STUDIES OF PHLEBOTOMINE SAND FLY (DIPTERA: PSYCHODIDAE) POPULATIONS IN LIMESTONE AREAS AND CAVES OF WESTERN MALAYSIA

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Abstract. Phlebotomine sand flies were collected using CO_2 baited CDC light trap in 2000 and 2001 in limestone areas and caves of western Malaysia. A total of 1,548 specimens were collected comprising 18 species from two genera: *Phlebotomus* (6 spp) and *Sergentomyia* (12 spp). *Phlebotomus major major* (38.9%) was the predominant species followed by *Sergentomyia perturbans* (20.1%), *P. stantoni* (15.3%) and others. Biting activity of the sand flies at the Gua Senyum caves, Gua Kota Gelanggi, Batu caves and Gua Kelam were observed using the bare leg landing catch (BLC) technique. Four *Phlebotomus* spp at Gua Senyum were found to bite humans with a unimodal biting peak (between 01:00 and 04:00 AM). At Gua Kota Gelanggi *P. major major* was observed to bite humans, but at Batu Caves and Gua Kelam no sand flies were observed to bite humans. *Sergentomyia* spp did not feed on humans even though high numbers were caught in light traps. The populations of phleobotomine sand flies fluctuated, with several peaks especially among *P. major major* which peaked in December and was low in February and August. *Phlebotomus stantoni* was abundant throughout 2001. Most species populations were weakly related to rainfall because they inhabited caves.

Keywords: *Phlebotomus, Sergentomyia*, phlebotomine sand fly, Malaysia

INTRODUCTION

Phlebotomine sand flies play an important part in the transmission of leishmaniasis. Malaysian phlebotomine sand flies are abundant in limestone areas (Quate, 1962a; Lewis and Wharton, 1963; Knudsen *et al*, 1979). Many species of phlebotomine sand flies from the genera *Sergentomyia* and *Phlebotomus*, including the important species of *Phlebotomus argentipes*, are found in limestone caves. *Phlebotomus argentipes* has been found in a few states of Indo-China (Quate, 1962b). Rudnick *et al* (1971) reported 22 species and subspecies of Phlebotominae from throughout Malaysia. Of these, 16 species belonged to the genus *Sergentomyia*, 4 species to *Phlebotomus* and 2 species to *Idiophlebotomus*. Knudsen *et al* (1979) observed *P. argentipes*, *Phlebotomus betisi* and *Phlebotomus kiangsuensis* to feed on humans in a primary forest in Gunong Besout Forest Reserve.

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The sand fly populations have been reported to be abundant during certain months (Rahman *et al*, 1986; Srinivasan, 1990; Boussaa *et al*, 2005). However, these studies were carried out in cattle sheds and human dwelling areas; no studies have been done in limestone and cave areas.

This paper reports a survey of phlebotomine sand fly species, their biting patterns and population density fluctuations in limestone caves in Malaysia. These caves were chosen due to their close association with human populations, such as temples in caves and nearby residential areas. No leishmaniasis cases were reported from these areas.

MATERIALS AND METHODS

A survey of phlebotomine sand fly populations was carried out in several limestone mountainous areas and caves of western Malaysia. The sites of survey were Gua Kelam, Perlis; Gunung Senyum, Temerloh, Pahang; Gua Kota Gelanggi, Jerantut, Pahang; Batu Caves, Gombak, Selangor; Gunung Lang, Ipoh, Perak; temples in caves located in Ipoh, Perak; Gua Musang railway station, Kelantan; Gua Nelayan in Gua Musang, Kelantan; Bukit Chuping, Chuping Perlis; Gunung Churuh Ipoh, Perak and limestone areas near a residential area in Ipoh Groove, Ipoh, Perak. These areas are shown in Table 1. Ten CDC (Centers for Disease Control) light trap baited with CO_2 from dry ice (200-250 g) were set up at each location from 7:00 PM to 7: 00 AM every two months during 2001 and 2002.

Sand fly human biting activity studies were carried out using a bare leg landing catch (BLC) method from 7:00 PM to 7:00 AM. The BLC method consisted of 2 persons, exposing their legs to the knees in the study areas. Trapping was carried out over three consecutive nights at Gua Kelam and Gua Kota Gelanggi in March and August, 2000, respectively. At Gunung Senyum and Batu Caves, six nights of trapping were carried out in February, April, June, August, October and December during 2000 and 2001. These limestone areas were chosen because they are popular tourist sites.

