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Assessing genetic variation in the Asian tiger mosquito *Aedes albopictus* based on DNA barcoding databases

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Aedes (Stegomyia) *albopictus* (Skuse, 1895) (Diptera: Culicidae) is an important mosquito vector for several viruses. This species has spread throughout tropical and temperate countries and has also been distributed from the Far East to the Western Hemisphere, Europe, and Africa, in which the spread in at least 28 countries was caused by international trade and ground transport. This research was conducted to assess the genetic variations in the 59 genome sequences for *Ae. albopictus* within 11 countries based on genetic data in the DNA barcoding databases Barcode of Life Data systems and National Center for Biotechnology Information GenBank. Analysis of the cytochrome c oxidase subunit 1 sequence (613 bp) in *Ae. albopictus* showed 26 haplotypes and 28 polymorphic sites. The total haplotype and nucleotide diversities in the 59 samples were 0.918 ± 0.022 S.D. and 0.004 ± 0.003 S.D., respectively. The population structure of *Ae. albopictus* in this study was analyzed using analysis of molecular variance and found ΦST = 0.302 ($P < 0.01$) of genetic structure value within populations. These results are especially useful to use as a basis for further control of the Aedes mosquito.

Keywords: *Aedes albopictus*, Asian tiger mosquito, DNA barcoding databases, genetic variation, infection.

INTRODUCTION

Aedes (Stegomyia) *albopictus* (Skuse 1895) (Diptera: Culicidae), also called the Asian tiger mosquito, is an important mosquito vector for the dengue and chikungunya viruses (Killick-Kendrick, 1996). The species is recognized as a secondary vector for the dengue virus and a primary vector for the chikungunya virus (Gratz, 2004; Pagès et al., 2009). The global incidence of dengue cases of infection has increased dramatically, especially in recent decades, and 50% of the world's population is at risk of infection (World Health Organization, 2019). Chikungunya virus originated in Africa and is now found in nearly 40 countries (World Health Organization, 2016). There were also reports that *Ae. albopictus* is a vector of the Zika, yellow fever, and West Nile viruses, which are the cause of deaths in several countries (Killick-Kendrick, 1996; Paixão et al., 2018). These data indicate that *Aedes* mosquito–borne diseases are important global health problems.

Aedes albopictus is a mosquito native to Southeast Asia (Cunze et al., 2016). The species spread throughout tropical and temperate countries and also distributed from the Asia to the New World, Europe, and Africa, the spread being caused by international trade and ground transport in at least 28 countries (Benedict et al., 2007; Braack et al., 2018). North and South America, *Ae. albopictus* was first found in North America (the USA) in 1985 (Sprenger and Wuithiranyagool, 1986) and spread to South America (Brazil) (Forattini, 2006). After its global spread, the species' adaptation to the terrains within the new countries that were different

from those of the original source might have caused its genetic diversity (Goubert et al., 2017). The genetic information, including the genetic structure used as the molecular basis by which to evaluate the genomic background of specific vectors and genetic diversity, is also an important parameter by which to evaluate interventions to control the species (Ayres et al., 2002).

There have been reports of genetic variation in *Ae. albopictus* in several countries (Ruiling et al., 2018) and these genetic data will be collected in a database. DNA-barcoding techniques are gaining popularity for use in identifying organisms and studying genetic variations (Beebe, 2018). This technique uses basic molecular biology methods to amplify a standardized short-sequence fragment (approximately 400–800 bp) from an unknown specimen and compare them to those in the DNA sequence libraries, such as The Barcode of Life Data systems (BOLD) and the National Center for Biotechnology Information (NCBI) GenBank for identification (Wilson et al., 2019). The mitochondrial gene cytochrome c oxidase subunit 1 (COI) is recognized as a representative for barcoding studies, including mosquitoes, because of the base substitution in the third codon position (Weeraratne et al., 2018). Previous research has evaluated the DNA barcoding based on the mitochondrial CO1 sequence in GenBank to analyze the variations among five overlooked *Culex* vectors of Japanese encephalitis—*Culex fuscocephala* Theobald, *Cx. gelidus* Theobald, *Cx. tritaeniorhynchus* Giles, *Cx. pseudovishnui* Colless and *Cx. vishnui* Theobald and found genetic variations in several countries (Karthika et al., 2018).

