DISTRIBUTION OF PHLEBOTOMINE SAND FLIES (DIPTERA:PSYCHODIDAE) IN LIMESTONE CAVES, KHAO PATHAWI, UTHAI THANI PROVINCE, THAILAND

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Abstract. This study investigated the species composition and density of the sand flies found inside four limestone caves at Khao Pathawi, Thap Than District, Uthai Thani Province. Sand flies were collected using Centers for Disease Control (CDC) light traps from October 2012 to September 2013. The sand flies were captured between 06:00 PM - 06:00 AM. A total of 11,817 sand flies were collected with a male:female ratio of 1.0:1.2 (5,325:6,492). The specimens were identified as eight species belonging to three genera *Phlebotomus*, *Sergentomyia, Chinius,* and comprised of *S. anodontis, P. argentipes*, *P. stantoni*, *S. barraudi*, *S. silvatica* , *S. gemmea, S. indica,* and *C. barbazani*. *Sergentomyia anodontis* (55.0%) was the predominant species followed by *P. argentipes* (33.6%) and others. Five species of sand fly were found throughout the year in this area: *P. argentipes, P. stantoni, S. anodontis, S. barraudi* and *S. gemmea*. The highest average density of sand flies was found in Ratree cave (35.0 sand flies per trap per night) and lowest in Bandai cave (29.0 sand flies per trap per night). The population of sand fly fluctuated from the highest peak in December (28.5%) to the lowest peak in May (2.3%). The distribution of sand fly species in attraction areas is important for the control program of infection risk of leishmaniasis.

Keywords: sand flies, CDC light traps, leishmaniasis, limestone cave, Thailand

INTRODUCTION

Leishmaniasis is a vector-borne disease caused by the protozoan parasite of *Leishmania* species. The parasite is transmitted by female sand flies, particularly members of genera *Lutzomyia* and *Phlebotomus*. Phlebotomine sand flies (Diptera:Psychodidae) are found in a wide range of habitats. The genus *Phlebotomus* is responsible for the transmission of leishmaniasis in the Old World. About 40 species of *Phlebotomus* spp in the Old World have been proven or suspected to be vectors of *Leishmania* spp (Alexander and Maroli, 2003). *Leishmania* spp have a wide geographic distribution. Some species of sand flies are adapted to a range

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Fig 1–Outside a limestone cave at Khao Pathawi, Thap Than District, Uthai Thani Province.

Fig 2–Inside a limestone cave at Khao Pathawi, Thap Than, District, Uthai Thani Province.

of environmental conditions with a wide distribution. About 30 species of sand flies are proven vectors of at least 20 *Leishmania* species (Emamia and Yazdib, 2008).

In Thailand, there are 5 genera (*Phlebotomus, Sergentomyia, Idiophlebotomus, Chinius* and *Nemopalpus*) including 27 species of sand flies reported from different habitats (Apiwathanasorn *et al*, 1989; 2011). Autochthonous cases of leishmaniasis are caused by *Leishmania donovani, L. infantum* and *L. siamensis* (Kongkaew *et al*, 2007; Maharom *et al*, 2008; Bualert *et al*, 2012). *L. siamensis* is a new species found in Thailand where it is the cause of visceral leishmaniasis (Sukmee *et al*, 2008; Bualert *et al*, 2012). *P. argentipes* is the species of sand flies involved in the transmission of leishmaniasis and it can be found in Thailand. At present, *Sergentomyia gemmea* may be a potential vector of *L. siamensis* which is the cause of visceral leishmaniasis in Thailand (Kanjanopas *et al*, 2013). The sand flies fauna is presented in different locations such as cave, forest, rural, urban, sylvatic and domestic areas (Magill *et al*, 1993). Previous studies have shown a diversity of sand flies in different parts of Thailand. Cave-dwelling phlebotomine sand flies are represented by different species in different sites (Polseela *et al*, 2007; 2011a,b; Shahar *et al*, 2011).

The objective of this study was to determine the species composition and seasonal fluctuations of sand fly species inside caves within a single mountain region. This may assist in planning for programs of prevention and control of leishmaniasis.

