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Origin of a High-Latitude Population of *Aedes aegypti* in Washington, DC

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Abstract. An overwintering population of *Aedes aegypti* has been documented in the Capitol Hill neighborhood of Washington, DC, since 2011. Mitochondrial cytochrome oxidase I (*mtCOI*) sequence data presented in a previous study traced the origin to the New World. Here, we use microsatellite and 14,071 single nucleotide polymorphisms along with mitochondrial DNA (mtDNA) sequences on Washington *Ae. aegypti* samples and samples from potential sources to further narrow the origin of this population. Genetically, Washington *Ae. aegypti* are closest to populations in Florida, meaning this is the most likely source. Florida experienced the first mosquito-borne transmission of dengue in the United States after decades of absence of this disease, as well as local transmission of chikungunya and Zika in recent years. This suggests that the Capitol Hill, Washington, DC population of *Ae. aegypti* is capable of transmitting viruses such as dengue, chikungunya, and Zika in modern US city environments.

INTRODUCTION

Aedes aegypti was given the common name of "the yellow fever mosquito" because it was identified as the primary urban vector of that devastating disease in the early twentieth century. Today, it is a major public health concern as the main global vector of dengue, chikungunya, and Zika viruses. Human populations at risk for these diseases coincide with the distribution of *Ae. aegypti*, thus factors affecting the distribution of this mosquito are of considerable medical importance.

The distribution of *Ae. aegypti* is temperature restricted. The generally accepted dogma is that permanent year-round breeding populations cannot exist where the mean temperature of the coldest month is less than 10°C.[1] In North America, this corresponds approximately to 33° N latitude. The report of a year-round breeding population in the Capitol Hill neighborhood of Washington, DC, was, therefore, unexpected.[2] Washington, DC, is at about 39° N, with a mean January (minimum month) temperature of 6°C. Data from Lima et al.[2] indicate that this population is temporally stable, suggesting an overwintering population. Although these data could also be consistent with an influx of large numbers of *Ae. aegypti* individuals from the same source to the same neighborhood in DC every year, this possibility is deemed unlikely. Evidence indicates that the population is overwintering, probably in subterranean refugia and engaging in limited breeding aboveground in summer and fall.[2]

Historical records of yellow fever in the northeastern United States indicate that temporary warm weather (summer/fall) introductions of *Ae. aegypti* have occurred for centuries;[3] however, this Capitol Hill population is unique in overwintering. Given its distinctive appearance and major health importance, it is likely that the population would have been noted had it existed for some time, thus it is probably a recent introduction. The question remains as to where it came from. Lima et al.[2] presented mitochondrial DNA (mtDNA) data consistent with the population originating within the Americas, but were not able to pinpoint a more precise location. Identifying the source of the Capitol Hill population can help to understand how *Ae. aegypti* may have arrived to Washington, DC, and to prevent similar introductions in the future, both to the area and to other naive regions. Furthermore, the ability of *Aedes* mosquitoes to transmit disease varies across geographic regions,[4–10] as it does insecticide resistance.[11–13] Determining the genetic affiliations of the Washington, DC, population of *Ae. aegypti* could guide control efforts and provide insights on the ability of these mosquitoes to transmit viruses with worldwide circulation.

Aedes aegypti populations are highly genetically differentiated across its range, displaying a strong and hierarchical genetic structure.[14–17] This pattern of geographic differentiation can be used as population "genetic signatures" to track the source of novel introductions, when compared with a reference panel.[15] In two previous cases, The Netherlands and California, we successfully used genetic approaches based on microsatellite markers to identify the origin of new introductions of *Ae. aegypti*.[18,19] Here, we apply these methods, supplemented by 14,071 single nucleotide polymorphisms (SNPs) and additional mtDNA sequencing, to narrow down the likely origin of the overwintering Capitol Hill, Washington, DC, population of *Ae. aegypti.*

MATERIALS AND METHODS

Mosquito samples. Collection information and specific locations for *Ae. aegypti* samples from the Capitol Hill neighborhood in Washington, DC, were previously described.[2] DNA preparations representing a subset of individuals from that study collected during 2014 were included in microsatellite and SNP chip genotyping assays.

Most *Ae. aegypti* collections used as reference for microsatellites and the SNP genotypes for this analysis were reported previously.[15,16,20–23] Collections not reported previously were obtained as eggs from oviposition traps or as adults sampled directly from the field. Eggs were hatched at Yale University and reared to adults for identification and preservation in 100% ethanol at –20°C. Collections included in this study are described in Table 1.

