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DNA barcoding of Sri Lankan Anopheline mosquitoes reveals its usefulness in identifying sibling species

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“DNA barcoding” is a powerful tool for species identification and used in studying evolutionary relationships among species. The objective of this study was to characterize *Cytochrome c Oxidase subunit I* (*COI*) and Internal Transcribed Spacer 2 (*ITS2*) sequences of morphologically identified *Anopheles* species collected from two different areas in Sri Lanka and to distinguish sibling species using DNA barcodes.

Anopheles peditaneatus, *An. nigerrimus*, *An. jamseii*, *An. tessellatus*, *An. annularis*, *An. pallidus*, *An. barbirostris*, *An. vagus*, *An. subpictus*, *An. culicifacies* and *An. varuna* were collected from Jaffna in the Northern (N) Province and Wariyapola in the North Western (NW) Province. *COI* sequences (52) of 3,3,7,1,2,6,1,6,8,8, and 6 and, *ITS2* sequences (21) 4,3,6,0, 1,5,0,2,0,0,0 were respectively generated from the PCR positive samples of DNA extracted from the above species (10 individuals from each species). The *COI* sequences showed high genetic variation compared to that of *ITS2*. Two of the morphologically identified *An. peditaneatus* specimens had sequences similar to that of *An. nigerrimus* which show high resemblance in morphological features to *An. peditaneatus*. Based on *COI* sequences, *An. subpictus* samples from N Province were identified as *An. subpictus* B and samples of *An. subpictus* from NW Province had sequences similar to sibling species A. Analysis of *COI* and *ITS2* sequences confirmed the presence of *An. annularis* sibling species A in the N Province. *An. barbirostris* sequence obtained from the N Province was different from the previously described sequences of *An. barbirostris* complex suggesting the existence of a different molecular type. Sequences of morphologically identified other anopheline species confirmed their identity with GenBank entries from South and Southeast Asian countries. However cytologically identified sibling species B and E of *An. culicifacies* could not be separated using *COI* and *ITS2* markers. The present study indicates that DNA barcoding is useful in identifying sibling species and proves that Sri Lankan *Anopheles* species have a close evolutionary relationship with the South and Southeast Asian counterparts.

Keywords: *Anopheles*, *COI*, DNA barcoding, *ITS2*, Sri Lanka

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