DISTRIBUTION OF CAVE-DWELLING PHLEBOTOMINE SAND FLIES AND THEIR NOCTURNAL AND DIURNAL ACTIVITY IN PHITSANULOK PROVINCE, THAILAND

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Abstract. An entomological survey of sand flies was conducted in Naresuan Cave in Noen Maprang District, Phitsanulok Province, during November 2009 to December 2010. A total of 10,115 cave-dwelling sand flies were collected with CDC light traps nocturnally (06:00 AM and 06:00 PM) and diurnally (06:00 PM and 06:00 AM). The ratio between male and female sand flies was 1:1.3 (4,363:5,752). The ratio between the number of sand flies caught nocturnally and diurnally was 2.6:1 (7,268:2,847). In this study, 13 species belonging to 4 genera were identified, of which 4 belonged to the genus *Phlebotomus*, 7 to *Sergentomyia*, 1 to *Nemopalpus* and 1 to *Chinius*. An abundance of species were observed: *Nemopalpus vietnamensis* (49.15%), *P. argentipes* (20.15%), *C. barbazani* (15.79%), *P. teshi* (9.53%), and *S. anodontis* (3.21%). Less common species (<1%) were *S. barraudi* (0.63%), *P. stantoni* (0.57%), *S. dentata* (0.49%), *S.quatei* (0.17%), *P. philippinensis gouldi* (0.12%), *S.silvatica* (0.10%), *S. gemmea* (0.05%), and *S. iyengari* (0.04%). The predominant species in the Naresuan Cave was *Nemopalpus vietnamensis* (49.15%). The data demonstrates variability in sand fly prevalence, species composition, and relative abundance in caves. *P. argentipes* was found throughout the day in the caves, which is important because it is believed to be the *Leishmania* spp vector. This study highlights the diurnal activity of the sand fly and the day-time risk of leishmaniasis. In conclusion, although leishmaniasis has not been reported in Phitsanulok, there should be heightened awareness of infection in these areas with vectors of the protozoa.

Keywords: sand fly, cave-dwelling, nocturnal, diurnal, distribution, Thailand

INTRODUCTION

Phlebotomine sand flies of genus *Phlebotomus* and *Lutzomyia* are known to transmit leishmaniasis in the Old and New Worlds. Species of genus *Phlebotomus* and *Lutzomyia* are proven vectors of vertebrate pathogens, at least 20 species of *Leishmania* spp, which affects people in more than 80 countries (Desjeux, 2004). There are estimated 2 million new cases occurring each year, and 350 million people are at risk of visceral leishmaniasis (Desjeux, 2001). In India, Nepal, and Bangladesh, *Leishmania donovani* is transmitted by *Phlebotomus argentipes* (Swaminath *et al*,

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1942; Kumar *et al*, 2001). The incidence of leishmaniasis varies with the local prevalence of different *Leishmania* spp and with the behavior of sand flies that transmit the parasite (Maria *et al*, 2000). Leishmaniasis is an emerging vector-borne disease in Thailand. At least 60 cases of visceral leishmaniasis and cutaneous leishmaniasis have been found in various regions of Thailand. The distribution of sand flies originally found in different areas, including in caves (Magill *et al,* 1993). The sand flies found in Thailand consist of 24 species recorded by many investigators in different areas. The diversity of sand fly species can play some roles in the maintenance of the transmission cycle.

This was the first study undertaken to report species composition and distribution patterns of cave-dwelling phlebotomine sand flies during nocturnal and diurnal activity. This study shall set a precedent for future sand fly survey designs for diurnal capturing in dark areas during day time.

