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Genetic diversity of the dengue vector *Aedes aegypti* assessed using DNA sequencing libraries

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Aedes (*Stegomyia*) *aegypti* (Linnaeus) is the main vector for several human viruses including yellow fever, dengue, chikungunya, and Zika viruses. We assessed the worldwide genetic diversity of the dengue vector, *Ae*. *aegypti* using cytochrome oxidase subunit I (COI) nucleotide sequences from DNA sequencing libraries. Fifty-three COI *Ae*. *aegypti* sequences from 10 countries Thailand, Pakistan, India, Singapore, Laos, Russia, France, Kenya, Ecuador, and Chile were selected. We identified 12 haplotypes and 18 polymorphic sites in the 53 COI sequences, with a total haplotype diversity of 0.625 ± 0.074 and nucleotide diversity of 0.006 ± 0.002. The *Ae*. *aegypti* population in Ecuador had the highest haplotype diversity (1.000 ± 0.500) and the highest nucleotide diversity (0.017 ± 0.013). A pairwise F_{ST} analysis indicated that there was significant genetic differentiation between 12 pairs of countries: France and Thailand; Kenya and India; Kenya and France; Ecuador and Thailand; Ecuador and Pakistan; Ecuador and India; Ecuador and Singapore; Ecuador and Laos; Ecuador and Russia; Ecuador and France; China and Kenya; and China and Ecuador ($p < 0.05$). The results of this study indicate that there are differences in *Ae*. *aegypti* populations in many countries around the world. Therefore, it appears that the environment directly affects the genetics of this dengue vector.

Keywords: Mosquito, *Aedes aegypti*, Genetic diversity, cytochrome oxidase subunit I

INTRODUCTION

Aedes (*Stegomyia*) *aegypti* (Linnaeus) is a mosquito species belonging to the tribe Aedini of the dipteran family Culicidae, genus *Aedes*, and subgenus Stegomyia and is the main vector of several human viruses including yellow fever, dengue, chikungunya, and Zika viruses (Service, 2008; Rattanarithikul et al., 2010). The *Ae*. *aegypti* mosquito originated in Africa and spread globally to tropical and subtropical areas of the world (Kraemer et al., 2015). Currently, dengue is the most important *Aedes*-borne viral disease and is a major public health problem worldwide, especially in subtropical and tropical regions, mainly in Southeast Asia, the Pacific, and the Americas (Guzman et al., 2010; Leta et al., 2018). The global incidence of dengue (including dengue fever and dengue hemorrhagic fever) has increased dramatically over the past few decades, and nearly half of the world's population, an estimated 2.5 billion people, is now at risk of dengue infection (Guzman et al., 2010; World Health Organization, 2014). The World Health Organization has stated that effective dengue prevention and control requires the implementation of effective *Aedes* mosquito control measures (World Health Organization, 2018). Planning of strategies to control the dengue vector requires knowledge about several aspects of the biology of the *Aedes* mosquitoes,

including genetic diversity, behavior and habitat (Chaiphongpachara and Laojun, 2019).

DNA-barcoding techniques are gaining popularity as a means to identify species and to study genetic variation in mosquitoes such as *Anopheles*, *Aedes*, *Culex*, and *Mansonia* (Rosero et al., 2012; Ruangsittichai et al., 2011; Sumruayphol et al., 2016; Gao et al., 2017; Beebe, 2018; Hernández-Triana et al., 2019). DNA barcoding is a molecular method that uses a short nucleotide sequence, approximately 400–800 base pairs, from a standard genetic locus, for comparison with sequences in DNA libraries. For example, a 650 base pair region at the 5′ end of the cytochrome oxidase subunit I (COI) gene has been widely used for barcoding studies in mosquitoes (Wilson et al., 2019). DNA-barcoding libraries such as The Barcode of Life Data System (BOLD: http://www.boldsystems.org/) and the National Centre for Biotechnology Information (NCBI) GenBank (https://www.ncbi.nlm.nih.gov/) are public databases that collect DNA sequences that researchers worldwide can use for species identification and the investigation of genetic variation (Kress and Erickson, 2012).

