

Molecular characterization and identification of members of the *Anopheles subpictus* complex in Sri Lanka

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Abstract

Background: *Anopheles subpictus* sensu lato is a major malaria vector in South and Southeast Asia. Based initially on polytene chromosome inversion polymorphism, and subsequently on morphological characterization, four sibling species A-D were reported from India. The present study uses molecular methods to further characterize and identify sibling species in Sri Lanka. **Methods.** Mosquitoes from Sri Lanka were morphologically identified to species and sequenced for the ribosomal internal transcribed spacer-2 (ITS2) and the mitochondrial cytochrome c oxidase subunit-I (COI) genes. These sequences, together with others from GenBank, were used to construct phylogenetic trees and parsimony haplotype networks and to test for genetic population structure. **Results:** Both ITS2 and COI sequences revealed two divergent clades indicating that the Subpictus complex in Sri Lanka is composed of two genetically distinct species that correspond to species A and species B from India. Phylogenetic analysis showed that species A and species B do not form a monophyletic clade but instead share genetic similarity with *Anopheles vagus* and *Anopheles sunaicus* s.l., respectively. An allele specific identification method based on ITS2 variation was developed for the reliable identification of species A and B in Sri Lanka. **Conclusion:** Further multidisciplinary studies are needed to establish the species status of all chromosomal forms in the Subpictus complex. This study emphasizes the difficulties in using morphological characters for species identification in *An. subpictus* s.l. in Sri Lanka and demonstrates the utility of an allele specific identification method that can be used to characterize the differential bio-ecological traits of species A and B in Sri Lanka.

Author keywords

Anopheles subpictus; *Anopheles sunaicus*; Cytochrome c oxidase subunit-I; ITS2; Malaria; Sibling species; Sri Lanka

Indexed keywords

EMTREE drug terms: cytochrome c oxidase; cytochrome c oxidase 1; internal transcribed spacer 2; unclassified drug

EMTREE medical terms: allele; *Anopheles*; *anopheles subpictus*; *anopheles sunaicus*; *Anopheles vagus*; article; cladistics; female; GenBank; gene sequence; genetic similarity; haplotype; India; mitochondrial gene; molecular phylogeny; morphological trait; nonhuman; nucleotide sequence; parsimony analysis; phylogenetic tree; population genetic structure; sequence alignment; sibling; species identification; Sri Lanka