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First report on the presence of morphospecies A and B of *Phlebotomus argentipes sensu lato* (Diptera: Psychodidae) in Sri Lanka – implications for leishmaniasis transmission

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Phlebotomus argentipes Annandale and Brunetti (Diptera : Psychodidae) is the major Indian vector of visceral leishmaniasis (VL), caused by *Leishmania donovani*. However, whilst the geographic range of *P. argentipes* extends from Iran and Afghanistan in the west to Malaysia and Indonesia in the southeast[1], VL is confined to northeastern and southern India, and neighbouring Nepal and Bangladesh[2,3]. Despite being located 32 km from the Indian state of Tamil Nadu, where VL is endemic, autochthonous cases of leishmaniasis were not reported in Sri Lanka until 1992. Prior to this leishmaniasis was detected only in persons who had contracted the disease while working in the middle east[4]. However, since 1992 the incidence has been rising steadily, with 65 cases documented by Siriwardena *et al*[5] in 2003. The parasite was identified in several Sri Lankan patients as *L. donovani* zymodeme MON-37[6]. However, this typically causes VL in India, in Sri Lanka the clinical manifestations are characteristic of cutaneous leishmaniasis.

Variations in feeding preference have been proposed as an explanation of the restricted range of VL transmission relative to vector distribution. VL is considered an anthroponosis and independent of a nonhuman reservoir; thus only anthropophagus *P. argentipes* are susceptible to *L. donovani*. Lewis and Killick-Kendrick[7] reported that *P. argentipes* was almost entirely zoophilic in its feeding behaviour in the southern part of its range (including Sri Lanka), explaining the lack of endemic VL transmission. Subsequently, however, prompted by unpublished reports of human biting[8], Lane *et al*[9] found anthropophagy in *P. argentipes* in central Sri Lanka, with human-biting rates similar to those reported from VL-endemic regions of India; and Surendran *et al* [10] collected flies using human-baited traps in the northern Sri Lanka.

P. argentipes shows geographic variation in several morphological characteristics[8]. The best-described of these is a difference in the length of the *Sensilla chaetica* (previously known as the antennal ascoids on antennal flagellomere II[2,3,7,8]. *Sensilla chaetica* have previously been used to differentiate between sand-fly species[11] . The length of the *s. chaetica* appears to correlate with VL distribution; sand-flies from VL-endemic areas in India have short *s. chaetica* (less than half the length of the flagellomere to which they are attached) whereas sand-flies from non-endemic areas have longer *s. chaetica*. Sympatric populations have also been recorded in India[2,12]. As a result of these and other studies (e.g. variation in cuticular hydrocarbons[13]) it has been suggested that *P. argentipes* exists as a species complex, with two morphological species of different vectorial capacity. *s. chaetica* are likely to

function in detecting mechanical and/or chemical stimuli[3] , triggers that are probably integral to mating. Different sensitivities to such stimuli could very well result in mate-recognition preferences.

Leishmaniasis is considered an emerging vector-borne disease in Sri Lanka and of public health significance. Hence, it is very likely that vector control measures will soon be implemented to reduce transmission. For optimal control it is crucial to understand the distribution of vector species and their contribution to transmission, particularly when the taxon exists as a species complex comprising two or more species with differential vector capacity. Therefore, a pilot study was initiated to investigate the *P. argentipes* population in Sri Lanka.

Sand-fly specimens were collected from a western area of Delft Island (9:32N 79:41E, 35 km off the northern coast of mainland Sri Lanka and 37 km from the coast of Tamil Nadu. Delft is located within the Palk Strait and in the dry zone of Sri Lanka. Pelawatta (6º 45' N 81º 10' E), a locality in the district of Moneragala in the intermediate rainfall zone of southern Sri Lanka (Fig. 1). Collections were made in May 2005. Human landing catches (HLC) and cattle-baited net collections (CBNC) were performed. In Delft both HLC and CBNC, and in Pelawatta only CBNC techniques were employed. The collected sand-flies were identifed using light microscopy (Olympus B × 51, Tokyo) in the laboratory using a standard key[1]. The length of labrum, flagellomere-I, *s. chaeticum* of flagellomere-II (proportion of flagellomere II length) and wing were measured. If the *s. chaeticum* of flagellomere-II was less than half (< 0.5) of the length of flagellomere-II then the species was recorded as morphospecies B and if it was greater than half (> 0.5) the species was recorded as morphospecies A, as mentioned in earlier reports[2,3].

