

1 **Global genetic diversity of *Aedes vexans***

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3 **North America Insect Invades the World: the Case of the Flood Mosquito, *Aedes vexans***
4 **(Diptera: Culicidae), from Mitochondrial DNA**

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14 **Abstract**

15 The flood mosquito, *Aedes vexans* (Diptera: Culicidae), native of Canada, and currently
16 present in all continents, has a vector competence for 30 arboviruses, being responsible for
17 transmitting diseases, like West Nile fever, Rift Valley fever, Saint Louis Encephalitis and
18 Eastern Equine Encephalitis. Hence, knowing the structure and gene flow of *A. vexans* is
19 important to develop adequate vector control strategies for this species. For this, from partial
20 sequences of the mitochondrial COI gene available in Bold and GenBank, it was possible to
21 determine the Haplotypic (*Hd*) and nucleotide (π) gene diversity, genetic structuring and gene
22 flow at global, continental, and country levels. In total, 1184 sequences were recovered,
23 distributed between America (88.60%), Europe (7.35%), Asia (3.89%), and Africa (0.17%).
24 From these, 395 haplotypes (H) were detected without presence of pseudogenes (NUMTs),
25 with H1 being the most frequent (24.58%) and between H12 - H395 the least frequent varying
26 between 0.93% (H12) and 0.08% (H395). Phylogenetically, the haplotypes were grouped into
27 six clades. Clade I grouped haplotypes from countries in America and Europe, while clades II
28 and III presented haplotypes exclusively from Asia and Europe; clade IV grouped only one
29 haplotype from Africa and the last ultimo clade V grouped haplotypes from America and
30 Africa. The global *Hd* and π was 0.92 and 0.01, respectively. In addition, evidence was
31 obtained of genetic structuring among continents (7.07%), countries (1.62%), and within
32 countries (91.30%; $F_{ST} = 0.08$, $p < 0.05$) and no isolation by distance was detected ($r = 0.003$,
33 $p > 0.05$). These results suggest that the mosquito populations that invaded other continents
34 originate directly from the American continent, where possibly transcontinental commercial
35 routes favored their long-distance dispersion.

36 **Key words:** Cytochrome oxidase subunit I, genetic diversity, gene flow, genetic structuring,
37 haplotypes, mitochondrial DNA, pseudogenes, vector competence, vector control.

39 **Introduction**

40 The flood mosquito, *Aedes vexans* (Meigen, 1830), is present in subtropical regions of all
41 continents, except for the Antarctic [1,2,3]. In nature this species manages to travel up to 17
42 km [4]; however, its global invasion was favored through passive transport mediated by
43 human activities [5]. Like other mosquitos of medical and veterinary importance [6], *A.*
44 *vexans* lays its eggs in moist sites with flood probability to guarantee their offspring [7] and
45 these can resist drying out and survive up to three years when kept moist [8]. In general, *A.*
46 *vexans* females and males feed on nectar, but to mature their ovaries and reproduce, the
47 females feed principally on mammals, like deer, horses, cattle and pigs [9,10,11]. Normally,
48 this species is found in rural zones, but when it inhabits suburban and urban zones, it prefers
49 humans as principal feeding source [12,13].

50 Morphological and molecular evidence suggest the existence of three subspecies of *A.*
51 *vexans* throughout the world: *Aedes vexans vexans* Meigen in eastern Asia and Oceania,
52 *Aedes vexans arabiensis* Patton in Africa and Europe, and *Aedes vexans nipponii* Theobald in
53 southeast Asia [1,14,15,16,17]. The flood mosquito has vector competence for 30 arbovirus
54 (Elizondo-Quiroga *et al.* 2018) and is involved in the transmission of important diseases, like
55 the West Nile fever, Rift Valley fever, Saint Louis encephalitis and Eastern Equine
56 encephalitis, as well as filarial nematodes [18,19].

57 Given the epidemiological and sanitary importance of *A. vexans*, understanding the
58 structuring patterns and gene flow of this species' populations is important to develop more-
59 adequate vector control programs [20], as well as understand the transmission of vectors
60 among the human population, given its influence on pathogen transfer and dissemination of
61 characteristics, like vector competence and resistance to insecticides [21,22]. For example,
62 studies on genetic structure in populations of *Aedes aegypti*, dengue, chikungunya, and Zika
63 vector, in two locations of Queensland, Australia, indicated that said locations were partially

64 isolated genetically, with these two sites being adequate for the release of mosquitos infected
65 with *Wolbachia pipientis* because it was important to restrict the strain released during the
66 initial implementation phases [23,24]. Thereafter, mosquitos infected with *Wolbachia*
67 *pipientis* were released with successful establishment in both locations, thus, suppressing
68 dengue transmission [25].

69 Molecular markers are widely used to understand the biology and population
70 dynamics of vector species of diseases [26]. Among the molecular markers used in population
71 genetics studies in *A. vexans*, the mitochondrial DNA (mtDNA) sequences are broadly used
72 due to properties, like their abundance in the organism, size and small genomic structure,
73 rapid rate of evolution, and exclusive maternal inheritance with low genetic recombination
74 [27]. However, one of the disadvantages of using mtDNA in population genetics and
75 phylogenetic studies is NUMTs presence, result of the translocation of mitochondrial
76 sequences of the mitochondrial genome for the nuclear genome [28]. Furthermore, this type
77 of information is freely available in the GenBank and Boldsystem databases. Particularly for
78 *A. vexans*, until now, nobody has analyzed the genetic information available within a global
79 context. This work sought to know the global panorama of genetic diversity and gene flow of
80 *A. vexans*, using mtDNA sequences available in GenBank and Boldsystem.

81 **Materials and Methods**

82 A prior search in GenBank permitted detecting that the mtDNA Cytochrome oxidase subunit I
83 (COI) gene was the most representative, and given that Boldsystem is this marker's
84 depository, it was also determined to work with it. For this, partial nucleotide sequences were
85 obtained from the mitochondrial (mtDNA) COI gene deposited in GenBank and Boldsystem
86 for *A. vexans*. The search criteria in GenBank used the words *Aedes vexans* AND COI, while
87 for Boldsystem, it only used *Aedes vexans*. The sequences obtained were analyzed by using
88 the BLAST tool in the NCBI website (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>) to confirm
89 identity with *A. vexans* and only sequences with identity percentage between 98% and 100%
90 were considered in our analysis. Thereafter, the sequences selected were classified according
91 to continent and country, so that analyses listed ahead were performed for each of them. In
92 addition, for each sequence, geographic data were extracted to later geo-reference it on a map;
93 sequences without geographic information were eliminated from the analysis. These data
94 were filtered and organized through the RStudio platform by using Bold packages version 0.9
95 [29] and Ape version 5.3 [30].

96 Then, the sequences were aligned by using the MAFFT software version 7 to later
97 detect haplotypes (H) [31]. Each haplotype was numbered based on its frequency, thus, the
98 most frequent was H1, the following H2, and so forth. To detect potential NUMTs among the
99 H detected, additional stop codons were searched for in the alignment [28]. In case of
100 detecting any NUMT, it was reported and removed from the analysis. With H without
101 NUMTs, the haplotype network was constructed through the Pegas package version 0.11 [32]
102 in the RStudio platform [33].

103 Diversity and neutrality tests were estimated by using the DnaSP program version 6.0
104 [34]. The analysis of molecular variance (AMOVA) was conducted by using the Arlequin

105 program version 3.5 [35], which evaluated the variation between continents and countries.
106 Population genetic structuring was tested by using the fixation index (F_{ST}) proposed by
107 Wright [36], and the gene flow (N_m) was calculated through the Arlequin program version
108 3.5 [35]. In cases in which statistically significant differences were obtained, the Bonferroni
109 correction was used to verify the existence of significant differences.

110 To test isolation by distance, Mantel's test was carried out to estimate the correlation
111 between genetic distance (F_{ST}) and geographic distance (Km) using the Vegan package
112 version 2.5 [37] on the RStudio platform [33]. The geographic distances were obtained by
113 calculating with the Geographic Distance Matrix Generator program version 1.2.3. To
114 estimate the genetic affinity of *A. vexans* populations, a phylogenetic reconstruction was
115 performed using maximum likelihood (ML) and Bayesian inference (BI) from the haplotypes
116 found. For this, we initially searched for the nucleotide substitution model that best fits our
117 data in the program jModelTest version 2.1.1, which selected the model with the lowest value
118 from the Akaike information criterion, AIC, [38]. Then, the model selected was used for the
119 phylogenetic reconstruction under the ML and BI approach. The ML analysis was conducted
120 with the RAxML software [39], under the following parameters: ML+ thorough Bootstrap
121 and 1,000 boot replicas. In turn, the BI analysis was conducted in the Mr.Bayes program
122 version 3.2.7, under the following parameters: number of generations = 2,000,000, with $\sigma <$
123 0.01 of the frequencies to indicate robustness of the phylogenetic hypothesis [40]. The
124 visualization and editing of the phylogenetic trees obtained was carried out in Mr.Ent version
125 2.5 [41].

