SPECIES DIVERSITY AND SEASONALITY OF PHLEBOTOMINE SAND FLIES (DIPTERA: PSYCHODIDAE) IN SATUN PROVINCE, THAILAND

Amonrat Panthawong[1], Theeraphap Chareonviriyaphap[2] and Jumnongjit Phasuk[1]

[1]Department of Parasitology, Faculty of Veterinary Medicine, [2]Department of Entomology, Faculty of Agriculture, Kasetsart University, Bangkok, Thailand

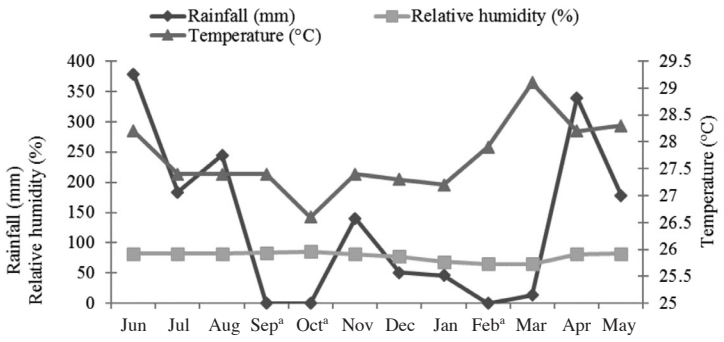
Abstract. Leishmaniasis is prevalent mainly in the southern provinces of Thailand where sand flies are considered to be an important vector. Sand flies were collected using Centers for Disease Control (CDC) light traps in Satun Province from June 2013 to July 2014. A total of 1,982 sand flies (1,228 females and 754 males) were collected. Only female sand flies were identified to the species level and were tested for *Leishmania* infection using polymerase chain reaction (PCR). Morphological identification revealed 2 genera and 9 species: *Phlebotomus stantoni*, *P. argentipes, Sergentomyia gemmea, S. indica*, *S. barraudi*, *S. iyengari*, *S. bailyi*, *S. perturbans*, and *S. silvatica*. *S. gemmea* (57.2%) was the most abundant species. The diversity of sand flies was highest in Thung Wa District. The sand flies were most abundant late in the hot season and early in the rainy season (April to June). The highest number of sand flies was collected in June. Significant correlations between the number of female sand flies and rainfall and between *S. gemmea* and rainfall were found. Of the female sand flies tested, none were positive for *Leishmania* spp.

Keywords: *Leishmania*, sand flies, seasonality, diversity

INTRODUCTION

Leishmaniasis is a zoonotic disease caused by parasitic protozoans of the genus *Leishmania* that are transmitted by the bite of female sand flies belonging to the subfamily Phlebotominae in the family Psychodidae (Desjeux, 1996; Herwaldt, 1999; Kobets *et al*, 2012). Leishmaniasis is non-endemic in Thailand, it is sporadic. Most imported cases of cutaneous and visceral leishmaniasis have been reported in Thai workers returning from the Middle East since 1960 (Laohapaibol and Siampakdi, 1960; Suttinont *et al*, 1987; Viriyavejakul *et al*, 1997). During 1996 and 2010, 58 leishmaniasis cases were reported from 10 provinces in Thailand. Most of these cases were in southern Thailand (Thisyakorn *et al*, 1999; Kongkaew *et al*, 2007; Sukmee *et al*, 2008; 2011). Until recently, 6 cases of autochthonous disseminated dermal and visceral leishmaniasis have been reported from different provinces in Thailand (Sukmee *et al*, 2008; Bualert *et al*, 2012; Polseela, 2012). Species of sand flies such as *Phlebotomus argentipes*, a natural vector of *Leishmania donovani* in India, Nepal, and Bangladesh have been identified in Thailand (Sukra *et al*, 2013). *P. major major*,

Correspondence: Jumnongjit Phasuk, Department of Parasitology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand.
Tel: 66 (0) 2942 8438; Fax: 66 (0) 2942 8438
E-mail: fvetjjp@ku.ac.th



[a]No rainfall data available

Fig 1–Mean monthly rainfall, relative humidity and temperature in the study area.