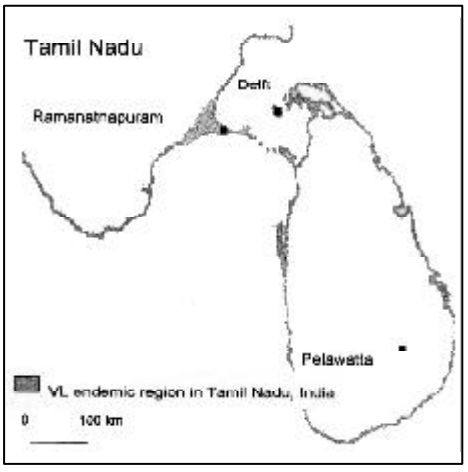

Fig. 1: Geographic closeness between VL endemic region in Tamil Nadu and sample collected localities in Sri Lanka

The sand-flies identifed as *P. argentipes* were used for sibling species identification. Selected morphometric characteristics of these two species are given in Table 1. There were no significant differences noted other than in the *s. chaetica*/flagellomere-II ratio. Forty-one flies from Delft Island and 4 flies from Pelawatta were analysed for the relative length of the *s. chaetica* compared with flagellomere-II. All 4 specimens from Pelawatta had long *s. chaetica* (Fig. 2a) resembling morphospecies A of Ilango[2,3] and all specimens from Delft

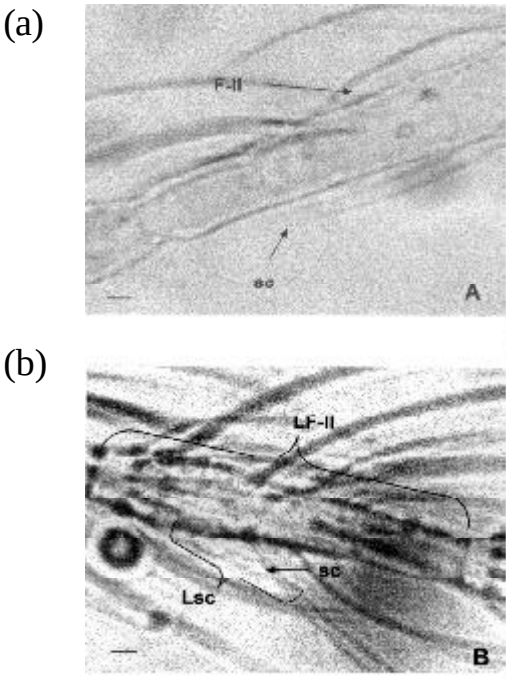

Fig. 2: Relative length of *s. chaeticum* (SC) on flagellomere II (F-II) of *P. argentipes* collected from Pelawatta (a) and Delft (b). (Bar = 0.01 mm). Lsc – length of *S. chaeticum*; LF-II —length of flagellomere II

Table 1. Morphometric characteristics of *P. argentipes* from Delft and Pelawatta in Sri Lanka

Local	Collection technique	Morpho-species	No. of specimen	Ratio of *s. chaeticum* and F-II length	Labrum length	F-I length (mm)	Wing length (mm)
Delft	Human landing catches	B	10	0.42 ± 0.04	0.24 ± 0.04	0.219 ± 0.01	2.12 ± 0.18
	Cattle-baited net collection	B	31	0.42 ± 0.10	0.24 ± 0.01	0.211 ± 0.06	2.15 ± 0.07
Pelawatta	- do-	A	4	0.66 ± 0.12*	0.25 ± 0.01	0.203 ± 0.01	2.18 ± 0.09

F — Flagellomere; *Fisher's exact test p = 0.00000671 between all Delft and Pelawatta samples.

Island had short *s. chaetica* (Fig. 2b) resembling morphospecies B. This distribution was highly significant, Fisher's exact test p=0.00000671 between the Delft and Pelawatta collections, and remained significant when the human-biting collection was excluded, p=0.0000191.

P. argentipes, throughout its geographic range, exhibits its morphological variation in the relative length of *s. chaetica* on the antennal flagellomere-II. This was first described by Lewis and Killick-Kendrick[7] and in greater detail by Lane[8], who found the relative length of *s. chaeticum* to be shorter in the population of VL-endemic regions whilst those elsewhere are longer. In Tamil Nadu, Ilango *et al*[2] reported two morphologically distinct species—morphospecies A with longer *s. chaetica* and morphospecies B with shorter *s. chaetica*. The morphological variation in *s. chaetica*[2] and the variations in cuticular-wax hydrocarbons[13] supported the existence of two genetically distinct populations—*P. argentipes* exists as a species complex. Ilango *et al*[2] further suggested that morphological variation in *s. chaetica* could be used to identify vector species of VL in the *P. argentipes* complex.

In Sri Lanka only specimens with long *s. chaetica* (morphospecies A) have previously been reported[7,8] . This study reports for the first time the identification of both morphospecies in Sri Lanka, with apparent allopatric distribution. However, definitive conclusions on allopatric distribution cannot be drawn from the small sample size from Pelawatta in southern Sri Lanka. More collections with greater geographical distribution need to be examined.

Morphospecies B with shorter *s. chaetica* was the only sibling species identified from Delft Island which lies in Palk Bay in the north of Sri Lanka, close to the southern state of India. It has a resident population of only 5700, but is used by many returning refugee Tamils in transit from India to mainland in northern Sri Lanka. Of the 65 identified cutaneous leishmaniasis cases in Sri Lanka majority are from the northeast (20) and north-central (34) provinces[5] ; only 8 cases originated from southern Sri Lanka. Carter and Antonipulle[14] identified *P. argentipes* as the main species (97% of a large collection) on Delft Island in 1949, and its continued prevalence was reported by Surendran *et al*[10]. Although no case of leishmaniasis had been reported from permanent Delft Island residents, the potential for infection of the vector population on Delft Island with *L. donovani* arriving from India, and transmission from there to Sri Lanka mainland, is relatively high. Morphospecies B is linked with VL-endemicity, although whether this is due to innate differences in vectorial capacity is unknown. It is feasible to speculate that the higher incidence of leishmaniasis in northern Sri Lanka is at least partly due to the prevalence of morphospecies B, whether alone or in sympatry with morphospecies A.