MATERIALS AND METHODS

Study area

The entomological survey was carried out from November 2009 to December 2010 at Naresuan Cave, Noen Maprang, District, Phitsanulok Province, Thailand. The limestone cave is situated on a hill at 163º 02′ 71″N, 1003º 97′ 49″E and consists of two large caverns. This cave is 100-200 m long 1-10 m wide and 1-15 m high. The site is a mountainous area 78 m above sea level. Normally, it is dry, but in the rainy season, it becomes moist and wet inside the cave. There is a mountainside area, surrounded by various plants. Inside the cave, there are many sand fly hosts including bats. This cave is opened to tourists. Rainfall data was provided by the Thai Meteorological Department. Daily temperature and relative humidity were recorded monthly with a hydro-hygrometer when the trap was set for investigation.

Specimen collection and identification

Miniature light traps from the Centers for Disease Control and Prevention (CDC), USA were used to collect adult sand flies every month. The traps were set up at about 30-50 cm above ground level (Sudia and Chamberin, 1962). The diurnal capture was undertaken from 06:00 AM - 06:00 PM and the nocturnal capture was from 06:00 PM - 06:00 AM. The sand flies were captured at the same location for the same period of time in the dark zone of the cave. Ten trap locations were selected in different areas in the cave about 10-20 m apart in each collection. The collected sand flies were preserved in 80% alcohol and only female sand flies were mounted on glass slides with Hoyer's medium. The males of many species are useful for confirming the identification of females (Lewis, 1978). The identification was based on the morphology of the female spermatheca and pharynx and by taxonomic keys, particularly those of Lewis (1978), Artemiev (1980), Lewis (1982), Killick-Kendrick *et al* (1991), and other related keys.

RESULTS

A total of 10,115 phlebotomine sand flies were obtained from the collection sites. The highest peak was found during March and April, and lowest point was in August (Table 1, 2). Thirteen species were identified of which 4 belonged to the genus *Phlebotomus*, 7 to the genus *Sergentomyia*, 1 to the genus *Nemopulpus*, and 1 to the genus *Chinius* (Table 2, 3). The most abundant of the main species was *Nemopulpus vietnamensis* (49.15%) during diurnal and nocturnal capture while

P. argentipes, the proven vector of *Leishmania donovani*, accounting for 20.15% (Table 5). The greatest number of specimens were collected in March while *N. vietnamensis*, *P. argentipes*, *P. teshi*, and *C. barbazani* were trapped throughout the whole year (Table 3). Both male and female flies were attracted to the light. The male to female ratio was 1:1.3. The highest number of *N. vietnamensis* were found in the cave in April, and *P. argentipes* was found in July (Fig 1, 2). During diurnal capture, 11 species were found, and 13 species of sand flies were observed during nocturnal activity (Table 3, 4). In all months, the number of sand flies captured diurnally was lower than at nocturnal times (Table 1, 2).

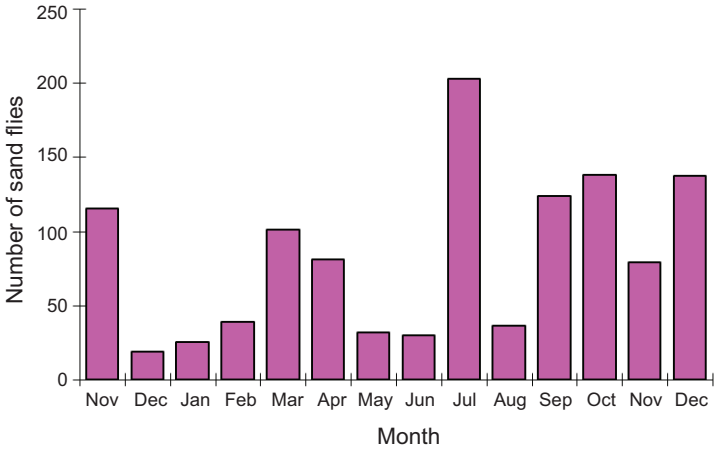

Fig 1–Number of *Phlebotomus argentipes* captured in Naresuan Cave, in Noen Maprang District, Phitsanulok Province from November 2009 to December 2010.

