found in Taiwan and H37 was in New Jersey and Texas, USA. The two most common haplotypes, H67 and H68 collected from CR were directly connected to H17 and H37, respectively. These results suggest the Costa Rican *Ae. albopictus* population to be closely related with populations from Taiwan and Eastern USA. By contrast, H72, a new haplotype found in our samples from the Atlantic basin of Panamá (Arco Iris, Provincia de Colón) was linked with H03, the most common haplotype reported for long COI sequences, which has been found in China, Japan, Taiwan, Italy and the west coast of USA (Zhong et al. 2013). Nevertheless, H03 was not present in our samples from Panamá and CR. The lack of connection between the two Panamanian haplotypes *Ae. albopictus* suggests that introgression of this mosquito into Panamá occurred two times (Clement et al. 2000), H72, being a haplotype whose spread might be limited to the Atlantic basin of the country. In this sense our results partially support the expectation of more likely introductions in Panamá, as expected under a higher propagule pressure (Lounibos 2002). Nevertheless, although more than 10000 houses were sampled in Panamá, *Ae. albopictus* was present in only 18, of

353 which only 16 samples were analyzable, thus rendering impossible more
354 statistically powerful comparisons about diversity that would have benefited from
355 a larger sample size from Panamá.
356 *Ae. albopictus* populations in Panamá and CR are likely not expanding, and in a
357 genetic equilibrium, as indicated by a Tajima's D not different from 0 (Tajima
358 1989). The K_{st} analysis showed that all populations were differentiated, even
359 samples from CR and Panamá.
360 A limitation of our study was the heterogeneity in the mosquito sampling
361 protocol, which ultimately reflects different procedures for entomological
362 surveillance by the Costa Rican and Panamanian Ministries of Health.
363 Nevertheless, this limitation came at the expense of cooperation for a better
364 understanding of the phylogeography of a medically and economically important
365 invasive mosquito vector species, *Ae. albopictus*. Although detected in both CR
366 and Panamá, *Ae. albopictus* has not been directly implicated in dengue
367 transmission in any of the two countries (Morice Trejos et al. 2010, Espino et al.
368 2011, Marín Rodríguez et al. 2013).Nevertheless, a study from Panamá City
369 found up to 47% of *Ae. albopictus* pools positive to Serotype 2 Dengue virus,
370 employing molecular markers for Flavivirus and RT-PCR (Espino et al. 2011),
371 highlighting the potential for this mosquito species to become a major Dengue
372 virus vector in Central America.
373 The new vector control strategies targeting both *Ae. albopictus* and *Ae.*
374 *aegypti* might increase the costs for epidemic containment (Vazquez-Prokopec

et al. 2010) and require a better understanding of *Ae. albopictus* ecology in the neotropics, mainly to improve entomological surveillance and control practices exclusively designed for *Ae. aegypti* (Morice Trejos et al. 2010, San Martín et al. 2010, Díaz 2012). Also, preliminary studies on *Ae. albopictus* larval ecology have shown its co-occurrence with many mosquito species unique to the Neotropics, e.g., *Limatus durhanmi* Theobald, *Haemagogus regalis* Dyar and Knab, *Trichoprosopon compressum* Lutz among other species with a wider distribution (Marín et al. 2009, Calderón Arguedas et al. 2012), and inquiring about the potential of these species to interact or even regulate the expansion of *Ae. albopictus* is a clear research priority. This is especially necessary in light of the need to control *Ae. albopictus* in a pesticide free manner, given the added value of organically grown products (Perfecto and Vandermeer 2008), like the pineapples of the farm where Costa Rican samples were collected. Similarly, modeling *Ae. albopictus* population dynamics in tropical environments is necessary to untangle any role of climate change that may be playing in the expansion of *Ae. albopictus* and its interaction with *Ae. aegypti* (Tsuda and Takagi 2001, Chaves et al. 2012, Chaves et al. 2014).

Finally, our study highlights the need to further strengthen the regional cooperation in Central America to monitor the potential impacts of *Ae. albopictus* in the changing epidemiological patterns of dengue transmission, and to also formulate new control methods aimed at tackling the challenges that arise from the co-occurrence of *Ae. albopictus* and *Ae. aegypti* in a dengue endemic

multinational region.

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Figure legends.

