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Light trap collection of blood sucking dipterans from selected sites in hambantota district which is an endemic cutaneous leishmaniasis area in Sri Lanka and morphometric study of collected *Phlebotomus argentipes* sand flies

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Abstract
Cutaneous leishmanisis (CL) patients were reported from Hambantota district, Sri Lanka. Causative organism of CL is *Leishmania donovani* and the potential vector *is Phlebotomus argentipes.* Current study was designed to identify the potential vectors in CL infected sites in Hambantota district, Sri Lanka.
Ten households of CL postive patients were selected and dipterans were captured using the CDC light, sticky traps. Sampling of dipterans was continued one night per month from January, 2016 to January, 2017. Collected insects were preserved in 70% Ethanol. Presrved insects were identified using taxonomic keys and mounted heads and genitalia of sand flies were used for morphological identification. Student t-test and principal component analysis were used to identify the different morphological characters. Blood sucking dipterans such as biting midges (n= 358) and sand flies (n=120) were captured. *Phlebotomus argentipes, P.salehi* and *P.stantoni* and *Sergentomyia* species such as S. *babu*, S. *clydei*, *S. indica*, *S.punjabensis* and *S.zeylanica* were identified. Findings indicated that some morphological characters were statistically different in sibling species such as *P.argentipes s.s.*, *P.annandalei* and *P.glaucus* of *Phlebotomus argentipes* species complex in study sites. PCA analysis also pointed out that there were different clusters for different sibling species of *P.argentipes* species complex collected from study sites in Hambantota district, Sri Lanka.

Keywords: *Leishmania donovani*, *Sergentomyia* spp., biting midges, sibling species, hoyer's medium

Introduction
Leishmaniasis is still a negligible disease especially in developing countries and 350 million people are at risk of infection and 2 million cases (0.5 million of visceral leishmaniasis and l.5 million of cutaneous leishmaniasis) are reported annually. Visceral leishmaniasis causes an estimated over 50,000 deaths annually. This disease is caused by an intracellular protozoan parasite named *Leishmania* species. Cutaneous leishmaniasis (CL), visceral leishmaniasis (VL) and muco-cutaneous leishmaniasis (MCL) are three main clinical forms of the disease which are important to humans [1].
First autochthonous CL case was reported in 1992 from Hambantota district in Sri Lanka where the main clinical form is CL [2]. The causative species in CL cases in Sri Lanka is *Leishmania donovani* MON 37 [3, 4]. Since 2001, more than 6500 CL cases have been reported with few MCL and VL cases from Sri Lanka and thereafter the disease was considered as a significant health problem in the country [5]. At present north-western, north-central and southern regions of Sri Lanka are endemic for CL and temperature, humidity and wind speed in these areas contribute to the transmission of CL [6, 7].
The parasite, *Leishmania* sp. has a simple life cycle altering between a mammalian host and insect vectors of phlebotomine sand flies (Diptera: Psychodidae, subfamily: Phlebotominae) without sexual stage. Ovoid, non-motile and an intracellular stage can be seen in vertebrate hosts while elongated, motile and an extracellular stage is found in the digestive tract of insect vector [8]. The phlebotomine sandfly is found throughout the world's inter-tropical and temperate regions. The old-world sandfly species live in desert or semi-arid ecosystems while the new world species are in forest dwellings. The only proven vectors of human leishmaniases are species and subspecies of *Phlebotomus* in the Old World and *Lutzomyia* sp. in the New World. *Phlebotomus argentipes* is the known vector of *L.donovani* in VL cases in the north-east Indian subcontinent. This species is composed of two or more populations, sibling species with different vectorial capacities due to the differences in morphology and cuticular hydrocarbons between geographically defined populations [1].
Lane *et al.*, (1990) [9] reported that *P. argentipes* is widely distributed throughout the Indian subcontinent and South East Asia. According to Lane, (1988) [10] *P. argentipes* exists as a species complex with two or more morphological species, namely morpho- species A and B, with different vectorial capacity which might depend on their feeding preference. There are only a few studies related to the bionomics of vector of CL transmission in Sri Lanka. Among them, Lewis, (1978) [11] and Ozbel *et al.*, (2011) [12] identified two species of *Phlebotomus* (*P.argentipes* & *P.statoni*), in Sri Lanka. Except Psychodidae, Ceratopogonidae, Simulidae and Culicidae dipeterans are also blood sucking dipterans (Order Diptera, Suborder: Nematocera) and they transmit human diseases. According to the findings of Gajapathi *et al.*, (2013) [13] *P.argentipes* is considered as the potential vector of CL in Sri Lanka.

