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Morphometric and meristic characterization of *Phlebotomus argentipes* species complex in northern Sri Lanka: evidence for the presence of potential leishmaniasis vectors in the country

Gajapathy, K.*, Jude, P.J. and Surendran, S.N.
Department of Zoology, Faculty of Science, University of Jaffna, Jaffna 40000
* Corresponding author email: gajapathyk@jfn.ac.lk or gayan156@gmail.com
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Abstract. The transmission of cutaneous leishmaniasis (CL) is of public health concern in Sri Lanka. The parasite *Leishmania donovani* is reported to be the causative agent for CL in Sri Lanka. However there is no report on the vector of CL in the country. *Phlebotomus argentipes* sensu lato is the well known vector of *L. donovani* which causes visceral leishmaniasis (VL) in the nearby South India. The taxon *Ph. argentipes* previously reported to occur as a species complex comprising of two morphospecies namely A and B. The taxonomy of the Argentipes complex was reassessed recently and reported to have three species viz. *Phlebotomus glaucus*, *Ph. argentipes* sensu stricto and *Ph. annandalei*. A study was carried out in Jaffna mainland, where three CL patients have been recorded, and two associated islands in northern Sri Lanka to record the presence of the members of the Argentipes complex. Sandflies were collected using human landing and cattle baited collections. Collected samples were analyzed based on reported morphometric and meristic characteristics. The study revealed the presence of all three members of the complex in which *Ph. glaucus* and *Ph. argentipes* s.s. are reported for the first time in Sri Lanka.

INTRODUCTION

Leishmaniasis was considered as an exotic disease for decades in Sri Lanka (Navaratna *et al.*, 2007) until the first autochthonous case of cutaneous leishmaniasis (CL) which was identified in 1992 (Athukoralle *et al.*, 1992). Since then, the number of cases identified has increased dramatically. More than 400 cases were identified for CL from 2001 to 2005 (Siriwardana *et al.*, 2007). Around 2000 cases have been reported in the last eight years from different parts of the country including the northern Jaffna Peninsula where three CL patients have been recorded (Siriwardana *et al.*, 2010). The first case of visceral leishmaniasis (VL) was identified from North Central Province of the country in 2006 (Abeygunasekara *et al.*, 2007). *Leishmania donovani* zymodeme MON -37 is identified as the parasite responsible for CL in Sri Lanka (Karunaweera *et al.*, 2003). However in India, *L. donovani* is associated with VL and the vector is *Phlebotomus (Euphlebotomus) argentipes* Annandale & Brunette, 1908 (Diptera, Psychodidae) (Ilango, 2000).

Phlebotomus argentipes s.l. (sensu lato) is suspected as a potential vector of CL in Sri Lanka. It shows geographic variation which is associated with several morphological characteristics (Lane, 1988). The best described of these is the difference in the length of the sensilla chaetica (s. chaetica, previously known as the antennal ascoids; Ilango, 2000) on antennal flagellomere II (Ilango *et al.*, 1994; Ilango, 2000). The morphology of s. chaetica has previously been used to differentiate sandfly species (Lane & Fritz, 1986). The length of the s. chaetica appears to correlate with VL

distribution; sandflies from VL-endemic areas in India have short s. chaetica (less than half the length of the flagellomere which they are attached to) whereas sandflies from non-endemic areas have longer s. chaetica. Sympatric populations have also been recorded in India (Ilango *et al*., 1994). As a result of this and other studies (such as variation in cuticular hydrocarbons, Kamhawi *et al*., 1992), it has been suggested that *Ph. argentipes* s.l. exists as a species complex.

Previously the species complex was described to be composed of two morphospecies, viz. A and B based on the length ratio of s. chaetica and second antennal flagellomere (Ilango, 2000). The populations with long s. chaetica in the second antennal flagellomere (more than 50% of the length of the flagellomere II) were referred as morphospecies A and those with short s. chaetica (less than 50% of the length of the flagellomere II) as morphospecies B (Ilango, 2000). The presence of morphospecies B is associated with CL endemic areas in India (Ilango, 2000).

