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# Species composition and population dynamics of phlebotomine sand flies in a *Leishmania* infected area of Chiang Mai, Thailand

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**Abstract.** Phlebotomine sand flies are established vectors of leishmaniasis in humans. In Thailand, *Leishmania martiniquensis* and "*Leishmania siamensis*" have been described as causative agents of leishmaniasis. In this study, a survey of sand flies in the *Leishmania* infected area of Hang Dong district, Chiang Mai, Thailand was performed using CDC light traps for eight consecutive months, from January to August 2016. A total of 661 sand flies were collected, and of 280 female sand flies, four species of the genus *Sergentomyia* including *Sergentomyia gemmea*, *S. barraudi*, *S. indica*, and *S. hivernus* and one species of the genus *Phlebotomus*, *Phlebotomus stantoni*, were identified. *S. gemmea* and *S. hivernus* were found in Chiang Mai for the first time. The density of captured female sand flies was high in warm and humid periods from June to August, with temperatures of around 26°C and relative humidity about 74%. In addition, *S. gemmea* was the most predominant species in the area. Further studies as to whether or not these sand fly species could be a vector of *Leishmaniasis* in Thailand are required.

## INTRODUCTION

Leishmaniases are a group of diseases caused by *Leishmania* parasites occurring in three main forms in humans: visceral leishmaniasis (VL), cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis (MCL). Related health problems exist in both tropical and subtropical areas, with an estimated 1.3 million new cases and 20,000 to 30,000 deaths occurring annually (WHO, 2016). In Thailand *Leishmania martiniquensis* and "*Leishmania siamensis*" have been described as causative agents of leishmaniasis and a leishmaniasis case has been reported in the Hang Dong district, Chiang Mai, Thailand (Chiewchanvit *et al.*, 2015). Sand flies have been suspected for some time of being vectors of leishmaniasis in the country (Kanjanopas *et al.*, 2013; Chusri *et al.*, 2014).

Survey studies of sand fly species distribution have been conducted in the central, western, northern, and northeastern regions of Thailand, where four genera, i.e., *Sergentomyia*, *Phlebotomus*, *Chinius* and *Idiophlebotomus* have been identified. Of the four genera, *Sergentomyia* has been the most predominant genus found in all of the study areas. To date, at least 29 sand fly species in several provinces of Thailand have been reported, i.e., *Chinius barbazani*, *Idiophlebotomus teshi*, *Phlebotomus argentipes*, *P. asperulus*, *P. barguesae*, *P.*

*betisi*, *P. hoepplii*, *P. major*, *P. mascomai*, *P. phillipinensis*, *P. pholetor*, *P. stantoni*, *Sergentomyia anodontis*, *S. bailyi*, *S. barraudi*, *S. brevicaulis*, *S. dentata*, *S. gemmea*, *S. hodgsoni*, *S. indica*, *S. iyengari*, *S. perturbans*, *S. phasukae*, *S. punjabensis*, *S. quatei*, *S. sylvatica* (reviewed by Polseela *et al.*, 2016b), *S. phadongensis* (Polseela *et al.*, 2016a), *S. hivernus* and *S. khawi* (Phumee *et al.*, 2016). Eight of these species have been reported in Chiang Mai, i.e., *P. argentipes*, *P. stantoni*, *S. anodontis*, *S. bailyi*, *S. barraudi*, *S. indica*, *S. iyengari* and *S. sylvatica* (Polseela, 2012). However, little is known about the distribution of sand fly species in Hang Dong, Chiang Mai, Thailand, where leishmaniasis has been reported. Thus, the objective of this study was to identify the distribution of sand fly species and their population dynamics in the infected area.

## MATERIALS AND METHODS

### Study area

The Hang Dong District, Chiang Mai, Thailand (18°41'15"N et 98°55'28"E) was selected as the study area because a leishmaniasis patient had been reported there previously (Chiewchanvit *et al.*, 2015) (Fig. 1).