Feeding preference of *P.argentipes* complex in Sri Lanka is mainly zoophilic however, anthropophagous *P.argentipes* sand flies were also reported in Kandy district, the central part of the Country [9]. Surendran *et al.*, (2005) [14] also reported that higher CL incidences in northern Sri Lanka due to the prevalence of *P.argentipes* morpho-species B whether alone or in sympatric with morpho species A.

The distribution of CL is dynamic. Climatic, socio-economic and other environmental changes could expand the geographical range of the vectors and CL transmission in the future [1]. Hence, before launching vector control programme it is needed to identify the vector sand fly species complex and associated dipterans and then breeding and feeding habitats of these sand-fly species in the region. The current study was carried out to identify the sand fly species and other blood sucking dipterans which will be the potential vectors that transmit the *Leishmania* parasites in Hambantota district.

Materials and methods

Study area

Present study was carried out in Hambantota district (6° 7' 28.5348" N and 81° 6' 3.8664" E) of Southern province in Sri Lanka and it is margined by Western and Uwa provinces. Its' land area is 2609 km^2 and total human population is 599,903 individuals. Annual average rainfall is 1000-1250mm whereas annual average temperature is 30^{0}C. Hambantota district has divided into 12 District Secretary Divisions (DSD) for the administrative purposes and more DSDs are rural areas [15] (figure 1).

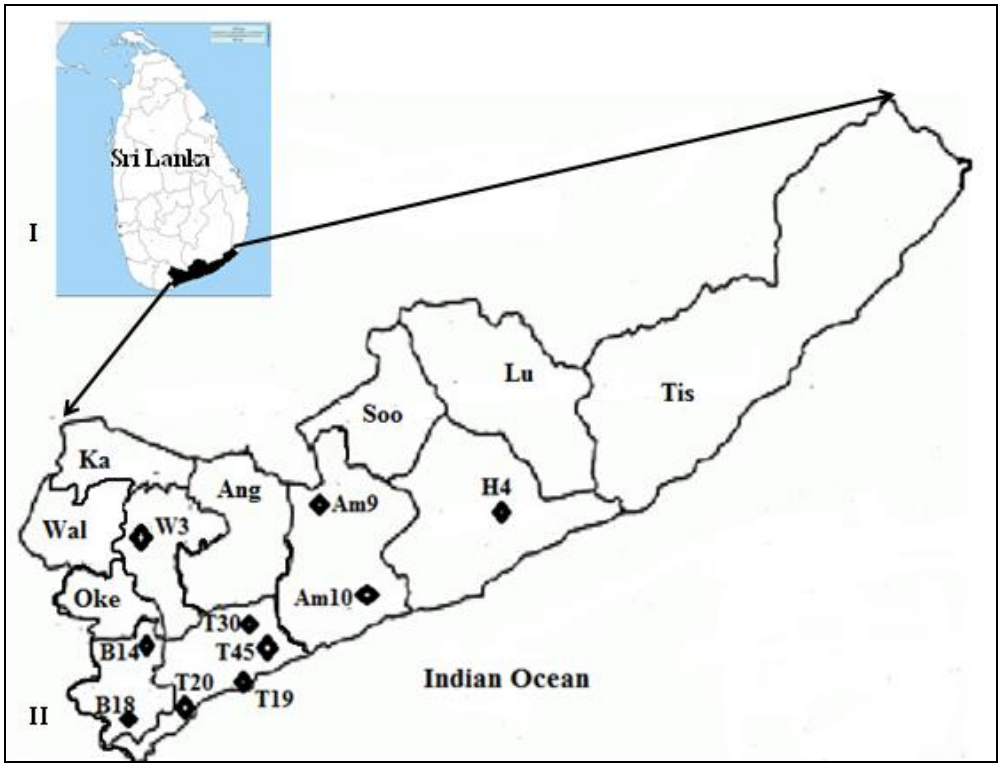

Fig 1: I: Map of Sri Lanka with Hambantota district shaded. II. District Secretary Divisions. DSDs are Ka: Katuwana, Wa: Walasmulla, W: Weeraketiya, Oke: Okewela, B: Beliatta, T: Tangalle, Am:Ambalantota, Ang: Angunakolapelessa, H: Hambantota, Soo: Sooriyawewa, Tis: Tissamaharamaya, Lu: Lunugamwehera. The symbol indicates the sampling sites of current study. (Source: Adapted from Google maps)

