CAVERNICOLOUS SPECIES OF PHLEBOTOMINE SAND FLIES FROM KANCHANABURI PROVINCE, WITH AN UPDATED SPECIES LIST FOR THAILAND

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Abstract. During 2008-2009 2,401 Phlebotomine sand flies were collected in 14 limestone caves in Kanchanaburi Province, Thailand to determine the prevalence and type of cavernicolous species that have the potential to be leishmaniasis vectors. Twenty species belonging to the genera *Chinius, Nemopalpus*, *Phlebotomus* and *Sergentomyia* were identified. An additional man-biting species, *P. major major* was recorded for the first time in Thailand. Ecological observations of the habitats were made. It is expected the diversity of cavernicolous sand flies is more than currently known. An updated list of 26 phlebotomine species for Thailand is provided.

Keywords: phlebotomine sand flies, cavernicolous species, anthropophilic species

INTRODUCTION

Phlebotomine sand flies are increasingly important in Thailand due to an increase in reports of indigenous visceral leishmaniasis cases (Suankratay *et al*, 2010). They have a scanty erratic distribution within arid southern Thailand. Attempts have been made to elucidate the natural transmission cycle of the species. Recent field studies revealed ubiquitous distribution, high density and diversity of phlebotomine sand flies inhabiting caves and a rarity of anthropophilic and zoophilic species (Apiwathnasorn *et al*, 1989, 1993; Polseela *et al*, 2007; Sukmee *et al*, 2008). With the growth of ecotourism, including visits to caves, the threat to the health of the visitors is a matter of concern. This led us to undertake this survey of cavernicolous phlebotomine sand flies and their vector potential, to identify potential health risk to people living in close proximity to the caves and to visitors. This information would lead to further public health recommendations to protect the public. The purpose of this study was to contribute to the knowledge of the cave fauna of Phlebotomidae in Thailand.

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MATERIALS AND METHODS

Sand flies were captured during peak periods of seasonal activity (Polseela *et al*, 2007); one day per collection site monthly during October-December 2008 and June-August 2009 using aspirators with three collectors for

one hour during the daytime for sand flies resting on the ceilings and walls of the cave and those attracted to or that bit catchers and using four CDC light traps per night (06:00 PM - 06:00 AM). The CDC miniature light traps were hoisted on wooden poles leaning against the walls of the cave to a height of approximately 1-2 meters.

The collections were conducted at 16 limestone caves which are popular tourist attractions in Lum Sum Sub-district of Kanchanaburi Province: Badan Cave, Chaloei Cave, Da-Wa-Dung Cave, Klang Cave, Kuan Im Goddess Cave, Lawa Cave, Ma Ha Mong Kon Cave, Ma Ha Mongkon Hed Kon Cave, Nam Cave, Nam Thip Cave, Prong Fa Cave, Pu Toei Cave, Thep Pa Tan Porn Cave, Wanon Cave, Wat Benjarat Cave and Wat Phrom Lok Cave. Most caves are surrounded by mixed deciduous and bamboo forest and access to the entrance is by steep descent through the forest. Annual temperatures inside most caves range from 26-28°C and tend to be stable and independent of outdoor variations in temperature, relative humidity (75-80%) or annual rainfall (800 to 2,000 mm).

All sand flies captured were preserved in 90% alcohol for at least 3 days, then cleared in a 30% solution of chloral hydrate in glacial acetic acid for 5 minutes. Each specimen was then mounted in a few drops of Hoyer's medium on a microscope slide. The heads of both male and female specimens were cut off and placed upside down to expose the cibarial and pharyngeal regions and female abdomens were removed and set in a position to expose the spermathecae. Identification was made based mainly on the keys and species descriptions provided by Lewis (1978, 1982).

