NOCTURNAL ACTIVITY OF PHLEBOTOMINE SAND FLIES IN SATUN PROVINCE, THAILAND

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Abstract. Phlebotomine sand flies are proven vectors of leishmaniasis in several countries. The main vector species in Thailand is still unknown and knowledge of nocturnal activity patterns of phlebotomine sand flies is very limited. Phlebotomine sand flies were collected using Center for Disease Control (CDC) incandescent light and black light traps were set up outdoors at two hourly intervals from 18:00 to 06:00 hours in Satun Province, southern Thailand. In total, 6,739 sand flies, 2,069 males and 4,670 females, were collected during June 2015, comprising six species belonging to 2 genera, *Phlebotomus* and *Sergentomyia*. *S. indica* (72.8%) and *S. gemmea* (26.6%) were the most common species caught in both types of traps. The number of phlebotomine sand flies collected in the CDC black light traps is significantly higher than in the incandescent light traps. The numbers of female *S. gemmea* differs significantly among the time intervals, whereas *S. indica* shows no significant differences. The number of phlebotomine sand flies is significantly correlated with temperature and relative humidity.

Keywords: *Phlebotomus*, *Sergentomyia*, light trap, nocturnal activity, sand fly, Thailand

INTRODUCTION

Phlebotomine sand flies are blood-sucking insects belonging to the order Diptera, family Psychodidae and subfamily Phlebotominae (Killick-Kendrick, 1999). As with mosquitoes, male sand flies do not bite but adult females require blood for egg maturation. *Leishmania* parasites are transmitted by the bite of infected female sand flies. About 98/800 species of phlebotomine sand flies are proven or suspected vectors of leishmaniasis (Maroli *et al*, 2013). In Thailand, at least 30 species of phlebotomine sand flies belonging to 4 genera (*Chinius*, *Idiophlebotomus*, *Phlebotomus* and *Sergentomyia*) have been recorded (Apiwathnasorn *et al*, 1989; Depaquit *et al*, 2006; Muller *et al*, 2007; Depaquit *et al*, 2009; Apiwathnasorn *et al*, 2011; Curler, 2011; Polseela *et al*, 2016a, b; Phumee *et al*, 2017), and among them *P. argentipes*, *P. major*, *S. gemmea* and *S. barraudi* are potential vectors for leishmaniasis (Kanjanopas *et al*, 2013; Chusri *et al*, 2014; Suankratay, 2014; Leelayoova *et al*, 2017).

Phlebotomine sand flies are not strong fliers but fly close to the ground in short hops, searching for blood and sugar meals, mates or resting and breeding sites

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(Yuval and Schlein, 1986). Only host-seeking or unfed females typically travel a few kilometers from their breeding site, while others rarely move more than a few hundred meters (Ready, 2013). Such behavior is governed by temperature, relative humidity, rain and wind speed (Oliveira *et al*, 2013). The biting activity of phlebotomine sand flies is usually crepuscular or nocturnal, but some species may bite during the day in darkened rooms or in forests on overcast days (Service, 2000).

Centers for Disease Control (CDC) light traps are used extensively for trapping and monitoring sand flies (Alexander, 2000). The nocturnal activity of sand flies shows a preference for different colored lights (Hoel *et al*, 2007). Knowledge on nocturnal activity is important for reducing contact between phlebotomine sand flies and humans or animals, and in planning control programs for leishmaniasis. Little is known regarding the nocturnal activity of phlebotomine sand fly species in Thailand, especially where leishmaniasis has been reported. The present study determined the nocturnal activity of phlebotomine sand flies by means of light traps in Satun Province, one of the leishmaniasis affected areas of southern Thailand.

MATERIALS AND METHODS

Study area

Field sites were randomly selected in Ban Sai Ngam (06º 37'07.19" N, 100º 03'27.90" E; 3 m above sea level), Mueang Satun District, Satun Province, Thailand. The population size of the village is approximately 1,932 inhabitants, the majority of whom are farmers and live in close association with domestic animals. Among the collection sites there were domestic animals (cat, cattle, chicken, duck, goat, and rabbit) and wild birds. Rubber plantations and termite mounds surround human habitations.

