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SHORT COMMUNICATION

Report of the presence of *Phlebotomus (Phlebotomus) salehi* Mesghali in Sri Lanka: a potential cutaneous leishmaniasis vector

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Abstract: Cutaneous leishmaniasis (CL) is emerging as a serious public health threat in Sri Lanka and more than 2000 cases have been reported during the past nine years. The parasite responsible for CL has been identified as *Leishmania donovani* zymodeme MON -37. *Phlebotomus (Phlebotomus) salehi* is a proven vector of oriental sore or CL in India. *Phlebotomus (Euphlebotomus) argentipes* sensu lato, which is a vector of *L. donovani* that causes visceral leishmaniasis (VL) in India, is suspected to be a vector of CL in Sri Lanka. But the vector of CL in Sri lanka is yet to be determined. Cattle- baited collection and hand picking techniques were used for sandfly collection in three CL endemic localities, namely, Vavuniya, Anuradhapura and Trincomalee. Female *Ph. (Phl.) salehi* flies from the collections were identified based on morphological and published morphometric characters. This is the first record of the presence of *Ph. (Phl.) salehi* in the country.

Keywords: Leishmaniasis, *Phlebotomus (Phlebotomus) salehi*, sandfly, Sri Lanka, vector.

INTRODUCTION

Leishmaniasis is a global health threat affecting 88 countries in tropical and warm temperate regions (Ilango, 2011). Sandflies (Diptera: Psychodidae: Phlebotominae) are the vectors of leishmaniasis and the taxonomy of the sandflies are being updated regularly. In recent time, new species have been reported and the taxonomy of previously reported taxa has been reassessed. Among the 700 species of sandflies reported so far in the world, more than 30 are proven to be the vectors for leishmaniasis (Ilango, 2011).

Leishmaniasis is a major public health concern in Sri Lanka since the first report of the autochthonous case of cutaneous leishmaniasis (CL) in 1992 (Athukorale *et al*., 1992). Since then the number of cases has been increased dramatically. More than 2000 cases have been reported from the country in the last nine years (Siriwardana *et al*., 2010). The first case of visceral leishmaniasis (VL) was identified from the North Central Province of the country in 2006 (Abeygunasekara *et al*., 2007).

Leishmania donovani zymodeme MON -37 was identified as the causative agent for CL in Sri Lanka (Karunaweera *et al*., 2003). The vector for the disease however, is yet to be confirmed apart from a recent study showing the vectorial capacity of *Phlebotomus (Euphlebotomus) argentipes* sensu lato (Senanayake *et al*., 2011). This species harbours a closely related strain of *L. donovani*, which causes VL in India. This is the proven vector for VL in many parts of India including the Southern State of India (Ilango, 2011), which lies in close proximity to Sri Lanka. In addition to this vector, other species such as *Phlebotomus papatasi* Scopoli and *Ph. salehi* Mesghali are proven vectors of CL or oriental sore in India, which is caused by *L. major*.

As leishmaniasis is emerging as a major public health threat in Sri Lanka (Navaratna *et al.*, 2007), proper identification of the sandfly vector species is of paramount importance. With this background, a survey was carried out at some CL prevailing localities in Sri Lanka to record the presence of any potential CL vectors.

METHODS AND MATERIALS

Phlebotomine sandflies were collected using cattle-baited collection (CBC) and hand picking techniques. Samplings were done using a mouth aspirator from

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1800 - 0600 h, during the period of May 2010 to February 2011 at different sites in three districts, namely, Vavuniya (Chettikulam, Pambaimadu, Maniarkulam villages), Trincomalee (Nilaveli, Padavi Siripura) and Anuradhapura (Padaviya, Anuradhapura) Districts located in the Dry Zone of Sri Lanka (Figure 1). Two samplings were done in each site in the Trincomalee District and five samplings each from the sites in the other districts. Baits were setup in selected locations (with accessibility and convenience in setting up the bait) after monitoring for the presence of sandfly.

Sandflies were preserved using conventional techniques (Lewis, 1978; Ko *et al*., 2008). Microscopic analysis was done with Kyowa Model SE- L (Japan), Olympus Model BX 51 (Japan) and a monocular light microscope (Kyowa, Japan) equipped with a moving vernier scale and an ocular micrometer. Photographs of the taxonomic features were taken with a Nikon Coolpix Digital Camera (10 megapixels).

Morphometric and meristic characters were analysed and identification of species was done with the published taxonomic keys (Lewis, 1978; Kakarsulemankhel, 2003; Lane, 1993). Morphological features were compared with the existing literature from the South-East Asian region (Kakarsulemankhel, 2003) (Table1).

