

เอกสารอ้างอิง (References)

- Adler S, Theodor O. Transmission of disease agents by phlebotominae sand flies. *Ann Rev Entomol* 1957; 2:203-26.
- Apiwathnasorn C, Sucharit S, Rongriyam Y, *et al.* A brief survey of Phlebotominae sand flies in Thailand. *Southeast Asian J Trop Med Public Health* 1989;20:429-31.
- Apiwathnasorn C, Sucharit S, Surathi K, Deesin T. Anthropophilic and zoophilic phlebotomine sand flies (Diptera:Psychodidae) from Thailand. *J Am Mosq Contro.* 1993;9:135-7.
- Brown HW. *Phlebotomus* sand flies, *Basic Clinical Parasitology* 1969;59-68.
- Busvine JR. Sand flies (Phlebotominae, Psychodidae) *Insects and Hygines* 3th edition. 1962;174-7.
- Causey OR. *Phlebotomus* of Siam with a description of new variety. *Am J Hyg* 1938;28:487-9.
- Desjeux P. Leishmaniasis: current situation and new perspectives. *Comp. Immunol Microbiol infect Dis* 1996;27:305-18.
- Eiko K, Christopher OA, Rajindar KS, *et al.* Phlebotomine sand flies (Diptera: Psychodidae) collected in Mukusu, Machakos District, Kenya and their nocturnal flight activity. *Appl Entomol Zool* 2004; 39:651-9.
- Emanuel R, John LF. *Pathology*. Second edition. J.B. Lippincott company Philadelphia 1994:431-3.
- Erika S, Leonard EM, Cristina F. Estimating Carrying Capacity in a Newly Colonized Sand Fly *Lutzomyia serrana* (Diptera: Psychodidae) *J Econom Entomol* 2002;95:149-54.
- Guernaoui S, Boussaa S, Pesson B, *et al.* Nocturnal activity of phlebotomine sand flies (Diptera: Psychodidae) in a cutaneous leishmaniasis focus in Chichaoua Morocco. *Parasitol Res* 2005;184-8.
- Hermes WB. *Medical Entomology*. The Macmillan company. London 1969;156.
- Kalra NL, Bong YH. *Manual on entomology in visceral leishmaniasis*. World Health Organization 1988; 2-3.
- Killick-Kendrick R. The biology and control of phlebotomine sand flies. *Clinics in dermatology* 1999; 17:279-89.
- Killick-Kendrick R, Rioux JA, Bailly M, *et al.* Ecology of leishmaniasis in the south of France 20. Dispersal of *Phlebotomus ariasi* Tonnoir, 1921 as a vector in the spread of visceral leishmaniasis in the Cevennes. *Annales de Parasitologie humaine et compare* 1984;59:555-72.
- Kishore K, Kumar V, Kesari S, *et al.* Vector control of leishmaniasis. *Indian J Med Res* 2006;123:467-72.
- Kongkaew W, Siriarayaporn P, Leelayoova S, *et al.* Autochthonous visceral leishmaniasis; a report of a second case in Thailand. *Southeast Asian J Trop Med Public Health* 2007;38:8-12.

- Lane RP, Crosskey RW. Medical insects and Arachnids. The natural History Museum 1993;6-102.
- Leishmaniasis [online].2008 [cited 2008 January 17]. Available from URL: <http://www.who.int/tdr/dw/leish2004.htm>
- Leishmaniasis: Susceptible species [online].2008 [cited 2008 January 5]. Available from URL: www.Leishmaniasis.htm
- Lewis DJ. The biology of Phlebotomidae in relation to leishmaniasis. *Ann Rev Entomol* 1974;19:363-84.
- Lewis DJ. Phlebotomine sand flies (Diptera: Psychodidae) from the Oriental Region. *Systematic* 1987;12:163-80.
- Mary LS. Laboratory Culture of two *Phlebotomus* Species, *P. papatasi* and *P. orientalis* Bull Org mond Sante and Bull Wld Hlth Org 1964;31:577-8.
- Morton I, Ward RD. Laboratory response of female *Lutzomyia longipalpis* sand flies to a host and male pheromone source over distance. *Med Vet Entomol* 1989;3:219-23.
- Muller F, Depaquit J, Leger N. *Phlebotomus (Euphlebotomus) mascomai* n. sp. (Diptera: Psychodidae). *Parasitol Res* 2007;101:1597-602.
- Polseela R, Apiwathanasorn C, Samung Y. Seasonal Variation of Cave-Dwelling Phlebotomine Sand Flies (Diptera: Psychodidae) in Phra Phothisat Cave, Saraburi Province, Thailand. *Southeast Asian J Trop Med Public Health* 2007;38:1011-1015.
- Polseela, R., Apiwathnasorn, C, Samung, Y.. Seasonal distribution of phlebotomine sand flies (Diptera: Psychodidae) in Tham Phra Phothisat temple, Saraburi province, Thailand. *Tropical Biomedicine* 2011;28(2): 366–375.
- Quate LW. A Review of the Indo-Chinese Phlebotominae (Diptera: Psychodidae). *Pacific Insect* 1962;4: 251-67.
- Raynal J, Gaschen H. Sur La presence de phlebotomus dans lenord de l'Indochine, *Bulletin of Society of Medical. Chir. Indochine* 1934;12:531.
- Samir SS, Muhamad SS, Haroun MK, *et al.* Phlebotomine sand flies (Diptera: ychodidae) of the Palestinian West Bank: Potential vectors of leishmaniasis. *J Med Entomol* 2003;40:321-8.
- Sanyal RK, Alam SN, Kaul SM, Wattal BL. Some observations on the epidemiology of current outbreak of Kala azar in Bihar. *J Commun Dis* 1979;11:170-82.
- Service MW. A Guide to medical Entomology. Macmillan International college editions 1980;77.
- Service MW. Medical entomology for student. Second edition. Cambridge University press 2000; 91-100.
- Sukmee T, Siripattanapipong S, Mungthin M, *et al.* A suspected new species of *Leishmania*, the causative