Collection of sand flies and other blood sucking dipterans

Ten households of CL positive patients from five DSDs (Walasmulla, Beliatta, Tangalle, Ambalantota and Hambantota) in Hambantota district were selected as study sites (figure 1). Based on the number of CL positive patients in our earlier section of the study, four houses from Tangalle, two houses from Beliatta and Ambalantotata and one house each from Hambantota and Weeraketiya DSDs were selected. Sand flies and other dipterans were captured using the CDC green-light traps (height: 38cm, diameter of the open: 23cm) with Castor oil coated paper strips (29.5cm x 6.5cm). One trap was hanged from 6.00 p.m. to next day 6.00 a.m. in a tree in each site in a less windy location. The distance from the house to the hanged trap was 1m to 5m. This sampling was continued one night per month from January, 2016 to January, 2017. Captured sand flies and other dipterans were washed in 90% ethanol and transferred to 70% ethanol. Heads and genitalia of sand flies were separated and genitalia were kept in 5% KOH for about 01 hour. These specimens were mounted in Hoyer's' medium.

Identification of sand flies and other blood sucking dipteran

All collected dipterans were identified using standard dipteran keys [16, 17, 18]. Identification of species of sand flies based on morphological characteristics of pharynx, cibarium, wings and male and female genitalia using the standard keys [11, 19, 20].

The length of labrum, maxillary palps, pharynx, length of antennae and flagellomere I and II (FI & FII), *sensilla chaetica (SC)* on flagellomere II, length of wings, wing venation, length of halters, length and width of head, length of eye, length of thorax and abdomen, length of femur, tibia and basitarsal segments, length and shape of gonocoxite, gonostyle, paramere, aedegal sheath, genital pump and aedegal filaments in male genitalia and shape, length and width of spermathecae of female genitalia were measured.

Ratios of *sensilla chaeticum* II to flagellomere II and wing index for all *P. argentipes* sand flies and ratio of gonocoxite length to gonostyle length of male sand flies were calculated. All measurements were done with a calibrated eye-piece in an Leica Light microscope under suitable magnifications. Based on the recent findings of Illango (2000 & 2010)[21, 22], Surendran *et al.,* (2005) [14], Gajapathy *et al.,* (2011)[23] and Ranasinghe *et al.,* (2012)[24], the mean values of selected morphometric characteristics were used to separate *P.argentipes* species complex in Hambantota district.

Statistical analysis

Selected morphometric characteristics of *P.argentipes* species, were analyzed using Students t-test (SPSS version 20.0, USA) and compared with the recently published descriptions to separate the members of *P. argentipes* species complex. Principal Component Analysis (PCA) was also performed separately for morphometric characteristics of males and females of *P.argentipes* sand flies and the component error rotated using the Varimax method using Minitab software (Minitab 17). Eigen values and proportions (variability) of first 3 principle components were obtained and Eigen values higher than 1 were considered as statistically significant. Scores of first two components were plotted for males and females separately to display the differentiation of sibling species of *P.argentipes* complex.

Ethical approval

Ethical approval for the study was granted by the Ethics Review Committee of the Faculty of Medicine, University of Ruhuna, Matara, Sri Lanka.

Results and discussion

Cutaneous leishmaniasis (CL) is an established public health problem in north-central and southern provinces in Sri Lanka. Epidemiology data on CL cases revealed that the highest number of CL patients reported from Anuradhapura district and the highest incidence per 100,000 persons was reported from Hambantota district [7]. Cattle-baited net traps or human- baited traps and manual collection with aspirators had been used to collect potential vectors of CL in majority of studies [25, 26]. Yet, in current study, traps with Caster oil coated sticky paper and CDC green light were selected as a feasible and low cost trapping method to collect sand flies and other dipterans in ten sites on the same day.

Households of CL positive patients were selected for this study based on the findings of our previous study. Ten study sites which were households of CL positive patients located in Beliatta, Tangalle, Weeraketiya, Ambalantota and Hambantota DSDs in Hambantota district. Among them, four (4) study sites in Tangalle were located in urban areas and all other study sites (6) were located in rural areas.

During the present study, blood sucking dipterans belonged to families of Ceratopogonidae and Psychodidae were collected using the above mentioned traps from ten (10) households of CL positive patients in Hambantota district and it was illustrated in table 1.

