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Screening of sibling species of *Anopheles culicifacies* Giles in Sri Lanka, using DNA based methods and determining population structure of sibling species E

by

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CERTIFICATION OF SUPERVISORS

We certify that the candidate has incorporated all corrections, additions, and amendments recommended by the examiners.

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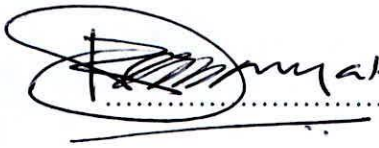

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DECLARATION

The work described in this thesis was carried out by me under the supervision of Prof. B. G. D. N. K. De Silva, Department of Zoology, Faculty of Applied Sciences, University of Sri Jayewardenepura and Prof. R.S. Dassanayake, Department of Chemistry, Faculty of Science, University of Colombo 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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24-03-2016

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

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Prof. B. G. D. N. K. De Silva

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Prof. R.S. Dassanayake

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ABBREVIATIONS

μ ..... Microsatellite mutation rate

A ..... Adenine

A ..... number of alleles

Anu ..... Anuradhapura

Mon ..... Monaragala

Tha ..... Thanamalwila

Kan ..... Kandy

Kat ..... Kataragama

Nik ..... Nikaweratiya

Ae ..... *Aedes*

AFLP ..... Amplified Fragment Length Polymorphism

AMC ..... Anti Malaria Campaign

AMOVA ..... Analysis of Molecular Variance

An. ..... *Anopheles*

AT ..... Adenine + Thymine

BC ..... Before Christ

BPB ..... Bromo phenol blue

Bti ..... *Bacillus thurigiensis* var *israelensis*

BYM ..... Tess software specific admixture model

C ..... Cytocine

CAR ..... Conditionally Auto Regressive admixture model

cDNA ..... Complementary DNA

COI ..... Cytochrome oxidase I

COII ..... Cytochrome oxidase II

Cyt-b ..... Cytochrome b

D2 ..... Domain 2 of ribosomal DNA

D3 ..... Domain 3 of ribosomal DNA

DDT ..... Dichlorodiphenyltrichloroethane

DIC ..... Deviance Information Criterion

DNA ..... Deoxyribonucleic acid

dNTP ..... Deoxy Nucleotide triphosphate

DVS ..... Dominant Vector Species / species complexes

EDTA ..... Ethylene-diamine-tetra-acetic acid

EMBL ..... European Molecular Biology Laboratory

EtBr ..... Ethidium bromide

ETS ..... External transcribed spacer

F_1 ..... First filial

F_{IS} ..... Inbreeding coefficient

F_{ST} ..... Fixation index

G ..... Guanine

GC ..... Guanine + Cytocine

G_{ST} ..... Analogue to the fixation index

H_e ..... Expected heterozygosity

He ..... Heterozygosity from allele frequency data

Heq ..... Heteroztgosity from number of alleles and sample sizes

H_o ..... Observed heterozygosity

HWE ..... Hardy-Weinberg Equilibrium

IAM ..... Infinite allele model

IBD ..... Isolation by distance

IGS ..... Intergenic Spacer

IRS ..... Indoor residual spraying

ITNs ..... Insecticide treated nets

ITS 1 ..... Internal Transcribed Spacer 1

ITS 2 ..... Internal Transcribed Spacer 2

K ..... gene pool clusters

Ldh ..... Lactate dehydrogenase

M ..... Genetic similarity

ML ..... Maximum likelihood

MP ..... Maximum parsimony

MCMC ..... Marcov chain monte carlo method

MDE ..... Mutation drift equilibrium

$MgCl_2$ ..... Magnesium Chloride

mRNA ..... Messenger RNA

mtDNA ..... Mitochondrial DNA

NCBI ..... National Center for Biotechnology Informaion

NJ ..... Neighbor joining

ND ..... *NADH dehydrogenase subunit*

Ne ..... Effective population size

NORs ..... Nucleolar Organizer Regions

N_{ST} ..... Analogue to the fixation index

NTS ..... Non transcribed spacer

ORF ..... Open Reading Frame

OTU ..... Operational taxonomic unit

PCD ..... Passive case detection

PCR ..... Polymerase Chain Reaction