Nuclear DNA genotyping and analyses. Total nucleic acids were extracted from individual mosquitoes with the DNeasy Blood and Tissue kit (Qiagen), according to manufacturer instructions, including an optional treatment with 4 µL of RNase A (Qiagen, Hilden, Germany). Samples were stored at –20°C until further analysis.

Microsatellite markers. We used 12 highly variable microsatellites (~10 alleles per locus, > 50% heterozygosity),

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TABLE 1

Aedes aegypti collections included in this study

Population	Region	Year*	N_μ†	Reference	N_{SNP}‡	Reference
Dakar, SE	2005	Africa	20	[15]	–	–
Lunyo, UG	2013	Africa	20	[15]	–	–
Yaounde, CM	2014	Africa	20	[15]	–	–
Francesville, GA	2014	Africa	20	[15]	–	–
Johannesburg, ZA	2015	Africa	20	[15]	–	–
Bijagos, GW	2009	Africa	20	This study	–	–
Nairobi, KE	2012	Africa	20	[15]	–	–
Ho Chi Minh, VT	2013	Asia	54	[15]	19	This study
Hanoi, VT	2013	Asia	54	[15]	22	This study
Cebu City, PH	2013	Asia	108	[15]	8	This study
Pakistan, PK	2010	Asia	49	[15]	17	This study
Jeddah, SA	2012	Asia	84	[15]	11	This study
Rayong,TH	2006	Asia	48	[16]	–	–
Prachuabkhirikan, TH	2009	Asia	47	[16]	–	–
Townsville, AU	2009	Asia	47	[16]	–	–
Cairns, AU	2009	Asia	–	–	12	[20]
Cairns, AU	2015	Asia	24	[22]	–	–
Bangkok, TH	2012	Asia	54	This study	11	This study
Bangkok, TH	2013	Asia	–	–	–	–
Sri Lanka, SL	2014	Asia	7	[15]	5	This study
Patillas, PR	2014	Caribbean	54	[15]	12	[20]
Trinidad, TT	2014	Caribbean	51	[15]	12	This study
Tobago, TT	2015	Caribbean	–	–	8	This study
Saint Vincent, VC	2015	Caribbean	–	–	12	This study
Dominica, DM	2009	Caribbean	95	[15]	–	–
Dominica, DM	2017	Caribbean	–	–	13	This study
Siquirres, CR	2014	Central America	51	[15]	6	This study
Madeira, PO	2012	Europe	66	[15]	6	This study
Maricopa County, AZ, USA	2013	North America	53	[15]	–	–
Tucson, AZ, USA	2012	North America	–	–	12	This study
Tijuana, BC, MEX	2013	North America	20	[15]	10	This study
Madera, CA, USA	2013	North America	50	[15]	24	This study
Clovis, CA, USA	2013	North America	60	[15]	11	This study
Fresno, CA, USA	2015	North America	–	–	12	This study
Houston, TX, USA	2011	North America	19	[15]	8	[20]
Pijijiapan, CHP, MEX	2008	North America	47	[15]	–	–
Miami, FL, USA	2011	North America	47	[15]	–	–
Las Palomas, GRO, MEX	2012	North America	54	[15]	–	–
Lomas de Zapatero, GRO, MEX	2012	North America	51	[15]	–	–
Iguala, GRO, MEX	2012	North America	54	[15]	–	–
Mazatan, CHP, MEX	2012	North America	45	[15]	–	–
Columbus, GA, USA	2012	North America	55	[15]	8	This study
Tapachula Norte, CHP, MEX	2012	North America	54	[15]	12	[20]
San Mateo, CA, USA	2013	North America	21	[15]	–	–
Hermosillo, SON, MEX	2013	North America	50	[15]	–	–
Nogales, SON, MEX	2013	North America	51	[15]	9	This study
Chetumal, QRO, MEX	2013	North America	54	[15]	–	–
Rio, FL, USA	2014	North America	51	[15]	–	–
Conch Key, FL, USA	2006	North America	42	[15]	–	–
Palm Beach, FL, USA	2006	North America	42	[15]	–	–
Cameron, TX, USA	2015	North America	60	This study	–	–
Dallas, TX, USA	2015	North America	60	[23]	–	–
El Paso, TX, USA	2015	North America	–	–	8	This study
Las Cruces, TX, USA	2015	North America	54	This study	–	–
Vaca Key, FL, USA	2015	North America	48	[22]	–	–
Key West, FL, USA	2016	North America	38	[22]	12	[22]
New Orleans, LA, USA	2015	North America	24	[22]	12	[22]
Amacuzac, MOR, MEX	2016	North America	52	This study	60	This study
Washington, DC, USA	2014	North America	27	This study	16	This study
Hawaii, USA	2009	Pacific	25	This study	6	[20]
Hawaii, USA	2016	Pacific	–	–	7	This study
Tahiti, FP	2010	Pacific	48	[15]	12	[20]
Cali, CO	2013	South America	80	[15]	12	This study
Bolivar, VEN	2004	South America	48	[15]	–	–
Cachoeiro, BR	2012	South America	47	[15]	–	–
Zulia, VEN	2004	South America	47	[15]	–	–
Maraba, BR	2010	South America	48	[15]	12	This study
Natal, BR	2010	South America	47	[15]	–	–
Jacobina, BR	2013	South America	94	[15]	40	[20]
Rio de Janeiro, BR	2014	South America	39	[15]	7	This study
Total			2,821	–	484	–