Thus, our current research was conducted to assess the genetic variations in *Ae. albopictus* from several countries based on genetic data found in BOLD and NCBI GenBank. The results of this genetic study on *Ae. albopictus* can provide important information to help better understand this vector and for further control of the species.

MATERIALS AND METHODS

Aedes albopictus selection

COI sequences of *Ae. albopictus* were publicly available from BOLD and NCBI GenBank and were used in this study. Fifty-nine genetic sequences from 11 countries (China, Thailand, Pakistan, India, South Korea, Vietnam, Spain, Italy, Turkey, Cameroon, and the USA) were selected for genetic analyses (Table 1). All sequence data have been examined again using the Basic Local Alignment Search Tool in BOLD and NCBI GenBank to prevent errors in species identification.

Table 1. Information on COI in *Ae. albopictus* sequences used in this study.

Continent	Country	Database	No	Accession no.
Asia	China	NCBI	12	KX886340.1, KX886341.1, KX266663.1, KX981866.1, KX981867.1, KX981868.1, KX981869.1, KX266699.1, KX266706.1, KX266721.1, KX266724.1, KX266726.1
	Thailand	NCBI	6	KP843396.1, KP843397.1, KP843398.1, KP843399.1, KP843400.1, KP843401.1
	Pakistan	NCBI	7	KF406572.1, KF406573.1, KF406574.1, KF406575.1, KF406576.1, KF406577.1, KF406579.1
	India	NCBI	5	EU259306.1, KC970275.1, KC970276.1, KJ410335.1, KR817732.1
	South Korea	NCBI	3	KT358457.1, KT358458.1, KT358459.1
	Viet Nam	NCBI	2	HQ398900.1, HQ398901.1
Europe	Spain	NCBI	7	KU319443.1, KU319445.1, KU319446.1, KU319447.1, KU319448.1, KU319449.1, KU319450.1
	Italy	NCBI	6	JX679381.1, JX679382.1, JX679383.1, JX679384.1, JX679385.1, JX679386.1
	Turkey	NCBI	3	JQ412504.1, JQ412505.1, JQ412506.1
Africa	Cameroon	NCBI	6	MH025950.1, MH921568.1, MH921569.1 MH921570.1, MH921571.1, MH921572.1
North America	USA	BOLD	1	ACMIP154-07.COI-5P
	USA	NCBI	1	MF622084.1

Statistical analyses

The selected COI sequences of *Ae. albopictus* from the public databases were aligned using Clustal X (Larkin et al., 2007) and manually edited. The numbers of haplotypes (N) and polymorphic sites and the nucleotide (π) diversity and haplotype

(h) diversity were calculated using DnaSP ver. 6 (Rozas et al., 2017) to assess genetic variations in *Ae. albopictus* populations.

A hierarchical analysis of molecular variance (AMOVA) based on 10,000 permutations was used to estimate the population structure of the species using Arlequin ver. 3.5.2.2 (Excoffier and Lischer, 2010). For population analyses, groups of *Ae. albopictus* were organized according to their continent. The genetic differentiation between each pair of populations was examined using Pairwise Fst based on 10,000 permutations using Arlequin ver. 3.5.2.2 (Excoffier and Lischer, 2010). The neutrality test was assessed by Tajima's D and Fu's Fs per population also using Arlequin ver. 3.5.2.2.

For phylogenetic relationships, maximum-likelihood (ML) analyses were used in PHYML using Mega ver. 7 (Kumar et al. 2016) to create the polygenetic tree. A minimum spanning network tree was produced using Arlequin ver. 3.5.2.2 (Excoffier and Lischer, 2010) and then created using Network ver. 5.0.1.1 (freely available at http://www.fluxus-engineering.com) to examine the relationship among the haplotypes.