### Sand fly collections

Sand flies were collected monthly from January to August 2016 using five CDC light-traps from 6 pm to 6 am (overnight) for two consecutive nights. Annual temperature and relative humidity in the area ranged from 17 to 36°C and 32 to 74.4%, respectively. Collected sand flies were transferred to the laboratory at the Department of Parasitology, Faculty of Medicine, Chiang Mai University, for species identification and investigation for entomopathogenic nematodes.

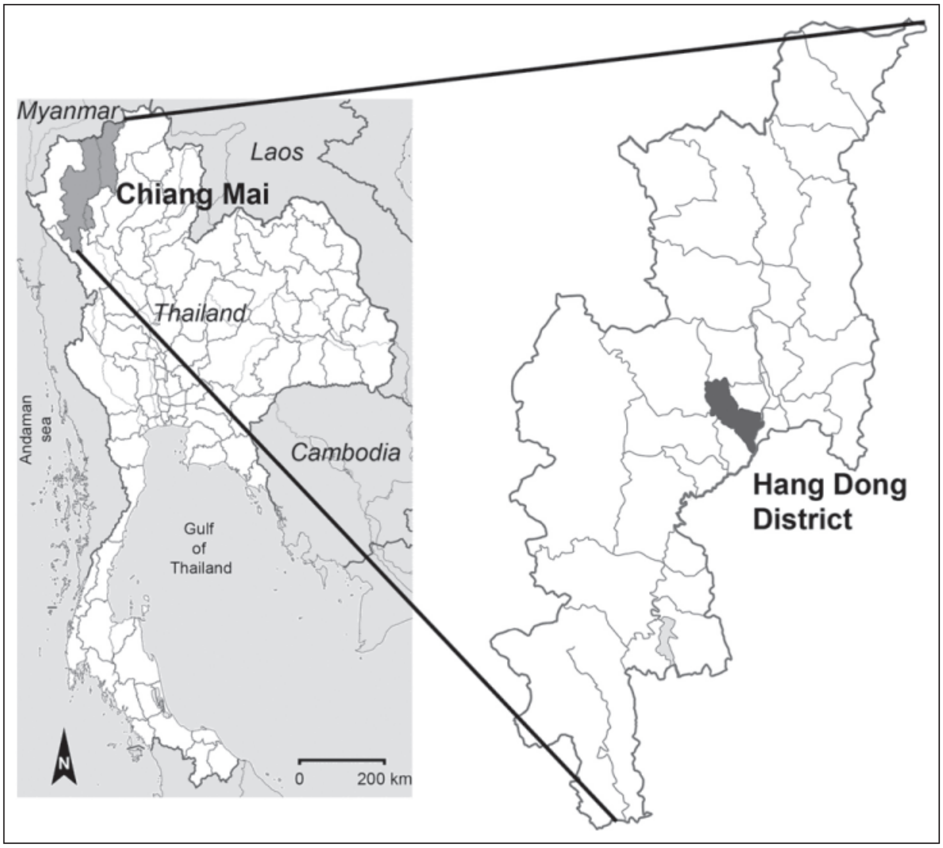


Figure 1. Map of Chiang Mai, northern Thailand showing the geographic location where sand flies were collected.

**Sand fly identification**

All of the sand flies were separated by sex and the numbers of males and females were recorded. Then, they were preserved in 75% ethanol until identification. For sand fly identification, the samples were dissected in Phosphate Buffer Saline (PBS; 10 mM sodium phosphate, 145 mM sodium chloride, pH 7.2) under a dissecting binocular microscope. However, only the female heads and segments 8-11 were cut and mounted in Hoyer's medium. Sand fly identification was performed using the following keys and articles (Theodor, 1938; Quate, 1962; Lewis, 1978, 1987).

**Light microscopy**

The samples were observed under a light microscope and photographed using an OLYMPUS microscopy camera using DP2-SAL Firmware Ver.3.3.1.198 (Tokyo, Japan).

