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Genetic characterization of *Aedes aegypti* (Diptera: Culicidae) in Sri Lanka based on *COI* gene

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ABSTRACT

Background & objectives: Aedes aegypti is the most prominent vector for dengue virus worldwide. Accurate identification of the species and understanding its colonization pattern are essential prerequisites in vector control. Thus, the present study was aimed to genetically characterize *Ae. aegypti* mosquitoes collected from different regions of Sri Lanka based on mitochondrial *COI* gene.

Methods: Thirty-three *Ae. aegypti* larval samples were collected from 19 districts. A 735bp region of the mitochondrial *COI* gene was amplified and analyzed for genetic diversity indices. Phylogenetic trees were constructed using Sri Lankan samples and also including mosquito samples reported from other parts of the world.

Results: High genetic diversity was observed within the samples analysed (gene diversity: 0.949; average number of nucleotide differences: 6.371). There were 20 haplotypes presented within the 19 localities investigated. The phylogenetic tree derived two main clades. However, no distinguishable clustering pattern was observed in the phylogenetic tree except for the districts in the northern corner indicating extensive admixing among different populations. When samples from other countries were included in the phylogenetic tree, Anuradhapura, and Mannar samples were clustered together with samples from India, Venezuela, USA, Portugal and Cambodia while Rathnapura was clustered with Bolivia and France.

Interpretation & conclusion: Our results suggest that Sri Lanka has undergone multiple invasions of *Ae. aegypti* from various parts of the world over an extensive period. Further, the mosquito control campaigns had not caused a significant effect on the *Ae. aegypti* populations which is existing in mutation-drift equilibrium.

Key words *Aedes aegypti*; COI gene; genetic characterization; genetic diversity; vector control; population admixture

INTRODUCTION

The threat delineates by mosquitoes has grown rapidly all over the world during the past few decades. Enhanced tolerance towards environmental stress along with progressive gain of insecticide-resistance is partly responsible for their enhanced vectorial capacities. The adaptive changes exhibited by different isolates/populations of the same mosquito species found in different parts of the world are found to be strikingly different. As a result, successful vector mitigation strategies are likely to rely on genetic characterization of vector populations, which would provide valuable information on their genetic makeup, vectorial capacity, temporal stability and the processes relevant to disease transmission, including movement of the vectors across various regions[1].

Due to its many desirable characteristics as a molecular marker[2–3], at present, frequently used barcode region for animals is a 5'-segment of the mitochondrial gene Cytochrome Oxidase I (*COI*) which is referred to as the 'Universal' or 'Folmer' region. This region is the standard marker chosen by the Barcode of Life Data System (*www.boldsystems.org*) (BOLD), an online platform for assembling and curating DNA barcoding data from all over the world. While most of mosquito barcoding studies use this region, some studies have used different areas of *COI* [4].

Several previous studies have successfully attempted the use of *COI* gene in genetic characterization of *Aedes aegypti,* a vector for many arboviral diseases including dengue and chikungunya[5], at a regional scale. For example, Scarpassa *et al* in 2008, showed the existence of two mitochondrial lineages separated by eight fixed mutations, distributed among 14 localities in Brazil, based on 852-bp region of *COI*. The use of both *COI* and *NADH4* together, replicated the same results for Brazilian *Aedes aegypt*i populations[6] indicating the relative robustness of *COI* region in phylogenetic analysis. Another study[7] conducted with *COI* region, covering the north, southeast, northeast and central regions of South India, has reported the presence of three genetic lineages within the 31 localities studied, but with limited genetic diversity.

An effort to use *COI* region to investigate Sri Lank-

an *Aedes aegypti* mosquitoes was conducted recently[8]. However, this study has covered only five administrative districts and thus provides only a limited understanding of the phylogenetic relationships of mosquito samples countrywide. Hence, the current study was designed for molecular characterization of the *Aedes aegypti* mosquitoes collected from all parts of Sri Lanka based on mitochondrial *COI* to provide a wider understanding of the phylogenetic relationships of *Ae. aegypti* mosquitoes that exist throughout Sri Lanka. The genetic relatedness between Sri Lankan mosquitoes and those reported from other parts of the world was also investigated to understand historical migratory routes.