Although presence of *Ph. argentipes* s.l. was reported for many years in Sri Lanka (Carter & Antonipulle, 1949; Lewis, 1978; Lane *et al*., 1990), the absence of any form of leishmaniasis was related to the zoophagic nature of the sandfly populations in the country. However Surendran *et al.* (2005a) reported the presence of anthropophagic and zoophagic *Ph. argentipes* s.l. populations on Delft island of northern Sri Lanka and later reported the presence of both morphospecies A and B in the country (Surendran *et al*., 2005b). However, the taxonomy of *Ph. argentipes* species complex was reassessed recently (Ilango, 2010) and reported to be composed of three members namely *Ph. argentipes* sensu stricto Annandale & Brunette 1908, *Phlebotomus glaucus* Mitra & Roy 1953 and *Phlebotomus annandalei* Sinton 1923 after considering the relative length of s. chaetica compared to that of the antennal flagellomere in the second antennal flagellomere, wing index, wing overlap and the length of the common spermathecal duct for females and the gonocoxite and gonostyle ratio for males based on statistical analysis.

Leishmaniasis is emerging as an important vector-borne disease in Sri Lanka (Nawaratna *et al.*, 2007). One of the options to control the disease transmission is to implement an optimum vector control measure. The perfect identification of vectors and establishment of their bionomics are important for a successful vector control programme when the vector taxon exists as a species complex. Presence of two or more sibling species in a particular area would conceal the real transmission pattern of the disease and lead to inaccurate assessment in the vector control programme.

With this background and considering the recent taxonomic reassessment, the morphometric characters of the *Ph. argentipes* sandflies from northern Sri Lanka were studied and the results are reported in this article.

MATERIALS AND METHODS

Sandfly collection

Phlebotomine sandflies were collected using human landings catches (HLC) and cattle baited collection (CBC) techniques. Samplings were done with a mouth aspirator from 1900 – 0100 hrs during the period between January and April 2010 from three locations namely Delft island (9 º 28' 60 N 79º 40' 0" E), Pungudutheevu (9º 34' 60" N, 79º 47' 60" E) and Chunnakam (9º 45' 0 N, 80º 1' 0" E) in the northern province of Sri Lanka (Fig. 1). Samples were collected on a monthly basis and collected samples were brought to Zoology Laboratory of the University of Jaffna. Sandflies 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 sandflies were identified using published keys of Lewis (1978) and Lane (1993). The collected flies were preserved in 70% ethyl alcohol for later analysis.

Morphometric and meristic analysis

Whole specimens were temporarily mounted in distilled water to study the morphometric and meristic characteristics. The flies were

Figure 1. Collection sites in northern Sri Lanka (D – Delft, P – Pungudutheevu, C – Chunnakam)

observed under a stereo microscope (Kyowa Model SE- L, Japan) and a monocular light microscope (Kyowa, Japan) equipped with a moving Vernier scale and an ocular micrometer. Permanent mounting of the whole specimen were done for some flies in Canada balsam after alcohol treatment (Ko, 2008).

Photographs of the morphometric structures like the wing veins, genitalia, mouth parts and appendages were taken with the Nikon Coolpix Digital Camera fixed to the microscopes. Camera Lucida was used to draw images of the structures like sensilla chaetica in some specimen, and mainly for the assessment of the teeth of the maxilla and for the dimension of spermathecae. The measurement of the structures such as wing (length and maximum width), wing venation (Radial (R) R_2, R_3, R_{2+3} and R Overlap), length of halter, lengths of head, eye, labium, maxillary palp, antennal flagellomere (AF)-I,II and III, s. chaetica on AFII , thorax, femur, tibial and basitarsal segments of each appendages, abdomen, coxa and style (clasper), paramere, aedegal sheath, genital pump and aedegal filament was done with the ocular micrometer and the number of teeth found in both ventral and lateral sides of the maxillae were counted under the light microscope equipped with a camera (Olympus Model BX 51, Japan).

Analysis of data

The wing index (R_2/R_{2+3}), wing overlap (R_1 overlap/ R_2) and the ratios between wing length and width, second s. chaetica and AFII, (AFIII+AFIII) and AFI, gonocoxite and gonostyle, genital pump and aedegal filament, length of head and eye, length of appendage segments of each thoracic appendage, and maxillary palp segments were calculated and recorded. Species were recognized primarily according to published keys (Ilango, 2010) and their characteristics were then analyzed separately. The selected morphometric parameters were analyzed using Student's *t*-test (Microsoft Office Excel 2003, USA) and compared with the published description for the members of the *Argentipes* species complex (Ilango, 2010). Principal component analysis (PCA) was performed for males and females separately. The log transformed values of the measured morphometric characters were used. Covariance matrix based PCA was performed using Minitab software (Minitab Release 14.12.0). Eigen values and proportions (variability) were obtained for the first 5 principal components.