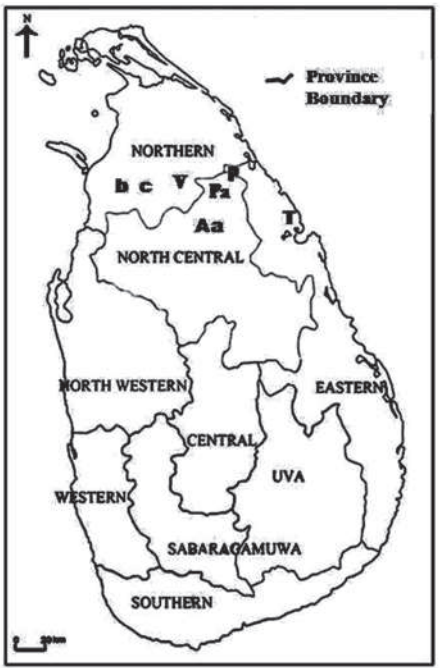

Figure 1: Sample collection sites (letters A, T and V denote districts of Anuradhapura, Trincomalee and Vavuniya, respectively and letters Pa, P, a, b and c denote collection sites Padavi Siripura, Padaviya, Anuradhapura, Maniarkulam and Chettikulam, respectively)

Table 1: Comparison of the morphological characters used in the identification of female *Phlebotomus (Phlebotomus) salehi* Mesghali (all measurements are in µm)

Character	Kakarsulemankhel (2003) in Pakistan	Present study in Sri Lanka
Wing length	1600−1680	1700−1750
Wing breadth	464−544	480
Wing index (R_2/R_{2+3})	1.56−1.6	1.875
AIII length	180−210	180
AIII/Labrum	1.0	0.86
AIII/AIV+AV	AIII<=AIV+AV	1.17 AIII>AIV+AV
Labrum length	260−300	210−216
AaIV/AIV	0.459	0.7
Cibarium	5−6 small denticles scattered and few lateral teeth	Scattered 6 small denticles on anterior without pigment patch
Pharynx length/breadth	2.61−2.64	3.0
Spermatheca	Conical , 7−8 segmented with larger last segment, narrow ducts all over the place	8 segments with large last segment and tip with projections. Ducts are large and narrow. Chitinous furca is present

RESULTS

Only 02 female *Ph. (Phl.) salehi* Mesghali flies were found in the collection in Maniarkulam village in Vavuniya along with *Phlebotomus (Euphlebotomus) argentipes* Annandale and Brunette species complex (Gajapathy *et al*., 2011) and 14 species belong to *Sergentomyia* Franca and Parrot (Table 2) (Gajapathy & Surendran, 2011a,b). *Phlebotomus* Rondani & Berte was distinguished, based on the absence of prominent cibarial teeth and pigment patch and the presence of erect hairs in hind abdominal sockets. They were distinguished as subgenus *Phlebotomus* Rondani & Berte by the presence of ridges and scales in the pharyngeal armature and equally thick spermathecal segments (especially the last spermathecal segment) (Lewis, 1978; Lane, 1993; Kakarsulemankhel, 2003). Taxonomic characters such as the relative length of third antennal segment (AIII) compared with that of labrum (× 0.8) and AIV+V (>×1.0); presence of small denticles in cibarium; number of spermathecal segments (7−8) with broader anterior segment, were used in the identification of *Ph. (Phl.) salehi* Mesghali (Kakarsulemankhel, 2003).

Table 2: The list of identified sandfly species during 2010−11 from the districts of Anuradhapura, Vavuniya, Trincomalee and Jaffna

Genus	Subgenus	Species
Phlebotomus	*Euphlebotomus*	*argentipes* Annandalae ‡
Phlebotomus	*Euphlebotomus*	*annandalei* Sinton ‡
Phlebotomus	*Euphlebotomus*	*glaucus* Mitra & Roy ‡
Phlebotomus	*Phlebotomus*	*salehi* Mesghali
Sergentomyia	*Parrotomyia*	*barraudi* Sinton ¥
Sergentomyia	*Parrotomyia*	*babu insularis* Theodor,
Sergentomyia	*Parrotomyia*	*rudnicki* Lewis ¥
Sergentomyia	*Parrotomyia*	*baghdadis* Adler & Theodor
Sergentomyia	*Parrotomyia*	*species* A Kaul, Dhanda & Modi ¥
Sergentomyia	*Parrotomyia*	*modii* Lewis ¥
Sergentomyia	*Parrotomyia*	*grekovi* Khodukin ¥
Sergentomyia	*Parrotomyia*	*bailyi* Sinton ¥
Sergentomyia	*Sergentomyia*	*punjabiensis* Sinton ±
Sergentomyia	*Sergentomyia*	*pondicherriensis* Srinivasan & Jambulingam ±
Sergentomyia	*Sergentomyia*	*dentata* Sinton ±
Sergentomyia	*Grassomyia*	*dreyfussi turkestanica* Theodor & Mesghali ¥
Sergentomyia	*Grassomyia*	*indica Theodor* ¥
Sergentomyia	*Neophlebotomus*	*malayae* Lewis ¥

‡ Gajapathy *et al*., 2011
¥ Gajapathy and Surendran, 2011b
± Gajapathy and Surendran, 2011a

The morphological features of the identified *Ph. (Phl.) salehi* Mesghali flies were as follows: thorax + abdomen length 1830−1850 µm; thorax + abdomen breadth 260−265 µm; head length 380−390 µm; head breadth 340−350 µm; eye length 210 µm; eye breadth 140 µm; eye separation 90 µm; wing length 1700−1750 µm; wing breadth 480 µm; halter length 17 µm.