MATERIAL & METHODS

Sample collection

The fourth stage larval samples of *Aedes aegypti* were collected from different regions of Sri Lanka using ovitraps or standard dipping method. Initially, a total of 50 sampling sites were selected from all the 25 administrative districts (2 sites per district) for sample collection with a considerable distance (at least 10 km) between the two sites. However, only 33 sites representing 19 districts were positive for *Ae. aegypti* larvae and were thus included in the study (Fig. 1 and Table 1). All samples were examined under a light microscope and morphologically identified to species level using taxonomic keys[9]. Identified larvae were preserved in 95% ethanol. Only one larval sample from each site was used for the study.

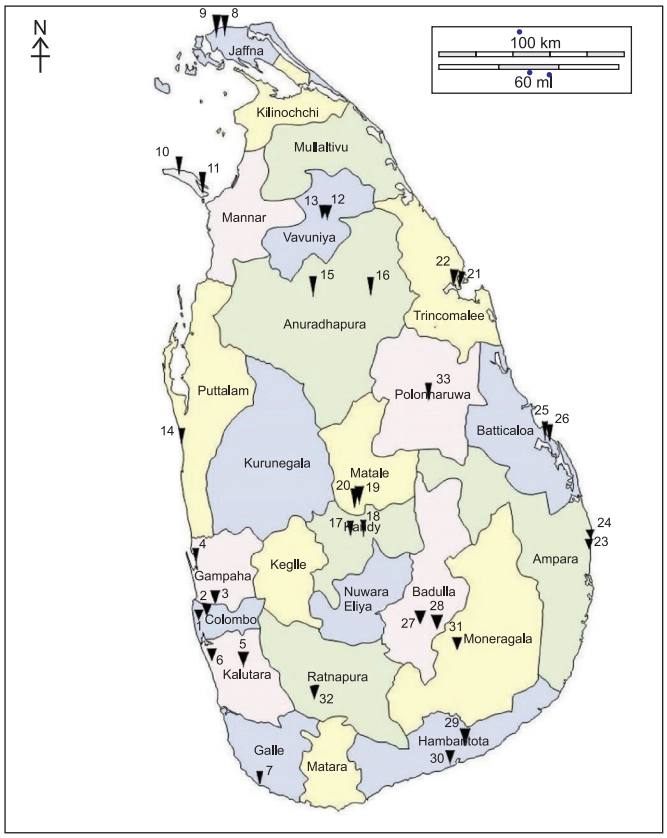

Fig. 1: District map of Sri Lanka showing sample collection locations (1-33 denote the collection sites).

PCR amplification and sequencing

Genomic DNA was extracted from the samples using Wizard® Genomic DNA Purification Kit. A 735bp region of the mitochondrial *COI* gene was amplified using oligonucleotide primers as described by Gupta *et al* (2016). PCR products were run on 2% agarose and the excised amplicon were purified using Wizard® SV gel and PCR clean up system (Promega, USA). Bidirectional sequencing of amplified fragments was conducted with the Big Dye® Terminator v3.1 cycle sequencing kit and 3500Dx Genetic analyzer (Thermo Fisher Scientific, USA).

Data analysis

The consensus sequence was generated for each sample using BioEdit version 6.0.7 and were searched over the GenBank database using Basic Local Alignment Search Tool (BLAST) against the *Ae. aegypti* genomes in GenBank. The complete sequences were deposited in GenBank (Table 1).

Local samples were compared with the *Ae. aegypti* found from other countries (Table 2) using Multiple Sequence Alignment (MSA) based on the sequences available in GenBank. Phylogenetic trees were built using maximum likelihood method (with 1000 bootstraps) with Kimura a cluster containing >50% bootstrap support, was considered significant. All sequences were analyzed with DnaSP version 5.10.01 (Librado and Rozas, 2009) for nucleotide diversity indices (the number of monomorphic sites, polymorphic sites, singleton sites and parsimony informative sites) and for the indices of haplotype diversity (the number of haplotypes, haplotype diversity and the average number of nucleotide differences).

