

Temporal distribution and behaviour of sand flies (Diptera: Psychodidae) in a cutaneous leishmaniasis focus of the Kani Tribe settlements in the Western Ghats, India



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ABSTRACT

The temporal distribution of sand flies in relation to environmental factors was studied in the Kani tribe settlements located on the southernmost part of the Western Ghats, Kerala, India, between June 2012 and May 2013. This area is known for occurrence of cutaneous leishmaniasis (CL) cases. Employing hand-held aspirator, light trap and sticky-trap collection methods, a total of 7874 sand fly specimens, comprising 19 species was collected. *Sergentomyia baghdadis* was predominant species, followed by *Phlebotomus argentipes*. Sand fly abundance was significantly higher indoors ($\chi^2 = 9241.8$; $p = 0.0001$) than outdoors. Mean density of *P. argentipes* in human dwellings, cattle sheds and outdoors was 7.2 ± 2.9 , 27.33 ± 21.1 and 0.64 ± 0.2 females/per man-hour (MHR), respectively. No sand fly species other than *P. argentipes* was obtained from cattle sheds. Although, sand fly populations were prevalent throughout the year, their abundance fluctuated with seasonal changes. Multiple regression analysis with backward elimination indicated that the increase in precipitation and relative humidity contributed to a significant positive association with the increase in sand fly abundance, while the increase in temperature showed no association. Fully engorged female sand flies tested for blood meal source showed multiple host-blood feeding. Analysis of resting populations of sand flies collected from human shelters indicated that the populations were found maximum on interior walls at 6–8 and >8 ft height, including ceiling during summer ($F = 83.7$, $df = 6$, $p = 0.001$) and at the lower half of the wall at 0 and 0–2 ft height, during monsoon season ($F = 41.4$, $df = 6$, $p = 0.001$). In cooler months, no preference to any height level ($F = 1.67$, $df = 6$, $p = 0.2$) was observed. Proportion of females sand flies with Sella's classification of abdominal stages, namely full-fed, half-gravid and gravid females did not vary significantly ($t = 1.98$, $p = 0.13827$) indoors, confirming their endophilic behaviour. Risk of CL transmission in these tribal settlements is discussed.

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1. Introduction

Leishmaniasis are complex infectious diseases caused by the parasites of the genus *Leishmania* Ross (Kinetoplastida: Trypanosomatidae) and transmitted by Phlebotomine sand fly species (Diptera: Psychodidae). Of the approximately known 800 species of sand flies, 98 have been implicated in the transmission of 40 different species of *Leishmania* parasites (Maroli et al., 2013). Leishmaniasis are found in 98 countries, both in tropical and subtropical regions. Today, more than 350 million people are at risk of contracting leishmaniasis, an estimated 12 million cases exist worldwide,

with an estimated number of 1.5–2 million new cases occurring annually; of which 1–1.5 million cases are cutaneous leishmaniasis (CL) and 0.5 million cases are visceral leishmaniasis (VL) (WHO, 2010). These diseases are distributed in widely scattered foci in many countries (Alvar et al., 2012), as the climatic conditions with high humidity and rainfall coupled with optimum temperature and environmental factors such as the nature of landform, land usage pattern, soil moisture and soil pH facilitate sand fly propagation, survival and abundance (Valderrama et al., 2011; Srinivasan et al., 2013).

In India distribution of sand flies has been reported from several areas of northern region (Dhanda and Modi, 1971; Kumar et al., 1992; Sharma et al., 2005), eastern coastal region (Ilango et al., 1994; Srinivasan and Jambulingam, 2010, 2011; Srinivasan et al., 2013, 2014) and western coastal region (Kaul, 1993; Kaul and Jain,

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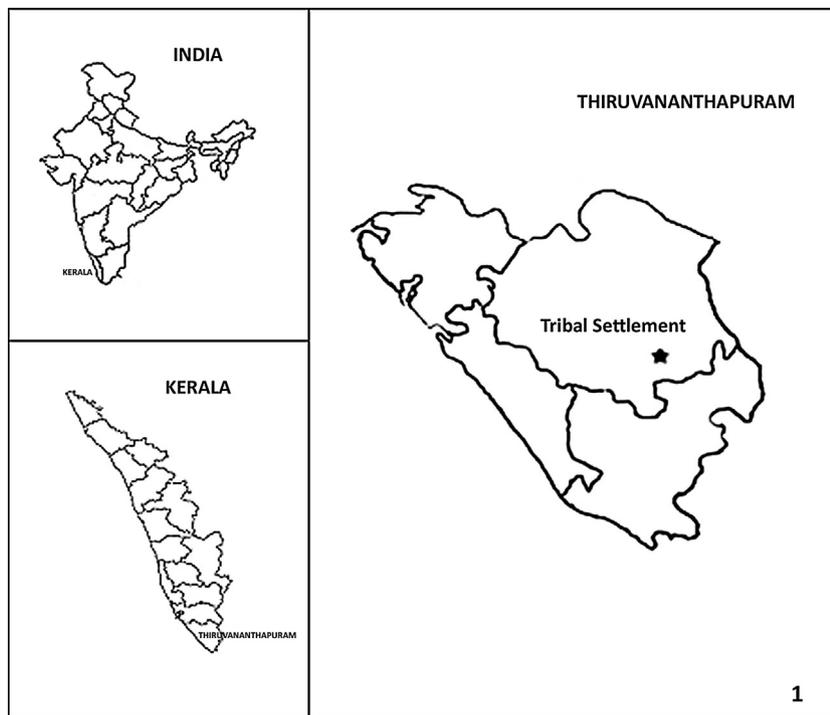


Fig. 1. Study area – Kani tribe settlements located at the southernmost part of the Western Ghats, Thiruvananthapuram, Kerala state, India.