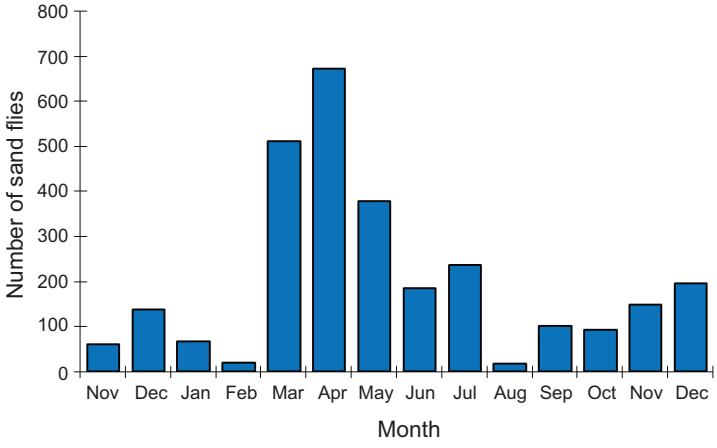

Fig 2–Number of *Nemopulpus vietnamensis* captured in Naresuan Cave in Noen Maprang District, Phitsanulok Province from November 2009 to December 2010.

The recorded minimum and maximum monthly temperature and relative humidity values ranged between 24.4-32.3ºC and 64-95%, respectively. The total annual rainfall recorded in the study area ranged from 0 mm to 308 mm. The overall population reached a peak during March and April and then decreased to a minimum during August (Table 1). The minimum and maximum temperature and relative humidity values ranged between 28.8-29.4ºC, and 48.8-75.0% in March and April. The known vector, *P. argentipes*, has its peak in July (Fig 1). In July, the minimum and maximum temperature and relative humidity values ranged between 27.5-29.5ºC, and 75.0-85.0%. The highest peak of *N. vietnamensis* was found in April (Fig 2). There are populations of different species with different seasonal patterns.

DISCUSSION

An entomological survey of sand fly populations was conducted for a 14 month

Table 1
The monthly distribution of sand flies captured at Naresuan Cave in Noen Maprang District, Phitsanulok Province from November 2009 to December 2010.

	Month														Total	(%)
	2009		2010													
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
Female	337	190	132	200	819	870	442	275	556	107	432	417	377	598	5,752	56.87
Male	336	161	136	233	560	477	288	233	356	112	396	455	212	408	4,363	43.13
Total	673	351	268	433	1,379	1,347	730	508	912	219	828	872	589	1,006	10,115	100.00

Table 2
The monthly diurnal activity and nocturnal activity of sand flies captured at Naresuan Cave in Noen Maprang District, Phitsanulok Province from November 2009 to December 2010.

	Month														Total	(%)
	2009		2010													
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
Diurnal	92	148	47	54	469	512	289	234	278	57	116	164	242	145	2,847	28.15
Nocturnal	581	203	221	379	910	835	441	274	634	162	712	708	347	861	7,268	71.85
Total	673	351	268	433	1,379	1,347	730	508	912	219	828	872	589	1,006	10,115	100.00

Table 3
The diurnal activity of female sand fly species captured in Naresuan Cave in Noen Maprang District, Phitsanulok Province from November 2009 to December 2010.

