Field evaluation of four commercial light traps, trap placement, and effect of carbon dioxide on phlebotomine sand fly collection in Thailand

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Abstract

Background

Several light trap devices have been invented and developed to assess the abundance of sand flies. Traps available in the market have different designs and attractant combinations to catch sand fly vectors. We evaluated the efficacy of four commercial light traps and determined the effect of trap placement and carbon dioxide (CO_2) on sand fly collection in northern Thailand.

Methods

Trap evaluations were conducted at two natural caves located in Chiang Rai province, Thailand. In the first part of the study, the efficacies of four trap types including the Centers for Disease Control miniature light trap (CDC LT), Encephalitis Vector Survey trap (EVS), CDC Updraft Blacklight trap (CDC UB), and Laika trap (LK) were evaluated and compared using a Latin square experimental design. The second half of the study evaluated the influence of trap placement and CO_2 on sand fly collection. Additionally, CDC LT were placed inside, outside, and at the entrance of caves to compare the number of sand flies collected.

Results

For the trap efficacy experiment, a total of 11,876 phlebotomine sand flies were collected over 32 trap-nights. Results demonstrated that CDC LT, CDC UB, and LK collected significantly more sand flies than EVS ($P > 0.05$). However, there were no significant differences between the numbers of sand flies collected by CDC LT, CDC UB, and LK. A total of 6,698 sand flies were collected from the trap placement and CO_2 experiment over 72 trap-nights. Results showed that CO_2 did not influence the numbers of sand flies captured ($P < 0.05$), whereas trap placement at the entrance of the caves resulted in collection of significantly more sand flies than traps placed inside and outside of the caves.

Conclusion

We found the CDC LT, CDC UB, and LK without CO_2 captured the greatest amount of sand flies. This was particularly observed when traps were placed at the entrance of a cave, perhaps because of the greater passage of stimuli caused by wind flow at the entrance of the cave. The light traps in this study can be used effectively to collect sand fly vectors in northern Thailand.

Background

Phlebotomine sand flies are important vectors of parasitic diseases such as leishmaniasis. In Thailand, leishmaniasis was historically considered a rare disease. Scattered cases were reported from 1960 to 1996, but these were considered imported since they were mainly observed in returning Thai laborers who had been working in Middle Eastern countries. Since 1996, reported autochthonous leishmaniasis cases have been continuously increasing. To date, at least 21 cases of autochthonous leishmaniasis have been reported across Thailand [1–3]. Therefore, adult sand fly monitoring and surveillance has become necessary for disease control. Adult sand flies are small, fragile, and nocturnally active insects with weak flight capacity. They shelter during the day in dark, humid places, i.e. soil, tree-holes, under rocks or animal burrows [4–6]. Sampling of adult sand flies should therefore be tailored to their habitat or resting site. In Thailand, several field studies have shown that phlebotomine sand fly populations have a high density and diversity in enclosed places like caves [7–9]. Trapping programs have been explored using commercial light traps. The Centers for Disease Control and Prevention light trap (CDC LT) is the most commonly used and the gold standard for blood-seeking insect trapping [10]. The CDC LT has been widely used to capture mosquitoes, phlebotomine sand flies, and other nocturnal blood-seeking insects. CDC LTs are commonly operated with a portable 6-volt battery, incandescent lights, and CO_2 as a bait [4, 6, 11]. The CDC LT can be modified by replacing the incandescent light with a long-wave ultraviolet, CDC Updraft Blacklight trap (CDC UB), in an attempt to attract and capture more mosquitoes and sand flies [12–14]. The CDC LT is widely used in field studies, but their electrical power requirements and bulkiness of size and weight are drawbacks. Recently, light-emitting diode (LED) technology in combination with the light traps, has been demonstrated to improve sand fly collection and simultaneously reduce the power consumption, resulting in longer battery life [15–17].