1995). However, VL (Prasad, 1987) and CL cases (Sharma et al., 2003, 2005) were found restricted only to northern parts of this country. Recently, CL cases have been reported from the southernmost part of the Western Ghats, where the Kani tribes, one of the Indian ethnicity or social groups were settled (Pradeep Kumar et al., 2015). The Western Ghats range receives heavy rainfall and supports rich fauna and flora, including endangered species. Hence, this region has been designated as one of the World's "biodiversity hotspots", with at least 325 globally threatened species (Myers et al., 2000; Clara Lewis, 2012). A new sand fly species, *Sergentomyia (Neophlebotomus) monticola* was reported from the southernmost part of the Western Ghats (Srinivasan et al., 2014). Despite, several aspects of sand flies in this region remain unknown. In view of the occurrence of CL cases among Kani tribes, a study was undertaken to investigate temporal distribution, seasonal abundance and behaviour of sand flies in this forest ecosystem.

2. Materials and methods

Study area: The Western Ghats is a mountain range, runs north to south, through the western edge of the Deccan Plateau of India and separates the plateau from a narrow coastal plain, called Konkan, along the Arabian Sea. This mountain range has created a barrier across the path of the southwest monsoon, resulting in the formation of a significant climatic variation with abundant rainfall on the windward side and a dry belt on the lee-ward eastern side. Therefore, there were great varieties of vegetation, all along the Ghats: scrub jungles, dry and moist deciduous forests, semi-evergreen, evergreen forests and grassland (Parthasarathy, 1999).

The Kani tribes had scattered in 28 settlements, over the southernmost part of the Western Ghats. Settlements are distributed at different altitudes, ranging from 267 to 2425 ft. These tribes were the hereditary proprietor of land (Arun Vijayan et al., 2007) (Fig. 1). Each tribal settlement was spreading within 1.5–2 km² area and located 5–10 km away from others. The tribes in these settlements inhabiting in hut dwellings (72.6%) made of natural materials such as dry leaves, wooden logs, grasses, bamboo and mud, while

the remaining tribes in houses made of brick and cement (23.8%). Houses/huts within a settlement were located about 100–500 mt apart. Geographic co-ordinates for each settlement were measured employing hand-held Garmin GPS map 76 in each settlement.

There were no transport and communication facilities to these settlements. However, a Primary Health Centre and schools for tribal children were located in the foothill area ~35 km down from a central point of the tribal settlement. The main source of water was from streams/brooks from top hills. The Government made provisions to distribute rice under subsidiary rate to the tribes. Traditional occupations of the tribes included collection of non-timber forest products, such as honey, bee-wax, medicinal plants, gums etc. Besides the wild varieties of plants, rubber, jack, coconut, plantain, pepper and teak were also found in settlements, cultivated by the tribes.

Considering feasibility and logistics, 10 settlements were chosen, for the longitudinal study, based on a stratified sampling technique and population proportion size (PPS) method, using the data obtained during a cross-sectional survey, conducted in all the 28 Kani tribe settlements (Srinivasan and Subramanian, 2015). Details of the settlements chosen for the study were given in Table 1. One of the settlements, Kunnadi located at foothill had cattle population ($N=8$), while in other settlements, no cattle was found. However, there were many dogs in all settlements, domesticated by the tribes for alerting them from untoward activities of wild animals.

2.1. Sand fly collection

Sand flies were sampled every month, at each study settlement, from June 2012 to May 2013, employing hand-held aspirators, light traps and sticky traps, indoors (human dwellings and cattle-sheds) and outdoors (tree-holes, tree-butresses, rock holes, rodent burrows). In each settlement, 10 sampling units (5 units each from indoors and outdoors) were chosen based on random sampling method. Resting collection of sand flies was made spending one man-hour, each indoors and outdoors, spending 12 min in each

Table 1
Details of the study settlements with population.

Sl. no.	Settlements	North latitude	East longitude	Altitude (ft)	No. of dwellings	Male	Female	Total
1	Mulamoodu	08° 35' 29.3"	077° 09' 32.2"	389	15	31	36	67
2	Kunnadi	08° 35' 11.3"	077° 07' 51.1"	480	10	21	20	41
3	Chonampara	08° 35' 03.5"	077° 09' 57.2"	621	17	41	43	84
4	Podiyam	08° 36' 36.1"	077° 10' 18.7"	625	24	39	43	82
5	Kombidi	08° 36' 43.9"	077° 11' 22.5"	653	24	33	38	71
6	Kamalakom	08° 36' 54.7"	077° 09' 44.3"	716	43	52	60	112
7	Keezheamala	08° 33' 10.8"	077° 12' 07.2"	733	7	16	20	36
8	Ayiramkal	08° 33' 17.9"	077° 12' 48.4"	751	14	18	23	41
9	Melaamala	08° 33' 52.8"	077° 11' 29.7"	1221	9	18	23	41
10	Cherumankal	08° 36' 16.3"	077° 11' 58.4"	2425	13	18	21	39

sampling unit, between 09.00 and 12.00 h, using aspirator and employing trained insect collectors/technical personnel. Light traps (3 numbers each indoors and outdoors) were installed and activated between 17.00 (previous day) and 10.00 h (next day), using 12 W batteries. Sticky traps, (5 numbers each indoors and outdoors) made of white sheet (30 cm × 21 cm), smeared with castor oil were placed, indoors and outdoors at suspected breeding sites of sand flies. Due to difficulty in transport and communication, apart from the threat of wild animals, sand flies sampling time were altered, depending upon the local situation. Both male and female sand fly specimens collected from all types of collections were identified referring standard keys (Lewis, 1978; Kalra and Bang, 1988; Artemiev, 1978). Density of female sandflies from handheld aspirator was expressed as the number of females collected per Man-Hour (MHR), while the density of sandflies obtained from light traps and sticky traps was stated as the number of females per trap night and the number of females per sticky trap, respectively.