Cave-dwelling species	Month														Total	(%)
	2009		2010													
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
N. vietnamensis	7	71	9	5	196	117	166	128	165	10	39	57	119	65	1,154	68.77
C. barbazani	5	5	4	8	26	88	5	7	0	3	8	27	23	11	220	13.11
P. argentipes	11	5	5	8	16	43	17	14	19	12	19	14	14	19	216	12.87
P. teshi	8	3	4	4	9	11	2	0	0	0	0	9	7	3	60	3.58
S. anodontis	2	0	1	7	2	1	0	0	0	0	0	0	1	0	14	0.83
P. stantoni	1	0	0	0	0	0	0	0	0	0	0	2	0	0	3	0.18
S. dentata	0	0	0	0	0	2	0	0	1	0	0	0	0	0	3	0.18
S. quatei	0	0	0	0	0	0	0	0	0	0	3	0	0	0	3	0.18
S. barraudi	1	0	0	0	0	1	0	0	0	0	0	0	0	0	2	0.12
S. silvatica	0	0	0	0	0	0	0	0	2	0	0	0	0	0	2	0.12
P. philippinensis gouldi	0	0	0	0	0	1	0	0	0	0	0	0	0	0	1	0.06
Total	35	84	23	32	249	264	190	149	187	25	69	109	164	98	1,678	100.00

Table 4
The nocturnal activity of female sand fly species captured in Naresuan Cave in Noen Maprang District, Phitsanulok Province from November 2009 to December 2010.

Cave-dwelling species	Month														Total	(%)
	2009		2010													
	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec		
N. vietnamensis	53	66	57	15	315	557	213	58	72	8	63	36	30	130	1,673	41.06
P. argentipes	104	14	20	31	85	38	15	16	184	24	105	124	65	118	943	23.15
C. barbazani	78	6	7	45	52	4	13	39	47	33	124	82	51	107	688	16.89
P. teshi	41	15	12	12	69	7	9	7	12	4	52	53	50	145	488	11.98
S. anodontis	19	0	11	61	41	0	2	0	13	5	12	2	5	0	171	4.20
S. barraudi	2	0	0	1	2	0	0	0	23	6	0	0	0	0	34	0.84
P. stantoni	2	2	1	0	2	0	0	1	10	0	1	4	7	0	30	0.74
S. dentata	2	2	1	2	1	0	0	1	4	1	2	4	5	0	25	0.60
S. quatei	0	0	0	0	0	0	0	3	0	0	4	0	0	0	7	0.17
P. philippinensis gouldi	1	0	0	0	1	0	0	0	1	1	0	2	0	0	6	0.15
S. silvatica	0	0	0	0	0	0	0	0	3	0	0	1	0	0	4	0.10
S. gemmea	0	0	0	1	2	0	0	0	0	0	0	0	0	0	3	0.07
S. iyengari	0	1	0	0	0	0	0	1	0	0	0	0	0	0	2	0.05
Total	302	106	109	168	570	606	252	126	369	82	363	308	213	500	4,074	100.00

Table 5
The diurnal and nocturnal activity of sand fly species captured in Naresuan Cave in Noen Maprang District, Phitsanulok Province from November 2009 to December 2010.

Cave-dwelling species	Diurnal activity	Nocturnal activity	Total	%
N. vietnamensis	1,154	1,673	2,827	49.15
P. argentipes	216	943	1,159	20.15
C. barbazani	220	688	908	15.79
P. teshi	60	488	548	9.53
S. anodontis	14	171	185	3.21
S. barraudi	2	34	36	0.63
P. stantoni	3	30	33	0.57
S. dentata	3	25	28	0.49
S. quatei	3	7	10	0.17
P. philippinensis gouldi	1	6	7	0.12
S. silvatica	2	4	6	0.10
S. gemmea	0	3	3	0.05
S. iyengari	0	2	2	0.04
Total	1,678	4,074	5,752	100.00