RESULTS

Haplotype and nucleotide diversities and the neutrality test

The 59 COI sequence analyses (613 bp) of *Ae. albopictus* revealed 26 haplotypes and 28 polymorphic sites (Table 2).

Table 2: Haplotype and nucleotide diversity and neutrality test results for *Ae. albopictus* .

Countries	No	No. haplotypes	No. Polymorphic sites	Haplotype Diversity (h) (mean ± S.D.)	Nucleotide diversity (π) (mean ± S.D.)	Tajima's D	Fu's Fs
China	12	9	15	0.909 ± 0.079	0.006 ± 0.004	-1.189 (0.113)	-3.331 (0.024)*
Thailand	6	2	1	0.533 ± 0.172	0.001 ± 0.001	0.850 (0.877)	0.625 (0.482)
Pakistan	7	3	2	0.524 ± 0.209	0.001 ± 0.001	-0.274 (0.386)	-0.438 (0.158)
India	5	3	2	0.800 ± 0.164	0.002 ± 0.000	0.243 (0.716)	-0.475 (0.193)
South Korea	3	1	0	0.000 ± 0.000	0.000 ± 0.000	0.000 (1.000)	0.000 (N.A.)
Vietnam	2	2	2	1.000 ± 0.500	0.003 ± 0.003	0.000 (1.000)	0.693 (0.375)
Spain	7	6	5	0.952 ± 0.096	0.002 ± 0.002	-1.486 (0.043)*	-4.257 (0.000)*
Italy	6	2	2	0.333 ± 0.215	0.001 ± 0.001	-1.132 (0.153)	0.952 (0.606)
Turkey	3	1	0	0.000 ± 0.000	0.000 ± 0.000	0.000 (1.000)	0.000 (N.A.)
Cameroon	6	5	4	0.933 ± 0.122	0.003 ± 0.002	-0.057 (0.466)	-2.429 (0.016)*
USA	2	1	0	0.000 ± 0.000	0.000 ± 0.000	0.000 (1.000)	0.000 (N.A.)
Total	59	26	28	0.918 ± 0.022	0.004 ± 0.003	-1.984 (0.006)*	*-22.342* (0.000)*

The total haplotype diversity and nucleotide diversity in the 59 samples were 0.918 ± 0.022 S.D. and 0.004 ± 0.003 S.D., respectively (Table 2). The Vietnam population had the highest haplotype diversity at 1.000 ± 0.500 S.D. The China population had the highest nucleotide diversity at 0.006 ± 0.004 S.D. In contrast, the South Korea population had the lowest of both the haplotype and nucleotide diversities at 0.000 ± 0.000 S.D. As shown in Table 2, the total Tajima's D value was -1.984 ($P < 0.05$), while the total Fu's Fs value was -22.342 ($P < 0.05$). Twenty six haplotypes were found that consisted of eight shared haplotypes—H1 (shared with China, Thailand, India, Vietnam, and Cameroon), H2 (two shared haplotypes from Cameroon), H3 (five shared haplotypes from Pakistan), H5 (two shared haplotypes from Thailand), H6 (shared with China, India, Spain, Turkey, and USA), H8 (five shared haplotypes from Italy), H22 (shared with Spain and Italy), and H25 (three shared haplotypes from South Korea), and 18 singular haplotypes comprising H4, H7, H9, H10, H11, H12, H13, H14, H15, H16, H17, H18, H19, H20, H21, H23, H24, and H26 (Table 3).

Genetic structure of *Ae. Albopictus* population

The population structure of Ae. albopictus in this study was analyzed using AMOVA and the results revealed ΦST = 0.302 ($P < 0.01$) of the genetic structure value within populations (Table 4). The pairwise genetic distances between haplotypes of *Ae. albopictus* populations are presented in Table 5. Pairwise FST analysis revealed significant genetic differences between 24 pairs—China and Thailand, China and Pakistan, China and South Korea, China and Italy, Thailand and Pakistan, Thailand and South Korea, Thailand and Spain, Thailand and Italy, Thailand and Turkey, Pakistan and India, Pakistan and South Korea, Pakistan and Spain, Pakistan and Italy, Pakistan and Turkey, Pakistan and Cameroon, Pakistan and the USA, India and South Korea, India and Italy, South Korea and Spain, South Korea and Cameroon, Spain and Italy, Italy and Turkey, Italy and Cameroon, and Italy and the USA ($P < 0.05$) (Table 4).