the proven vector of *L. infantum* in the Mediterranean region was mostly found in caves in Thailand (Apiwathnasorn *et al*, 1989). In addition, *Sergentomyia gemmea* and *S. barraudi* might be potential vectors of *L. siamensis*, the causative agent of autochthonous visceral leishmaniasis in southern Thailand (Kanjanopas *et al*, 2013; Chusri *et al*, 2014). The distribution and diversity of sand fly species can play roles in the maintenance of the transmission cycles of *Leishmania* spp (Polseela *et al*, 2011b). However, little is known about the distribution of sand fly species and potential sand fly vectors of leishmaniasis in the southern region of Thailand. The aims of this study were to investigate the seasonal abundance and diversity of sand flies and the detection of *Leishmania* DNA in sand flies in Satun Province, one of the affected areas of leishmaniasis in Thailand.

MATERIALS AND METHODS

Study areas

Sand flies were collected between June 2013 and July 2014 in 7 districts of Satun Province: Mueang, Tha Phae, Khuan Don, Khuan Kalong, Thung Wa, Manang, and La-ngu. The collection sites included peridomestic areas; domestic animal shelters for dogs, cats, cattle, and goats; and rubber and/or palm plantations. The weather in the collection areas is marked by 3 seasons: a hot season from mid-February to mid-May, a rainy season from mid-May to mid-October, and a colder season from mid-October to mid-February. The average annual rainfall is 179 mm. The temperature range is 27-29°C with 65-85% relative humidity. These data were obtained from the Thai Meteorological Department weather station in Satun Province (station: 570201) (Fig 1).

Sand fly collection

Sand flies were collected using Center for Disease Control (CDC) light traps and directly preserved in 70% ethanol. Traps were hung from trees approximately 50 cm above the ground between 6:00 PM and 6:00 AM. Sand flies were collected between June 2013 and July 2014 in seven districts of Satun Province. In Mueang District, collections were made monthly from June 2013 to May 2014, except during October, using 2 CDC light traps for 2 consecutive nights. For the remaining six districts, sand flies were collected for at least 2 consecutive nights (4 traps per night) in one month: Tha Phae (June 2013), Khuan Don (July 2013), Khuan Kalong (August 2013), Thung Wa (October 2013), Manang, (November 2013), and La-ngu (December 2013).

In four districts, the numbers of flies collected were low, so additional samples were taken using 3 traps per night for 2

nights: Khuan Kalong and Manang (June 2014), and Thung Wa and La-ngu (July 2014). Therfore, there were 116 trap nights in the seven districts: Mueang (44 nights), Tha Phae (8 nights), Khuan Don (8 nights), Khuan Kalong, Thung Wa, Manang, and La-ngu (14 nights each).

Sand fly identification

Sand flies were examined using a stereomicroscope, separated from other insects and identified according to sex. For species identification, the head and the abdominal segments of 8-11 females were dissected and mounted on slides using Hoyer's medium. Sand flies were identified based on the morphology of cibarium teeth and spermatheca following the taxonomic key of Lewis (1978). The remaining body parts were kept in 70% ethanol until used for the detection of *Leishmania* DNA.

Detection of *Leishmania* DNA in sand flies

One-to-10 specimens of female sand flies belonging to the same species, date, and site of capture were pooled. One hundred sixty-six pools were tested for *Leishmania* parasites. Extraction of genomic DNA from each pool was performed using the EZNA® tissue DNA kit (Omega Bio-Tek, Norcross, GA). Detection of *Leishmania* DNA was performed using PCR analysis. The internal transcribed spacer 1 (ITS1) region of genomic DNA was amplified with LITSR and L5.8S primers (El Tai *et al*, 2001). The PCR analyses were performed in 20 μl volumes of reaction mix containing 2 μl of genomic DNA, 1X buffer [10 mM Tris-HCl pH 8.8, 50 mM KCl and 0.1% (v/v) Triton X-100], 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 2.5 U of *Taq* DNA polymerase, and 1 pmol of each primer. The PCR reactions were processed on a MyCyclerTM Thermal Cycler® (BioRad Laboratories, Hercules, CA). The thermal cycling conditions were initial denaturation at 95ºC for 2 minutes followed by 45 amplification cycles of denaturation at 95ºC for 20 seconds, annealing at 54ºC for 30 seconds, and extension at 72ºC for 1 minute, and a final extension step at 72ºC for 6 minutes. PCR products were checked using electrophoresis in 1% agarose gel (SeaKem ME; FMC, Rockland, ME).

