

COMPENSATORY BASE CHANGES DIFFERENTIATE THE *ANOPHELES SUBPICTUS* (DIPTERA:CULICIDAE) SPECIES COMPLEX IN SRI LANKA

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Anopheles subpictus sensu lato is an important secondary vector of malaria in Sri Lanka. Molecular phylogenetic studies using Internal Transcribed Spacer 2 (ITS2) and Cytochrome *c* Oxidase subunit I (*COI*) gene have identified two sibling species *An. subpictus* A and B in Sri Lanka. The objective of the present study was to further investigate and confirm the status of *An. subpictus* species complex in Sri Lanka exploiting Compensatory Base Changes (CBCs), based on the reproductive isolation in sibling species. CBCs are double-sided base changes that occur in nucleotide pairs of helices of ITS2 secondary structures. Universal folding pattern of ITS2 region, depicting a eukaryote-universal core structure is preserved by the occurrence of CBCs. The CBC species concept is that having at least one CBC between two organisms denotes sexual incompatibility and hence the organisms belong to two different species. However, it is a one-way tool that its absence does not necessarily mean sexual compatibility and it may or may not belong to the same biological species. Morphologically identified *An. subpictus* specimens ($n=29$) from five localities in Sri Lanka were sequenced for ITS2. The full sequences of ITS2 were subjected to annotation by ITS2 Database and analysis by Software RNAstructure and 4SALE. The most stable ITS2 secondary structures having hallmark characteristics were selected and used as template for homology modeling. The generated ITS2 secondary structures were analyzed for the presence of CBCs. Eight distinct ITS2 sequence haplotypes were obtained and among the respective ITS2 secondary structures, two distinct secondary structure patterns were revealed. These two ITS2 secondary structure patterns were found to have two CBCs each depicting two sexually incompatible sets of individuals among the *An. subpictus* specimens. The two distinct types of ITS2 secondary structures each having two CBCs, clearly corresponded to sibling species A and B revealed from previous studies. However, noting the one-way nature of CBC approach, it can be concluded that, this study confirms the presence of at least two distinct sibling species in *An. subpictus* species complex, which is consistent with the previous studies as *An. subpictus* A and B in Sri Lanka.

Keywords: Malaria, *Anopheles subpictus*, sibling species, ITS2 secondary structures, Compensatory Base Changes