126

127 **Results**

128 Between April and May 2020, the study recovered 2,420 sequences from the Boldsystem
129 (82.64%) and GenBank (17.35%) databases, distributed among America (94.50%), Europe
130 (3.68%), Asia (1.23%), and Africa (0.58%) with median length of 467 bp, varying between
131 114 and 879 bp. Nevertheless, once the alignment was made, 1,184 sequences were selected
132 from these with a length of 340 bp each, all distributed among the continents mentioned.
133 Among the sequences selected, the American continent had the highest representation
134 (88.60%), followed by Europe (7.35%), Asia (3.89%), and Africa (0.17%). The American
135 continent was represented with sequences from Canada (64.10%) and the United States
136 (24.49%). Europe was represented by Sweden (2.87%), Belgium (1.35%), Spain (1.27%),
137 Netherlands (0.84%), Austria (1.35%), Germany (0.17%), Rumania (0.17%), Germany
138 (0.17%), Kosovo (0.17%), Hungary (0.17 %), and Turkey (0.17%). Asia was represented by
139 Japan (1.10%), China (1.10%), Iran (0.59%), Russia (0.42%), Singapore (0.25%), South
140 Korea (0.25%), and India (0.17%). Finally, Africa was represented only by South Africa
141 (0.17%).

142 Table 1 shows the global distribution of the haplotypes observed. In total, 395 H were
143 observed, with H1 being the most frequent (24.58%), followed by H2 (7.77%), H3 (4.39%),
144 H4 (3.38%), H5 (2.28%), H6 - H11 ranging between 1.86% (H6) and 1.10% (H11), and H112
145 - H395 varying between 0.93% (H112) and 0.08% (H395). Although H1 was the most
146 frequent, it was only observed in Canada and the USA. Nevertheless, the haplotype with the
147 greatest distribution was H7, which was present in eight countries, that is, Austria (11.76%),
148 Belgium (17.64%), Hungary (11.76%), Kosovo (5.88%), Netherlands (17.64%), Russia
149 (5.88%), Spain (11.76%), and Sweden (17.64%). None of the H had presence of NUMTs
150 (Supplementary material). Figure 1 displays the locations where the genetic material analyzed
151 was extracted (Fig. 1A), as well as the phylogenetic relationships between populations, as

152 arrows observed in the haplotype network (Fig. 1B). From this, it may be stated that native
 153 populations of *A. vexans* from America invaded some countries in Africa and Europe, and
 154 from there established themselves in other countries in these continents and in Asia.

Haplotype	Continent				Access code	Reference
	America	Africa	Asia	Europe		
H1	291	0	0	0	ACMC131-04	[42]
H2	92	0	0	0	ACMC277-04	[42]
H3	52	0	0	0	CNEIF006-12	[43]
H4	40	0	0	0	ACMC276-04	[42]
H5	27	0	0	0	CNGSD2112-15	[43]
H6	0	0	0	22	CULBE143-14	[44]
H7	0	0	0	17	GBMIN56980-17	[44]
H8	15	0	0	0	ASDIP113-15	http://www.boldsystems.org/
H9	13	0	0	0	ACMC289-04	[42]
H10	12	0	0	0	BBDCP596-10	http://www.boldsystems.org/
H11	13	0	0	0	CNPAD261-13	[43]
H12	11	0	0	0	CNEIF841-12	[43]
H13	10	0	0	0	CNEIF861-12	[43]
H14	9	0	0	0	ASDMT244-11	[43]
H15	9	0	0	0	ACMC290-04	[42]
H16	7	0	0	0	CNGSD6857-15	[43]
H17	7	0	0	0	CNGSD860-15	[43]
H18	7	0	0	0	CNJAE1246-12	[43]
H19	7	0	0	0	CNPAF983-13	[43]
H20	7	0	0	0	SSJAE8713-13	[43]
H21	6	0	0	0	CNJAE1248-12	[43]
H22	0	0	8	0	GBDP47430-19	[45]
H23	5	0	0	0	CNEIF812-12	[43]
H24	5	0	0	0	MBIOI074-13	[43]
H25	5	0	0	0	RRSSA3576-15	[49]
H26	5	0	0	0	SSEIC6245-13	[43]
H27	5	0	0	0	SSJAE8835-13	[43]
H28	0	0	0	4	CULBE132-14	[44]
H29	4	0	0	0	ACMC104-04	[42]
H30	4	0	0	0	CNEIG153-12	[43]
H31	4	0	0	0	CNGSD1517-15	[43]
H32	4	0	0	0	CNPAD244-13	[43]
H33	4	0	0	0	PHJUL2502-11	[43]
H34	4	0	0	0	SSEIC5403-13	[43]
H35	4	0	0	0	SSEIC5955-13	[43]
H36	4	0	0	0	SSEIC6896-13	[43]
H37	4	0	0	0	SSJAE8703-13	[43]

H38	4	0	0	0	SSJAE9027-13	[43]
H39	0	0	0	4	GBDP48120-19	[48]
H40	3	0	0	0	CNEIF014-12	[43]
H41	3	0	0	0	CNEIF817-12	[43]
H42	3	0	0	0	CNEIF830-12	[43]
H43	3	0	0	0	CNEIG182-12	[43]
H44	3	0	0	0	CNGSI1668-15	[43]
H45	3	0	0	0	CNRME208-12	[43]
H46	3	0	0	0	PHAUG1312-11	[43]
H47	3	0	0	0	SMTPJ4980-14	[43]
H48	3	0	0	0	SSEIA4548-13	[43]
H49	3	0	0	0	SSEIA5279-13	[43]
H50	3	0	0	0	SSEIA5341-13	[43]
H51	3	0	0	0	SSJAF9177-13	[43]
H52	3	0	0	0	SSPAC12518-13	[43]
H53	3	0	0	0	SSPPA9368-15	[43]
H54	0	0	3	0	MK962499	[50]
H55	0	0	3	0	AB738099	[45]
H56	0	0	0	3	GBMIN56959-17	[48]
H57	3	0	0	0	IUP1017-14	http://www.boldsystems.org/
H58	0	0	0	2	CULBE140-14	[44]
H59	2	0	0	0	ACMC033-04	[42]
H60	2	0	0	0	BBDCM210-10	[43]
H61	2	0	0	0	CNEIF002-12	[43]
H62	2	0	0	0	CNEIF798-12	[43]
H63	2	0	0	0	CNGSE2411-15	[43]
H64	2	0	0	0	CNGSI1097-15	[43]
H65	2	0	0	0	CNPKO3332-14	[43]
H66	2	0	0	0	CNRME207-12	[43]
H67	2	0	0	0	CNRME2449-12	[43]
H68	2	0	0	0	CNRME4093-12	[43]
H69	2	0	0	0	CNRMG626-12	[43]
H70	2	0	0	0	CNTIH462-15	[43]
H71	2	0	0	0	GMOKH968-15	[43]
H72	2	0	0	0	SSEIA3424-13	[43]
H73	2	0	0	0	SSEIA3430-13	[43]
H74	2	0	0	0	SSEIA5215-13	[43]
H75	2	0	0	0	SSEIA5266-13	[43]
H76	2	0	0	0	SSJAE1181-13	[43]
H77	2	0	0	0	SSJAE8706-13	[43]
H78	2	0	0	0	SSJAF9208-13	[43]
H79	2	0	0	0	SSPAB8912-13	[43]
H80	2	0	0	0	SSPAC12020-13	[43]
H81	2	0	0	0	SSPPA5271-15	[43]
H82	2	0	0	0	SSPPA5483-15	[43]

H83	2	0	0	0	SSPPA9400-15	[43]
H84	0	0	2	0	GBDP47423-19	[45]
H85	0	0	2	0	AB738168-	[45]
H86	0	0	0	2	GBMIN56950-17	[46]
H87	0	0	1	1	LRMQS005-17	[47]
H88	0	0	1	1	GBDP48149-19	[48]
H89	0	0	1	1	GBDP48185-19	[48]
H90	0	0	1	1	GBDP48490-19	[48]
H91	0	0	0	2	GBMIN56961-17	[48]
H92	2	0	0	0	MOSN025-17	http://www.boldsystems.org/
H93	2	0	0	0	MOSN050-17	http://www.boldsystems.org/
H94	2	0	0	0	MOSN1411-18	http://www.boldsystems.org/
H95	1	0	0	0	MOSN1957-18	http://www.boldsystems.org/
H96	1	0	0	0	MOSN2259-19	http://www.boldsystems.org/
H97	1	0	0	0	MOSN2970-19	http://www.boldsystems.org/
H98	0	0	0	1	CULBE141-14	[44]
H99	0	0	0	1	CULBE145-14	[44]
H100	0	0	0	1	GMOXH2148-15	[43]
H101	0	0	0	1	CULBE136-14	[44]
H102	0	0	0	1	CULBE138-14	[44]
H103	0	0	0	1	CULBE139-14	[44]
H104	1	0	0	0	ACMC053-04	[42]
H105	1	0	0	0	ACMC288-04	[42]
H106	1	0	0	0	ASDMT1059-11	[43]
H107	1	0	0	0	BBDCN133-10	[43]
H108	1	0	0	0	BBDCN233-10	[43]
H109	1	0	0	0	BBDCP593-10	[43]
H110	1	0	0	0	CNEIF015-12	[43]
H111	1	0	0	0	CNEIF023-12	[43]
H112	1	0	0	0	CNEIF3339-12	[43]
H113	1	0	0	0	CNEIF793-12	[43]
H114	1	0	0	0	CNEIF813-12	[43]
H115	1	0	0	0	CNEIF849-12	[43]
H116	1	0	0	0	CNEIG1456-12	[43]
H117	1	0	0	0	CNEIG152-12	[43]
H118	1	0	0	0	CNFNH1033-14	[43]
H119	1	0	0	0	CNGRD1138-12	[43]
H120	1	0	0	0	CNGSD1018-15	[43]
H121	1	0	0	0	CNGSD132-15	[43]
H122	1	0	0	0	CNGSD1611-15	[43]
H123	1	0	0	0	CNGSD263-15	[43]
H124	1	0	0	0	CNGSE1406-15	[43]
H125	1	0	0	0	CNGSE2070-15	[43]
H126	1	0	0	0	CNGSE2173-15	[43]
H127	1	0	0	0	CNGSG1207-15	[43]