## RESULTS

In this study, a total of 661 phlebotomine sand flies were found belonging to two genera, *Sergentomyia* and *Phlebotomus* with a female:male ratio of 1:1.36. Five species were identified from 280 females, i.e., *S. gemmea* (35.36%), *P. stantoni* (28.21%), *S. barraudi* (18.57%), *S. indica* (17.14%) and *S. hivernus* (0.71%) (Table 1). *S. gemmea* and *S. hivernus* were found in Chiang Mai for the first time. At this site, *S. gemmea* was found to be the most abundant species whereas the least common species was *S. hivernus*.

Figure 2 shows the morphological characteristics of the cibarium of the females from the species: *P. stantoni*, *S. gemmea*, *S. barraudi*, *S. indica* and *S. hivernus* which were used for species identification in this study. The cibarium of *S. gemmea* contained 10-12 hind teeth with two or three rows of fore teeth (Fig. 2a) whereas the cibarium of *S. hivernus* contained 13-14 hine teeth and one row of fore teeth (Fig. 2b). The cibarium of *S. indica* had 40 teeth in a convex plate with a brown pigment patch (Fig. 2c). The number of teeth of *S. barraudi* appeared to vary from about 45 to 70 teeth in a straight plate and the pigment patch was bifid (Fig. 2d). *P. stantoni* had a cibarium with about 15 pointed denticles of various lengths, irregularly arranged, the two or three median ones longer and stouter than the others (Figure 2e). Species identification was also performed using the spermathecae of the females of *P. stantoni*, *S. gemmea*, *S. barraudi*, *S. indica* and *S. hivernus* (Fig. 3). Spermathecae of *S. gemmea* had a narrow shape with knob in a deep narrow pit (Fig. 3a) but spermatheca of *S. hivernus* had a long, smooth and tubular shape (Fig. 3b). *S. indica* had a round finely speculate capsule spermatheca with minute projections at its tip (Fig. 3c). Spermathecae of *S. barraudi* were ovoid and smooth without setae (Fig. 3d). Spermathecae of *P. stantoni* were fusiform, with 15 of 16 rings, the neck was thick and short, the head more or less oblong (Fig. 3e).

Figure 4 shows variation of the sand fly density in each month in the study area. The density of captured female sand flies was high in warm and humid periods from June to August. The greatest number of collected specimens was in June with the temperature around 26°C and relative humidity around 74%. However, *P. stantoni*, *S. gemmea* and *S. barraudi* were found throughout this study.

## DISCUSSION

Results in the current study provided information on the distribution of sand fly species in Hang Dong District, Chiang Mai, Thailand where the leishmaniasis case was reported (Chiewchanvit *et al.*, 2015). Eight sand fly species have been reported in Chiang Mai but the suspected vector *S. gemmea* and *S. hivernus*, have not been found before (reviewed by Polseela, 2012). In this study, the identification of sand flies species was performed by using morphological characteristics of abdominal hairs, cibarium teeth and spermathecae. The abdominal hairs were used to differentiate between *Phlebotomus* and *Sergentomyia* species. *S. gemmea* and *S. hivernus* had similar cibarium hind teeth but they were

Table 1. Species identification numbers of 280 female sand flies in this study from January to August 2016

| Species | Month | | | | | | | | | Percentage |
|---|---|---|---|---|---|---|---|---|---|---|
| | Jan | Feb | Mar | Apr | May | Jun | Jul | Aug | Total | |
| *P. stantoni* | 3 | 5 | 4 | 7 | 15 | 23 | 5 | 17 | 79 | 28.22 |
| *S. gemmea* | 9 | 9 | 5 | 13 | 6 | 24 | 20 | 13 | 99 | 35.36 |
| *S. barraudi* | 2 | 2 | 2 | 4 | 6 | 11 | 10 | 15 | 52 | 18.57 |
| *S. indica* | 0 | 2 | 7 | 9 | 3 | 21 | 3 | 3 | 48 | 17.14 |
| *S. hivernus* | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 2 | 0.71 |
| Total | 14 | 18 | 19 | 33 | 30 | 79 | 39 | 48 | 280 | 100 |