Collection of sand flies and other blood-seeking insects can be augmented with CO_2 in the form of dry ice [4, 6, 18]. CO_2 is one of the most important attractants required by blood-seeking insects to find hosts. Traps combined with CO_2 can therefore increase the number of sand flies collected [19–21]. However, the use of dry ice is inconvenient as it is difficult to obtain in some locations due to the weight of the insulated CO_2 container and transport handling. The study by Obenauer and colleagues showed there were no significant differences between efficacy of CO_2 baited and CO_2 unbaited CDC LT for *Phlebotomus papatasi* collected in Libya [22]. In addition, there are several variable factors affecting the productivity of sand fly collections, such as trap placement, temperature and humidity, type of light source and environmental conditions during the collection [6]. Due to the increase of autochthonous leishmaniasis cases in Thailand, sand fly monitoring activities have been substantially enhanced in recent years but there is a lack of studies relating to the efficacy of different trap types in this region. The aim of the current study was to evaluate the efficacies of four commercial light traps and determine the effect of trap placement and CO_2 on sand fly collection in northern Thailand. Findings from this study will be useful to improve the sand fly surveillance in Thailand for public health officers and researchers.

Materials And Methods

Study areas

Field experiments were conducted at Pha Thong cave (20° 5'28.89"N, 99°54'13.09"E) and Phra cave (19°55'3.00"N, 99°47'20.30"E), located in Chiang Rai province, northern Thailand (Fig. 1). The experiment comparing trap type was conducted at Pha Thong cave during June and July 2019. Experiments to determine the effect of CO_2 and trap placement were conducted at both caves from July to August 2018. These caves are listed as tourism attractions and are visited by Thai and international tourists. The average temperature and relative humidity from June to August is 25°C (range 25.5-26.9°C) and 80% RH (range 89.5–93.8%). This corresponds to the sand fly season in the study area.

Trap Description

Four different traps (CDC LT, CDC UB, EVS, and LK) were evaluated to determine the most efficacious trap for sand fly collection. (Fig. 2). The CDC LT (Model 1012, John W. Hock Company, Gainesville, FL, USA) with a standard incandescent light bulb was used as the standard trap. CDC UB (Model 1312, John W. Hock Company, Gainesville, FL, USA) equipped with 4-watt blue-blacklight (320–420 nm) was used as an alternative. The EVS light trap (Model 2801A, BioQuip Products, Inc., Rancho Dominguez, CA) equipped with a “grain of wheat” lamp was used along with LK traps (Laika Trap 4.0, Laika→Lab, Pozzuolo, Italy) connected with LED white light (455 nm) and UV LED (395 nm). All traps were operated according to the manufacturers’ instructions.

Study Design And Sand Fly Collection

Comparative field evaluation of four commercial light traps

The four commercial light traps were placed and operated at Pha Thong cave using a 4 × 4 Latin square configured experiment. CO_2 and other supplemental attractants were not used. The study site was divided into a set of four cluster areas. Cluster areas were separated by distances of at least 20 m. In each cluster area two devices of the same trap type, separated by approximately 5 m, were operated from 1800 to 0600 hrs. A total of eight traps was tested during each collection night. Each trap type was rotated counterclockwise among four cluster areas in order to avoid position effects. A total of 32 trap-nights were performed. All captured sand flies were morphologically separated from other insects under the stereomicroscopes followed by sex sorting and number counting [23].

Effect of CO_2 and trap placement

A CDC LT, baited either with dry ice (1 kg/trap) or without dry ice, was used to determine the effect of CO_2 and trap placement on the number of sand flies collected. This experiment was carried out at both Pha Thong cave and Phra cave. Traps were placed approximately 0.5 m height above the ground in three locations of each cave; entrance of the cave, outside of the cave (approximately 30 m from the entrance), and inside the cave (approximately 20 m from the cave entrance) (Fig. 3). At each location, three replicates of the trap were placed at least 10 m apart to reduce the possibility of mutual interference. The

experiment was randomly conducted 4 nights with both CO_2 baited and CO_2 non-baited CDC LT. Therefore, there were a total of 72 trap nights in this experiment. Traps were operated from 1800 to 0600 hrs the following morning. All captured sand flies were morphologically identified and sorted by sex [23]. The total numbers of male and female sand flies collected from each location were counted and recorded.

Data analysis

All statistical analyses were performed using IBM SPSS statistical software, version 25. The numbers of captured sand flies from each trap type in both experiments were analyzed by generalized linear mixed model (GLMM) with negative binomial distribution and log link function. For trap type evaluation, variables of trap type, sex of sand flies, and their interactions were included as fixed effects in the model. Random effects were trap replicate, night of study, and cluster area. To determine the effects of trap placement and CO_2, factorial terms of trap placement, addition of CO_2, and sex of sand fly were included as fixed effects. The variables of trap name nested within a study cave were random effects in this experiment. Estimations of coefficients were performed using the most parsimonious models for both experiments. Incident rate ratios (IRR) were calculated from the exponential of estimated coefficients to determine effects of significant variables.