Seasonal fluctuations in population were studies at Gunung Senyum and Batu Caves using densities obtained from CDC light trap collections. Relative density was expressed as the number of sand flies/light trap/night. Species composition abundance and total numbers collected once every two months for two years were compared using Kruskal-Wallis ANOVA.

Mounting

Anatomical structures, particularly heads, wings, and abdomens, were mounted using Hoyer's medium on a glass slide. Only the head was mounted ventrally to aid in proper identification of the cibarium and pharynx.

Species identification

Sand flies were identified using taxonomic keys by Lewis (1978) and the Manual on Entomology in Visceral Leishmaniasis (WHO, 1988). *Phlebotomus betisi* identification was also based on Shahar *et al* (2008).

RESULTS

All 11 survey sites were positive for sand flies of both *Phlebotomus* and *Sergentomyia* genera. Eighteen species of sand flies were found: 6 species of *Phlebotomus* and 12 species of *Sergentomyia* (Table 2). A total of 1,548 sand flies were collected. Of these, 1,041 (67.2%) were *Phlebotomus* spp and 507 (32.8%) were *Sergentomyia* spp. Most female sand flies (65.9%) were collected using a CDC light

Table 1
Locations and descriptions of study areas.

No.	Name of sites	Coordinates position			Avg %RH at cave (5:00 -8:00 PM)		Avg Temp in cave (5:00-8:00 PM)		Climatic condition
		Longitude	Latitude	Altitude (m)	In-side	Out-side	In-side	Out-side	
1	Gua Kelam, Perlis	E100°12′5.2″	N6°38′39.4″	53	94	95	23.2	24.5	Damp, humid
2	Bukit Chuping, Perlis	E100°15′41.51″	N6°29′59.86″	64	94	89	25.5	24.3	Damp, humid
3	Gunung Churuh, Perak	E101°5′2.27″	N4°36′28.47″	72	92	89	24.2	25.7	Damp, humid
4	Gunung Lang, Perak	E101°5′22.9″	N4°37′38.0″	68	-	92	-	24.5	Damp, humid
5	Ipoh Grove cave, Perak	E101°5′9.06″	N4°37′46.86″	70	-	90	-	25.5	Damp, humid
6	Ipoh cave temples, Perak	E101°6′49.28″	N4°34′2.94″	72	91	87	26.4	27.7	Damp, humid
7	Gua Nelayan, Kelantan	E102°13′10.2″	N5°05′37.6″	92	93	92	23.2	23.5	Damp, humid
8	Gua Musang, Kelantan	E101°57′38.9″	N4°51′49.8″	80	-	89	-	26.5	Damp, humid
9	Gunung Senyum, Pahang	E102°25′42.4″	N3°41′42.4″	97	94	92	23.3	25.5	Damp, humid
10	Gua Kota Gelanggi, Pahang	E102°21′59.81″	N3°56′3.18″	95	93	89	23.4	24.6	Damp, humid
11	Batu Caves, Selangor	E101°41′0.3″	N3°14′9.8″	67	96	89	26.2	27.9	Damp, humid

Avg %RH, average percent relative humidity
Avg Temp, average temperature in degrees Celsius

trap. For *Phlebotomus* species, 67% were females and 33% were males. For *Sergentomyia* species, 62% were females.

The *Phlebotomus* species collected were *P. (Euphlebotomus) argentipes*, *P. (Larroussius) major major*, *P. (Idiophlebotomus) asperulus*, *P. (Anaphlebotomus) stantoni*, *P. (Idiophlebotomus) frondifer* and *P.* (*Larroussius*) *betisi*. *Phlebotomus argentipes* was found in nearly all sites. These species made up 68% of the total sand flies caught.

Of the 11 study areas, Gunung Senyum, in Temerloh Pahang had the greatest number of phlebotomine species (12): 5 *Phlebotomus* and 7 *Sergentomyia* species (Table 3). This was followed by Gua Kota Gelanggi; Gua Kelam; Bukit Chuping; Batu Caves and Gunung Churuh; Ipoh Groove; Gua Musang and Gua Nelayan; and lastly Gunung Lang cave. Of the 10 light trap locations, Gua Kota Gelanggi had the greatest number of sand flies followed by Bukit Chuping and Gunung Senyum (Fig 1). Other limestone areas

Table 2
Species composition of phlebotomine sand flies in limestone caves and areas of West Malaysia.