Ethical statement

This study was approved by the Ethics Review Committee of Institute of Biology, Sri Lanka (ERC IOBSL 122 04 15)

RESULTS

Sequence diversity

The sequence analysis was carried out using a 683bp fragment of the amplified region of *COI* gene using a total of 33 samples derived from the present study representing

Table 1. Sample collection locations and GenBank accession numbers

Sample number	Sample Abbreviations	Location	GPS coordinates of location	District	GenBank accession numbers
1	CLA1	Dematagoda	6.93 N/ 79.88 E	Colombo	MG004695
2	CLA2	Thummulla	6.89 N / 79.85 E	Colombo	MG004696 (CLA4*)
3	GMA1	Dalugama	6.97 N /79.92 E	Gampaha	MG004697
4	GMA2	Negombo	7.20 N/ 79.87 E	Gampaha	MG004698
5	KTA1	Horana	6.71 N/ 80.07 E	Kaluthara	MF993579 (AH12*)
6	KTA2	Kalamulla	6.58 N/ 79.98 E	Kaluthara	MF993580 (AK19*)
7	GLA1	Galle Fort	6.02 N/ 80.21E	Galle	MG384710
8	JFA1	Manipai	9.72 N/ 79.99 E	Jaffna	MG004699
9	JFA2	Sandilipay Uyanapulam	9.74 N/ 79.98 E	Jaffna	MG004700
10	MNA1	Pesalai	9.08 N /79.81 E	Mannar	MG004705 (MNA2*)
11	MNA2	Uppakulam	8.97 N/ 79.91 E	Mannar	MG004706 (MNA4*)
12	VVA1	1st Cross Street	8.75 N / 81.21 E	Vavuniya	MG004713
13	VVA2	Kurumankadu North	8.75 N / 80.47 E	Vavuniya	MG004714
14	PTA1	Chilaw	7.56 N/ 79.80 E	Puttalam	MG004709
15	ANA1	New Town	8.33 N /80.41 E	Anuradhapura	MG004689
16	ANA2	Kahatagasdigiliya	8.42 N / 80.69 E	Anuradhapura	MG004690
17	KDA1	University of Peradeniya	7.25 N /80.59 E	Kandy	MG004703
18	KDA2	Thalwatte	7.29 N/ 80.65 E	Kandy	MG004704
19	MTA1	Matale	7.46 N/ 80.62 E	Matale	MG004707
20	MTA2	Ukuwela	7.42 N/ 80.63 E	Matale	MG004708
21	TRA1	Manayaveli	8.69 N / 81.18 E	Trincomalee	MG004711
22	TRA2	Orr' Hills	8.57 N / 81.21 E	Trincomalee	MG004712
23	AMA1	Sainthamaruthu	7.39 N / 81.84 E	Kalmunai	MG004701 (KLA1*)
24	AMA2	Kalmunai North	7.42 N/ 81.82 E	Kalmunai	MG004702 (KLA2*)
25	BTA1	Koolawady	7.70 N / 81.71 E	Batticaloa	MG004693
26	BTA2	Kathankudy	7.68 N / 81.72 E	Batticaloa	MG004694
27	BDA1	Kailagoda	6.99 N/ 81.05 E	Badulla	MG004691
28	BDA2	Passara	6.93 N/ 81.15 E	Badulla	MG004692
29	HMA1	Thissamaharamaya	6.27 N /81.28 E	Hambantota	MG384711
30	HMA2	Hambantota Town	6.14 N/ 81.12 E	Hambantota	MG384712
31	MOA1	Monaragala Town	6.89 N /81.34 E	Monaragala	MG384713
32	RPA1	Good Shed road	6.68 N / 80.40 E	Rathnapura	MG004710
33	POA1	Hingurakkoda	8.04 N / 80.94 E	Polonnaruwa	MG384714

*Sample name/abbreviation given for the sequence during GenBank submission

19 districts. All sequences were deposited in GenBank repository (*https://www.ncbi.nlm.nih.gov/ genbank/*) and the accession numbers are given in Table 1.

The sequences consisted of 661 monomorphic sites (96.78%), 22 polymorphic sites (3.22%), 4 singleton sites (0.59%) and 18 parsimony informative sites (2.63%). This diversity was observed to generate 20 haplotypes as shown in Table 3. The haplotype (gene) diversity and average number of nucleotide differences were 0.949 and 6.371 respectively. The Tajima's D value for Sri Lankan isolates was 0.60607; $p > 0.05$.