Table 3: Haplotype distributions within *Aedes albopictus* populations.

Haplotype	Country											Total
	CHN	THA	PAK	IND	KOR	VIE	SPN	ITA	TUR	CMR	USA	
H1	1	4	-	2	-	1	-	-	-	1	-	9
H2	-	-	-	-	-	-	-	-	-	2	-	2
H3	-	-	5	-	-	-	-	-	-	-	-	5
H4	-	-	-	-	-	-	-	-	-	1	-	1
H5	-	2	-	-	-	-	-	-	-	-	-	2
H6	4	-	-	2	-	-	2	-	3	-	2	13
H7	-	-	-	-	-	-	-	-	-	1	-	1
H8	-	-	-	-	-	-	-	5	-	-	-	5
H9	-	-	1	-	-	-	-	-	-	-	-	1
H10	1	-	-	-	-	-	-	-	-	-	-	1
H11	1	-	-	-	-	-	-	-	-	-	-	1
H12	1	-	-	-	-	-	-	-	-	-	-	1
H13	-	-	-	-	-	-	1	-	-	-	-	1
H14	1	-	-	-	-	-	-	-	-	-	-	1
H15	-	-	-	-	-	-	1	-	-	-	-	1
H16	-	-	-	-	-	1	-	-	-	-	-	1
H17	-	-	-	1	-	-	-	-	-	-	-	1
H18	1	-	-	-	-	-	-	-	-	-	-	1
H19	-	-	-	-	-	-	1	-	-	-	-	1
H20	-	-	-	-	-	-	-	-	-	1	-	1
H21	1	-	-	-	-	-	-	-	-	-	-	1
H22	-	-	-	-	-	-	1	1	-	-	-	2
H23	1	-	-	-	-	-	-	-	-	-	-	1
H24	1	-	-	-	-	-	-	-	-	-	-	1
H25	-	-	-	-	3	-	-	-	-	-	-	3
H26	-	-	-	-	-	-	1	-	-	-	-	1
Total	13	6	6	5	3	2	7	6	3	6	2	59

Table 4: Results of an analysis of molecular variation (AMOVA) among 11 populations of *Aedes Albopictus*.

Source of variation	df	Sum of squares	Variance components	Percentage of variation	Φ	P-value
Among groups	2	2.113	0.009Va	-1.93	Φ_{CT} = -0.019	0.481
Among populations	8	8.486	0.145Vb	30.84	Φ_{SC}= 0.302*	< 0.001
Within populations	48	16.029	0.333Vc	71.10	Φ_{ST} = 0.302*	< 0.001
Total	58	26.627	0.200			

Table 5: Pairwise genetic differences between *Aedes albopictus* populations.

Country	CHN	THA	PAK	IND	KOR	VIE	SPN	ITA	TUR	CMR	USA
China	-										
Thailand	0.207 (0.002)*	-									
Pakistan	0.260 (0.001)*	0.472 (0.001)*	-								
India	-0.033 (0.800)	0.099 (0.169)	0.353 (0.004)*	-							
South Korea	0.355 (0.002)*	0.641 (0.011)*	0.634 (0.009)*	0.502 (0.017)*	-						
Vietnam	0.026 (0.558)	-0.015 (0.634)	0.362 (0.164)	-0.082 (0.697)	0.647 (0.097)	-					
Spain	-0.027 (0.100)	0.248 (0.001)*	0.262 (0.011)*	0.005 (0.424)	0.371 (0.009)*	0.338 (0.611)	-				
Italy	0.326 (0.000)*	0.567 (0.002)*	0.565 (0.004)*	0.450 (0.008)*	0.771 (0.013)	0.510 (0.098)	0.326 (0.005)*	-			
Turkey	0.104 (0.206)	0.642 (0.010)*	0.634 (0.010)*	0.205 (0.281)	1.000 (0.099)	0.647 (0.099)	0.160 (0.168)	0.770 (0.012)*	-		
Cameroon	0.067 (0.100)	0.175 (0.069)	0.280 (0.015)*	0.068 (0.208)	0.400 (0.013)*	-0.041 (0.854)	0.056 (0.161)	0.367 (0.009)*	0.400 (0.125)	-	
USA	0.008 (0.472)	0.586 (0.072)	0.584 (0.030)*	0.080 (0.426)	1.000 (0.106)	0.500 (0.331)	0.050 (0.436)	0.734 (0.036)*	0.000 (0.100)	0.311 (0.068)	-

