ENTOMOLOGY

# An entomological survey of phlebotomine sand flies (Diptera: Psychodidae) in different areas of Thailand

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## Abstract

Sand flies (Diptera: Psychodidae) are significant medical vectors of pathogens that cause illness in humans and animals. The most significant of these is the genus *Leishmania* (Kinetoplastida: Trypanosomatidae). Leishmaniasis is an important vector-borne disease. They are spread throughout the Old and New Worlds by the bites of female phlebotomine sand flies. Despite this importance, certain provinces where autochthonous leishmaniasis has historically existed and is re-emerging are lacking current data regarding their sand fly fauna. To provide a current update on the local sand fly fauna, a comprehensive entomological survey encompassing eight provinces was conducted subsequent to an analysis of past data regarding the species composition and distribution of sand flies in the area. Thus, the purpose of this study was to ascertain the species composition in various locations across eight provinces spread across four regions in Thailand. In order to gather information about the distribution of the phlebotomine sand fly, researchers used modified Centers for Disease Control and Prevention light traps between February 2021 and February 2022. At these 30 trappings in each province, we conducted 240 trapping nights in total. Collected sand flies were morphologically identified. The adult sand flies collected in this study were 8314 and represented 24 species of four genera, including 16 of *Sergentomyia*, 6 of *Phlebotomus*, 1 of *Idiophlebotomus*, and 1 of *Chinius*. Among these species, *Sergentomyia anodontis* had the largest population (25.71%), and population peaks were observed in Uthaithani province (51.81%). The collected sand flies' male-to-female ratio was 1.21:1.00 (4,556:3,758). The study also discovered that *Phlebotomus argentipes*, a vector of *Leishmania* spp., was captured in Phayao province, and *Phlebotomus major major* was majorly found in Uthaithani province. The widespread distribution of sand fly populations in this study may indicate the epidemiologic importance of vector ecology in this habitat. Our results confirm the presence of several sand fly species in different provinces of Thailand and provide valuable information about the distribution and behavior of phlebotomine sand flies, which can be used to develop effective control strategies for the prevention of sand fly-borne diseases. In several Thai provinces, the study also tracked the nocturnal movements of both male and female sand flies. The results showed that phlebotomine sand flies are more active at night, peaking just after dusk.

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## Introduction

The phlebotomine sand fly is a member of the Diptera: Psychodidae family of flies. There are over 950 species of sand flies that have been described and classified, with about 100 species found in the Old and New World. These flies are known to be the main vectors of various pathogens, including those that infect humans: bacteria *Bartonella bacilliformis*, viruses carried by sand

flies, and, most importantly, parasitic protozoa of the genus *Leishmania*, which are the cause of leishmaniases, a major risk to health (Munstermann, 2018).

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 and is included in the list of neglected tropical diseases (Alvar *et al.*, 2006) and has a strong link to poverty. *Leishmania* spp. illustrate a wide range of geographic distribution. The disease has been reported in 88 countries in five continents – Africa, Asia, Europe, North America, and South America (22 in the New World and 66 in the Old World) (Sharma & Singh, 2008). Regarding *Leishmania* spp. 30 species are known and approximately 20 are pathogenic for humans. These species generally present different epidemiological and clinical characteristics related to different genetic and phenotypic profiles (Bhargaval & Singh, 2012). About 98 species of sand flies are considered proven or suspected vectors of human leishmaniases; these include 42 *Phlebotomus* species in the Old World and 56 *Lutzomyia* species in the New World (Maroli *et al.*, 2013). The role of species belonging to the genus *Sergentomyia* in *Leishmania* spp. transmission among mammal hosts needs to be elucidated (Mukherjee *et al.*, 1997). *Leishmania martiniquensis* and *L. orientalis* (formerly *L. siamensis*) are causative agents of cutaneous leishmaniasis and visceral leishmaniasis in Thailand (Jariyapan *et al.*, 2018). Only adult female sand flies feed on blood, required for the nutrients to develop their eggs and transmit the protozoa to the host (Killick-Kendrick, 1999). In Thailand, phlebotomine sand flies are principally represented at different locations from different provinces in a wide variety. Based on literature reviews from 1934 and 2012, sand flies have been recorded from 37 provinces in Thailand (Apiwathanasorn *et al.*, 2011; Polseela *et al.*, 2016). Most sand flies are nocturnally active or crepuscular along with the highest activity after sunset (Dinesh *et al.*, 2001; Sawalha *et al.*, 2003). The nocturnal behavior of sand flies has been studied in several countries where leishmaniasis is endemic (Guernaoui *et al.*, 2006; Kasap *et al.*, 2009). Previous studies in Thailand show the presence of nocturnal activity of sand flies; the nocturnal activity of cave-dwelling sand flies at different time intervals and determined species composition and seasonal variation was investigated. The highest peak of collection was recorded at the time interval of 00:01-02:00, followed by 22:01-00:00 (Jaturas *et al.*, 2018). A recent review of sand fly distribution in Thailand indicated that at least 34 species of the genera *Sergentomyia*, *Phlebotomus*, *Idiophlebotomus* and *Chinius* have been identified (Apiwathanasorn *et al.*, 2011). Therefore, *Sergentomyia gemmea* has been considered a potential vector of *L. orientalis* (Kanjanopas *et al.*, 2013).