Identification of members of the Argentipes complex

Members of the Argentipes complex were identified based on the descriptions of Ilango (2010). Female flies were identified according to the ratio of SCII/AFII: for *Ph. annandalei*, *Ph. argentipes* s.s., and *Ph. glaucus* the ratio is <0.4, 0.4< >0.5, and >0.7 respectively (Fig. 2). Male flies were identified based on the ratio between gonocoxite and gonostyle: for *Ph. glaucus*, *Ph. argentipes* s.s. and *Ph. annandalei*, the ratio is <1.5, 1.65< >1.75 and >1.75 respectively.

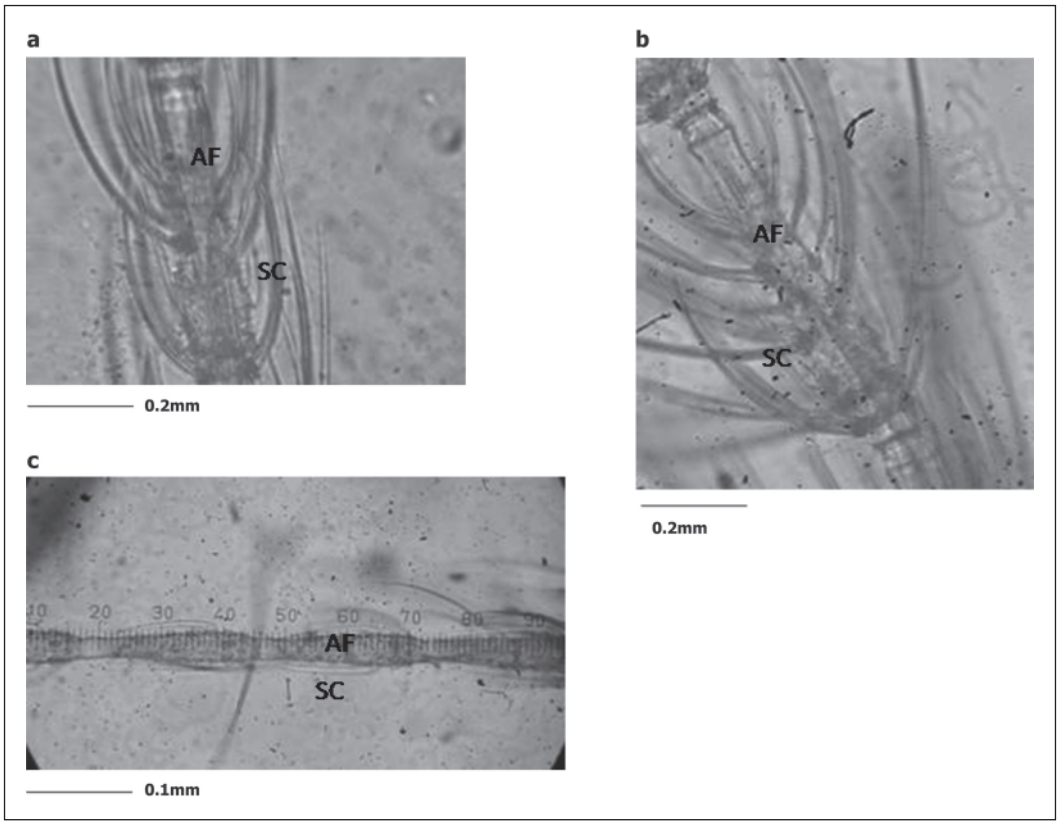

Figure 2. The sensilla chaetica length corresponding to the length of AFII in, a) *Phlebotomus annandalei* b) *Ph. argentipes* s.s. and c) *Ph. glaucus*

RESULTS AND DISCUSSION

A total of 120 flies were collected during the study period. All collected flies were identified as Phlebotomines (*EuPhlebotomus*) and as *Ph. argentipes* s.l.. The collected flies were identified to species level based on the morphometric diagnostic features of male and female flies. In addition, other morphometric characters listed above were also compared with the description of Ilango (2010) for the members of the Argentipes complex. The analysis revealed the presence of species *Ph. argentipes* s.s., *Ph. annandalei* and *Ph. glaucus* in all three collected localities (Table 1).