* Year of collection.

† No. of individuals genotyped with microsatellite loci.

‡ No. of individuals genotyped with SNPs.

validated in previous studies addressing the global genetic diversity of *Ae. aegypti*[15,16,20] and used to identify the origin of introductions in other studies.[18,19] Genotyping was performed as described in those publications.

SNP genotyping. A custom designed SNP chip[20] was used on a subset of the samples genotyped for microsatellites. Approximately 200 ng of genomic DNA from individual mosquitoes were sent to the Functional Genomics Core at the University of North Carolina, Chapel Hill, for hybridization with the Axiom aegypti1 SNP chip (Life Technologies Corporation CAT#550481) and production of genotypes. The Affymetrix Genotyping Console and the R package SNPolisher v1.4 (both from Affymetrix, Inc., Santa Clara, CA/now Life Technologies-Thermo Fisher Scientific, Waltham MA, USA) were used to generate and process genotype calls. While the SNP chip contains probes for > 27,000 well-validated biallelic SNPs (e.g., tested for Mendelian inheritance[20]), not all were variable in our samples. Additional pruning based on a linkage disequilibrium cutoff was performed using PLINK v.1.9[24] at a window size of 50 bp with a 10 bp window shift and considering a variance inflation factor threshold of 1.5, as recommended for small sample sizes.[24] The final dataset containing 14,071 SNPs was the basis for our analyses.

Analytic methods. The total number of alleles, average allele richness (AR), and private allele richness were calculated in HPRARE,[25] which uses rarefaction to correct for unequal sample sizes. Average observed heterozygosities (H_o) were estimated using GenAlEx.[26]

Population structure and assignments of individuals from the Washington, DC, population to specific genetic clusters were performed for microsatellite markers via the Bayesian clustering method implemented by the software STRUCTURE v. 2.3.[27] Each population was represented by 20 randomly chosen individuals. STRUCTURE identifies genetic clusters and assigns individuals to these clusters with no a priori information of the sample location. The most likely number of clusters (K) was determined by conducting 20 independent runs from K = 1 to 10 on 20 random individuals from each population. Each run assumed an admixture model and correlated allele frequencies using a burn-in value of 100,000 iterations followed by 500,000 repetitions. The optimal number of K clusters was determined following the guidelines of Pritchard et al.[28] and the Delta K method.[29,30] Results were plotted with the program DISTRUCT v.1.1.[31]

The program ADMIXTURE[32] was used to explore the population structure and genetic cluster assignment with the SNP dataset. This program uses maximum likelihood (ML) to estimate ancestral allele frequencies of unrelated individuals, in a similar manner as STRUCTURE, but runs more efficiently when analyzing large numbers of markers, such as our SNP dataset, thanks to the implementation of an expectation-maximization algorithm. Clustering algorithms are known to be greatly influenced by uneven sampling,[33,34] thus, each population was represented by 6–8 individuals (median = 8) chosen at random from the main dataset. This number of individuals has been shown to be sufficient to obtain accurate estimates of genetic diversity and differentiation when large numbers of SNPs are used (> 1,000 SNPs).[35,36]