In this study, collections of sand flies were obtained to investigate their distribution across different areas in four regions of Thailand. Additionally, the study aimed to determine the species composition and sex ratios of these sand flies, providing valuable insights into the characteristics of the population under investigation.

## Materials and Methods

### Study area

Sand flies were collected between January 2021 and February 2022 in eight provinces distributed across four regions of Thailand. In the northeastern region, the provinces of Sakon Nakhon (SKI) and Buengkan (BKN) were included. The northern region consisted of Chiang Rai (CRI) and Phayao (PYO) provinces. The southern region included Chumphon (CPN) and Ranong (RNG) provinces. Lastly, the central region comprised Uthaithani (UTI) and Lopburi (LRI) provinces (Figure 1). The selection of study sites was based on their proximity to tourist attractions such as near the national park, conservation areas, temples, houses, mountainsides, and limestone caves. Sand flies were collected in eight districts, including the outdoor areas surrounding houses. Surveillance was also conducted inside and outside limestone caves, namely Tham Prakayang (RNG), Tham Air, Tham Chang phung, Tham Kho Tha Noy (CPN), Tham Laung (PYO), and Tham Morragot (CRI). These limestone caves were all located within mixed deciduous forests (Figure 2). During the study, temperature and relative humidity were recorded by a Hygro thermometer (DHT1). The location coordinates of the study sites were recorded by a global positioning system (Gamin MAP65s) (*Supplementary Table S1*).

### Sand fly collection and identification

Adult sand flies were collected using modified Center for Disease Control (CDC) light traps (Cohnstaedt *et al.*, 2008). The modified CDC light traps (30 traps per province per night) were set up at about 50-100 cm above the ground level in different locations per province. Each trapping was set at the chosen collection area at sunset (6.00 PM) and collected specimens before sunrise the next morning (06.00 AM) respectively.