RESULTS

Cave inhabitants included bats (mainly *Hipposideros* species *eg*, *H. armiger* or the great round leaf bat and *Craseonycteris thonglongyai* or Kitti's hog-nosed bat), porcupines, reptiles (geckos and lizards), toads, snakes, various arthropods and insects. More than 3,000 specimens of cave-dwelling arthropods are commonly found in the caves: mosquitoes (*Aedes cavaticus*, *Ae. albopictus*, *Tripteroides* sp, *Uranotaenia sumethi*), Phlebotomine sand flies, cockroaches (*Diploptera punctata, Ergaula capucina, Neostylopyga rhombifolia, Periplaneta americana, Polyphaga obscura* and *Pycnoscelus surinamensis*), bat flies, bat bugs (*Leptocimex*), ants (*Camponotus* sp, *Pheidole* sp), beetles (cave beetles, *Cercyon* sp), camel crickets (*Diestrammena* sp), reptile ticks (*Aponomma* sp), mites, spiders (whip spider, *Phrynichus orientalis*), cave centipedes (*Scutigera* sp), millipedes (cave millipedes, *Glyphiulus* sp; pill millipedes, *Armadillidium* sp), and springtails.

A total of 2,401 Phlebotomine sand flies (1,461 from light traps and 940 from aspirators) were collected. The male-to-female ratio was 1:1.1 for light traps and 1:0.6 for aspirators. The number of sand flies captured each night varied throughout the study ranging from 12 to 333 sand flies per light trap/night and from 3 to 207 sand flies by hand catch. There were 20 species and two unrecognized species belonging to four genera (*Chinius*, *Nemopalpus*, *Phlebotomus* and *Sergentomyia*). They were *C. barbazani, N. vietnamensis, P. asperulus, P. barguesae, P. major major, P. philippinensis gouldi, P. pholetor, P. stantoni, P. teshi, S. anodontis, S. bailyi, S. barraudi, S. brevicaulis, S. dentata, S. gemmea, S. hodgsoni hodgsoni, S. iyengari, S. perturbans, S. quatei* and *S. silvatica.* The most common species seen (in more than 60% of the collection

sites) were *P. bargusae*, *S. hodgsoni hodgsoni* and *S. anodontis*, of which the former two were the most abundant, accounting for more than 30% of the samples collected. Wanon Cave was rich in sand fly diversity but low in density. Lawa Cave had a high diversity and density of Phlebotomines. Single specimens of *N. vietnamensis*, *S. quatei* and *S. brevicaulis* were discovered in Lawa and Wanon Caves. Therefore, the list of species confirmed for Thailand has increased to 26 species.

The updated list of sand fly species for Thailand is: *C. barbazani*, *N. vietnamensis*, *P. asperulus*, *P. barguesae*, *P. betisi*, *P. hoepplii*, *P. major major*, *P. mascomai*, *P. philippinensis gouldi*, *P. pholetor*, *P. stantoni*, *P. teshi*, *S. anodontis*, *S. bailyi*, *S. barraudi*, *S. brevicaulis*, *S. dentata*, *S. gemmea*, *S. hodgsoni hodgsoni*, *S. indica*, *S. iyengari*, *S. perturbans*, *S. phasukae*, *S. punjabensis*, *S. quatei* and *S. silvatica.*

Sand flies commonly rest on cave ceilings and walls in substantial numbers in close proximity to bat colonies. A sand fly was found feeding on a cave gecko sheltering inside the rock crevices at night. Some sand flies were unusual in that they bit the persons collecting them at rest during the daytime. *Anopheles dirus* was collected simultaneously with *P. major major*. Biting activity occurred over a short period of time (08:00-11:00 PM) at a density of <4 sand flies per person-hour with an indistinct pattern.