No.	Species	Number	Percent
1	*Phlebotomus argentipes*	113	7.3
2	*Phlebotomus major major*	598	38.6
3	*Phlebotomus asperulus*	92	5.9
4	*Phlebotomus stantoni*	236	15.3
5	*Phlebotomus frondifer*	2	0.1
6	*Sergentomyia rudnicki*	48	3.1
7	*Sergentomyia indica*	39	2.5
8	*Sergentomyia hodgsoni*	5	0.3
9	*Sergentomyia quatei*	1	0.1
10	*Sergentomyia perturbans*	317	20.5
11	*Sergentomyia sepilok*	2	0.1
12	*Sergentomyia iyengari*	2	0.1
13	*Sergentomyia gemmea*	44	2.8
14	*Sergentomyia linearis*	36	2.3
15	*Sergentomyia reidi*	2	0.1
16	*Sergentomyia cheongi*	1	0.1
17	*Sergentomyia tambori*	3	0.2
18	*Sergentomyia anodontis*	7	0.5
	Total	1,548	100

yielded less than 100 *Phlebotomus* sand flies per night. For *Sergentomyia* species, Gunung Churuh had the greatest number of specimens.

Biting activity

Biting activity of the sand flies was recorded at Gunung Senyum and Gua Kota Gelanggi (Fig 2). All *Phlebotomus* species at Gunung Senyum were observed to bite humans. The species observed to bite humans were *P. argentipes*, *P. major major*, *P. asperulus* and *P. stantoni*. Several species of *Sergentomyia* at Gunung Senyum did not bite humans: *S. rudnicki*, *S. indica*, *S. linearis*, *S. perturbans*, *S. hogsoni*, *S. reidi* and *S. cheongi*. *Phlebotomus argentipes* and *P. major major* were the only species observed to bite humans at Gua Kota Gelanggi. None of the *Sergentomyia* species found at Gua Kota Gelanggi bit humans.

At Gunung Senyum, *Phlebotomus* spp biting started at 8:00 PM, peaked at 1:00 AM. and nearly ceased by 4:00 AM (Fig 2), with a minor peak in biting activity found between 6:00 AM and 7:00 AM. Biting activity at Gua Kota Gelanggi was minor and occurred between 7:00 PM and 9:00 PM. This observation is similar to that found in Lalla Aziza Village, Morocco, which peaked at 7:00 PM to 9:00 PM and ceased by 5:00 AM. (Guernaoui *et al*, 2006). At Batu Caves and Gua Kelam, no human biting activity was observed. No biting was seen at Gua Kelam or Batu Caves, although, a large number of *Phlebotomus* spp and *Sergentomyia* spp were caught by light trap.

Seasonal fluctuation

The seasonal abundance in sand fly populations at Gunung Senyum and Batu Caves is shown in Figs 3 to 6. The monthly rainfall is compared with the number of sand flies collected. The rainfall data for the districts of Temerloh, Pahang and Gombak, Selangor was obtained from the Malaysian Meteorological Service, Malaysia.

Phlebotomus major major at Gunung Senyum (Fig 3) had a greater abundance in June and December compared to other months. The seasonal abundance of sand flies at Gunung Senyum was significantly different (p=0.02) from that of the Batu Caves (Fig 4). The seasonal abundance of *P. major major* at Gunung Senyum peaked in December. In February and August, *P. major major* numbers were lower. The number of *P. major* and other species increased between February and June (Fig 3). The number of *P. major major* specimens collected increased significantly (p=0.02) when rainfall decreased. There was a

Table 3
Number and types of phlebotomine species collected by site.

