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Seasonal distribution of phlebotomine sand flies (Diptera: Psychodidae) in Tham Phra Phothisat temple, Saraburi province, Thailand

Polseela, R.[1*], Apiwathnasorn, C.[2] and Samung, Y.[2]
[1] Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok,
[2] Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand
[*] Corresponding author email: raxsinap@nu.ac.th, polseela@hotmail.com
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Abstract. Phlebotomine sand flies have long been incriminated as vectors of leishmaniasis in various parts of both the Old and New World. Prompted by recent indigenous cases of leishmaniasis in Thailand, a bionomic study of sand flies was undertaken in Tham Phra Phothisat temple, Saraburi province. In this study, sand flies were collected using Centers for Disease Control (CDC) light traps, to clarify the activity patterns and species composition of the sand flies. Traps were laid from August 2005 to July 2006. The insects were collected monthly between 1800-0600 hours. A total of 8,131 sand flies were collected with a female:male ratio of 1.9:1. Sixteen species were identified, of which 5 belonged to the genus *Phlebotomus*, 9 to *Sergentomyia* and 1 to *Chinius*. Species comprised the abundant species (*Sergentomyia silvatica* 35.6%, *Sergentomyia barraudi* 18.1%, *Sergentomyia anodontis*, 17.1%, *Sergentomyia iyengari* 11.9%, and *Sergentomyia gemmea* 11.2%); the less common species (<2%) were *Sergentomyia dentata* 1.8%, *Phlebotomus stantoni* 1.1%, *Sergentomyia indica* 1.0%, *Phlebotomus argentipes* 0.8%, *Sergentomyia perturbans* 0.4%, *Chinius barbazani* 0.3%, *Phlebotomus asperulus* 0.2%, *Phlebotomus philippinensis gouldi* 0.1%, *Phlebotomus major major* 0.1%, *Sergentomyia quatei* 0.1% and *Sergentomyia bailyi* 0.1%. The results revealed seasonal variation in sand fly prevalence, with the highest peak in July. Soil samples collected were characterized by alkaline (pH 7.6).

INTRODUCTION

Phlebotomine sand flies are known to transmit leishmaniasis, bacteria and viruses that affect humans and animals in many countries worldwide. Phlebotomine sand flies are small bloodsucking insects of which there are about 700 species and around 30 of these are considered to be disease vectors. Species of the old world genus *Phlebotomus* are vectors of vertebrate pathogens, including zoonotic *Leishmania* spp., which affects people in more than 80 countries (Desjeuk, 2004). An estimated 200 million people are at risk of kala-azar and 500,000 new human kala-azar cases are registered annually (Desjeuk, 2001). Visceral leishmaniasis or Kala-azar is a severe and often fatal in the Mediterranean, Central Asia, Africa, South and Central America. In India, Nepal, and Bangladesh, *Leishmania donovani* is transmitted exclusively by *Phlebotomus argentipes*, a sand fly that is considered anthroponotic (Swaminath *et al.*, 1942; Kumar *et al.*, 2001). In Thailand, leishmaniasis is a growing public health problem and recently, at least 10 cases of autochthonous visceral leishmaniasis have been found in various regions of the country. Population dynamic of sand flies in their habitats can be in different areas, including rain forests, desert, rural, urban, sylvatic and domestic areas in Asia, Africa, Europe and South America (Magill *et al.*, 1993).

In Asia, phlebotomine sand flies were collected in Pakistan, Malaysia, Bangladesh,

and India (Lewis, 1967; Rudnick *et al*., 1971; Ismill *et al*., 1993; Singh & Singh, 2009). The sand flies vector found in Thailand comprise 24 species from 1934-2009 including *Phlebotomus argentipes* in difference areas. The distribution and abundance of sand flies seem to be influenced by edaphic factors.

The present study was undertaken to understand the seasonal distribution of phlebotomine sand flies. Breeding sites and soil characterization, was studied in variations of soil and its relationship with soil pH conditions relevant to breeding. In this way, the study will be on the alert of sand fly distribution in this area.