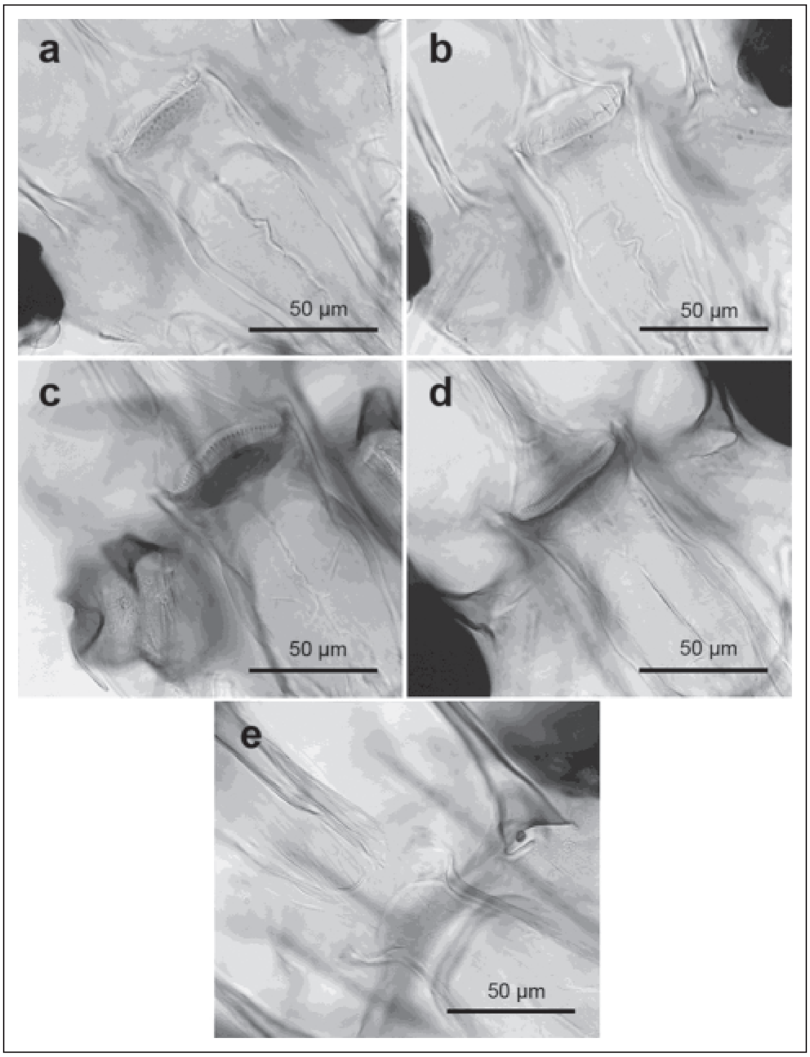


Figure 2. Morphological characteristics of cibarium teeth and pigment patches of female sand flies. (a) *S. gemmea* (b) *S. hivernus* (c) *S. indica* (d) *S. barraudi* (e) *P. stantoni*.