Results

Comparative field evaluation of four commercial light traps

A total of 11,876 adult sand flies were collected over 32 trap-nights comprising 3,962 (33.36%) females and 7,914 (66.64%) males (Table 1). Almost all sand flies collected from this study belong to the genera *Phlebotomus* and *Sergentomyia*. The number of captured sand flies using four different trap types is presented in Table 1. The GLMM results showed that 2 variables including trap type ($F_{3,56}$ = 3.45; P< 0.05) and sex of sand fly ($F_{1,56}$ = 3.45; P< 0.01) affected number of captured sand flies in this experiment. No significant interaction was found between sex of sand fly and the trap type ($F_{3,56}$ = 0.32; P= 0.81). The parameter estimation showed total sand flies captured by EVS trap was significantly lower than CDC LT (Table 2, IRR = 0.24, P< 0.01). The number of sand flies collected by the conventional CDC LT was not significantly different to CDC UB and LK (Table 2). Regardless of effects from different trap types, significantly more male sand flies were collected than females (Table 1, Fig. 4).

Table 1
Numbers of captured adult sand flies from effect of CO_2 and trap placement experiment during July-August 2018 and comparative commercial trap experiment during June-July 2019.

Trap	n	No. female	No. male	Total	% Total
Comparative commercial trap					
CDC LT	8	1,675	2,358	4,033	33.96
CDC UB	8	1,206	2,708	3,914	32.96
EVS	8	355	687	1,042	8.77
LK	8	726	2,161	2,887	24.31
Total	32	3,962	7,914	11,876	
Effect of CO_2 and trap placement					
Inside w/ CO_2	12	314	170	484	7.23
Inside w/o CO_2	12	395	309	704	10.51
Entrance w/ CO_2	12	797	744	1,541	23.01
Entrance w/o CO_2	12	1,229	857	2,086	31.14
Outside w/ CO_2	12	129	136	265	3.96
Outside w/o CO_2	12	753	865	1,618	24.16
Total	72	3,617	3,081	6,698	

Effect Of CO_2 And Trap Placement

A total of 6,698 adult sand flies were collected over 72 trap nights, 3,617 (54.01%) females and 3,081 (45.99%) males (Table 1). Statistical analysis by GLMM revealed significant results for variables of trap placement ($F_{2,132} = 3.98$; $P < 0.05$) and addition of CO_2 ($F_{1,132} = 10.94$; $P < 0.01$). Terms of all interactions and variables of sex were not affected in this experiment. Therefore, these terms were omitted from coefficients estimation to determine effects of variables as summarized in Table 2 and Fig. 5. The number of captured sand flies at the entrance of the cave was significantly greater than inside the cave (Table 2, IRR = 3.29, $P < 0.05$). Whereas, there was no significant difference between sand flies captured inside and outside of the cave (Table 2). In this experiment, non-baited CO_2 traps significantly collected more sand flies than traps augmented with CO_2, regardless of effect from trap placement (Table 2, Fig. 4, IRR = 1.71, $P < 0.01$).

Table 2
The coefficient estimation of significant variables which influenced number of sand fly collection from GLMM analyses of comparative commercial trap and effect of CO_2 and trap placement experiments.

Trap	Coefficient	SE	t	IRR	95% CI	*P*-value
Comparative commercial trap						
CDC LT vs CDC UB	-0.04	0.50	-0.07	0.97	0.35–2.63	0.94
CDC LT vs LK	-0.40	0.50	-0.80	0.67	0.25–1.82	0.42
CDC LT vs EVS	-1.41	0.37	-2.81	0.24	0.09–0.67	0.01
Female vs Male	0.71	0.23	3.09	2.04	1.28–3.23	0.00
Effect of CO_2 and trap placement						
Inside vs Entrance	1.19	0.55	2.15	3.29	1.10–9.81	0.03
Inside vs Outside	-0.22	0.55	-0.39	0.80	0.27–2.41	0.69
CO_2 baited vs CO_2 Unbaited	0.54	0.18	2.94	1.71	1.19–2.45	0.00

