SEASONAL VARIATION OF CAVE-DWELLING PHLEBOTOMINE SANDFLIES (DIPTERA:PSYCHODIDAE) IN PHRA PHOTHISAT CAVE, SARABURI PROVINCE, THAILAND

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Abstract. Phlebotomine sandflies are widely distributed in Thailand. In view of recent occurrence of indigenous cases of leishmaniasis in Thailand, a bionomic study of sandflies was undertaken in Phra Phothisat cave, Saraburi Province, Thailand from August 2005 to July 2006. The insects were collected monthly by CDC light traps between 06:00 PM and 06:00 AM. They were preserved in 80% alcohol and mounted with Hoyer's medium for species identification. A total of 5,514 sandflies were collected with a female:male ratio of 2.5:1. The collected sandflies belong to 13 species in the genera *Phlebotomus* and *Sergentomyia*, namely *S. silvatica* (43.5%), *S. anodontis* (31.5%), *S. dentata* (15.3%), *S. barraudi* (3.3%), *P. argentipes* (2.0%), *P. philippinensis gouldi* (1.0%), *P. stantoni* (0.5%), *S. gemmea* (0.5%), *P. major major* (0.1%), *S. perturbans* (0.1%), *S. iyengari* (0.1%), *S. bailyi* (0.1%), and *P. teshi* (0.1%). The results revealed seasonal variation in sandflies with the highest peak in July (436 sandflies/trap-night). Some of the sandflies could not be identified and were assumed to be new species. Soil samples inside the cave were analyzed for chemical characteristics. The soil was characterized by moderate acidity (pH 5.8) with various amount of chemicals and nutrients.

INTRODUCTION

Phlebotomine sandflies have a wide distribution throughout the world, mainly in the tropics and subtropics (Adler and Theodor, 1957). Phlebotomine sandflies are small bloodsucking flies that are vectors of leishmaniasis. There are about 700 species of phlebotomine sandflies, of which about 70 species are considered to be disease vectors.

In Thailand, leishmaniasis is seen as an imported disease, with the number of cases increasing in the last 20 years. Recently leishmaniasis has received increasing attention. There were three cases of autochthonous visceral leishmaniasis reported in 1996, 2005, and 2006 (Juthbhuddhi, 1985; Suttinont *et al*, 1987). Little is known about Thai sandflies, however, Apiwathnasorn *et al* (1989) conducted extensive sandfly surveys and reported *Phlebotomus major major* as a cow biting species and the most common cave dweller was *P. argentipes.* In 1993, *P. hoepplii* was found to be a man-biting species (Apiwathnasorn *et al*, 1993). The present study was undertaken to understand the bionomics of cave-dwelling sandflies.

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MATERIALS AND METHODS

Study area

Our investigation was carried out from August 2005 to July 2006 at Phra Phothisat cave, Thap Kwang Subdistrict, Kaeng Khoi District, Saraburi Province, Thailand. The cave is located within the precincts of Tham Phra

Phothisat temple, approximately 140 km from Bangkok. This limestone cave is 70 m long, 2-25 m wide and 2-20 m high and located 165 m above sea level. Caves are commonly found in calcareous areas. Normally it is dry, but in rainy periods transient water may occur. The cave is depicted in bas-reliefs of the Buddha preaching the dhamma. The Fine Art Department registered the cave as a national ancient monument in 1965.

Specimen collection

Adult sandflies were captured monthly by operating CDC light traps overnight (06:00 PM-06:00 AM) for consecutive nights. Twelve trap locations were selected along the interior of the cave and in areas with abundant crevices and chambers. The collected sandflies were preserved in 80% alcohol; only female sandflies were mounted on glass slides using Hoyer's medium for identification. Sandflies were identified by taxonomic keys, particularly that of Lewis (1978).

Soil samples

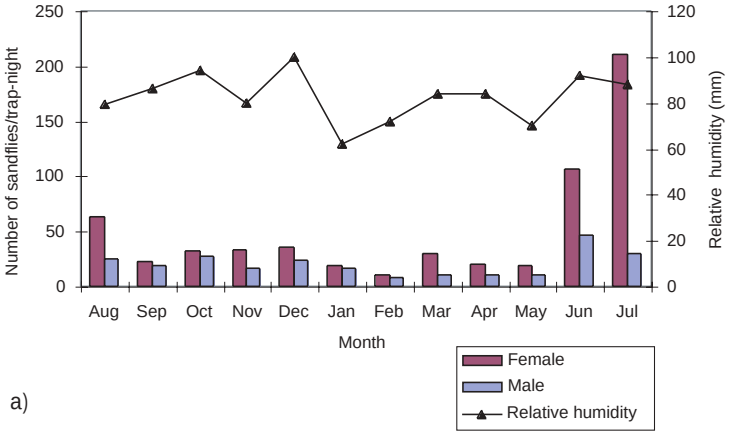
Soil samples were collected from the cave following the LaMotte Soil Handbook with some modifications (LaMotte, 2001). A shovel was used to collect samples of the top soil layer to a depth of approximately 30 cm. Four soil samples were collected from each location to produce average samples of 1 kg (Kankaew, 2005). Soil samples were mixed together. Roots, stones and other foreign materials were removed. Air-dried soil samples were ground by a stone pestle and screened through a wire sifter (80 mesh size). Fine soil samples were extracted and tested following the LaMotte Model STH series instruction manual with some modifications.

