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Analysis of helices of the ITS2 secondary structures of *Anopheles culicifacies* sibling species complex

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Anopheles culicifacies Giles s.l., the primary vector of malaria in Sri Lanka comprises a complex of five sibling species as A,B,C,D and E. Among them only sibling species B and E are found in Sri Lanka. Sibling species of the complex are morphologically identical and varies in vectorial capacity. Molecular characterization of the vector species is important in malaria control programs. Thus, in this study, secondary structures of Internal Transcribed Spacer 2 (ITS2) region of rDNA of the five sibling species were analyzed and further compared with the universal eukaryotic secondary structure. To obtain an in depth understanding of secondary structures different types of loops were examined. Genomic DNA of *An. culicifacies* sibling species B and E collected from six different locations in Sri Lanka were amplified for ITS2 region and sequenced. NCBI GenBank deposited ITS2 sequences of sibling species of *An. culicifacies* were retrieved for comparison. The length of the ITS2 region was 370 bp for A and D sibling species whereas this was 372 bp for B, C and E sibling species. Secondary structure analysis showed that all the sibling species possessed three helices as Helix I, Helix II and Helix III. Pyrimidine-pyrimidine mismatch in Helix II and a UUUGG motif at 5' of Helix III were displayed by sibling species B, C and E while these characters were absent in sibling species A and D. However, all five sibling species displayed a CGGC motif at 5' of Helix III. Five different types of loops were observed in all five sibling species. Out of five types of loops, interior and exterior loops were found to be more conserved than multibranching, hairpin and bulge loops. Bulge loops were highest in number. This study concluded that the secondary structures of ITS2 regions are highly conserved in the complex despite the vectorial capacity or geographical locations.

Keywords: *Anopheles culicifacies*, ITS2, sibling species, secondary structures

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