Fig. 1 Haplotype number for the long COI gene as function of the short COI gene by sampled location. For site code see Table 2. In the plot character size is proportional to the number of sampled mosquitoes and the solid line indicates the estimated linear regression. The regression equation is presented inside the plot, where the number of long sequence COI haplotypes (NCOI (1390 bp)) is a function of the number of short sequence COI haplotypes (NCOI (558 bp)) minus one. The one is subtracted from NCOI (558 bp) in order to interpret the intercept as the NCOI (1390 bp) when there is one haplotype in NCOI (558 bp). Parameter estimates for the intercept, *a*, the slope, *b*, and the error variance VAR(ε), are also presented in the figure. The error was assumed to be normally distributed. In the linear regression weights proportional to the inverse of the samples used to estimate the number of haplotypes were used, following the assumption that haplotype sampling has an error proportional to sample size.

Fig. 2 Haplotype network based on mitochondrial COI (1390bp) of *Aedesalbopictus* with all individuals collected in Costa Rica, Panamá and 12 populations reported by Zhong et al. (2013). Small black dots indicate hypothetical haplotypes not observed across the samples. Circle size is proportional to haplotype frequency, lines between haplotypes indicate a mutational change. For haplotype codes, indicated by an H followed by two numbers, and population codes, indicated with colors in the figure, please refer to the text and Table 2, respectively. Further details about haplotype codes are presented in the online only Table S3.

Figure 1.

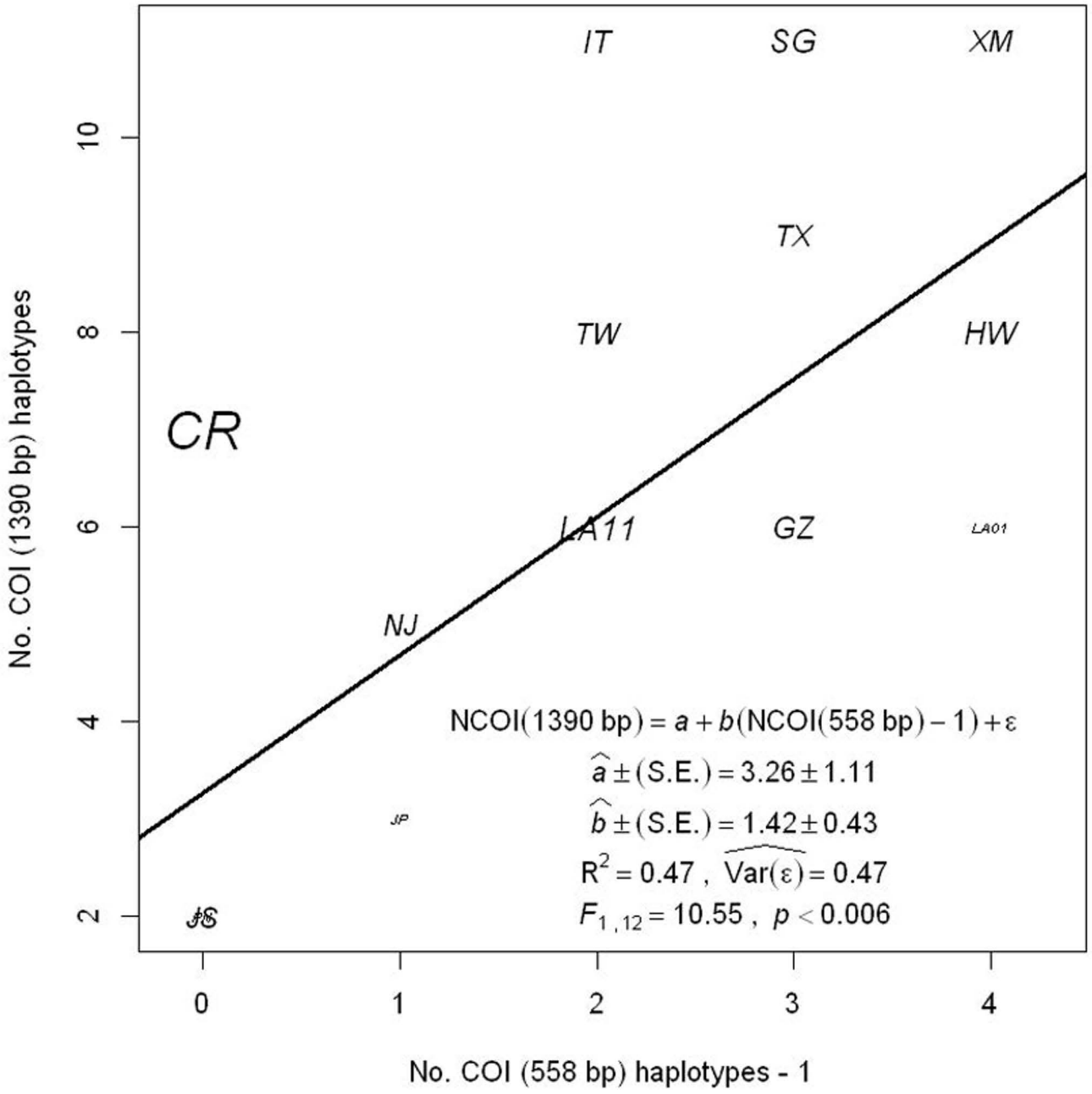
IT
SG
XM
TX
TW
HW
CR
LA11
GZ
LA01
NJ
JP
JS
$\mathrm{NCOI(1390\ bp)} = a + b(\mathrm{NCOI(558\ bp)} - 1) + \varepsilon$
$\widehat{a} \pm (\mathrm{S.E.}) = 3.26 \pm 1.11$
$\widehat{b} \pm (\mathrm{S.E.}) = 1.42 \pm 0.43$
$R^2 = 0.47$, $\widehat{\mathrm{Var}(\varepsilon)} = 0.47$
$F_{1,12} = 10.55$, $p < 0.006$
No. COI (1390 bp) haplotypes
No. COI (558 bp) haplotypes - 1
2
4
6
8
10
0
1
2
3
4