The number of studies on COI in *Ae*. *aegypti* is increasing, resulting in ever-increasing amounts of data for COI in *Ae*. *aegypti* being deposited in the DNA-barcoding libraries. Previous research has used DNA-barcoding data based on COI nucleotide sequences in the NCBI GenBank to assess genetic variation among five *Culex* mosquito vectors of Japanese encephalitis: *Cx. fuscocephala* (Theobald), *Cx*. *gelidus* (Theobald), *Cx*. *tritaeniorhynchus* (Giles), *Cx*. *pseudovishnui* (Colless), and *Cx*. *vishnui* Theobald. These researchers found genetic differences in and among populations in many countries (Theobald).

In the study reported here, we assessed the worldwide genetic diversity of the dengue vector, *Ae*. *aegypti* based on COI nucleotide sequences from the BOLD and NCBI GenBank databases. The results of this study allow us to better understand the dengue vector, leading to the possibility of developing effective controls for *Ae*. *aegypti* populations.

MATERIALS AND METHODS

Ae. albopictus sequences

In this study, COI sequences of *Ae*. *aegypti* were retrieved from GenBank and BOLD for analysis. Fifty-three COI sequences of *Ae*. *aegypti* from Thailand, Pakistan, India, Singapore, Laos, Russia, France, Kenya, Ecuador, and Chile were selected (Table 1). All sequence data were verified using BLAST against GenBank and BOLD to prevent errors in species identification.

Table 1. Information about the COI *Ae*. *aegypti* sequences used in this study.

Continents	Countries	Databases	Accession no.
Asia	Thailand	NCBI	KP843391.1
Asia	Thailand	NCBI	KP843390.1
Asia	Thailand	NCBI	KP843389.1
Asia	Thailand	NCBI	KP843388.1
Asia	Thailand	NCBI	KP843387.1
Asia	Thailand	NCBI	KP843386.1
Asia	Pakistan	NCBI	KF406396.1
Asia	Pakistan	NCBI	KF406395.1
Asia	Pakistan	NCBI	KF406394.1
Asia	Pakistan	NCBI	KF406393.1
Asia	Pakistan	NCBI	KF406380.1
Asia	Pakistan	NCBI	KF406378.1
Asia	India	NCBI	KC970271.1
Asia	India	NCBI	KC970270.1
Asia	India	NCBI	KT339669.1
Asia	India	NCBI	KT339672.1
Asia	India	NCBI	KT339674.1
Asia	Singapore	NCBI	KX420469.1
Asia	Singapore	NCBI	KX420443.1
Asia	Laos	BOLD	ENTJR113-08.COI-5P
Asia	Laos	BOLD	ENTJR115-08.COI-5P
Asia	Laos	BOLD	ENTJR116-08.COI-5P
Europe	Russia	NCBI	MH251911.1

Europe	Russia	NCBI	MH251910.1
Europe	Russia	NCBI	MG198594.2
Europe	Russia	NCBI	MG198593.2
Europe	Russia	NCBI	MG198592.2
Europe	Russia	NCBI	MG198591.2
Europe	Russia	NCBI	MG198588.2
Europe	Russia	NCBI	MG198587.2
Europe	French	NCBI	MF172260.1
Europe	French	NCBI	MF172259.1
Europe	French	NCBI	MF172258.1
Europe	French	NCBI	KX051587.1
Europe	French	NCBI	KX051584.1
Africa	Kenya	NCBI	KX420491.1
Africa	Kenya	NCBI	KX420490.1
Africa	Kenya	NCBI	KX420489.1
Africa	Kenya	NCBI	KX420488.1
Africa	Kenya	NCBI	KX420487.1
Africa	Kenya	NCBI	KX420486.1
Africa	Kenya	NCBI	KX420485.1
Africa	Kenya	NCBI	KX420484.1
Africa	Kenya	NCBI	KX420483.1
Africa	Kenya	NCBI	KX420482.1
Africa	Kenya	NCBI	KX420481.1
South America	Ecuador	NCBI	KX420476.1
South America	Ecuador	NCBI	KX420439.1
South America	Chile	NCBI	HQ991722.1
South America	Chile	NCBI	HQ991721.1
South America	Chile	NCBI	HQ991720.1
South America	Chile	NCBI	HQ991719.1
South America	Chile	NCBI	HQ991718.1

Statistical analysis

The COI sequences were aligned using Clustal X (Larkin et al., 2007) and manually edited. To assess the genetic variation of *Ae*. *aegypti* populations in each country, the numbers of haplotypes (N), polymorphic sites, nucleotide diversity (π), and haplotype diversity (h) were analyzed using the software DnaSP version 6 (Rozas et al., 2017).