Fore femur: fore tibia: fore basi tarsi 6.5:19:15; mid femur: mid tibia: mid basi tarsi 7:19:16; hind femur: hind tibia: hind basi tarsi 8:18:20.

Mouth parts: The labrum is a chitinized pointed structure with three apical sensilla. The hypopharynx is with marginal teeth and the mandible is with serrated teeth at the edge. Maxillary blade has visible teeth with 22 ventral and 11 laterals. Cibarium is with small denticles scattered from without a pigment patch. Pharynx is broader at base, with network like ridges and scales at the anterior. Pharynx is three times its breadth.

Labrum length 210−216 µm; maxillary palp length 4200 µm; palp formula (1,4,2,3,5) with a ratio of 3:9:14:5.5:10.5; proboscis length 220 µm; third antennal segment (AIII) 180 µm; AIV 78 µm; AV 76 µm; antennal ascoid/ sensilla chaetica on third antennal segment (AaIII) 50 µm; AaIV 54 µm; AaV 52 µm.

Wing venation: First radial vein (R1) 690−700 µm; R2 450−456 µm; R3 560−580 µm; R 2+3 240 µm; R1 overlap 24 µm.

Genitalia: Segmented spermatheca has 8 segments. The last segment is broader and with a small projection. The individual ducts are fused to form a common duct and then lead into the genital atrium. Chitinous furca present. Spermatheca length is 30−32 µm; breadth is 16 µm at the broadest part.

DISCUSSION AND CONCLUSION

This is the first report of the presence of *Phlebotomus (Phlebotomus) salehi* Mesghali from Sri Lanka, a species in the subgenus *Phlebotomus*. The other two species reported in the subgenus, *Ph. (Phl.) papatasi* Scopoli and *Ph. (Phl.) bergeroti* Parrot have not been reported from Sri Lanka. The flies were collected from Maniarkulam village of Vavuniya District, which is considered as an area with the risk of CL (Siriwardana *et al*., 2010).

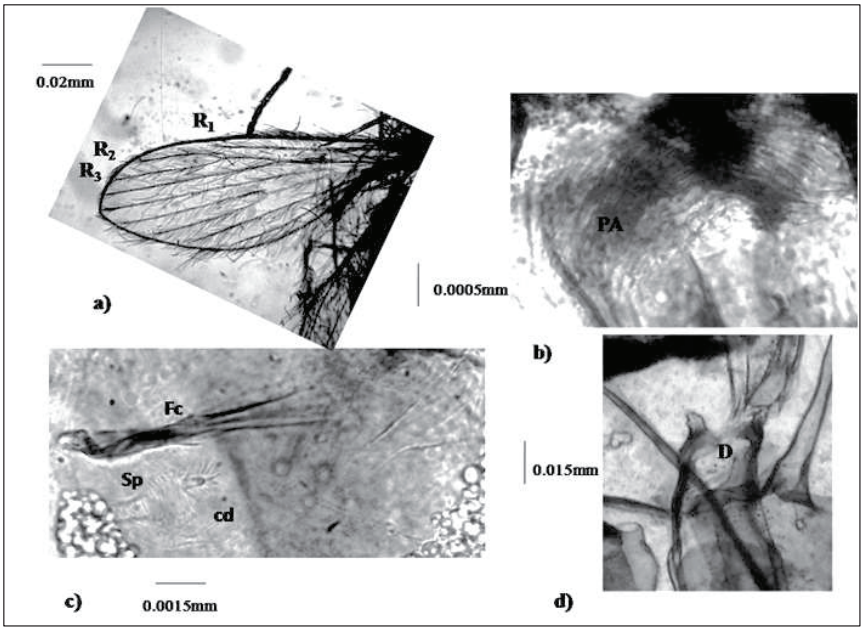

Figure 2: Images of female *Phlebotomus (Phlebotomus) salehi* Mesghali. a) Wing showing radial (R_1, R_2 and R_3) veins; b) pharyngeal armature (PA); c) spermatheca (Sp), furca (Fc) and common duct (cd); d) cibarium and denticles in scatter (D)

The morphology of the Sri Lankan flies is similar to that of the flies in Pakistan, described by Kakarsulemankhel (2003), except for some minor deviations. The flies in Sri Lanka have comparatively larger AIII and higher wing index (R2/R 2+3) values. The antennal ascoid (s*ensilla chaetica*) of the AIV is also comparatively larger.

The presence of *Ph. (Phl.) salehi* Mesghali in Sri Lanka especially in CL prevailing locality, necessitates a detailed analysis on the species richness and diversity of sandflies and their role in leishmaniasis transmission in the country. Presence of more than one insect vector species for a pathogen is a well established concept in epidemiology. A detailed entomological survey along with vector incrimination is warranted to establish the epidemiology of leishmaniasis in Sri Lanka.

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