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**Cytochrome Oxidase II region of mitochondrial DNA to differentiate the sibling species of *Anopheles culicifacies* in Sri Lanka**

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*Anopheles culicifacies* is the major vector of malaria. It is a species complex of five sibling species. Two-step multiplex PCR assay based on sequence differences within the CO II region is the latest method for distinguishing all five sibling species present in India. This BCE-PCR (PCR to distinguish sibling species B, C & E) based method distinguishes the sibling species of *An. culicifacies* in Sri Lanka while it gives an aberrant banding pattern for some mosquito DNA samples (n = 3). The CO II region of the mosquitoes (mitotically karyotyped, BCE-PCR based identified and aberrant) was amplified and sequenced. Genbank deposited CO II sequences of *An. culicifacies* from India and Sri Lanka were obtained. Sequences of sibling species B, E and aberrant sample were aligned with Genbank deposited sequences using ClustalW multiple sequence alignment. Sibling species B and E in Sri Lanka were identified using two methods; Mitotic karyotyping and BCE-PCR. There were two sequences for karyotyped sibling species E. BCE-PCR based identified sibling species B and E in Sri Lanka were identical in CO II sequence and one nucleotide position difference from Indian sibling species E. A 1.5% sequence variation in karyotyped sibling species E was observed using BCE-PCR. These variable sequences showed seven point substitutions from Indian species E. The sequence of karyotyped sibling species B was not identical to that of sibling species B in India. Multiple sequence alignment among the sibling species B showed 99.6% sequence similarity. It was found that the sequence similarity with aberrant sample and other sibling species of *An. culicifacies* was only 91.9%. A different banding pattern was shown for sibling species B, E in this study and the aberrant sample in RAPD analysis. CO II is highly variable among sibling species of *An. culicifacies* in Sri Lanka and it is not a suitable marker to differentiate these sibling species. A novel technique or another region has to be focused on for differentiation of sibling species in this species in Sri Lanka. The aberrant sample found from the BCE-PCR may be a variation of sibling species B or E as it could be differentiated by RAPD-PCR.

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