r ..... null allele frequency

RAPD ..... Random Amplified Polymorphic DNA

rDNA ..... Ribosomal DNA

RFLP ..... Restriction fragment length polymorphism

RPM ..... Rounds per minute

rRNA ..... Ribosomal RNA

Rs ..... allele richness

R_{ST} ..... Analogue to the fixation index

SAMOVA ..... Spatial Analysis of Molecular Variance

SDS ..... Sodium dodecyl sulfate

SIT ..... Sterile insect technique

SMM ..... Stepwise mutation model

SNP ..... Single nucleotide polymorphism

SPR ..... Subtree pruning and redrafting

SSR ..... Simple sequence repeat

STR ..... Short tandem repeat

T ..... Thymine

TAE ..... Tris Acetate EDTA

TBE ..... Tris Borate EDTA

TE..... Tris EDTA (Ethylenediaminetetraacetic acid)

TEMED..... NNN'N' – tetramethyl-ethylenediamine

TPM..... Two phase mutation model

tRNA..... Transfer RNA

UV..... Ultra violet

VNTR..... Variable number of tandem repeat

WHO..... World Health Organization

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ABSTRACT

Anopheles culicifacies, major vector of malaria in Sri Lanka is a complex of five sibling species designated as A, B, C, D and E. Sri Lanka is reported to have B and E, among them sibling species E is the major vector. Precise identification and characterization of sibling species and the understanding of the genetic structure of vector population is prerequisite in malaria vector control and prevention of re-occurrence programs. Therefore, the study was designed to investigate the potential of mitochondrial and ribosomal DNA regions to distinguish the *An. culicifacies* sibling species in Sri Lanka and to determine the population genetic structure of sibling species E in Sri Lanka. Mosquito samples were collected from six localities of Sri Lanka during 2009-2012 and the genomic DNA was extracted. BCE-PCR assay developed in India was used to test its utility in sibling species identification in Sri Lanka. Sequencing of mitochondrial and ribosomal DNA regions were carried out to investigate the genetic diversity in these regions of Sri Lankan sibling species. Then the cytogenetically identified sibling species E mosquitoes (n=193) were genotyped using microsatellite markers to analyze the population genetic structure using standard analyses, genetic differentiation, Hardy-Weinberg equilibrium, linkage disequilibrium, demographic stability, clustering analysis, AMOVA, SAMOVA, gene flow and isolation by distance. BCE-PCR profiles

of some sibling species of B and E were different from the profile reported in India limiting its utility in Sri Lanka suggesting existence of genetic variants. None of the DNA region was able to distinguish two sibling species because of the heterogeneity in mitochondrial regions and the similarity in ribosomal DNA regions in two sibling species. Further, non-synonymous substitutions were detected in *COII* gene amongst sibling species E populations in Sri Lanka (04%). In the genetic structure analysis, five microsatellite loci were highly polymorphic across populations with high allelic richness (4.963 – 9.830). Deviation from Hardy-Weinberg Equilibrium was observed in four loci in pooled population and in five subpopulations ($p<0.006$) with heterozygosity deficits. The population was observed in mutation drift equilibrium ($p<0.0062$) with a trend towards a recent population expansion creating a risk of spreading disease if there is an indigenous *Plasmodium* parasite population. Genetic differentiation was observed (F_{ST} 0.03331 - 0.23184) in some population pairs with reduced gene flow which were not supported by the isolation by distance model ($r^2 = 0.3057$, $p<0.0180$). Bayesian clustering analysis identified the presence of three sympatric clusters in the studied population. Further, the central hill region of the country was found to be act as a possible barrier to the gene flow among population.