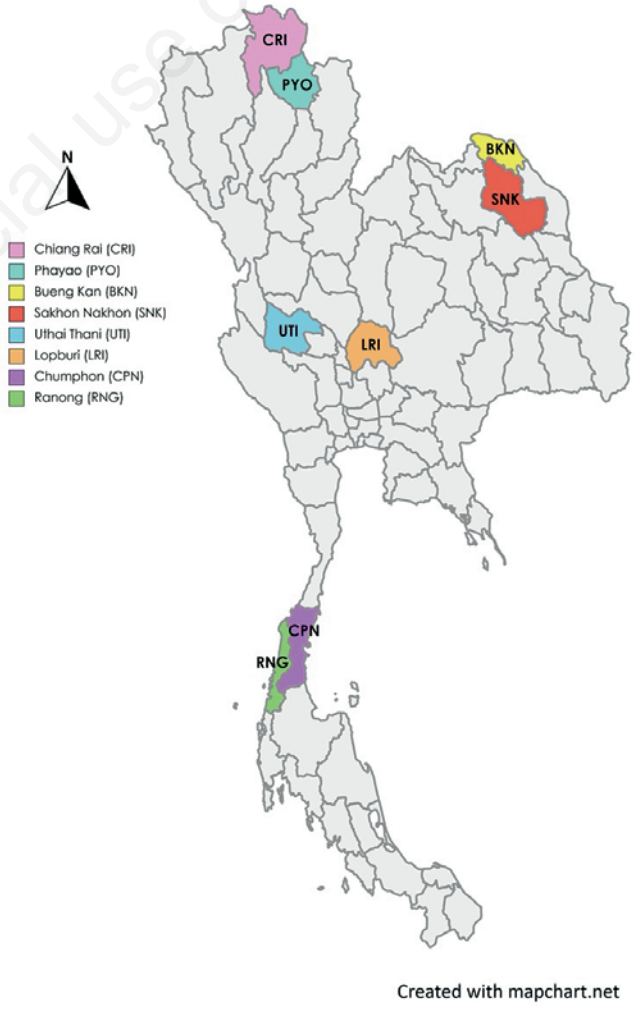


**Figure 1.** Map showing the locations of the eight provinces in Thailand where the surveillance of sand flies was conducted: Sakon Nakhon (SKI), Buengkan (BKN), Chiang Rai (CRI), Phayao (PYO), Chumphon (CPN), Ranong (RNG), Uthaithani (UTI), and Lopburi (LRI) province.


**Figure 2.** The study area in eight provinces in different parts of Thailand. a) Sakon Nakhon; b) Buengkan; c) Chiang Rai; d) Phayao; e) Chumphon; f) Ranong; g) Uthaithani; h) Lopburi.

Each trapping operated continuously for 12 hours. Specimens were kept in dry ice and transported to the laboratory of the Department of Microbiology and Parasitology, Naresuan University, Phitsanulok, Thailand. The sand flies from each trap were separated from other insects and stored in 80% ethanol for external and internal morphological identification. The sand fly specimens were separated by sex under a stereo-microscope. For species identification, each female was dissected into two parts (heads and thorax with abdomen) and mounted on glass slides using Hoyer's medium. Sand fly species were identified under a compound microscope principally based on morphologic characteristics: mainly internal structures, such as the spermatheca, cibarium, and pharynx for females using different taxonomic keys (Lewis 1978, 1987; Depaquit *et al.*, 2009; Muller *et al.*, 2007; Polseela *et al.*, 2016).

### Statistical analyses

The chi-square test was performed for proportional comparison among multiple groups and undertaken using Stata 12.0. Hierarchical clustering using Pearson's correlation was performed in Heatmapper (http://www.heatmapper.ca/) to group both study areas and species of sand flies.

## Results

### Sand fly species composition and sex ratio

An overall 8314 phlebotomine sand flies were collected using 240 light traps. The highest number was recorded from UTI province (4307 specimens) followed by PYO province (944 specimens), BKN province (899 specimens), RNG province (745 specimens), CPN province (705 specimens), LRI province (332 specimens), SKI province (267 specimens), and CRI province (115 specimens), as shown in Table 1. 24 species inhabiting different areas were identified, 16 of which belonged to the genus *Sergentomyia*, 6 to the genus *Phlebotomus*, 1 to the genus *Idiophlebotomus*, and 1 to the genus *Chinius* (Table 2). *Sergentomyia anodontis* was the most common accounted for 25.71% (Table 2). The total male and female ratio was 1.21:1.00 (4556:3758). The number of females was lower than males (Table 1). *P. asperulus, P. argentipes, P. major major, S. tambori*, and *Idiophlebotomus longiforceps* were trapped in one province (Table 2). Both male and female flies were attracted by the light. The average density of sand flies was 3.83-143.57 sand flies per light trap per night. The highest average density of sand flies was found in UTI province (143.47 sand flies per trap night) and the lowest in CRI province (3.83 sand flies per trap night). The minimum and maximum temperature and relative humidity values ranged between 11.6-37.1°C and 48-87%.

