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RESEARCH ARTICLE

Global phylogeography of the flood mosquito, *Aedes vexans* (Diptera: Culicidae), from mitochondrial DNA

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https://zoobank.org/7EEE1430-EAFF-4A30-A820-B4BECF87F6BA

ABSTRACT. This contribution endeavored to investigate the genetic structure and gene flow of the flood mosquito, *Aedes vexans* (Meigen, 1830). Using partial sequences of the mitochondrial COI gene, available from BOLD Systems and GenBank, the Haplotypic (Hd) and nucleotide (ϖ) gene diversity, genetic structuring and gene flow of *A. vexans* at the global, continental, and country levels were calculated. In total, 1,184 sequences were obtained, distributed among America (88.60%; represented by EUA and Canada), Europe (7.35%), Asia (3.89%), and Africa (0.17%). From these, 395 haplotypes (H) without presence of pseudogenes (NUMTs) were detected. The cluster analyses grouped the haplotypes into six clades. Clade I includes haplotypes from countries in America and Europe, while clades II and III include haplotypes exclusively from Asia and Europe; clade IV grouped only one haplotype from Africa and clade V grouped haplotypes from America and Africa. The global Hd and ϖ were 0.92 and 0.01, respectively. In addition, there is evidence of genetic structuring among continents (7.07%), countries (1.62%), and within countries (91.30%; $F_{ST} = 0.08$, $p < 0.05$) and no isolation by distance was detected ($r = 0.003$, $p > 0.05$). The genetic diversity of *A. vexans* was found to be greater in North America than in other continents. Although this provisional conclusion might be influenced by a sample bias, since 88.60% of the sequences are from America, is also plausible to consider that America corresponds to the ancestral distribution area of the flood mosquito. This hypothesis needs further testing, using a more comprehensive sample from other continents. Additionally, the six clusters found and their geographical distribution do not support previous proposals of splitting the genus into three subspecies confined to certain geographical areas.

KEY WORDS. Cytochrome oxidase subunit I, genetic diversity, gene flow, genetic structuring, haplotypes, mitochondrial DNA, pseudogenes, vector control.

INTRODUCTION

The flood mosquito, *Aedes vexans* (Meigen, 1830), is present in the subtropical regions of all continents, except the Antarctic (Reinert 1973, Johansen et al. 2005, Szalanski et al. 2006). In nature, this species can travel up to 17 km (Briegel et al. 2001), and has invaded areas beyond its putative native range through air transport (Ward 1984, Joyce and Nakagawa 1963). Like the females of other mosquitos of medical and veterinary importance, flood mosquito females lay their eggs in moist sites that are likely to flood (Strickman 1982). These eggs are very resistant, and will stay viable for up to three years, waiting for the optimal conditions to hatch (James and Harwood 1969). The food source of the flood mosquito is nectar, but the female needs mammal blood to complete the maturation of its ovaries (Edman 1977, Brust 1980, Nasci 1984). Although these mosquitoes are more often found in rural zones, they also inhabit suburban and urban areas. When humans are present in the environment, flood mosquito females give preference to them for their blood meals (Thompson and Dicke 1965, O'Donnell et al. 2017).

Based on morphological and molecular evidence, *A. vexans* has been subdivided into three subspecies: *Aedes vexans vexans* (Meigen, 1830) from eastern Asia and Oceania, *Aedes vexans arabiensis* (Patton, 1905) from Africa and Europe, and *Aedes vexans nipponii* (Theobald, 1907) from southeast Asia (Reinert 1973, Reinert et al. 2004, Fall et al. 2012, Francuski et al. 2016, Sanborn et al. 2019). The vector competence of the flood mosquito is 30 arboviruses. Among these viruses, some are important to human public health, for instance the causal agents of West Nile fever, Rift Valley fever, Saint Louis encephalitis and Eastern

Equine encephalitis, as well as filarial nematodes (Turell et al. 2005, Ndiaye et al. 2016).

Understanding the structure patterns and gene flow of the populations of *A. vexans* and other mosquitoes is important in the development of more adequate vector control programs (Baldacchino et al. 2015). Another important aspect to investigate is the transmission of pathogens to humans, and the resistance these insects develop to insecticides (Becker and Ludwig 1993, Dunbar et al. 2018). For example, a population genetic study was carried out in two locations in Queensland (Australia), to determine the viability of releasing *Aedes aegypti* (Linnaeus, 1762) infected with *Wolbachia pipientis* Hertig, 1936 to control the transmission of the dengue arbovirus. That study, concluded that there were two partially isolated populations (Endersby et al. 2009, 2011). Based in those findings, mosquitos infected with *W. pipientis* were released on the two populations, and the transmission of the dengue virus was successfully suppressed (Hoffmann et al. 2011).

Molecular markers are widely used to understand the biology and population dynamics of arbovirus vectors (Rašić et al. 2014). Among the molecular markers used in population genetics studies of mosquitoes is the mitochondrial DNA (mtDNA). It has several advantages over other genes: it is widely available, is small, has simple genomic structure, rapid rate of evolution, and is passed on exclusively by the females, with low genetic recombination rates (Avise et al. 1987).

Since no previous study has analyzed the genetic information available worldwide concerning the flood mosquito, we endeavored to map the global mtDNA diversity and gene flow of *A. vexans*, using sequences available on GenBank and BOLD Systems.

MATERIAL AND METHODS

A GenBank search revealed that the mtDNA Cytochrome oxidase subunit I (COI) gene was the most representative, and because BOLD Systems is the official repository of COI, sequences downloaded from there were also included here. The search criteria in GenBank included two words *Aedes vexans* AND COI, while for BOLD Systems only the species name, *Aedes vexans*, was used. To confirm the identity of the species, the sequences were analyzed using the BLAST tool in the NCBI website (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Only sequences that matched *A. vexans* sequences at 98–100% match with were used in our analysis. The selected sequences were separated according to the continent and country of origin. In addition, geographic data were extracted for each sequence, to geo-reference on a map; sequences without geographic information were eliminated from the analysis. These data were filtered and organized in the RStudio platform using Bold packages version 0.9 (Chamberlain 2017) and Ape version 5.3 (Paradis and Schliep 2018).