2.2. Distribution in relation to height of resting shelter

Distribution of sand flies in the interior walls of human dwellings, at different height intervals was determined based on the number of sand flies obtained, at each particular height interval. To begin with, 2 huts and 2 brick houses were randomly selected for sand fly collection in each settlement. Height of the wall from ground level to the ceiling, both in huts and brick houses were divided into six levels viz., 0 ft, 0–2 ft, 2–4 ft, 4–6 ft, 6–8 ft and >8 ft including ceiling. Using hand-held mechanical aspirator, sand flies resting on interior walls were collected simultaneously by six insect collectors/technical personnel, at all height intervals. Care was taken to collect sand flies resting at each specific height interval, without overlapping to adjacent levels.

Table 2
Species composition (%) of sand flies.

Sl no.	Sand fly species	Indoor (n = 6956)	Outdoor (n = 917)
1	<i>Ph. (Eup.) argentipes</i>	24.52	0.83
2	<i>Ph. (Ana.) colabaensis</i>	6.30	–
3	<i>Ph. (Ana.) stantoni</i>	0.49	0.15
4	<i>Se. (Neo.) zeylanica</i>	10.38	2.12
5	<i>Se. (Neo.) monticola</i>	5.80	1.48
6	<i>Se. (Neo.) malabarica</i>	0.40	0.42
7	<i>Se. (Neo.) dhandai</i>	0.30	0.01
8	<i>Se. (Neo.) arbores</i>	0.04	0.25
9	<i>Se. (nic nic gr.) bailyi</i>	0.20	0.18
10	<i>Se. (Par.) baghdadis</i>	31.86	3.12
11	<i>Se. (Par.) babu babu</i>	3.64	1.70
12	<i>Se. (Par.) shorttii</i>	3.00	0.04
13	<i>Se. (Par.) babu insularis</i>	0.32	0.23
14	<i>Se. (Par.) barraudi</i>	0.30	–
15	<i>Se. (Par.) rectangulata</i>	0.22	0.16
16	<i>Se. (Par.) jerighatiensis</i>	0.18	0.55
17	<i>Se. (Par.) kauli</i>	0.06	0.41
18	<i>Se. (Sin.) eadithae</i>	0.29	–
19	<i>Se. (Sin.) hospitii</i>	0.04	–

2.3. Resting habit and blood meal source

Abdominal conditions of female sand flies collected from indoors and outdoors using hand-held aspirators were classified into un-fed (UF), full-fed (FF), half-gravid (HG) and Gravid (G), following Sella's method, described for mosquitoes (WHO, 1975). Based on the proportion of females with different abdominal stages in the human dwelling and outdoor habitats, the endophilic and/or exophilic behaviour was ascertained. Blood meal sources of wild caught female sand flies were detected subjecting the gut contents of freshly engorged specimens to agar gel immuno diffusion test, described by WHO (1987) and Crans (1969); and modified by Bheema Rao (1984). Antisera of human, bovine, canine, avian, and murine (rodent) used in the assay, were obtained from M/S Sigma-Aldrich, Steinheim.

3. Result

A total of 7874 sand fly specimens, comprising 1666 males and 6208 females was collected, employing hand-held aspirators, light traps and sticky traps. Of the three methods employed in the sand fly survey, hand-held aspirator collection yielded significantly more number sand fly specimens (87.7%), followed by light traps (8.5%) and sticky-traps (3.8%). The entire collections yielded 19 species of sand flies, which were given below in order of abundance: *Sergentomyia (Paratomyia) baghdadis* (34.99%), *Phlebotomus (Euphlebotomus) argentipes* (25.35%), *Sergentomyia (Neophlebotomus) zeylanica* (12.5%), *Sergentomyia (Neophlebotomus) monticola* (7.28%), *Phlebotomus (Anaphlebotomus) colabaensis* (6.30%), *Sergentomyia (Paratomyia) babu* (5.34%), *Sergentomyia (Paratomyia) shorttii* (3.04%), *Sergentomyia (Neophlebotomus) malabarica* (0.82%), *Sergentomyia (Paratomyia) jerighatiensis* (0.73%), *Phlebotomus (Anaphlebotomus) stantoni* (0.64%), *Sergentomyia (Paratomyia) insularis* (0.55%), *Sergentomyia (Paratomyia) kauli* (0.47%), *Sergentomyia (nic nic gr.) bailyi* (0.38%), *Sergentomyia (Paratomyia) rectangulata* (0.38%), *Sergentomyia (Neophlebotomus) dhandai* (0.31%), *Sergentomyia (Paratomyia) barraudi* (0.3%), *Sergentomyia (Neophlebotomus) arbores* (0.29%), *Sergentomyia (Sintonius) eadithae* (0.29%) and *Sergentomyia (Sintonius) hospitii* (0.04%).

Density of *P. argentipes* (mean ± SD) was 7.2 ± 2.9 (range: 2.9–12.0) females/MHR, in human dwellings (Fig. 2a) and 0.64 ± 0.21 (range: 0.3 and 1.0) females/MHR outdoors (Fig. 2b) in hand-held aspirator collection. Density of sand fly species other than *P. argentipes* was 34.25 ± 11.84 (range: 12.9–62.8) females/MHR, in human dwellings (Fig. 3a) and 3.7 ± 1.24 (range: 2.3–6.7) females/MHR, outdoors (Fig. 3b). In cattle sheds, *P. argentipes* alone was recorded and its density was 27.33 ± 21.08 (range: 4.0–66.0) females/PMR (Fig. 4). Sand flies density measured using hand-held aspirator collections, showed a peak during October in human dwellings and cattle sheds, while during October/November, outdoors. Sex ratio of males and females obtained in hand-held aspirator collections was 1:5.4.