MATERIALS AND METHODS

Study area

The entomological survey was carried out from August 2005 to July 2006 at Tham Phra Phothisat temple, Thap Kwang Subdistrict, Kaeng Khoi District, Saraburi Province, Thailand which is 140 km north of Bangkok and is situated near Namphu hill at 73% 11'12"N,

161% 25'47"E (Fig. 1). Besides many visitors, there are many sand fly hosts including dogs, monkeys, cats and rodents. The site is a mountainous area 165 meter above sea level and covered with vegetation typical of a tropical rain forest further humidified by a waterfall and canals. Rainfall data were provided by the Thai Meteorological Department. Daily temperature and relative humidity were recorded hourly from 06:00PM to 06:00AM by hydro-hygrometer. Used only during one night in each month when the trap were set to investigation.

Specimen collection and Identification

Adult sand flies were collected one night per month by Centers for Disease Control CDC miniature light traps overnight (06:00PM-06:00AM) and set up about 1.5 m above ground (Sudia & Chamberlain, 1962). The sand flies were captured at the same location and period. Twelve trap locations (100-300

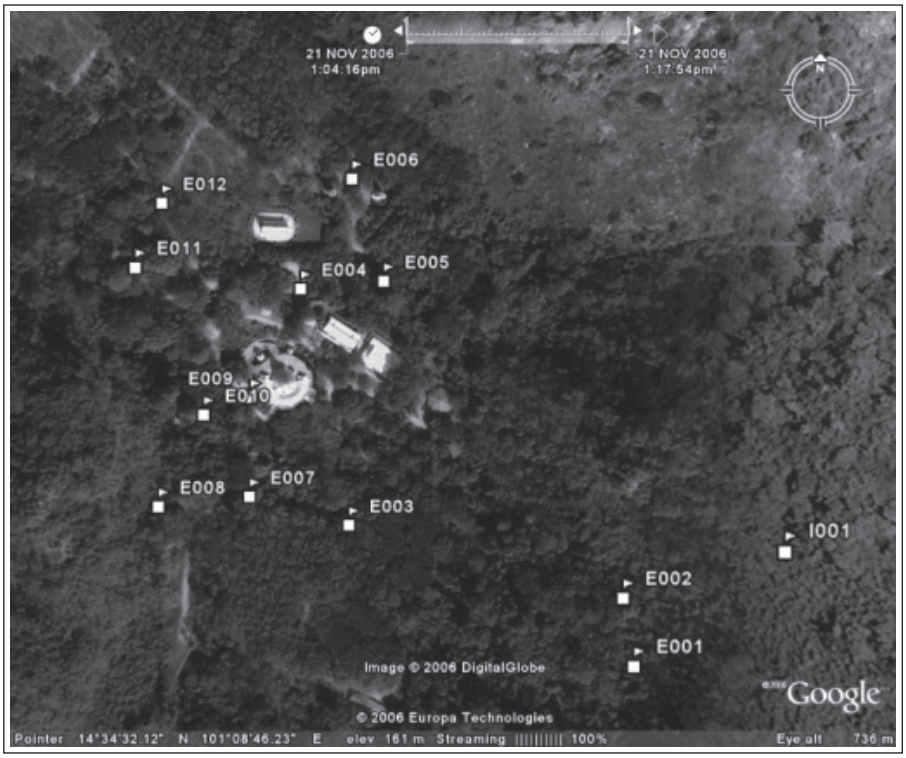

Figure 1. Satellite map showing locations of collected adult sand flies by CDC light trap at Tham Phra Phothisat temple (E001-E012)

m apart) were selected in differences areas such as; near toilet (E001), rock fissure (E002), near tree hole (E003), near kitchen (E004), monkey habitation (E005), near old wood (E006), animal burrows (E007), under up stair (E008), near dog cage (E009), near bridge with cracks in the ground (E010), beside the canal (E011) and termite mounds (E012). The collected sand flies were preserved in 80% alcohol and only female sand flies were mounted on glass slides using Hoyer's medium. The males of many species are useful for confirming the identification of females and provide no other useful information (Lewis, 1978). Identification was based on the morphology of the female spermatheca and pharynx and by taxonomic keys, particularly those of Theodor (1958), Lewis (1978), Artemiev (1980), Lewis (1982), Killick-Kendrick *et al.* (1991) and other related keys.