H128	1	0	0	0	CNGSG122-15	[43]
H129	1	0	0	0	CNGSI1101-15	[43]
H130	1	0	0	0	CNGSI1218-15	[43]
H131	1	0	0	0	CNGSI1988-15	[43]
H132	1	0	0	0	CNGSI246-15	[43]
H133	1	0	0	0	CNGSI326-15	[43]
H134	1	0	0	0	CNGSI582-15	[43]
H135	1	0	0	0	CNJAE1277-12	[43]
H136	1	0	0	0	CNJAE965-12	[43]
H137	1	0	0	0	CNJAF2111-12	[43]
H138	1	0	0	0	CNJAF2114-12	[43]
H139	1	0	0	0	CNJAF2142-12	[43]
H140	1	0	0	0	CNPAD242-13	[43]
H141	1	0	0	0	CNPAD260-13	[43]
H142	1	0	0	0	CNPPC1400-12	[43]
H143	1	0	0	0	CNPPC1722-12	[43]
H144	1	0	0	0	CNRME198-12	[43]
H145	1	0	0	0	CNRME2450-12	[43]
H146	1	0	0	0	CNRMG169-12	[43]
H147	1	0	0	0	CNROH071-13	[43]
H148	1	0	0	0	CNTIH336-15	[43]
H149	1	0	0	0	CNWBC188-13	[43]
H150	1	0	0	0	CNWBH228-13	[43]
H151	1	0	0	0	CNWBH672-13	[43]
H152	1	0	0	0	GMOKH2148-15	[43]
H153	1	0	0	0	JSJUN1501-11	[43]
H154	1	0	0	0	MSQ002-04	http://www.boldsystems.org/
H155	1	0	0	0	MSQ003-04	http://www.boldsystems.org/
H156	1	0	0	0	MSQ015-04	http://www.boldsystems.org/
H157	1	0	0	0	MSQ022-04	http://www.boldsystems.org/
H158	1	0	0	0	NCCA889-11	[43]
H159	1	0	0	0	NCCA895-11	[43]
H160	1	0	0	0	PHAUG1277-11	[43]
H161	1	0	0	0	PHAUG1318-11	[43]
H162	1	0	0	0	PHSEP1211-11	[43]
H163	1	0	0	0	PREXP286-14	http://www.boldsystems.org/
H164	1	0	0	0	RRINV213-15	[49]
H165	1	0	0	0	RRINV3712-15	[49]
H166	1	0	0	0	RRINV3721-15	[49]
H167	1	0	0	0	RRINV3723-15	[49]
H168	1	0	0	0	RRINV3798-15	[49]
H169	1	0	0	0	RRMF14275-15	[49]
H170	1	0	0	0	RRSSA425-15	[49]
H171	1	0	0	0	SMTPI2660-14	[43]
H172	1	0	0	0	SMTPI3303-14	[43]
H173	1	0	0	0	SSBAE5601-13	[43]

H174	1	0	0	0	SSBAE5713-13	[43]
H175	1	0	0	0	SSEIA3425-13	[43]
H176	1	0	0	0	SSEIA4525-13	[43]
H177	1	0	0	0	SSEIA5135-13	[43]
H178	1	0	0	0	SSEIA5184-13	[43]
H179	1	0	0	0	SSEIA5193-13	[43]
H180	1	0	0	0	SSEIA5225-13	[43]
H181	1	0	0	0	SSEIA5296-13	[43]
H182	1	0	0	0	SSEIA5301-13	[43]
H183	1	0	0	0	SSEIA5335-13	[43]
H184	1	0	0	0	SSEIC5215-13	[43]
H185	1	0	0	0	SSEIC5397-13	[43]
H186	1	0	0	0	SSEIC5788-13	[43]
H187	1	0	0	0	SSEIC5981-13	[43]
H188	1	0	0	0	SSEIC6590-13	[43]
H189	1	0	0	0	SSEIC6788-13	[43]
H190	1	0	0	0	SSEIC6792-13	[43]
H191	1	0	0	0	SSEIC6946-13	[43]
H192	1	0	0	0	SSEID823-13	[43]
H193	1	0	0	0	SSJAE10960-13	[43]
H194	1	0	0	0	SSJAE11005-13	[43]
H195	1	0	0	0	SSJAE5456-13	[43]
H196	1	0	0	0	SSJAE6982-13	[43]
H197	1	0	0	0	SSJAE7467-13	[43]
H198	1	0	0	0	SSJAE8697-13	[43]
H199	1	0	0	0	SSJAE8710-13	[43]
H200	1	0	0	0	SSJAE8721-13	[43]
H201	1	0	0	0	SSJAE8767-13	[43]
H202	1	0	0	0	SSJAE8770-13	[43]
H203	1	0	0	0	SSJAE8773-13	[43]
H204	1	0	0	0	SSJAE8790-13	[43]
H205	1	0	0	0	SSJAE8802-13	[43]
H206	1	0	0	0	SSJAE8804-13	[43]
H207	1	0	0	0	SSJAE8810-13	[43]
H208	1	0	0	0	SSJAE8842-13	[43]
H209	1	0	0	0	SSJAE8865-13	[43]
H210	1	0	0	0	SSJAE8905-13	[43]
H211	1	0	0	0	SSJAE8909-13	[43]
H212	1	0	0	0	SSJAE8962-13	[43]
H213	1	0	0	0	SSJAE8972-13	[43]
H214	1	0	0	0	SSJAE8997-13	[43]
H215	1	0	0	0	SSJAE9009-13	[43]
H216	1	0	0	0	SSJAE9068-13	[43]
H217	1	0	0	0	SSJAF10000-13	[43]
H218	1	0	0	0	SSJAF10014-13	[43]
H219	1	0	0	0	SSJAF10300-13	[43]

H220	1	0	0	0	SSJAF10307-13	[43]
H221	1	0	0	0	SSJAF10358-13	[43]
H222	1	0	0	0	SSJAF10367-13	[43]
H223	1	0	0	0	SSJAF259-13	[43]
H224	1	0	0	0	SSJAF5420-13	[43]
H225	1	0	0	0	SSJAF9159-13	[43]
H226	1	0	0	0	SSJAF9168-13	[43]
H227	1	0	0	0	SSJAF9715-13	[43]
H228	1	0	0	0	SSJAF9963-13	[43]
H229	1	0	0	0	SSPAA9388-13	[43]
H230	1	0	0	0	SSPAA9453-13	[43]
H231	1	0	0	0	SSPAA9670-13	[43]
H232	1	0	0	0	SSPAA9697-13	[43]
H233	1	0	0	0	SSPAA9698-13	[43]
H234	1	0	0	0	SSPAC11523-13	[43]
H235	1	0	0	0	SSPAC11881-13	[43]
H236	1	0	0	0	SSPAC12030-13	[43]
H237	1	0	0	0	SSPAC12040-13	[43]
H238	1	0	0	0	SSPAC12053-13	[43]
H239	1	0	0	0	SSPAC12068-13	[43]
H240	1	0	0	0	SSPAC12075-13	[43]
H241	1	0	0	0	SSPAC12104-13	[43]
H242	1	0	0	0	SSPAC12117-13	[43]
H243	1	0	0	0	SSPAC12132-13	[43]
H244	1	0	0	0	SSPAC12142-13	[43]
H245	1	0	0	0	SSPAC12161-13	[43]
H246	1	0	0	0	SSPAC12173-13	[43]
H247	1	0	0	0	SSPAC12503-13	[43]
H248	1	0	0	0	SSPAC1637-13	[43]
H249	1	0	0	0	SSPPA3871-15	[43]
H250	1	0	0	0	SSPPA4038-15	[43]
H251	1	0	0	0	SSPPA4045-15	[43]
H252	1	0	0	0	SSPPA4260-15	[43]
H253	1	0	0	0	SSPPA4386-15	[43]
H254	1	0	0	0	SSPPA5066-15	[43]
H255	1	0	0	0	SSPPA5187-15	[43]
H256	1	0	0	0	SSPPA5299-15	[43]
H257	1	0	0	0	SSPPA5324-15	[43]
H258	1	0	0	0	SSPPA5593-15	[43]
H259	1	0	0	0	SSPPA5622-15	[43]
H260	1	0	0	0	SSPPA8632-15	[43]
H261	1	0	0	0	SSPPA8661-15	[43]
H262	1	0	0	0	SSPPA8754-15	[43]