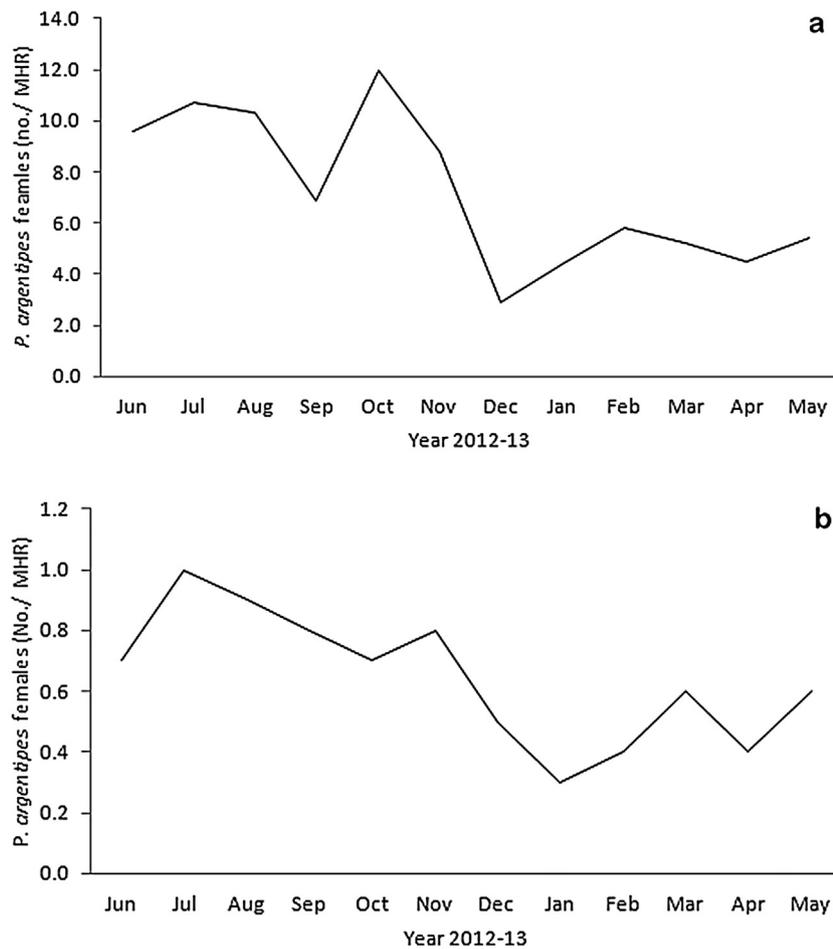


Fig. 2. (a) Density of *P. argentipes* in human dwellings of tribal settlements of the Western Ghats. (b) Density of *P. argentipes* outdoors of tribal settlements of the Western Ghats.

Sand fly density obtained from light traps and sticky traps were given in Table 3. Density of *P. argentipes* in a cattle shed was 7.0 ± 4.4 females/light trap/night, while in human dwellings and outdoors its density was very low. Sticky trap collections yielded a meagre number of sand fly specimens. Both in light trap and sticky trap collections, male specimens outnumbered females. Male to female sex ratio in light trap and sticky trap collections was 1:0.67 and 1:0.56 respectively.

The climate of the study area remains within a narrow fluctuation. The period between March and May is summer, which is having a hot and humid climate. The months from June to September receive south-west monsoon, while October and November experience northeast monsoon. The duration between December and February is relatively cool. Meteorological data for the last decade indicate that the mean minimum temperature was 24.09°C (range: 22.2°C in January– 30.2°C in September) and the mean maximum temperature was 32.1°C (range: 31.1°C in August– 33.8°C in March) (Fig. 5a). Average monthly rainfall

varied between 15.2 mm (February) and 386.9 mm (October), with a mean annual rainfall, 2102.8 mm (Fig. 5b). Relative humidity ranges between 79.1 and 88.5% at 8.30 h and 62.9 and 79.1% at 17.30 h (Fig. 5c).

Sand fly populations were prevalent throughout the year in all the tribal settlements. However, the populations were found to be dynamic and their abundance fluctuated with a change in climatic conditions. *P. argentipes* population showed a declining trend during the latter half of the study period, yielding a trend line $y = -18.706x + 464.09$; $R^2 = 0.3245$, where y = predicted population and x = time period. Multiple regression analysis was carried out with average monthly minimum and maximum temperature, relative humidity at 08.30 h, and at 17.30 h and precipitation as independent variables and *P. argentipes* population as the dependent variable. Backward elimination of the variables indicated that the average monthly precipitation and relative humidity (08.30 h) contributed significantly to the prediction of abundance of *P. argentipes* population ($F = 23.37$; $R^2 = 0.84$, $df = 2, 9$,

Table 3
Sand fly density in human dwellings, cattle sheds and outdoor habitats.

Collection Methods	Species	Habitat	No. of sand flies/light trap/night		No. of sand flies/sticky trap	
			Mean(\pm SD)	Range (min–max)	Mean(\pm SD)	Range (min–max)
Light trap	<i>P. argentipes</i>	Human dwellings	0.2 ± 0.1	0.5–0.1	0.033 ± 0.006	0–0.017
		Outdoors	0.02 ± 0.05	0–2	0.01 ± 0.01	0–0.03
		Cattle sheds	7.0 ± 4.4	17–3	0.01 ± 0.01	0–0.04
Sticky trap	Other species	Human dwellings	0.78 ± 1.1	1.7–0.2	0.036 ± 0.035	0–0.13
		Outdoors	0.32 ± 0.18	0.6–0.2	0.091 ± 0.05	0–0.21
		Cattle sheds	–	–	–	–