GENECLASS2[37] was used to perform individual assignment tests on the Washington, DC, population against the reference population dataset that included all collections from America and the Caribbean listed in Table 1, using the Bayesian criteria for likelihood estimation to determine the population assignment ranking.[38] The entire microsatellite dataset was run at once, but due to software constrains limiting the number of markers that can be analyzed, SNP-assignment tests were conducted as 10 independent runs of 4,000 randomly selected SNPs drawn from the same individuals. Self-assignment tests on the microsatellite reference dataset resulted in 76.2% of individuals assigned to the correct population. In addition, individual assignment tests of 1 or 2 random individuals from each of the reference populations were performed to further evaluate the accuracy of the assignment method. Based on population assignment ranking, 77% of the individuals were assigned to their population of origin, whereas for 91% of the individuals the population of origin was included within the top three highest assignment probabilities or scores, respectively. Self-assignment tests on the SNP reference dataset resulted in 97.1% ± 0.3% of the individuals assigned to the correct population, with most misassignments pointing to a geographically and genetically close population.

Pairwise genetic distances[39] were calculated with the ADEGENET package in R v. 3.3.2.[40,41] Principal component analysis (PCA) and construction of the Neighbor Joining tree on the genetic distance matrix generated, were performed in the same package.

PCA on the SNP dataset was conducted with the package LEA,[42] available for the R software v. 3.3.2.[41]

Five individuals from each of the American and Caribbean populations were used to build a ML tree from the SNP data in RaxML v.8[43] using the GTRCAT model of rate heterogeneity, with ascertainment bias correction and midpoint rooting. Support for the branches was evaluated by running 1,000 independent bootstraps using the same package.

Mitochondrial cytochrome oxidase I (*mtCOI*) haplotype genotyping and analysis. DNA samples representing randomly selected subsets of individuals (N = 10) from three locations in Florida (Key West, Miami, and Palm Beach), one location in western Georgia (Columbus), a southern Mexico population (Tapachula), and a population from Central America (Siquirres, Costa Rica) were subjected to *mtCOI* haplotype genotyping, as previously described.[2] These populations were selected based on results from the microsatellite and SNP data analysis to narrow down the possible origin of the Washington, DC, population. Briefly, 710-bp *mtCOI* amplicons were obtained using 25-μL polymerase chain reaction (PCR) mixtures containing 1X Taq buffer (50 mM KCl, 10 mM Tris pH 9.0, 0.1% Triton X), 1.5 mM $MgCl_2$, 200 μM each dNTP, 5 pmoles of each primer, 1 unit of Taq DNA polymerase, and 1 μL of DNA template (some samples were diluted from 1:10 to 1:100 in sterile water). PCR products were size fractionated by electrophoresis in 2% agarose gels stained with ethidium bromide and visualized under ultraviolet light. PCR products were purified using the E.Z.N.A. Cycle Pure Kit (Omega Bio-Tek, Norcross, GA) and sequenced using an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA). The same primer sequences were used for amplification and sequencing: LCO1490: GGTCAACAAATCATAAAGATATTGG and HCO2199: AAACTTCAGGGTGACCAAAAAATCA.[44]

Sequences were aligned in Clustal Omega v1.2.4.[45,46] Clustal Omega. Available at: http://www.ebi.ac.uk). These sequences, along with a subset of *Ae. aegypti mtCOI* sequences from various geographic regions of the world used previously, were trimmed to a 497 base pair consensus and used to construct a phylogenetic tree in MEGA7[47] using a *mtCOI* sequence for *Aedes albopictus* (GenBank accession no. KC690960) as an outgroup.

Data availability. Microsatellite and SNP genotypes were deposited in VectorBase[48,49] PopBio projects: VBP0000201 (new data), and VBP0000138, VBP0000176-177 (previously published data). Sequencing data were deposited in NCBI under accession numbers MF371160–MF371174 and MG241351–MG241354.

RESULTS

Microsatellite markers. Average AR of the Washington, DC, collection was similar to the average AR of the American and Caribbean samples across all loci (3.73 and 3.84 [range: 2.92–4.76], respectively). The average number of private alleles across all loci in Washington, DC, was 0.06, whereas the average across American and Caribbean samples was 0.03 (0–0.16). Observed heterozygosity (H_o) was 0.493 compared with an average H_o of 0.532 across the American and Caribbean populations. These data suggest that the Washington, DC, population has not undergone an extreme bottleneck, which would suggest the reestablishment of the population from a few founders every year. Instead, the observed levels of genetic diversity, similar to those of other field populations, are consistent with an overwintering population. AR values and heterozygosities for all collections are shown in Supplemental Table 1.