Sites	No. of species by genera		Total	Species
	Phlebotomus	*Sergentomyia*		
Gunung Senyum	5	7	12	*P. argentipes, P. major major, P. asperulus, P. stantoni, P. betisi, S. rudnicki, S. indica, S. linearis, S. perturbans, S. hodgsoni, S. reidi, S. cheongi*
Gua Kota Gelanggi	6	5	11	*P. argentipes, P. major major, P. asperulus, P. stantoni, P. frondifer, P. betisi, S. rudnicki, S. tambori, S. linearis, S. perturbans, S. anodontis*
Gua Kelam	5	4	9	*P. argentipes, P. major major, P. asperulus, P. stantoni, P. betisi, S. rudnicki, S. indica, S. hodgsoni, S. quatei*
Bukit Chuping	5	3	8	*P. argentipes, P. major major, P. asperulus, P. stantoni, S. rudnicki, S. indica, S. perturbans*
Batu Caves	4	3	7	*P. major, P. asperulus, P. stantoni S. indica, S. linearis, S. pertubans*
Gunung Churuh	4	3	7	*P. argentipes, P. major major, P. asperulus, P. stantoni, S. indica, S. pertubans, S. gemmea*
Ipoh Groove	2	3	5	*P. major, P. stantoni, S. indica, S. sepilok, S. iyengari*
Gua Musang	3	1	4	*P. major, P. stantoni, P. asperulus S. pertubans*
Gua Nelayan	3	1	4	*P. major major, P. stantoni, P. asperulus, S. pertubans*
Gunung Lang	2	1	3	*P. major major, P. stantoni, S. sepilok*

greater number of specimens collected and a greater variety of species collected when the rainfall was 100-200 mm (June, October and December).

At Batu Caves, *P. stantoni* was the dominant population throughout 2001 (Fig 4). The number of *P. stantoni* specimens collected at Batu Caves was higher throughout the year (p=0.04) than at Gunung Senyum. *Phlebotomus stantoni* increased when more rainfall was recorded (p=0.04). The greatest number of *P. stantoni* specimens were collected in October, 2001 during a period of high rainfall (September to November) where a range of 350-500 mm was recorded.

At Gunung Senyum, *P. argentipes* was not found in February 2000 but a month later, 2 specimens were caught and the numbers increased to 35 and 32 in October and December, respectively (Fig 3). The number of *Phlebotomus argentipes* in Gunung Senyum increased in April, had a further increase in June and August and

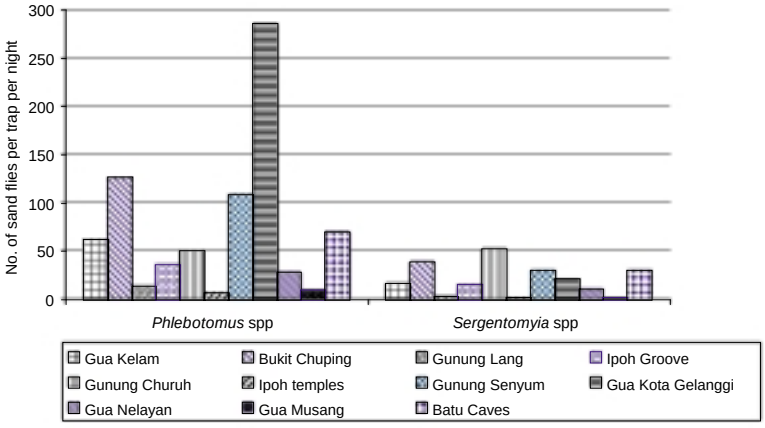

Fig 1–Number of *Phlebotomus* sp and *Sergentomyia* sp caught in the light trap by location.

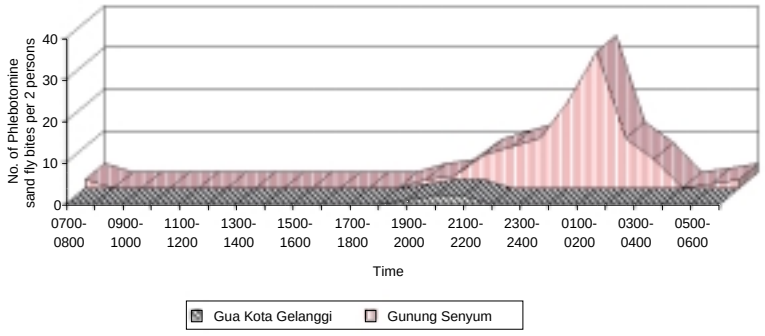

Fig 2–Biting activity by Phlebotomine sand flies by location.