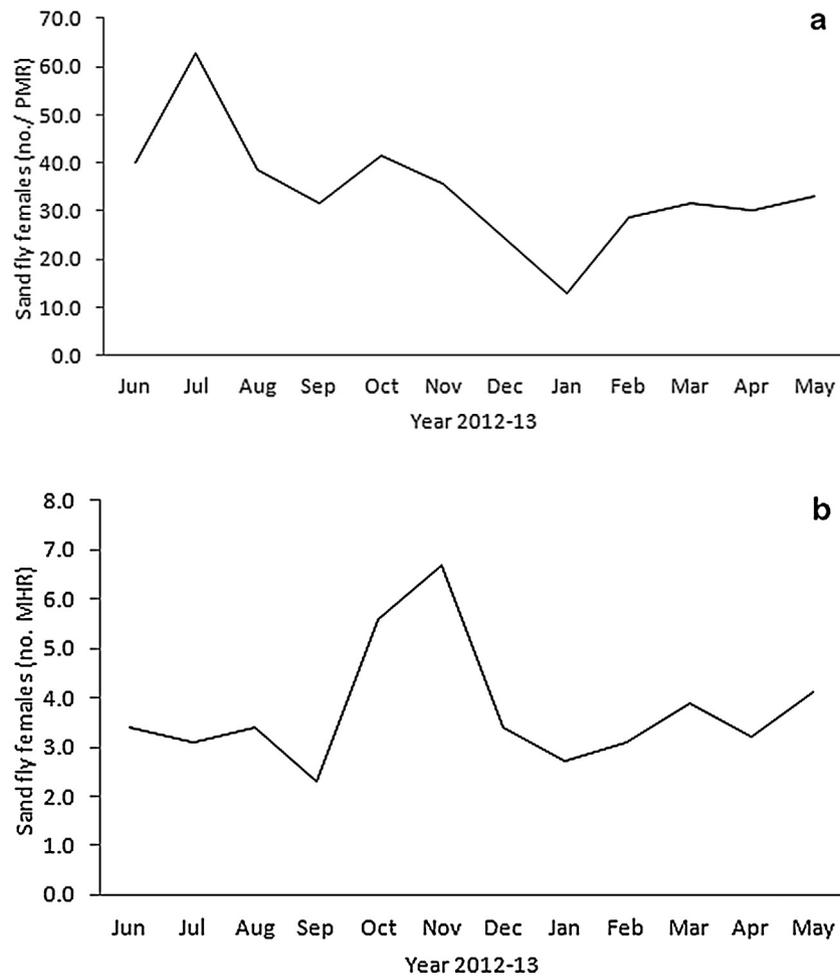


Fig. 3. (a) Density of sand flies other than *P. argentipes* indoors of tribal settlements of the Western Ghats. (b) Density of sand flies other than *P. argentipes* outdoors of tribal settlements of the Western Ghats.

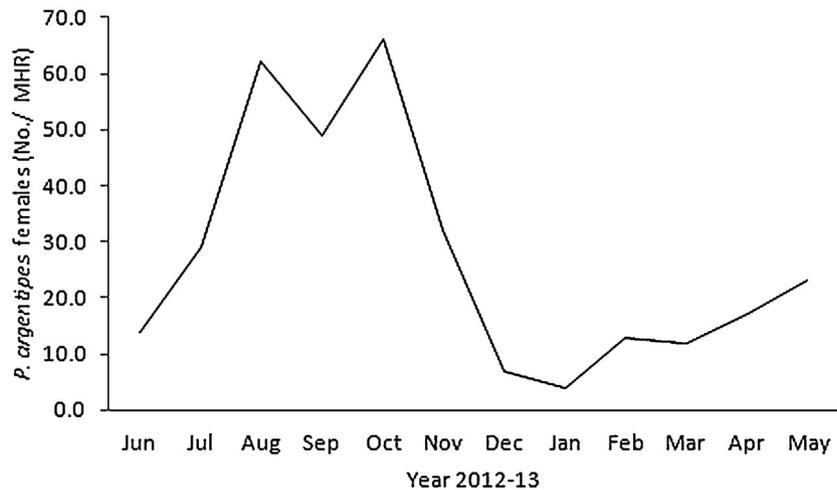


Fig. 4. *P. argentipes* density in cattle shed in tribal settlements of the Western Ghats.

$p=0.001$). These two variables yielded a regression equation $y = -0.350.21 + 0.246x_1 + 4.768x_2$, where y is the density of *P. argentipes* obtained per month, x_1 is the precipitation and x_2 is the relative humidity at 08.30h, respectively. Slopes of these two variables showed a positive correlation with *P. argentipes* population (precipitation: $t = 3.53$, $p = 0.006$; relative humidity at 08.30 h: $t = 2.677$, $p = 0.022$). There was no significant correlation with minimum and maximum temperature and relative humidity at 17.30 h ($p > 0.2$).

Sand fly species other than *P. argentipes* population gave a trend line $y = -6.0105x + 111.15$; $R^2 = 0.5374$ and these populations were stable with a little fluctuation during different seasons. Multiple regression analysis was also carried out with the average monthly minimum and maximum temperature, relative humidity both at 08.30 h and at 17.30 h and precipitation as independent variables and sand fly populations other than *P. argentipes* as dependent variable. Backward elimination indicated that the average monthly precipitation and relative humidity, collected during