*$P < 0.05$

Phylogenetic tree and median-joining haplotype network

Phylogenetic analyses of *Ae. albopictus* populations were calculated using the maximum likelihood method with the 1,000 bootstrap replicates (Figure 1). The maximum likelihood tree presents the phylogenetic relationships among the *Ae. albopictus* populations. Three *Ae. albopictus* populations Italy, Pakistan, and South Korea were clustered from other countries, while those in other countries were not separated (Figure 1). The relationships of the 26 haplotypes in the *Ae. albopictus* populations were illustrated by the median-joining haplotype network (Figure 2), which can be separated into two groups.

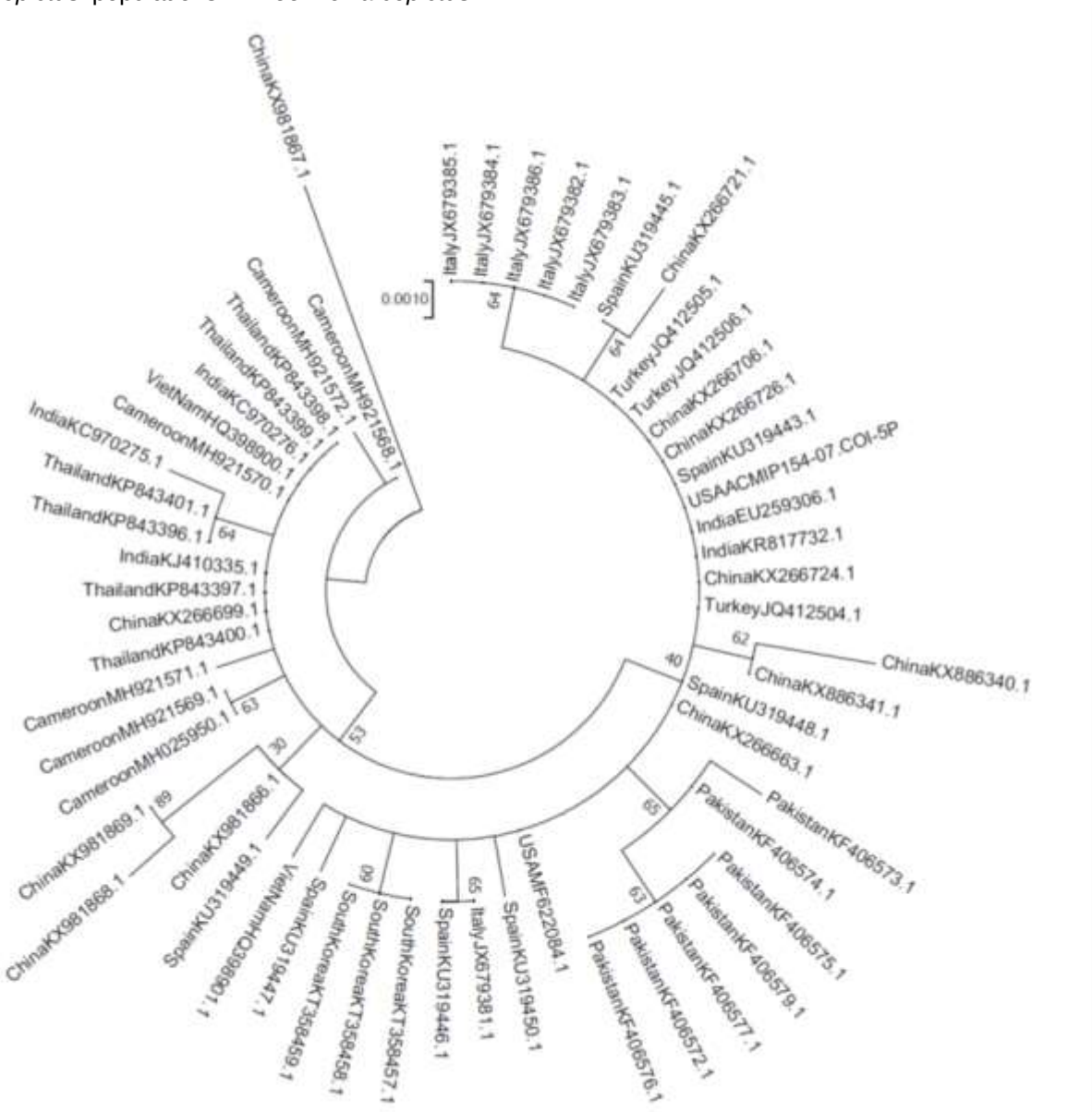

Figure 1: A maximum likelihood tree constructed based on 59 cytochrome c oxidase subunit 1 sequences from sequence data in the Barcode of Life Data and National Center for Biotechnology Information GenBank.

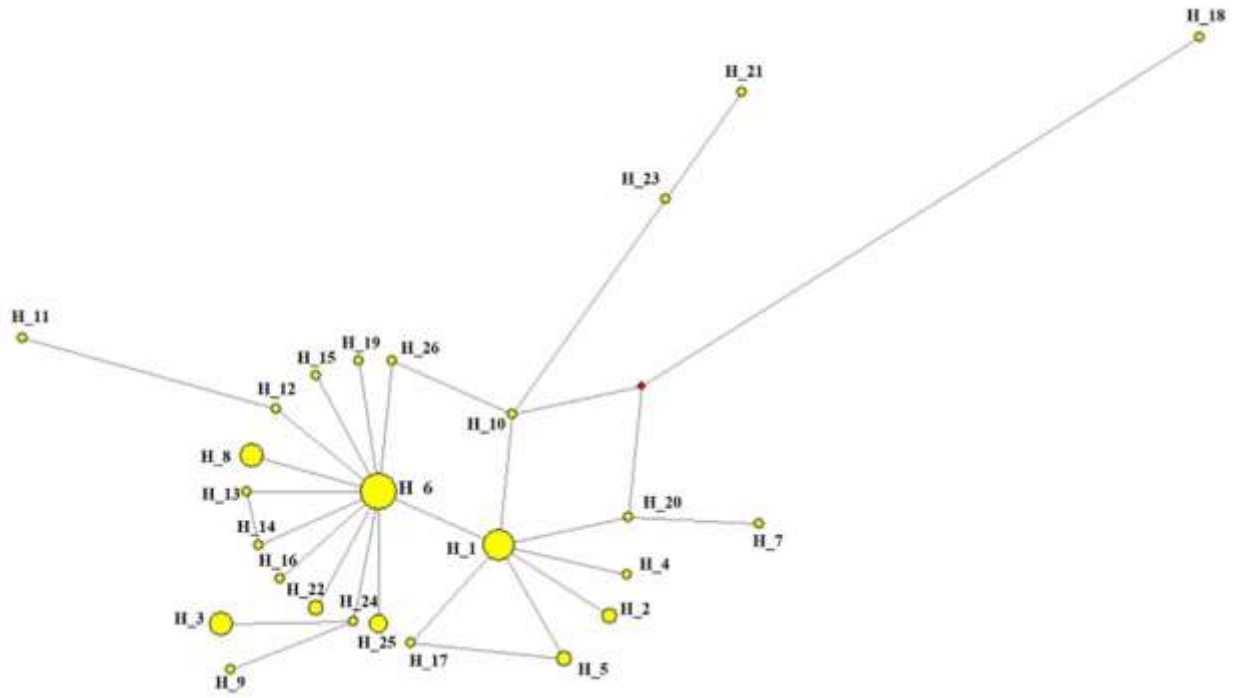

Figure 2: A minimum spanning network tree constructed based on cytochrome c oxidase subunit 1 sequences from publicly available sequence data in Barcode of Life Data systems and National Center for Biotechnology Information GenBank. Each haplotype is represented by a circle and the size of the circle is proportional to the number of individuals with that haplotype.