Soil samples

Soil samples were collected from the temple following the LaMotte Soil Handbook with some modifications (LaMotte, 2001). The criteria to select the variables of the moisture soil for study must be that it should be near CDC light traps. A shovel was used to collect samples of the top soil layer to a depth of approximately 30 cm. Four soil samples were collected from each area from twelve locations to produce average samples of 1 kilogram/location (Kankaew, 2005). Each soil sample was broken down together. The foreign materials removed and air-dried. The samples were then ground by a stone pestle and screened through a wire sifter (80 mesh sizes). Fine soil samples were extracted and tested following the LaMotte Model STH series instruction manual with some modifications.

RESULTS

A total of 8,131 phlebotomine sand flies were obtained from the collection site (Table 1). Sixteen species were identified, of which five species belong to the genus *Phlebotomus*, ten species to the genus *Sergentomyia* and one species to the genus *Chinius*. The relative abundance of the main species was *Sergentomyia silvatica* 35.6% while *P. argentipes* the proven vector of *L. donovani* accounted for 0.8%. The greatest number of specimens were collected in March while *S. silvatica*, *Sergentomyia anodontis*, *Sergentomyia barraudi*, *Sergentomyia gemmea* and *Sergentomyia iyengari* were trapped throughout the year (Table 2). A female to male ratio of 1.9:1 was seen. During July 6 times more females than males were captured. In March, the peak month, was the only month with more males than females and captured the most number of *S. anodontis*. The greatest number of specimens were collected from location near rock fissures (Table 1). *S. anodontis*, *S. barraudi*, *S. gemmea*, *S. iyengari*, *Sergentomyia dentata*, *Sergentomyia indica*, *Phlebotomus stantoni* and *P. argentipes* were found in all locations whereas *S. silvatica* were found in almost locations (Table 3).

The recorded minimum and maximum monthly temperature and relative humidity values ranged between 17.0-33.0°C, and 64-92%, respectively. Total annual rainfall received in the study area was 86 mm ranging from 0 mm to 455 mm. The overall population reached a peak during March and then decreased to a minimum during June (Table 1). The minimum and maximum temperature and relative humidity values ranged between 27.0-33.0°C, and 67.0-72.0% in March. The trap siting appears to be important because some traps, although gathering large numbers of insects, contained few or no sand flies (Table 1). The highest peak of *S. silvatica* was found in July (Fig. 2). There are populations of different species with different seasonal patterns. *Phlebotomus argentipes*, the known vector has its peak in October (Fig. 3). In October, the minimum and maximum temperature and relative humidity values ranged between 23.0-26.0°C, and 90.0-92.0%.

The character of the soil samples from twelve locations hanging CDC light traps were silt clay loam, loam, sandy loam, silt loam, sand loamy, loamy sand, and loamy fine sand with medium to fine texture. The colors observed were light to dark brown with roots, stones, and other foreign materials.

Table 1. The monthly distribution of sand flies captured from various locations in Tham Phra Phothisat temple, Saraburi Province from August 2005 to July 2006

	Month												Male	Female	Total (%)
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul			
Near toilet	23	24	41	35	52	37	115	281	60	123	12	25	398	430	828 (10.2)
Rock fissure	125	368	283	61	27	216	245	146	38	70	11	416	765	1241	2006 (24.7)
Near tree hole	5	7	59	7	28	12	0	51	38	39	15	129	89	301	390 (4.8)
Near kitchen	14	2	17	7	5	0	53	24	31	24	52	72	52	249	301 (3.7)
Monkey habitation	29	32	31	36	3	38	28	177	22	7	2	0	151	254	405 (5.0)
Near old wooden	35	23	18	80	42	122	100	372	73	132	116	43	450	706	1156 (14.2)
Animal burrows	34	23	35	17	12	5	4	169	41	16	17	72	157	288	445 (5.5)
Under upstair	69	38	205	27	29	33	25	168	12	27	42	60	224	511	735 (9.0)
Near dog cage	20	23	94	16	7	7	32	59	38	64	6	3	77	292	369 (4.5)
Near bridge	105	21	10	8	55	8	2	250	45	13	0	28	246	299	545 (6.7)
Beside canal	40	70	7	4	23	22	18	51	35	10	8	15	60	243	303 (3.7)
Termite mounds	72	24	27	28	69	48	109	32	19	148	16	56	163	485	648 (8.0)
Female	365	394	675	240	308	297	413	796	311	456	250	794	–	5,299	5,299 (65.17)
Male	206	261	152	86	44	251	318	984	141	217	47	125	2,832	–	2,832 (34.83)
Total	571	655	786	326	352	548	731	1780	452	673	297	919	–	–	8,131 (100.0)