Specimen collection

Nocturnal activity of phlebotomine sand flies was determined for 8 nights in June 2015. Collections were made using two CDC incandescent light and two CDC black light traps per site. Traps were hung from tripods approximately 50 cm above the ground and were in operation for four consecutive nights (June 8-11) and later again on June 22-25. In order to minimize positional bias, the two types of light traps were operated alternatively. Collections were made at two hourly intervals from 18:00 to 06:00 hours, and temperature and relative humidity were recorded every two hours using a digital thermometer (Qingdao Tlead International, Shan Dong, China). Captured specimens were directly preserved in 95% ethanol.

Species identification

Captured insects were examined using a stereomicroscope to separate phlebotomine sand flies from other insects and to determine the sex based on the genitalia. Female head and genitalia were cleaned and mounted for morphological identification using standard taxonomic keys (Quate, 1962; Lewis, 1978; Phumee *et al*, 2017).

Data analysis

Prior to data analysis, data were log-transformed [log (n+1)] to normalize the distribution. The numbers of phlebotomine sand flies collected using the incandescent and black light traps were compared using an independent *t*-test. The differences between the two hourly intervals were compared using a one-way analysis of variance (ANOVA). When ANOVA is significant, means are separated by least significant difference

Table 1
Numbers of phlebotomine sand flies collected in Mueang Satun District, Satun Province, Thailand.

Species	CDC trap type		Total	(%)
	Incandescent light	Black light		
Phlebotomus stantoni	0	6	6	0.13
Sergentomyia barraudi	6	3	9	0.19
Sergentomyia gemmea	459	784	1,243	26.62
Sergentomyia hivernus	1	6	7	0.15
Sergentomyia indica	892	2,508	3,400	72.81
Sergentomyia perturbans	2	3	5	0.11
Female	1,360	3,310	4,670	69.30
Male	542	1,527	2,069	30.70

(LSD) *post hoc* analysis. Pearson correlation coefficient (r) was used to estimate relationships between phlebotomine sand fly abundance and temperature and relative humidity. Statistical significance level is set at $p < 0.05$. Data were analyzed using R program version 3.4.1 (R Core Team, 2017).

RESULTS

In total, 6,739 phlebotomine sand flies were captured, comprising 2,069 males (30.7%) and 4,670 females (69.3%). Six species belonging to two genera (*Phlebotomus* and *Sergentomyia*) were identified to the species level as *P. stantoni*, *S. barraudi*, *S. gemmea* (26.6%), *S. hivernus*, *S. indica* (72.8%) and *S. perturbans* (Table 1).

Both incandescent light and black light traps caught more females than males throughout the collection period, with female:male ratio of 2.5:1 and 2.2:1, respectively. CDC black light trap was more efficient than incandescent light in capturing phlebotomine sand fly males (t = -3.248, p = 0.003), females (t = -2.698, p = 0.011) and total number (t = -3.048, p = 0.005).

The mean number of female *S. indica* collected is not significantly different among the time intervals using CDC incandescent light trap (F = 1.173, df = 5, 90; p= 0.329) or using CDC black light trap (F = 0.425, df = 5, 90; p= 0.830) (Table 2). However, collection of *S. indica* in incandescent light trap peaked during 22:00-24:00 hours and during 24:00-02:00 hours in black light trap. On the other hand, there is a significant difference in the numbers of *S. gemmea* collected in both types of light traps over the time intervals (F = 3.420, df = 5, 90, $p < 0.05$ and F = 3.471, df = 5.90, $p < 0.05$, respectively) with the highest numbers collected during 22:00-24:00 hours. With both types of light traps, significantly less *S. gemmea* is collected during 04:00-06:00 hours than during all other periods of trapping time ($p< 0.05$) but no significant difference between 02:00-04:00 and 04:00-06:00 hours ($p> 0.05$).

As regards environmental factors, there is a significantly negative correlation between the total number of phle-

Table 2
Mean number (±SE) of phlebotomine sand fly species collected during 2-hourly intervals in CDC traps in Mueang Satun District, Satun Province, Thailand.