DISCUSSION

Phlebotomine sand flies are common in Thailand, but have not been known to cause health problems to humans here yet, but have received more attention recently due to an increase in autochthonous cases of visceral leishmaniasis in Thailand. Tree holes, rock crevices and human dwellings did not yield significant numbers of sand flies compared to caves in a previous study carried out by Apiwathnasorn *et al* (1989). Sand flies are medically important cavernicoles and a significant part of the cave ecosystem. A previous study increased the list of cave-inhabiting sand fly species from 13 to 20 (Polseela *et al*, 2007) and our study brings the number of sand fly species in Thailand to 26. Genus *Chinius* was the third genus of Old World phlebotomines proposed by Leng (1987). *C. barbazani*, *P. barguesae*, *P. mascomai* and *S. phasukae* are new species recently described by Depaquit *et al* (2006, 2009), Muller *et al* (2007) and Curler (2011), respectively. The most frequent cave species found in this study were *P. major major* and *S. anodontis.* Most caves in this country are unexplored, and it is expected many more species remain undiscovered.

Although *P. argentipes*, a well known vector of kala-azar in India, is the most common cave-dweller in northeastern Thailand (Apiwathnasorn *et al*, 1989), it was absent from Kanchanaburi caves. This could be attributed to different cave environments, such as temperature and humidity, essential for both oviposition and resting sites and the coexistence of the animals on which they feed (Kirk and Lewis, 1951; Mutinga *et al*, 1986). This could explain why sand flies were collected in large abundance in one habitat but not in other habitats. *Sergentomyia* species were the most common collected and are known to have a preference for lizards (Mutinga *et al*, 1990). An unidentified sand fly was seen to be feeding on a gecko. *Sergentomyia* species are usually seen in association with lizards (Asimeng, 1992). Besides mammals, lizards are known to harbor *Leishmania* parasites. Promastigote flagellates have been isolated from various species of lizards and other reptiles,

which could be a reservoir for mammalian leishmaniasis (Belova 1971; Seyedi-Rashti *et al* 1994). Bats and swiftlets abound in limestone caves and are also suggested as blood sources for sand flies (Quate and Fairchild, 1961; Lampo *et al*, 2000). Mutinga (1975) found 3 out of 104 cave bats in Kenya harbored *L. donovani* sensu lato. The status of these cave dwellers as blood sources for *Leishmania* vectors has possible implications for maintenance and transmission of leishmaniasis. Further investigations are needed to determine the importance of reptiles and bats as a reservoir for leishmaniasis in the caves of Thailand.

P. major major, has been previously recorded as a cow biter (Apiwathnasorn *et al*, 1993), was noted to have anthropophilic behavior in this study, providing additional evidence for its potential as a vector. Cows and domestic cats have been reported as possible natural reservoir hosts as determined by direct agglutination test (DAT) (Suankratay *et al*, 2010). *P. major major* is a proven vector of visceral leishmaniasis in the Mediterranean (Lewis, 1974). In Iran, it was found to be a probable vector of visceral leishmaniasis caused by *Leishmania infantum* (Sahabi *et al* 1992). Until recently, the manner in which leishmaniasis was transmitted was unclear. *P. major major* may serve as enzootic or zooanthroponotic vectors of parasites causing visceral leishmaniasis in Thailand.

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REFERENCES

Apiwathnasorn C, Sucharit S, Rongsriyam Y, *et al*. A brief survey of phlebotomine sand flies in Thailand. *Southeast Asian J Trop Med Public Health* 1989; 20: 429-32.

Apiwathnasorn C, Sucharit S, Surathin K, Deesin T. Anthropophilic and zoophilic phlebotomine sand flies (Diptera: Psychodidae) from Thailand. *J Am Mosq Control Assoc* 1993; 9: 135-7.

Asimeng EJ. Natural habitats of phlebotomine sandflies in Northern Nigeria. *Insect Sci Soc* 1992; 13: 113-9.

Belova EM. Reptiles and their importance in the epidemiology of leishmaniasis. *Bull WHO* 1971; 44: 553-60.

Curler GR. Records of phlebotomine sand flies (Diptera, Psychodidae, Phlebotominae) with a description of a new species of *Sergentomyia* Franca & Parrot from Khao Yai National Park, Thailand. *Zootaxa* 2011; 2808: 60-8.