Morphometric analysis of specific features for the identified sibling species showed variations in statistical analysis (Table 2). While the student's two-tailed *t*- test analysis of wing index, wing overlap and SCII/AFII analysis for males of *Ph. argentipes* s.s. and *Ph. annandalei* showed insignificant difference (p values of 0.679, 0.139 and 0.323 respectively) *Ph. annandalei* and *Ph. glaucus* showed significant difference between them in the SCII/AFII (p = 0.043) and less significance in wing over lap (p= 0.335) and wing index (p=0.780). *Phlebotomus argentipes* s.s. and *Ph. glaucus* showed significant difference between them in the SCII/AFII (p=0.059) and less significant in wing index (p= 0.221) and wing overlap (p= 0.327) (Table 2). As only two female flies were identified as *Ph. annandalei* during the study period, similar analysis could not be performed to show the usefulness of morphometric characteristics, which were applied for male flies.

However females of *Ph. glaucus* showed significant difference in wing index (p= 0.05) and SCII/AFII (p= 0.00) and insignificant difference in wing overlap (p= 0.119) in comparison with samples identified as *Ph.*

Table 1. Description of collected sandflies from the study sites

Locality	*Ph. annandalei*				*Ph.argentipes* s.s.				*Ph. glaucus*			
	male	female	CBC	HLC	male	female	CBC	HLC	male	female	CBC	HLC
Delft	19	02	09	12	12	06	12	06	09	17	26	00
Pungudutheevu	16	01	13	04	11	00	09	02	00	01	01	00
Chunnakam	03	00	03	00	06	00	06	00	08	09	17	00

HLC – human landing catches
CBC – cattle baited collections

Table 2. Results of the statistical analysis of selected morphometric parameters applicable to the collected members of the Argentipes complex

Characters	*Ph. annandalei*			*Ph. argentipes* s.s.			*Ph. glaucus*		
	Mean	st. dev.	P value vs *P. argentipes* s.s.	vs *P. glaucus*	Mean	st.dev.	P value vs *P. glaucus*	Mean	st.dev.
Length of eye/head (male)	0.5406	0.0525	0.6150	0.4430	0.5716	0.0916	0.9890	0.5853	0.0576
Length of eye/head (female)	0.4619	0.0217	0.1540	0.0070	0.4958	0.0409	0.1390	0.5300	0.0734
Lengthof AFI/(AFII+AFIII) (male)	1.1647	0.0437	0.1550	0.5000	1.2029	0.2272	0.1180	1.1923	0.0846
Lengthof AFI/(AFII+AFIII) (female)	1.0879	0.1411	0.2040	0.3030	1.2408	0.0256	0.0830	1.2028	0.0973
Length of labium/head (male)	0.5278	0.0360	0.3360	0.7270	0.5359	0.1333	0.4420	0.5469	0.0616
Length of labium/head (female)	0.6355	0.0484	0.9380	0.6330	0.6388	0.0688	0.5690	0.6161	0.1433
Length of maxilla/head (male)	1.2472	0.0752	0.1120	0.5590	1.2708	0.1909	0.4080	1.2878	0.1940
Length of maxilla/head (female)	1.2227	0.0323	0.0860	0.0270	1.2881	0.0632	0.7910	1.2968	0.0969
Length of coxa/style (male)	1.8236	0.0572	0.0000	0.0000	1.6578	0.0536	0.0000	1.3911	0.1035
Genital pump/genitalfilament (male)	0.4006	0.0385	0.2060	0.3020	0.4066	0.0438	0.7190	0.4048	0.0358
Paramere/coxa (male)	0.7306	0.0469	0.8670	0.0110	0.7520	0.1270	0.0260	0.8500	0.1160
Wing index (male)	1.7864	0.2076	0.1390	0.7800	2.0590	1.3010	0.2210	1.7727	0.3557
Wing index (female)	1.9300	–	–	–	2.0350	0.1910	0.0500	1.8167	0.2437
Wing overlap (male)	0.1312	0.0594	0.3230	0.3350	0.1821	0.1492	0.3270	0.1726	0.0574
Wing overlap (female)	0.1670	–	–	–	0.1710	0.0580	0.1190	0.1400	0.0420
SCII/AFII (male)	0.4490	0.0538	0.6790	0.0430	0.4493	0.0513	0.0590	0.4969	0.0685
SCII/AFII (female)	0.4000	0.0424	–	–	0.4483	0.0313	0.0000	0.6004	0.0626

argentipes s.s.. Interestingly *Ph. glaucus* differ significantly in the ratio between eye and head length with that of the *Ph. argentipes* s.s. (p= 0.018). According to Ilango (2010) the SCII/AFII ratio is > 0.7 for *Ph. glaucus*. This description is similar to the previously reported morphospecies A of the Argentipes complex in which s. chaetica is more than half the length of the second antennal flagellomere (Ilango *et al.*, 1994; Ilango, 2000). Further, Ilango (2010) described female *Ph. glaucus* to have wing index < 0.2 and the wing over lap < 0.16 (along with SCII/AFII > 0.7). Considering the above descriptions, the present study suggests that it is appropriate to use the SCII/AFII as > 0.5 for *Ph. glaucus*.