Discussion

This study evaluated the effect of CO_2 baited CDC LT, trap placement and compared four commercial light trap types as surveillance tools for routine sand fly sampling in Chiang Rai province, Thailand. CO_2 is a well-known attractant of blood-seeking insects, especially for female phlebotomine sand flies. It is often employed to improve sand fly trap yields [4, 19–21, 24]. In contrast, our finding demonstrated that CO_2 baited CDC LT did not positively influence but actually significantly decreased the numbers of sand fly collected in this study. Only two other previous studies showed that CO_2 did not have a positive influence on the numbers of sand fly collected. Obenauer and colleagues revealed that CDC LT baited with or without CO_2 showed no difference in *Phlebotomus papatasi* collection in Libya [22]. Another study found that CDC LT and CO_2 light trap had similar efficiency of sand fly collection in Iran [25]. It is noteworthy that CO_2 acts as a long range attractant for sand flies and mosquitoes, whereas light is probably perceived by sand flies at much closer range [4]. This might suggest that only light intensity from CDC LT is sufficiently attractive to sand flies.

Trap placement at the entrance of the cave resulted in more sand fly collection when compared to inside and outside of the cave. The caves in this particular study are characterized as limestone caves surrounded by human residences with a humid environment and an abundance of bats. The study result was perhaps predictable, based on sand fly behavior through cave openings, and also could be explained by the pattern of wind flow in the front side of the cave, which permits the passage of stimuli such as heat and odor plumes from bats, thereby attracting most of the host seeking sand flies. The results of a

previous study by Padro and colleagues [26] showed that *Lutzomyia longiflocosa* sand flies have house entering behavior preferences through large openings, especially at the front side of a house rather than at the rear side. They explained this by two reasons; first sand flies prefer to enter a house through the large front openings because of the absence of obstacles in the large openings and second the increased odor plume at the front side further attracts the host seeking sand flies. However, there were several factors affecting the success of sampling methods, such as different sand fly species, habitat behavior preferences, urban and/or rural areas, and sheltered and/or open areas [6]. Moreover, sand fly flight activity is limited by rain, wind flow, temperature, and attraction to bait animals, depending on the odor plume direction [6]. Trap placement inside of the cave was a zone of virtually no wind flow. This might explain the number of captured sand flies being lower than that the number captured at the entrance of the cave. Furthermore, moonlight or the lunar cycle may interfere with the light intensity of the traps which affects sand fly collection [27–29]. Sand flies exhibit phototaxis, therefore, increased intensity of moon illumination may adversely affect light trap attraction. This could be the explanation that trap placement at the outside the caves showed less numbers of captured sand flies than the others.

Conclusion

Since CO_2 showed a negative effect on sand fly collection, in this study CO_2 was not used for trap type comparison. The EVS light trap showed the lowest performance for sand fly capture and there were no significant differences among the other three traps. Although there is no published data on the performance of EVS traps for capturing sand flies, EVS traps have been investigated with mosquitoes [30–31]. Both studies showed EVS traps had either less or no significant differences in mosquito capture compared to CDC LT, either baited or unbaited with CO_2 [30–31]. The EVS trap with the black trap body was operated with three 3-D cell batteries (4.5 volts) and a two-bladed propeller, whereas CDC LT with transparent trap body was operated with a 6-volt battery and a three-bladed propeller. Based on these specifications, the CDC LT would be expected to have a substantially greater light distribution and wind flow than the EVS trap.

The CDC LT and CDC UB traps showed similar performance for sand fly collection in the current study. Previous studies with sand flies showed the CDC LT equipped with UV lamp was more effective than CDC LT equipped with an incandescent lamp [13–14, 32]. Insects exhibit phototaxis and are sensitive to a broad UV spectrum, by virtue of their trichromatic vision with photoreceptor sensitivities detecting the UV, green and blue wavebands [33]. Mellor and Hamilton studied the effects of different light wavelengths on *Lutzomyia longipalpis*. They showed that *Lu. longipalpis* is very attracted to UV light with a secondary blue-green region [34]. The CDC UB captured large numbers of sand flies; however, massive numbers of non-target insects were also trapped. Moreover, insects captured by the CDC UB tended to be in worse condition than those caught by the downdraft trap, presumably because of repeated contact with dead and moribund specimens and the fan blades at the bottom of the trap.