Figure 2.

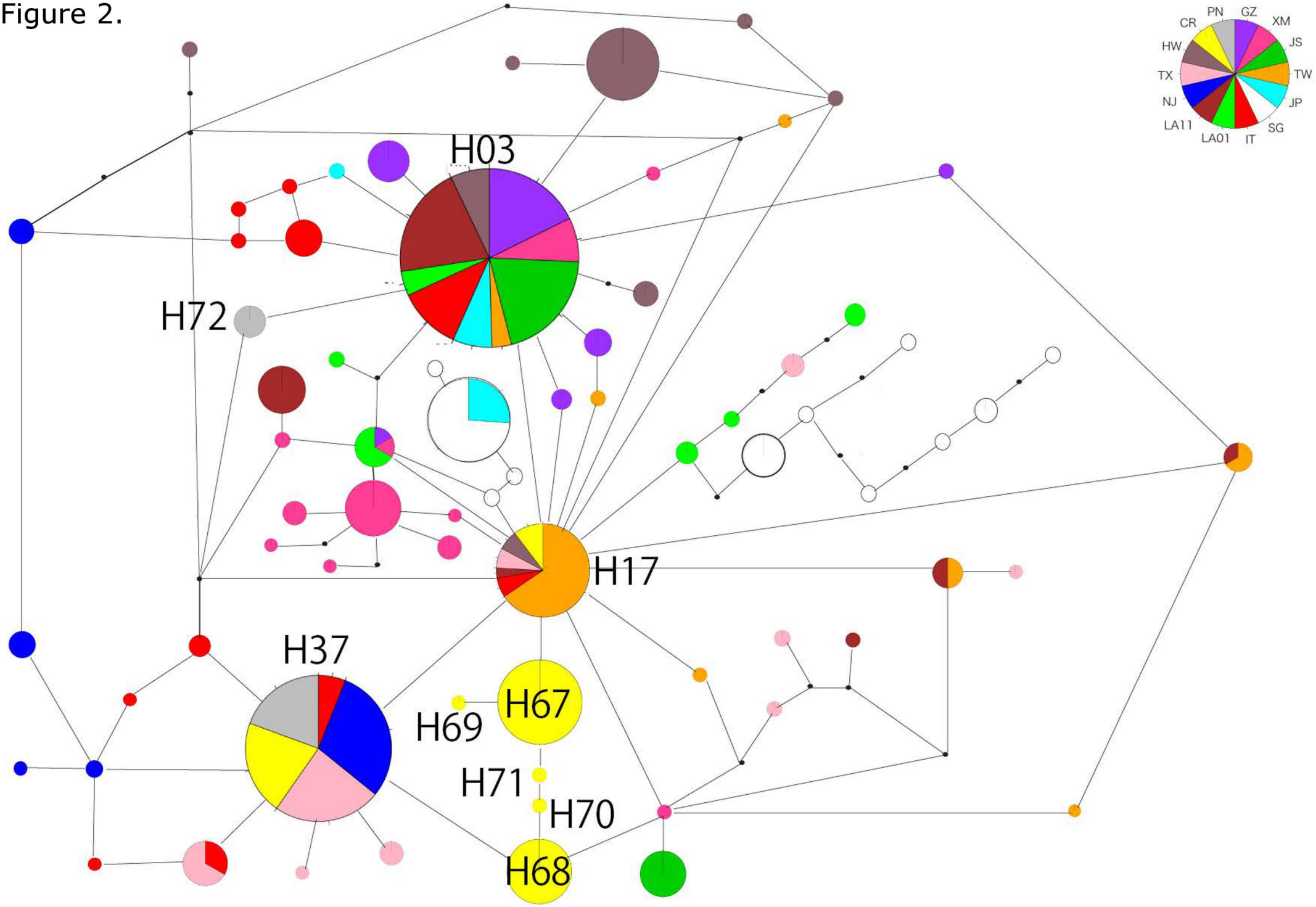
PN
GZ
XM
JS
TW
JP
SG
IT
LA01
LA11
NJ
TX
HW
CR
H03
H72
H17
H37
H67
H69
H71
H70
H68

