

Multiplex PCR assay for malaria vector *Anopheles minimus* and four related species in the *Myzomyia* Series from Southeast Asia

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Abstract: Mosquitoes (Diptera: Culicidae) of the *Anopheles* (*Cellia*) *Myzomyia* Series are important malaria vectors in Africa, India and Southeast Asia. Among 10 named species of *Myzomyia* known from the Oriental Region, seven form the *An. minimus* group. Even for expert taxonomists, the adults of these species remain difficult to identify morphologically. For technical staff of malaria control programmes, confusion may extend to misidentification of species that are not formally within the *minimus* group. For identification of specimens from Indochina (Cambodia, Laos, Vietnam), we describe a multiplex polymerase chain reaction (PCR) assay, based on rDNA internal transcribed spacer 2 (ITS2) sequences, that employs a cocktail of primers to identify *An. minimus* Theobald sibling species A and C (sensu; Green et al., 1990) and three other species in the *An. minimus* group (*An. aconitus* Dönitz, *An. pampanai* Büttiker & Beales, *An. varuna* Iyengar), as well as *An. jeyporiensis* James, also belonging to the *Myzomyia* Series. As the test is DNA-based, it can be applied to all life stages of these mosquitoes for ecological investigations and vector incrimination studies. This PCR assay is simpler, quicker, cheaper and more readily interpreted than previous assays.

Author Keywords: *An. jeyporiensis*; *An. minimus*; *An. varuna*; *An. pampanai*; *Anopheles aconitus*; Cambodia; Laos; Malaria vectors; *Myzomyia*; Polymerase chain reaction assay; rDNA ITS2; Sibling species; Southeast Asia; Species identification; Vietnam

Index Keywords: ribosome DNA; identification method; mosquito; polymerase chain reaction; taxonomy; animal; animal disease; *Anopheles*; article; chemistry; classification; disease carrier; disease transmission; female; gene; genetics; human; malaria; male; methodology; nucleotide sequence; polymerase chain reaction; sensitivity and specificity; Southeast Asia; species difference; time; Animals; *Anopheles*; Asia, Southeastern; Base Sequence; DNA, Ribosomal; Female; Genes, Insect; Humans; Insect Vectors; Malaria; Male; Polymerase Chain Reaction; Sensitivity and Specificity; Species Specificity; Time Factors; Asia; Eurasia; Southeast Asia; Animalia; *Anopheles aconitus*; *Anopheles jeyporiensis*; *Anopheles minimus*; *Anopheles pampanai*; Culicidae; Diptera; Insecta; *minimus* group; *Myzomyia*; Varuna

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