- agent of visceral leishmaniasis in a Thai patient. *Int J Parasitol* 2008;38:617-22.
- Symes CB, Thompson RCM, Busvine JR. *Insect control in Public Health*. Elsevier Publishing Companies, Amsterdam 1962;136.
- Theodor O. On sand flies *Phlebotomus* from Ceylon, Siam and Malay. *Indian J Med Res* 1938;26-261.
- Ward RD, Phillips A, Burnet B, *et al.* The *Lutzomyia longipalpis* complex: reproduction and distribution. *Biosystematics of haematophagous insects*.xi. Clarendon Press, Oxford 1988;57-69.
- World Health Organization. Control of leishmaniasis. Reports of a WHO Expert Committee. WHO Technical Report 1990;739,793.
- World Health Organization. Manual on Entomology in Visceral Leishmaniasis by Kalra NL and Bang YH. SEA/VBC 1988;1-85.

กิตติกรรมประกาศ

คณะผู้วิจัยขอขอบคุณ มหาวิทยาลัยนเรศวร ที่ได้สนับสนุนทุนวิจัยจากเงินงบประมาณแผ่นดิน ประจำปีงบประมาณ 2553 ทำให้โครงการวิจัยนี้สามารถดำเนินการจนเสร็จสมบูรณ์ ขอขอบคุณ รศ. ดร. ชำนาญ อภิวัฒน์สร คณะเวชศาสตร์เขตร้อน มหาวิทยาลัยมหิดล ที่ให้คำปรึกษาตลอดโครงการ ขอขอบคุณ กรมอุทยานแห่งชาติ สัตว์ป่า และพันธุ์พืช กระทรวงทรัพยากรธรรมชาติและสิ่งแวดล้อม ที่ได้อนุญาตให้เข้าทำการสำรวจในพื้นที่ของเขตของอุทยาน อีกทั้งหัวหน้าเขตห้ามล่าสัตว์ป่าถ้ำผาท่าพล นายชาญวิทย์ แสงสร้อย และหัวหน้าเขตอุทยานแห่งชาติทุ่งแสลงหลวง นายสุชาติ วัฒนะกาญจน์ ที่ได้อำนวยความสะดวกและช่วยเหลือในการสำรวจในพื้นที่ด้วยดี รวมทั้ง นายแพทย์ การสมดี เจ้าหน้าที่ของเขตห้ามล่าสัตว์ป่าถ้ำผาท่าพล และเจ้าหน้าที่ของเขตหน่วยพิทักษ์อุทยานแห่งชาติที่ สล.6 บ้านมุง ทุกท่านที่ให้ความอนุเคราะห์และช่วยในการเก็บข้อมูลในพื้นที่ที่ทำการศึกษา ซึ่งทีมงานประกอบด้วยนายสันติภาพ ศิริ นายณัฐสรณ์ พุกอูด และนายมนัส สีแก้ว ขอขอบคุณผู้ใหญ่บ้านหมู่ที่ 1 บ้านมุง ตำบลบ้านมุง อำเภอเนินมะปราง จังหวัดพิษณุโลก ที่ให้ความอนุเคราะห์ในการเก็บรีนฝอยทรายในเขตหมู่บ้าน รวมทั้งนายสวาท สุขสวัสดิ์ ที่ได้ประสานงานและช่วยทำการเก็บตัวอย่างรีนฝอยทรายด้วยวิธีการใช้กับดักแสงไฟ การใช้สัตว์เป็นเหยื่อล่อและ human landing capture และประชาชนในหมู่บ้านที่ให้ความร่วมมือในการวางกับดักในบริเวณที่อยู่อาศัย ขอขอบคุณ ผู้ช่วยศาสตราจารย์แสงชัย นทีวรรณด ที่มีส่วนในการดักรีนฝอยทราย และขอขอบคุณผู้ช่วยวิจัยโดยเฉพาะอย่างยิ่ง นายบรรจง ดีเหลือที่มีส่วนช่วยทั้งแรงกายแรงใจในการวางแผนและดำเนินการเก็บตัวอย่างรีนฝอยทรายทุก 2 ชั่วโมงตลอดการศึกษา อีกทั้งดูแลทีมงานและเครื่องมือที่ใช้ทำการศึกษาให้มีประสิทธิภาพตลอดการศึกษาวิจัยครั้งนี้ นอกจากนี้ขอขอบคุณภาควิชาจุลชีววิทยาและปรสิตวิทยา และคณะวิทยาศาสตร์การแพทย์ที่ให้ความอนุเคราะห์ครุภัณฑ์และสถานที่ที่จำเป็นในการวิจัยตลอดโครงการจนสำเร็จลุล่วงไปด้วยดี รวมทั้งขอขอบคุณนิสิตสาขาวิชาจุลชีววิทยาที่ได้มีส่วนในการเก็บรีนฝอยทรายคือ นายพีระวิทย์ กิจดิยะพงศ์ และนายณัฐพงศ์ บุญเจริญ นอกจากนี้ขอขอบคุณเพื่อน พี่ และน้องๆ ทุกท่านที่ได้มีส่วนในการศึกษาค้นคว้าครั้งนี้สำเร็จ ซึ่งผลการศึกษาค้นคว้าครั้งนี้จะเป็นข้อมูลที่มีประโยชน์ต่อประชาชนในพื้นที่ สังคมและประเทศชาติในการเฝ้าระวังโรค ไลชมาเนียต่อไป

