Phylogeographic and Population Genetic Structure, and '*kdr*' type gene mutations of *Aedes aegypti* in Sri Lanka

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Ph.D **2017**

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By

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Thesis submitted to the University of Sri Jayewardenepura, Sri Lanka for the award of the Degree of Doctor of Philosophy in Zoology on 2017

Declaration

The work described in this thesis was carried out by me under the supervision of Prof. B. G. D. N. K. De Silva and Dr. Menaka Hapugoda and a report on this has not been submitted in whole or in part to any university or any other institution for another Degree/Diploma.

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Declaration

We certify that the above statement made by the candidate is true and that this thesis is suitable for submission to the University for the purpose of evaluation.

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We certify that the candidate has incorporated all corrections, additions and amendments recommended by the examiners to this version of the Ph.D thesis.

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LIST OF ABBREVIATIONS

A	Adenine
AGE	Agarose Gel Electrophoresis
AD	*Amno Domini*
AS-PCR	Allele-Specific Polymerase Chain Reaction
ATP	Adenosine Triphosphate
BG	BioGents
bp	base pair
C	Cytosine
CO_2	Carbon Dioxide
COI	Cytochrome C Oxidase subunit I
DDT	Dichlorodiphenyltrichloroethane
DENV	Dengue Virus
DF	Dengue Fever
DHF	Dengue Hemorrhagic Fever
DNA	Deoxyribonucleic Acid
dNTP	Deoxy Nucleotide Triphosphate
DSS	Dengue Shock Syndrome
E	Envelop protein
EDTA	Ethylenediaminetetraacetic Acid
F1534C	Phenylalanine1534Cysteine
g	gram

G	Guanine
GABA	Gamma-Aminobutyric acid
GIS	Geographical Information System
Gly923Val	Glycine923Valine
GPS	Global Positioning System
h	haplotype
Hd	Haplotype diversity
H_E	Expected Heterozygosities
H_O	Observed Heterozygosities
HWE	Hardy- Weinberg Equilibrium
IDT	Integrated DNA Technologies
Ile1011Met	Isoleucine1011Methionine
Ile1011Val	Isoleucine1011Valine
k	Average number of nucleotide differences
Kb	Kilo bases
kdr	knockdown resistance
km	kilometre
L	Litre
LD	Linkage Disequilibrium
Leu982Trp	Leucine982Trptophan
M	Molar

m	metre
μg	Microgram
μl	Microlitre
mA	milli Ampere
ml	millilitre
mM	millimolar
mtDNA	mitochondrial DNA
NADH	Nicotinamide Adenine Dinucleotide
NaV	Sodium Voltage Channel
ND4	NADH dehydrogenase subunit 4
ng	nanogram
nm	nanometre
no.	number
NS	Non Structural
NUMTs	nuclear mtDNA
^{O}C	Centigrade
PCR	Polymerase Chain Reaction
pmol	picomole
RNA	Ribonucleic Acid
rpm	revolutions per minute

R_S	Allelic Richness
RT	Room Temperature
SDS	Sodium Dodecyl Sulfate
SNP	Single Nucletode Polymorphism
SSR	Simple Sequence Repeats
STR	Short Tandem Repeats
T	Thymine
TBE	Tris-borate-EDTA
Tris	Tris(hydroxymethyl)aminomethane
TE	Tris EDTA
U.S.A	United States of America
UV	Ultra Violet
V	Volt
Val1016Gly	Valine1016Glycine
Val1016Ile	Valine1016Isoleucine
WHO	World Health Organization
yr	years
π	Nucleotide diversity
π_a	placement substitution site
π_s	synonymous substitution site

ACKNOWLEDGMENTS

I would like to extend my sincere gratitude to my supervisor Prof. B.G.D.N.K. De Silva, Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura, Sri Lanka for guidance, timely comments and encouragement given to me throughout the period of the study. I am also extremely grateful to him for providing me with an extremely valuable opportunity to go for a study visit to Colorado State University, USA.

I am also very much thankful to my co-supervisor Dr. Menaka Hapugoda, Molecular Medicine Unit, Faculty of Medicine, University of Kelaniya for her kind guidance and encouragement during the period of my study especially during the thesis writing.

I am also very much thankful to Prof. William C. Black IV, Department of Microbiology, Immunology and Pathology, Arthropod-borne and Infectious Disease Laboratory, Colorado State University, USA for his immense help in analyzing the results generated from my study conducted in Sri Lanka. I am immensely grateful to him for building up my knowledge in analytical methods and my theoretical knowledge in phylogenetic and population genetic studies. I am very much thankful to him for prioritizing me and my work during my two months visit at his laboratory despite his very busy schedule.

I am really grateful to Dr. Rushika Perera, Assistant Professor, Department of Microbiology, Immunology and Pathology, Arthropod-borne and Infectious Disease Laboratory, Colorado State University, USA for housing me at her place for two whole months and giving me a wonderful experience which I will remember for the rest of my life. I am also really grateful to her for teaching me cell culture and virological techniques at her laboratory in every possible movement. I am especially thankful to her for turning a mentor for me and giving me advice and courage to pursue my dreams in the field.

