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Identification of sibling species of *Anopheles culicifacies s.l.* present in Sri Lanka using multiplex BCE-PCR in the CO II region of the mitochondrial DNA

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Anopheles culicifacies is the primary vector of malaria in Sri Lanka and Indian subcontinent. Presence of sibling species complexes within a single species causes more complexities as a vector in terms of vector control. Sibling species show differences in vectorial capacity, insecticidal resistance, and host feeding preference. There are five sibling species provisionally designated as A, B, C, D and E in *An. culicifacies* complex. Therefore, precise identification becomes more critical in vector control and disease prevalence. Along with the development and sophistication of technologies, use of ovarian polytene chromosome in semi gravid females, differences in the mitotic Y-chromosome among males of 3rd or early 4th instar larvae and use of cuticular hydrocarbon profiles are now outdated as the methods are inconvenienced. DNA based, PCR associated techniques provide more reliable answers in such studies than any of previous methods. Wild caught mosquitoes collected from malaria endemic areas (Monaragala, Kataragama and Kandy) of Sri Lanka were used for extract DNA. DNA from individual mosquitoes was extracted using a phenol-chloroform extraction method. A BCE-PCR was performed using 50ng of genomic DNA in PCR mixture to differentiate the sibling species on the basis of the variations in the cytochrome oxidase II subunit. PCR products were visualized by agarose gel electrophoresis in a 1.5% agarose gel. BCE- PCR assay accurately identified two sibling species B and E recorded in Sri Lanka. Presence of sibling species B was indicated by a single PCR band of 248bp. Sibling species E showed two bands of 248bp and 178bp. This banding pattern comparable with the previous study of identification of sibling species present in India. Therefore the sibling species B and E present in Sri Lanka can be identified by using this BCE-PCR assay and they are similar to the species recorded from India. In this study we came across with some variations of above observed results. There were some mosquitoes showing a single band between 100bp and 200bp in the same PCR assay. It may be due to a variation of sibling species. Further clarification of these samples is in progress.

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