Bayesian clustering analysis on a subsample of 37 representative world populations confirmed that the Washington, DC, samples were of the *Ae. aegypti aegypti* type (Supplemental Figure 1A). Further analysis including 53 populations outside of Africa identified three main genetic clusters (Supplemental Figure 1B), consistent with previous work.[15] Within these clusters, the Washington, DC, population was identified as an admixed population between the two clusters that included North and South America and the Caribbean (Supplemental Figure 1B). Subsequent hierarchical Bayesian clustering analyses suggest that the Washington, DC, population has a mixed ancestry, similar to populations from Georgia, Texas, and Florida (Figure 1A and B). PCA on genetic distances between the American populations was not conclusive (Supplemental Figure 2A and B). The neighbor-joining tree constructed from the same genetic distances positions the Washington, DC, population close to Georgia and Florida (Supplemental Figure 2C). Genetic assignment of the Washington, DC, individuals to the reference dataset of American populations (including the Caribbean) resulted in 13 out of 27 individuals assigned to a Florida population with the highest score, with the rest being mostly assigned to either Georgia or Arizona (Figure 1C, Supplemental Table 2).

SNP chip. PCA on the American and Caribbean populations using the SNP markers positioned the Washington, DC, population nearby Tapachula, Mexico; Key West, FL; and Siquirres, Costa Rica (Figure 2A). This relationship is also evident by inspecting the ML tree in Figure 2B. In the ML tree, the Washington, DC, clade is sister to a weakly supported clade that includes samples from Siquirres, Costa Rica; Key West, FL; and Tapachula, Mexico. These two clades belong to a larger clade that includes the rest of the North American populations (Figure 2B). Hierarchical clustering analysis on the SNP dataset includes representative populations outside Africa and clusters Washington, DC, within America (American continent and the Caribbean; Supplemental Figure 3). Subsequent clustering within this group positions Washington, DC, with Siquirres, Costa Rica; Tapachula, Mexico; and Key West, FL (Figure 2C). Genetic assignment tests connected 77.5% ± 11.5% of the Washington individuals to Florida and 17.4% ± 11.2% to Georgia (Figure 2D, Supplemental Table 3).

Cytochrome oxidase I (*COI*) mtDNA. We identified eight *COI* mtDNA haplotypes from 60 individuals collected at three locations in Florida, one location in Georgia, Costa Rica, and south Mexico (Table 2, Supplemental Figure 4); the three regions identified as possible origins of the Washington, DC, *Ae. aegypti* population by SNPs and microsatellite markers. Our sequence data for representative haplotypes identified at each location were submitted to GenBank under accession numbers MF371160–MF371174 and MG241351–MG241354. Both Washington, DC, haplotypes (A and B) were shared with Key West, Miami, and Palm Beach samples from Florida, but only haplotype A was present in the Columbus, GA; Siquirres, Costa Rica; and Tapachula, Mexico samples. None of the remaining seven haplotypes recovered (B–H) were represented at each of the six sample locations. Haplotype G was only found in Siquirres, Costa Rica, and Haplotype H only in Tapachula, Mexico. Phylogenetic comparisons with representative haplotype sequences of a larger group of *mtCOI* haplotypes from other geographical regions reported in Lima et al.[2] are consistent with those results (Supplemental Figure 4).

Results summary. Microsatellite and SNP data points to Florida, southern Mexico (Tapachula), or Costa Rica, as the likely source of the Washington, DC, population (Figures 1 and 2). Florida is the stronger candidate based on genetic assignment tests using SNPs and on complementary analysis of mitochondrial haplotypes. Both of the two mtDNA haplotypes present in Washington, DC, are found in a number of Florida populations (Table 2), whereas only one of them is shared with Georgia, south Mexico, or Costa Rica.

DISCUSSION

As summarized previously, our results from nuclear genetic markers and mtDNA consistently point to Florida as the most likely source of the Capitol Hill, Washington, DC, overwintering population of *Ae. aegypti*. Given the geographic locations and transportation connections, this is not surprising. Washington has two major airports, one ~50 km from its center (Dulles) and one in the downtown area (Reagan) close to the *Ae. aegypti* population. Miami has 12 daily nonstop commercial flights to Reagan airport. In addition, a busy interstate highway (US1/I-95) originates in Key West and is a major shipping corridor along the East Coast of the US, passing through Washington. Washington and Miami are also directly connected by railroad with both passenger and freight traffic.