Phylogenetic analysis of Aedes aegypti

The analysis of evolutionary divergence within Sri Lankan samples of *Ae. aegypti,* resulted in two main clades with the second clade bearing two subclades as shown in Fig. 2. The first clade includes more samples from the southern part of the country (i.e. Hambantota, Colombo, Gampaha, Monaragala and Rathnapura) while second clade carries more from the northern region (i.e. Mannar, Vavuniya, Ampara, Jaffna, Anuradhapura, Puttalam, Batticaloa). Interestingly, all districts in the northern corner of the country i.e. Jaffna, Vavuniya, Ampara and

Table 2. GenBank Accession numbers of previously published *Aedes aegypti COI* sequences used in the current analysis

Country	Accession number
Bolivia	JQ926679.1
Brazil	JQ926703.1
Cambodia	HQ688294.1
Cameroon	JQ926702.1
France	HQ688296.1
Guinea	JQ926700.1
Ennore,Tamil Nadu, India	DQ424949.1
Puthur, Andhra Pradesh, India	HM807261.1
Mamulapusi, Odisha, India	HM807269.1
Thiruvananthapuram, Kerala, India	HM807268.1
Thirumala, Andhra Pradesh, India	HM807266.1
Ivory Coast	JQ926694.1
Madagascar	HQ688298.1
Mexico	JQ926698.1
Portugal	KF909122.1
Tanzania	JQ926704.1
Thailand	JQ926692.1
USA	JQ926684.1
Venezuela	JQ926701.1
Vietnam	JQ926687.1

Mannar have clustered separately. Even though samples from Gampaha, Colombo, Hambantota, Kandy and Batticaloa districts were distributed among both main clades, samples from other districts were found within only one main clade.

An effort to understand the relationship between Sri Lankan *Ae. aegypti* samples and samples reported from other countries, were also made by constructing a phylogenetic tree that consisted of 16 other *COI* sequences from GenBank database (Fig. 3). Since India is the closest country to Sri Lanka, with frequent cargo and passenger transportation throughout history, it is likely that passive transportation of mosquitoes between the two countries has resulted in a higher genetic similarity among the samples derived from the two countries. To analyse this possibility, five *COI* sequences representing five different areas of India is also included in the phylogenetic tree. As shown, Sri Lankan samples of the present study did not cluster separately when analyzed together with the samples collected from different regions of the world, but showed varying degrees of relationship with them.

In the phylogenetic tree derived from this analysis, two main clades were observed where Madagascar sample was separated from all other countries in a separate clade. Anuradhapura, and Mannar samples were clustered together with isolates from India, Venezuela, USA, Portu-

Table 3. Haplotype diversity, frequency and distribution in different districts in Sri Lanka.

Haplotype	Sequence	District	Frequency
1	CCTTCATAGGGGTTGAGAATCTTATTATAATTCGAAGACACTAA	Anuradhapura	1
2	..............G.G...C...G.C.............T...T.	Anuradhapura, Kaluthara	2
3	................G.......G...C...........T.....	Badulla, Gampaha	2
4	.......T.......G.......G...C...........T.....	Badulla	1
5	................G.......G..............T...T.	Batticaloa, Matale, Vavuniya	3
6	.T.C.G...A.....G.G...C......G.C.T.G...T.T...	Batticaloa, Gampaha, Monaragala, Rathnapura	4
7	.T.C.G...A.....G.G...C......G.C.T.G...T.T.T.	Colombo	1
8	.......T.......G.......G..............T.....	Colombo	1
9	...........A...G.......G............A.T.....	Galle	1
10	.T...G...A.....G.......G..............T.T...	Hambantota	1
11	.T.C.G...A.....G.....C........C.T.....T.T...	Hambantota	1
12	................G.......G..............T.....	Jaffna, Ampara, Mannar, Vavuniya	6
13	................G.......G..............T.....	Kandy, Puttalam	2
14	.T.C.G...A.....G.....C........C.T.....T.T.T.	Kandy	1
15	..............G.G.......G..............T.....	Kaluthara	1
16	..............G.G...C...G.C............T.....	Mannar	1
17	................G.......G......C.......T...T.	Matale	1
18	.T.C.G...A.....G.G...C........C.T.....T.T...	Polonnaruwa	1
19	.T...G...A.....G.....C........C.T.....T.T...	Trincomalee	1
20	.T...G...A.....G.....C........C.T.....T.T.T.	Trincomalee	1