ความก้าวหน้าตามตัวชี้วัดเพื่อประเมินผลสำเร็จของโครงการ

| ระดับ ผลงาน | ประเภทของผลงาน | จำนวน |
|----------------|---|----------|
| A | 1. จดอนุสิทธิบัตร สิทธิบัตร หรือจดทะเบียนทรัพย์สินทางปัญญา อื่นๆ | - ผลงาน |
| | 2. ตีพิมพ์ในวารสารระดับนานาชาติที่มีค่า Impact Factor | 1 ผลงาน |
| B | 3. ตีพิมพ์ในวารสารระดับนานาชาติ (ไม่มีค่า Impact Factor) | - เรื่อง |
| | 4. ตีพิมพ์ในวารสารระดับประเทศ | - เรื่อง |
| | 5. นำเสนอในการประชุมวิชาการที่มีการตีพิมพ์บทความบน Proceedings | - เรื่อง |
| | 6. ถ่ายทอดผลงานวิจัย / เทคโนโลยีสู่กลุ่มเป้าหมาย | - คน |
| | 7. ได้สิ่งประดิษฐ์ อุปกรณ์ เครื่องมือ หรืออื่นๆ เช่น ฐานข้อมูล Software ที่สามารถนำไปใช้ประโยชน์ได้ต่อไป | - ชิ้น |
| C | 8. นำเสนอผลงานในที่ประชุมวิชาการ ที่มีการตีพิมพ์เฉพาะ Abstract | - เรื่อง |

Output ที่ได้จากโครงการ

1. ส่งผลงานตีพิมพ์ในวารสารนานาชาติ Tropical Biomedicine ในชื่อเรื่อง
The Nocturnal Flight Activity of the Phlebotomine Sand Fly (Diptera:Psychodidae) in Phadang Cave, Phitsanulok, Thailand (ได้รับการตอบรับแล้ว ตามเอกสารแนบและอยู่ระหว่างการแก้ไขตามคำแนะนำ)
2. ทำการวิจัยต่อเนื่องเรื่อง “การจำแนกชนิดของริ้นฝอยทรายโดยใช้วิธี PCR และ Morphology ในเขตภาคเหนือตอนล่างของไทย” และ “ลำดับนิวคลีโอไทด์ และความสัมพันธ์ทางสายวิวัฒนาการของริ้นฝอยทรายในประเทศไทย”

ภาคผนวก

The Nocturnal Flight Activity of the Phlebotomine Sand Fly (Diptera:Psychodidae) in Phadang Cave, Phitsanulok, Thailand

POLSEELA, R. *¹, VITTA, A. ¹, SAMUNG Y. ² AND APIWATHANASORN, C. ²

¹Department of Microbiology and Parasitology, Faculty of Medical Science, Naresuan University, Phitsanulok, Thailand

²Department of Medical Entomology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand.

^{1*}Corresponding author: e-mail:raxsinap@nu.ac.th,polseela@hotmail.com

Abstract

A study investigating the nocturnal flight activities of the phlebotomine sand fly was conducted from February 2010 to January 2011. Sand flies were collected by CDC light traps in Phadang Cave, Noen Maphrang, Phitsanulok, changing the bag at 2 hour intervals. Adult sand flies (totaling 18,709), represented fourteen species of three genera (9 of *Sergentomyia*, 4 of *Phlebotomus*, and 1 of *Chinius*). The population of *Sergentomyia dentata* (29.19%) was the largest among these fourteen species found in the cave. Population peaks in May and July were observed for these sand flies species and declined in December. The maximum numbers of sand flies were active during two periods; 2200-2400 and 2400-0200 hours, respectively. A male to female ratio of 1.35:1 was recorded for the phlebotomine sand flies. Examination of the activity conditions of sand flies collected showed that almost all male and females took place all night. *Phlebotomus argentipes* were found in the trap monthly through the course of the year. A population peak was observed for *P. argentipes* at intervals of 2200-2400 and 2400-0200 hours. This suggests that phlebotomine sand flies show stronger nocturnal flight activity. The result of the trap collection suggests that the sand flies are nocturnally active with the highest flight activity level after sunset. The widespread distribution and interval activity of sand flies may be an indicator of the epidemiologic importance of the vector sand flies in this habitat.

Key words: sand flies, CDC light traps, interval, nocturnal activity

INTRODUCTION

Phlebotomine sand flies (Diptera:Psychodidae) are an important vector of leishmaniasis, several arboviruses, bartonellosis, and sand fly fever (Young & Duncan, 1994). Genus *Lutzomyia* and *Phlebotomus* are known to transmit the protozoan parasites responsible for leishmaniasis. Leishmaniasis is one of the most important vector-borne diseases of humans. The disease is endemic in 88 countries; 66 are in the Old World and 22 in the New World (Desjeuk, 2004; Sharma & Singh, 2006). Species of the *Leishmania* have a wide geographic distribution. In Thailand, cases of visceral leishmaniasis are caused by *L. donovani*, *L. infantum*, and *L. saimensis* (Sukmee *et al.*, 2008). In the Old World, about 40 species of *Phlebotomus* spp. have been proven or suspected vectors of *Leishmania* spp. (Alexander & Maroli, 2003). Only adult female sand flies feed on blood required for the nutrients to develop their eggs and transmit the protozoa to the host (Lane, 1987; Killick-Kendrick, 1999). Sand flies occurred in a wide range of habitat. In Thailand, the sand flies fauna is represented in different locations. Most sand flies are nocturnally active or crepuscular, with activity peaks after sunset (Merrison *et al.*, 1995; Killick-Kendrick, 1999; Dinesh *et al.*, 2001, Sawalha *et al.* 2003). The nocturnal activity of sand flies has been studied in several countries where leishmaniasis is endemic (Kan *et al.*, 2004; Guernaoui *et al.*, 2006; Fahmy *et al.*, 2009; Kasap *et al.*, 2009). Previous studies in Thailand show the presence of nocturnal activity of sand flies from the different regions. Sand flies displayed nocturnal activity with different patterns (Badry, 2008). Predominantly, the sand fly is found in densely populated settlements. Sand flies as a vector of leishmaniasis in Thailand are a possibility.

In this study, collections of sand flies were obtained to determine the characteristics of nocturnal flight activity, and to specify the risk period of leishmaniasis in Phadang Cave. The species composition and sex ratios were also determined.