Data analysis

The Shannon-Wiener species diversity index (Krebs, 1999) was calculated using the formula $H = -\sum_{i=1}^{s} (p_i) (\ln p_i)$, where H is the species diversity index, s is the number of species and p_i is the proportion of the total sample belonging to i^{th} species. The correlations between the numbers of sand flies and temperature, relative humidity, and rainfall were assessed using the Pearson product-moment correlation coefficient. The SYSTAT® computer package version 8.0 (SYSTAT Software, San Jose, CA) was used for statistical analysis. A $p<0.05$ was considered significant.

RESULTS

A total of 1,982 sand flies (62.0% females and 38.0% males) were collected during the study period (Table 1). The ratio of females to males was 1.6: 1. The 1,228 female sand flies were identified as belonging to 2 genera and 9 species, with 2 species of *Phlebotomus* (*P. stantoni* and *P. argentipes*) and 7 species of *Sergentomyia* (*S. gemmea, S. indica, S. barraudi*, *S. iyengari*, *S. bailyi*, *S. perturbans,* and *S. silvatica*). *Sergentomyia gemmea* was the most abundant species (57.2%) followed by *S. indica* (26.9%), *S. barraudi* (6.3%), *P. stantoni* (4.1%), and *S. iyengari* (3.5%), respectively. The remaining species were present but less abundant: *S. bailyi* (0.9%), *S. perturbans* (0.6%), *S. silvatica*

Table 1
Numbers of male and female sand flies collected in 7 districts of Satun Province.

Sex	Collection sites (district)							
	Tha Phae	Khuan Don	Khuan Kalong	Thung Wa	Manang	La-ngu	Mueang	Total (%)
Female, *n* (%)	253 (74.6)	60 (52.2)	87 (58.4)	275 (47.8)	34 (55.7)	40 (54.0)	479 (71.6)	1,228 (62.0)
Male, *n* (%)	86 (25.4)	55 (47.8)	62 (41.6)	300 (52.2)	27 (44.3)	34 (45.9)	190 (28.4)	754 (38.0)

(0.4%), and *P. argentipes* (0.1%) (Table 2). *S. gemmea* and *S. indica* were found at all collection sites. The former of these 2 species was the most abundant at all collection sites. The highest values of diversity and species richness were found in Thung Wa (1.74 and 8, respectively) followed by Khuan Kalong (1.26 and 6, respectively) and Manang (1.18 and 4, respectively). The highest dominance level was 0.97 in Khuan Don followed by Tha Phae (0.91) whereas the lowest value was in Thung Wa (0.31).

The sand flies were most abundant late in the hot season and early in the rainy season (April to June), with a marked population increase between 2 rainfall peaks (Table 3 and Fig 1). The highest number of sand flies was collected in June. *S. gemmea* occurred almost throughout the year with a peak in June. *S. indica* was sampled throughout the year except in August and December, with a peak in May. *P. stantoni* was collected only in April, May, June, July, and September with fewer than 5 specimens collected each month. *S. barraudi* was found only in January, April, June, August, and December. *S. iyengari* and *S. perturbans* were collected in April and June. *P. argentipes* was only collected in April, but this month provided the highest number of species (7). Single specimens collected each month represented the latter 4 species. The data analysis showed a significant correlation between the number of female sand flies and rainfall ($r = 0.722$, $p = 0.014$) and a significant correlation between *S. gemmea* and rainfall ($r = 0.736$, $p = 0.012$) (Table 4). None of the 1,228 females tested were positive for *Leishmania* spp.

