

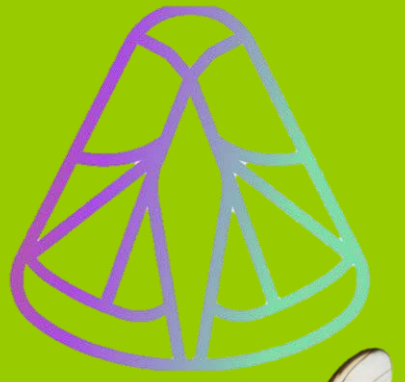
# Identification of the taxonomic status of fruit flies (Diptera: Tephritidae) in Sri Lanka; An initiation to establish a Tephritid barcoding library.

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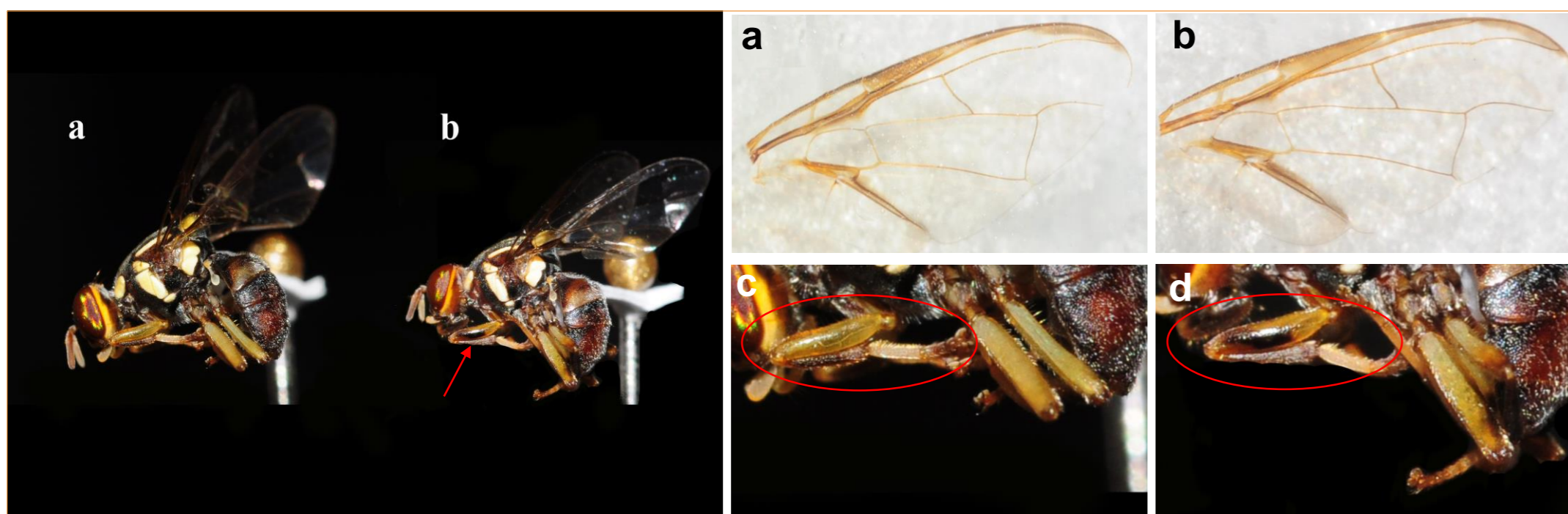
## Introduction:

### Background of the Study:

Fruit flies (Diptera: Tephritidae) are important insect pests in the commercial fruit and vegetable industry worldwide. In the Sri Lankan scenario, the majority of tephritid taxonomic studies relied on morphological features and that modus faces some phenotypic confusions and limitations over the presence of closely related species having cryptic or hybrid speciation. **DNA barcoding concept** was introduced as an alternative solution.