Table 1: Number of collected dipterans from study sites in Hambantota district during the current study period.

Study site	Dipterans	
	No. of Sand flies	No. of biting midges
1. B14	12	62
2. B18	03	93
3. W3	02	02
4. T30	25	12
5. T45	14	09
6. Am9	31	55
7. Am10	03	46
8. H1	05	11
9. T19	07	62
10. T20	18	06
Total	120	358
Blood fed	-	21
Gravid	02	-

Identified ceratopogonides namely, biting midges (*Culicoides* spp.) were collected from all study sites in Hambantota district and majority of them were caught from study site B18 which was located at Beliatta DSD where two hen houses were found. Braverman (1994) [27] and Lassen *et al.,* (2012) [28] reported that biting midges transmit parasites of bird and lizard haematozoa, horse onchocercosis, wild animal filariasis and mansonelliasis in humans. Lassen *et al.,* (2012) [28] indicated that some *Culicoides* species in Denmark are opportunistic and feed on variety of mammals and birds. Due to lack of studies on these biting midges in the country there is no information on medical and/or veterinary importance of them. Hence, identification of species of these biting midges and their blood meal analysis are very important to identify their hosts and disease transmission in Hambantota district.

In total, 120 sand flies were collected from ten households of CL positive patients in Hambantota district. Out of them, 50 specimens belong to the genus *Phlebotomus* (41.7% of specimens) of 3 species such as *P. argentipes, P. salehi and P. stantoni*. Sex ratio of *P.argentpes* was 1.6 males to 1female. *P.argentipes* is the only sand fly species found in all study sites except B18. Two gravid females of *P.argentipes* were collected and the length and width of their eggs were 0.38±0.01 and 0.12 ±0.02 mm respectively and eggs were blackish brown in color. Further, five species named, S*ergentomyia babu, S. clydei, S. indica, S. punjabensis* and *S. zeylanica* belonged to genus *Sergentomyia* (n=70, 58.3% of specimens) were identified and total numbers of males and females are shown in figure 2. The sex ratio of male to female of *Sergentomyia* spp. was 1 to 2.5.

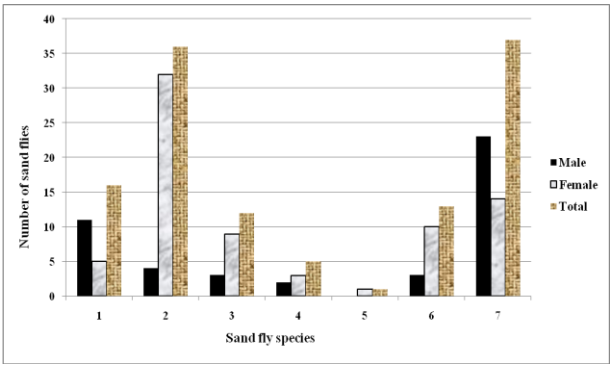

Fig 2: Number of sand fly species found from Hambantota district during the study. **1**: *Sergentomyia babu*, **2**: *S. clydei,* **3**: *S. indica*, **4**: *S.punjabensis*, **5**:*S.zeylanica*, **6**: *P. stantoni* & *P. salehi*, **7**: *P.argentipes*

Several studies on CL vector collection in different parts of Sri Lanka indicated that *P.argentipes* and *Sergentomyia* species as potential vectors of CL in the country. Earlier, in 1990 Lane *et al.*,[9] found *P.argentipes* in central highlands of Sri Lanka, near Kandy and Surendran *et al.,* (2005)[14] collected *P.argentipes* species from a western area of Delft Island, 35km off the northern coast of mainland Sri Lanka and 37km from the coast of Tamil Nadu in India. Ozbel *et al.,* (2011)[12] reported that *P.argentipes* and *Sergentomyia* sand flies were widely distributed throughout the country including southern part and *P. stantoni* were also caught from four districts of Sri Lanka. Morphologically distinct species of *P. argentipes* were found from CL positive areas in central part of Sri Lanka in 2012 [24]. In 2013, Gajapathy *et al*.,[13] identified two morpho species of *P.argentipes* species using molecular techniques and *Sergentomyia* species which were collected from different parts of the country. The first report of the presence of *P. salehi* Mesghali in Sri Lanka was done by Gajapathy and Surndran in 2012. Premachandra *et al.,* (2012) [29] have found only *Sergentomyia zeylanica* sand flies from Dickewella District Secretary Division, Matara district in Southern Sri Lanka. In 2015, Senanayake *et al.,* [26] collected *P. argentipes* and *S. zeylanica* from three districts including Hambantota district. During the present study, three species of *Phlebotomus* such as *P. argentipes*, *P. salehi* and *P.stantoni* were identified from study sites in Hambantota district and the highest number was *P.argentipes.*