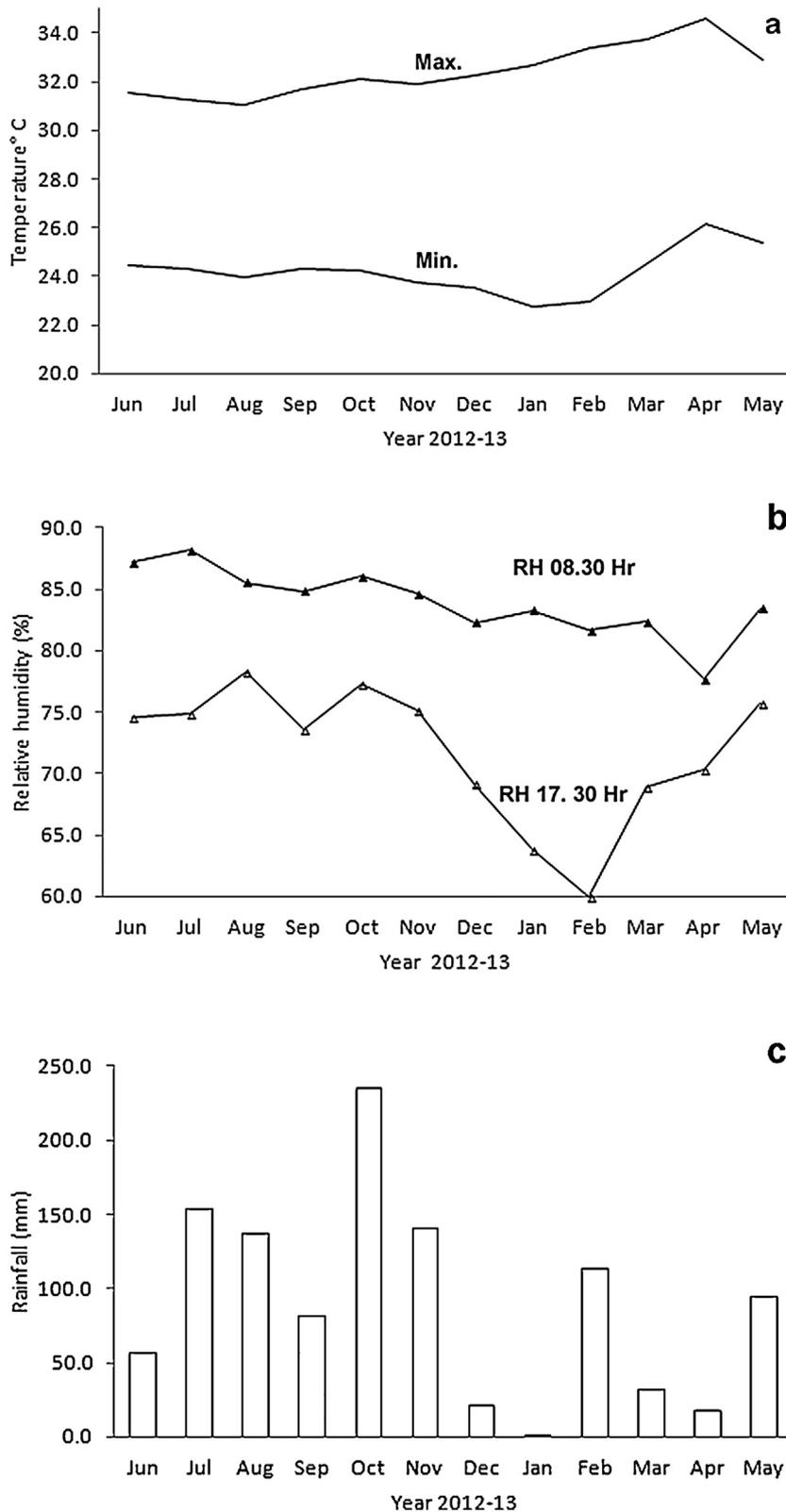


Fig. 5. (a) Temperature (mean minimum and mean maximum) recorded during the study period in tribal settlements of the Western Ghats. (b) Relative humidity (08.30 h and 17.30 h) recorded during the study period in tribal settlements of the Western Ghats. (c) Rainfall recorded during the study period in tribal settlements of the Western Ghats.

morning hours (08.30 h) contributed significantly to the prediction of abundance of sand fly species other than *P. argentipes* population ($F=8.14$; $R^2=0.0.80$, $df=2, 9$, $p=0.01$). These two variables yielded a regression equation $y = -3892.88 + 61.72x_1 + 32.966x_2$, where y is the density of sand fly population other than *P. argentipes* obtained

per month during the study period, x_1 is the precipitation and x_2 is the relative humidity at 08.30 h, respectively. Slopes of these two variables showed a positive correlation with sand fly population other than *P. argentipes* (precipitation: $t=2.45$, $p=0.037$; relative humidity at 08.30 h: $t=3.75$, $p=0.005$). As observed in

Table 4
Host feeding pattern sand fly females in Tribal settlements of the Western Ghats ($n = 160$).

No. of females Fed on	Antiserum tested	Species of sand fly females tested blood meal source					
		<i>P. argentipes</i>	<i>P. colabaensis</i>	<i>S. baghdadis</i>	<i>S. babu</i>	<i>S. zeylonica</i>	<i>S. shorttii</i>
Single host ($n = 113$)	Human	16.7	1.8	2.7	8.0	13.3	–
	Cattle	23.9	–	–	–	–	–
	Dog	15.0	–	–	–	–	–
	Rodent	2.7	3.5	–	2.7	9.7	–
	Fowl	–	–	–	–	–	–
Double host ($n = 39$)	Human & dog	30.7	2.6	–	15.4	7.7	12.8
	Bovine & rat	2.6	–	7.7	–	–	–
	Human & rat	5.1	–	2.6	–	–	2.6
	Rat & dog	–	–	–	10.2	–	–
Triple host ($n = 8$)	Human, rat & dog	12.5	37.5	–	12.5	–	12.5
	Rat, dog & fowl	–	12.5	–	12.5	–	–

P. argentipes population, there was no significant correlation with other variables such as minimum and maximum temperature and relative humidity at 17.30 h ($p > 0.2$) to sandfly species other than *P. argentipes*.