I am also very much thankful to Ms. Karla Saavedra-Rodriguez, Ms. Selene G-Luna, Ms. Farah Vera Maloof and Ms. Ashley Janich, Department of Microbiology, Immunology and Pathology, Arthropod-borne and Infectious Disease Laboratory, Colorado State University, USA for helping me in the laboratory studies and providing me with the most wonderful time period of my life at Colorado.

I am thankful to Mr. Dinesh (Puttalum), Mr. Vajira Upeksha (Trincomalee) and Mr. Nirmal Subasinghe (Hambanthota), Entomological Assistants attached to Anti-Malaria Campaign, Sri Lanka for providing me mosquito samples.

I also wish to acknowledge Department of Zoology, University of Sri Jayewardenepura, Sri Lanka for providing me with this opportunity and all the technical staff for helping me in various aspects of my study.

I am really grateful to Ms. Dilakshini Dayananda, Ms. Iresha Harischandra and Ms. Pamoda Jayatunga, Department of Zoology, University of Sri Jayewardenepura, Sri Lanka for their encouragement and great help during my study period.

Last but not least my heartfelt gratitude goes to my beloved sister and my mother whose encouragement made this thesis a reality. I am really in gratitude to them for standing with me through my tears and difficulties always encouraging me to do my best. This thesis is dedicated to them and my late father.

Phylogeographic and Population Genetic Structure of *Aedes aegypti* in Sri Lanka

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ABSTRACT

Mosquitoes as disease causing vectors, are of major relevance as insect invaders, due to their ability to transmit pathogens to humans and their ability to adapt to human built environment. *Aedes aegypti* (Linneaus), major vector for Dengue Viruses (DENV), originated as a canopy dwelling species in Africa, feeding on non-human primates, invaded the tropics and subtropics of the world through globalization and trade. In Sri Lanka, dengue is the most serious arboviral infection accounting for severe number of deaths annually. The present study was conducted to investigate the evolutionary history of *Ae. aegpti* in Sri Lanka, and to study the present genetic structure of *Ae. aegypti* mosquito populations within the country.

Ae. aegypti mosquitoes were sampled from eight districts during the period of 2013-2015 in Sri Lanka. The phylogeographic relationships of Sri Lankan *Ae. aegypti* was studied in an macro-evolutionary time frame using two mitochondrial Deoxy Ribonucleic Acid (DNA) markers named Cytocrome C Oxidase 1 (*COI*) and NADH subunit 4 (*ND4*) in PART 1 of the study. The population genetic structure of *Ae. aegypti* in the island was studied using eleven microsatellite loci in PART 2 of the study and the results were used to interpret gene flow patterns and population structuring of the

species in the island. The presence and distribution of knockdown resistant (*kdr*) alleles were studied, hypothesizing a resistant mosquito population for the most commonly used insecticide in the country, pyrethroids, in PART 3 of the study. Here three most commonly occurring point mutations, Phenylalanine 1534 Cysteine (F1534C), Valine 1016 Isoleucine (Val1016Ile) and Valine 1016 Glycine (Val1016Gly) in the sodium voltage gated channel was genotyped through Allele Specific Polymerase Chain Reaction (AS-PCR) and real time PCR assays.

The phylogeographic study in PART 1 of the study recorded 14 *COI* and 40 *ND4* haplotypes for the first time in Sri Lanka, with the highest genetic diversity recording for *ND4* gene. Presence of two mitochondrial lineages of *Ae. aegypti* was recorded among Sri Lankan mosquitoes. These two Sri Lankan lineages were related respectively to East and West African specimens and the analysis of gene flow patterns revealed abundant gene flow between South-East Asian countries and Sri Lanka. The population structure analysis in the PART 2 of the study revealed high genetic diversity in Sri Lankan *Ae. aegypti* with all the studied loci being polymorphic in all populations. The software STRUCTURE estimated three genetic clusters with the presence of weak isolation by distance pattern. A non-equilibrium analysis of gene flow rates and patterns indicated abundant bidirectional gene flow among all samples collected. The study of *kdr* allele mutations in the PART 3 of the study revealed the presence of F1534C mutation in Sri Lanka. The mutant allele was found to be wide spread in the island and it can be hypothesized that there is a resistant mosquito population for the insecticide. However V1016I and V1016G mutations were not recorded in the present study.

The efficiency of transmitting DENV and the ability to resist insecticides is dependent upon the genetic composition of the *Ae. aegypti* population. The identification of the presence of *Ae. aegypti* belonging to the two clades, representing the origins of East and West Africa suggests differences in flavivirus susceptibility and resistance to insecticides. Population genetic structure of the Sri Lankan *Ae. aegypti* revealed highly genetically differentiated populations due to genetic drift. Vector control through source reduction and insecticide treatment of breeding sites along with the seasonal changes may create periodic population reductions leading to genetic drift. Genetic drift may result in an increase of selectable genetic variation and could lead to the selection of insecticide resistance genes thus making the newly found population resistance to commonly used insecticides. Thus the results of the present study are of utmost importance to in understanding the corridors and barriers for mosquito movement and in turn, develop the strategies for controlling DENV vector mosquitoes more effectively.

Key words: *Aedes aegypti*, phylogeographic structure, mitochondrial DNA, population genetic structure, microsatellites, *kdr*