Previous studies done for CL vector identification in the country, *S. zeylanica* [26, 29] and *S.punjabensis* [30] had been collected. Yet, this is the first study which reports the presence of *S. clydei* and *S. babu* in Hambantota district and *S.clydei* was the most abundant sand fly species in study sites in Hambantota District.

Large number of *Sergentomyia* species feed preferably on cold-blooded vertebrates and hence, they are proven vectors of *Leishmania* species of reptiles. Some indicative data which was provided by the detection of *Leishmania* DNA in several *Sergentomyia* species, support for the potential role of *Sergentomyia* as a vector. *L. major* has been detected in *S. clydei* and *S. minuta* in Tunisia (Rondani, 1843) and *L. donovani* DNA has been isolated from *S. babu* in India [31]. Some researchers [32, 33, 34, 35] found that *S. clydei*, *S. darling*, *S. minuta* and *S. schwetzi* have fed on mammals including man and infected with *Leishmania* spp. which may be pathogenic to humans. Despite all these facts, one of the important factors to be a potential vector of a given region is the ability of *Sergentomyia* spp. to transmit Old world *Leishmania* spp. to a native mammal host [36].

Previous studies indicated the occurrence of morphologically different two or three sibling species of *P.argentipes* from different districts of Sri Lanka [24, 13].

Present study also reports the presence of three sibling species of *P.argentipes* copmplex in Habmbatota district. *P.argentipes* complex belonged to the subgenus *Phlebotomus* (*Euphlebotomus*) Theodor 1948, and genus *Phlebotomus* Rondani & Berte 1840. Morphological characteristics of male flies of *P. argentipes* are absence of a lobe on the gonocoxite, gonostyle with five spines and trilobes paramere. Specific characteristics found in females of them are presence of group of spicules in the pharynx and a spermathecae with a large apical segment.

Within the collected *P.argentipes* species, some morphological characters were significantly different. As stated by Illango,(2010) [22] significant differences of selected morphometric characteristics of collected female *P.argentipes* species complex were tested using independent sample t-test and it was found that length and width of wings ($P< 0.001$ & $P= 0.011$), length of Antennal flagellomere II (AF2) ($P< 0.001$), ratio of *sensilla chaeticum* II to Antennal flagellomere II ($P< 0.001$), lengths of pharynx ($P< 0.001$), head ($P= 0.010$), eye ($P= 0.011$) and halters ($P= 0.001$) of *P.argentipes* sensu strico were statistically different from those characters of *P.annandalei*. Meanwhile P. *annandalei* and *P.glaucus* showed significant differences between them in length and width of wing ($P<0.001$), length of antennal flagellomere1 (AF1) ($P <0.001$), ratio of *sensilla chaeticum* II to AF2, ($P <0.001$) and lengths of labrum ($P = 0.016$), pharynx ($P = 0.018$) and eye ($P=0.012$). Female *P.argentipes* s. s. and *P.glaucus* were significantly different from each other by the ratio of *sensilla chaeticum* II to AF2 ($P <0.001$).

According to the morphometric characteristics of males of *P.argentipes* complex, they belong to *P.argentipes s.s.* and *P.annandalei* sibling species and morphometric characters such as ratio of *sensilla chaeticum*II to AF2 ($P <0.001$), lengths of head ($P =0.012$), eye ($P=0.029$), halters ($P =0.003$), and lengths of gonocoxite ($P=0.04$), gonostyle ($P=0.002$), paramere ($P =0.007$), aedegal sheath ($P =0.015$) and genital pump ($P <0.001$) were showed significant differences between males of *P.argentipes s.s.* and *P.annandalei*. There was no significant difference between males of *P.argentipes s.s.* and *P.annandalei* in ratio of gonocoxite and gonostyle lengths ($P=0.627$) and ratio of genital pump and genital filament lengths ($P=0.230$). Males of *P. glaucus* were not found at the present study area. The mean and standard deviations of means (S.D.) of the selected morphometric characteristics of sibling species of *P.argentipes* species complex were given in table 2. As indicated in table 2, the ratio of *sensilla chaeticum* II to Antennal Flagellomere II values were 0.626 in females and 0.507 in males for *P.argentipes s.s.,* 0.296 in females and 0.312 in males for *P.annandalei* and 0.725 in males of *P.glaucus.*

Table 2: Selected morphometric characteristics of sibling species of collected *P.argentipes* complex from study sites in Hambantota district, Sri Lanka.