### Objective of the Study:

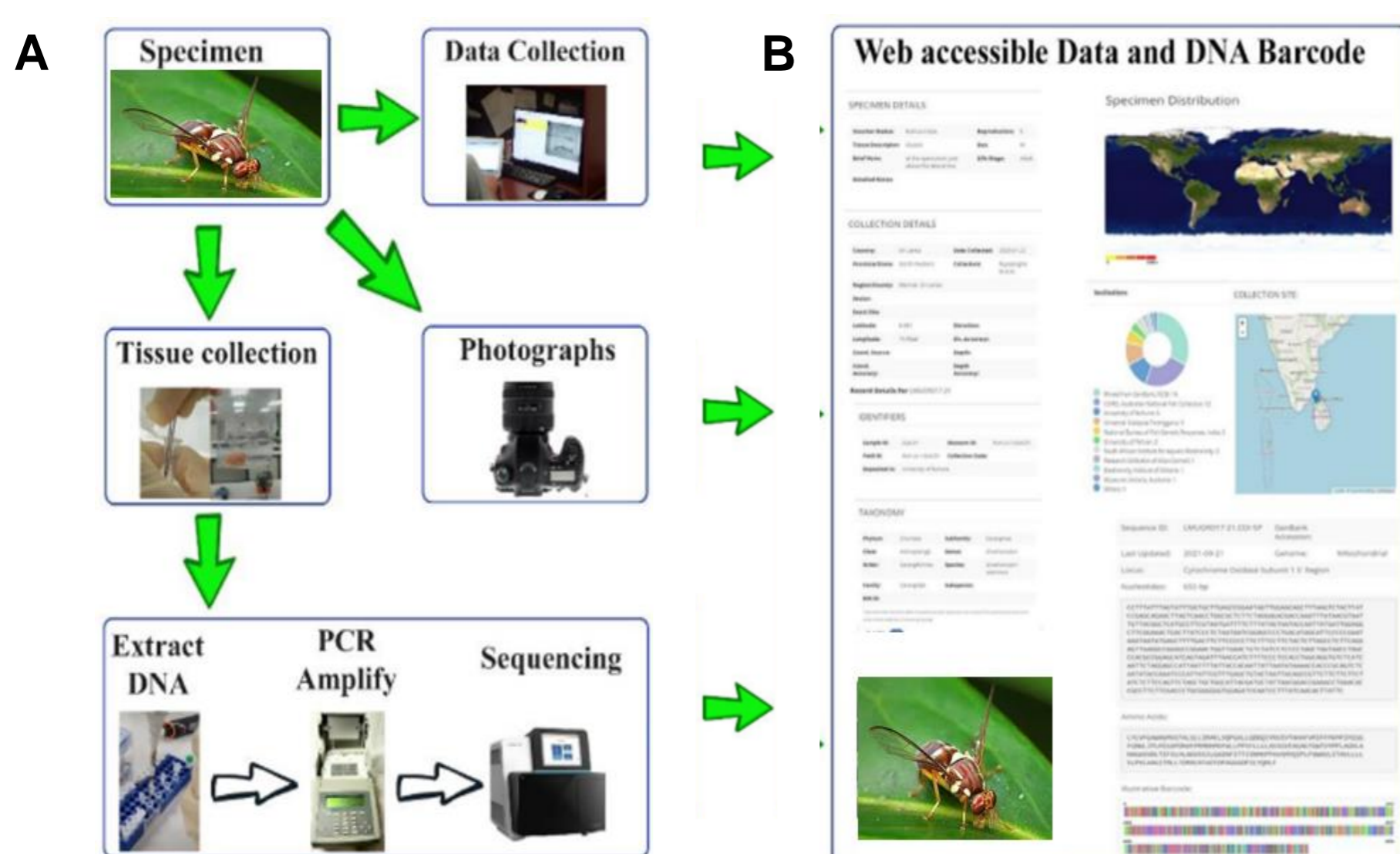
To establish the first Sri Lankan barcode library of tephritid fruit flies as an informative platform to facilitate reference data acquisition, storage and analysis.



**Figure 1.** Lateral view of two cryptic *Bactrocera* fruit fly species in Sri Lanka (Left); a: *B. dorsalis* and *B. kandiensis* (endemic\*). Phenotypic similarities representing taxonomic ambiguities between the two species (Right).

## Materials & Methods:

- As an initiation, mitochondrial (~710bp) COI barcoding gene region of *B. dorsalis* and *B. kandiensis* was PCR amplified (HCO2198/LCO1490 primer pair).
- Sequenced and maximum compatibility was compared with the previously published reference sequences under the blastn algorithm (NCBI).
- Utility of the COI barcoding in the species delimitation was assessed by the Pairwise *P*-distance method and simultaneous phylogenetic tree analysis.
- The reference library, TEPSL: Taxonomic Identification of Fruit flies (Diptera: Tephritidae) in Sri Lanka was established.



**Figure 2.** Overview of the barcode library; TEPSL at the Barcode of Life Database (BOLD). A: Workflow of the establishment; B: Appearance of the specimen and sequence data pages.