To compare frequencies of sand fly species distributed among various areas, a chi-square test was conducted. Overall, significant levels of different relative frequencies of sand flies were observed for studying at UTI, PYO, BKN, RNG, CPN, LRI, SKN, and CRI with a P-value smaller than 0.001 (chi-square test). Especially, percentage of sand flies in UTI was found to be the highest level compared to other areas studied like PYO [95% confidence interval (CI), 0.05 to 0.23], BKN (95% CI, 0.05 to 0.23), CPN (95% CI, 0.04 to 0.20), LRI (95% CI, 0.04 to 0.20), and SKN (95% CI, 0.01 to 0.09) (Table 1).

To conduct multi-group comparison tests, we categorized sand fly species as *S. anodontis, S. iyengari, S. khawi, S. barraudi, P. stantoni*, and other species (prevalence lower than 5%). Overall, the percentage of various sand fly species was statistically significant (chi-square test). The largest proportion of sand fly species was *S. anodontis* and significantly greater than that of *P. stantoni* (P<0.001; 95% CI 2.52 to 17.60). While a relative proportion between *S. iyengari* and *S. khawi* was not statistically different with a P-value higher than 0.05 (95% CI 0.47 to 1.87). We also found a statistical significance difference between percentages of *S. iyengari* and *S. khawi* versus that of *P. stantoni* with P-values of 0.002 and 0.003, respectively (95% CI 1.76 to 12.75; 1.64 to 12.01, respectively) (Figure 3).

As shown in Table 2, only the number and percentages of each sand fly species were described. We present a coupled two-way clustering approach to understand the correlation of sand fly species and the provinces studied (Figure 4). Based on propositional profiles of sand fly species detected in each studied province, two clusters were found in both first- and second-way clustering dendrograms. In vertical clustering, studied area-cluster I consisted of CRI, PYO, RNG, and CPN. While studied area-cluster II included UTI, BKN, LRI, and SKI. For horizontal way, sand fly species could be grouped as sand fly-cluster I (11 species as highlighted in green) and sand fly-cluster II (13 species as highlighted in red) as represented in Figure 4.

The signature sand fly species in studied area-cluster I was *S. anodontis* that high percentages ranging from 28.57-54.93% were found. While *S. anodontis* in studied area-cluster II was

**Table 1.** The number of sand flies collected by the modified Centers for Disease Control and Prevention light traps in eight provinces from February 2021 to February 2022.

| Locations (province) | Number of males | Number of females | Total number | % | Total traps | Catch rate/trap/night | Sex ratio male:female |
|---|---|---|---|---|---|---|---|
| Uthaithani | 2860 | 1447 | 4307 | 51.81* | 30 | 143.57 | 1.98:1.00 |
| Phayao | 511 | 433 | 944 | 11.35 | 30 | 31.47 | 1.18:1.00 |
| Buengkan | 309 | 590 | 899 | 10.81 | 30 | 29.97 | 1.00:1.91 |
| Ranong | 315 | 430 | 745 | 8.96 | 30 | 24.83 | 2.29:1.00 |
| Chumphon | 168 | 537 | 705 | 8.48 | 30 | 23.50 | 1.00:3.20 |
| Lopburi | 160 | 172 | 332 | 4.00 | 30 | 11.07 | 1.00:1.08 |
| Sakon Nakhon | 153 | 114 | 267 | 3.21 | 30 | 8.90 | 1.34:1.00 |
| Chiang Rai | 80 | 35 | 115 | 1.38 | 30 | 3.83 | 2.29:1.00 |
| Total | 4556 | 3758 | 8314 | 100.00 | 240 | - | 1.21:1.00 |
| Catch rate/trap/night | 18.98 | 15.66 | 34.64 | - | - | - | - |

*The P-value to compare proportions among eight areas studied was conducted with a chi-square test, and was less than 0.001.

detected in only SKI (10.53%) and UTI (19.42%) with lower proportions compared to cluster II. Likewise, the major of sand fly species in studied area-cluster II tend to be *S. barraudi*. This sand fly species could be collected and identified in all four provinces of studied area-cluster II (9.68-49.42%), while percentages of *S. barraudi* in studied area-cluster I ranged from 2.79% to 16.63% and tended to be lower than that in studied area-cluster II.