Lengths (mm)	*P. argentipes*		*P. annandalei*		*P. glaucas*
	(♀= 8) Mean ± S.D.	(♂ =19) Mean± S.D.	(♀= 3) Mean ± S.D.	(♂ =3) Mean± S.D.	(♀= 4) Mean± S.D.
Antennal flagellomere I (A1)	0.801±0.04	0.798±0.20	0.467±0.04	1.003±0.84	0.828±0.03
Antennal flagellomere II (A2)	0.331± 0.02	0.330±0.08	0.297±0.06	0.470±0.23	0.335±0.01
Ratio of Sensilla chaeticum/A2	0.626±0.032	0.507±0.13	0.296±0.01	0.312±0.08	0.725±0.03
Labrum length	0.210±0.04	0.181±.014	0.173±0.03	0.183±0.09	0.253±0.03
Maxillary palps length	0.414±0. 26	0.432±0.24	0.107±0.18	0.420±0.37	0.448±0.30
Head length	0.458±0.05	0.451±0.05	0.347±0.06	0.353±0.08	0.423±0.05
Eye length	0.263±0.04	0.256±0.05	0.180±0.03	0.180±0.04	0.248±0.02
Pharynx length	0.708±0.07	0.647±0.1	0.287±0.25	0.587±0.13	0.728±0.05

Wing length	0.748 ±0.07	0.653± 0.16	0.543 ± 0.03	0.583±0.06	0.813±0.03
Wing width	0.214±0.04	0.191±0.06	0.140± 0. 01	0.150±0.05	0.250±0.08
Wing index	1.034±0.64	1.729±0.58	0.567±0.49	1.272±0.89	1.365±0.93
Halter length	0.107±0.02	0.097±0.02	0.150±0.03	0.047±0.04	0.103±0.03
Gonocoxite length	-	0.883±0.29	-	0.470±0.4	-
Gonostyle length	-	0.566±0.16	-	0.197±0.17	-
Aedeagal sheath length	-	0.317±0.11	-	0.133±0.12	-
Paramere length	-	0.764 ±0.20	-	0.357±0.31	-
Ratio of genital coxite / genital style	-	1.633±0.55	-	1.830±0.58	-
Ratio of genital pump / genital filament	-	0.527±0.2	-	0.287±0.25	-

In accordance with the statistical analysis (Student t-test) the signicantly different factors which were used to separate females of three siblng species of *P.argentipes* complex were length and width of wings and lengths of pharynx, head, labrum and eye. Common character for three sibling species was the ratio of *sensilla chaeticum* II (ScII) to antennal flagellomere II (AF2) which was different in each subspecies. Morphological characters which separate males of *P.argentipes* and *P. annandalei* were ratio of *sensilla chaeticum* II to Antenna Flagellomere II and lengths of head, paramere, gonocoxite, gonostyle, aedegal sheath and genital pump.

Further, Principal Component Analysis (PCA) was performed to separate female morphological characters of three sibling species and male morphological characters in two sibling species separately. Females of *P.argentipes* s.s., *P.annandalei* and *P.glaucus* were separated from each other with Eigen values of PC1 by 7.1348 (accounting for 59.5 % of variability) and PC2 by 1.2739 (accounting for 10.6 % of variability). The first rotated component included a strong positive loading for wing length, component2 had a strong positive loading for wing index, component3 included a strong positive loading for halter length and component4 had a strong negative loading for labrum length (table 3). Plotting of first two rotated components against one another (PC1 on x-axis and PC2 in Y- axis) for females resulted in three separate groups for three sibling species, *P. argentipes* s.s., *P. annandalei* and *P.glaucus* and the clusters of two sibling species of *P. argentipes s.s.* and *P.glaucus* were closed to each other (figure 3a). The score plot of first two components of morphological characters of males showed two separate clusters for *P.argentipes* and *P.annandalei* sibling species (figure 3b). The first rotated component of male *P.argetipes* and *P.annandalei* sibling species included a strong positive loading for paramere length, component2 had a strong positive loading for wing width and component3 included a strong negative loading for head length. Plotting of first two rotated components against one another (PC1 on x-axis and PC2 on Y-axis). Table 3 shows the Component loadings for Varimax rotating components of female and male morphological characters of *P.argentipes* subspecies collected during the current study.