MATERIALS AND METHODS

Study area

The study collected sand flies from October 2012 to September 2013 from four limestone caves in Khao Pathawi,

Thap Than District, Uthai Thani Province: Ratree cave, Bandai cave, Aunag cave, and Phuttha Prawat cave. These caves situated on mountainsides at 15°28′40.1″N, 099°45′54.7″E (Figs 1 and 2). All caves are about 10-50 m wide, 15-50 m long and 5-30 m high. There are some birds and bats inside caves. The caves are surrounded by green trees and cover mainly by forest vegetation. The caves are near a village. In this area, measures of temperature and relative humidity were recorded monthly with a digital hygro-thermometer. The average temperature during the study were 25.0-32.4°C. The annual average relative humidity is 54-94%. Rainfall data were provided by the Thai Meteorological Department.

Sand fly collection and identification

A survey of sand flies population was made using 32 miniature CDC light traps one night per month (Sudia and Chamberin,1962). CDC light traps were placed inside the cave at 5-8 m from each location. The trap were set near crack of rock. The traps were operated continuously for 12 hours (06:00 PM to 06:00 AM) and the insects were collected after 12 hours period. During each month of survey in these caves, the sand flies were captured at the same site.

The sand flies were separated from other arthropods under a stereo-microscope and stored in 80% alcohol for identification. Head and thorax of sand flies were separated and mounted with Hoyer's medium on glass slides. Taxonomic keys were used for identifications base on the morphology of female spermatheca, pharyngx, and male genitalia (Lewis, 1978).

RESULTS

Sand fly species composition and sex ratio

A total of 11,817 phlebotomine sand flies were captured from the four caves. The highest peak of activity was found during December and then decreased to the lowest point in May (Tables 1 and 2). Eight species were identified of which 5 belonged to the genus *Sergentomyia*, 2 to the genus *Phlebotomus*, 1 to the genus *Chinius* (Table 2). The greatest number of specimens was collected in December, while *P. argentipes, P. stantoni, S. barraudi,* and *S. anodontis* were trapped throughout the whole year (Table 2). Both male and female flies were attracted by the light. The male to female ratio was 1.0:1.2 (6,492:5,325). The average density was 29.03-35.05 sand flies per light trap per night. The highest average density was found in Ratree cave (35.05 sand flies per trap per night) and the lowest in Bandai cave (29.03 sand flies per trap per night). The known vector, *P. argentipes* had its peak in December (Fig 3). The monthly variation of sand fly density is presented in Fig 4. The minimum and maximum temperature and relative humidity values ranged between 22.5-34.5ºC, and 54-94%, respectively.

DISCUSSION

A survey of sand fly activity was conducted for a 12 month period inside four limestone caves using CDC light traps. The nocturnal activity among the different species of sand flies was studied (Apiwathnasorn *et al*, 1989, 1993; Polseela *et al*, 2007, 2011). The greatest numbers of specimens were collected in December. In this study, 8 species were identified: *P.argentipes, P. stantoni, Sergentomyia anodontis, S. barraudi, S. silvatica, S. gemmea, S. indica,* and *Chinius barbazani.* In Thailand, previous captures of sand flies documented 27 species (Apiwathnasorn *et al*, 1989; 1993; 2011). There are some

Table 1
The number of sand flies captured by CDC light traps in limestone caves at Khao Pathawi, Thap Than District, Uthai Thani Province from October 2012 to September 2013.

Cave	2012			2013									Total	%
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep		
Bandai														
Male	209	483	245	248	57	26	109	74	39	41	52	49	1,632	
Female	294	440	301	280	36	28	91	65	42	52	160	63	1,852	
Total	503	923	546	528	93	54	200	139	81	93	212	112	3,484	29.5
Phuttha Prawat														
Male	169	152	286	248	58	17	52	38	27	39	30	321	1,437	
Female	395	410	544	290	32	18	53	38	48	33	99	210	2,170	
Total	564	562	830	538	90	35	105	76	75	72	129	531	3,607	30.5
Aunag														
Male	90	148	380	66	58	116	18	13	52	31	27	12	1,011	
Female	101	113	506	113	19	142	7	15	53	39	49	34	1,191	
Total	191	261	886	179	77	258	25	28	105	70	76	46	2,202	18.6
Ratree														
Female	110	147	602	118	13	30	14	10	65	62	61	13	1,245	
Male	197	120	509	144	18	43	15	16	37	75	84	21	1,279	
Total	307	267	1,111	262	31	73	29	26	102	137	145	34	2,524	21.4
Total														
Female	578	930	1,513	680	186	189	193	135	183	173	170	395	5,325	45.1
Male	987	1,083	1,860	827	105	231	166	134	180	199	392	328	6,492	54.9
Total	1,565	2,013	3,373	1,507	291	420	359	269	363	372	562	723	11,817	100.0
%	13.2	17.0	28.5	12.8	2.5	3.6	3.0	2.3	3.1	3.1	4.8	6.1	100	
Male:Female													1.0:1.2	