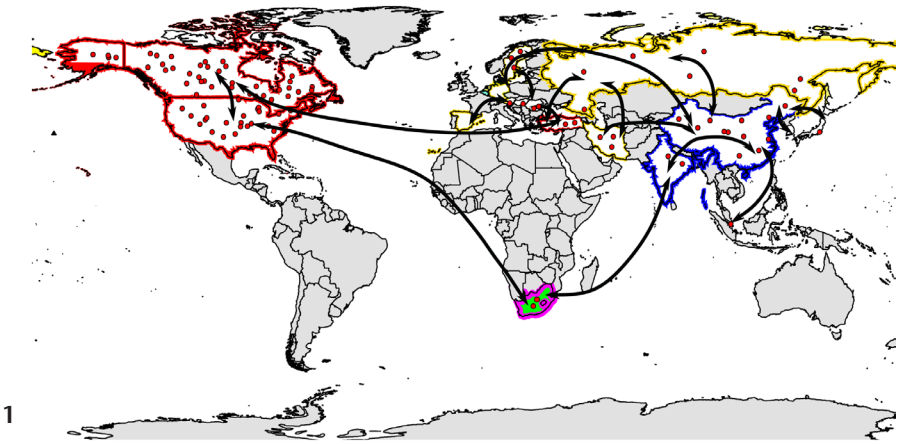
The sequences were aligned using MAFFT version 7 (Katoh and Standley 2013). Each haplotype (H) detected was numbered based on its frequency. For instance, the most frequent haplotype was named H1, the second most frequent was named H2, and so forth. To detect potential NUMTs, we searched for additional stop codons in the alignment (Haran et al. 2015). When a NUMT was detected, the sequence was removed from the analysis. After eliminating the sequences that were NUMTs, the haplotype network was constructed by median-joining methods using the Pegas package version 0.11 (Paradis 2010) in the Rstudio platform (Rstudio Team 2020).

Diversity and neutrality were estimated using the DnaSP program version 6.0 (Rozas et al. 2017). The analysis of molecular variance (AMOVA) was conducted using the Arlequin program version 3.5 (Excoffier and Lischer 2010), which evaluated the genetic variation between populations in continents and countries. Population genetic structuring was tested using the fixation index (F_{ST}) proposed by Wright (1921), and gene flow (Nm) was calculated through the Arlequin program version 3.5 (Excoffier and Lischer 2010) followed by the Bonferroni correction.

To test isolation by distance, the Mantel test was used to estimate the correlation between genetic (F_{ST}) and geographic (Km) distances, using the Vegan package version 2.5 (Oksanen et al. 2017) on the Rstudio platform (Rstudio Team 2020). Geographic distances were obtained from Google Earth. To estimate the genetic affinity between *A. vexans* populations, a clustering analysis was performed using maximum likelihood (ML) and Bayesian inference (BI). For this, we initially searched for the nucleotide substitution model that best fits our data in the jModelTest program version 2.1.1, which selected the model with the lowest value from the Akaike information criterion, AIC (Darriba et al. 2012). Then, the model selected was used in the ML and BI analyses. The ML analysis was conducted using the RaxML software (Stamatakis 2006), under the following parameters: ML+ thorough Bootstrap and 1,000 boot replicas. In turn, the BI analysis was conducted in the Mr.Bayes program version 3.2.7, under the following parameters: number of generations = 2,000,000, with $\sigma < 0.01$ of the frequencies to indicate robustness of the hypothesis (Ronquist et al. 2012). Visualization and editing of the clusters obtained were carried out in Mr.Ent version 2.5 (Zuccon and Zuccon 2014).

RESULTS

We obtained 2,420 sequences from the Bold System (82.64%) and GenBank (17.35%) databases, distributed among America (94.50%; represented by EUA and Canada), Europe (3.68%), Asia (1.23%), and Africa (0.58%). The median length of these sequences was 467 bp, varying between 114 and 879 bp. Nevertheless, after the alignment was completed, 1,184 sequences were selected, each 340 bp (the shorter sequences were excluded and the longer ones were trimmed), all distributed among the continents mentioned. Among the sequences that had to be trimmed, most were from EUA and Canada (88.60%), followed by Europe (7.35%), Asia (3.89%), and Africa (0.17%).



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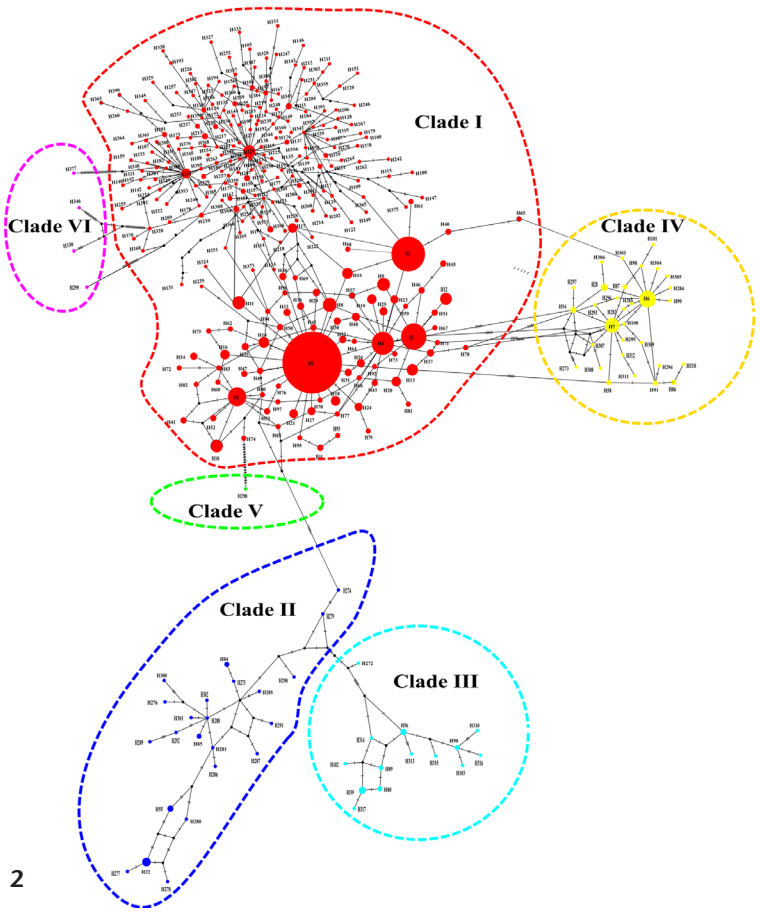