In addition to the ratio between the gonocoxite and gonostyle lengths, the ratio between paramere lobe and gonocoxite could be used to distinguish the males of *Ph. glaucus* from *Ph. argentipes* s.s. (p = 0.05) and *Ph. annandalei* (p= 0.011). However the applicability of the ratio between paramere lobe and gonocoxite to distinguish *Ph. annandalei* from *Ph. argentipes* s.s. was insignificant (p= 0.867). Similarly, the SCII/AFII ratio could also be used to differentiate the *Ph. glaucus* from *Ph. argentipes* s.s. (p= 0.059) and *Ph. annandalei* (p= 0.043) (Table 2).

Phlebotomus glaucus had on average of 9 lateral and 17 ventral teeth. However both *Ph .argentipes* s.s. and *Ph. annandalei* had

on average of 8 lateral and 15 ventral teeth (Fig. 3). This was found to be in accordance with Ilango (2010).

The pharyngeal armature pattern also vary within *Ph. glaucus* and *Ph. annandalei* as the former have a more concentric pattern with more depth along the length of pharynx of armature than the later (Fig. 4). The other measured parameters did not show significant variation among the three species (Table 2). The colouration of the three members was in accordance with Ilango (2010). The species *Ph. argentipes* s.s. and *Ph. annandalei* are lighter compared to *Ph. glaucus*.

Phlebotomus annandalei and *Ph. argentipes* s.s., both of which had a SCII/AFII ratio <0.5, which is a morphologically significant character of the previously described morphospecies B (associated with the transmission of *L. donovani*), were collected by both CBC and HLC. This suggests their probable zoophagic and anthropophagic nature, though no females were among the collection from human landing catches. However no *Ph. glaucus* (either male or female) was collected from human landing catches which suggests its zoophagic nature (Table 1).

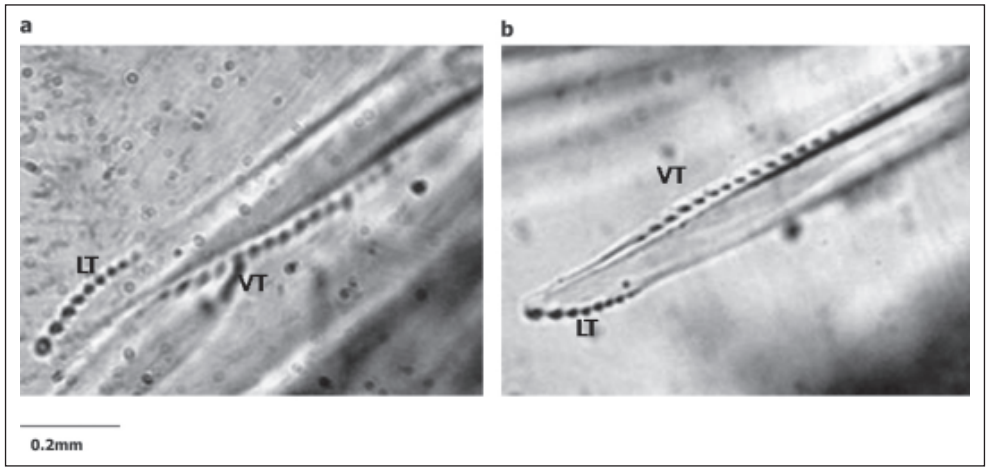

Figure 3. The maxillary teeth of a) *Phlebotomus annandalei* and b) *Ph. glaucus* (LT - lateral teeth, VT - ventral teeth)

