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Use of *white* gene sequences to further diversify the *Anopheles subpictus* (Diptera: Culicidae) species complex in Sri Lanka

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Vector control is an essential component in elimination of malaria in Sri Lanka in which the proper identification of vector mosquitoes from non-vector mosquitoes is insisted. *Anopheles subpictus*, an important secondary vector of malaria in Sri Lanka, was recently identified as a species complex of two sibling species, *An. subpictus* A and B. The objective of this study was to further investigate and confirm the status of *An. subpictus* species complex in Sri Lanka based on *white* gene sequences. This single copy nuclear gene *white* is used in systematics studies in constructing phylogenetic relationships at species level. This study reports sequence comparisons of the *white* gene in *An. subpictus* in Sri Lanka for the first time. Adult F₁ mosquitoes of *An. subpictus* from different isofemale progenies belonging to five different localities in the island were used as specimens (n=30). A partial region of *white* gene was PCR amplified and sequenced. There were fourteen haplotypes of *white* gene which were submitted to GenBank under accession numbers, KP733780-KP733793. Phylogenetic relationships among the mosquitoes were estimated using PhyML v3.1 and MrBayes v3.2.2 software. The Maximum Likelihood and Bayesian analyses revealed similar tree topologies with two distinct, strongly supported, monophyletic clades which were consistent with previous studies, as Species A clade and Species B clade. However, this putatively conserved single copy nuclear DNA marker *white*, reliably indicated additional polymorphism in terms of further clustering of the two main clades. In this context, bayesian inference gave more robust support on these sub clades with all posterior probabilities exceeding 0.78. Present investigations on *An. subpictus* species complex in Sri Lanka confirm the presence of two distinct sibling species A and B based on *white* gene sequences. Furthermore, the *white* gene phylogeny together with the fourteen *white* single copy nuclear gene haplotypes unravels clues of a hidden diversity that may exist in the species complex. In conclusion, molecular evidence from this study indicates the likelihood of the presence of a yet undiscovered sibling species for *An. subpictus* s.l. in Sri Lanka.

Keywords: *Anopheles subpictus*, sibling species, *white* gene

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