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Figures 1–2. Map (1) showing the origin of COI gene sequences for *Aedes vexans*, and haplotype network (2) based on COI sequences showing the genetic relationship between populations. On the map (1), the circles indicate the location of the sequences used and the arrows, the genetic relationships between the populations. In the haplotypes network (2), the size of the circles is proportional to the haplotype frequency and each circle in color belongs to a haplotype that is respectively numbered; black docks represent ancestral haplotypes. On the map (1) and in the haplotype network (2), the following colors, group the cluster observed on the network and in the ML and BI trees (see Figs 4, 5 for details): (●) America (Canada and USA) + Europe (Turkey); (●) Asia (China, India, Japan, Singapore and South Korea); (●) Europe (Sweden and Belgium) + Asia (China); (●) Eurasia (Romania, Sweden, Belgium, Russia, Kosovo, the Netherlands, China, Spain, Germany, Iran, Austria and Hungary); (●) Africa (South Africa); (●) America (USA) + Africa (South Africa).

The Americas were represented by sequences from Canada (64.10%) and USA (24.49%). Europe was represented by Sweden (2.87%), Belgium (1.35%), Spain (1.27%), Netherlands (0.84%), Austria (1.35%), Germany (0.17%), Rumania (0.17%), Germany (0.17%), Kosovo (0.17%), Hungary (0.17%), and Turkey (0.17%). Asia was represented by Japan (1.10%), China (1.10%), Iran (0.59%), Russia (0.42%), Singapore (0.25%), South Korea (0.25%), and India (0.17%). Finally, Africa was represented only by South Africa (0.17%).

Table S1 (Supplementary material S1) shows the global distribution of the haplotypes while the Table S2 (Supplementary material S2) shows that none of the them have NUMTs. In total, 395 H were observed, with H1 being the most frequent (24.58%), followed by H2 (7.77%), H3 (4.39%), H4 (3.38%), H5 (2.28%), H6–H11 ranging between 1.86% (H6) and 1.10% (H11), and H112–H395 varying between 0.93% (H112) and 0.08% (H395). Although H1 was the most frequent, it was only observed in Canada and the USA. Nevertheless, the haplotype with the widest distribution was H7, present in eight countries: Austria (11.76%), Belgium (17.64%), Hungary (11.76%), Kosovo (5.88%), Netherlands (17.64%), Russia (5.88%), Spain (11.76%), and Sweden (17.64%). Figures 1 and 2 displays the origin of the genetic material extracted (Fig. 1), and the haplotype network (Fig. 2). The highest Hd was found in America (EUA and Canada), followed by Africa and Europe.

Table 1 shows, by continent and countries, the results of Hd, ϖ and the different neutrality tests. In general, the global Hd was 0.92. Haplotypic diversity varied between 0.90 (Europe) and 1.0 (Africa). The Hd by countries was between 0.0 (Austria, Turkey, Hungary, Singapore, and India) and 1.0 (Germany, Kosovo, Rumania, Russia, South Korea, and South Africa). In turn, the global ϖ was 0.01, varing between 0.005 (Europe) and 0.08

Table 1. Haplotype diversity (Hd), nucleotide diversity (ϖ) and neutrality test results calculated for the flood mosquito, *Aedes vexans*, by country and continent.

Countries by continent	Number of sequences	Genetic diversity			Neutrality tests	
		Number of haplotypes	Hd	ϖ	Tajimas' D	Fus's F
Africa	2	2	1	0.08511	N/A	N/A
South Africa	2	2	1	0.08511	N/A	N/A
America	1049	325	0.973913	0.02994	-2.463612*	-5.65202*
Canada	759	191	0.90113	0.00518	-2.435669*	-5.13490*
USA	290	134	0.93120	0.01088	-2.306334*	-4.96645*
Asia	46	29	0.91259	0.02135	0.7167907	-0.55008
China	13	9	0.98717	0.02515	-0.0748804	-0.51062
South Korea	3	3	1	0.01176	N/A	N/A
Iran	7	3	0.80952	0.00405	0.4024933	0.4229
India	2	1	0	0	N/A	N/A
Japan	13	8	0.94871	0.01481	-0.3390129	-0.52121
Russia	5	4	1	0.00588	0.2734498	0.27834
Singapore	3	1	0	0	N/A	N/A
Europe	85	39	0.90983	0.00598	-0.2722376	-165434
Germany	2	2	1	0.00294	N/A	N/A
Austria	2	1	0	0	N/A	N/A
Belgium	16	8	0.89166	0.01455	-116233	-0.48408
Spain	15	7	0.88571	0.00577	-1619073	-229068
Hungary	2	1	0.0000	0	N/A	N/A
Kosovo	2	2	1	0.00294	N/A	N/A
Netherlands	10	4	0.71111	0.00320	0.0964613	0.17394
Rumania	2	1	1	0.01176	N/A	N/A
Sweden	34	12	0.95365	0.02759	1301434	0.12606
Turkey	2	1	0	0	N/A	N/A
Total	1182	395	0.92853	0.01099	-2.208676*	-5.22367*

N/A = Not available, *p < 0.05.

Table 2. Analysis of molecular variance (AMOVA) of populations of *Aedes vexans* at continental level, by countries, and within them.

Variation source	d.f.	Sum of Squares	Variation components	Variation percentage	F_{st}	p - value*
Between continents	3	10.820	0.03508 Va	7.07	0.08697	0.04059+-0.00196
Between countries within continents	16	11.450	0.00804 Vb	1.62		
Within countries	1164	526.956	0.45271 Vc	91.30		
Total	1183	549.226	0.49584			

*Value obtained from 10000 random permutations.