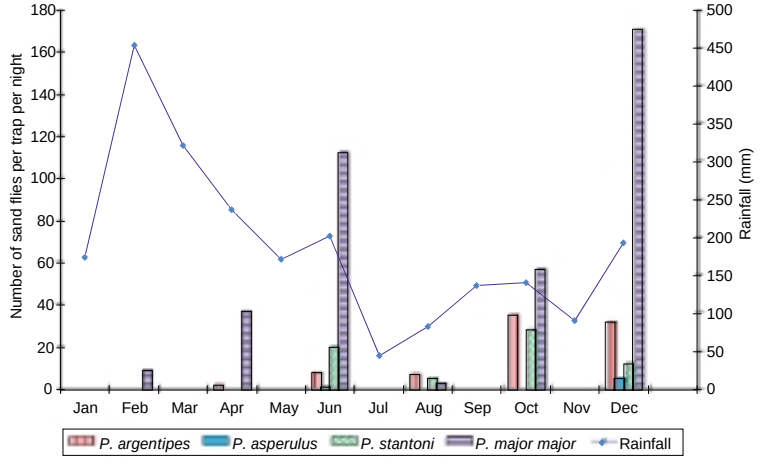

Fig 3–Seasonal abundance of *Phlebotomus* spp at Gunung Senyum, Temerloh Pahang during 2000 compared to rainfall.

peaked in October; then subsequently, decreased in December and became nil in February. Thus, *P. argentipes* at Gunung Senyum was abundant in October and December, when the rainfall was between 50 and 100 mm (Fig 3). At Batu Caves no *P. argentipes* were observed throughout 2001 (Fig 4).

At Gunung Senyum, *S. perturbans* was the dominant species, with a peak in June and a decrease in August (Fig 5). The species increased in October and peaked in December. A negative association in the number of *S. perturbans* specimens was seen with high rainfall in February (453.3 mm) and April (237.3 mm) in Gunung Senyum. In June, several species of *Sergentomyia* were caught: *S. linearis, S. hodgsoni, S. reidi, S. cheongi, S. indica* and *S. perturbans*. Six species of *Sergentomyia* were observed in June, when the rainfall was 202.5 mm. At Batu Caves, *S. perturbans* was more abundant than the other *Sergentomyia* species (Fig 6). The number of *S. perturbans* species was high (35) in February, when the rainfall was low (80.3 mm). The number of sand flies was also high with a large amount of rainfall in October

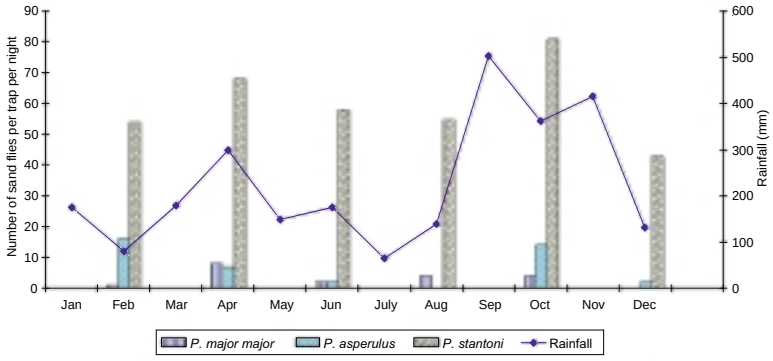

Fig 4–Seasonal abundance of *Phlebotomus* spp at Batu Caves, Selangor during 2001 compared to rainfall.

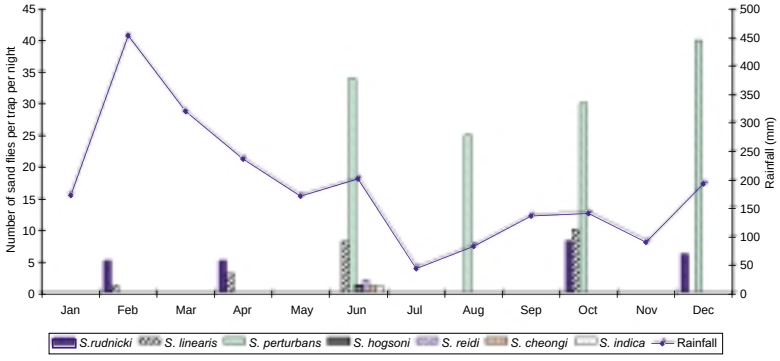

Fig 5–Seasonal abundance of *Sergentomyia* spp at Gunung Senyum, Temerloh Pahang during 2000 compared to rainfall.