Depaquit J, Léger N, Beales P. *Chinius barbazani* n. sp. de Thaïlande (Diptera: Psychodidae). *Parasite* 2006; 13: 151-8.

Depaquit J, Muller F, Léger N. *Phlebotomus* (*Euphlebotomus*) *barguesae* n. sp. from Thailand (Diptera - Psychodidae). *Parasit Vectors* 2009; 2: 5.

Kirk R, Lewis DJ. The Phlebotominae of the Ethiopian region. *Trans R Soc Lond* 1951; 102: 383-509.

Lampo M, Feliciangeli MD, Márquez LM, Bastidas C, Lau P. A possible role of bats as a blood source for the Leishmania vector *Lutzomyia longipalpis* (Diptera: Psychodidae). *Am J Trop Med Hyg* 2000; 62: 718-9.

Leng YJ. A preliminary survey of phlebotomine sandflies in limestone caves of Sichuan and Guizhou Provinces, south-west China, and description and discussion of a primitive new genus *Chinius*. *Ann Trop Med Parasitol* 1987; 81: 311-7.

Lewis DJ. The biology of Phlebotomidae in relation to leishmaniasis. *Ann Rev Entomol* 1974; 19: 363-84.

Lewis DJ. The phlebotomine sandflies (Diptera: Psychodidae) of the Oriental Region. *Bull*

Br Mus Nat Hist (Entomol) 1978; 37: 217-343.

Lewis DJ. A taxonomic review of the genus *Phlebotomus* (Diptera: Psychodidae). *Bull Br Mus Nat Hist (Entomol)* 1982; 45: 121-209.

Lewis DJ, Lane RP. A taxonomic review of *Phtebolomus* (*Idiophlebotomus*) (Psychodidae). *Syst Entomol* 1976; 1: 53-60.

Muller F, Depaquit J, Léger N. *Phlebotomus* (*Euphlebotomus*) *mascomai* n. sp (Diptera-Psychodidae). *Parasitol Res* 2007; 101: 1597-602.

Mutinga MJ. The animal reservoir of cutaneous leishmaniasis on Mount Elgon, Kenya. *East Afr Med J* 1975; 52*:* 142-51.

Mutinga MJ, Kainau CC, Kyai PM. Investigations the epidemiology of leishmaniasis Kenya-IV. Breeding habitat of *Phlebotomus duboscqi* (Diptera: Psychodidae) of *Leishmania major* in Marigat, Baringo District, Kenya. *Insect Sci Applic* 1986; 7: 727-9.

Mutinga MJ. Basimike M, Kamau CC, Mutero CM. Epidemiology of leishmaniasis in Kenya. Natural host preference of wild-caught phlebotomine sandflies in Baringo District, Kenya. *East Afr Med J* 1990; 67: 319-27.

Polseela R, Apiwathnasorn 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*:* 1011-5.

Quate LW. Fairchild GB. *Phlebotomus* sandflies of Malaya and Borneo. *Pacific Insects* 1961; 3: 203-22.

Sahabi Z, Seyadi Rashti MA, Nadim A, Javadian E, Kazemeini M, Abai MR. A preliminary report on the natural leptomonad infection of *Phlebotomus major* in an endemic focus of V.L. in Fars Province, south of Iran. *Iranian J Public Health* 1992; 21: 87-93.

Seyedi-Rashti MA, Agh-Atabay MD, Mohebali M. Natural promastigote infection of *Sergentomyia sintoni*, its seasonal variation and reservoir host in Turkemen-Sahara, Iran. *Iranian J Public Health* 1994; 23: 41-50.

Suankratay C, Suwanpimolkul G, Wilde H, Siriyasatien P. Case report: Autochthonous visceral leishmaniasis in a human immunodeficiency virus (HIV)-infected patient: The first in Thailand and review of the literature *Am J Trop Med Hyg* 2010; 82: 4-8.

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.