

**IDENTIFICATION OF *BACTROCERA DORSALIS* AND *BACTROCERA KANDIENSIS*
VIA *COI* BAR-RFLP**

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Bactrocera dorsalis (Oriental fruit fly) and *Bactrocera kandiensis* (an endemic) are two common fruit flies in Sri Lanka. When discriminating these two species using their external morphology, there were many taxonomic impediments over the phenotypic mutualism. Conservation and management practices are thoroughly influenced by their overlapping ranges and limited taxonomic approaches. Thus, we present a consolidated molecular tool for identifying these two fruit fly species. It is based on polymerase chain reaction (PCR) amplification of the partial mitochondrial *cytochrome oxidase subunit I (COI)* gene, followed by restriction fragment length polymorphism (RFLP) assay, with banding patterns resolved *via* agarose gel electrophoresis. The assay was designed using a large training data set obtained from Genbank. These training data were evaluated using an independent test panel of 12 individuals for both species collected from Kandy, Matara, Galle, and Jaffna using pheromone traps (methyl eugenol), for which species assignments were determined *via* phylogenetic comparison to reference sequences. *Drosophila melanogaster* (Common fruit fly) was considered for high-resolution results of the analysis. Theoretical RFLP interpretations and mapping were performed by the NEBcutterV2.0; New England BioLab[®] Inc. software. After refining the interpretive framework, the PCR-RFLP assay under *Mbo*II enzyme treatment was shown to identify the two co-occurring species accurately. The pairwise genetic divergence within species (intraspecific) ranged from 0.00 - 1.80%, and between species (interspecific) ranged from 6.10 - 7.60%. The average interspecific nucleotide divergence was greater than the intraspecific divergence, and it followed the 2% conceptual divergence of discriminating two individuals as two species. Furthermore, the phylogenetic isolation of two distinct clusters into corresponding species groups revealed the utility of *COI* as a barcoding gene. The *COI* Bar-RFLP assay is both cost and time-efficient and will help to improve taxonomic identifications over conventional sequencing methods.

Keywords: *COI*Bar-RFLP, Fruit flies, Identification, Mitochondrial, Phylogeny