DISCUSSION

A total of 1,982 sand flies were collected in the study with a higher propor-

Table 2
Species and distribution of female sand flies collected in 7 districts of Satun Province, Thailand.

Genus	Species	Tha Phae		Khuan Don		Khuan Kalong		Thung Wa	
		Individuals	%	Individuals	%	Individuals	%	Individuals	%
Phlebotomus	*stantoni*	3	1.2	-	-	8	9.2	24	8.7
	argentipes	-	-	-	-	-	-	-	-
Sergentomyia	*gemmea*	**229**	**90.5**	**58**	**96.7**	**38**	**43.7**	**85**	**30.9**
	indica	11	4.3	2	3.3	33	37.9	55	20
	iyengari	1	0.4	-	-	3	3.4	32	11.6
	barraudi	9	3.6	-	-	4	4.6	59	21.5
	perturbans	-	-	-	-	1	1.2	4	1.5
	bailyi	-	-	-	-	-	-	11	4
	silvatica	-	-	-	-	-	-	5	1.8
Total number of individuals (*N*)		253	100	60	100	87	100	275	100
Total number of samples (*n*)		8		8		14		14	
Mean number of individuals (*N*/*n*)		31.63		7.5		6.21		19.64	
Total number of species		5		2		6		8	
Diversity (*H*)		0.42		0.14		1.26		1.74	
Dominance (*N*max/*N*)		0.91		0.97		0.44		0.31	

Genus	Species	Manang		La-ngu		Mueang		Total	%
		Individuals	%	Individuals	%	Individuals	%		
Phlebotomus	*stantoni*	2	5.9	2	5	11	2.3	50	4.1
	argentipes	-	-	-	-	1	0.2	1	0.1
Sergentomyia	*gemmea*	**15**	**44.1**	**22**	**55**	**256**	**53.5**	**703**	**57.2**
	indica	12	35.3	16	40	202	42.2	331	26.9
	iyengari	5	14.7	-	-	2	0.4	43	3.5
	barraudi	-	-	-	-	5	1	77	6.3
	perturbans	-	-	-	-	2	0.4	7	0.6
	bailyi	-	-	-	-	-	-	11	0.9
	silvatica	-	-	-	-	-	-	5	0.4
Total number of individuals (*N*)		34	100	40	100	479	100	1,228	100
Total number of samples (*n*)		14		14		44		116	
Mean number of individuals (*N*/*n*)		2.43		2.86		10.89		10.59	
Total number of species		4		3		7		9	
Diversity (H)		1.18		0.85		0.89		1.2	
Dominance (*N*max/*N*)		0.44		0.55		0.53		0.57	

Nmax, number of individuals in the dominant species, bold type.
H, Shannon-Wiener species diversity index.

Table 3
Numbers of female sand flies collected each month in Mueang District, Satun Province.

Species	Month											Total (%)
	Jun	Jul	Aug	Sep	Nov	Dec	Jan	Feb	Mar	Apr	May	
P. stantoni	4	1	0	1	0	0	0	0	0	3	2	11 (2.4)
P. argentipes	0	0	0	0	0	0	0	0	0	1	0	1 (0.2)
S. gemmea	115	29	8	2	13	5	1	2	6	30	45	256 (53.4)
S. indica	27	4	0	1	3	0	1	1	14	61	90	202 (42.2)
S. iyengari	1	0	0	0	0	0	0	0	0	1	0	2 (0.4)
S. barraudi	1	0	1	0	0	1	1	0	0	1	0	5 (1)
S. perturbans	1	0	0	0	0	0	0	0	0	1	0	2 (0.4)
Total	149	34	9	4	16	6	3	3	20	98	137	479 (100)
Total number of species	6	3	2	3	2	2	3	2	2	7	3	

Table 4
Pearson product-moment correlation coefficient values (r) between total numbers of individuals and main species of sand flies captured and climatological data of Satun Province.