H263	1	0	0	0	SSPPA8797-15	[43]
H264	1	0	0	0	SSPPA8894-15	[43]
H265	1	0	0	0	SSPPA8942-15	[43]
H266	1	0	0	0	SSPPA8947-15	[43]
H267	1	0	0	0	SSPPA9373-15	[43]
H268	1	0	0	0	SSPPA9424-15	[43]
H269	1	0	0	0	SSPPA9526-15	[43]
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H275	0	0	1	0	GBDP47421-19	[45]
H276	0	0	1	0	GBDP47422-19	[45]
H277	0	0	1	0	GBDP47424-19	[45]
H278	0	0	1	0	GBDP47425-19	[45]
H279	0	0	1	0	GBDP47427-19	[45]
H280	0	0	1	0	GBDP47428-19	[45]
H281	0	0	1	0	GBDP47429-19	[45]
H282	0	0	0	1	GBMIN56977-17	[45]
H283	0	0	0	1	GBMIN56977-18	[49]
H284	0	0	1	0	MK962500	[50]
H285	0	0	1	0	AB738152	[45]
H286	0	0	1	0	AB738264	[45]
H287	0	0	1	0	LC054400	[51]
H288	0	0	1	0	LC054401	[51]
H289	0	0	1	0	LC054402	[51]
H290	0	0	1	0	LC054403	[51]
H291	0	0	1	0	LC054404	[51]
H292	0	0	1	0	GBDCU003-12	[52]
H293	0	0	0	1	GBMNA38704-19	[52]
H294	0	0	0	1	MK403546	[53]
H295	0	0	0	1	GBMIN56951-17	[46]
H296	0	0	1	0	GBDP14515-13	[52]
H297	0	0	1	0	GBDP14517-13	[52]
H298	0	1	0	0	CULSA041-19	http://www.boldsystems.org/
H299	0	1	0	0	CULSA043-19	http://www.boldsystems.org/
H300	0	0	1	0	GBDP48513-19	[45]
H301	0	0	1	0	GBMIN56978-17	[44]
H302	0	0	1	0	GBMIN56979-17	[44]
H303	0	0	0	1	LRMQS002-17	[47]
H304	0	0	0	1	LRMQS035-17	[47]
H305	0	0	0	1	LRMQS066-17	[47]
H306	0	0	0	1	MLQSR050-17	[47]
H307	0	0	0	1	MLQSR051-17	[47]

H308	0	0	0	1	MLQSR055-17	[47]
H309	0	0	0	1	GBDP48491-19	[48]
H310	0	0	0	1	GBDP48493-19	[48]
H311	0	0	0	1	GBMIN56957-17	[48]
H312	0	0	0	1	GBMIN56958-17	[48]
H313	0	0	0	1	GBMIN56960-17	[48]
H314	0	0	0	1	GBMIN56965-17	[48]
H315	0	0	0	1	GBMIN56967-17	[48]
H316	0	0	0	1	GBMIN56969-17	[48]
H317	0	0	0	1	GBMIN56972-17	[48]
H318	0	0	0	1	GBMIN56974-17	[48]
H319	1	0	0	0	BBDIT1116-11	http://www.boldsystems.org/
H320	1	0	0	0	BBDIT1121-11	http://www.boldsystems.org/
H321	1	0	0	0	BBDIT1122-11	http://www.boldsystems.org/
H322	1	0	0	0	BBDIT1123-11	http://www.boldsystems.org/
H323	1	0	0	0	BBDIV1558-12	http://www.boldsystems.org/
H324	1	0	0	0	GMGAA514-13	http://www.boldsystems.org/
H325	1	0	0	0	GMGCC029-13	http://www.boldsystems.org/
H326	1	0	0	0	GMGDD139-13	http://www.boldsystems.org/
H327	1	0	0	0	IUP1014-14	http://www.boldsystems.org/
H328	1	0	0	0	IUP1016-14	http://www.boldsystems.org/
H329	1	0	0	0	IUP1018-14	http://www.boldsystems.org/
H330	1	0	0	0	IUP675-14	http://www.boldsystems.org/
H331	1	0	0	0	IUP885-14	http://www.boldsystems.org/
H332	1	0	0	0	MOSN019-17	http://www.boldsystems.org/
H333	1	0	0	0	MOSN044-17	http://www.boldsystems.org/
H334	1	0	0	0	MOSN088-17	http://www.boldsystems.org/
H335	1	0	0	0	MOSN1014-18	http://www.boldsystems.org/
H336	1	0	0	0	MOSN1083-18	http://www.boldsystems.org/
H337	1	0	0	0	MOSN1374-18	http://www.boldsystems.org/
H338	1	0	0	0	MOSN1382-18	http://www.boldsystems.org/
H339	1	0	0	0	MOSN1421-18	http://www.boldsystems.org/
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H341	1	0	0	0	MOSN1425-18	http://www.boldsystems.org/
H342	1	0	0	0	MOSN1428-18	http://www.boldsystems.org/
H343	1	0	0	0	MOSN1615-18	http://www.boldsystems.org/
H344	1	0	0	0	MOSN1652-18	http://www.boldsystems.org/
H345	1	0	0	0	MOSN179-18	http://www.boldsystems.org/
H346	1	0	0	0	MOSN1869-18	http://www.boldsystems.org/
H347	1	0	0	0	MOSN2115-19	http://www.boldsystems.org/
H348	1	0	0	0	MOSN2116-19	http://www.boldsystems.org/
H349	1	0	0	0	MOSN2480-19	http://www.boldsystems.org/
H350	1	0	0	0	MOSN2487-19	http://www.boldsystems.org/
H351	1	0	0	0	MOSN2647-19	http://www.boldsystems.org/

H352	1	0	0	0	MOSN2649-19	http://www.boldsystems.org/
H353	1	0	0	0	MOSN2655-19	http://www.boldsystems.org/
H354	1	0	0	0	MOSN288-18	http://www.boldsystems.org/
H355	1	0	0	0	MOSN2897-19	http://www.boldsystems.org/
H356	1	0	0	0	MOSN2909-19	http://www.boldsystems.org/
H357	1	0	0	0	MOSN2913-19	http://www.boldsystems.org/
H358	1	0	0	0	MOSN292-18	http://www.boldsystems.org/
H359	1	0	0	0	MOSN2953-19	http://www.boldsystems.org/
H360	1	0	0	0	MOSN2978-19	http://www.boldsystems.org/
H361	1	0	0	0	MOSN3117-19	http://www.boldsystems.org/
H362	1	0	0	0	MOSN3126-19	http://www.boldsystems.org/
H363	1	0	0	0	MOSN3127-19	http://www.boldsystems.org/
H364	1	0	0	0	MOSN3136-19	http://www.boldsystems.org/
H365	1	0	0	0	MOSN3165-19	http://www.boldsystems.org/
H366	1	0	0	0	MOSN3182-19	http://www.boldsystems.org/
H367	1	0	0	0	MOSN3228-19	http://www.boldsystems.org/
H368	1	0	0	0	MOSN3318-19	http://www.boldsystems.org/
H369	1	0	0	0	MOSN3319-19	http://www.boldsystems.org/
H370	1	0	0	0	MOSN3321-19	http://www.boldsystems.org/
H371	1	0	0	0	MOSN3381-19	http://www.boldsystems.org/
H372	1	0	0	0	MOSN3402-19	http://www.boldsystems.org/
H373	1	0	0	0	MOSN3403-19	http://www.boldsystems.org/
H374	1	0	0	0	MOSN3490-19	http://www.boldsystems.org/
H375	1	0	0	0	MOSN3499-19	http://www.boldsystems.org/
H376	1	0	0	0	MOSN3523-19	http://www.boldsystems.org/
H377	1	0	0	0	MOSN360-18	http://www.boldsystems.org/
H378	1	0	0	0	MOSN436-18	http://www.boldsystems.org/
H379	1	0	0	0	MOSN491-18	http://www.boldsystems.org/
H380	1	0	0	0	MOSN503-18	http://www.boldsystems.org/
H381	1	0	0	0	MOSN848-18	http://www.boldsystems.org/
H382	1	0	0	0	MOSN852-18	http://www.boldsystems.org/
H383	1	0	0	0	MOSN853-18	http://www.boldsystems.org/
H384	1	0	0	0	MOSN858-18	http://www.boldsystems.org/
H385	1	0	0	0	MOSN968-18	http://www.boldsystems.org/
H386	1	0	0	0	MOSN972-18	http://www.boldsystems.org/
H387	1	0	0	0	MOSN976-18	http://www.boldsystems.org/
H388	1	0	0	0	MOSN991-18	http://www.boldsystems.org/
H389	1	0	0	0	NEONT275-10	[53]
H390	1	0	0	0	NEONU128-11	http://www.boldsystems.org/
H391	1	0	0	0	NEONU130-11	http://www.boldsystems.org/
H392	1	0	0	0	NEONU309-11	http://www.boldsystems.org/
H393	1	0	0	0	TDWGB127-10	http://www.boldsystems.org/
H394	1	0	0	0	TDWGB705-10	http://www.boldsystems.org/
H395	1	0	0	0	USDIQ1433-10	http://www.boldsystems.org/
Total	1049	2	46	87	1184	