Plotting of first and second Principal components (Principle Component Analysis) also demonstrated that there were three separate clusters of *P.argentipes* females and two separate clusters of male *P.argentipes*. This finding is somewhat similar to the finding of Gajapathy *et al*.,(2011)[23] in northern Sri Lanka. During the current study, males of *P.glaucus* were not found and the numbers of males and females of *P.annandalei* sibling speices were also lower. Therefore further studies are needed to get a clear conclusion of morpho characterisrtices of sibling species of *P.argentipes* species complex.

Table 3: Component loadings for four Varimex-rotaed components extracted from 12 morphological characters in females and 20 chatares in males of *Phlebotomus argentipes*, *Phlebotomus annandalei* and *Phlebotomus glaucus* in the present study.

Character	Female Components			Male Components		
	1	2	3	1	2	3
1.Wing length	0.353266	0.178115	-0.407943	-0.059878	0.369284	0.371558
2. Wing width	0.346157	0.148831	0.176118	-0.047623	0.415082	0.094484
3. Wing index	0.120276	0.673993	0.243611	-0.085708	0.404761	-0.030942
4. Ant. flagellomere I	0.350693	0.008003	-0.004961	-0.269274	0.284173	-0.065279
5. Ante.flagell. II	0.177099	0.493205	-0.446405	-0.291969	0.240508	0.012251
6. Ratio of Sen. Cha.2/Ante. Flag. II	0.350943	0.057108	-0.092593	0.096469	0.359420	0.330816
7. Labrum length	0.260984	-0.104967	0.342449	-0.257979	0.197123	-0.287728
8. Maxi.palp length	0.214089	-0.170293	-0.388158	0.167754	0.128557	0.295754
9. Pharyn. Length	0.319735	-0.302589	0.124720	-0.052645	0.109082	-0.200463
10. Head width	0.295715	-0.285334	0.071160	0.100714	0.216597	-0.500911
11. Eye length	0.310285	-0.057417	0.348945	0.097048	0.231982	-0.411796
12. Halter length	-0.251993	0.170267	0.537720	0.199423	0.102802	0.129128
13. Coxite length				0.296523	0.050494	-0.026401
14. Style length				0.325755	0.142702	-0.175871
15. Aedegal length				0.274649	0.134662	-0.089338
16. Paramere length				0.336327	0.086853	-0.145587
17. Genital pump length				0.303649	0.048531	0.131418
18. Genital fila. Length				0.324299	-0.001100	0.067752
19. Ratio of Ge. Co.len./Ge. Sty. Len.				-0.013672	-0.166264	-0.070257
20. Ratio of Ge. Pum. Len. /Gen. fil. Leng.				0.287739	0.050509	-0.054803

