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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Thesis submitted to the University of Sri Jayewardenepura for the award of the Degree of Master of Philosophy in Zoology

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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. culicifacies 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.
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