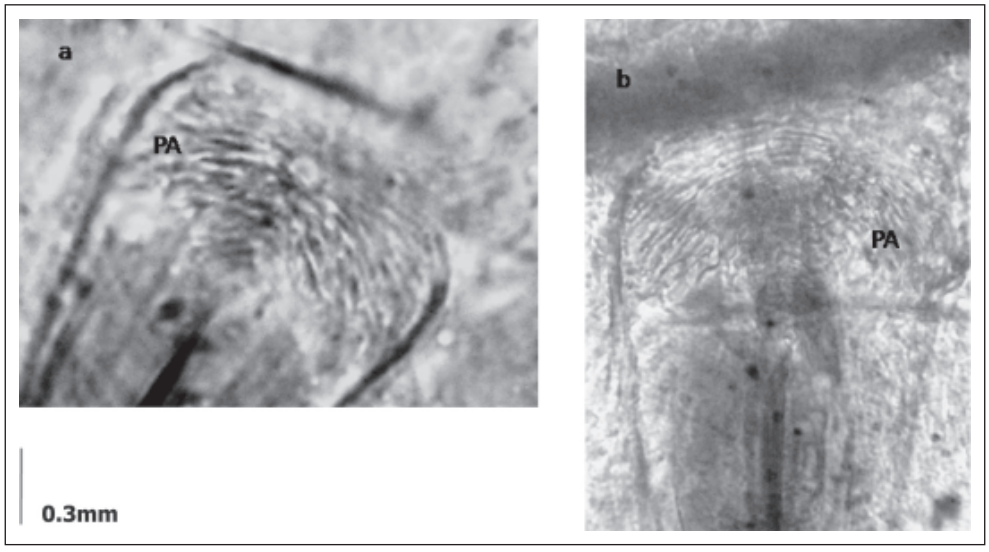

Figure 4. Pharyngeal armature (PA) pattern in a) *Phlebotomus annandalei*, b) *Ph. glaucus*

Table 3. The measured morphometric characters of the members of the Argentipes complex (AI-III- first to third antennal flagellomere, AaII- 2nd antennal ascoid, Lab- labrum, Mp- maxillary palp, L-length, W-width, Wi L-wing length, Wi W-wing width, Hal-halter, $R_{2,3}$- 2nd and 3rd radial vein, R_1O.L- R1overlap, R_{2+3}- Radial vein 2+3, A. sheath-aedegal sheath, P. lobe- paramere lobe, G. p-genital pump and G.F- genital filament; all lengths are in mm)

Sex	lengths	*Ph. annandalei*			*Ph. argentipes*			*Ph. glaucas*		
		minimum	maximum	mean	minimum	maximum	mean	minimum	maximum	mean
Female	**A I**	0.1680	0.1800	0.1740	0.1740	0.1950	0.1855	0.1560	0.2100	0.1826
Female	**A II**	0.0760	0.0960	0.0863	0.0720	0.0780	0.0748	0.0675	0.0840	0.0752
Female	**A III**	0.0750	0.0960	0.0855	0.0690	0.0780	0.0748	0.0675	0.0890	0.0768
Female	**Aa II**	0.0305	0.0355	0.0330	0.0330	0.0375	0.0335	0.0368	0.0570	0.0452
Female	**Lab**	0.0355	0.2400	0.2250	0.1995	0.2370	0.2226	0.1800	0.2520	0.2161
Female	**Mp**	0.0408	0.4170	0.4125	0.4320	0.4680	0.4500	0.3840	0.5040	0.4405
Female	**Head L**	0.3240	0.3480	0.3360	0.3240	0.3720	0.3500	0.2820	0.3960	0.3412
Female	**Eye L**	0.1560	0.1620	0.1590	0.1620	0.1800	0.1730	0.1440	0.2160	0.9800
Female	**Wi L**	1.4200	1.5200	1.4700	1.6200	1.9800	1.7625	1.4700	1.9800	1.6848
Female	**Wi W**	0.4000	0.4510	0.4200	0.4200	0.5700	0.4950	0.3900	0.5850	0.5019
Female	**Hal**	0.2200	0.2470	0.2350	0.2300	0.2500	0.2400	0.1560	0.3000	0.2250
Female	**R_2**	0.3170	0.3400	0.3240	0.3600	0.4200	0.3960	0.3000	0.5040	0.3880
Female	**R_3**	0.4100	0.4530	0.4440	0.4920	0.5400	0.5100	0.3960	0.6000	0.5019
Female	**R1 O.L**	0.0003	0.0004	0.0003	0.0004	0.0012	0.0007	0.0002	0.0010	0.0006
Female	**R_{2+3}**	16.800	20.000	18.667	22.800	19.800	16.800	16.200	25.200	21.450
Male	**A I**	0.1545	0.2260	0.1926	0.1650	0.2310	0.1965	0.1770	0.2310	0.2019
Male	**A II**	0.0750	0.1050	0.0832	0.0690	0.1080	0.0844	0.0780	0.0900	0.0845
Male	**A III**	0.0660	0.1080	0.0838	0.0720	0.0960	0.0843	0.0780	0.0900	0.0850
Male	**Aa II**	0.0150	0.0480	0.0357	0.0284	0.0480	0.0371	0.0320	0.0480	0.0418
Male	**Lab**	0.1350	0.2640	0.1752	0.1440	0.2160	0.1797	0.1500	0.2040	0.1795
Male	**Mp**	0.3840	0.4680	0.4196	0.3120	0.5160	0.4113	0.3640	0.4800	0.4208
Male	**Head L**	0.2760	0.3840	0.3322	0.2190	0.3960	0.3276	0.2640	0.3720	0.3305
Male	**Eye L**	0.1320	0.2400	0.1810	0.1320	0.2400	0.1857	0.1440	0.2400	0.1934
Male	**Wi L**	1.2750	1.8000	1.5587	0.7800	1.8000	1.5732	1.3200	1.8600	1.6038
Male	**Wi W**	0.3900	0.5400	0.4727	0.3000	0.5500	0.4762	0.4500	0.6000	0.5160
Male	**Hal**	0.0960	0.2640	0.2132	0.1200	0.2520	0.2037	0.1200	0.2160	0.1748
Male	**R_2**	0.1440	0.4800	0.3386	0.2160	0.7080	0.4006	0.1560	0.4680	0.3692
Male	**R_3**	0.2240	0.5400	0.4206	0.2280	0.7680	0.4556	0.1920	0.5640	0.4726
Male	**R1 O.L**	0.0120	0.5460	0.2061	0.0120	0.5280	0.2031	0.0180	6.0900	0.5275
Male	**R_{2+3}**	22.400	54.600	45.944	18.000	22.800	19.611	17.400	20.400	19.114
Male	**Coxa**	0.0120	0.2670	0.1783	0.0270	0.2650	0.1796	0.1530	0.2340	0.2092
Male	**Style**	0.0930	0.2670	0.1765	0.1200	0.2580	0.1755	0.1170	0.1710	0.1500
Male	**A Sheath**	0.0750	0.1560	0.1145	0.0660	0.1560	0.1226	0.0630	0.1440	0.1093
Male	**P. Lobe**	0.0510	0.2190	0.1431	0.0450	0.2160	0.1352	0.1350	0.2010	0.1756
Male	**G.p**	0.0900	0.1925	0.1397	0.0960	0.2160	0.1468	0.1080	0.1320	0.1203
Male	**G.F**	0.1080	0.3360	0.1709	0.0960	0.2520	0.1592	0.1530	0.2220	0.1905