156 **Figure 1.** Locations where genetic material was extracted for the *A. vexans*
 157 populations analyzed (2A), as well as phylogenetic relations among populations, as arrows
 158 observed in the haplotype network (2B). In both figures the colors below denote the
 159 continents and their respective countries. ● America + Europe: Canada, United States and
 160 Turkey. ● Asia: China, India, Japan, Singapore and South Korea. ● Europe + Asia: Sweden,
 161 Belgium and China. ● Europe + Asia: Romania, Sweden, Belgium, Russia, Kosovo, the
 162 Netherlands, China, Spain, Germany, Iran, Austria and Hungary. ● Africa: South Africa. ●
 163 America + Africa: South Africa and the United States.

164 Table 2 shows, by continent and countries, the results of Hd , π and the different
 165 neutrality tests. In general, the global Hd was 0.92, while by continents it varied between 0.90
 166 (Europe) and 1.0 (Africa). The Hd by countries was between 0.0 (Austria, Turkey, Hungary,
 167 Singapore, and India) and 1.0 (Germany, Kosovo, Rumania, Russia, South Korea, and South
 168 Africa). In turn, the global π was 0.01, while by continents it varied between 0.005 (Europe)
 169 and 0.08 (Africa). The π among countries ranged between 0.0 (Austria, Hungary, Turkey,
 170 Singapore, and India) and 0.08 (South Africa). Neutrality tests, Tajima's D, and Fu's F, at
 171 global level, were negatively significant ($D = -2.20$, $p < 0.001$; $F = -5.22$, $p < 0.02$); by
 172 continent and countries, America ($D = -2.46$, $p < 0.05$; $F = -5.65$, $p < 0.02$) and all its countries
 173 (Canada $D = -2.43$, $p < 0.05$; $F = -5.13$, $p < 0.02$ and USA $D = -2.30$, $p < 0.05$; $F = -4.96$, $p <$
 174 0.02) were statistically significant and with negative values.

Table 2. Results of genetic diversity and neutrality tests at global level and by countries for *A. vexans*.

Contine nt	Countri es	Number of sequences	Genetic diversity			Neutrality tests	
			Number of haplotypes	Hd	π	Tajimas' D	Fus's F

America		1049		0.97391	0.0299		
			325	3	4	-2.463612*	-5.65202*
	Canada	759	191	0.90113	0.0051	-2.435669*	-5.13490*
					8		
	USA	290	134	0.93120	0.0108	-2.306334*	-4.96645*
					8		
Africa		2		1.0000	0.0851	N/A	N/A
			2		1		
	South Africa	2	2	1.0000	0.0851	N/A	N/A
					1		
Asia		46		0.91259	0.0213	0.7167907	-0.55008
			29		5		
	China	13	9	0.98717	0.0251	-0.07488041	-0.51062
					5		
	South Korea	3	3	1.0000	0.0117	N/A	N/A
					6		
	Iran	7	3	0.80952	0.0040	0.4024933	0.4229
					5		
	India	2	1	0	0	N/A	N/A
	Japan	13	8	0.94871	0.0148	-0.3390129	-0.52121
					1		
	Russia	5	4	1.0000	0.0058	0.2734498	0.27834
					8		
	Singapore	3	1	0	0	N/A	N/A
Europe		85		0.90983	0.0059	-0.2722376	-1.65434
			39		8		
	Germany	2	2	1.0000	0.0029	N/A	N/A
					4		
	Austria	2	1	0	0	N/A	N/A
	Belgium	16	8	0.89166	0.0145	-1.16233	-0.48408
					5		
	Spain	15	7	0.88571	0.0057	-1.619073	-2.29068
					7		
	Hungary	2	1	0.0000	0	N/A	N/A
	Kosovo	2		1.0000	0.0029	N/A	N/A
			2		4		

Netherlands	10	4	0.71111	0.0032 0	0.0964613	0.17394
Rumania	2	1	1.0000	0.0117 6	N/A	N/A
Sweden	34	12	0.95365	0.0275 9	1.301434	0.12606
Turkey	2	1	0	0	N/A	N/A
Total	1182	395	0.92853	0.0109 9	-2.208676*	-5.22367*

Hd = Haplotype diversity; π = Nucleotide diversity; N/A = Not available; * = $p < 0.05$.

175 In Table 3, the AMOVA indicated the existence of genetic structuring at continent and
 176 country levels and within countries ($F_{ST} = 0.08$, $p < 0.05$), where the highest variation
 177 percentage was observed among *A. vexans* individuals within countries (91.30%), followed by
 178 7.07% among continents and 1.62% among countries in the same continent. In Figure 2,
 179 Mantel's test indicated no isolation by distance ($r = 0.003$, $p > 0.05$).

Table 3. Analysis of molecular variance (AMOVA) of populations of *A. vexans* at continental level, by countries, and within them.

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

^aValue obtained from 10000 random permutations

180 **Figure 2.** Global Mantel’s correlation test for genetic and geographic distance of the
 181 *A. vexans* populations analyzed.

182 Table 4 shows peer-to-peer comparisons among countries after Bonferroni’s
 183 correction. For these, significant genetic structuring was detected within Canada with respect
 184 to Belgium, Sweden, Spain, Hungary, and Netherlands. The USA with respect to Austria,
 185 Belgium, Sweden, Spain, Hungary, and Netherlands. China with respect to Netherlands, the
 186 USA, and Canada. India with respect to the USA and Canada. Iran with respect to the USA
 187 and Canada. Japan with respect to Belgium, Sweden, Spain, and Netherlands. Russia with
 188 respect to the USA and Canada. Singapore with respect to Belgium, Sweden, and Spain.

Table 4. Values by countries of genetic differentiation by peers (F_{ST}) and geographic distance (Km) among *A. vexans* populations. Values in bold are genetically structured populations.

	Au	Bel	Tu	Sw	Sp	Ru	Neth	Ko	Hu	Ger	Ca	The	Chi	Indi	Iran	Jap	Ru	Sin	Sout	Sout
	stri	giu	rke	ede	Sp	ma	erlan	sov	nga	ma	na	United	na	a	Iran	an	ssi	gap	h	h
	a	m	y	n	ain	nia	ds	o	ry	ny	da	States	na	a		a	a	Kore	Afric	
Austria	0	80	19	14	16	813	839.	74	375	503	72	8353.2	714	643	368	916	15	985	8624	8731
		6.8	20.	28.	56.	.21	29	0.2	.66	.23	60.	8	5.3	7.5	5.8	6.6	80.	2.86	.48	.87
		1	67	80	86		7				85		8	3	4	2	06			
Belgium	0.1		27	13	12	159		15	116		65		763	717	446	937	12	105		
		0	24.	91.	86.	6.5	190.	29.	0.2	426	69.	7562.3	5.7	9.4	8.5	5.4	82.	62.1	8930	9205
	6		38	91	86	6	10	55	7		97	6	3	4	1	2	1	5	.74	.65
Turkey	1.0	0.2		26	33	114		12	156	235	88		595	463	181	853	30	808	7798	7843
	0	9	0	26.	15.	5.4	2727	72.	7.4	7.3	47.	10193.	6.5	6.3	4.9	7.2	26.	3.65	.83	.71
				41	95	7	.37	91	2	5	73	06	4	6	4	6	34			
Sweden	0.1	0.0	0.2		26	163		19	144	112	62		638	641	400	798	46	962	7557	1010
				0	78.	3.0	1211	56.	4.4	0.6	21.	7676.0	5.7	1.6	5.7	4.0	6.4	0.94	.36	2.98
	8	1	3		56	1	.49	96	2	6	33	5	1	8	8	5	2			
Spain	0.2	-			239			20	200	161	70		879	795	512	106	25	113	1019	8371
	0	0.0	0.2	0.0	0	4.3	1470	60.	1.7	7.1	67.	7597.4	8.1	0.5	9.7	60.	35.	96.1	6.42	.54
			9	1		.75		65	2	9	6	2	1	5	5	56	95	4		

