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Analysis of the genetic variation of the *An. culicifacies* and *An. subpictus* complexes in Sri Lanka using DNA based techniques

By

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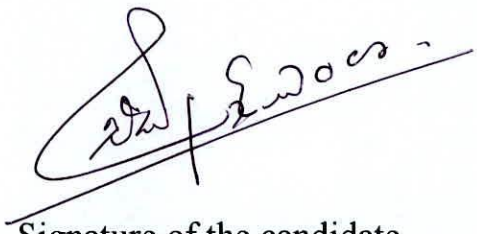
Declaration by the candidate

The work described in this thesis was carried out by me under the supervision of Dr. B. G. D. N. K. de Silva, Prof. E. H. Karunanayeke and Prof. S. Fernando 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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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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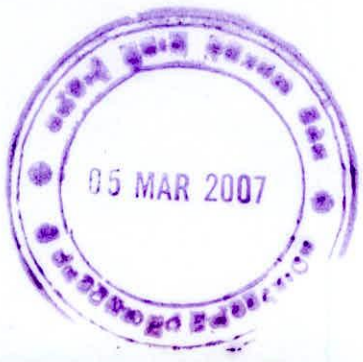

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A.M.C	Anti Malaria Campaign
ATP	Adenosine Tri Phosphate
BSA	Bovine Serum Albumin
$CaCl_2$	Calcium Cloride
CDC	Centre for Disease Control
DDT	Dichloro diphenyl trichloroethane
DTT	Dithiothreitol
DMSO	Dimethylsulphoxide
DNA	Deoxyribose Nucleic Acid
dNTP	Deoxy Nucleotide Tri Phosphate
DoD	Department of Defence(USA)
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme Linked Immunosorbant Assay
GMT	Greenwich Median Time
hr	hour
IAA	Isoamyl Alcohol
IPTG	Isopropyl beta-D-thiogalactopyranoside
ITS2	2^{nd} Internal Transcribed Spacer
Kb	Kilo base
LB	Luria Bertani medium
$MgCl_2$	Magnesium Chloride

$MgSO_4$	Magnesium Sulphate
mM	Mili Molar
NaCl	Sodium Chloride
ng	Nano Grams
NIAID	National Institute of Allergy and Infectious Diseases
NTS	Non Transcribed Spacer
OD	Optical Density
PBS	Plasmid Bluescript
RNA	Ribose Nucleic Acid
SDS	Sodium Dodecyle Sulphate
SSC	Standard Saline Citrate
TAE	Tris Acetate EDTA
TBE	Tris Borate EDTA
TE	Tris EDTA
TEMED	N, N, N --tetramethylethylenediamine
Tris	Tris (hydroxymethyl) aminomethane
Rp	Repetitive (sequence)
rpm	revolutions per minute
U	Unit
USAID	United States Agency for International Development
WHO	World Health Organisation
X-gal	5-bromo-4-chloro-3-indolyl beta-D-galacto pyranoside
µg	Microgram
µl	Micro litres

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ABSTRACT

Anopheles culicifacies s.l. is the major vector while *Anopheles subpictus s.l.* is the most important secondary vector of malaria in Sri Lanka. *An. culicifacies* is known to exist as a complex of five sibling species A, B, C, D and E in India, the neighbouring country. Karyotyping has revealed the presence of *An. culicifacies* B and E sympatric in Sri Lanka. Previous studies revealed that in Sri Lanka, sp. E is predominant and act as the vector while sp. B is less common and act as a non vector or is having a least vector potential. *An. subpictus* complex consists at least of four sibling sp. naming A, B, C and D those who could be differentiated by morphological characteristics of eggs, larvae and adults. All the members of this complex are present in Sri Lanka. Sp. B prefers saline water and sp. D prefers fresh water while sp. A and C don't show any preferences.

Cloned Polymerase Chain Reaction (PCR) fragments of the ribosomal Deoxyribonucleic Acid (DNA) second internal transcribed spacer of available members of above species complexes were sequenced. Sequences of two complexes were analysed separately using Bio Edit Sequence Alignment Editor 6.0.5. Previously developed DNA probes were manipulated to check any difference in hybridization between *An. culicifacies* B and E DNA.

An. culicifacies B and E had identical ITS2 sequences. Phylogenetic tree generated using ITS2 sequences of *An. culicifacies* complex available in the web revealed that members of the complex evolving in two different lines: sp. A and D in a one lineage and sp. B, C and E in the other. Secondary structure predictions from their ITS2 region showed identical folding patterns among B, C and E as well as very similar secondary structures of A and D. Structural analogy of those secondary structures showed a functional stability of ITS2 region among *An. culicifaies* complex which led to a slow evolution rate of that region. Therefore, it is difficult to display genetic variation through analysis of ITS2 of *An. culicifacies* complex. Also hybridization with the DNA probe exhibited a similar pattern between sp. B and E.

An. subpictus complex could be categorized into two groups based on PCR assay: sp. A and C into one group and B and D into the other. Different ITS2 sequences could be seen among members. *Sp.* B could be clearly separated from sp. A, C and D based on the ITS2 sequence dissimilarities. Phylogenetic tree showed that *An. subpictus* B is evolving in a separate evolutionary line and could easily distinguish from other members of the complex.