An analysis of molecular variance (AMOVA) based on 10,000 permutations was used to analyze the genetic structure of the *Ae*. *aegypti* populations, using the software Arlequin version 3.5.2.2 (Excoffier and Lischer, 2010). *Ae*. *aegypti* populations were grouped according to continent in order to assess differences in genetic structure. Genetic differentiation in *Ae*. *aegypti populations* between countries were assessed by Pairwise F_{st} based on 10,000 permutations, using the software Arlequin version 3.5.2.2 (Excoffier and Lischer, 2010).

Phylogenetic relationships

Maximum-likelihood analyses based on 10,000 permutations were used for the identification of phylogenetic relationships and to build a phylogenetic tree using Mega version 7 (Kumar et al., 2016). A minimum spanning network tree was also built, to examine the relationships of haplotypes, using the software Arlequin version 3.5.2.2 (Excoffier and Lischer, 2010) and the Network software Version 5.0.1.1, which is freely available at http://www.fluxus-engineering.com.

RESULTS

Genetic diversity: haplotype and nucleotide diversities

Fifty-three COI sequences of *Ae*. *aegypti* from Thailand, Pakistan, India, Singapore, Laos, Russia, France, Kenya, Ecuador, and Chile were obtained from GenBank and BOLD. Twelve haplotypes and 18 polymorphic sites were identified (Table 2). The total haplotype diversity was 0.625 ± 0.074, and nucleotide diversity was 0.006 ± 0.002 (Table 2).

Table 2. Haplotype diversity and nucleotide diversity of *Ae. aegypti* populations.

Countries	No.	No. haplotypes	No. Polymorphic sites	Haplotype Diversity (h) (mean ± S.D.)	Nucleotide diversity (π) (mean ±S.D.)
Thailand	6	3	11	0.733±0.155	0.010±0.004
Pakistan	6	2	1	0.333±0.215	0.000±0.001
India	5	1	0	0.000±0.000	0.000±0.000
Singapore	2	1	0	0.000±0.000	0.000±0.000
Lao	3	2	10	0.667±0.134	0.011±0.007
Russia	8	2	1	0.429±0.169	0.001±0.001
French	5	1	0	0.000±0.000	0.000±0.000
Kenya	11	5	13	0.709±0.137	0.009±0.003
Ecuador	2	2	10	1.000±0.500	0.017±0.013
Chile	5	1	0	0.000±0.000	0.000±0.000
Total	53	12	18	0.625±0.074	0.006±0.002

Table 3. Haplotype distributions of *Ae. aegypti* populations.

Haplotypes	Countries										Total
	THA	PAK	IND	SIN	LAO	RUS	FAR	KEN	ECU	CHI	
H1	2	5	5	2	2	6	5	-	-	5	32
H2	-	1	-	-	-	-	-	-	-	-	1
H3	-	-	-	-	-	-	-	2	-	-	2
H4	3	-	-	-	-	-	-	-	-	-	3
H5	1	-	-	-	-	-	-	-	-	-	1
H6	-	-	-	-	-	2	-	-	-	-	2
H7	-	-	-	-	-	-	-	1	-	-	1
H8	-	-	-	-	-	-	-	1	-	-	1
H9	-	-	-	-	-	-	-	6	-	-	6
H10	-	-	-	-	-	-	-	-	1	-	1
H11	-	-	-	-	-	-	-	1	-	-	1
H12	-	-	-	-	1	-	-	-	1	-	2

Table 4. Analysis of molecular variance (AMOVA) among 10 populations of *Ae. aegypti*.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Φ	p
Among groups	2	2.588	0.023Va	6.95	Φ_{CT} = 0.069	0.308
Among populations	7	4.778	0.101Vb	30.68	Φ_{SC} = 0.376	0.000*
Within populations	43	8.879	0.206Vc	62.38	Φ_{ST} = 0.329	0.000*
total	52	16.245	0.331			

The *Ae*. *aegypti* population in Ecuador had the highest haplotype diversity (1.000 ± 0.500) and the highest nucleotide diversity (0.017 ± 0.013). The 12 haplotypes found in this study consisted of six shared haplotypes: H1, shared with Thailand, Pakistan, India, Singapore, Laos, Russia, France, and Chile; H3 with two shared haplotypes in Kenya; H4 with three shared haplotypes in Thailand; H6 with two shared haplotypes in Russia; H9 with six shared haplotypes in Kenya; and H12 shared with two countries, Laos and Ecuador. There were six singular haplotypes, H2, H5, H7, H8, H10, and H11 (Table 3).