Rumania	0.5	0.0	0.5	0.0	0.0	0	1585	49	121	78	9055.9	648	562	287	871	19	904	8097	8518	
	0	8	0	2	8		.82	3.0	439	4.3	00.	3.4	7.0	5.3	7.0	67.	9.68	.49	.7	
								2	3	24	9	9	5	2	8	74				
Netherlands	0.2	-	0.4	0.0	-	0.2	0	15	116	64	7514.4	749	711	443	919	10	104	8760	9364	
	2	0.0	3	6	0.0	1	0	78.	1.4	60.	9	4.7	8.4	9.9	5.3	92.	80.8	.3	.94	
		2			1			53	4	97		9	1	2	5	23	1			
Kosovo	0.0	-	0.5	-	0.0	0.0	0.07	0	123	79	9089.5	692	589	308	920	22	933	8577	8147	
	0	0.0	0	0.0	0.0	0.0	0	519	8.8	83.	9	5.2	3.5	4.7	6.7	16.	6.84	.55	.03	
		1		1					9	27			5	4	8	33				
Hungary	0.0	0.1	1.0	0.1	0.2	0.5	0.22	0.0	74	794	8661.7	682	606	331	893	16	947	8357	8658	
	0	6	0	8	0	0	0	0	84.	56	5	1.4	1.8	4.8	4.0	97.	8.4	.86	.83	
									73			7	7	5	7					
Germany	0.5	0.0	0.5	0.0	0.0	0.0	0.21	0.0	67	58.	7870.2	723	675	406	905	11	101	8579	9179	
	0	8	0	3	8	0	0	0	0	28	8	1.9	9.7	8.9	8.5	53.	36.4	.29	.84	
												8	5	8	5	09	5			
Canada	0.2	0.1	0.2	0.0	0.1	0.0	0.17	0.0	0.2	0.0	2264.0	939	114	999	809	58	130	8588	1520	
	6	0	3	7	0	7	0.17	7	6	7	7	6.3	81.	8.9	1.7	36.	82.5	.54	1.01	
												7	96	1	7	54	8			
The United States	0.2	0.0	0.2	0.0	0.0	0.0	0.15	0.0	0.2	0.0	0	116	135	116	101	72	153	1075	1441	
	4	8	3	5	8	5	0.15	5	4	5	0	60.	91.	70.	61.	28.	07.0	4.68	4.9	
												31	88	95	81	03	3			
China	0.2	0.0	0.2	0.0	0.0	0.0	0.14	0.0	0.2	0.0	0.0		298	461	305	67	384	2120	1124	
	3	6	3	3	6	0	0.14	0	3	0	6	0.04	0	7.1	7.4	0.1	50.	1.78	.74	9.63
													4	8	7	64				
India	10.	0.2	1.0	0.2	0.2	0.5	0.43	0.5	1.0	0.5	0.2	0.1	283	596	68	344	5022	8264		
	00	9	0	3	9	0	0.43	0	0	0	6	0.25	0	0.9	5.9	74.	7.51	.33	.28	
													2	8	4					
Iran	0.3	0.0	0.3	0.0	0.1	0.1	0.18	0.1	0.3	0.1	0.1	0.0	0.3		752	44	627	6664	7724	
	8	8	8	8	0	3	0.18	3	8	3	3	0.11	9	8	0	3.9	63.	7.06	.19	.52
															4	71				
Japan	0.2	0.0	0.2	0.0	0.0	0.0	0.16	0.0	0.2	0.0	0.0	0.06	0.0	0.2	0.1		81	525	943.	1410
	6	8	6	4	8	3	0.16	3	6	3	7	0.06	3	6	1	0	83.	5.67	81	2.62
																22				
Russia	0.1	-	0.2	-	-	0.0	0.00	-	0.1	0.0	0.0	0.0	0.2	0.0	0.0		100	7814	1031	
	3	0.0	8	0.0	0.0	0	0.00	0.1	3	0	6	0.04	0	8	1	2	0	60.8	.24	1.88
		5		2	3			1									3			

Singapore	1.0	0.3	1.0	0.2	0.3	0.6		0.6	1.0	0.6	0.3		0.2	0.0	0.4	0.3	0.3		4572	9223
	0	5	0	9	5	4	0.48	4	0	4	0	0.29	5	0	5	2	8	0	.22	.14
South Korea	0.3	0.0	0.3	0.0	0.0	0.0		0.0	0.3	0.0	0.0		0.0	0.3	0.1	0.0	0.0			1318
	6	7	6	3	7	0	0.19	0	6	0	6	0.04	0	6	1	3	0	0.50	0	8
South Africa	0.5	0.0	0.5	0.0	0.0	0.0		0.0	0.5	0.0	0.0		0.0	0.5	0.1	0.0	0.0			
	0	8	0	3	8	0	0.21	0	0	0	7	0.05	0	0	3	3	0	0.64	0.00	0

189

190 Figure 3 shows the results of the phylogenetic analyses by using BI (Fig. 3A) and ML
 191 (Fig. 3B); both analyses recovered the same six clades, but with distinct topologies: clade I
 192 grouped mosquito populations from the United States, Canada, and Turkey (n = 322 H); clade
 193 II, populations from China, Japan, Singapore, and South Korea (n = 23); clade III, populations
 194 from Sweden, Belgium, and China (n = 14 H); clade IV, mosquito populations from Rumania,
 195 Sweden, Belgium, Russia, Kosovo, Netherlands, China, Spain, Germany, Iran, Austria, and
 196 Hungary (n = 31 H); clade V, populations from South Africa (n = 1 H); and clade VI,
 197 populations from the United States and South Africa (n = 4 H). Similar results were observed
 198 in the haplotype network (Figure 1B).

199 **Figure 3.** Phylogenetic tree for *Aedes vexans* populations constructed from 395
 200 haplotypes from the COI gene by using Bayesian Inference, BI, (3A) and Maximum
 201 Likelihood, ML, (3B). The evolutionary history for both analyses was inferred by using the
 202 GTR + G model, as suggested by jModelTest version 2.1.10. The BI tree was obtained by
 203 using 2-million generations, while the ML used 1,000 replicas. For BI, the support of the
 204 branches is indicated by the subsequent probability values, while for ML the bootstrap values
 205 are shown. Numbers in blue represent sequences from the *A. nipponii* subspecies. In both
 206 figures the colors below denote the continents and their respective countries. ● America +
 207 Europe: Canada, United States and Turkey. ● Asia: China, India, Japan, Singapore and South

208 Korea. ● Europe + Asia: Sweden, Belgium and China. ● Europe + Asia: Romania, Sweden,
209 Belgium, Russia, Kosovo, the Netherlands, China, Spain, Germany, Iran, Austria and
210 Hungary. ● Africa: South Africa. ● America + Africa: South Africa and the United States.

211

212

213 Discussion

214 To our knowledge, this is the first study on the genetic structure of *A. vexans* using all the
215 genetic information available for the COI gene from the GenBank and Boldsystem databases.
216 From this information, both the haplotype network and the phylogenetic tree revealed the
217 existence of six clades; clade I grouped mosquito populations from America and Europe;
218 clade II, populations from Asia; clades III and IV, populations from Europe and Asia; clade
219 V, populations from Africa; and clade VI, populations from America and Africa. For *A.*
220 *vexans*, existence is suggested of three subspecies: *A. vexans vexans*, *A. vexans arabiensis*,
221 and *A. vexans nipponii*, from which it was possible to include in our natural population
222 analyses of *A. vexans* and *A. vexans nipponii*. *A. vexans vexans* has been reported for east
223 Asia and Oceania, *A. vexans arabiensis* in Africa and Europe, and *A. vexans nipponii* in
224 southeast Asia [1,14,2,42,15,16,17]. However, our results do not suggest the existence of
225 three subspecies or the geographic relations observed. Nonetheless, it is interesting that with
226 *A. vexans* being considered native of America [13] its invasion is not hypothesized to other
227 latitudes, a pattern observed even with *A. vexans nipponii* terminals registered in Asia, as
228 suggested by our results. This is why the subspecies observed in countries different from
229 America would probably be populations from their place of origin through passive transport
230 (*i.e.*, maritime, air, or land transport), as already observed for other Culicidae invaders
231 [54,55,56,57,58], including *A. vexans* [59]. Future studies should include information at
232 genome level to try to solve the possible existence of subspecies, given that using a single
233 marker is not sufficient to define the species [60], as was observed even for this species by
234 using the COI gene [48].

235 Haplotype diversity and number of haplotypes observed in the American continent
236 ($Hd = 0.97$; $H = 325$) were higher than in other continents; for example, Europe ($Hd = 0.90$; H
237 $= 39$). Various studies suggest that native species have higher genetic diversity when

238 compared with places different from their native area [61,62,63]. For example, a global study
239 on the Cosmopolitan Asian mosquito, *Aedes albopictus*, principal dengue, Zika and
240 Chikungunya vector in Asia and Europe, observed greater genetic diversity ($Hd = 0.94$, $\pi =$
241 1.60) in its native area with respect to all the areas it has invaded, showing – therein – lower
242 diversity indices (lower Hd in Netherlands ($0.059 = \pi = 0.011$) and higher Hd in China (0.946
243 $= \pi = 1.609$)) [63]. The aforementioned supports our hypothesis that *A. vexans* populations
244 may have invaded other latitudes.