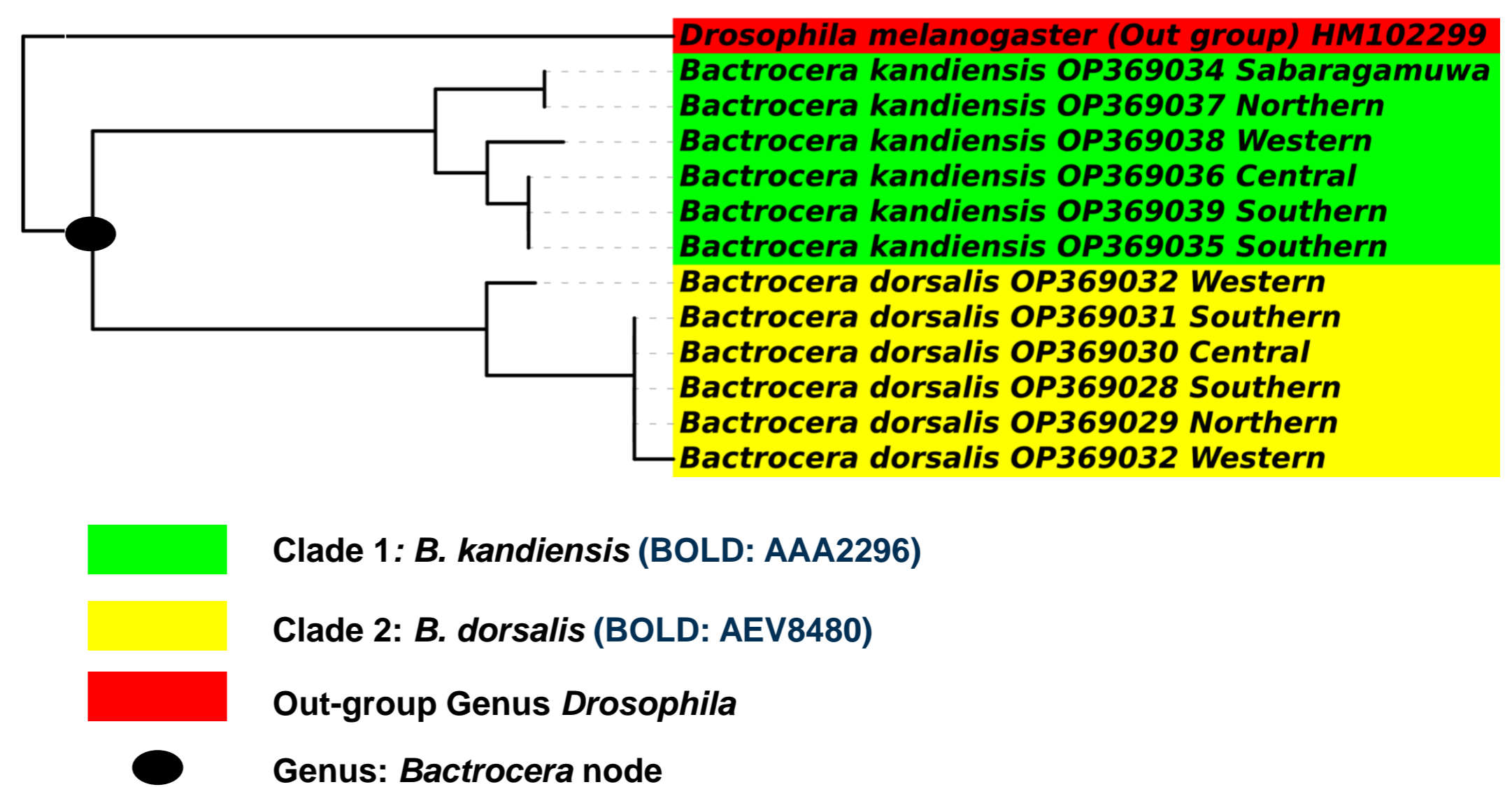
## Results:

- Consensus sequences were compatible (more than 99 %) with the initial phenotypic identification of species (Blastn).
- The average interspecific divergence is greater than the intraspecific divergence and follows the 2 % conceptual divergence (Table 1).
- BIN clustering: two distant BIN clusters: *B. dorsalis* (BOLD: AEV8480), *B. kandiensis* (BOLD: AAA2296).

Table 1. Average nucleotide divergence values (pairwise *P*- distance).

Divergence	<i>P</i> - distance range	marginal
Intraspecific	0.00 % to 1.80 %	< 2 %
Interspecific	6.10 % to 7.60 %	> 2 %

\*2% conceptual divergence of discriminating two individuals as two species.



**Figure 3.** Maximum likelihood tree (bootstrap value 1000); separation of the two *Bactrocera* species into separate clades which is independent from their phenotypic mutualism.

## Conclusions:

- COI divergences are consistent with the reported divergence values for *Bactrocera* species.
- Study results provide an excellent initiative for the Tephritids barcoding library in Sri Lanka.
- This system reflects the “**matching hypothesis**”, DNA sequences are more similar among individuals of the same species compared to that of another species.
- Valid when identifying a species during different developmental stages – adults and larvae etc.
- Further studies will be expanded to place the remaining fruit fly species in this library.

## Acknowledgement:

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## References :

- Costa, et al. *The Barcode of Life Initiative*. 2007;3 (2): 29–40.
- Hebert, et al. *Biological identifications through DNA barcodes*. 2003; 270(1512), 313–321.



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