(Africa). The ϖ among countries ranged between 0.0 (Austria, Hungary, Turkey, Singapore, and India) and 0.08 (South Africa). Neutrality tests, Tajima's D, and Fu's F, at the global level, presented negative and statistically significant values (D = -2.20, p < 0.001; F = -5.22, p < 0.02); by continent and countries, America (EUA and Canada) (D = -2.46, p < 0.05; F= -5.65, p < 0.02) and all its countries (Canada D = -2.43, p < 0.05; F = -5.13, p < 0.02 and USA D = -2.30, p < 0.05; F = -4.96, p < 0.02) were statistically significant and with negative values.

In Table 2, the AMOVA indicated genetic structuring at the continental and country levels and within countries (F_{ST} = 0.08, p < 0.05), where the highest percentage of variation was observed among *A. vexans* individuals within countries (91.30%), followed by 7.07% among continents and 1.62% among countries in the same continent. In Fig. 3, the Mantel test indicated that Genetic distance (F_{ST}) and geographic distance (km) were not correlated (Fig. 3; Mantel, R^2 = 0.003; p > 0.05).

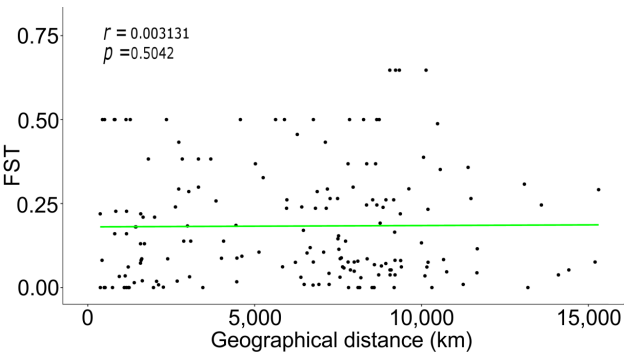


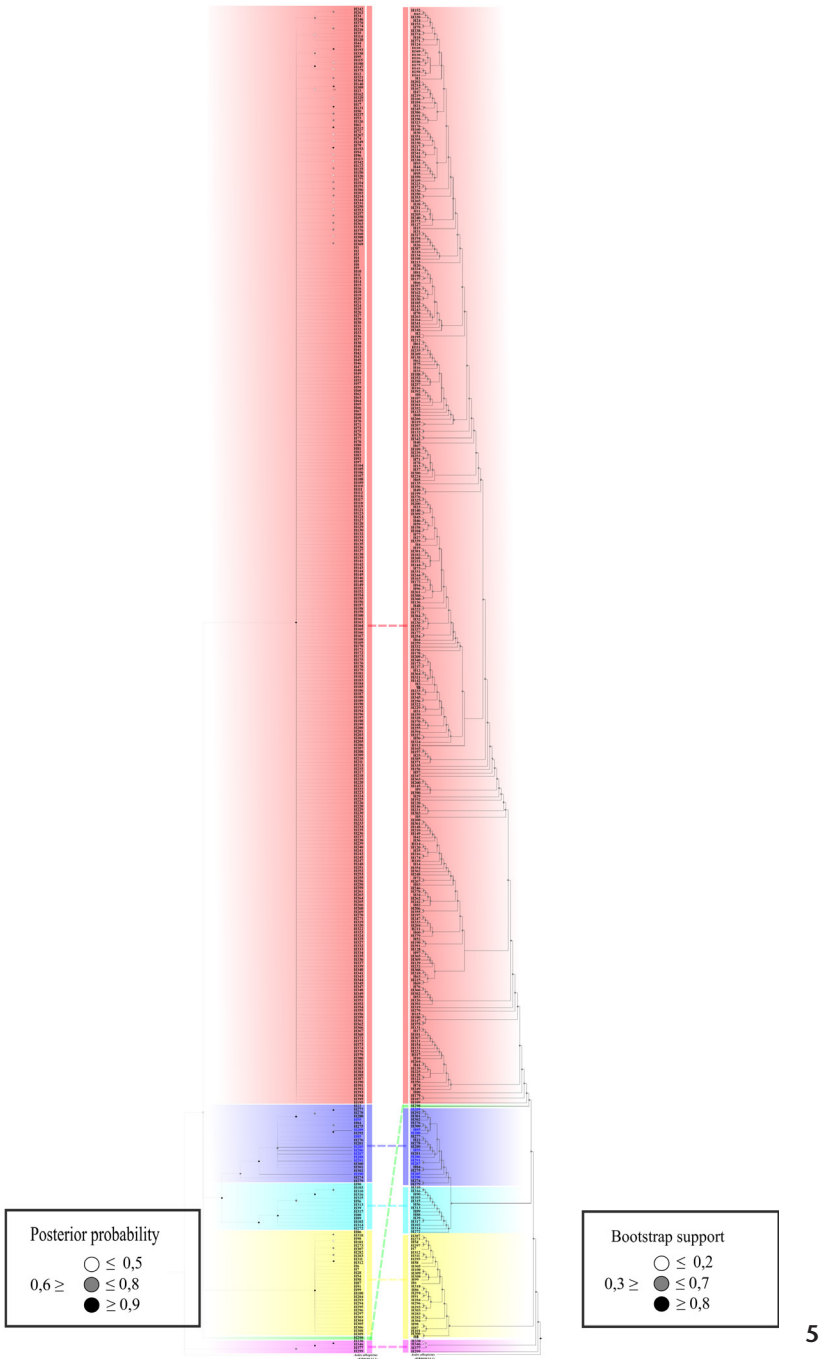
Figure 3. Global Mantel's correlation test for genetic and geographic distance of the *Aedes vexans* populations analyzed.

Table 3 shows pairwise comparisons between countries after the Bonferroni's correction. For these, significant genetic structuring was detected between Canada and the following countries: Belgium, Sweden, Spain, Hungary, and the Netherlands; between the USA and: Austria, Belgium, Sweden, Spain, Hungary, and the Netherlands; between China and: Netherlands, the USA, and Canada; between India and: the USA and Canada; between Iran and: the USA and Canada; between Japan and: Belgium, Sweden, Spain, and Netherlands; between Russia and: the USA and Canada; between Singapore and: countries Belgium, Sweden, and Spain.