Fully engorged female sand flies ($n = 189$) subjected to blood meal identification, 84.7% ($n = 160$) showed reaction with the antiserum of different vertebrate host and formed a precipitin band, while 15.3% ($n = 29$) did not show any reaction. Among the blood samples which showed a reaction, 70.6% ($n = 113$) were positive for a single host blood meal, while 24.4% ($n = 39$) for double and 5.0% ($n = 8$) for a triple host blood meal (Table 4). Blood samples which showed reaction to the single host blood meal on further analyses, 42.5% were found positive to human blood antiserum, 23.9% of bovine, 15.0% of dog, 18.6% to rodent and none to avian. When the validity of the result was tested using χ^2 test, the number of females found engorged with human blood was significantly higher ($p < 0.05$) than that fed on other hosts, indicating that the majority of the sand flies species prevalent in tribal settlements were anthropophilic in behaviour (Table 4).

Resting populations of sand flies were obtained from interior walls, at all the height intervals, both in huts and RCC houses. In RCC houses, sand flies were also collected from the interior ceiling. However, no sand fly was seen resting either on furniture or hanging objects, including cloth materials. There was no significant difference ($t = 11.07$, $p = 0.0001$) in the number of sand flies collected from both huts and RCC houses. Overall, sand flies abundance was significantly higher indoors (88.35%) (7.8 females/MHR in huts and 6.0 females/MHR IN RCC houses) than outdoors (11.65%) (Table 2). *P. argentipes* population collected from indoors constituted a more number of females ($n = 893$; males = 18.7% and females = 81.3%), while outdoors ($n = 52$; males 78.8% and females = 21.2%) constituted a number of males. The corresponding figure for sand fly species other than *P. argentipes* were $n = 2736$ (males 25.4% and females 74.6%) indoors and $n = 941$ (males 59.3% and females 40.7%), outdoor, respectively.

The proportion of females with different Sella's stages of abdominal conditions (UF, FF, HG and G) showed a significant variation in relation to the height of the resting surfaces ($\chi^2 = 32.4$, $p = 0.006$). Density of resting population of *P. argentipes* (no. of females/PMR) at various height intervals in human dwellings differed significantly with various height levels during summer (one-way ANOVA: $F = 83.7$; $df = 5.12$; $p = 0.001$) and rainy seasons (one-way ANOVA: $F = 41.4$; $df = 5.24$; $p = 0.001$) but not in cooler months (one-way ANOVA: $F = 1.67$; $df = 5.18$; $p = 0.2$). Post-hoc ANOVA test based on least significant difference (LSD) showed that the density was significantly higher ($p < 0.05$ for all comparisons) at the height levels of 6–8 ft (mean \pm SD: 25.7 ± 4.0) and >8 ft including ceiling (48.3 ± 5.1) than that of the other height levels viz., 0 ft (6.7 ± 3.2), 0–2 ft (4.0 ± 3.0), 2–4 ft (0.7 ± 1.2), 4–6 ft (5.3 ± 3.2) in interior walls, during summer season. A similar analysis showed that during rainy

season the density was significantly higher ($p < 0.05$ for all comparisons) at the height levels of 0 ft (33.6 ± 7.2) and 0–2 ft (20.0 ± 7.5) than that of the height levels such as 2–4 ft (7.4 ± 3.6), 4–6 ft (2.2 ± 1.3), 6–8 ft (1.0 ± 1.0) and >8 ft (1.6 ± 1.5). In cooler months, the density (mean \pm SD) at various height levels was as follows, 0 ft (7.5 ± 2.6), 0–2 ft (6.3 ± 4.7), 2–4 ft (2.5 ± 1.9), 4–6 ft (3.8 ± 2.4), 6–8 ft (5.8 ± 1.9) and >8 ft (5.5 ± 2.1).

A total of 3161 female specimens collected from both indoors ($n = 2767$) and outdoors ($n = 394$), were examined for the abdominal conditions based on Sella's stage classification. Composition of UF, FF, HG and G stages of females obtained from indoors were 25.1%, 39.7%, 22.5% and 12.7% respectively and the corresponding figures of females, obtained from outdoors were 65.1%, 2.7%, 7.5% and 24.7% respectively. Prevalence of females with all stages of abdominal conditions indoors, indicate the majority of females spent the entire gonotrophic cycle indoors. The distribution of abdominal conditions of *P. argentipes* females differed significantly between indoors and outdoors (χ^2 test, $p < 0.0001$ for all seasons). While, unfed females were significantly higher (χ^2 test, $p < 0.0001$) outdoor than indoor, FF and HG were higher indoor than outdoor in all seasons (χ^2 test, $p < 0.0001$). Although gravid females were higher (χ^2 test, $p < 0.0001$) outdoor than indoor in monsoon and summer seasons, they were significantly higher indoor than outdoor in cooler months (χ^2 test, $p < 0.05$). Perhaps, newly enclosed females that emerge from adjoining breeding habitats and gravid females frequenting breeding habitats to deposit eggs were predominant outdoors.

4. Discussion

Several sampling methods are being employed to monitor the sand fly density, (Hati et al., 1987; Rotureau et al., 2006; Dinesh et al., 2008; Poché et al., 2011). However, the types of sampling methods influences the diversity of sand fly species captured, as a standardized technique to compare between sand fly abundance and climatic variations differs with the situation (WHO, 1984). Killick-Kendrick (1987) has documented that human-landing, sticky-trap and light-trap collections were the standard sampling techniques for adult sand fly survey. Das and Ramaiah (2002) disagreed that human-landing collection of medically important insects is regarded unethical, due to risk of volunteers being infected with vector-borne diseases. Both sticky-trap and light-trap methods have been recommended for monitoring sand fly abundance (Hoel et al., 2007; Orshan et al., 2010). Yet, Burkett et al. (2007) has emphasized that use of sticky trap and light trap attracted sand flies only from their immediate breeding sites and accordingly tend to yield a relatively low number of sand fly specimens. Alexander (2000) has documented that sand flies that show positive phototropism alone were attracted to light traps. Despite, light trap was reported to be effective in capturing sand flies in

habitats which were difficult to reach (Rotureau et al., 2006). Hati et al. (1987) compared the efficacy of hand-capture technique, human landing collection, sticky-trap and light trap in sand fly collection, in West Bengal, India and found that hand-capture technique using an aspirator was effective method, in measuring the sand fly density. In the present, study hand-held aspirator collection was found to yield significantly more number of sand fly specimens ($p=0.0001$) than light traps and sticky traps.