The calculated mean values of the measured morphometric characters (15 characters for females and 21 for males) are given in Table 3. The results of the principal component analysis (PCA) are given in Table 4. In the PCA, the males of the three species were separated from each other with Eigen values of 0.0033643 (54.4% variability), 0.0021121 (34.1% variability) and 0.0007132 (11.5% variability) respectively for the first three principal components (PCs). The females of the three species were separated from each other with the Eigen values of 0.030650 (57.2% variability), 0.010749 (20.1% variability), 0.003564 (6.7% variability) for

Table 4. The Eigen values and proportion or variability in percentage for the Principal components for males and females (results from the principal component analysis using a covariance matrix; Minitab Release 14.12.0)

Values	male (for 3 genital characters)	female
Eigen value for PC1	0.0033643	0.030650
Eigen value for PC2	0.0021121	0.010749
Eigen value for PC3	0.0007132	0.003564
Eigen value for PC4	_	0.002518
Eigen value for PC5	_	0.001938
% variance for PC1	54.4	57.2
% variance for PC2	34.1	20.1
% variance for PC3	11.5	6.7
% variance for PC4	_	4.7
% variance for PC5	_	3.6

the first three PCs. The score plot was obtained with the PC1 on x axis and the PC2 on y axis for males (12 sample from each species) and females (with at least three from each species). The chart for the males shows three distinct groups when only the lengths of coxa, style and genital pumps were used in the analysis (Fig. 5a). For females, three distinct groups were observed when all the measured characters were used in the analysis (Fig. 5b). There was overlap in the chart of the males when all the measured characters were used in the analysis (data not shown). The results indicate that a