LED technology has been reported to improve sand fly trapping [15–16, 35]. Contrary to this we found that the Laika trap 4.0, an LED light trap, showed no significant difference in capture competence

compared to CDC LT and CDC UB. Our finding is in agreement with the result of Gaglio and colleagues in which they employed a Laika trap 3.0 model equipped with the same white light LED and UV LED to Laika trap 4.0 model [17]. In contrast, reports of sand fly collections using LED lamp with different colors, have shown variable responses. Red LED, UV LED and blue or green LED were the most attractive for sand fly trapping in southern Egypt [15], in the Mediterranean [36] and in northeastern Brazil [35], respectively. However, incandescent light has increased attraction to sand flies either the same or more than various color LED [35, 37]. LED technology has advantages, resulting in lighter and handier lamps with reduced power consumption while also being less attractive to non-target insects [16]. As a matter of fact, the LK trap was the most suitable for sand fly collection in this area. For further studies, we are going to focus on the effect of different color LED and their attractiveness to sand flies.

The present study showed the use of CO_2 baited traps is not necessary for sand fly trapping and that optimal trap placement is at the entrance of caves. Findings from the trap efficacy experiment showed the EVS trap had the lowest performance, while CDC LT, CDC UB, and LK traps were not significantly different for collecting sand flies. However, we think the LK (Laika Trap 4.0) was the most suitable due to its being light and easy to use, has reduced power consumption, and is less attractive to non-target insects. This kind of trap is recommended for general monitoring surveillance of the local sand fly vectors in northern Thailand.

Abbreviations

CDC LT
Centers for Disease Control and Prevention miniature light trap
CDC UB
CDC Updraft Blacklight trap
EVS
Encephalitis Vector Survey trap
LK
Laika trap
LED
light-emitting diodes
GLMM
Generalized linear mixed model
IRR
Incident rate ratios

Declarations

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Authors' contributions

PS, PK, SD and APL designed experiments. PS, PK, NP and APL performed the field experiments. PS, PK, and NP identified sand fly samples. APS analyzed data. PS, PK, SD and APL wrote the manuscript. All authors read and approved the final version of the manuscript.

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Availability of data and materials

Data supporting the conclusions of this article are included in the published article.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Material has been reviewed by the Walter Reed Army Institute of Research. There is no objection to its publication. The opinions or assertions contained herein are the private views of the author, and are not to be construed as official, or as reflecting true views of the Department of the Army or the Department of Defense.

Competing interests

The authors declare that they have no competing interests.