MATERIALS AND METHODS

Study area

Field studies were undertaken between February, 2010 and January, 2011 in Phadang Cave in Noen Maprang, Phitsanulok Province, located in the lower northern part of Thailand at $16^{\circ}30'58.8''\text{N}$, $100^{\circ}40'00.5''\text{E}$. This limestone cave is situated in the foothills of the mountains and presently consists of two asymmetrical caverns. This cave is estimated to be 15-80 m wide, 80-100 m long, and 1-20 m high. The area is mountainous, 108 meters above sea level. The cave may be wet or dry depending on the season and the amount of sunlight it receives. The white material on the walls is a calcium deposit. There are many bats for supporting sand fly hosts. This cave is open and suitable to the traveler. Many houses have been built on hillsides near the cave, about 2-3 kilometers in this area. Basic environmental conditions including temperature and relative humidity were recorded monthly with a hydro-hygrometer as part of the investigation. Rainfall data was kindly provided by the Thai Meteorological Department.

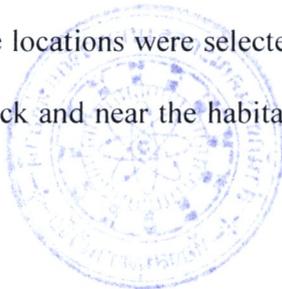




Figure 1. Inside Phadang cave, Noen Maprang, Phitsanulok Province

Specimen collection

Adult sand flies were collected using ten miniature light traps from the Center for Disease Control (CDC), USA one night per month (Sudia & Chamberin, 1962). The traps were set up at about 50-100 cm above the ground inside the cave. Traps were set before sunset till after sunrise. The capture was undertaken from 1800 to 0600 hours. The collection bag of each trap was changed at two hour intervals. A total of six collections per night were used to clarify the characteristics of nocturnal activity rhythm. During each month of the survey the sand flies were captured at the same locations. The locations were selected in different areas; holes in the rocks, abundant crevices, cracks in the rock and near the habitats of bats. About 8-15 m apart in each collection site.



Sand fly identification

Sand flies collected from each trap were stored in 80% alcohol for external and internal morphological identification. The sand fly specimens were separated by sex under a stereomicroscope. Only female sand flies were identified and mounted on glass slides with Hoyer's medium. The male sand flies are useful for conformation of female identification (Lewis, 1978). Identifications were based on the morphology of female spermatheca and pharynx using the taxonomic keys of Lewis (1978;1982), Killick-Kendrick *et al.* (1991), and related keys.

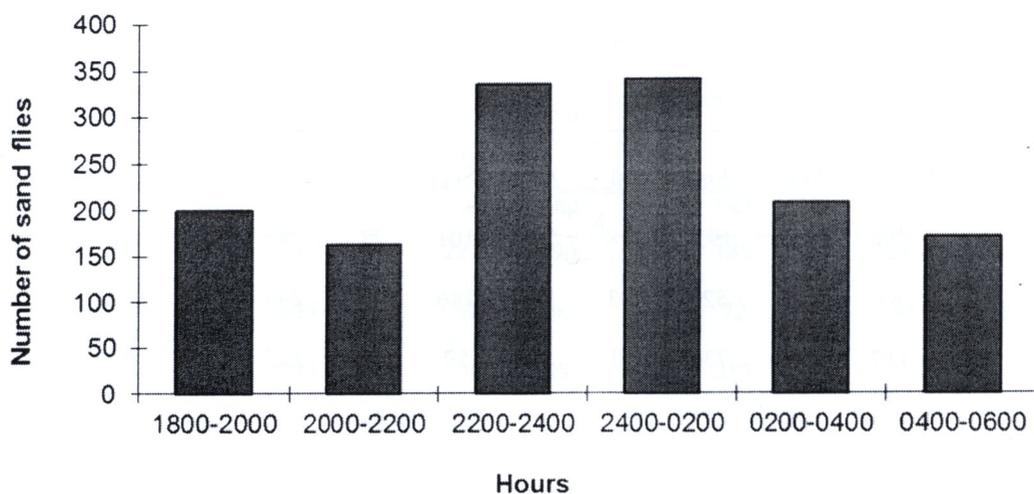


Figure 2. Nocturnal activity of *Phlebotomus argentipes* captured by CDC light traps at Phadang Cave, in Noen Maprang, Phitsanulok Province from February 2010 to January 2011