Figures 4 and 5 shows the results of the clustering analyses using BI (Fig. 4) and ML (Fig. 5); both analyses recovered the same six clades, but with distinct topologies: clade I grouped mosquito populations from USA, Canada, and Turkey (n = 322 H); clade II, populations from China, Japan, Singapore, and South Korea (n = 23); clade III, populations from Sweden, Belgium, and China (n = 14 H); clade IV, mosquito populations from Rumania, Sweden, Belgium, Russia, Kosovo, the Netherlands,

Table 3. Fixation index (F_{ST}; lower half) and geographic distance (Km; upper half) pairwise comparison between populations of the flood mosquito, *Aedes vexans*, from the different countries sampled. Values in bold indicate genetically structured populations.

	Austria	Belgium	Turkey	Sweden	Spain	Rumania	Netherlands	Kosovo	Hungary	Germany	Canada	USA	China	India	Iran	Japan	Russia	Singapore	South Korea	South Africa
Austria	0	806.81	1920.67	1428.80	1656.86	813.21	839.29	740.27	375.66	503.23	7260.85	8353.28	7145.38	6437.53	3685.84	9166.62	1580.06	9852.86	8624.48	8731.87
Belgium	0.16	0	2724.38	1391.91	1286.86	1596.56	190.10	1529.55	1160.27	426.81	6569.97	7562.36	7635.73	7179.44	4468.51	9375.42	1282.1	10562.15	8930.74	9205.65
Turkey	1.00	0.29	0	2626.41	3315.95	1145.47	2727.37	1272.91	1567.42	2357.35	8847.73	10193.06	5956.54	4636.36	1814.94	8537.26	3026.34	8083.65	7798.83	7843.71
Sweden	0.18	0.01	0.23	0	2678.56	1633.01	1211.49	1956.96	1444.42	1120.66	6221.33	7676.05	6385.71	6411.68	4005.78	7984.05	466.42	9620.94	7557.36	10102.98
Spain	0.20	-0.02	0.29	0.01	0	2394.31	1470.75	2060.65	2001.72	1617.19	7067.6	7597.42	8798.11	7950.55	5129.75	10660.56	2535.95	11396.14	10196.42	8371.54
Rumania	0.50	0.08	0.50	0.02	0.08	0	1585.82	493.02	439.61	1214.33	7800.24	9055.99	6483.49	5627.05	2875.32	8717.08	1967.74	9049.68	8097.49	8518.7
Netherlands	0.22	-0.02	0.43	0.06	-0.01	0.21	0	1578.53	1161.44	372.23	6460.97	7514.49	7494.79	7118.41	4439.92	9195.35	1092.23	10480.81	8760.3	9364.94
Kosovo	0.00	-0.01	0.50	-0.01	0.01	0.0	0.07	0	519.44	1238.89	7983.27	9089.5	6925.2	5893.55	3084.74	9206.78	2216.33	9336.84	8577.55	8147.03
Hungary	0.00	0.16	1.00	0.18	0.20	0.50	0.22	0.00	0	794.56	7484.73	8661.75	6821.47	6061.87	3314.8	8934.05	1697.7	9478.4	8357.86	8658.83
Germany	0.50	0.08	0.50	0.03	0.08	0.00	0.21	0.00	0.50	0	6758.28	7870.28	7231.98	6759.75	4068.98	9058.55	1153.09	10136.45	8579.29	9179.84
Canada	0.26	**0.10**	0.23	**0.07**	**0.10**	0.07	**0.17**	0.07	**0.26**	0.07	0	2264.07	9396.37	11481.96	9998.91	8091.77	5836.54	13082.58	8588.54	15201.01
USA	**0.24**	**0.08**	0.23	**0.05**	**0.08**	0.05	**0.15**	0.05	**0.24**	0.05	0.00	0	11660.31	13591.88	11670.95	10161.81	7228.03	15307.03	10754.68	14414.9
China	0.23	0.06	0.23	0.03	0.06	0.00	**0.14**	0.00	0.23	0.00	**0.06**	**0.04**	0	2987.14	4617.48	3050.17	6750.64	3841.78	2120.74	11249.63
India	10.00	0.29	1.00	0.23	0.29	0.50	0.43	0.50	1.00	0.50	**0.26**	**0.25**	0.18	0	2830.92	5965.98	6874.4	3447.51	5022.33	8264.28
Iran	0.38	0.08	0.38	0.08	0.10	0.13	0.18	0.13	0.38	0.13	**0.13**	**0.11**	0.09	0.38	0	7523.94	4463.71	6277.06	6664.19	7724.52
Japan	0.26	**0.08**	0.26	**0.04**	**0.08**	0.03	**0.16**	0.03	0.26	0.03	0.07	0.06	0.03	0.26	0.11	0	8183.22	5255.67	943.81	14102.62
Russia	0.13	-0.05	0.28	-0.02	-0.03	0.00	0.00	-0.11	0.13	0.00	**0.06**	**0.04**	0.00	0.28	0.01	0.02	0	10060.83	7814.24	10311.88
Singapore	1.00	**0.35**	1.00	**0.29**	**0.35**	0.64	0.48	0.64	1.00	0.64	0.30	0.29	0.25	0.00	0.45	0.32	0.38	0	4572.22	9223.14
South Korea	0.36	0.07	0.36	0.03	0.07	0.00	0.19	0.00	0.36	0.00	0.06	0.04	0.00	0.36	0.11	0.03	0.00	0.50	0	13188
South Africa	0.50	0.08	0.50	0.03	0.08	0.00	0.21	0.00	0.50	0.00	0.07	0.05	0.00	0.50	0.13	0.03	0.00	0.64	0.00	0



Figures 4–5. Phylogenetic tree for *Aedes vexans* populations constructed from 395 haplotypes from the COI gene by using Bayesian Inference, BI (4) and Maximum Likelihood, ML (5). The evolutionary history for both analyses was inferred by using the GTR + G model, as suggested by jModelTest version 2.1.10. The BI tree was obtained by using 2-million generations, while the ML used 1,000 replicas. For BI, the support of the branches is indicated by the subsequent probability values, while for ML the bootstrap values are shown. Numbers in blue represent sequences from the *A. nipponii* subspecies. In both figures the colors below denote the continents and their respective countries: (●) America (Canada and USA) + Europe (Turkey); (●) Asia (China, India, Japan, Singapore and South Korea); (●) Europe (Sweden and Belgium) + Asia (China); (●) Eurasia (Romania, Sweden, Belgium, Russia, Kosovo, the Netherlands, China, Spain, Germany, Iran, Austria and Hungary); (●) Africa (South Africa); (●) America (USA) + Africa (South Africa).