## Discussion

The research identified a total of 24 different species during the study period: *Phlebotomus stantoni, P. kiangsuensis, P. asperulus*, *P. argentipes, P. barguesae, P. major major, Sergentomyia anodontis, S. khawi, S. hodgsoni, S. barraudi, S. tambori, S. rudnicki*, *S. iyengari*, *S. bailyi*, *S. indica*, *S. sylvatica*, *S. phadangensis, S. mahadevani, Idiophlebotomus longiforceps*, *Chinius barbazani,* and other unidentified species belonging to *Sergentomyia* spp. (*Sergentomyia* spp.a, *Sergentomyia* spp.b, *Sergentomyia* spp.c, and *Sergentomyia* spp.d).

In Thailand, previous captures of sand flies documented 34 species (Apiwathnasorn *et al.,* 1989; 1993; 2011; Depaquit *et al.*, 2009; Polseela *et al.*, 2016). The nocturnal activity among the different species of sand flies was studied for a significant period in different parts of Thailand (Apiwathnasorn *et al.*, 1989; 1993; Polseela *et al.*, 2007; 2011a). The greatest number of specimens

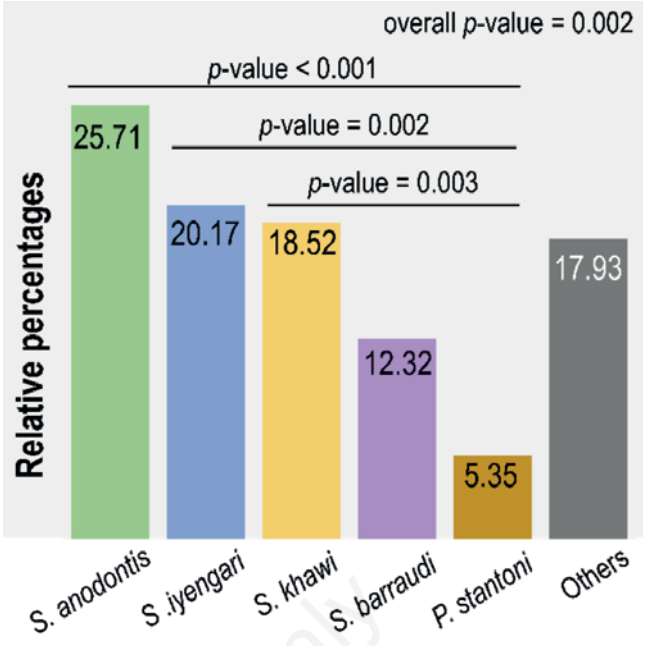


**Figure 3.** Statistical comparisons of the relative percentage of sand fly species distributed in various studied areas of the eight provinces.

**Table 2.** The distribution of female sand fly species collected by modified the Centers for Disease Control and Prevention light traps in eight provinces from February 2021 to January 2022.