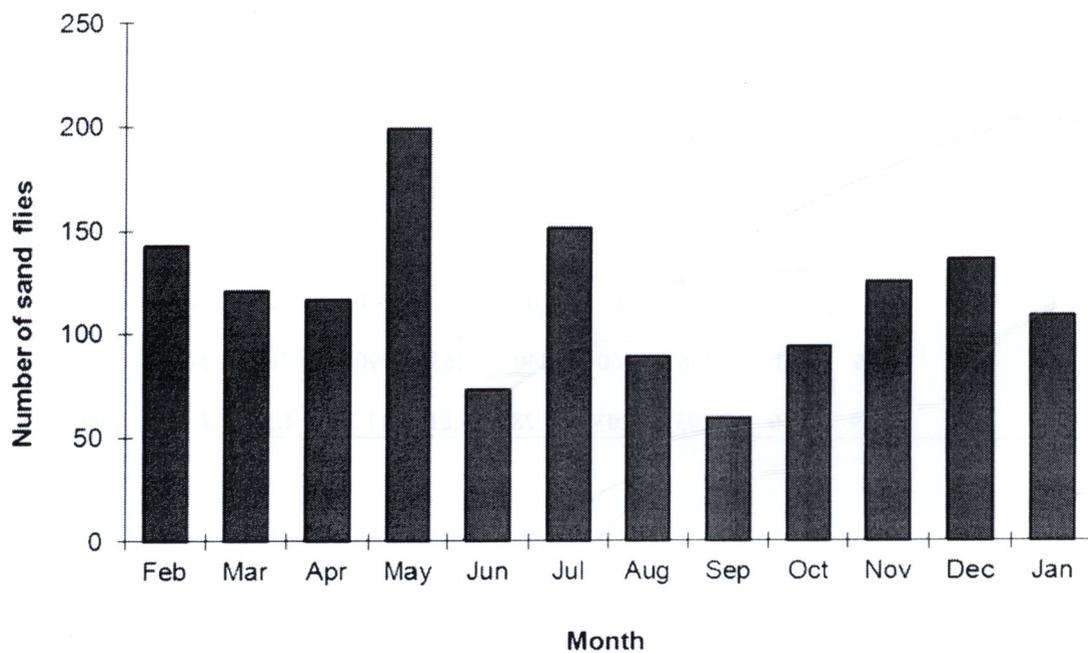


Figure 3. Number of *Phlebotomus argentipes* captured by CDC light traps at Phadang Cave, in Noen Maprang, Phitsanulok Province from February 2010 to January 2011

Sand fly species composition and sex ratio

An overall total of 18,709 phlebotomine sand flies were captured from the cave. The highest peak was found during May and July, the lowest point was in December (Table 1). Fourteen species were identified of which 9 belonged to the genus *Sergentomyia*, 4 to the genus *Phlebotomus*, and 1 to the genus *Chinius* (Table 2). The most abundant of the main species was *S. dentata* (29.19%). The remainder species were distributed as follows: *S. anodontis* (21.40%), *P. argentipes* (17.77%), *C. barbazani* (9.21%), *S. barraudi* (5.06%), *S. gemmea* (4.04%), *S. indica* (3.46%), *S. silvatica* (3.39%), *S. quatei* (2.99%), *P. teshi* (2.02%); the less common species (<1%) were *S. iyengari* (0.74%), *P. stantoni* (0.50%), *S. perturbans* (0.13%), and *P. philippinensis gouldi* (0.11%) (Table 2). The greatest number of specimens was collected in May and July, while *S. dentata*, *S. anodontis*, *P. argentipes*, *C. barbazani*, *S. barraudi*, and *P. teshi* were trapped throughout the whole year (Table 2). Both male and female flies were attracted to the light. The total male to female ratio was 1.35:1 (10,740:7,969). The number of males was lower than females in January, February, November, and December. The male to female ratio of the phlebotomine sand flies varied from a high in June (2.24:1) to a low (0.59:1) in January (Table 1). The highest number of *S. dentata*, and *P. argentipes* were found in May (Table 2).

Nocturnal flight activity

In all months, the number of sand flies captured at nocturnal times from 1800 to 0600 hours is shown in Table 1 and 2. Sand flies were active and stronger during 1800-0600 hours. The maximum peak activity of adult sand flies was during two periods; 2200-2400 and 2400-0200 hours. Results with respect to the nocturnal activity indicated that even though the number of captured declined between 0200-0600 hours. The minimum peak was 0400-0600 hours (Table 1). The known vector, *P. argentipes* had its peak in 2200-2400 and 2400-0200 hours (Fig. 2).

The recorded minimum and maximum monthly temperature and relative humidity values ranged between 22.3-36.1°C and 40-80%, respectively. The total annual rainfall recorded in the study area ranged from 0 mm to 308.7 mm. The overall population reached a peak during May and July, and then decreased to a minimum during December (Table 1). *P. argentipes* had its peak in May (Fig.3). The minimum and maximum temperature and relative humidity values ranged between 27.8-32.2°C, and 65.3-75.0% in May and July. In December, the minimum and maximum temperature and relative humidity values ranged between 25.2-33.4°C, and 60.5-75.1%.

DISCUSSION

A survey of sand fly nocturnal flight activity was conducted for a 12 month period at Phadang Cave. This is the first report of interval capture of sand flies in this area. The sand flies were collected by using CDC light traps. In this study, 14 species were identified: *P. argentipes*, *P. teshi*, *P. philippinensis gouldi*, *P. stantoni*, *Sergentomyia anodontis*, *S. barraudi*, *S. dentata*, *S. quatei*, *S. silvatica*, *S. gemmea*, *S. indica*, *S. iyengari*, *S. perturbans*, and *Chinius barbazani*. In Thailand, previous captures of sand flies documented 24 species (Causey, 1938; Theodor, 1938; Quate, 1962; Apiwathnasorn *et al.*, 1989;1993; Depaquit *et al.*, 2006;2009; Muller *et al.*, 2007; 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 May and July. There are some species present in this area and others have disappeared, perhaps due to different environmental aspects in each area. All traps were positive for sand flies throughout the studied period. *S. dentata* was the most abundant of the main species (29.19%). *P. argentipes*, the suspected vector of *Leishmania donovani*, accounting for 17.77%. Our findings; cave-dwelling *P. argentipes* showed its highest peak in May. Sand flies are found

frequently in the natural ecotope in the cave. The greatest number of specimens was collected from a location near abundant crevices in the cave. In this way, a clear demarcation of the geographic limits of sand fly species and activity was demonstrated, according to the type of cave.

In Thailand, the results revealed nocturnal activity among the different species of sand flies in PhraPhothisat Cave and Tham Phra Pho Thisat Temple that had their highest peak in July and March. Thirteen species were also found in PhraPhothisat Cave, and 16 species were found in Tham Phra Pho Thisat Temple, Saraburi Province. There are some differences in the species composition based on regional differences such as *S. bailyi*, *P. asperulus* and *P. major major*. However, *P. argentipes* was found in Saraburi Province about 0.8-2.2% (Polseela *et al.*, 2007;2011). In January and February 2006, the sand fly was apparently absent in Saudi Arabia (Badry, 2008). Coleman *et al.* (2006) reported sand fly activity periods 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 15 °C and 32 °C. The species differed in their activity period. *P. sergenti*, was active especially during the period of April, May and June (Boussaa *et al.*, 2005). In northern Israel, sand fly populations were prevalent from April to November and peaked between June and August (Kravchenko *et al.*, 2004). These seasonal patterns should be related to different environmental factors, especially climatologically ones.