Population genetic structure of *Ae*. *aegypti*

Table 4 shows the results of the analysis of genetic structure variation based on an AMOVA. According to the variations tested for 10 *Ae*. *aegypti* populations, significant variation was observed among and within populations, indicating the existence of genetic structure differences both among and within populations ($p < 0.01$).

The pairwise comparison of *Ae*. *aegypti* population differences is presented in Table 5. The pairwise F_{ST} analysis indicated significant genetic difference between 12 pairs of countries: France and Thailand; Kenya and India; Kenya and France; Ecuador and Thailand; Ecuador and Pakistan; Ecuador and India; Ecuador and Singapore; Ecuador and Laos; Ecuador and Russia; Ecuador and France; China and Kenya; and China and Ecuador ($p < 0.05$).

Phylogenetic tree and median-joining haplotype network

Phylogenetic analysis of 10 *Ae*. *aegypti* populations were assessed using a maximum-likelihood analysis with 10,000 bootstraps (Figure 1). The maximum-likelihood identifies the phylogenetic relationships among the *Ae*. *aegypti* populations but cannot separate each *Ae*. *aegypti* population. The relationships between 12 haplotypes in *Ae*. *aegypti* populations are shown in the median-joining haplotype network (Figure 2).

Table 5.Pairwise genetic differences between 10 *Ae*. *aegypti* populations.

	THA	PAK	IND	SIN	LAO	RUS	FAR	KEN	ECU	CHI
THA	-									
PAK	0.261 (0.108)	-								
IND	0.413 (0.065)	-0.034 (0.100)	-							
SIN	0.205 (0.424)	-0.304 (0.100)	0.000 (0.100)	-						
LAO	0.091 (0.356)	-0.059 (0.100)	0.189 (0.381)	-0.200 (0.100)	-					
RUS	0.413 (0.060)	-0.034 (0.100)	0.000 (0.100)	0.000 (0.100)	0.189 (0.369)	-				
FAR	0.240 (**0.035**)*	-0.021 (0.481)	0.062 (0.480)	-0.170 (0.100)	-0.043 (0.665)	0.062 (0.486)	-			
KEN	0.192 (0.250)	0.510 (0.110)	0.773 (0.040)*	0.500 (0.325)	0.045 (0.617)	0.773 (0.051)	0.448 (0.049)*	-		
ECU	0.281 (0.002)*	0.443 (0.000)*	0.544 (0.000)*	0.430 (0.028)*	0.304 (0.021)*	0.544 (0.000)*	0.418 (0.000)*	0.222 (0.138)	-	
CHI	0.412 (0.062)	-0.034 (0.100)	0.000 (0.100)	0.000 (0.100)	0.189 (0.375)	0.000 (0.100)	0.062 (0.485)	0.773 (0.045)*	0.544 (0.000)*	-