Period (hrs)	Incandescent light		Black light	
	Sergentomyia gemmea	*Sergentomyia indica*	*Sergentomyia gemmea*	*Sergentomyia indica*
18.00 - 20.00	6.4 ± 3a	7.4 ± 2.5a	11 ± 3.7ab	24.6 ± 6.8a
20.00 - 22.00	5.9 ± 1.9a	10.1 ± 3.7a	11.1 ± 3.9ab	22.4 ± 6.6a
22.00 - 24.00	6.6 ± 1.9a	12.5 ± 3.7a	11.4 ± 2.4a	28.5 ± 7.7a
24.00 - 02.00	5.6 ± 1a	12 ± 3.1a	8.4 ± 2.2ab	32.8 ± 9.8a
02.00 - 04.00	2.9 ± 0.8ab	9.3 ± 1.9a	4.3 ± 1.4bc	31.4 ± 9a
04.00 - 06.00	1.3 ± 0.4b	4.6 ± 1.1a	2.8 ± 0.8c	17.1 ± 3.8a
ANOVA	$F = 3.420$	$F = 1.173$	$F = 3.471$	$F = 0.425$
$df = 5, 90$	$p = 0.007$	$p = 0.329$	$p = 0.006$	$p = 0.830$

Column means followed by the same lowercase letter in each column are not significantly different, LSD test, $p < 0.05$. Untransformed data shown.

botomine sand flies collected and relative humidity ($r = -0.459$, $p = 0.001$), while there is a significant positive correlation with temperature ($r = 0.328$, $p = 0.023$) (Fig 1). The correlation of total number of male phlebotomine sand flies trapped with relative humidity was negative ($r = -0.676$, $p = 0.000$) and positive with temperature ($r = 0.500$, $p = 0.000$). There are no significant correlations between total number of female phlebotomine sand flies collected with either relative humidity ($r = -0.256$, $p = 0.079$) or temperature ($r = 0.157$, $p = 0.286$). The number of female *S. gemmea* shows a significant negative correlation with relative humidity ($r = -0.526$, $p = 0.000$) and a significant positive correlation with temperature ($r = 0.446$, $p = 0.002$), while for *S. indica* there is no significant correlations with either relative humidity ($r = -0.118$, $p = 0.426$) or temperature ($r = 0.04$, $p = 0.979$).

DISCUSSION

In our study, one species of *Phlebotomus* (*P. stantoni*) and five species of *Sergentomyia* were identified (*S. barraudi*, *S. gemmea*, *S. hivernus*, *S. indica* and *S. perturbans*). A previous study in this area by Panthawong *et al* (2015) found two species of *Phlebotomus* (*P. argentipes* and *P. stantoni*) and five species of *Sergentomyia* (*S. barraudi*, *S. gemmea*, *S. indica*, *S. iyengari* and *S. perturbans*) with *S. gemmea* being the most abundant species (53.5%) followed by *S. indica* (42.2%). Several other surveys in southern Thailand reported *S. gemmea* the most abundant species (Kanjanopas *et al*, 2013; Sukra *et al*, 2013; Chusri *et al*, 2014), while the present study revealed the most abundant species as being *S. indica*, followed by *S. gemmea*. Differences in abundance and sand fly species among the studies could be due to variations in local conditions, such as wind speed, tem-