The nocturnal flight activity of sand flies in the cave was also investigated by the present light trap collection. In this study, the maximum peak activities of adult sand flies were 2200-2400 and 2400-0200 hours. The vector, *P. argentipes* had its peak in the same period. Similar

data was observed by Fahmy *et al.* (2009) for the sand flies peak at 2400-0200 hours. Both sand fly species, *P. sergenti* and *P. papatasi*, exhibited nocturnal activity and peaked after midnight (2400-0200 hours). Coleman *et al.* (2006) showed that in Southern Iraq, the peak activity period shifted from early evening (2000-2200 hours) in April and October to late night (2200-2400 hours) in May and June. In Morocco, the sand fly activity was found to be continuous all night in August, whereas in October the activity declined rapidly after midnight. The activity of *P. sergenti* was nocturnal and stronger at 1900-2100 hours. *P. perniciosus* peaked at 2000-2200 hours (Guernaoui *et al.*, 2006). Some studies reporting on the nocturnal flight activity of sand flies in different interval nature have been studied. In the collection, *S. bedfordi* were all female and were captured in the second half (2400-0600 hours) by Kan *et al.* (2004). Its duration was directly dependent on the photoperiod. One possible reason is that sand flies are active at night and because of the number of bats in the cave is less at night, a lot of sand flies will come to the light trap (Dyce, 1991). These results confirm the association between the activity of sand flies and the ecosystems of the cave. When phlebotomine sand flies are active, they fly to disperse, to feed, to find mates and to search for oviposition sites (Fahmy *et al.*, 2009). It seems possible that the lights of the checkpoint may be the key factor in attracting both male and female flies in such great density away from their emergence sites (Hanafi *et al.*, 2007).

Both sexes of the sand fly were found to have nocturnal activity in this study. The male to female ratio was 1.35:1. This suggests that both sexes of the sand fly are attracted to the light traps. CDC light traps could attract additional phototropic sand flies (Wheeler *et al.*, 1996). Similarly, many previous studies have shown the number of male sand flies captured using CDC light traps was significantly higher than that of female. Ratio of sex (male:female) was reported as 2.5:1 and 1.9:1 in Saraburi, Thailand, respectively (Polseela *et al.*, 2007;2011). According to

the results of a study conducted in Bihar, the Indian State, males and non-fed females of *Phlebotomus argentipes* comprised > 60% of the total collections (Dinesh *et al.*, 2008). Males were found to be 4.7 times more abundant than females in Southern Cukurova, Turkey (Kasap *et al.*, 2009). In different areas, the number of males captured was higher than that of females, similar to the sex ratio reported by Badry *et al.* (2008) (1.12:1), Emamia *et al.* (2008) (2:1), Hanafi *et al.* (2007) (3.4:1) and Boussaa *et al.* (2005) (2.3:1). The higher proportion of males in the captures with CDC light traps could be explained by a natural behavior in which the males follow the females to assure fertilization during their displacements (Barata *et al.*, 2004). Male sand flies are understood to have a weaker dispersal tendency than females and limit their flight to questing for food plants (Yuval & Schlein, 1986; Janini *et al.*, 1995). It is known that male sand flies arrive first, and waits for the female for mating (Merrison *et al.*, 1995; Killick-Kendrick, 1999). Males attract the females by emitting pheromones and wing vibrations and compete for mating privileges (Ward *et al.*, 1990). The hopping behavior has given rise to the assumption that they do not disperse far from breeding site (Killick-Kendrick, 1999).

For this study's month-by-month collection, the highest peak for capture was in May and July, and lowest point was in December. The total annual rainfall recorded in the study area ranged from 0 mm to 308.7 mm. The cave temperature was 22.3-36.1°C with 40-80% relative humidity (RH). Temperature and humidity are factors closely related to duration of metamorphosis (Theodor, 1936). However, the optimum rearing temperatures depend on the different species of sand flies. The reports of successful sand fly *P. kazaruni* rearing, in an insectary maintained at 26°C and 90% RH, in southern Sinai, Egypt (Hanafi *et al.*, 1999). In Northern-Sinai, Egypt, the peak of sand flies is probably due to the fact that this study was performed during the month of May when temperatures remain 30 °C to 35 °C almost till

midnight (Fahmy *et al.*, 2009). Guernaoui *et al.* (2006) reported that variations in the nocturnal activity rhythm are related to the variations in temperature and relative humidity. The most favorable temperatures for development of sand flies are between 25 °C and 30 °C (Safyanova, 1964). It is clear that the activity of sand flies is continuous through the night and that relative humidity has a significant effect on the nocturnal activity. However, many investigations demonstrated the existence of seasonal variations in the nocturnal activity of sand flies.

Understanding the monthly and interval nocturnal activities of sand flies is important in determining the period of maximum risk of *Leishmania* transmission, and for implementation of a control program to prevent human-sand fly contact.

Acknowledgements

This study was conducted under the financial support of Naresuan University, Phitsanulok Province, Thailand. We would like to thank Mr. Chanwit Sangsoi, the Tham Pha Tha Phon non-hunting area, Department of National Parks, Wildlife and Plant Conservation for his kind cooperation during the field study. Also, a special thanks to Mr. Banjong Deelua for the collection specimens from the cave.