| Species | Ranong | Chumphon | Uthaithani | Lopburi | Phayao | Chiang Rai | Sakon Nakhon | Buengkan | Total | % |
|---|---|---|---|---|---|---|---|---|---|---|
| *S. anodontis* | 221 | 295 | 281 | 0 | 147 | 10 | 12 | 0 | 966 | 25.71 |
| *S. iyengari* | 4 | 0 | 474 | 0 | 0 | 0 | 26 | 254 | 758 | 20.17 |
| *S. khawi* | 128 | 35 | 381 | 0 | 0 | 0 | 4 | 148 | 696 | 18.52 |
| *S. barraudi* | 12 | 20 | 140 | 85 | 72 | 0 | 55 | 79 | 463 | 12.32 |
| *P. stantoni* | 14 | 36 | 54 | 4 | 42 | 0 | 7 | 44 | 201 | 5.35 |
| *S. hodgsoni* | 49 | 48 | 6 | 0 | 0 | 6 | 3 | 0 | 112 | 2.98 |
| *P. barguesae* | 0 | 0 | 6 | 0 | 88 | 0 | 2 | 0 | 96 | 2.55 |
| *S. bailyi* | 0 | 4 | 45 | 34 | 0 | 0 | 0 | 0 | 83 | 2.21 |
| *S. silvatica* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 55 | 55 | 1.46 |
| *P. kiangsuensis* | 0 | 54 | 0 | 0 | 0 | 0 | 0 | 0 | 54 | 1.44 |
| *S. indica* | 0 | 0 | 18 | 24 | 0 | 0 | 0 | 0 | 42 | 1.12 |
| *S. mahadevani* | 0 | 0 | 0 | 0 | 32 | 3 | 0 | 0 | 35 | 0.93 |
| *Chinius barbazani* | 2 | 3 | 24 | 4 | 2 | 0 | 0 | 0 | 35 | 0.93 |
| *P. asperulus* | 0 | 34 | 0 | 0 | 0 | 0 | 0 | 0 | 34 | 0.90 |
| *Sergentomyia* spp.b | 0 | 0 | 0 | 0 | 21 | 0 | 1 | 10 | 32 | 0.85 |
| *S. phadangensis* | 0 | 0 | 0 | 21 | 0 | 0 | 0 | 0 | 21 | 0.56 |
| *P. major major* | 0 | 0 | 18 | 0 | 0 | 0 | 0 | 0 | 18 | 0.48 |
| *S. rudnicki* | 0 | 5 | 0 | 0 | 13 | 0 | 0 | 0 | 18 | 0.48 |
| *Sergentomyia* spp.c | 0 | 0 | 0 | 0 | 14 | 1 | 0 | 0 | 15 | 0.40 |
| *Sergentomyia* spp.a | 0 | 0 | 0 | 0 | 0 | 14 | 0 | 0 | 14 | 0.37 |
| *S. tambori* | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0.08 |
| *Sergentomyia* spp.d | 0 | 0 | 0 | 0 | 0 | 1 | 2 | 0 | 3 | 0.08 |
| *Idiophlebotomus longiforceps* | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 2 | 0.05 |
| *P. argentipes* | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 2 | 0.05 |
| Total | 430 | 537 | 1447 | 172 | 433 | 35 | 114 | 590 | 3758 | 100.00 |
| % | 11.44 | 14.29 | 38.50 | 4.58 | 11.52 | 0.93 | 3.03 | 15.70 | 100.00 | - |

was collected in UTI province. Some species were found to be abundant in this area while others were absent, perhaps due to different environmental aspects in each area. All traps were positive for sand flies throughout the studied period, especially *Sergentomyia anodontis, which* was the most abundant (25.71%), and *P. argentipes,* which is a proven vector of *Leishmania donovani* accounted for 0.05%. Based on the morphological identification of female specimens, *S. anodontis* was the predominant species in several of the provinces investigated in this study, especially CPN, UTI, PYO, and RNG provinces. From our findings, sand flies are found frequently in the natural ecotone in different areas.

The greatest number of specimens was collected from a location near plenty of crevices inside and outside the cave. In this way, as per different types of caves, a clear demarcation of the geographic limits of sand fly species and activity was demonstrated.

Our findings differed from those of Jaturas *et al*. (2018), who discovered that *S. phadangensis* accounted for 31.9% of all species in the province of Phitsanulok. In most of these six provinces, *Sergentomyia iyengari* predominated and was discovered at every collection site (Phuphisut *et al.*, 2021). According to Chittsamart *et al*. (2015), the most common species in Lang Ga Jiew Island's edible-nest swiftlet cave, which makes up 94.7% of the cave, is *Sergentomyia hodgsoni*. In the affected region of southern Thailand, *S. gemmea* (81.4%) predominated (Sukra *et al.*, 2013). *S. anodontis*, however, is the most common (26.8%) in Satun province (Thammapalo *et al.*, 2020). Khao Pathawi in the province of UTI discovered that our study and *S. anodontis* (55.0%) (Polseela *et al.*, 2015) were similar.