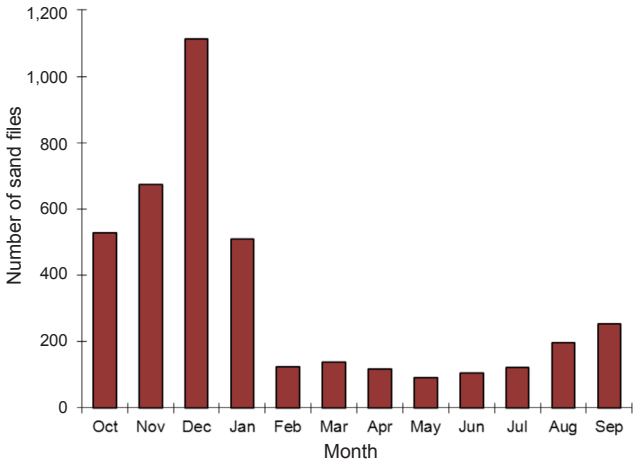

Fig 3–Nocturnal activity of *Phlebotomus argentipes* captured by CDC light traps in limestone caves at Khao Pathawi, Thap Than, Uthai Thani Province from October 2012 to September 2013.

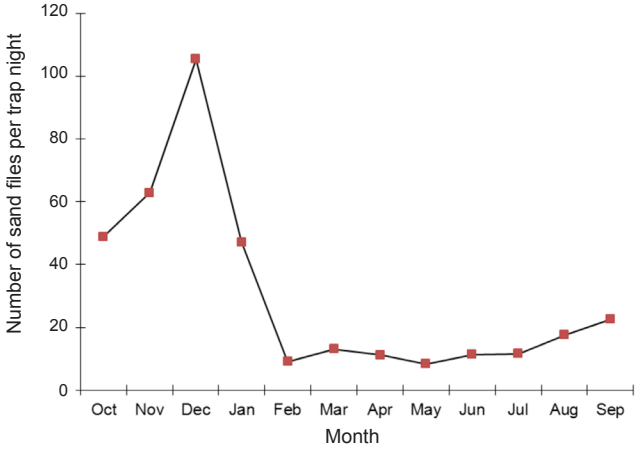

Fig 4–Monthly variation of sand fly density captured by CDC light traps in limestone caves at Khao Pathawi, Thap Than, Uthai Thani Province from October 2012 to September 2013.

species present in this area and others have disappeared, perhaps due to different environmental aspects in each area. *S. barraudi* was the most abundant species (55.0%). *P. argentipes,* a proven vector of *Leishmania donovani*, accounted for 33.6%. *P. argentipes* showed its highest peak in December. The greatest number of specimens was collected from a location near rock fissure in the cave. The density variation of sand fly observed in this study may indicate the present of sand flies to survive in the different caves. Sand flies in Phra Phothisat cave in Saraburi Province had their highest peak in July (436 sand flies per light trap per night) (Polseela *et al*, 2007). Currently there are two sand fly vectors of visceral leishmaniasis in other countries known in Thailand: *P. argentipes* and *P. major major* (Swaminath *et al*, 2006; Azizi *et al*, 2008). Of these, we found *P. argentipes* sand fly in this study. Thus a clear demarcation of the geographic limits of sand fly species and activity was demonstrated, according to the type of cave and different environments of this area. The findings also indicate a health risk for both locals and visitors to the caves in this area.

Our previous study in Phra Phothisat cave and Tham Phra Pho Thisat Temple that had their highest peak in July and March (Polseela *et al*, 2007, 2011a). Thirteen species were found in Phra Phothisat cave, and 16 species were found in Tham Phra Pho Thisat Temple, Saraburi Province. There are some differences in the species composition found in difference area. *P. argentipes* was found at the level of 0.8-2.2% in Saraburi Province and 20.2% in Naresuan cave, Phitsanulok (Polseela *et al*, 2007; 2011a, b). In northern Israel, sand fly populations were prevalent from April to November and peaked between June and August (Kravchenko

Table 2
The monthly distribution of sand flies species captured by CDC light in limestone caves at Khao Pathawi, Thap Than, Uthai Thani Province from October 2012 to September 2013.