In Delft Island morphospecies B were successfully

collected from both CBNC and from HLC. This indicates that morphospecies B is at least partially anthropophilic, supporting a link with leishmaniasis transmission. Human biting in southern Sri Lanka was not tested in this study, but Lane *et al* [9] found anthropophagy near Kandy, 90 km further northwest of Pelawatta. Leishmaniasis transmission has been documented in the vicinity of Kandy[15]. It will be of interest to determine the incidence of anthropophily in the sibling species.

It is clear that larger and more detailed studies on the prevalence of the two morphospecies of *P. argentipes* throughout Sri Lanka are warranted, including the development of a simpler tool than light microscopy to distinguish between them. This will facilitate the development of an optimal public health measure to control the spread of leishmaniasis before it becomes a major public health problem in Sri Lanka.

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References

1. Lewis DJ. The phlebotomine sand-flies (Diptera: Psychodidae) of the oriental region. *Bull Bri Mus* (Natural History—Entomology) 1978; *37:* 217–343.
2. Ilango K, Srinivasan VDR, Sadanand AV, Lane RP. Phlebotomine sandflies (Diptera: Psychodidae) of Tamil Nadu and Pondicherry, southern India, in relation to visceral leishmaniasis. *Ann Trop Med Parasitol* 1994; *88:* 413–31.
3. Ilango, K. Morphological characteristics of the antennal flagellum and its *Sensilla chaetica* with character displacement in the sandfly *Phlebotomus argentipes* Annandale and Brunetti *sensu lato* (Diptera: Psychodidae). *J Biosci* 2000; *25:* 163–72.
4. Athukorale DN, Seneviratne JKK, Ihalamulla RL, Premaratne UN. Locally acquired cutaneous leishmaniasis in Sri Lanka. *J Trop Med Hyg* 1992; *95:* 432–3.
5. Siriwardena HVYD, Udagedara CU, Karunaweere ND. Clinical features, risk factors and efficacy of cryotherapy in cutaneous leishmaniasis in Sri Lanka. *Cey Med J* 2003; *48:* 10–2.
6. Karunaweera ND, Pratlong F, Siriwardena HVYD, Ihalamulla RL, Dedet JP. Sri Lankan cutaneous leishmaniasis is caused by *Leishmania donovani* zymodeme MON-37. *Trans R Soc Trop Med Hyg* 2003; *97:* 380–1.
7. Lewis DJ, Killick-Kendrick R. Some phlebotomid sandflies and other Diptera of Malaysia and Sri Lanka. *Trans R Soc Trop Med Hyg* 1973; *67:* 4–5.
8. Lane RP. Geographic variation in old world phlebotomine sandflies. In: Service MW editor. *Biosystematics of haematophagous arthropods*. Oxford University Press, 1988; p. 77–90.
9. Lane RP, Pile MM, Amerasinghe FP. Anthropophagy and aggregation behaviour of the sandfly *Phlebotomus argentipes* in Sri Lanka. *Med Vet Entomol* 1990; 4: 79–88.
10. Surendran SN, Karunaratne SHPP, Adams Z, Hemingway J, Hawkes NJ. Molecular and biochemical characterization of a sandfly population from Sri Lanka: evidence for insecticide resistance due to altered esterases and insensitive acetylcholinesterase. *Bull Entomol Res* 2005; *95:* 371–80.
11. Lane RP, Fritz G. The differentiation of the leishmaniasis vector *Phlebotomus papatasi* from the suspected vector *P. bergeroti* (Diptera: Phlebotominae). *Syst Ent* 1986; *11:* 439–45.
12. Lane RP, Rahman SJ. Variation in the ascoids of the sandfly *Phlebotomus argentipes* in a population from Patna, northern India. *J Com Dis* 1980; *124:* 216–8.
13. Kamhawi S, Lane RP, Cameron M, Phillips A, Milligan P, Molyneux DH. The cuticular hydrocarbons of *Phlebotomus argentipes* (Diptera: Phlebotominae) from field populations in northern India and Sri Lanka, and their change with laboratory colonization. *Bull Entomol Res* 1992; *82:* 209–12.
14. Carter HF, Antonipulle P. Observation on sandflies (Phlebotomus) in Delft Island, North Ceylon. *Ann Trop Med Parasitol* 1949; *43:* 62–73.
15. Seneviratne, JKK, Siriwardena S, Ranatunga N, Wijesundera MdeS. Locally acquired cutaneous leishmaniasis from central Sri Lanka: a case report. *Kandy Med J* 1995; *4:* 54–6.

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