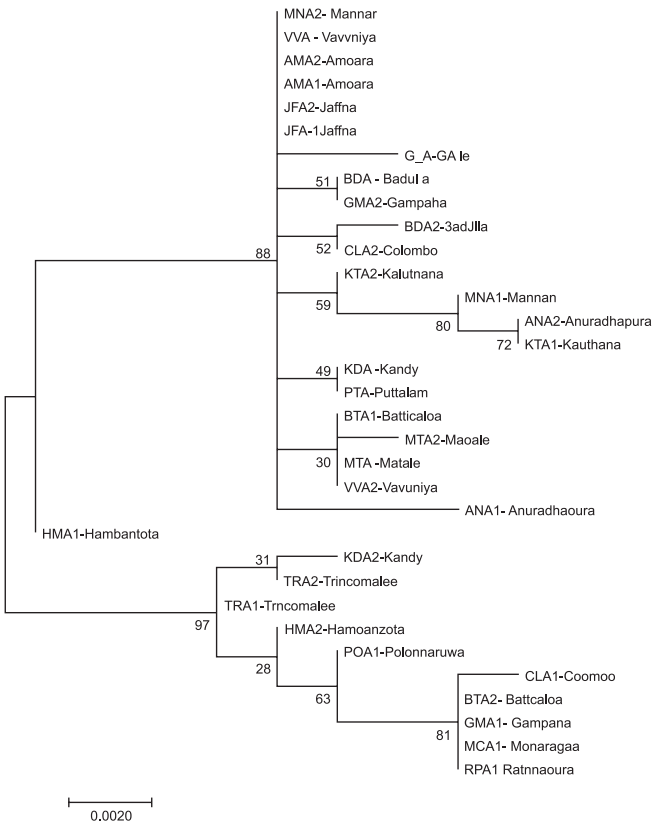

Fig. 2: Phylogenetic tree showing the relationship of *COI* sequence of Sri Lankan *Ae. aegypti* samples collected from different districts.

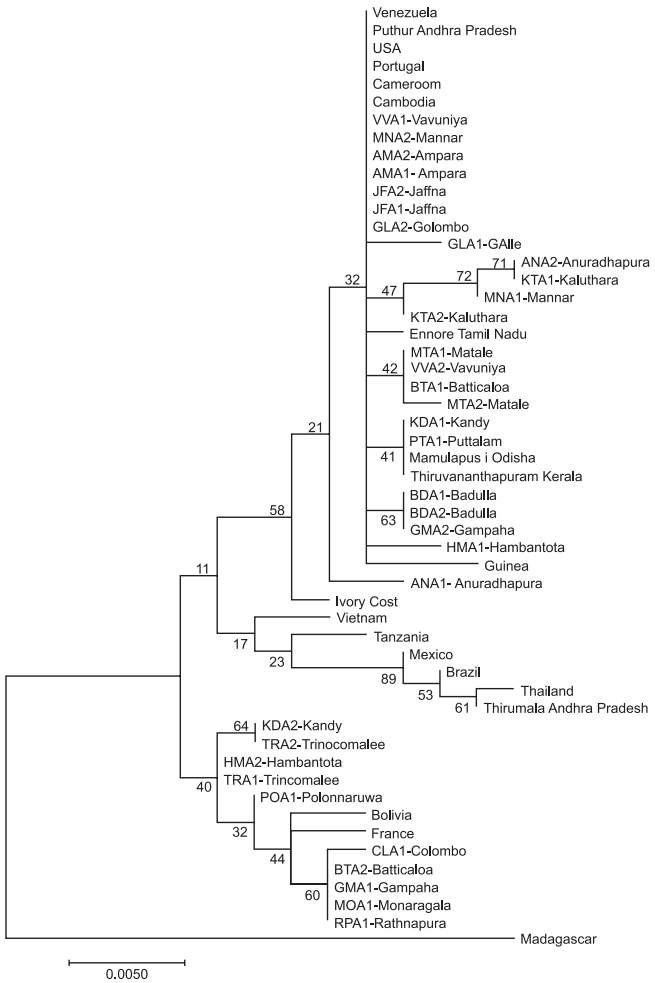

Fig. 3: Phylogenetic tree showing the relationship among *COI* sequence of Sri Lankan samples and samples of *Ae. aegypti* derived from various parts of the world

gal and Cambodia. Sample from Rathnapura district that was clustered with Bolivia and France was found in the second clade.