245 The *A. vexans* mosquito showed significant genetic divergence among some populations from
246 the American continent with respect to some European and Asian populations (Table 4). [64],
247 analyzing natural *A. vexans* populations from the United States and Germany, found that these
248 do not share a common gene pool, proposing that the geographic barriers formed by the
249 Atlantic and Pacific Oceans impede gene flow and cause genetic changes in the evolutionary
250 lineages of *A. vexans*. However, our results suggest no existence of geographic and genetic
251 isolation. Additionally, for most of the populations, the results of the neutrality tests, Tajima's
252 D and Fu's FS were negative (Table 2), suggesting that these have experienced recent
253 bottlenecks and population expansion [65]. This may be due to recent vector-control actions
254 and colonization events, phenomena commonly observed in mosquitos of medical and
255 veterinary importance [66]. In the first case, these are used to diminish the population size of
256 *A. vexans* and other vector species of diseases and, consequently, curtail the epidemiologic
257 transmission of the diseases it transmits [67,68,69]. In the second case, recent colonization
258 events may take place in areas from where the *A. vexans* populations are lost during harsh
259 winters or after vector-control actions and can be re-colonized by surviving individuals from
260 neighboring areas [3,70,58].

261 **Conclusions**

262 Finally, our results suggest that the *A. vexans* populations that invaded other continents
263 originate directly from America, where possibly transcontinental commercial routes favored
264 their long-distance dispersion. Moreover, we consider this study as the base for future
265 taxonomic research that address the problem of the existence of subspecies within *A. vexans*,
266 given that our results did not recover any of the subspecies suggested by the literature.

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270 **Ethical considerations**

271 This work did not experiment with humans or other living beings; its data were obtained from

272 genetic databases freely available on line.

273 **Conflicts of interest**

274 The authors have no conflict of interest to declare.

275

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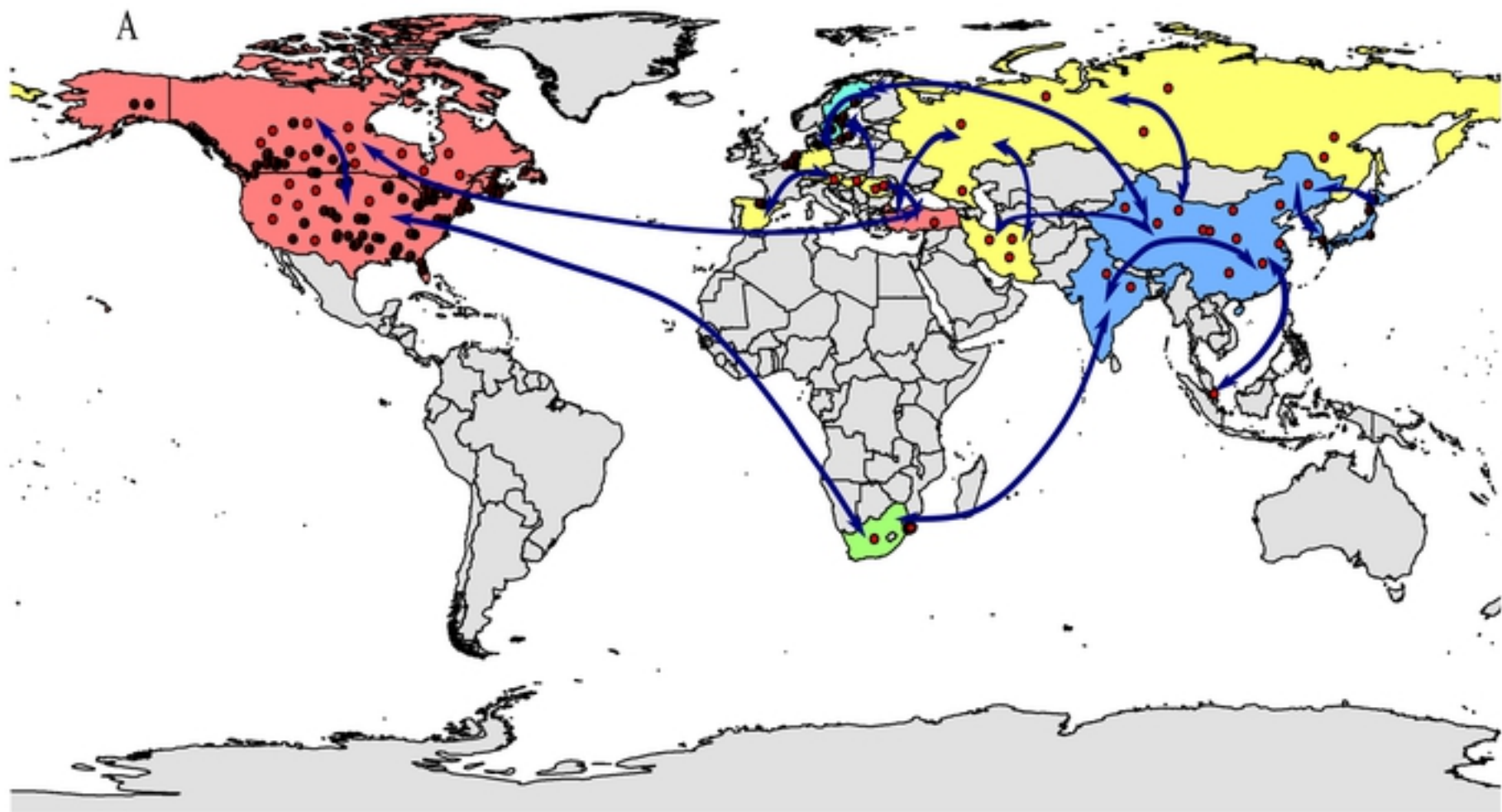
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489 **Supporting information**

490 **S1.** Nucleotide diversity of the COI gene for *A. vexans* populations. Mafft alignment to
491 compare the haplotypes from this study. The amino acid sequence translated is represented by
492 capital letters in blue, over the first nucleotide of its corresponding codon. Invariable sites are
493 indicated with points, contrary to the alternative nucleotide, and spaces with (-).