From a health perspective, it is relevant that in 2009, Key West, FL, was the location of the first transmitted cases of dengue fever in the US for > 60 years.[50] The first time that chikungunya was reported transmitted in the US was in Florida in 2014 (ArboNET-CDC[51]). Likewise, in 2016, Miami Dade County, FL, was the first US mainland locality to report Zika virus transmission.[52] This indicates that Florida populations of *Ae. aegypti* are capable of transmitting dengue, chikungunya, and Zika viruses in modern US urban environments.

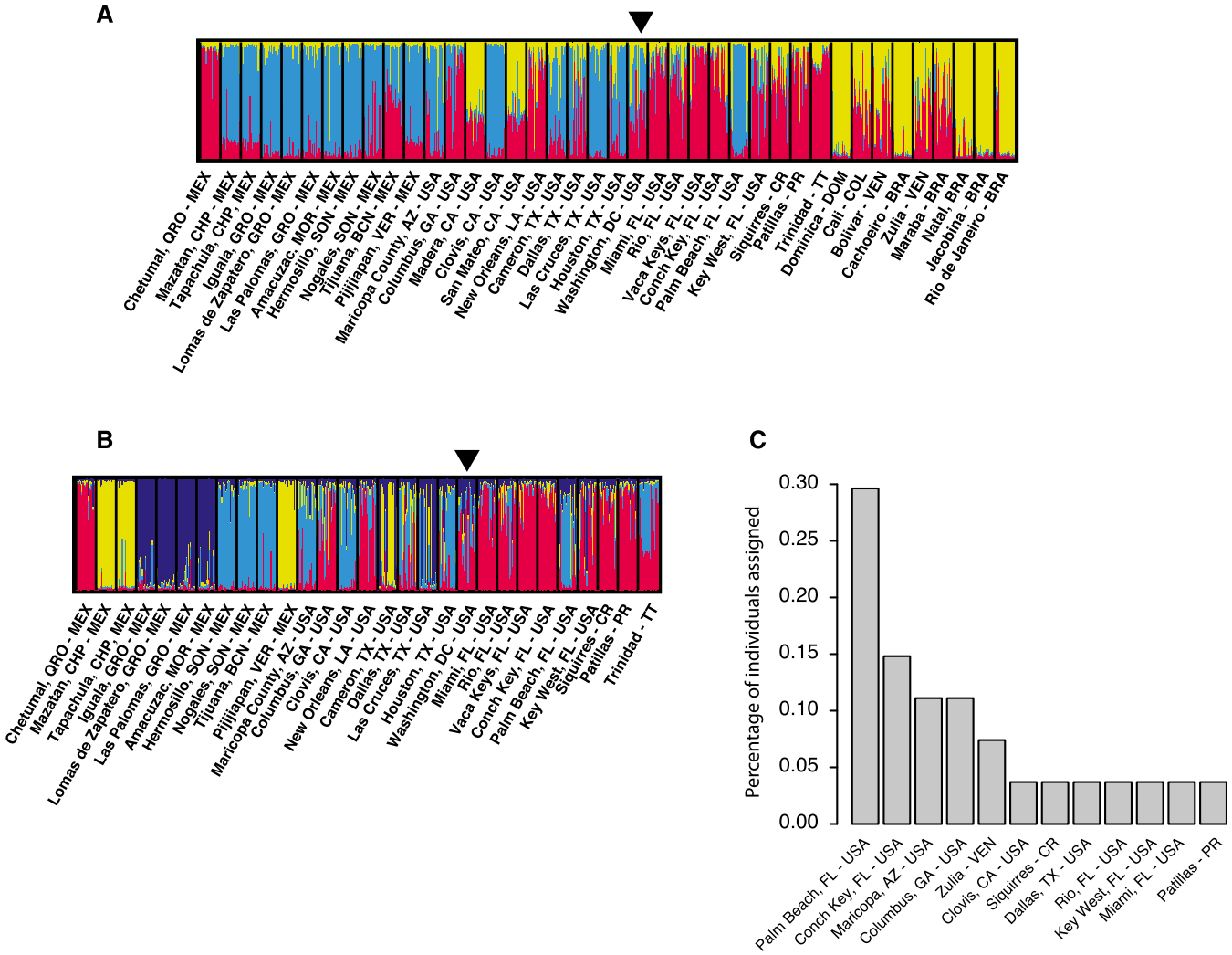

FIGURE 1. Analyses of the *Aedes aegypti* from Capitol Hill, Washington, DC, using 12 microsatellite markers. (**A, B**) STRUCTURE plots[28] illustrating the genetic structure of *Aedes aegypti* in America. Each vertical bar represents an individual. The height of each bar is the probability of assignment to each of *K* genetic clusters (indicated by different colors), as determined using the Delta *K* method.[29] $K = 3$ for America (**A**) and $K = 4$ for a subset of North American populations (**B**). An arrow at the top of the plot highlights the location of the Washington, DC, population. (**C**) Percentage of individuals from Washington, DC, assigned to each of the reference populations depicted in the *Y*-axis with the highest score. Only populations to which at least one individual was assigned are shown. Assignments were performed using Bayesian criteria for likelihood estimation with GENECLASS 2.0.[37] This figure appears in color at www.ajtmh.org.

Washington, being an international center for visitors from around the world, routinely reports imported cases of *Ae. aegypti*-borne diseases. Since 2015, there have been 43 reported cases of imported Zika infections and 15 cases of dengue in Washington, DC, to date (CDC[51] and USGS[53] disease maps).