DISCUSSION

The overall objective of this study was to genetically characterize the *Aedes aegypti* mosquitoes collected from different regions of Sri Lanka in relation to *Ae. aegypti* mosquitoes found from various parts of the world based on 683bp fragment of mitochondrial *COI* gene. Sequences of 33 mosquito samples from 19 administrative regions were analysed for genetic diversity and phylogenetic relationships. Our results suggest that Sri Lanka has undergone multiple invasions of *Ae. aegypti* from various parts of the world over on extensive period. The mosquito population is existing in mutation-drift equilibrium unrestrained by breeding habitat removal or chemical control.

According to our results 20 haplotypes were present within the 19 localities investigated. Although the number of samples analysed does not allow the detection of the pattern of haplotype distribution, the presence of 20 haplotypes among the 33 mosquito isolates provides evidence for extensive genetic diversity. Additionally, this observation supports the suggestion made previously on the relative high variability of *Ae. aegypti COI* gene. According to Mousson *et al* (2005), *Ae. aegypti COI* gene is more variable than the *ND5* (nicotinamide adenine dinucleotide dehydrogenase subunit 5) or *Cytb* (cytochrome b) genes in Brazilian samples. The high haplotype diversity values (ranging from 0.2 to 0.8) observed among the Brazilian samples supported this suggestion. The value we have observed in the present study is even greater suggesting an extensive genetic diversity of the mosquitoes. This diversity might be indicative of several facts such as a large effective population size of mosquitoes within the island, high gene flow among local populations and favorable environmental factors for mosquito breeding, irrespective of the ongoing mosquito control campaigns. The observed nucleotide diversity (Pi=0.0093) and the

average number of nucleotide differences (K=6.371) was also relatively high and higher than what had been reported from Brazil for the same gene region (Pi=0.0065; K=5.546). This implies the presence of relatively large differences between haplotypes suggesting a long-established mosquito population within the country that has not suffered recent bottlenecks. However, it is equally possible that the presence of genetically distant haplotypes in sympatry to produce the same observations.

The Tajima D value calculated is also in favour of this hypothesis. Tajima's D test distinguishes between DNA sequences evolving randomly (neutrally) from one's that are evolving under a non-random process. Statistically non-significant Tajima's D value obtained for the present study accepts the null hypothesis of neutral evolution. This indicates that the mosquito populations within the country is at mutation-drift equilibrium maintaining a stable large population.

When the phylogenetic tree drawn for the Sri Lankan samples from the 19 districts were considered, there was no distinguishable clustering observed except for the three districts of the northern corner [Mannar, Vavunia (one sample each), Jaffna and Ampara]. They clustered together when analysed along with 20 samples around the globe including the five samples from different regions in India. All other isolates which represented various districts in Sri Lanka were randomly distributed over the phylogenetic tree. However, one would expect mosquito samples from geographically closer regions to be more genetically similar than those collected from far apart when considering their dispersal distances. Honório *et al* (2003), reported *Ae. aegypti* to have a general dispersal distance around 800m. This suggests of restricted genetic mixing of the mosquitoes collected from several hundred kilometers away from each other. Contradictory results observed in the present study to this assumption suggest mosquitoes were able to surpass their biological dispersal barrier perhaps through the means of passive transportation. It is possible that passenger and cargo transportation through various motor vehicles among different districts would have served as an ideal passage for both the adult, larvae and mosquitoes eggs from one district to another allowing extensive admixing. The limited admixture observed between the mosquitoes of northern most districts and the rest of the Sri Lanka is likely to have been caused by the civil war that lasted for decades until it ended in 2009. The main routes of transportation were re-opened for civil usage since then, and this might have caused some amount of mosquito migrations between northern area and others. The grouping of two northern samples (MNA1 and VVA2) with samples from western and central provinces of the country could have resulted from these recent advances in transportation. This observation agrees with the previous reports of low levels of genetic differentiation among *Aedes aegypti* samples from different regions of Sri Lanka based on microsatellite markers[14].