RESULTS

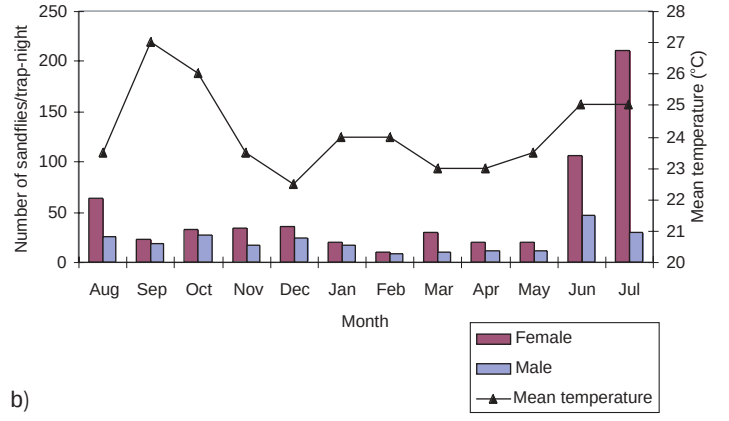
The Phra Phothisat cave is occupied by various species of phlebotomine sandflies. A total of 5,514 sandflies were collected with a female to male ratio of 2.5:1. Thirteen species

Table 1
The monthly distribution of sandflies captured in Phra Phothisat cave, Saraburi Province from August 2005 to July 2006.

Cave-dwelling	Month													
	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Jul	Total	%
P. argentipes	20	13	14	7	3	5	1	2	2	5	2	4	78	2.0
P. philippinensis gouldi	0	30	9	0	1	0	0	0	0	0	0	1	41	1.0
P. stantoni	2	2	1	0	2	2	2	0	1	1	3	4	20	0.5
P. major major	1	0	0	0	1	0	0	2	0	0	0	0	4	0.1
P. teshi	2	0	0	0	0	0	0	0	0	0	0	0	2	0.1
S. silvatica	190	36	135	54	1	2	0	0	1	39	249	999	1,706	43.5
S. anodontis	88	14	10	88	125	63	48	145	92	59	305	199	1,236	31.5
S. dentata	61	35	18	49	88	47	13	32	26	18	113	100	600	15.3
S. barraudi	7	10	4	17	9	7	4	11	4	1	10	47	131	3.3
S. gemmea	0	2	0	0	0	0	0	2	0	2	0	12	18	0.5
S. perturbans	0	0	0	0	0	0	0	0	0	0	2	0	2	0.1
S. iyengari	0	0	0	0	0	0	0	0	0	2	0	0	2	0.1
S. bailyi	0	2	0	0	0	0	0	0	0	0	0	0	2	0.1
Unidentified	40	1	17	2	1	0	0	0	4	1	5	4	75	1.9
Female	411	145	208	217	231	126	68	194	130	128	689	1,370	3,917	71.0
Male	164	123	176	106	156	112	58	67	72	69	301	193	1,597	29.0
Total	575	268	384	323	387	238	126	261	202	197	990	1,563	5,514	100.0



a)



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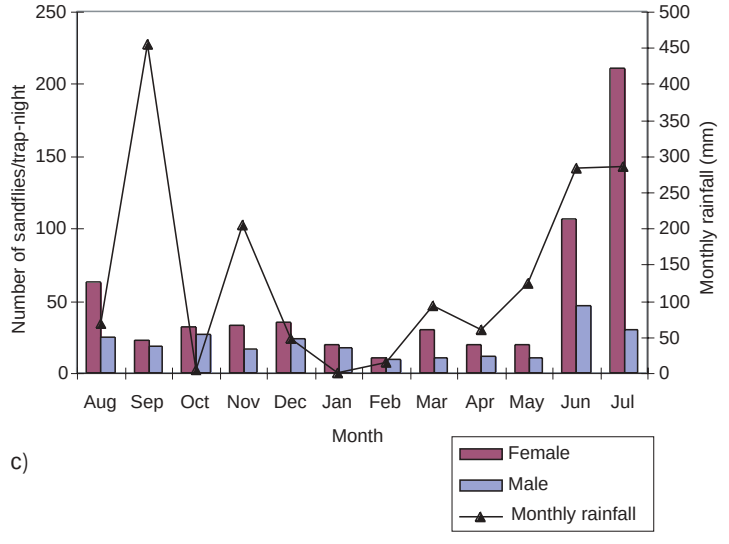

c)