Table 2. The monthly distribution of female sand flies species captured in Tham Phra Phothisat temple, Saraburi Province from August 2005 to July 2006

Species	Month												Total	%
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul		
S. silvatica	197	195	372	142	0	0	1	3	35	203	101	640	1889	35.6
S. barraudi	60	88	69	37	105	101	118	179	91	39	56	15	958	18.1
S. anodontis	23	21	49	3	87	84	176	369	38	31	15	10	906	17.1
S. iyengari	53	9	48	21	22	48	47	158	86	86	29	23	630	11.9
S. gemmea	17	53	68	21	62	42	26	76	26	83	36	84	594	11.2
S. dentata	3	7	44	4	19	2	15	0	3	0	0	5	102	1.9
P. stantoni	7	15	3	0	12	2	9	3	0	5	2	0	58	1.1
S. indica	0	0	2	5	0	11	9	3	22	1	1	0	54	1.0
P. argentipes	3	2	16	2	0	0	8	1	7	1	0	2	42	0.8
S. perturbans	2	0	4	3	0	0	0	0	0	2	2	10	23	0.4
C. barbazani	0	0	0	0	0	4	2	2	2	1	5	4	20	0.3
P. asperulus	0	0	0	0	0	3	1	0	0	4	2	1	11	0.2
P. philippinensis gouldi	0	3	0	2	0	0	0	1	0	0	0	0	6	0.1
P. major major	0	0	0	0	0	0	0	1	1	0	0	0	2	0.1
S. bailyi	0	1	0	0	1	0	0	0	0	0	0	0	2	0.1
S. quatei	0	0	0	0	0	0	1	0	0	0	1	0	2	0.1
Total	365	394	675	240	308	297	413	796	311	456	250	794	5,299	100.00

Table 3. The locations distribution of female sand flies species captured in Tham Phra Phothisat temple, Saraburi Province from August 2005 to July 2006

Species	Locations												Total	%
	Near toilet	Rock fissure	Near tree hole	Near kitchen	Monkey habitation	Near old woden	Animal burrows	Under upstair	Near dog cage	Near bridge	Beside the canal	Termite mounds		
S. silvatica	118	725	98	59	50	158	77	259	87	66	64	128	1889	35.6
S. barraudi	82	148	56	29	59	185	75	52	38	64	61	109	958	18.1
S. anodontis	106	168	28	40	62	167	38	46	62	50	35	104	906	17.1
S. iyengari	39	36	43	48	37	103	39	71	44	71	33	66	630	11.9
S. gemmea	57	92	61	51	34	50	36	62	38	36	30	47	594	11.2
S. dentata	17	35	3	1	1	7	10	5	14	4	3	2	102	1.9
P. stantoni	1	9	2	4	3	2	3	7	2	3	6	16	58	1.1
S. indica	1	3	1	11	1	17	3	2	1	2	7	5	54	1.0
P. argentipes	6	12	2	1	3	1	5	3	4	3	1	1	42	0.8
S. perturbans	0	5	7	4	1	3	0	1	2	0	0	0	23	0.4
C. barbazani	2	3	0	0	1	8	1	3	0	0	0	2	20	0.3
P. asperulus	1	2	0	1	1	3	0	0	0	0	1	2	11	0.2
P. philippinensis gouldi	0	3	0	0	0	0	0	0	0	0	1	2	6	0.1
P. major major	0	0	0	0	1	1	0	0	0	0	0	0	2	0.1
S. bailyi	0	0	0	0	0	0	1	0	0	0	0	1	2	0.1
S. quatei	0	0	0	0	0	1	0	0	0	0	1	0	2	0.1
Female	430	1241	301	249	254	706	288	511	292	299	243	485	5,299	100.00
Male	398	765	89	52	151	450	157	224	77	246	60	163	2,832	
Total	828	2006	390	301	405	1156	445	735	369	545	303	648	8,131	