Despite the extensive admixing observed among Sri Lankan samples, two main clades were identified in the phylogenetic tree. This pattern of clustering was consistent when the samples were analysed with samples derived from various parts of the world. Interestingly, Venuzuela, Andhra Pradesh in India, USA, Portugal, Cameroon and Cambodia samples clustered together with the samples derived from the northern parts of Sri Lanka. Vietnam, Tanzania, Mexico, Brazil, Thailand and Thirumala, Andhra Pradesh in India made a separate subclade which did not carry a single Sri Lankan sample indicating a subtle but distinct genetic difference between the Sri Lankan samples and samples from these countries. The second main clade of Sri Lankan phylogenetic tree clustered with samples of Bolivia and France shows the close genetic similarity of some of the Sri Lankan samples to these countries. Unfortunately, the phylogenetic tree with global samples does not have sufficient bootstrap values to make strong inferences, probably due to the low sample numbers used in the analysis. Nevertheless, it is highly likely that Sri Lanka received multiple invasions from *Ae. aegypti* mosquitoes from various parts of the world as indicated in the phylogenetic tree (Vietnam, Tanzania, Mexico, Brazil, Thailand, Thirumala, Andhra Pradesh in India, Bolivia and France). This agrees with the observation made previously by other authors upon the recurrent spreading of mosquitoes over various continents producing genetically similar mosquito samples among geographically faraway regions like South America, Asia and Africa[15].

The extensive genetic mixing of *Ae. aegypti* population in Sri Lanka and around the world as evident from our results has important implications on mosquito control and disease transmission. Firstly, high genetic diversity and extensive genetic mixing increases the evolutionary fitness of the mosquitoes and stabilizes their populations. This would be important for mosquitoes for surviving in an environment where numerous environmental stresses are constantly available due to the continuous mosquito control measures. Secondly, such genetic mixing would favour the spread of evolutionary important traits in mosquitoes such as insecticide resistance and salinity

tolerance over a wider geographic area enhancing their adaptive fitness. As an invasive species, this would make their status even stronger. Thirdly, as a highly successful vector of many arboviral diseases, extensive dispersal of mosquitoes indicates spreading of diseases over larger geographic area resulting in emergence of mosquito borne diseases in new localities. Thus, the passive long distance migrations of *Aedes aegypti* indicated in our results highlights the necessity to prevent human-aided mosquito dispersals to ensure successful dengue control.

However, it is important to understand the relativity of these inferences in the context of genetic stability of different marker regions in the genome. In a study[6] where both *ND4* and *COI* genes of *Aedes aegypti* have been used to measure the nucleotide diversity, *COI* gene showed significantly lower diversity compared to the *ND4* gene. It was postulated that this difference may have arose due to the higher constraints (i.e. balancing selection) acting upon the mutation rate of the *COI* gene. This indicates that *COI* gene in *Ae. aegypti* is more conservative than the *ND4* gene. Thus, it is possible that a higher genetic diversity with a somewhat different phylogenetic picture is to be yielded, if *ND4* gene has been used in the analysis along with the *COI* gene.

Despite such limitations, knowledge on vector genetics and the gene flow between mosquito populations residing at various geographically distant localities is beneficial in designing effective vector control strategies. This is the first study that genetically characterized *Aedes aegypti* samples from all the 19 districts of Sri Lanka, where the species is routinely reported. Though this paper only presents the results of a preliminary investigation conducted using a limited number of samples and with a single genomic region, the results revealed are of significant epidemiological value. As a country which is extensively affected by dengue fever related morbidity and mortality, the need to look for means of reducing passive mosquito dispersal within the country would be of utmost importance to keep the disease transmission under control. The mosquito population with high evolutionary potential points at the necessity to revisit the vector control strategies to yield success. While more elaborate studies are required to delve into the role of control programs in manipulating the genetic composition of the mosquito populations, it is recommended to incorporate the information on other genetic markers such as mitochondrial NADH dehydrogenase subunit 4 (ND4) in such analysis to improve the robustness of the reported observations.