period at Naresuan Cave. The sand flies were collected by using CDC light traps, and the sand fly species were documented. This is the first report of diurnal capture in a dark area of a cave in Thailand. In this study, 13 species were identified. In Thailand, previous captures of sand flies documented 24 species: *P. argentipes, P. asperulus, P. barguesae, P. hoepplii, P. major major, P. mascomai, P. philippinensis gouldi, P. stantoni, P. teshi, Sergentomyia anodontis, S. barraudi, S. bailyi, S. dentata, S. quatei, S. silvatica, S. gemmea, S. hodgsoni hodgsoni, S. iyengari, S. indica, S. mahadevani, S. perturbans, S. punjabensis, Chinius barbazani,* and *Nemopalpus vietnamensis* (Causey, 1938; Theodor, 1938; Quate, 1962; Apiwathnasorn *et al*, 1989, 1993; Depaquit *et al*, 2006, 2009; Muller *et al*, 2007; Polseela *et al*, 2007). *P. argentipes* is the potential vector of leishmaniasis found in this area. In Asia, phlebotomine sand flies have also been reported in Bangladesh, Pakistan, India, Malaysia and Thailand (Lewis, 1967; Sanyal *et al,* 1979; Rudnick *et al*, 1971; Apiwathnasorn *et al*, 1989, 1993; Ismill *et al*, 1993; Shakila *et al*, 2006; Singh and Singh, 2009). There are some species present in this area and others have disappeared, maybe due to different environmental aspects in each area.

The most abundant of the main species was *N. vietnamensis* (49.15%). *P. argentipes,*the proven vector of *L. donovani*, accounting for 20.15%. In this study, cave-dwelling *P. argentipes* showed its highest peak in July, and *P. argentipes* were trapped throughout the year. In Assam, India, *P. argentipes* was absent during the winter season (Sanyal *et al*, 1979). The greatest number of specimens was collected from a location near rock fissures in the cave. Nocturnal activity among the different species of sand flies in Phra Phothisat Cave was studied by Polseela *et al* (2007). Thirteen species were also found in

Phra Phothisat Cave in Saraburi Province. The results revealed the highest peak was in March. Diurnal flight activity of sand flies has been reported by several authors. The previous diurnal study at Pitangueiras Cave and Gaucho Cave in Brazil found *Lutzomyia almerioi* in dark and twilight zones of the caves (Eunice *et al*, 2003). The nocturnal rhythm could be a result of the decline in the diurnal temperature and increase in the relative humidity (Cross and Hyams 1996). Guernaoui *et al* (2006) in Morocco studied the sand fly nocturnal activity. It was significant in that it revealed the best time to collect and study the flies. The activity was nocturnal and stronger at twilight. It started at sunset and stopped immediately after sunrise.

In this study, the male to female ratio was 1:1.3. This suggests that both sexes of the sand flies are attracted to the light trap. The sand flies exhibited positive phototaxis and were attracted to the light trap (Santos *et al*, 2002). It is known that male sand flies arrive first and wait for the female for mating (Morrison *et al*, 1995; Killick-Kendrick, 1999). The actual proportion of females to males is unknown in the natural population by species. The abundance of *P. argentipes* is related to the biology or leishmaniasis risk in this area. Increasing numbers of female sand flies may be due to the greater opportunity for them to feed on the blood of the cave-dwelling bats. In all months, the number of sand flies captured diurnally was lower than at nocturnal times. Only female sand flies may feed on the blood of bats in caves because bats are normally inactive during the day. Adult bats normally abandon the caves at night when sand flies are feeding, and some bats stay inside throughout the night (Lampo *et al*, 2000). One possible reason is that bats are active and feed at night. The number of bats in the cave is therefore less at night, and a lot of sand flies will come to the light trap.

For this study's monthly collection, the highest peak for capture was in March and April, and lowest point was in August. The cave temperature was 24.4-32.3ºC with 64-95% relative humidity (RH). Tropical sand flies tend to feed at a temperature of at least 20ºC and a RH of 75-80% (Lane, 1987). Temperature and humidity are factors closely related to the duration of metamorphosis of sand flies (Theodor, 1936). Sand flies activity is sensitive to environmental conditions such as temperature, habitat availability geographical, and distribution of abundant hosts (Cross *et al*, 1996; Ghosh *et al*, 1999).

Understanding the diurnal and nocturnal activity of sand flies is important for determining the period of risk of *Leishmania* transmission. Study on sand fly's habits improves and strengthens the planning to avoid human-sand fly contact.

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