Studies on identification of *Anopheles culicifacies*, Giles, sibling species complex using DNA based techniques, and associated field investigations

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ABSTRACT

Anopheles culicifacies is the major vector of malaria in Sri Lanka. It is known to exist as a complex of four sibling species A, B, C and D within its geographical distribution. The four species have been found to be different in their pattern of distribution, behaviour, and in malarial vector competency, hence, precise identification of member species of a vector complex is important for accurate epidemiological studies as well as for the successful implementation of control programmes. Three repetitive DNA sequences (Rp36, Rp217 and Rp234) isolated from *An.culicifacies* species B genome were characterized in this study. The cloned DNA sequences were specific only to *An.culicifacies* mosquitoes and were found at a higher copy number in species B and C than in species A, when analyzed by Dot-blot and Southern blot hybridization assays. Hence, these sequences could be used as DNA probes to detect *An.culicifacies* from other mosquitoes, as well as to distinguish species A from B and C, using a 200 fold dilution of a single mosquito/head DNA extract in a dot blot hybridization assay. The sequence Rp217 was further developed as a non-radioactive DNA probe to be used in the field employing a simplified squash blot hybridization assay. The copy number of Rp36 was found to be approximately 50,000 which represents approximately 5% of the *An.culicifacies* species B genome. Rp36 and Rp217 were completely sequenced. The sequence Rp36 is devoid of any internal repeats. However, analysis of Rp217 revealed the presence of two related tandemly repeating core sequences of 13 and 16bp. The partial sequence data of Rp234 revealed the presence of a sequence homologous (94%) to Rp36 in Rp234. Analysis of mosquitoes (n = 1119) collected from various malaria endemic regions in Sri Lanka using the above DNA probes, did not reveal the presence of species A. Studies on vector bionomics were also carried out in Gomadiyagala, a village in the North Western Province of Sri Lanka for a period of 3 years. *An.culicifacies* originating from this area, was established as a forced mating colony at the University of Sri Jayewardenepura and was identified as belonging to species B. The distribution of *An.culicifacies* B was unimodal, peaking around the North-East monsoon / post monsoon periods during November to April.

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