In Thailand, the results revealed nocturnal activity among the different species of sand flies. Sixteen species were also found in Tham Phra Pho Thisat Temple, and 13 species were found in Phra Phothisat Cave, Saraburi Province. In Phitsanulok, 13 species were found in Naresuan cave (Polseela *et al.*, 2011b), eight species in Khao Phatawi, UTI province (Polseela *et al.*, 2015). Four species in Hang Dong District, Chiang Mai, Thailand (Sor-Suwan *et al.*, 2017), six species in the affected areas of leishmaniasis, South Thailand (Sukra *et al.*, 2013). Some remarkable differences were pointed out in the species composition based on regional differences such as *P. argentipes*. However, *P. argentipes* was found in Saraburi province about 0.8-2.2% (Polseela *et al.*, 2007; 2011a); Phitsanulok province (20.15%) (Polseela *et al.*, 2011b); Khao Phatawi, UTI province (33.6%) (Polseela *et al.*,

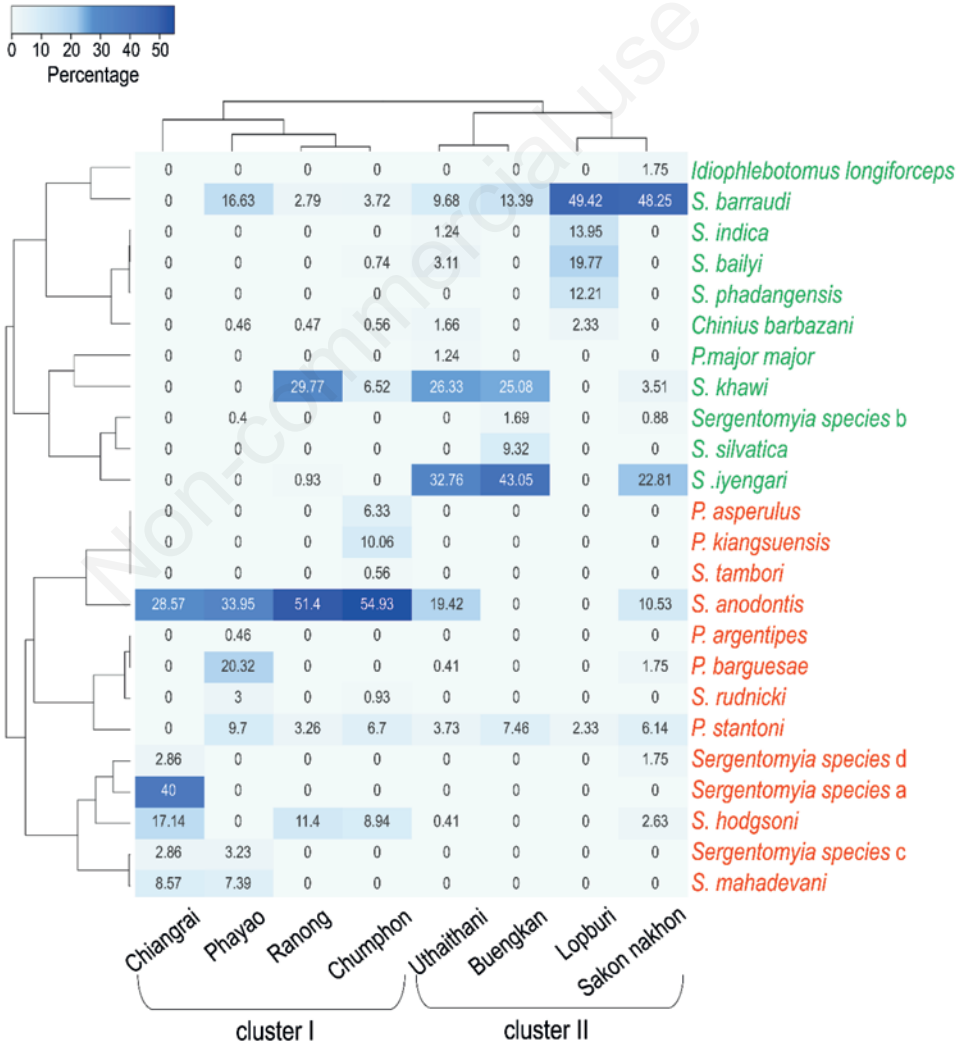


**Figure 4.** Two-way hierarchical clustering analysis displaying the relatedness between the different studied provinces and the collected sand fly species, respectively. Heatmaps show the relative proportions of each sand fly species identified in the various collection sites.