Lima et al.[2] suggest that cryptic underground habitats, such as storm drains or tunnels, are the likely site of overwintering by the Capitol Hill *Ae. aegypti* population. *Ae. aegypti* using storm drains as a larval site has been documented elsewhere in Brazil,[54] Mexico,[55] California,[56] and Arizona (K. Smith, personal communication; 2017). They have also been detected in septic tanks in Puerto Rico[57] and Trinidad,[58] a distinct subterranean larval site. However, all these subterranean breeding sites are in tropical or subtropical areas that also have year-round surface populations of this mosquito. The Washington, DC, population we studied is unique in being well-outside the normal year-round breeding range of *Ae. aegypti.*

Adult *Ae. aegypti* eclosing from septic tanks in Puerto Rico were found to be larger than those from surface larval habitats[59] but septic tank populations are genetically indistinguishable from nearby surface populations.[60] Thus, there is no evidence that subterranean breeding in *Ae. aegypti* has a genetic basis.

Is the establishment in Washington, DC, a harbinger of further expansion of the distribution of *Ae. aegypti* to higher latitudes? Will subterranean habitats become common overwintering sites? The epidemiological implications are immense. Should the northern distribution limits become today's 6°C minimum isotherm, the additional number of human populations at risk for *aegypti*-borne diseases would expand greatly, most of which have never experienced these diseases and are thus immunologically naive.