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Figures

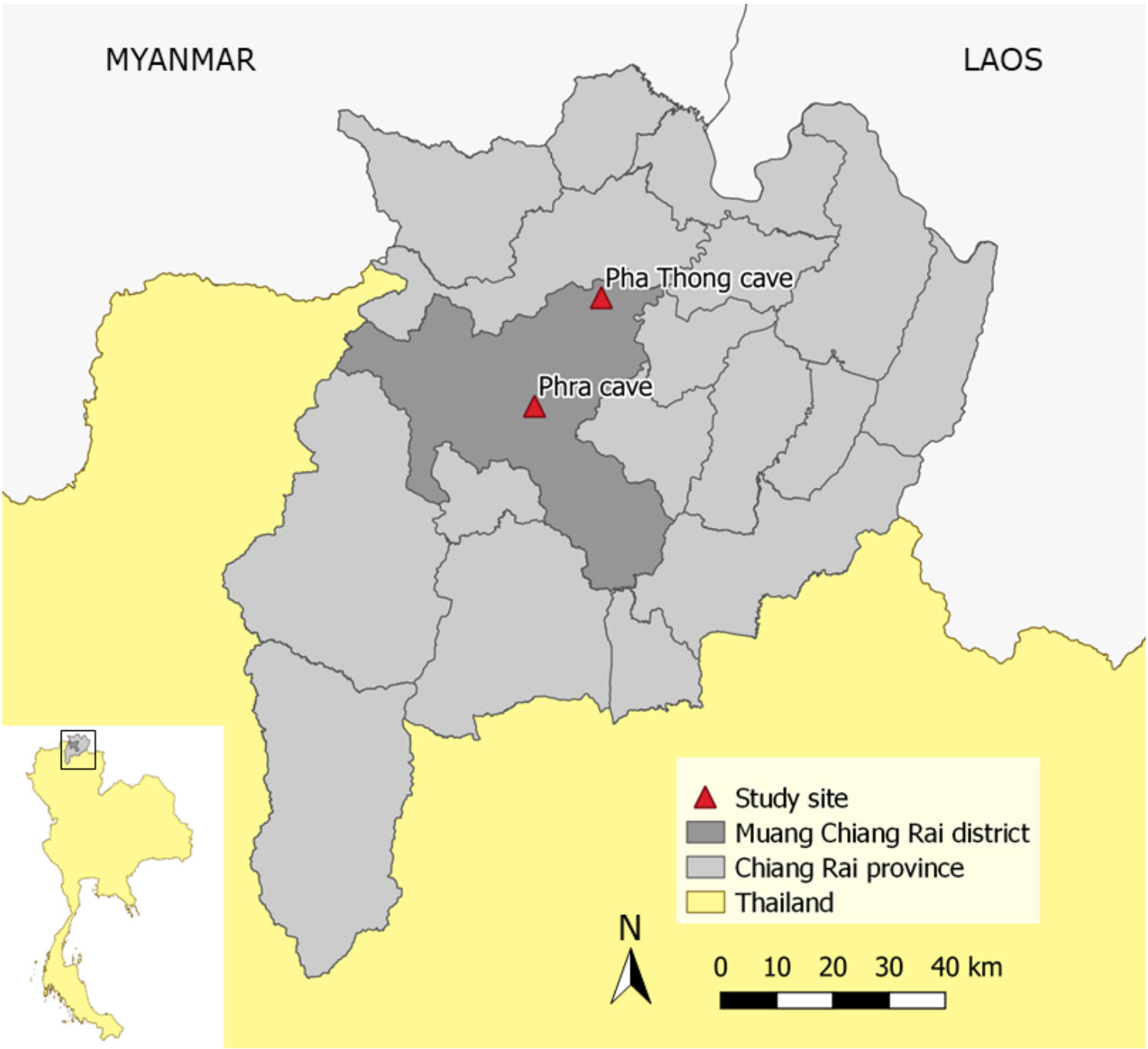

Figure 1

Sand fly collections were performed at Pha Thong cave and Phra cave located in Chiang Rai province, northern Thailand during July 2018 and July 2019.

Figure 2

Four different trap types used for the collection of adult sand flies: A. CDC LT, B. EVS trap, C. CDC Updraft Blacklight (UV), and D. Laika trap 4.0.

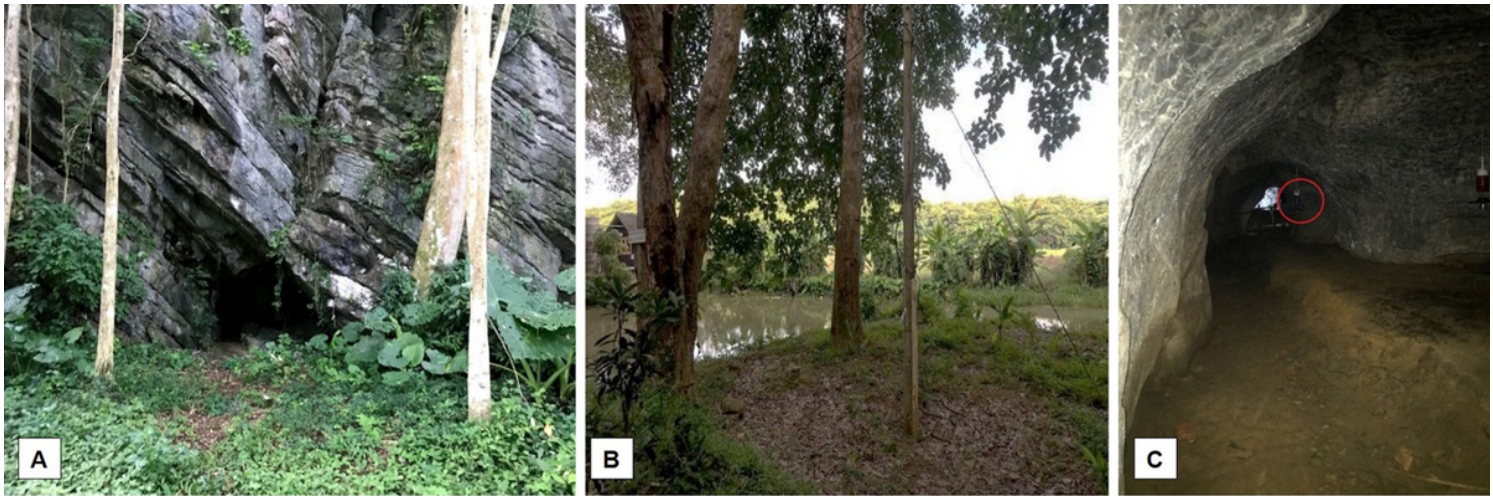

Figure 3

Trap setting at three different placements. A. entrance of the cave, B. outside of the cave, C. inside of the cave.