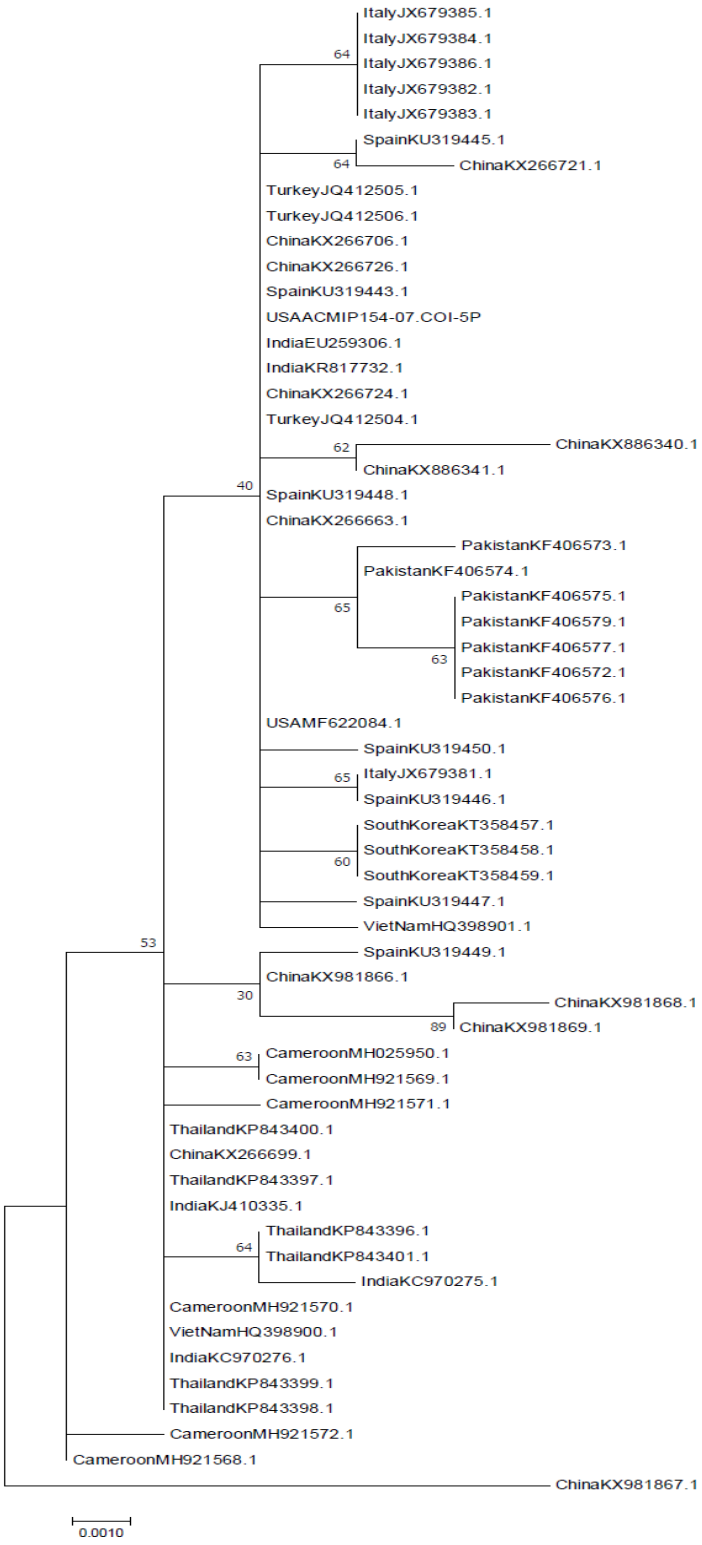
ItalyJX679385.1
ItalyJX679384.1
64
ItalyJX679386.1
ItalyJX679382.1
ItalyJX679383.1
SpainKU319445.1
64
ChinaKX266721.1
TurkeyJQ412505.1
TurkeyJQ412506.1
ChinaKX266706.1
ChinaKX266726.1
SpainKU319443.1
USAACMIP154-07.COI-5P
IndiaEU259306.1
IndiaKR817732.1
ChinaKX266724.1
TurkeyJQ412504.1
62
ChinaKX886340.1
ChinaKX886341.1
40
SpainKU319448.1
ChinaKX266663.1
PakistanKF406573.1
PakistanKF406574.1
PakistanKF406575.1
65
PakistanKF406579.1
PakistanKF406577.1
63
PakistanKF406572.1
PakistanKF406576.1
USAMF622084.1
SpainKU319450.1
ItalyJX679381.1
65
SpainKU319446.1
SouthKoreaKT358457.1
SouthKoreaKT358458.1
60
SouthKoreaKT358459.1
SpainKU319447.1
VietNamHQ398901.1
53
SpainKU319449.1
ChinaKX981866.1
30
ChinaKX981868.1
89
ChinaKX981869.1
63
CameroonMH025950.1
CameroonMH921569.1
CameroonMH921571.1
ThailandKP843400.1
ChinaKX266699.1
ThailandKP843397.1
IndiaKJ410335.1
ThailandKP843396.1
64
ThailandKP843401.1
IndiaKC970275.1
CameroonMH921570.1
VietNamHQ398900.1
IndiaKC970276.1
ThailandKP843399.1
ThailandKP843398.1
CameroonMH921572.1
CameroonMH921568.1
ChinaKX981867.1
0.0010

Figure 1. A maximum-likelihood tree constructed on the basis of 53 COI sequences of 10 *Ae. aegypti* populations from publicly available sequence data from GenBank and BOLD.