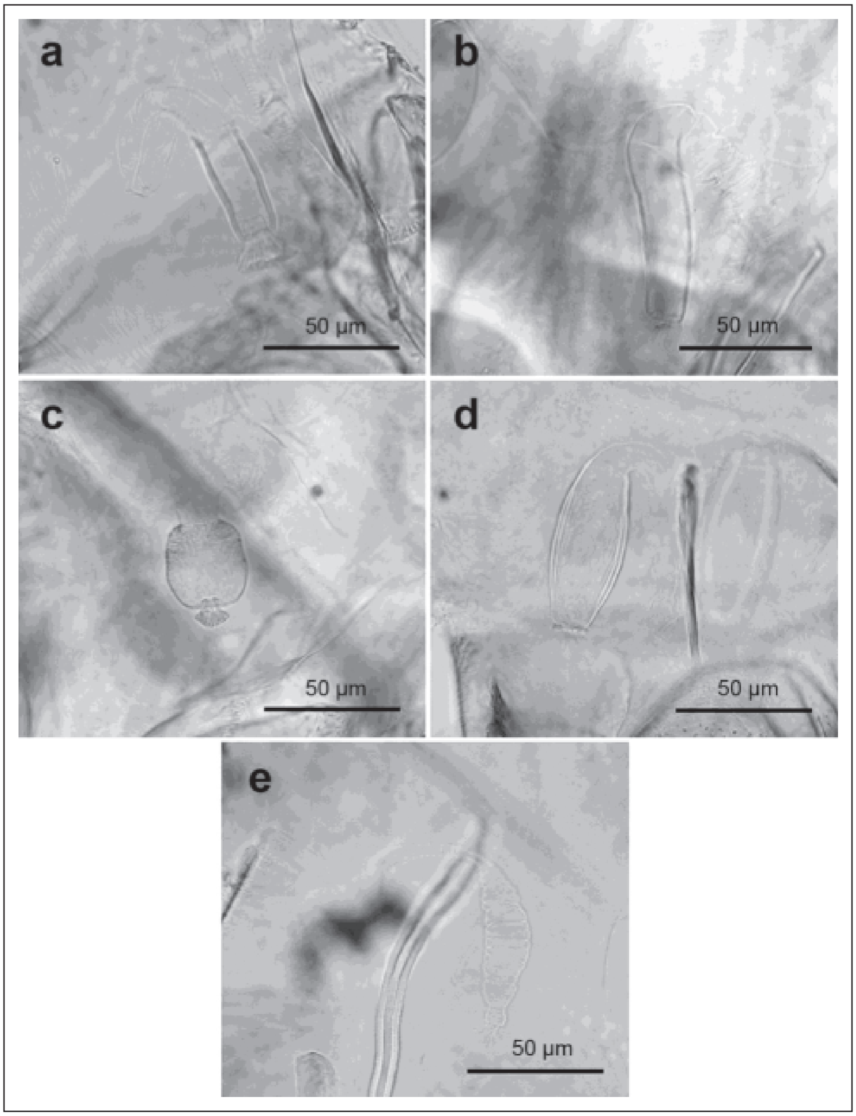


Figure 3. Morphological characteristics of spermatheca of female sand flies (a) *S. gemmea* (b) *S. hivernus* (c) *S. indica* (d) *S. barraudi* (e) *P. stantoni*.

distinguished clearly by the morphological characteristics of their spermathecae. Recently, Phumee *et al*. (2016) have distinguished *S. hivernus* and *S. iyengari* by the morphology of cibarial teeth and spermathecae. The cibarium of *S. hivernus* contains 13-14 hind teeth and one row of fore teeth and the spermathecae are smooth, long and tubular without obvious junctions between the body and duct. *S. iyengari* has a cibarium which contains 13-14 hind teeth, the central teeth are smaller than the rest, and fore teeth are absent. Its spermathecae are shorter with easily observed junctions between the body and duct. The morphological characteristics of spermathecae of *S. hivernus* collected in this current study correspond with the report of Phumee *et al.* (2016).

*S. gemmea* was the most abundant species in the area. This statistic is similar to that in previous studies in the south of Thailand, i.e., Phang-nga, Suratthani, Nakonsitammarat, Songkhla (Sukmee *et al.*,

