



# DNA barcoding of Sri Lankan phlebotomine sand flies using cytochrome c oxidase subunit I reveals the presence of cryptic species



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## ABSTRACT

Sri Lanka is known for high diversity of phlebotomine sand flies and prevalence of cutaneous and visceral leishmaniasis; a disease vectored by sand flies. The taxonomy of phlebotomine sand flies is complicated and often the diversity is over/underrated. The current study aims to use the cytochrome c oxidase gene subunit 1 (COI) sequence and formulate a barcode for the sand fly species in Sri Lanka. A total of 70 samples comprising seven species morphologically identified and collected from dry zone districts of Hambantota, Anuradhapura, Vavuniya, Trincomalee and Jaffna were processed. Neighbour-joining (NJ) tree created using the sequences revealed the species identity is compatible with the current morphology based identification. Further the analysis delineated morphologically identified *Se. bailyi*, *Se. babu babu* and *Se. babu insularis* into genetically distinct groups.

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## 1. Introduction

Generally phlebotomine sand flies are considered as vectors for leishmaniasis (Lewis, 1978). Sri Lanka is one of the South East Asian countries that have been affected by leishmaniasis in the recent past. The disease was earlier thought to be exotic with the first autochthonous case of cutaneous leishmaniasis reported in 1992 (Athukorale et al., 1992). Leishmaniasis is endemic in several dry zone localities of Sri Lanka and has become a notifiable disease in the country (Siriwardana et al., 2012). Phlebotomine sand flies are broadly classified into two groups, based on their geographical distribution, as Old World and New World species (Lewis, 1971). This distinction is proposed to be somewhat artificial (Ashford, 2000) and there is no universally accepted taxonomic key for the classification of sand flies. Several proposals have been put forward for the classification of the phlebotomine sand flies as a result of the interest in the role of these phlebotomine sand flies in disease transmission. There were only three species of phlebotomine sand flies known in 1905 (Lewis, 1978), but several new species have been identified during the last century. Over 500 species had been

reported by the 1970s (Lewis, 1971). More than 700 species have been recorded so far (Ilango, 2011). The addition of new species and the taxonomic reassessments of many taxa are being continued.

The members of the genus *Sergentomyia* (Franca and Parrot), the other major Old World genus, mostly prefer to feed on reptiles (Lewis, 1978; Artemev, 1978). However number of studies revealed that *Sergentomyia* spp. harbor different pathogens including *Leishmania* spp. (Geevarghese et al., 2004; Mukherjee et al., 1997; Maroli et al., 1988). *Leishmania tarentolae* have been isolated from *Sergentomyia minuta* in Italy (Maroli et al., 1988). All these suggest a possible role of *Sergentomyia* spp. in the transmission of leishmaniasis.

The studies on Sri Lankan phlebotomine sand flies are fragmentary even though they date back to the mid twentieth century (Carter and Antonipulle, 1949; Lewis, 1978). The diversity of the phlebotomine sand flies is high in the country and the taxonomy based on morphology makes it hard to discriminate species and sibling species, which is more abundant among several species of phlebotomine sand flies. Recent studies from Sri Lanka reveal the presence of rich diversity of phlebotomine sand flies (Gajapathy and Surendran 2011; Gajapathy and Surendran 2012a,b; Ozbek et al., 2011).

DNA barcoding is an approach widely used to assess the species delimitation. This has been used for insect vector species such as mosquitoes and phlebotomine sand flies (Besansky et al., 2003;

Abbreviations: bp, base pairs; COI, cytochrome c oxidase subunit 1; CL, cutaneous leishmaniasis; VL, visceral leishmaniasis.

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Fig. 1. Map of Sri Lanka; sample collection districts are indicated.

Azpurua et al., 2010; Jinbo et al., 2011; Kumar et al., 2012; Polseela et al., 2015). DNA bar coding has been used to answer few critical questions related to taxonomy and vector biology. Considering the recent advances in molecular techniques and the potential use of them in taxonomy, and to devise a simple method for discrimination and identification of sand flies species and species complex, Cytochrome c Oxidase gene subunit I (*COI*) was sequenced and DNA barcode was developed for Sri Lankan phlebotomine sand flies.

## 2. Material and methods

Ethical approval was obtained from Animal Ethics Committee of the University of Jaffna (AERC/2014/07). Approval was also obtained from Department of Wild Life Conservation of Sri Lanka for sand fly collections. Informed consent was obtained from land owners for collecting phlebotomine sand flies on private property.

### 2.1. Sample site selection and sampling efforts

Samples were collected on monthly basis from Jaffna, Anuradhapura, Hambantota, Vavuniya and Trincomalee districts (Fig. 1). The sampling locations were randomly to cover the whole area to make sure that all the phlebotomine sand fly species can be caught and identified. Phlebotomine sand flies were collected from different habitats that include human dwellings, burrow pits and cattle sheds. The CDC light traps, cattle baited nets and mouth aspirators were employed for sand fly collection to collect different species.

Collected samples were brought to the Zoology Laboratory of the University of Jaffna. Collected sand flies were categorized according to the collection site, host (human or cattle), and sex. Phenotypic characters such as colour and size of the flies were recorded and coded accordingly. Phlebotomine sand flies were identified using published keys of Lewis (1978) and Lane (1993). The collected flies were preserved in  $-20^{\circ}\text{C}$  in 70% ethyl alcohol for later analyses.

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