Species	2012			2013									Total (%)
	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	
S.anodontis	829	1,187	1,867	893	145	240	175	130	188	194	281	364	6,493 (55.0)
P.argentipes	528	674	1,113	509	124	138	118	91	105	122	196	253	3,971 (33.6)
S.barraudi	119	89	165	58	11	16	23	13	29	11	27	36	597 (5.0)
S.gemmea	31	16	113	30	6	9	12	8	24	14	30	21	314 (2.7)
P.stantoni	37	25	62	15	3	12	19	6	8	20	12	37	256 (2.2)
S.silvatica	21	10	47	0	2	5	8	7	9	4	16	12	141 (1.2)
S.indica	0	12	6	0	0	0	4	9	0	0	0	0	31 (0.3)
C.barbazani	0	0	0	2	0	0	0	5	0	7	0	0	14 (0.1)
Total	1,565	2,013	3,373	1,507	291	420	359	269	363	372	562	723	11,817 (100.0)
(%)	(13.2)	(17.0)	(28.5)	(12.7)	(2.5)	(3.6)	(3.0)	(2.3)	(3.1)	(3.2)	(4.8)	(6.1)	(100.0) -

et al, 2004). In January and February 2006, the sand fly was apparently absent in Saudi Arabia (Badry *et al*, 2008). Coleman *et al* (2006) reported sand fly activity periods in Iraq with the highest mean monthly captured rates in August, 2003 and July, 2004. Sand flies were active especially during the dry season (from May to November) in Marrakech, Morocco. The maximum of species richness was in May, when the daily temperature ranged between 13ºC and 26ºC. The species differed in their activity period; *P. sergenti*, was active especially during April, May and June (Boussaa *et al*, 2005). These seasonal patterns should be related to different environmental factors. Nocturnal activity of sand flies may result in transmitted pathogens to animals and humans. Those people living near the cave may be particularly at risk of exposure to such pathogens.

Both sexes of the sand fly were found to have nocturnal activity in this study. The male to female ratio was 1.0:1.2. This suggests that both sexes of the sand fly are attracted to the light traps. Previous studies have shown the number of male sand flies captured using CDC light traps was significantly higher than that of female with a male:female ratio of 2.5:1 and 1.9:1 in Saraburi, Thailand (Polseela *et al*, 2007; 2011a). According to a study conducted in Cukurova plain, Turkey, males were found to be 4.7 times more abundant than females (Kasap *et al*, 2009). In different areas, the number of males captured was higher than that of females, with the sex ratio of 2.3:1 reported by Boussaa *et al* (2005), 3.4:1 by Hanafi *et al* (2007), 1.12:1 by Badry *et al* (2008) and 2:1 by Emamia and Yazdib (2008). The

higher proportion of males in the captures with CDC light traps could be explained by the natural behavior of the males to follow the females to assure fertilization during their displacements (Barata *et al*, 2004). Male sand flies have a weaker dispersal tendency than females and limit their flight to questing for food plants (Yuval and Schlein, 1986; Janini *et al*, 1995). The hopping behavior leads to the assumption that they do not disperse far from the breeding site (Killick-Kendrick, 1999). However, only the female sand fly can feed on blood and be the vector for leishmaniasis.

For this study's month-by-month collection, the highest peak of capture was in December, and lowest in May. Temperature and humidity are factors closely related to duration of metamorphosis (Theodor, 1936). However, the optimum rearing temperatures differ among species of sand flies. Guernaoui *et al* (2006) reported that variations in the nocturnal activity rhythm are related to variations in temperature and relative humidity. The most favorable temperatures for development of sand flies are between 25 ºC and 30ºC (Safyanova, 1964). The activity of sand flies is continuous through the night and that relative humidity has a significant effect on the nocturnal activity. However, sand fly activity was closely related to climatic factors. Many investigations have demonstrated the existence of seasonal variations in the nocturnal activity of sand flies (Merrison *et al*, 1995; Polseela *et al*, 2011a).

According to our findings, sand flies are active in the different caves. The high number of flies are found in several sites in the cave. The continental climatic environment inside the cave provides a possible breeding site of the potential vector. Based on the occurrence of autochthonous human cases in Thailand and on the presence of the *Leishmania siamensis*, it is possible that *S.gemmea* phlebotomine sand fly might be involved in the cycle of leishmaniasis in Thailand. The study of population activity pattern might be helpful in the implementation of successful tactics for vector control and disease prevention.

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