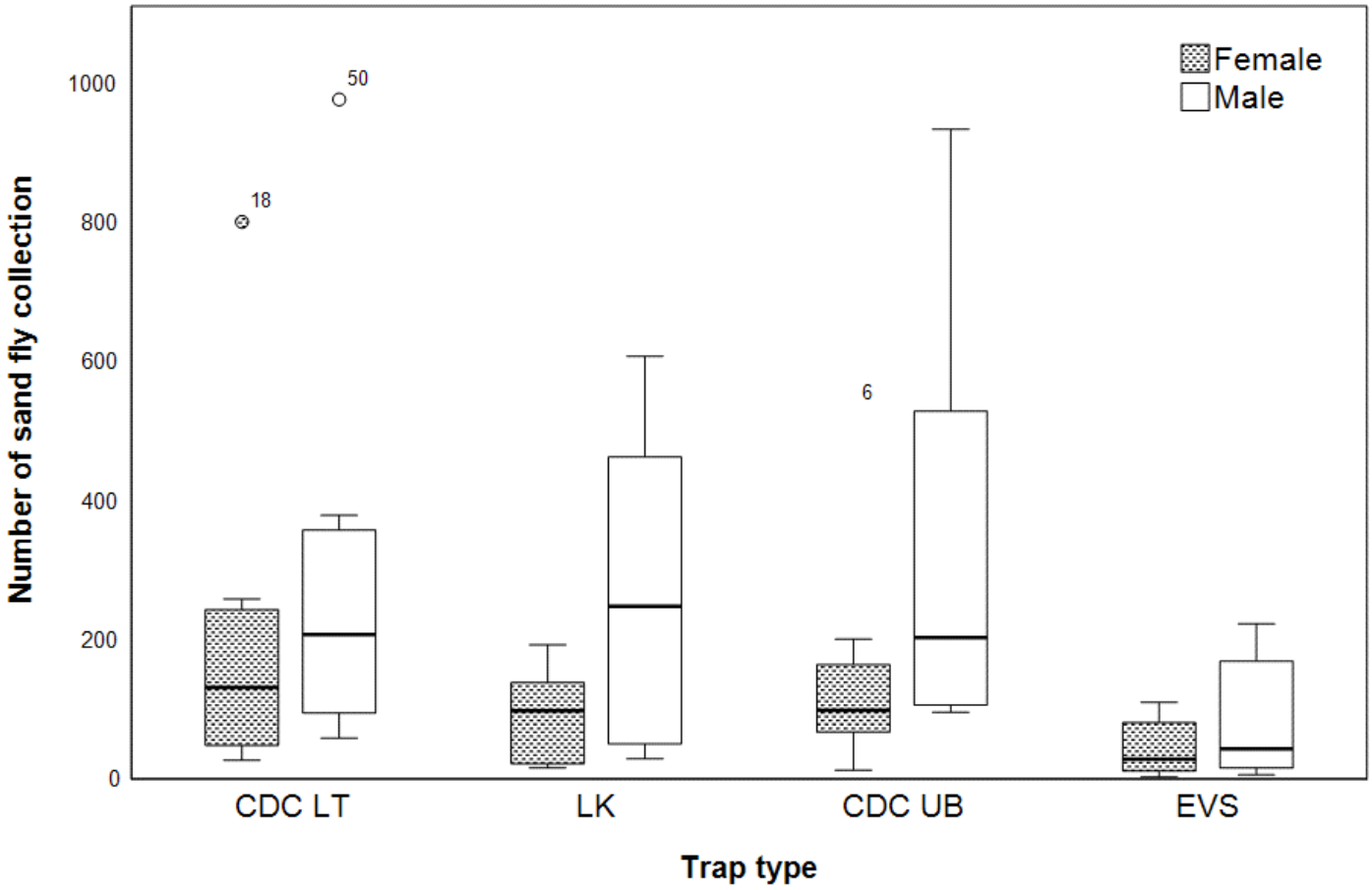

Figure 4

Box plot of number of female and male phlebotomine sand flies collected by different light traps.