Table 1 Codes and accession numbers for the long COI gene (1390 bp) haplotypes found Costa Rica (CR) and Panamá (PN).

Haplotype	GenBank access no.	CR	PN
H17	KC690912	3	0
H37	KC690932	14	13
H67	AB907796	24	0
H68	AB907797	13	0
H69	AB907798	1	0
H70	AB907799	1	0
H71	AB907800	1	0
H72	AB907801	0	3

Table 2 Haplotype (Hd) and nucleotide (π) diversity in long sequences of *Aedes albopictus* COI gene.

Site Code	Site Name	N	Variable sites	No. of Haplotypes	Hd (S.D.)	π x10^{-4}
GZ	Guanzhou, China	32	6	6	0.59 (0.09)	5
XM	Fujian, China	29	11	11	0.82 (0.05)	16
JS	Jiangsu, China	30	3	2	0.37 (0.08)	8
TW	Taiwan	30	8	8	0.59 (0.10)	6
JP	Japan	15	3	3	0.59 (0.08)	8
SG	Singapore	36	11	11	0.74 (0.07)	28
IT	Italy	32	7	11	0.81 (0.06)	14
LA01	Los Angeles 01, USA	15	9	6	0.83 (0.06)	21
LA11	Los Angeles 11, USA	34	8	6	0.51 (0.09)	13
NJ	New Jersey, USA	30	4	5	0.54 (0.10)	7
TX	Texas, USA	31	12	9	0.72 (0.08)	14
HW	Hawai, USA	32	8	8	0.70 (0.07)	10
CR	Costa Rica	57	4	7	0.72 (0.04)	10
PN	Panamá	16	3	2	0.33 (0.13)	7
--	All Areas	419	36	72	0.89 (0.01)	19

Table 3 Pairwise K_{st} estimates for the long (1390bp) COI gene sequences of *Aedes albopictus.* Pop indicates the population, with rows indicating the focal populations Costa Rica (CR) and Panamá (PN) and columns indicate the background populations. Codes for background populations are presented in Table 2.

Pop	GZ	XM	JS	TW	JP	SG	IT	LA01	LA11	NJ	TX	HW	CR	PN
CR	1.18*	1.69*	1.32*	1.22*	1.37*	2.38*	1.61*	1.74*	1.56*	1.26*	1.61*	1.41*	-	
PN	0.83*	1.78*	1.06*	0.88*	1.06*	2.99*	1.62*	1.92*	1.53*	0.96*	1.61*	1.24*	1.33*	-

* Significant value ($P < 0.05$).