China, Spain, Germany, Iran, Austria, and Hungary (n = 31 H); clade V, populations from South Africa (n = 1 H); and clade VI, populations from USA and South Africa (n = 4 H). Similar results were observed in the haplotype network (Fig. 2).

DISCUSSION

To our knowledge, this is the first study of the genetic structure of *A. vexans* using the genetic information available for the COI gene from the GenBank and Boldsystem databases (as of April and May 2020). Both the haplotype network and the clustering analysis recovered six clades; clade I grouped mosquito populations from America (EUA and Canada) and Europe; clade II, populations from Asia; clades III and IV, populations from Europe and Asia; clade V, populations from Africa; and clade VI, populations from America and Africa.

Aedes vexans has been previously subdivided into three subspecies: *A. vexans vexans*, *A. vexans arabiensis*, and *A. vexans nipponii*. *Aedes vexans vexans* has been reported from east Asia and Oceania, *A. vexans arabiensis* from Africa and Europe, and *A. vexans nipponii* from southeast Asia (Reinert 1973, Reinert et al. 2004, Johansen et al. 2005, Cywinska et al. 2006, Fall et al. 2012, Francuski et al. 2016, Sanborn 2019). Our results support neither the division of the genus into three subspecies, nor their putative geographic distributions. Further investigations including mitochondrial or nuclear genomes or some nuclear genes, are necessary to test the monophyly of the proposed subspecies (Lilja et al. 2018).

Haplotype diversity and the number of haplotypes were higher in EUA and Canada (Hd = 0.97; H = 325, with 245 being private haplotypes) than in other continents; for example, Europe (Hd = 0.90; H = 39, with 27 being private haplotypes). This result could be a function of sample bias, since 88.60% of the haplotypes in our sample came from this region. Another possible explanation, which needs further testing, is that North America may be the ancestral distribution *A. vexans*. There is evidence from other studies that genetic diversity is often higher in areas where it a species is native (Gloria-Soria et al. 2016, Ruiling et al. 2018). However, more genetic data from the other continents where *A. vexans* is presented are necessary to test this hypothesis.

The F_{ST} between the populations of the flood mosquito in the USA and Canada were more divergent among than the F_{ST} between populations of other two continents, Europe and Asia (Table 3). Krtinić et al. (2013), analyzing natural *A. vexans* populations from USA and Germany, found that these do not share a common gene pool, and proposed that the geographic barriers formed by the Atlantic and Pacific Oceans preclude gene flow between the evolutionary lineages of *A. vexans*. However, our results do not support this putative genetic isolation. Additionally, for most populations, the results of the neutrality tests, Tajima's D and Fu's FS were negative (Table 1), suggesting that these populations have experienced recent bottlenecks and population expansion (Zawani et al. 2014). This may be due to recent vector-control initiates, followed by colonization events, which are commonly observed in mosquitos of medical and veterinary importance (Harris et al. 2010). Mosquito populations are targeted to elimination, to decrease the epidemiologic transmission of the arboviruses they transmit (Ocampo and Wesson 2004, Koou et al. 2014, Zhu et al. 2016). Subsequently, mosquitoes from neighboring zones are able to re-colonize the areas from which they had been mostly eliminated (Szalanski et al. 2006, Monteiro et al. 2014, Díaz-Nieto et al. 2016).

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LITERATURE CITED

Avise J, Arnold J, Ball R, Bermingham E, Lamb T, Neigel J, Reeb C, Saunders N (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. Annual Review of Ecology and Systematics 18: 489–522.

Baldacchino F, Caputo B, Chandre F, Drago A, della Torre A, Montarsi F, Rizzoli A (2015) Control methods against invasive *Aedes* mosquitoes in Europe: a review. Pest Management Science 71: 1471–1485. https://doi.org/10.1002/ps.4044

Becker N, Ludwig M (1993) Investigations on possible resistance in *Aedes vexans* field populations after a 10-year application of *Bacillus thuringiensis israelensis*. Journal of the American Mosquito Control Association 9: 221–224.

Briegel H, Waltert A, Kuhn R (2001) Reproductive Physiology of *Aedes* (*Aedimorphus*) *vexans* (Diptera: Culicidae) in Relation to Flight Potential. Journal of Medical Entomology 38: 557–565. https://doi.org/10.1603/0022-2585-38.4.557

Brust R (1980) Dispersal behavior of adult *Aedes sticticus* and *Aedes vexans* (Diptera: Culicidae) in Manitoba. The Canadian Entomologist 112: 31–42. https://doi.org/10.4039/ent11231-1

Chamberlain S (2017) Bold: interface to Bold Systems 'API'. R package version 0.4.0. Available only at: https://CRAN.R-project.org/package=bold

Cywinska A, Hunter F, Hebert P (2006) Identifying Canadian mosquito species through DNA barcodes. Medical and Veterinary Entomology 20: 413–424. https://doi.org/10.1111/j.1365-2915.2006.00653.x

Darriba D, Taboada G, Doallo R, Posada D (2012) JModelTest 2: More models, new heuristics and parallel computing. Nature Methods 9: 772. https://doi.org/10.1038/nmeth.2109

Díaz-Nieto L, Chiappero M, Díaz de Astarloa C, Maciá A, Gardenal C, Berón C (2016) Genetic Evidence of Expansion by Passive Transport of *Aedes* (Stegomyia) *aegypti* in Eastern Argentina. PLoS Neglected Tropical Diseases 10: 10–15. https://doi.org/10.1371/journal.pntd.0004839

Dunbar M, Bachmann A, Varenhorst A (2018) Reduced Insecticide Susceptibility in *Aedes vexans* (Diptera: Culicidae) where Agricultural Pest Management Overlaps with Mosquito Abatement. Journal of Medical Entomology 55: 747–751. https://doi.org/10.1093/jme/tjx245