Weather conditions	Number of individuals	*Sergentomyia gemmea*	*Sergentomyia indica*
Rainfall (mm)	0.722[a]	0.736[a]	0.402
Relative humidity (%)	0.448	0.464	0.309
Temperature (°C)	0.523	0.307	0.53

[a]Significant at $p<0.05$.

tion of females (1,228) than males (754). Similar findings were reported by Polseela *et al* (2011a, b) and Sukra *et al* (2013). Both sexes are generally considered to be crepuscular and nocturnal in their activity (Lewis, 1971; El-Badry *et al*, 2008). Only female sand flies feed on the blood of various vertebrate animals (Lane, 1987). After feeding, females fly to mates and to search for oviposition sites. Therefore, the females were more likely to be caught than males in all traps (Eiko *et al*, 2004).

During 1938-2011, at least 27 species of sand flies were recorded by many investigators in different areas of Thailand (Causey, 1938; Quate, 1962; Apiwathnasorn *et al*, 1993; Polseela *et al*, 2007; Nilsang *et al*, 2010; Apiwathnasorn *et al*, 2011; Polseela *et al*, 2011b; Sukmee *et al*, 2011). A previous survey of sand flies in Satun Province found 10 species: *P. stantoni, P. papatasi, P. major major, P. argentipes, S. gemmea, S. barraudi, S. iyengari, S. perturbans, S. indica,* and *S. anodotis.* Of these 10 species, *S. barraudi* was the most abundant (Nilsang *et al*, 2010). In the present study, 9 species of sand fly were trapped. Additionally, *S. bailyi* and *S. silvatica* were

identified in this area. *S. gemmea* was the most abundant sand fly species. This result was consistent with previous findings that *S. gemmea* was the most predominant species in southern Thailand, the affected area of leishmaniasis caused by *L. siamensis* (Sukmee *et al*, 2008; Kanjanopas *et al*, 2013; Sukra *et al*, 2013). *Sergentomyia* species feed mainly on reptiles and some species of this genus bite humans, but rarely (Lane, 1993; Polseela *et al*, 2011a; Sadlova *et al*, 2013). Therefore, they were suspected as the potential vector of this disease in southern Thailand (Kanjanopas *et al*, 2013; Chusri *et al*, 2014). *P. argentipes*, a known vector of kala-aza in India was found in the present study but in low numbers. This finding was similar to previous survey in Phang-nga and Surat Thani Provinces in Thailand (Sukra *et al*, 2013). This species was found mainly in caves (Apiwathnasorn *et al*, 1989). However, *P. argentipes* is widely distributed in Thailand (Apiwathnasorn *et al*, 1993).

The highest values of diversity and species richness of sand flies were observed in Thung Wa District which might indicate that this area contains suitable breeding and resting sites and feeding sources for sand flies. In addition, the degree of conservation in the region, the use of agroecosystems, the forest covers and human population density may affect sand fly diversity and abundance (Pinto *et al*, 2010; Nieves *et al*, 2014; Ramos *et al*, 2014).

Monthly collection of sand flies in Mueang District showed sand fly numbers occurred throughout the year with peaks in June 2013 and April and May 2014. This finding was consistent with the results reported by Polseela *et al* (2007) that sand flies were more prominent during the early rainy season (June-August) in Saraburi Province, Thailand. The results of this study showed that high rainfall increased the number of sand flies caught. In addition, the temperature and the relative humidity may influence the density of sand fly caught (Rafatbakhsh-Iran *et al*, 2015). Sand flies were abundant in the warm and humid months when the average temperature was 28.2-28.3ºC and the relative humidity was 81-82%. *P. argentipes* was active throughout the year in Saraburi Province (Polseela *et al*, 2011a). In the present study, this species was caught only in April and *S. gemmea* was the most frequently identified species throughout the year followed by *S. indica*.

Although no *Leishmania* species among sand flies collected were detected in this study, people are at risk from infection if they live in a region where *Leishmania* species are found. In 2008, a case of visceral leishmaniasis caused by *L. siamensis* was reported in a 5-year old girl living in Satun Province (Osatakul *et al*, 2014). *S. gemmea* and *S. barraudi* (potential vectors for *L. siamensis*) and *P. argentipes* (a natural vector for *L. donovani*) were identified in this study. Further studies are needed to prove the potential sand flies vectors of leishmaniasis in Thailand.

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