Distribution of sand flies varies depending upon the environmental factors such as rainfall, temperature and humidity; and physical factors such as geographical barriers and habitat availability (Srinivasan et al., 2013). Besides, abundance of vegetation, vertebrate host (Ozbel et al., 2011) and human exploitation of the environment (Srinivasan and Subramanian, 2015) also influence the sand fly abundance. Distribution of sand flies has been reported from different physiographic zones of India by several workers and these studies mainly focus on species composition and relative density of sand flies in different seasons (Lodha et al., 1971; Sharma et al., 1973; Kalra and Lewis, 1976; Dhanda et al., 1983; Kalra et al., 1986; Kumar et al., 1992; Bansal et al., 1996; Srinivasan et al., 1993, 2013; Dinesh et al., 2005). In the present investigation, we have observed that rainfall and humidity were found to influence the sand fly abundance in the southernmost part of the Western Ghats in this country.

P. argentipes and other sand fly species were found to be abundant among tribal dwellings in the study areas and completed their gonotrophic cycle indoors. The dwellings which were made of mud and other natural plant materials were providing adequate moisture, warmth and protection from sunlight. Hence, sand fly females with all abdominal stages were found indoors, suggesting their endophilic behaviour in this region. Parallel observation conducted in kala azar endemic region of Bihar indicated that *P. argentipes* preferred cattle sheds than human dwellings for resting (Hati, 1983; Dinesh et al., 2001). Sanyal et al. (1979), Kumar et al. (1995) in kala azar endemic areas in India.

The resting behaviour of sand flies for a given landscape is crucial in designing site specific intervention measures. Hati et al. (1991) from West Bengal in India had reported that resting population of sand flies were found restricted to the lower half of the interior walls, indoor, during different seasons. Based on these results, the Directorate of National Vector Borne Disease Control Program (NVBDCP guidelines) has recommended, Indoor Residual Spraying (IRS) of chemical insecticides (IRS) on walls, in human dwellings, restricting up to 6 ft height from ground level, to control sand fly vectors, in kala azar endemic areas. Contrary to this finding, in the present study, sand flies were found resting on walls, at all heights, with a maximum number at height level 6–8 ft and >8 ft, including ceiling, in summer and on the lower half of the wall particularly at 0 and 0–2 ft height level in monsoon. The current report suggests while designing sand fly adult control measure involving indoor residual spraying (IRS) of insecticides for the control of CL transmission, the entire interior wall surface in human dwellings, including ceiling needs to be targeted for effective control of the vector population.

In the northern part of India, which has winter, monsoon and summer season, sand flies were abundant during monsoon season alone. Dhanda and Modi (1971) from Aurangabad district, Maharashtra and Joseph (1981) from Kala azar affected areas of northern districts of Bihar and Tiwary et al. (2013) from VL endemic areas of Muzaffarpur in Bihar, India substantiated that sand flies were abundant with a single peak in monsoon month of October. In the present investigation, sand flies including *P. argentipes* were found prevalent throughout the year, with two peaks, a minor peak in June/July which was in the south-west monsoon period and a major peak in October, in northeast monsoon season, where there was no winter season, however, the month the period between December

and February were relatively cooler in this region. Since the sand fly abundance has a direct relation to CL incident, information generated on the seasonal dynamics of sand flies in this study is of immense importance in deciding the time of implementation of intervention measures.

Picado et al. (2010) studied the seasonal pattern of sand fly population in Nepal and they have observed that sand flies were positively associated with temperature and negatively associated with rainfall. In a coffee agroecosystem in Mexico, sand flies of the genus *Lutzomyia* showed positive correlation with temperature and negative correlation with rainfall (Jeanneth Pérez et al., 2014). However, in the present study *P. argentipes*, the wet zone hygroscopic species, which absorb moisture from atmospheric air were abundant during monsoon season in these settlements. Moreover, this vector species was profuse in forest regions, coastal areas and Indo-Gangetic plains, having humid air; and rare in the desert region of western part of India which was semiarid, with little humidity. The vectors of *L. tropica* such as *Phlebotomus papatasi* and *P. salehi*, are thermophilic species which prefer dry weather, with little moisture in the air and abundant in the desert region of Rajasthan, are totally absent in this tribal belt. *P. argentipes*, which shows a predilection towards wet zone, was abundant in all the months, with a maximum during monsoon months in the reserve forest regions of southernmost part of the Western Ghats. Sand flies were reported to feed on hosts which were found in the vicinity of their breeding habitats (Ghosh et al., 1999). In Kani tribe settlements, although wild caught female sand flies were found engorged on human, dogs and rodents blood, significantly more number of *P. argentipes* females were found to have engorged with human blood, compared that reported from endemic areas of Kala azar in India (Dhanda et al., 1983). Sand fly abundance throughout the year with anthrophagous and endophilic behaviour, conducive climatic conditions with optimum temperature, high rainfall and relative humidity; and occurrence of CL cases among the Kani tribes indicate that the tribes are under risk of CL infection. However, no intervention measure exists in this area. In view of the occurrence of CL infection among the tribes, the comprehensive information generated on ecological aspects of sand flies in this complex environment provides the means and methods to design intervention measures.

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Conflict of interest

None.

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