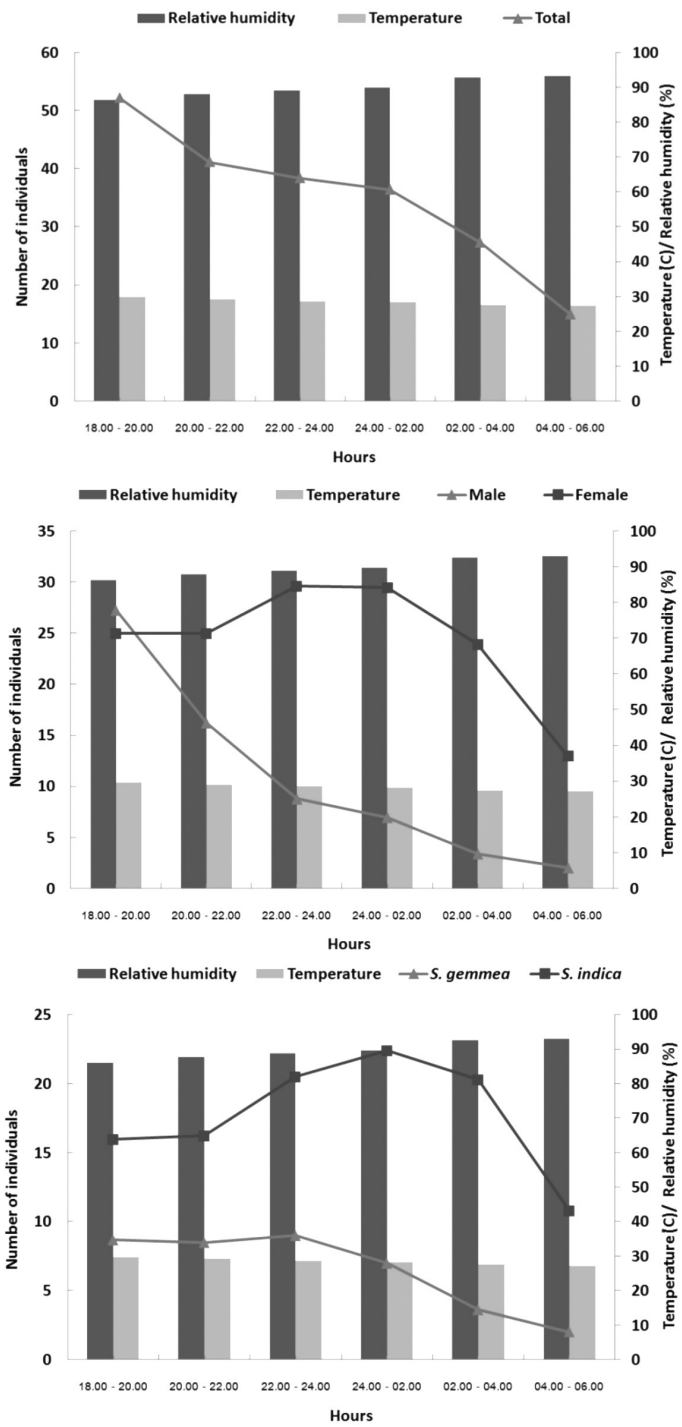

Fig 1–Nocturnal activity of phlebotomine sand fly with variations in relative humidity and temperature during June 2015.

perature, humidity, type of resting habitats and trap location. Srinivasan *et al* (2013) reported sand flies are most abundant in tree buttress, followed by tree hole, termite mound, cattle shed and human dwelling.

The present study collected more female than male phlebotomine sand flies using either incandescent or black light trap is similar to the findings of Polseela *et al* (2007, 2011a,b), Chittsamart *et al* (2015), Panthawong *et al* (2015) and Polseela *et al* (2015). Female phlebotomine sand flies not only have to take a blood meal but need also to find mates and to search for oviposition sites. Therefore, the females are more active and likely to be caught in traps than males (Kan *et al*, 2004).

Significantly more phlebotomine sand flies were attracted to the CDC black light than to CDC incandescent light traps in the present study is in line with observations from Iraq (Burkett *et al*, 2007). However, in Kenya CDC miniature light traps caught more *Phlebotomus* species than black light traps but no difference was found with *Sergentomyia* species (Kasili *et al*, 2010).

In Thailand the main vector species of leishmaniasis remains unknown. However, *Leishmania* DNA was detected in *S. barraudi* and *S. gemmea* in southern Thailand (Kanjanopas *et al*, 2013; Chusri *et al*, 2014). Further studies are needed to determine the vector competence for *Leishmania* transmission of these sand fly species. It is worth noting the nocturnal activities of *S. gemmea* were greatest from 18:00 to 04:00 hours, with relatively few collected from 04:00 to 06:00 hours, whereas *S. indica* was active throughout the night during June, similar to findings in Iraq where *Sergentomyia* spp are active throughout the night during June (Coleman *et al*, 2007), and in Turkey where sand fly activity is continuous throughout the night and decreases during 04:00-06:00 hours (Kasap *et al*, 2009).

Guernaoui *et al* (2006) found nocturnal activity among different species of sand flies in Morocco is correlated with temperature and relative humidity. In Turkey, the number of sand flies showed a significant negative correlation with relative humidity (Kasap *et al*, 2009). On the other hand, in Ethiopia nocturnal activity of female *P. orientalis* in the north-western lowland region exhibits a significant negative correlation with temperature and a significant positive correlation with relative humidity; however, in the highlands, mean temperature has a significant positive correlation with mean hourly activity, whereas there is no correlation of activity with relative humidity (Aklilu *et al*, 2017). It would appear that both high temperature and low relative humidity are the most important factors affecting sand fly activity or abundance, and other factors, such as light intensity, rain and wind speed have significant effects on nocturnal activity (Roberts, 1994; Brilhante *et al*, 2017).

In summary, our survey of phlebotomine sand fly nocturnal activity suggests that people reside in or visit leishmanias affected areas will be exposed to phlebotomine sand flies throughout the night. To avoid being bitten individuals should wear clothing covering exposed skin areas and sleep under bed nets.

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