2015). Therefore, this area is free from leishmaniasis because of the lack of parasite carriers. Sand flies collected in each habitat were combined for the analysis to determine the influence of environmental factors; a separate analysis was not made for *P. argentipes* owing to its low number. Previous study in Thailand, *S. khawi*, *S. gemmea, S. iyengari,* and *S. barraudi,* they have been found to be positive from *L. martiniquensis* and *L. orientalis* in reported leishmaniasis areas (Chusri *et al.*, 2014; Kanjanopas *et al.*, 2013; Siripattanapipong *et al.*, 2018; Srisuton *et al.*, 2019).

Both sexes of the sand fly were found to have nocturnal activity in this study. The male and female ratio was 1.21:1.00. This suggests that both sexes are attracted to the light traps. CDC light traps could allure additional phototropic sand flies (Wheeler *et al.*, 1996). Similarly, many previous studies have shown that the number of male sand flies captured using CDC light traps was significantly higher than that of females. The ratio of sex (male:female) was reported as 2.50:1.00 and 1.90:1.00 in Saraburi, Thailand, respectively (Polseela *et al.*, 2007; 2011a). 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 Hanafi *et al*. (2007) (3.40:1.00) and Badry *et al*. (2008) (1.12:1.00). The higher proportion of male sand flies captured by CDC light traps can be attributed to their natural behavior of following females to ensure fertilization during their movements (Barata *et al.*, 2004). Male sand flies are known to have a weaker tendency to disperse compared to females and primarily limit their flight to search for food plants (Janini *et al.*, 1995). It has been observed that male sand flies arrive at the mating site first and wait for females to mate (Morrison *et al.*, 1995; Killick-Kendrick, 1999). Males attract females through the release of pheromones and wing vibrations, engaging in competition for mating opportunities (Ward *et al.*, 1990). The hopping behavior of male sand flies has led to the assumption that they do not disperse far from their breeding sites (Killick-Kendrick, 1999)

For statistical analysis, the first three species – *S. anodontis*, *S. iyengari*, and *S. khawi* – were more relatively abundant than *P. stantoni* among the chosen five areas. Prior studies suggested a higher abundance of these species distributed in various provinces of Thailand and other countries (Srisuton *et al.*, 2019; Phuphisut *et al.*, 2021; Valdivia *et al.*, 2021). Moreover, a previous study reported that *S. khawi* and *S. anodontis* were found to be potential vectors carrying *Leishmania* and *Trypanosoma* (Srisuton *et al.*, 2019). This report together with our clustering analysis suggested that more than half of the study provinces might be considered as risk areas for the transmission of leishmaniasis.

Interestingly, two risk sand fly species were detected in RNG whereas molecular investigating sand fly diversity and their associated parasites need to be performed as well. In addition, our two-ways clustering analysis also demonstrated that *S. barraudi* was mostly abundant in two provinces and could be an indicator for transmitting *Leishmania* parasites (Kanjanopas *et al.*, 2013).

## Conclusions

The understanding of variations and distribution of sand flies in different areas is important to determine the responsible period of higher risk of *Leishmania* transmission. This research has the potential to enable the prediction of future threats posed by an increasing number of sand fly species, which could serve as potential vectors for the widespread dissemination of leishmaniasis in Thailand. Furthermore, these consecutive studies possess the capability to establish an interdisciplinary framework between the fields of medical sciences and public health, allowing them to collaborate under a unified platform such as an "Awareness program".

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*Online supplementary material:*

*Table S1. Temperature, relative humidity, and location coordinate of study sites.*