Conflict of interest: None

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REFERENCES

1. Brown JE, Obas V, Morley V, Powell JR. Phylogeography and spatio-temporal genetic variation of *Aedes aegypti* (diptera: culicidae) populations in the Florida keys. *J Med Entomol* 2013; *50*(2): 294–9.
2. Lunt DH, Zhang DX, Szymura JM, Hewltt OM. The insect cytochrome oxidase I gene: Evolutionary patterns and conserved primers for phylogenetic studies. *Insect Mol Biol* 1996; *5*(3): 153–65.
3. Scarpassa VM, Cardoza TB, Junior RPC. Population genetics and phylogeography of *Aedes aegypti* (Diptera: Culicidae) from Brazil. *Am J Trop Med Hyg* 2008; *78*(6): 895–03.
4. Batovska J, Blacket MJ, Brown K, Lynch SE. Molecular identification of mosquitoes (Diptera: Culicidae) in southeastern Australia. *Ecol Evol* 2016; *6*(9): 3001–11.
5. Gloria-Soria A, Ayala D, Bheecarry A, Calderon-Arguedas O, Chadee D, Chiappero M *et al*. Global genetic diversity of *Aedes aegypti*. *Mol Ecol* 2016; *25*(21): 5377–95.
6. Paduan KDS, Ribolla PEM. Mitochondrial DNA Polymorphism and heteroplasmy in populations of *Aedes aegypti* in Brazil. *J Med Entomol* 2008; *45*(1): 59–67.
7. Vadivalagan C, Karthika P, Murugan K, Panneerselvam C, Paulpandi M, Madhiyazhagan P, *et al*. Genetic deviation in geographically close populations of the dengue vector *Aedes aegypti* (Diptera: Culicidae): Influence of environmental barriers in South India. *Parasitol Res* 2015; *115*(3): 1149– 60.
8. Fernando HSD, Hapugoda MD, De Silva BGDNK. A preliminary study to analyze *Aedes aegypti* diversity in Sri Lanka using mitochondrial DNA variations. Proceedings of International Conference on Multidisciplinary Approaches 2015; University of Sri Jayewardenepura, Nugegoda.
9. Rattanarithikul R, Panthusiri P. Illustrated keys to the medically important mosquitoes of Thailand. *Southeast Asian J Trop Med Public Health* 1994; *25*(suppl 1): 66–9.
10. Gupta K, Dhawan R, Kajla M, Kumar S, Jnanasiddhy B, Naveen K, *et al*. Molecular identification of *Aedes aegypti* mosquitoes from Pilani region of Rajasthan, India. *J Vector Borne Dis* 2016; *53*(2): 149–55.
11. Librado P, Rozas J. DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. *Bioinformatics* 2009; *25*(11):1451–2.
12. Mousson L, Dauga C, Garrigues T, Schaffner F, Vazeille M, Failloux A. Phylogeography of *Aedes* (Stegomyia) *aegypti* (L.) and *Aedes* (Stegomyia) *albopictus* (Skuse) (Diptera: Culicidae) based on mitochondrial DNA variations. *Genet Res* 2005; *86*(1): 1–11.
13. Honório N, Silva W, Leite P, Gonçalves J, Lounibos L, Lourenço-de-Oliveira R. Dispersal of *Aedes aegypti* and *Aedes albopictus* (Diptera: Culicidae) in an urban endemic dengue area in the State of Rio de Janeiro, Brazil. *Mem Inst Oswaldo Cruz* 2003; *98*(2): 191–8.

14. Nirmani MD, Gangani PD, Perera KLN, Galhena GH. Long distance passive migrations may explain the low genetic differentiation in principal dengue vector (*Aedes aegypti*) in Sri Lanka. Proceedings of the 36th Annual Sessions of the Institute of Biology 2016.
15. Failloux AB, Vazeille M, Rodhain, F. Geographic genetic variation in populations of the dengue virus vector *Aedes aegypti*. *J Mol Evol* 2002; *55*(6): 653–63.

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