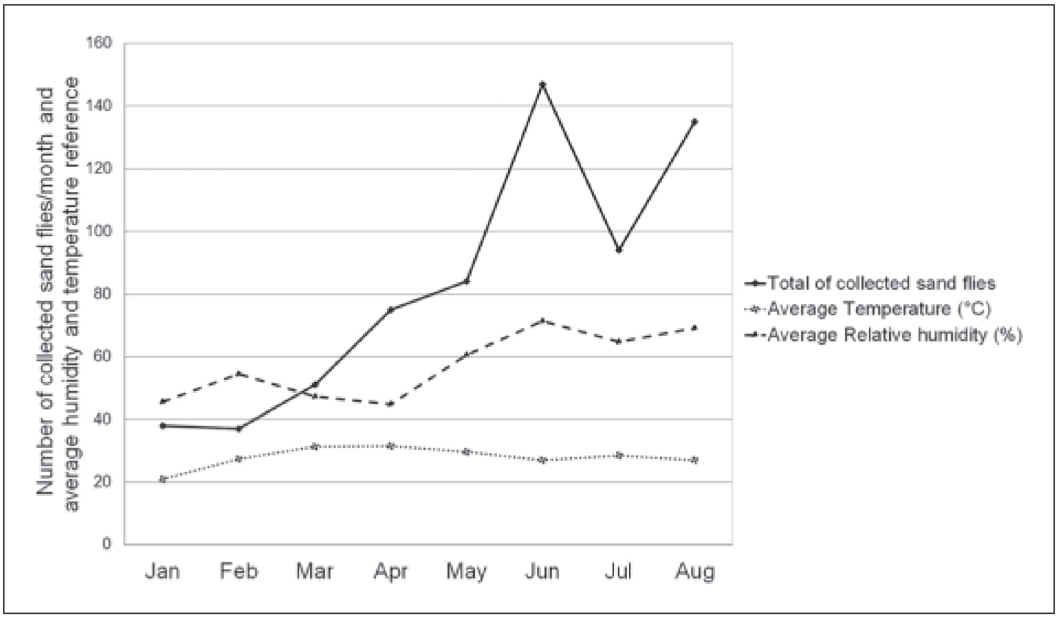


Figure 4. Relationship between diversity of collected sand flies and the climate parameters of relative humidity and temperature in the *Leishmania* infected area from January to August 2016.

2008; Sukra *et al.*, 2013; Chusri *et al.*, 2014), and Trang (Kanjanopas *et al.*, 2013). The other abundant species in the study area were *P. stantoni* and *S. barraudi*. It has been suggested that *S. gemmea* and *S. barraudi* might be possible vectors of the leishmaniasis in this area since Chusri *et al.* (2014) have detected *Leishmania* DNAs in both species. In addition, Phumee *et al.* (2016) have detected unknown *Trypanosoma* DNA in *P. stantoni* collected from southern Thailand. Although investigation of *Leishmania* or *Trypanosoma* parasites and DNA in unidentified sand flies in this study area has been performed in our laboratory, we have yet not found any parasites and/or DNA in the flies (unpublished data). More investigation of the vector status of *S. gemmea* and *S. barraudi* is required in all infected areas in Thailand.

Furthermore, *L. martiniquensis* and "*L. siamensis*" have been reported as members of the *Leishmania enriettii* complex (Bualert *et al.*, 2012; Leelayoova *et al.*, 2013; Kwakye-Nuako *et al.*, 2015). Dougall *et al.* (2011) have presented strong evidence that the biting midge *Forcipomyia* (*Lasiohelea*) sp.1 and possibly *Forcipomyia* (*Lasiohelea*) *peregrinator* may be transmitting Australian *Leishmania*, a member of the *L. enriettii* complex, in northern Australia. Seblova *et al.* (2015) have demonstrated that *L. enriettii* could develop late stage infection in the biting midge *Culicoides sonorensis* and that the late stage parasites could produce large, ulcerated, tumour-like lesions in hamsters. The studies have supported the hypothesis that some biting midges could be natural vectors of the *L. enriettii* complex. Therefore, investigation of the vector status of midges is required in all infected areas in Thailand.

Previous studies in many caves in Thailand have shown that *S. gemmea* has been a less common species (~0.5%) (Polseela *et al.*, 2007, 2011; Apiwathnasorn *et al.*, 2011). Caves in Thailand, generally, have low temperatures, around 24.4 to 32.3°C, with relative humidity around 64 to 95% (Polseela *et al.*, 2007; Polseela *et al.*, 2011; Apiwathnasorn *et al.*, 2011). The distribution information from our current study and the studies in southern Thailand suggest that *S. gemmea* is likely to be more successful in slightly warm and humid habitats near human living areas.

In conclusion, five species of sand flies collected from the *Leishmania*-infected area in Hang Dong, Chiang Mai, Thailand were identified. *S. gemmea* and *S. hivernus* were found in Chiang Mai for the first time. Also, *S. gemmea* was the most abundant species in the area. This finding provided information for further studies on the vector status of these sand fly species in Thailand.

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**Conflict of Interest**

The authors declare that they have no conflict of interest.

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