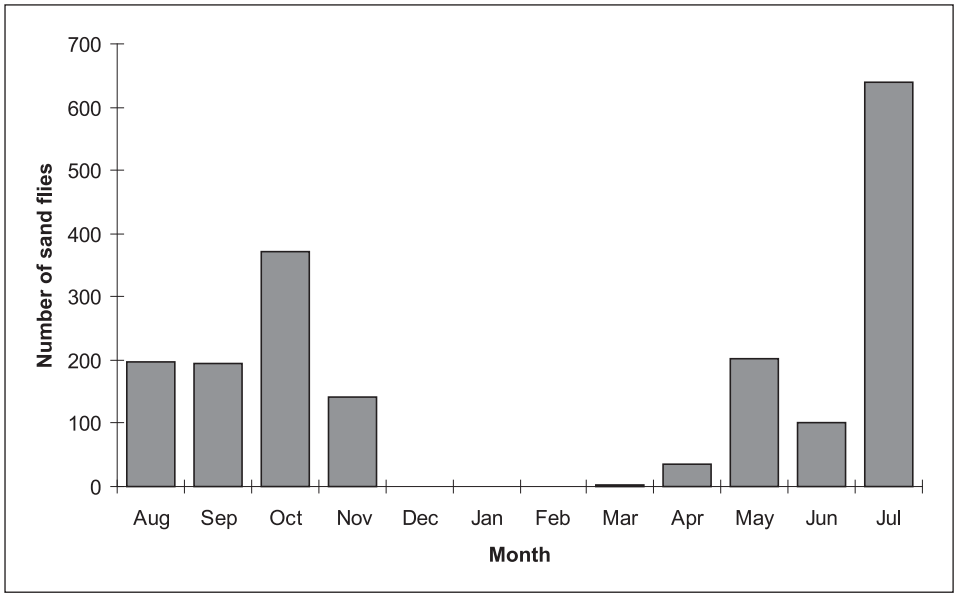

Figure 2. Charts showing the number of *Sergentomyia silvatica* captured in Tham Phra Phothisat temple, Saraburi Province from August 2005 to July 2006

Chemical characteristic of soil samples were determined for all twelve samples and showed high levels of nitrate, phosphorus, potassium, calcium, sulfate and low levels of aluminum, ferric iron, magnesium, manganese and humus (Table 4). The average pH of the soil was 7.6 (pH range= 7.1-7.9).

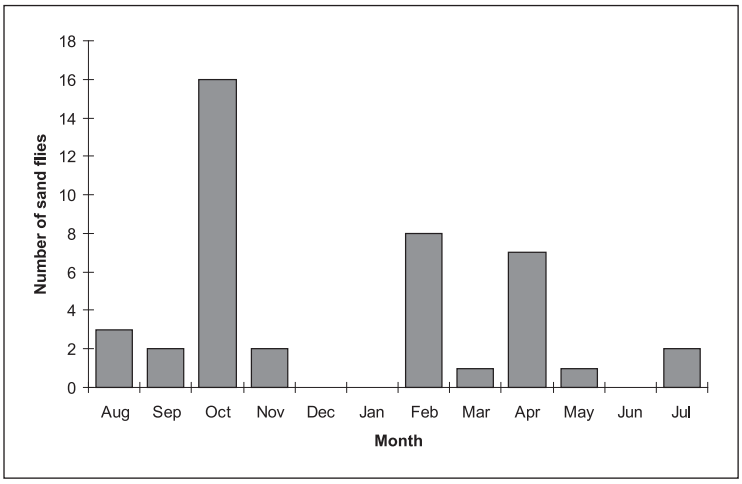

Figure 3. Charts showing the number of *Phlebotomus argentipes* captured in Tham Phra Phothisat temple, Saraburi Province from August 2005 to July 2006

