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A preliminary study on the Internal Transcribed Spacer 2 (ITS 2) of Ribosomal DNA (rDNA) of the Anopheles subpictus sibling species complex in Sri Lanka

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Anopheles subpictus is a potential vector of malaria and has been identified as a species complex consisting with four sibling species, which are designated as sibling A, B, C and D. Although there are some morphological differences in the eggs, larvae, pupae and adult of each sibling species, depending on morphological characteristics is not a reliable method to distinguish them. Some morphological characters may depend on environmental conditions, poor conditions of specimens and human errors also can be lead to misidentifications. Slight differences could be observed even among members of individual progenies and some characters are overlapped with those of other sibling species in the complex. Further morphological differences are minute and restricted to a particular stage in the life cycle. Therefore the primary structure of the Internal Transcribed Spacer 2 (ITS2) of rDNA of each sibling species was studied to test for genetic differences among them to find out the possibility off using this region in the genome, to develop DNA based method to distinguish each sibling species as this region has proven to be informative in such studies. ITS2 region rapidly undergoes divergence and therefore is a suitable region to detect differences between closely related species.

Mosquitoes were collected from Kurunegala, Puttalam and Monaragala and the study was carried out from March 2003 to November 2003. The sibling species were distinguished based on morphological variations and DNA was extracted from 3 individuals from each F_1 sibling species. ITS2 region was amplified in PCR from 2/3 individual DNA samples from each sibling species. That region was amplified using a primer pair that have been developed for An. gambiae, which bind to a highly conserved rRNA gene region 5.8s and 28s, flanking the non-coding ITS2 region. Amplified products were partially sequenced using 5.8s primer in chain termination sequencing reactions.

Agarose gel electrophoresis of PCR products indicated that An. subpictus species complex has two unique sets of length variants in the amplified region and it revealed that the amplified region of DNA of sibling A and C are sufficiently different in size (about 650 bp) from those of B and D (about 600 bp) to distinguish them without further manipulation. Partial sequence data were not adequate for discrimination between the sibling species. Further studies have to be carried out to obtain full length sequences of the amplified products and also It is essential to extend this study to a larger sample population of each sibling species to find out the possibility of developing sibling species specific primers.

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