DISCUSSION

DNA barcoding has become increasingly popular in genetic studies on an organism, including species identification and genetic variation (Kress and Erickson, 2008). DNA barcoding is a technique in molecular biology for species identification based on a standardized short sequence that is compared with a reference sequence database, such as BOLD, NCBI GenBank, and other public databases (Beebe, 2018). The present study was conducted to analyze the genetic variations in 49 COI sequences in *Ae. albopictus* populations from 11 countries—China, Thailand, Pakistan, India, South Korea, Vietnam, Spain, Italy, Turkey, Cameroon, and the USA—based on two public databases.

The results of the genetic analyses of *Ae. albopictus* revealed a 71.10% variation within each country; the difference was statistically significant ($P < 0.05$). In this study, the values of both haplotype and nucleotide diversities were moderate, which was consistent with the results of previous research on the population genetics of *Ae. albopictus* in Mexico, which found that *Ae. albopictus* populations in Mexico have moderate genetic structuring but low genetic variation (Pech-May et al., 2016). In this study, we found 26 haplotypes of *Ae. albopictus* populations—18 singular haplotypes and 8 shared haplotypes. These results might be caused by expansion of the local population, while the mix of haplotypes reflects a degree of gene flow among populations (Pech-May et al., 2016).

The analyses of the genetic structure of a population based on AMOVA revealed differences in the *Ae. albopictus* populations, and when comparing the differences between population pairs using Pairwise F_{ST} analysis, genetic differences were found between populations from China and Thailand, China and Pakistan, China and South Korea, China and Italy, Thailand and Pakistan, Thailand and South Korea, Thailand and Spain, Thailand and Italy, Thailand and Turkey, Pakistan and India, Pakistan and South Korea, Pakistan and Spain, Pakistan and Italy, Pakistan and Turkey, Pakistan and Cameroon, Pakistan and the USA, India and South Korea, India and Italy, South Korea and Spain, South Korea and Cameroon, Spain and Italy, Italy and Turkey, Italy and Cameroon, and Italy and the USA ($P < 0.05$).

The genetic differences among populations of the species used in this study might be the result of the geographical distances between the 11 countries and their different environmental conditions. The total of Tajima's D value was -1.984 in the populations undergoing demographic expansion or mutational selection (Tajima, 1989).

The examination of the genetic relationships based on maximum likelihood with 1,000 bootstraps indicated from the phylogenetic tree that it was not possible to separate populations from each country, except that those from Italy, Pakistan, and South Korea were clustered from other countries. The haplotype network showed a separation into two clades. These results are similar to those of previous research on the genetic analysis of 263 sequences of *A. albopictus* from 26 countries and that found that both the haplotype network and phylogenetic relationships can be separated into several clades (Ruiling et al., 2018). In addition, this study was also consistent with the results of the haplotype analysis from previous research that reported that H1 played an important role in the rapid spread of *Ae. albopictus* (Ruiling et al., 2018).

CONCLUSION

The present study revealed the genetic variation in *Ae. albopictus* based on 49 COI sequences from 11 countries noted in the BOLD and NCBI GenBank public databases. The genetic analyses of *Ae. albopictus* in this study might be caused by expansion of the local populations, while the mix of haplotypes reflects a degree of gene flow among populations. In addition, genetic-structure differences in *Ae. albopictus* populations might be caused by mosquito populations coming from different geographical regions and possible gene flow. All 11 countries are highly geographically separated from each other, which results in different environmental conditions. These results are especially useful for understanding and controlling this species to help abate the spread of several diseases for which it is a 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

SL and TC designed and performleed the experiments and wrote the manuscript. All authors read and approved the final version.

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