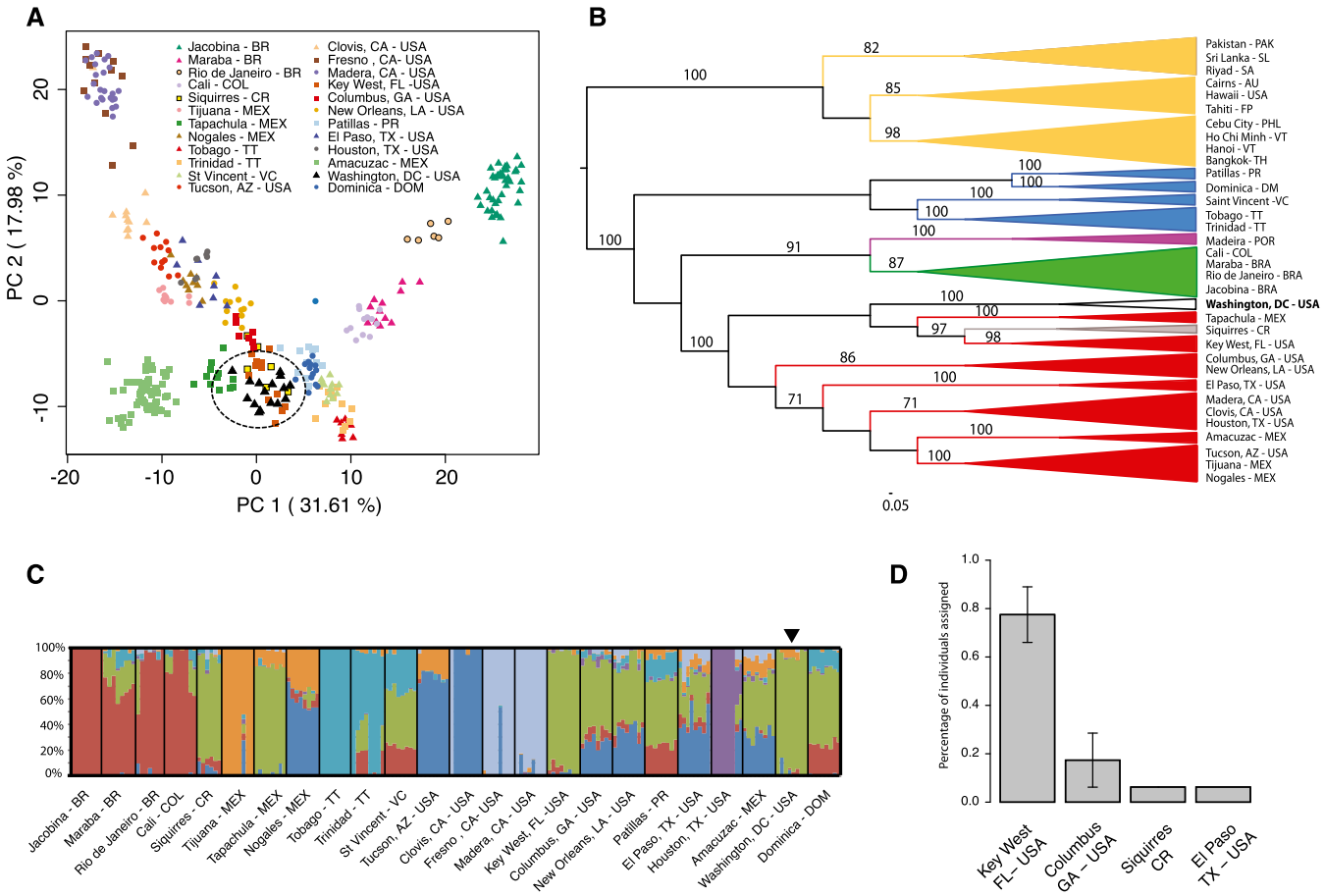

FIGURE 2. Analyses of the *Aedes aegypti* from Capitol Hill, Washington, DC, using 14,071 SNP markers. (**A**) Principal component analysis on allele frequencies. A dashed circle surrounds individuals from Washington, DC. (**B**) Midpoint rooted phylogenetic tree of *Ae. aegypti* populations outside Africa constructed using maximum likelihood in RAXML.[43] Yellow: Asia, Blue: Caribbean, Purple: Europe, Green: South America, Grey: Central America, Red: North America, and Grey: Capitol Hill, Washington, DC. Numbers on branches indicate bootstrap values (1,000 replicates). Bootstrap values below 70% are not shown. (**C**) and (**D**) are same as Figure 1B and C except using 14,071 SNPs as markers. This figure appears in color at www.ajtmh.org.

Finally, we point out the importance of the publicly available genetic databases of the sort we used in this study. The microsatellite database for *Ae. aegypti* now comprises genotypes for ~7,600 mosquitoes from 190 population samples taken in 40 countries in six continents; the SNP chip database has now ~2,000 genotyped mosquitoes from over 95 populations in 31 countries in six continents (VectorBase[48,49]). Such databases are especially important for tracing new introductions, which, history tells us, are inevitable with mosquitoes such as *Ae. aegypti.* Knowing the origin allows inference of the mode of transportation of the introduction, assessment of the health threat the introduction poses (e.g., did it come from a region where it is actively transmitting a disease?), and may guide efforts to control the population (e.g., did it come from a region known to have resistance to certain insecticides?).

TABLE 2

Aedes aegypti mtDNA *COI* haplotypes present in Washington, DC, and various localities considered as potential sources

A	B	C	D	E	F	G	H
Washington, DC†	Washington, DC†						
Miami, FL (2)	Miami, FL (1)		Miami, FL (7)				
Palm Beach, FL (3)	Palm Beach, FL (4)	Palm Beach, FL (1)	Palm Beach, FL (1)	Palm Beach, FL (1)			
Key West, FL (4)	Key West, FL (4)	Key West, FL (2)					
Columbus, GA (2)		Columbus, GA (2)	Columbus, GA (3)		Columbus, GA (3)		
Siquirres, CR (7)						Siquirres, CR (2)	
Tapachula, MEX (1)					Tapachula, MEX (6)		Tapachula, MEX (2)

* Haplotypes A–H were identified based on a 497 bp amplicon of the mitochondrial cytochrome oxidase I gene. The numbers in parentheses indicate the number of individuals with that haplotype observed per location.

† Previously identified.[2]

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Note: Supplemental tables and figures appear at www.ajtmh.org.

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