Table 4. Chemical characteristics of soil samples in Tham Phra Phothisat temple, Saraburi Province

Chemical	Mean	Min	Max
pH	7.6	7.1	7.9
Nitrate nitrogen (ppm)	15.4	10	75
Phosphorus (ppm)	25.4	5	100
Potassium (ppm)	364.17	220	400
Aluminum (ppm)	5	5	5
Calcium (ppm)	14,000	14,000	14,000
Ferric iron (ppm)	6.5	2.5	25
Humus (level)	4.4	3	5
Magnesium(ppm)	34.2	25	80
Manganese (ppm)	9.7	5	12
Sulfate (ppm)	50	50	50

DISCUSSION

This study reports the results of an entomological survey for sand fly populations conducted at Tham Phra Phothisat temple using CDC light traps during a 12 month period to document the sand flies species composition and soil chemical analysis. In this study, sixteen species were identified. However, there were no reported new species or new recorded of sand flies. Previous surveys of sand flies in Thailand found twenty-four species: *P. argentipes*, *Phlebotomus philippinensis gouldi*, *Phlebotomus asperulus*, *Phlebotomus hoepplii*, *P. stantoni*, *Phlebotomus major major*, *Phlebotomus teshi*, *Phlebotomus mascomai*, *Phlebotomus barguesae*, *S. barraudi*, *S. anodontis*, *Sergentomyia bailyi*, *S. dentata*, *S. iyengari*, *Sergentomyia mahadevani*, *S. silvatica*, *S. gemmea*, *Sergentomyia hodgsoni hodgsoni*, *S. indica*, *Sergentomyia perturbans*, *Sergentomyia quatei*, *Sergentomyia punjabensis*, *Nemopalpus vietnanmensis*, and *Chinius barbazani* (Raynal & Gaschen, 1934; Causey, 1938; Theodor, 1938; Quate, 1962; Apiwathnasorn *et al.*, 1989; 1993; Depaquit *et al.*, 2006; 2009; Muller *et al.*, 2007). *Phlebotomus argentipes* is the potential vector of leishmaniasis found in this area. Five species of sand flies previously recorded from Bangladesh that included *P. argentipes* and *S. barraudi* was found in Thailand (Ismill *et al.*, 1993). The sand flies vector found in Pakistan comprise 29 species of *Phlebotomus* and ten or more species may be expected to occur, and at least 20 species of *Sergentomyia* (Lewis, 1967). The sand flies that were found in Pakistan: *P. argentipes*, *P. major*, *S. punjabensis*, *S. dentata*, and *S. bailyi* were found in Thailand (Shakila *et al.*, 2006). There are some species present in this area but not others and this may be due to difference in environmental conditions of both areas. The relative abundance of the main species was *S. silvatica* 35.6%. *Phlebotomus argentipes* the proven vector

of *L. donovani* accounted for 0.8%. In this study, *P. argentipes* in temple showed highest peak in October (Rainy season). In Assam, *P. argentipes* was absent during the winter months, with the onset of warm weather, a gradual increase in density takes place till May-June, when decline is noticeable. This is followed by an increase, reaching a maximum during, and just after, the monsoon rains of August-October. A similar trend was noticed in Bihar by Sanyal *et al.* (1979).