REFERENCES

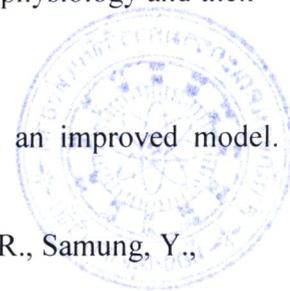
- Alexander, B. & Maroli, M. (2003). Control of Phlebotomine sand flies. *Medical and Veterinary Entomology* **17**: 1-18.
- Apiwathnasorn, C., Sucharit, S., Rongsriyam, Y., Leemingsawat, S., Kerdpibule, V., Deesin, T., Surathin, K., Vutikes, S. & Punavuthi, N. (1989). A brief survey of Phlebotominae sand flies in Thailand. *Southeast Asian Journal of Tropical Medicine and Public Health* **20**: 429-432.
- Apiwathnasorn, C., Sucharit, S., Surathi, K. & Deesin, T. (1993). Anthropophilic and zoophilic phlebotomine sand flies (Diptera:Psychodidae) from Thailand. *Journal of the American Mosquito Control Association* **9**: 135-137.
- Badry, A.E., Juhani, A.A., Ibrahim, E.K. & Zubiany, S.A. (2008). Distribution of sand flies in El-Nekheil province, in Al-Madinah Al-Munawwarah region, western of Saudi Arabia. *Parasitology Research* **103**: 151-156.
- Barata, R.A., Silva, J.C.F., Costa, R.T., Fortes-Dias, C.L., Silva, J.C., Paula, E.V., Prata, A., Monteiro, É.M. & Dias, E.S. (2004). Phlebotomine Sand Flies in Porteirinha, an Area of American Visceral Leishmaniasis Transmission in the State of Minas Gerais, Brazil. *Memórias do Instituto Oswaldo Cruz, Rio de Janeiro* **99**: 481-487.
- Boussaa, S., Guernaoui, S., Pesson, B. & Boumezzougha, A. (2005). Seasonal fluctuations of phlebotomine sand fly populations (Diptera:Psychodidae) in the urban area of Marrakech, Morocco. *Acta Tropica* **95**: 86-91.
- Causey, O.R. (1938). *Phlebotomus* of Siam with a description of new variety. *American Journal of Hygiene* **28**: 487-489.

- Coleman, R.E., Burkett, D.A., Putnam, J.L., Sherwood, V., Caci, J.B., Jennings, B.T., Hochberg, L.P., Spradling, S.L., Rowton, E.D., Blount, K., Ploch, J., Hopkins, G., Raymond, J.-L.W., O'Guinn, M.L., Lee, J.S. & Weina, P.J. (2006). Impact of Phlebotomine sand flies on U.S. military operations at Tallil Air Base, Iraq. 1. Background, military situation, and development of a Leishmaniasis Control Program. *Journal of Medical Entomology* **43**: 647-662.
- Depaquit, J., Leger, N. & Beales, P. (2006). *Chinius barbazani* n.sp. de Thailandae (Diptera: Psychodidae). *Parasite* **13**: 151-158.
- Depaquit, J., Muller, F. & Léger, N. (2009). *Phlebotomus (Euphlebotomus) barguesae* n. sp. From Thailand (Diptera - Psychodidae). *Parasites & Vectors* **2**: 5.
- Desjeux, P. (2004). Leishmaniasis: current situation and new perspectives. *Comparative Immunology, Microbiology and Infectious Diseases* **27**: 305-331.
- Dinesh, D.S., Das, P., Picado, A., Davies, C., Speybroeck, N., Boelaert, M. & Coosemans, M. (2008). The efficacy of indoor CDC light traps for collecting the sand fly *Phlebotomus argentipes*, vector of *Leishmania donovani*. *Journal of Medical Entomology* **22**: 120-123.
- Dinesh, D.S., Ranjan, A., Palit, A., Kishore, K. & Kar, S.K. (2001). Seasonal and nocturnal landing/biting behaviour of *Phlebotomus argentipes* (Diptera: Psychodidae). *Annals of Tropical Medicine and Parasitology* **95**: 197-202.
- Dyce, A.L. & Wellings, G. (1991). Phlebotomine sand flies (Diptera:Psychodidae) from caves in Australia. *Parasitologia* **33**: 193-198.
- Emamia, M.M. & Yazdib, M. (2008). Entomological survey of phlebotomine sand flies (Diptera:Psychodidae) in a focus of visceral leishmaniasis in central Iran. *Journal of Vector Borne Diseases* **45**: 38-43.

- Fahmy, A.R., Samy, A.M., Doha, S.A. & Shehata, M.G. (2009). Preliminary field investigations on Phlebotomine sand flies (Diptera: Psychodidae) from a recent cutaneous leishmaniasis focus in Northern-Sinai, Egypt. *Egyptian Academic Journal of Biological Sciences* **2**: 9-15.
- Guernaoui, S., Boussaa, S., Pesson, B. & Boumezzough, A. (2006). Nocturnal activity of phlebotomine sand flies (Diptera:Psychodidae) in a cutaneous leishmaniasis focus in Chichaoua, Morocco. *Parasitology Research* **98**: 184-188.
- Hanafi, H.A., Fryauff, D.J., Modi, G.B., Ibrahim, M.O. & Main A.J. (2007). Bionomics of phlebotomine sand flies at a peace keeping duty site in the north of Sinai, Egypt. *Acta Tropica* **101**: 106-114.
- Hanafi, H.A., Kanourjr, W. W., Beavers, G.M. & Tetreault, G.E. (1999). Colonization and bionomics of the sand fly *Phlebotomus kazeruni* from Sinai, Egypt. *Medical and Veterinary Entomology* **13**: 295-298.
- Janini, R., Saliba, E. & Kamhawi, S. (1995). Species composition of sand flies and population dynamics of *Phlebotomus papatasi* (Diptera: Psychodidae) in the southern Jordan Valley, an endemic focus of cutaneous leishmaniasis. *Journal of Medical Entomology* **32**: 822-826.
- Kan, E., Anjili, C.O., SainiR, K., Hidaka, T. & Githure, J.I. (2004). Phlebotomine sand flies (Diptera:Psychodidae) collected in Mukusu, Machakos District, Kenya and their nocturnal flight activity. *Applied Entomology and Zoology* **39**: 651-659.
- Kasap, O.E., Belen, A., Kaynas, S., Simsek, F.M., Biler, L., Ata, N. & Alten, B. (2009). Activity patterns of sand fly (Diptera: Psychodidae) species and comparative performance of different traps in an endemic cutaneous leishmaniasis focus in Cukurova Plain, Southern Anatolia, Turkey. *Acta Veterinaria Bron* **78**: 327-335.