494



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B

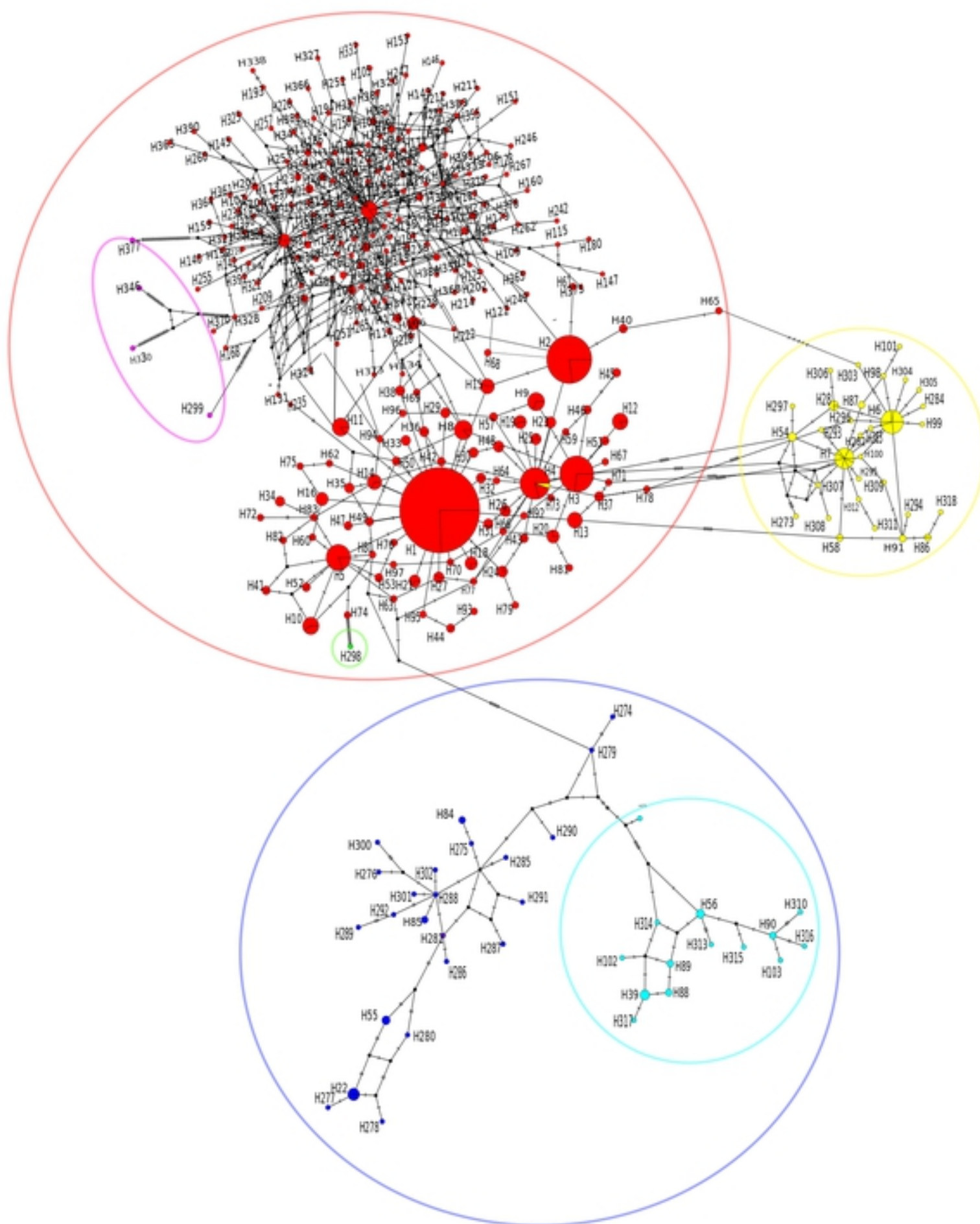


Figure 1

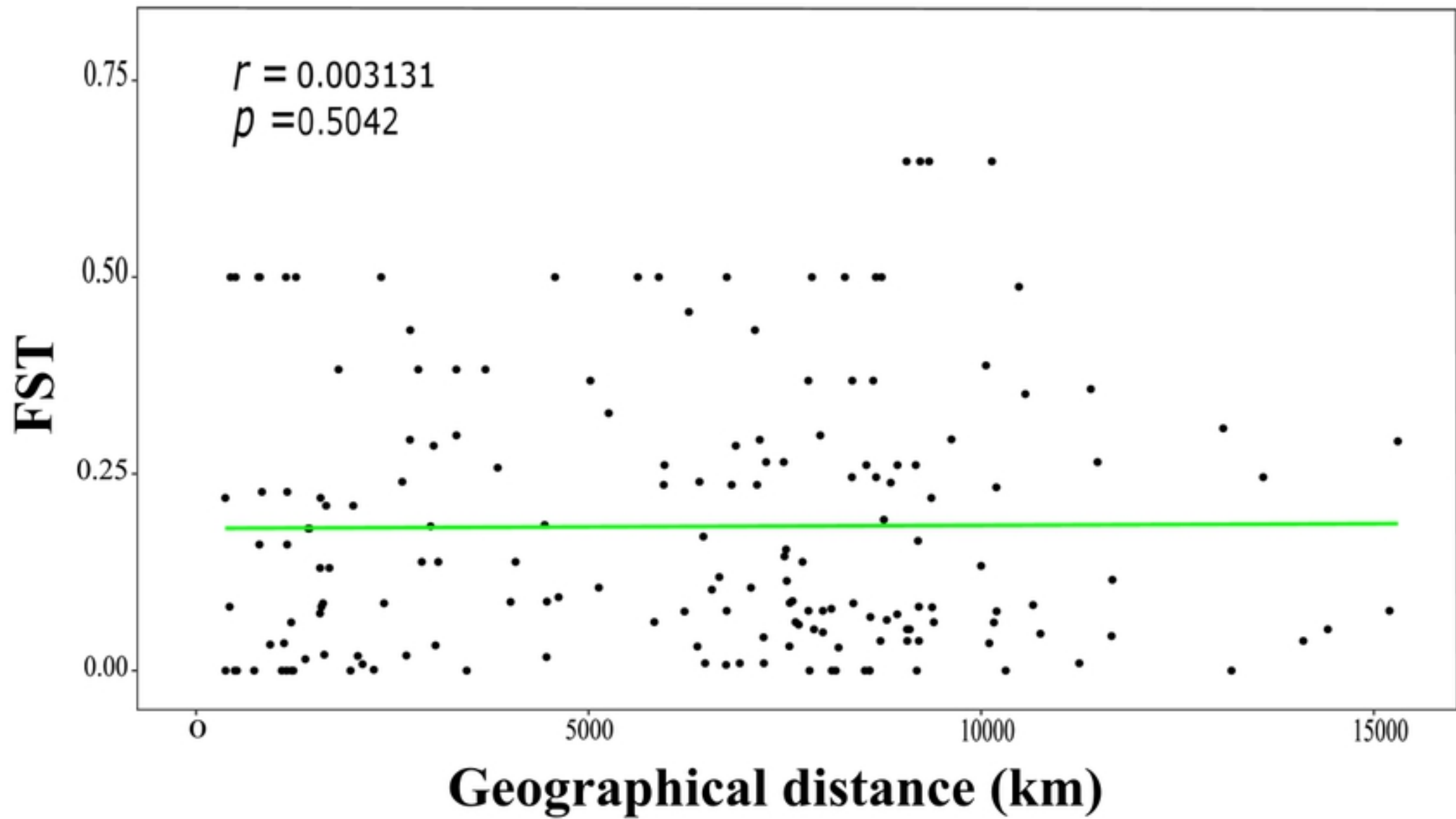


Figure 2

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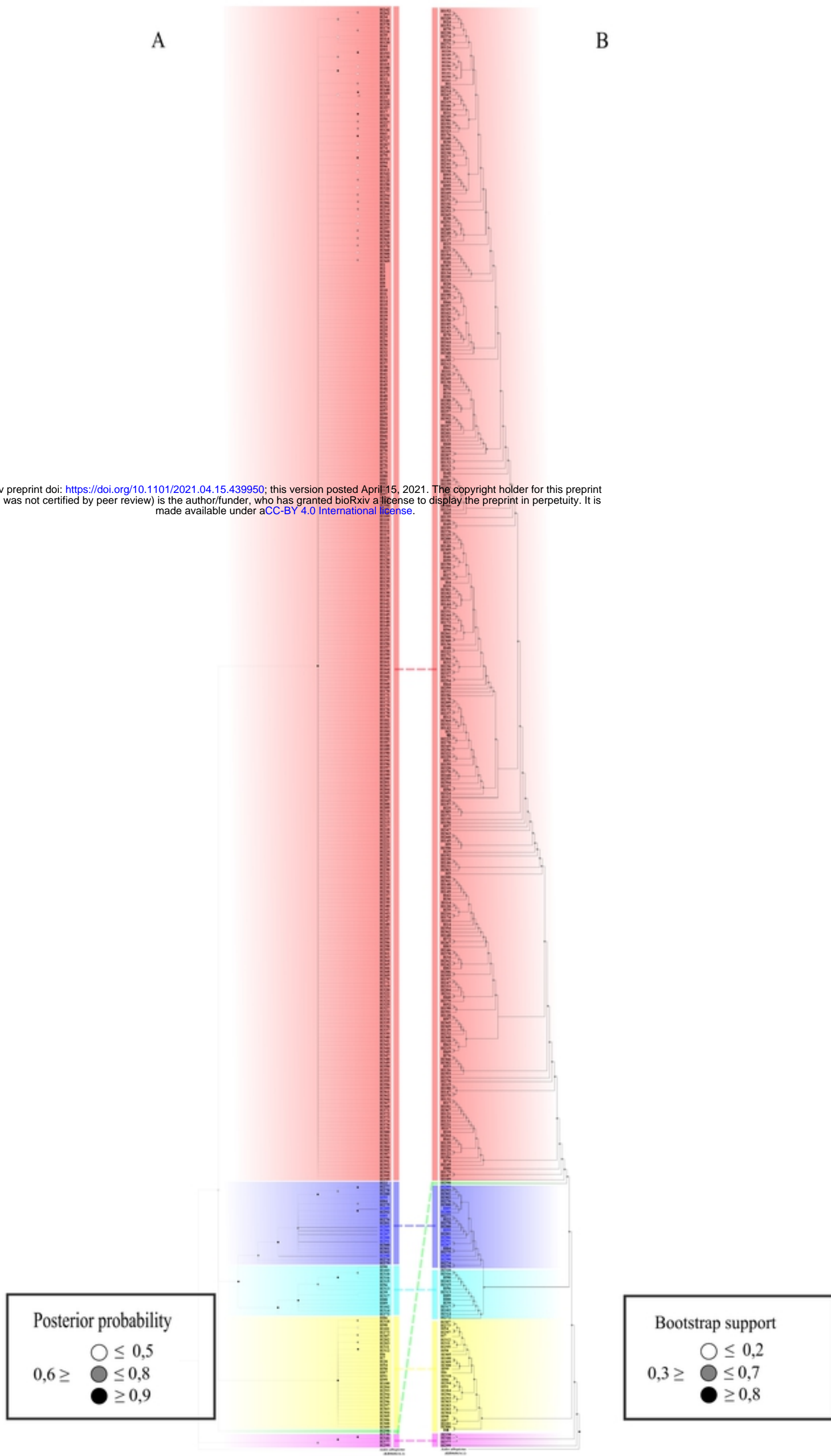


Figure 3