The abundance of *P. argentipes* is related to the biology or leishmaniasis risk in this area. All these factors are likely to influence the spread of leishmaniasis. During July, 6 times more females than males were captured. Dramatically increasing number of the female sand flies may be great opportunity for them to have more chance to suck blood which may be involved in the transmission. In March, the peak month, was the only month with more males than females and captured the most number of *S. anodontis*. However, male sand flies are not involved with transmission. Only female sand flies feed on blood of various vertebrate animals including domestic animals, dogs, urban and wild rodent, snakes, lizard and amphibians. A few species feed on birds whereas most species of *Sergentomyia* feed mainly on reptiles and rarely bite man. Alexander (2000) reported that a potential disadvantage of light traps was that they might preferentially sample females of certain species that are highly phototropic. Sand fly would exhibit positive phototaxis and be attracted to light trap (Santos *et al.*, 2002). Previous studies revealed that the effective range of CDC light traps were less than 5 m (Wheeler *et al.*, 1996). Furthermore, it is considered that the migration by flight is necessary for the female sand flies not only to have a blood meal but also to search for their oviposition sites. Therefore, the female may easily tend to be victims of any trap (Eiko *et al.*, 2004). It is known that male sand flies arrive on their host first, form an aggregation and wait for the female for mating (Morrison *et al.*, 1995; Killick-Kendrick, 1999). It is necessary for female sand flies to have a mating before a flight to search for oviposition sites. Anyway, if the actual proportion of females to males is unknown in the natural population by species.

Monthly collection of sand flies demonstrated they were more prominent during the summer (March-May), rainy season (June-October), and cold season (November-February). The highest peak found in March, and lowest peak found that in June. The temple temperature was 17.0-33.0°C with 64.0-92.0% relative humidity (RH). Tropical sand flies tend to feed at a temperature of at least 20°C and generally prefer a RH of 75-80% (Lane, 1987). *Sergentomyia silvatica* population falls to 0 for 2 months of the year when the numbers of *S. anodontis* are highest. On the other hand *S. barraudi* and some of the other species are constant. Optimum temperatures vary among different species of sand flies. In Saudi Arabia, adult sand flies are sensitive to high temperatures and low humidity. With the onset of the cold weather (January to February) there was an absence of adult sand fly population, as sand fly larvae undergo diapauses, permitting them to survive the winter and emerge as adults the following spring with a population increase beginning in most areas by April or May and declining in December (Killick-Kendrick *et al.*, 1985; Theodor 1934, 1936; Ayman *et al.*, 2008). Temperature and humidity are factors closely related to each other and the duration of metamorphosis is conditioned by them (Theodor, 1936). An observation accounted for by the predilection of sand flies to seek microhabitats offering high humidity and stable temperatures while avoiding high temperatures and low relative humidity (Kaul, 1991). The density of sand flies are not always correlated with the rainfall and temperature. Sand flies activity can vary within its geographical range depending on local factors, such as temperature, geographical barriers, habitat availability and the distribution and abundance of vertebrate hosts (Cross *et al.*, 1996; Ghosh *et al.*, 1999).

Soil pH plays an important role in sand fly larvae breeding. In this study, the average pH of the soil was alkaline 7.6 (7.1-7.9). However, in many previous studies soil pH were reported 7.4, 7.7, 7.8, and 7.2-8.5 in

Panama, Saudi Arabia, Italy, and India, respectively. (Rutledge & Mosser, 1972; Buttiker & Lewis, 1979; Bettini & Melis, 1988; Sudhakara *et al*., 2006). The importance of chemical properties of the soil governing the breeding and distribution of sand flies has been emphasized (Sivagnaname & Amalraj, 1997). *Phlebotomus argentipes* thus preferred to breed in the alkaline soil of cattle sheds in Bihar, India (Ram *et al*., 2008). The preference for breeding sites by the sand flies appeared to depend on soil pH. Chemical characteristic of soil samples showed a high levels of nitrate, phosphorus, potassium, calcium, and sulfate. They require moist soil rich in organic and nitrogenous matter to breed (Napier & Smith, 1926; Adler & Theodor, 1957).

Leishmaniasis is an emerging vector-borne disease in Thailand. Knowing the activity of sand flies is important in determining the period of maximum risk of *Leishmania* transmission and for the successful implementation of control program. Using the results of this investigation, health workers in this area may be better able to control and prevent leishmaniasis. At present, *P. argentipes* make up a very small proportion of sand flies and ill-considered proactive interference may disturb this balance in favour of this species and other species as potential vectors. Thus further study followed by the careful design of pilot control measures will be necessary.

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