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A preliminary study on the analysis of genetic variations between sibling species B and E of *Anopheles culicifacies* using RAPD markers

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Anopheles culicifacies Giles *sensu lato* (Diptera; Culicidae) is the predominant vector of malaria in Sri Lanka, India, Pakistan and parts of adjacent countries. Five sibling species of the *An. culicifacies* complex, distinguished by means of different polytene chromosomes banding sequences or metaphase karyotypes correlated with reproductive barriers, have been designated as species A, B, C, D and E. Polytene chromosome banding pattern studies on the *An. culicifacies* complex in Sri Lanka have previously been interpreted as showing the presence of only species B in Sri Lanka. Surveys using a specific DNA probe found no evidence of species A. Recently, metaphase karyotype studies have shown, for the first time, that Y-chromosome variation among natural populations of the *An. culicifacies* complex compatible with the presence of species B and E in Sri Lanka. Precise identification of anopheline mosquitoes is essential for a better understanding of their potential role in malaria transmission, as well as for improving the effectiveness of vector control strategies. Technical difficulties associated with the use of chromosome as population genetic markers, however, had led to a shift toward the use of molecular markers in population genetic studies, especially those that can be detected by PCR. We report here, for the first time, genetic variations of the *An. culicifacies* B and E using Random – Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) Method. Adult mosquito samples, from progenies of *An. culicifacies* sibling species B and E identified by mitotic karyotyping, were used to extract DNA for RAPD analysis. The reproducibility of RAPD analysis was checked by repeating each assay. The primer OPD 18 was tested with three individuals each of sibling species B and E and all three individuals of species E gave a unique band between 1.5 kbp and 2.0 kbp, which was absent in the three tested individuals of species B.

Since only one of 30 primers tested was able to discriminate between sibling species B and E and only a single unique band was observed between the two species by RAPD analysis, our study further confirms that B and E are very closely related. It is essential t perform similar RAPD analyses with other sibling species of the complex using the prime OPD 18 to test whether the band is unique to species E.

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