Edman J, Haeger J (1977) Host-feeding patterns of Florida mosquitoes *Wyeomyia*. Journal of Medical Entomology 14: 477–479. https://doi.org/10.1093/jmedent/14.4.477

Endersby N, Hoffmann A, White V, Lowenstein S, Ritchie S, Johnson P, Rapley L, Ryan P, Nam V, Yen N, Kittiyapong P, Weeks A (2009) Genetic Structure of *Aedes aegypti* in Australia and Vietnam Revealed by microsatellite and exon primed intron crossing markers suggests feasibility of local control options. Journal of Medical Entomology 46: 1074–1083. https://doi.org/10.1603/033.046.0514

Endersby N, Hoffmann A, White V, Ritchie S, Johnson P, Weeks A (2011) Changes in the Genetic Structure of *Aedes aegypti* (Diptera: Culicidae) Populations in Queensland, Australia, Across Two Seasons: Implications for Potential Mosquito Releases. Journal of Medical Entomology 48: 999–1007. https://doi.org/10.1603/me10264

Excoffier L, Lischer H (2010) Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10: 564–567. https://doi.org/10.1111/j.1755-0998.2010.02847.x

Fall A, Diaïté A, Etter E, Bouyer J, Ndiaye T, Konaté L (2012) The mosquito *Aedes* (*Aedimorphus*) *vexans arabiensis* as a probable vector bridging the West Nile virus between birds and horses in Barkedji (Ferlo, Senegal). Medical and Veterinary Entomology 26: 106–111. https://doi.org/10.1111/j.1365-2915.2011.00974.x

Francuski L, Milankov V, Ludoški J, Krtinić B, Lundström J, Kemenesi G, Ferenc J (2016) Genetic and phenotypic variation in central and northern European populations of *Aedes* (*Aedimorphus*) *vexans* (Meigen, 1830) (Diptera, Culicidae). Journal of Vector Ecology 41: 160–171. https://doi.org/10.1111/jvec.12208

Gloria-Soria A, Ayala D, Bheecarry A, Calderon-Arguedas O, Chadee D, Chiappero M, Coetzee M, Elahee KB, et al. (2016) Global genetic diversity of *Aedes aegypti*. Molecular Ecology 25: 5377–5395. https://doi.org/10.1111/mec.13866

Haran J, Koutroumpa F, Magnoux E, Roques A, Roux G (2015) Ghost mtDNA haplotypes generated by fortuitous NUMTs can deeply disturb infra-specific genetic diversity and phylogeographic pattern. Journal of Zoological Systematics and Evolutionary Research 53: 109–115. https://doi.org/10.1111/jzs.12095

Harris A, Rajatileka S, Ranson H (2010) Pyrethroid resistance in *Aedes aegypti* from Grand Cayman. American Journal of Tropical Medicine and Hygiene 83: 277–284. https://doi.org/10.4269/ajtmh.2010.09-0623

Hoffmann A, Montgomery B, Popovici J, Iturbe-Ormaetxe I, Johnson P, Muzzi F, Greenfield M, et al. (2011) Successful establishment of *Wolbachia* in *Aedes* populations to suppress dengue transmission. Nature 476: 454–459. https://doi.org/10.1038/nature10356

James M, Harwood R (1969) Herms's Medical Entomology. Macmillan Co, New York, 484 pp.

Johansen C, Lindsay M, Harrington S, Whelan P, Russell R, Broom A (2005) First Record of *Aedes* (*Aedimorphus*) *vexans vexans* (Meigen) in Australia. Journal of the American Mosquito Control Association 21: 222–224. https://doi.org/10.2987/8756-971X(2005)21[222:FROAAV]2.0.CO;2

Joyce C, Nakagawa P (1963) *Aedes vexans nocturnus* (Theobald) in Hawaii. Proceedings Hawaiian Entomological Society 18: 273–280.

Katoh K, Standley D (2013) MAFFT multiple sequence alignment software version 7: Improvements in performance and usability. Molecular Biology and Evolution 30: 772–780. https://doi.org/10.1093/molbev/mst010

Koou S, Chong C, Vythilingam I, Lee C, Ng L (2014) Insecticide resistance and its underlying mechanisms in field populations of *Aedes aegypti* adults (Diptera: Culicidae) in Singapore. Parasites and Vectors 7: 1–15. https://doi.org/10.1186/s13071-014-0471-0

Krtinić B, Francuski L, Petrić D, Milankov V (2013) Genetic diversity and differentiation between Palearctic and Nearctic populations of *Aedes vexans* (Meigen, 1830) (Diptera, Culicidae). Journal of Vector Ecology 38: 154–162. https://doi.org/10.1111/j.1948-7134.2013.12021.x

Lilja T, Troell K, Kirik H, Lindström A (2018) A distinct group of north European *Aedes vexans* as determined by mitochondrial and nuclear markers. Medical and Veterinary Entomology 32: 282–289. https://doi.org/10.1111/mve.12294

Monteiro F, Shama R, Martins A, Gloria-Soria A, Brown J, Powell J (2014) Genetic Diversity of Brazilian *Aedes aegypti*: Patterns following an Eradication Program. PLoS Neglected Tropical Diseases 8: 1–10. https://doi.org/10.1371/journal.pntd.0003167

Nasci R (1984) Variations in the blood-feeding patterns of *Aedes vexans* and *Aedes trivittatus* (Diptera: Culicidae). Journal of Medical Entomology 21: 95–99. https://doi.org/10.1093/jmedent/21.1.95

Ndiaye E, Fall G, Gaye A, Bob N, Talla C, Diagne C, et al. (2016) Vector competence of *Aedes vexans* (Meigen), *Culex poicilipes* (Theobald) and *Cx. quinquefasciatus* Say from Senegal for West and East African lineages of Rift Valley fever virus. Parasites and Vectors 9: 1–9. https://doi.org/10.1186/s13071-016-1383-y

O'Donnell K, Bixby M, Morin K, Bradley D, Vaughan J (2017) Potential of a northern population of *Aedes vexans* (Diptera: Culicidae) to transmit Zika virus. Journal of Medical Entomology 54: 1354–1359. https://doi.org/10.1093/jme/tjx087

Oksanen A, Blanchet F, Friendly M, Kindt R, Legendre P, Mcglinn D (2017) Package vegan. Community Ecology Package, version 2.6-2.