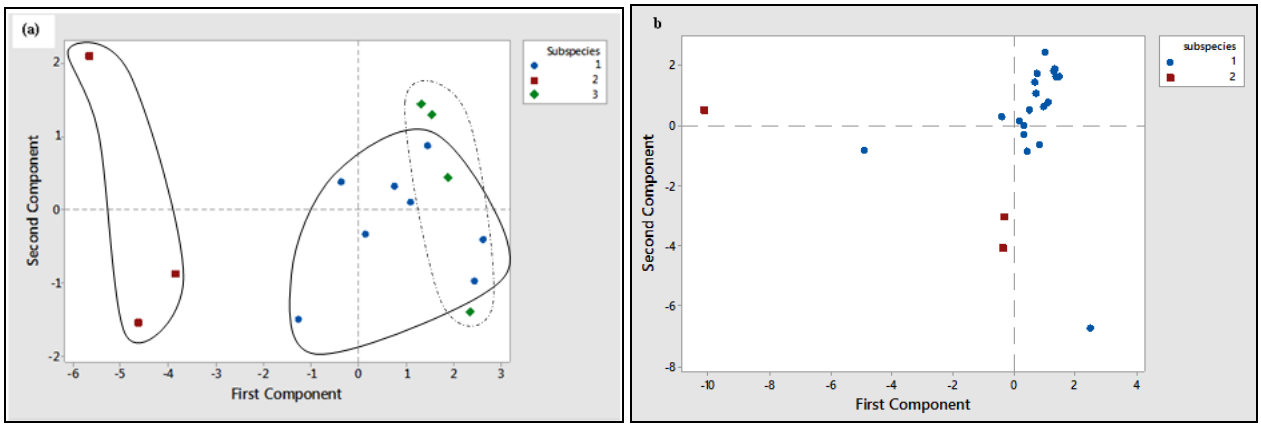

Fig 3: Score plots for Principal component (PC1) 1 and (PC2) 2 for (**a**) female morphometric characters and (**b**) male morphometric characters. (Sub species **1**: *Phlebotomus argentipes* s.s., **2**: *P. annandalei* and **3**: *P.glaucus*)

Other scientiest, Lane in 1988[10] described that length of *sensilla chaeticum* II is less than the half of the length of the flagomereII in sand fly populations of visceral leishmaniasis endemic regions while sand fly populations in other regions have longer *sensilla chaeticum* II. As described by Lewis and Killick-Kendrick (1973) [37], Lane (1988) [10], Surendran *et al*.,(2005) [14] and Illango (2010) [22] results of the present study indicated that there are two morphologically distinct species of *P.argentipes* complex in Hambantota district. They are morpho-species A with longer *sensilla chaticum* II in antennal flagomere II (more than 50% of the length of flagomere II) and morpho-species B with short *sensilla chaetium* II in antenneal flagomere II (less than 50% of the length of flagomere II). According to the conclusions of Lane (1988) [10], both morpho-species found in curent study may have sympartric distribution in adjacent DSDs in Hambantota district.

P.argentipes is the well-known vector of visceral leishmaniasis in India and members of this species complex are morphologically identical or nearly so, but differ in behaviors. Lane (1988) [10] indicated that ratio of *sensilla chaeticum* II length to the length of flagellomere II are shorter in sand flies of visceral leishmaniasis endemic regions while those elsewhere are longer. Structure of sensilla of *P.argentipes* provides information of host recognition, mate selection and blood feeding of sand fly vectors and support to develop control strategies using pheromone/kairomone traps for leishmaniasis vector control [21]. Further, Lewis & Kilick-Kendrick (1973) [37] indicated that *P.argentipes* also exhibits clinical variation in its biting preferences and almost entirely zoophilic in Sri Lanka and South East Asia. Previously it was reported the presence of morpho-species A with longer ascoids in Sri Lanka and Surendran *et al.,* (2005) [14] documented the presence of more anthropophagic morpho-species B with shorter ascoids in Delft Island. The present study highlights the presence of both morpho-species, but few number of morpho-species B in study sites in Hambantota district which is considered as one of the CL endemic areas in Sri Lanka indicating more zoophilic *P.aregntipes* vectors are than anthropophagic vectors. It is needed to thoroughly consider even the presence of less number of *P.argentipes* mopho-species B in Hambantota district which are identified as anthropophagic vectors of endemic visceral leishmaniasis in neighboring North East India, Nepal and Bangladesh. However, it is very important to collect more sand flies to gather more information on *P.argentipes* sibling species complex in Hambantota district. Initiatition of proper control measures based on these findings is essential to reduce the CL cases in Hambantota district before it is becoming a major public health problem in the area.

Conclusion

During the current study period blood sucking dipterans such as biting midges (*Culicoides* spp.) and *Phlebotomus* and *Sergentonyia* sand flies were captured using CDC light traps with castor oil coated sticky papers from selected households of CL positive patients in Hambantota district. This is the first study reports the presence of *Sergentonyia clydei* and *S. babu* in Hambantota district and majority of sandflies were *S.clydei*. Comparing the ratios of *sensilla chaticum* II to antennal flagomereII and several other morphological characters, there are three sibling species of *P. argentipes* species complex namely, *P.argentipes s.s*., *P. annandalei* and *P.glauses* in Hambantota district. These sibling species of *P.argentipes* belong to two morphologically distinct groups such as morpho-species A which includes *P.argentipes s.s*., and *P.glauses* with longer *sensilla chaticum* II in antennal flagomere II and morpho-species B includes *P.annandalei* with short *sensilla chaetium* II in antenneal flagomere II. More collection of sand fly speciemens are needed for a definite conclusions of distribution of morpho-species B in study area because it is the most available vector in visceral leishmaniasis endemic areas in Asian region.

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