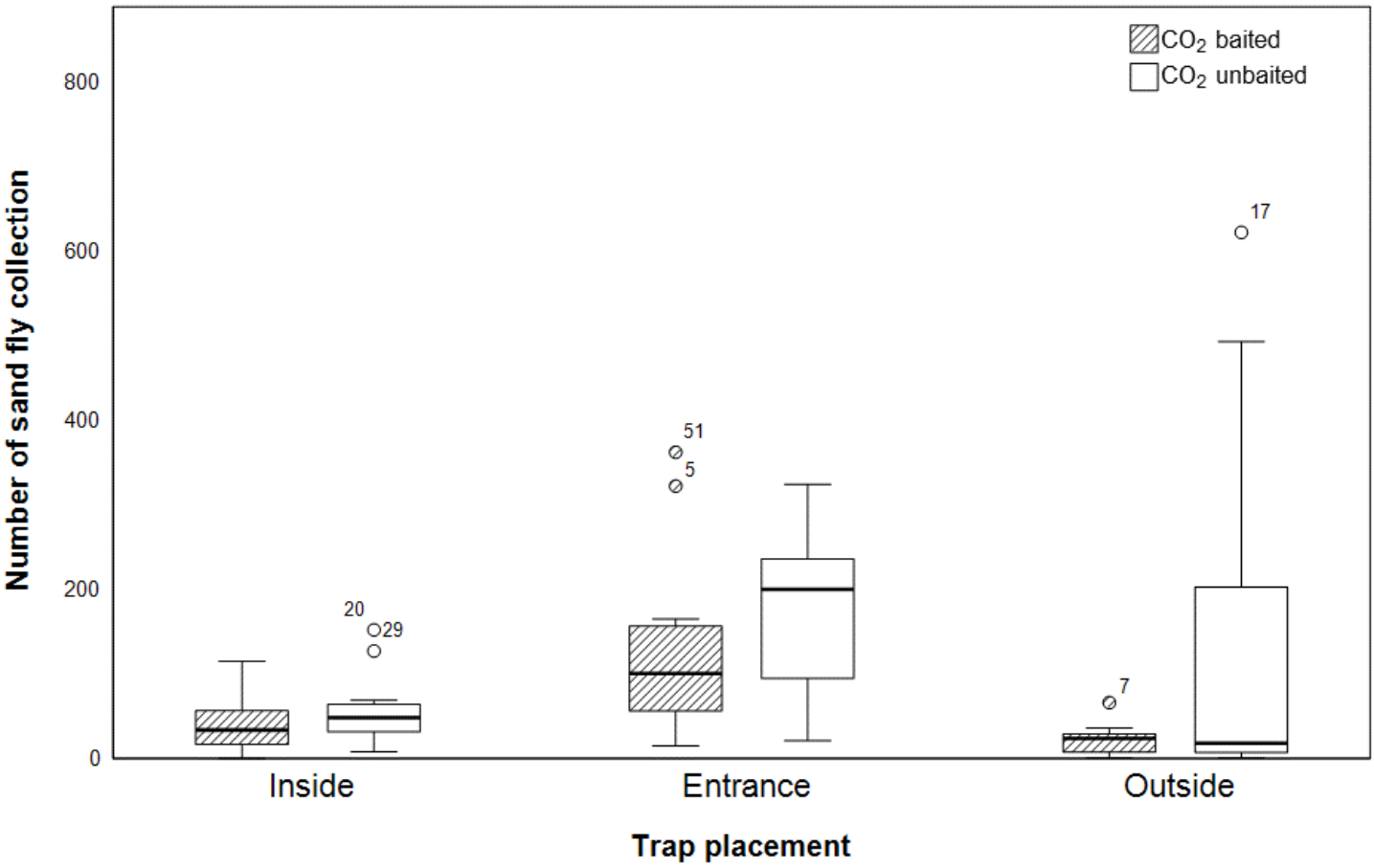

Figure 5

Box plot of number of sand fly collection at three positions and with or without CO2 bait.

Supplementary Files

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- Graphicalabstract.tif