Ocampo C, Wesson D (2004) Population dynamics of *Aedes aegypti* from a dengue hyperendemic urban setting in Colombia. American Journal of Tropical Medicine and Hygiene 71: 506–513. https://doi.org/10.4269/ajtmh.2004.71.506

Paradis E (2010) Pegas: an R package for population genetics with an integrated-modular approach. Bioinformatics 26: 419–20. https://doi.org/10.1093/bioinformatics/btp696

Paradis E, Schliep K (2018) Ape 5.0: an environment for modern phylogenetics and evolutionary analyses in R. Bioinformatics 35: 526–528. https://doi.org/10.1093/bioinformatics/bty633

Rašić G, Filipović I, Weeks A, Hoffmann A (2014) Genome-wide SNPs lead to strong signals of geographic structure and relatedness patterns in the major arbovirus vector, *Aedes aegypti*. BMC Genomics 15: 1–12. https://doi.org/10.1186/1471-2164-15-275

Reinert J (1973) Contributions to the mosquito fauna of Southeast Asia. XVI. Genus *Aedes* Meigen, subgenus *Aedimorphus* Theobald in Southeast Asia. Contributions of the American Entomological Institute 66: 37–39.

Reinert J, Harbach R, Kitching I (2004) Phylogeny and classification of Aedini (Diptera: Culicidae), based on morphological characters of all life stages. Zoological Journal of the Linnean Society 142: 289–368. https://doi.org/10.1111/j.1096-3642.2004.00144.x

Ronquist F, Teslenko M, Van Der Mark P, Ayres D, Darling A, Höhna S, et al. (2012) MrBayes 3.2: Efficient bayesian phylogenetic inference and model choice across a large model space. Systematic Biology 61: 539–542. https://doi.org/10.1093/sysbio/sys029

Rozas J, Ferrer-Mata A, Sanchez-DelBarrio J, Guirao-Rico S, Librado P, Ramos-Onsins S, Sanchez-Gracia A (2017) DnaSP 6: DNA sequence polymorphism analysis of large data sets. Molecular Biology and Evolution 34: 3299–3302. https://doi.org/10.1093/molbev/msx248

RStudio Team (2020) RStudio: Integrated Development for R. RStudio, PBC, Boston, Available online at: http://www.rstudio.com

Ruiling Z, Tongkai L, Dezhen M, Zhong Z (2018) Genetic characters of the globally spread tiger mosquito, *Aedes albopictus* (Diptera, Culicidae): implications from mitochondrial gene *COI*. Journal of Vector Ecology 43: 89–97. https://doi.org/10.1111/jvec.12287

Sanborn M, Klein T, Kim H, Fung C, Figueroa K, Yang Y, et al. (2019) Metagenomic analysis reveals three novel and prevalent mosquito viruses from a single pool of *Aedes vexans nipponii* collected in the republic of Korea. Viruses 11: 1–17. https://doi.org/10.3390/v11030222

Stamatakis A (2006) RAxML-VI-HPC: Maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22: 2688–2690. https://doi.org/10.1093/bioinformatics/btl446

Strickman D (1982) Stimuli affecting selection of oviposition sites by *Aedes vexans* (Diptera: Culicidae): moisture. Journal of Medical Entomology 19: 181–184. https://doi.org/10.1093/jmedent/19.2.181

Szalanski A, Owens C, Lewter J, Broce A (2006) Genetic Structure of *Aedes vexans* (Diptera: Culicidae) Populations from Central United States based on mitochondrial ND5 sequences. Annals of the Entomological Society of America 99: 157–163. https://doi.org/10.1603/0013

Thompson P, Dicke R (1965) Sampling studies with *Aedes vexans* and some other Wisconsin *Aedes* (Diptera: Culcidae). Annals of the Entomological Society of America 58: 927–930. https://doi.org/10.1093/aesa/58.6.927

Turell M, Dohm D, Sardelis M, O'Guinn M, Andreadis T, Blow J (2005) An update on the potential of North American mosquitoes (Diptera: Culicidae) to transmit West Nile virus. Journal of Medical Entomology 42: 57–62. https://doi.org/10.1093/jmedent/42.1.57

Ward R (1984) Mosquito fauna of Guam: case history of an introduced fauna. In: Laird M (Ed.) Commerce and the spread of pests and disease vectors. Praeger, New York, 143–162.

Wright S (1921) Systems of Mating. I. The biometric relations between parent and offspring. Genetics 6: 309–309. https://doi.org/10.1093/genetics/6.3.309

Zawani M, Abu H, Sazaly A, Zary S, Darlina M (2014) Population genetic structure of *Aedes albopictus* in Penang, Malaysia. Genetics and Molecular Research 13: 8184–8196. https://doi.org/10.4238/2014.October.7.13

Zhu F, Lavine L, O'Neal S, Lavine M, Foss C, Walsh D (2016) Insecticide resistance and management strategies in urban ecosystems. Insects 7: 1–26. https://doi.org/10.3390/insects7010002

Zuccon A, Zuccon D (2014) MrEnt: An editor for publication-quality phylogenetic tree illustrations. Molecular Ecology Resources 14: 1090–1094. https://doi.org/10.1111/1755-0998.12253

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Supplementary material 1

Table S1. Global distribution of *Aedes vexans* haplotypes. For each haplotype, the access code and its subsequent reference was selected based on the oldest sequence and referenced in some manuscript; on the contrary, it was assigned to any of the genetic bases used.

Authors: José H. Vargas-Espinosa, Oscar A. Aguirre-Obando

Data type: Haplotypes, the access code and its subsequent reference.

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Supplementary material 2

Table S2. Nucleotide diversity of the COI gene for *Aedes vexans* populations. Mafft alignment to compare the haplotypes from this study. The amino acid sequence translated is represented by capital letters in blue, over the first nucleotide of its corresponding codon. Invariable sites are indicated with points, contrary to the alternative nucleotide, and spaces with (–).

Authors: José H. Vargas-Espinosa, Oscar A. Aguirre-Obando

Data type: Haplotypes with their base pairs and their translation into amino acids.

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