Fig 1–Number of phlebotomine sandflies per trap-night and relative humidity (a), average temperature (b), and monthly rainfall (c) in Phra Phothisat cave, Saraburi Province from August 2005 to July 2006.

were identified, of which five species belong to the genus *Phlebotomus* and eight species to the genus *Sergentomyia.* Some sandflies were unidentified and may be new species. Cave dwelling species comprised the abundant species (*S. silvatica* 43.5%, *S. anodontis* 31.5%, and *S. dentata* 15.3%) and the less common species (<4%) (*P. argentipes, P. philippinensis gouldi*, *P. stantoni*, *P. major major, P. teshi*, *S. barraudi*, *S. gemmea, S. perturbans*, *S. iyengari* and *S. bailyi*). The greatest number of specimens were collected in July. *P. argentipes*, *S. anodontis*, *S. dentata*, and *S. barraudi*, were found in CDC light traps throughout the year (Table 1).

Soil samples were loamy fine sand with medium to fine texture. The colors observed were light to dark brown with roots, stones, and other foreign materials. Chemical characteristic of soil samples were determined for all twelve samples. Soil analysis for chemical characteristics showed a high level of nitrate nitrogen, phosphorus, potassium, calcium, sulfate and low levels of aluminum, ferric iron, magnesium, manganese and humus. The average pH of the soil was 5.8 (pH range = 5.5-6.2) (Table 2).

Table 2
Chemical characteristics of soil samples from Phra Phothisat cave, Saraburi Province.

Chemical	Mean	Min	Max
pH	5.8	5.5	6.2
Nitrate nitrogen (ppm)	56.3	50	75
Phosphorus (ppm)	100	100	100
Potassium (ppm)	400	400	400
Aluminum (ppm)	5	5	5
Calcium (ppm)	14,000	14,000	14,000
Ferric iron (ppm)	25	25	25
Humus (level)	0	0	0
Magnesium (ppm)	15	5	25
Manganese (ppm)	10.7	5	25
Sulfate (ppm)	1,916.7	1,000	2,000

DISCUSSION

A previous survey in Thailand of cave-dwelling sandflies found eight species: *P. argentipes*, *P. stantoni*, *P. teshi*, *S. anodontis*, *S. hodgsoni hodgsoni*, *S. iyengari*, *S. silvatica*, and *N. vietnamensis* (Apiwathnasorn *et al*, 1989).

More female phlebotomine sandflies were collected than males. It is necessary for female sandflies to have a blood meal before flight to search for oviposition sites. Therefore, females may be more likely to be captured in traps (Eiko *et al*, 2004).

Monthly collection of sandflies demonstreated they were more prominent during the early rainy season (June-August) (Fig 1). The greatest number (436 sandflies/trap-night) were captured in July. The cave temperature was 22.5-27.0ºC with 62-100% relative humidity (RH). Tropical sandflies tend to feed at a temperature of at least 20ºC and generally prefer a RH of 75-80% (Lane, 1987). The most favorable conditions for sandflies to reside in the cave found in this study were a temperature of 23.5-25ºC with 80-90%RH, which was during rainy season. An observation accounted for by the predilection of sandflies to seek microhabitats offering high humidity and stable temperatures, avoiding high temperatures and low relative humidity (Kaul, 1991). Modi and Tesh (1983) reported the successful rearing of *P. kezeruni* in an insectary maintained at 26ºC and 90%RH.

The soil acidity averaged pH 5.8, similar to the soil pH of the breeding place for *P. mascittii* reported by Grimm *et al* (1993). Sandfly larvae feed on dead organic matter containing macronutrients present in damp places, including cracks in walls or rocks (Lane, 1993). In Sardinia, soil analysis of the substrate soil for texture, pH, $CaCO_3$, organic matter and water content showed no correlation with the number of sandflies that emerged from the breeding site for *S. minuta, P. periliewi*, and *P. perniciosus* (Bettini *et al*, 1986; Bettini and Melis, 1988).

Our findings are beneficial for monitoring population dynamics of phlebotomine sandflies in relation to environmental conditions.

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