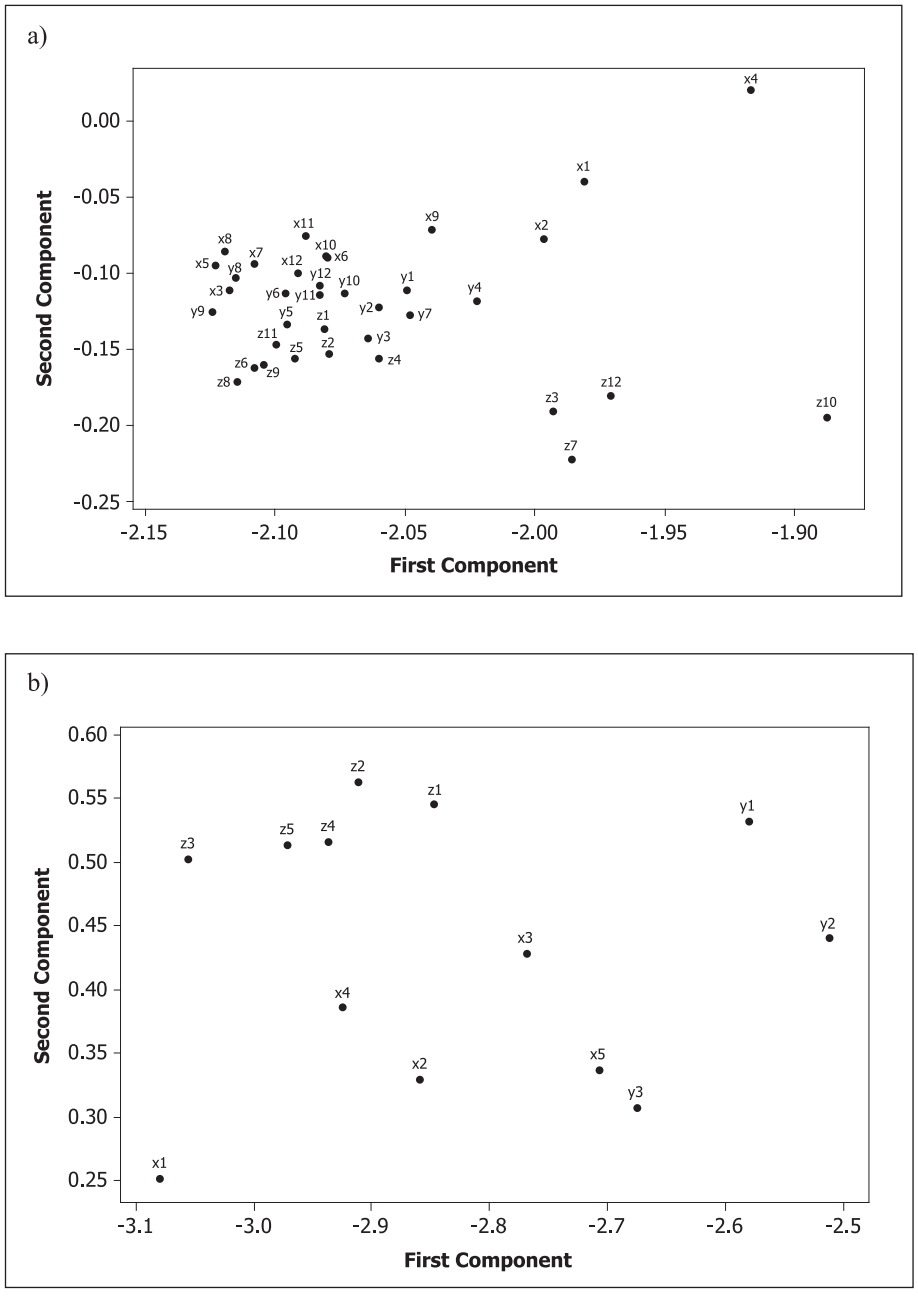

Figure 5. Score Plots for Principal component (PC) 1 and PC2 for a) male genital characters and b) all measured characters of females (x- *Phlebotomus annandalei*, y- *Ph.argentipes* s.s, z- *Ph.glaucas*, "." Data points)

morphometric as well as multivariate approach is possible for the identification of the members of the Argentipes species complex.

The presence of *Ph. annandalei* on the Delft Island was previously reported by Ilango (2010). The present study further reveals the presence of all three members of the Argentipes complex in the country including the potential vectors *Ph. argentipes* s.s. and *Ph. annandalei*. This is the first report of the presence of *Ph. glaucus* and *Ph. argentipes* s.s. in Sri Lanka.

The description and presence of all three members of the Argentipes complex in Sri Lanka warrants more extensive vector survey in the CL endemic localities in the country to establish their vector potentiality and bio-ecological traits. Considering the role of members of the Argentipes complex in the transmission of leishmaniasis and also the difficulties in identifying the vector based on the morphological characters, a simple reliable molecular tool is needed for the characterization of the members in the complex.

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