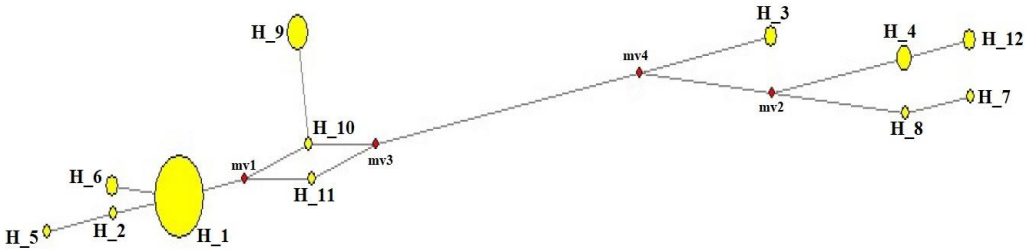

Figure 2. A minimum spanning network tree constructed using Network 5.0.1.1 based on 12 haplotypes of 53 COI sequences of 10 *Ae. aegypti* populations from publicly available sequence data in GenBank and BOLD. Each haplotype is represented by a circle, and the size of the circle is proportional to the number of individuals with each haplotype.

DISCUSSION

In this paper, we describe a genetic analysis of a 587 bp sequence of the COI gene in 10 *Ae*. *aegypti* populations. All sequences were retrieved from the public DNA databases NCBI GenBank and BOLD.

Haplotype diversity and nucleotide diversity were found to be moderate, with a total haplotype diversity of 0.625 ± 0.074 and nucleotide diversity of 0.006 ± 0.002. No genetic diversity was found in four countries: India, Singapore, France, and Chile. It is possible that this lack of diversity is due to the programs for the eradication of *Ae*. *aegypti* implemented in each area. Twelve haplotypes of 10 *Ae*. *aegypti* populations, consisting of six singular haplotypes and six shared haplotypes, were identified. A haplotype network based on the 12 haplotypes showed only one cluster, in which H1 was the dominant haplotype. This result indicates that H1 is important for the genetic diversity of *Ae*. *aegypti* populations.

The results of the population genetic structure analysis of *Ae*. *aegypti* showed that 62.38% of the genetic variation within populations was statistically significant based on AMOVA ($p < 0.05$). These results indicate that there is some degree of genetic differentiation within *Ae*. *aegypti* populations. The genetic differences between *Ae*. *aegypti* populations identified by Pairwise F_{ST} analysis revealed differences in 12 pairs of populations: France and Thailand; Kenya and India; Kenya and France; Ecuador and Thailand; Ecuador and Pakistan; Ecuador and India; Ecuador and Singapore; Ecuador and Laos; Ecuador and Russia; Ecuador and France; China and Kenya; and China and Ecuador ($p < 0.05$). Environmental differences between countries may be one of the factors that have influenced the genetics of *Ae*. *aegypti*. Joyce et al., (2018) previous research included a study of genetic variability of *Ae*. *aegypti* in six geographical regions of El Salvador and found statistically significant differences in some pairs of areas. In addition, ecological factors and human activities affect the genetic structure of *Ae*. *aegypti* populations.

Previous research has been conducted into the genetic diversity and structure of *Ae*. *aegypti* in five areas of Senegal, which differ in their ecological characteristics, including sylvatic and domestic populations (Huber et al., 2008). The researchers found that domestic *Ae*. *aegypti* populations showed decreased genetic diversity and lower genetic differentiation compared with sylvatic *Ae*. *aegypti* populations (Huber et al., 2008). The environment is an important factor that affects the genetics of *Ae*. *aegypti*. However, previous research in Thailand has explored the morphological variations of this mosquito in multiple geographical locations including coastal, residential, and cultivated areas and found statistically significant differences in wing size and shape between locations (Chaiphongpachara et al., 2018).

CONCLUSION

In conclusion, moderate values of genetic

diversity and structure were found among *Ae*. *aegypti* populations in Thailand, Pakistan, India, Singapore, Laos, Russia, France, Kenya, Ecuador, and Chile based on COI sequences obtained from public DNA sequencing libraries. The results of this study indicate that there are differences in *Ae*. *aegypti* populations in many countries around the world. Therefore, the environment appears to directly affect the genetics of this dengue vector.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

TC designed and performed the experiments and also wrote the manuscript. SL performed data analysis.

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