- Killick-Kendrick, R. (1999). The biology and control of phlebotomine sand flies. *Clinical Dermatology* **17**: 279-289.
- Killick-Kendrick, R., Tang, Y., Killick-Kendrick, M., Sang, D.K., Sirdar, M.K., Ke, L., Ashford, R.W., Schorscher, J. & Johnson, R.H. (1991). The identification of female sand flies of the subgenus *Larrousius* by the morphology of the spermathecal ducts. *Parasitologia* **33**: 337-347.
- Kravchenko, V., Wasserberg, G. & Warburg, A. (2004). Bionomics of phlebotomine sand flies in the Galilee focus of cutaneous leishmaniasis in northern Israel. *Journal of Medical Entomology* **18**: 418-428.
- Lane, R.P. (1987). Phlebotomine sand flies. In: Manson-Bahr PEC, Bell DR, eds. Manson's tropical disease. London: *BaillierTindall*: 1395-1404.
- Lewis, D.J. (1978). The phlebotomine sand flies (Diptera: Psychodidae) of the Oriental Region. *Bulletin of the British Museum (Natural History)* **37**: 217.
- Lewis, D.J. (1982). A taxonomic review of the genus *Phlebotomus* (Diptera:Psychodidae). *Bulletin of the British Museum (Natural History) (Entomology)* **45**: 121-129.
- Morrison, A.C., Ferro, C., Pardo, R., Torres, M., Wilson, M.L. & Tesh, R.B. (1995). Nocturnal activity patterns of *Lutzomyia longipalpis* (Diptera: Psychodidae) at an endemic focus of visceral leishmaniasis in Colombia. *Journal of Medical Entomology* **32**: 605-617.
- Muller, F., Depaquit, J & Leger, N. (2007). *Phlebotomus (Euphlebotomus) mascomai* n. sp. (Diptera:Psychodidae). *Parasitology Research* **101**: 1597-1602.
- Polseela, R., Apiwathnasorn, C. & Samung, Y. (2007). Seasonal variation of cave-dwelling phlebotomine sand flies (Diptera:Psychodidae) in PhraPhothisat cave, Saraburi, Province. *Southeast Asian Journal of Tropical Medicine and Public Health* **38**: 1011-1015.

- Polseela, R., Apiwathnasorn, C. & Samung, Y. (2011). Seasonal distribution of phlebotomine sand flies (Diptera:Psychodidae) in Tham Phra Phothisat temple, Saraburi province, Thailand. *Tropical Biomedicine* **28**: 366-375.
- Quate, L.W. (1962). A Review of the Indo-Chinese Phlebotominae (Diptera: Psychodidae). *Pacific Insects* **4**: 251-267.
- Sawalha, S.S., Shtayeh, M.S., Khanfar, H.M., Warburg, A. & Abdeen, Z.A. (2003). Phlebotomine Sand Flies (Diptera: Psychodidae) of the Palestinian West Bank: Potential Vectors of Leishmaniasis. *Journal of Medical Entomology* **40**: 321-328.
- Safyanova, V.M. (1964). Laboratory Cultivation of Sand flies (Diptera:Psychodidae). *Bulletin de l' Organisation Mondiale de la Santé , Bulletin of the World. Health Organization* **31**: 573-576.
- Sharma, U. & Singh, S. (2008). Insect vectors of *Leishmania*: distribution, physiology and their control. *Journal of Vector Borne Diseases* **45**: 255-272.
- Sudia, W.D. & Chamberlain, R.W. (1962). Battery-operated light trap, an improved model. *Mosquito News* **22**: 126-129.
- Sukmee, T., Siripattanapipong, S., Mungthin, M., Worapong, J., Rangsin, R., Samung, Y., Kongkaew, W., Bumrungsana, K., Chanachai, K., Apiwathanasorn, C., Rujirojindakul, P., Wattanasri, S., Ungchusak, K. & Leelayoova, S. (2008). A suspected new species of *Leishmania*, the causative agent of visceral leishmaniasis in a Thai patient. *International Journal for Parasitology* **38**: 617-622.
- Theodor, O. (1936). On the relation of *Phlebotomus papatasi* to the temperature and humidity of the environment. *Bulletin of Entomological Research* **27**:653-671.
- Theodor, O. (1938). On sand flies *Phlebotomus* from Ceylon, Siam and Malay. *Indian Journal of Medical Research* : 26-261.



- Ward, R.D., Morton I.E., Brazil. R.P., Trumper, S. & Falcã, A.L. (1990). Preliminary and laboratory field trials of a heated pheromone trap for the sand fly *Lutzomyia longipalpis* (Diptera:Psychodidae). *Memórias do Instituto Oswaldo Cruz* **85**: 445-452.
- Wheeler, A.S., Feliciangeli, M.D., Ward, R.D. & Maingon, R.D.C. (1996). Comparison of sticky-traps and CDC light-traps for sampling phlebotomine sand flies entering houses in Venezuela. *Medical and Veterinary Entomology* **10**: 295-298.
- Young, D. & Duncan, M. (1994). Guide to the identification and geographic distribution of *Lutzomyia* sand-flies in Mexico, West Indies, Central and South America (Diptera: Psychodidae). *Memoirs of the American Entomological Institute* **54**: 1-881.
- Yuval, B. & Schlein, Y. (1986). Leishmaniasis in the Jordan Valley. III. Nocturnal activity of *Phlebotomus papatasi* (Diptera:Psychodidae) in relation to nutrition and ovarian development. *Journal of Medical Entomology* **23**: 411-415.



เอกสารนี้มี CD-ROM

ติดต่อบรรณารักษ์