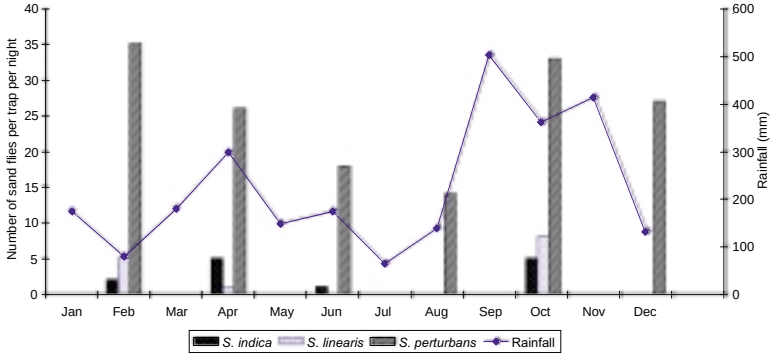

Fig 6–Seasonal abundance of *Sergentomyia* spp at Batu Caves, Selangor during 2001 compared to rainfall.

(361.6 mm). Thus, the abundance of *S. perturbans* at Batu Caves was not affected by rainfall in 2001.

DISCUSSION

Collection of sand flies from caves of West Malaysia indicates phlebotomines are probably indigenous in that environment. Eighteen species of phlebotomines were collected: 6 species of *Phlebotomus* and 12 species of *Sergentomyia.* Gunung Senyum in Pahang had the most species of sand flies, with 5 *Phlebotomus* and 7 *Sergentomyia* species.

Of the eighteen species found in the survey, *P. argentipes* was the most important species in regard to disease transmission (WHO, 1990). This species was found in Gua Kelam (2.5%), Bukit Chuping (4.2%), Gunung Churuh (0.9%), Gunung Senyum (10.2%), Gua Kota Gelanggi (5.6%) and Gua Nelayan (12.8%). There were no *P. argentipes* specimens collected from other limestone areas; the reason for this is unknown. Quate (1962a) reported *P. argentipes* from Batu Cave. However, later the species was not found (McClure *et al*, 1967). Human activity and environmental changes, such as cave wall painting and excessive use of cement in the cave,

might have made the cave less conducive for species to survive and multiply. Changes in the profile of phlebotomine species in the Batu Caves have not been reported since the studies of Quate (1962a) and McClure *et al* (1967).

In Thailand, *P. argentipes* was found abundantly in caves and forests along stream banks (Apiwatnasorn *et al*, 1989). Other species of sand flies have been found in temple caves: *P. stantoni, P. teshi, S. anodontis, S. hodgsoni hodgsoni* and *S. silvatica* (Apiwatnasorn *et al*, 1989). Only two species of sand flies were found in temple caves in Ipoh: *P. major major* and *P. asperulus*. The Gunung Churuh site, which is part of the Ipoh limestone range formerly served as a Hindu temple; has been found to be a habitat for *P. argentipes, P. major major, P. asperulus, S. indica, S. perturbans* and *S. gemmea. P. argentipes* is the main vector for visceral leishmaniasis in India, where the larvae can normally be found on cowshed floors (Hati, 1987); this species is primarily zoophilic in Southeast Asia (Lewis, 1971). The latter report postulated cave dwelling may be a step in the development of anthropophily. Thisyakorn *et al* (1999) reported the first indigenous case of leishmaniasis in Thailand was in a cattle farm area. *Phlebotomus major major* has been reported in Thailand as a cow biting species (Apiwathnasorn *et al*, 1993). Records of the Institute for Medical Research show *P. major major* is abundant in caves in Malaysia and it displays seasonal activity.

A recent study of leishmaniasis in Thailand screened blood samples from 41 dogs, 5 cats, 6 pigs and 3 cows in a village with a human case. Twenty percent of the cats and all the cows screened were positive with antibody titers of 1:100 and 1:100-1:200, respectively (Kongkaew *et al*, 2007). Of 118 sand flies collected in the case area, 85% were *Sergentomyia gemmea*, 15% *S. barraudi* and 2 were *Phlebotomus stantoni*.

A study by Srinivasan *et al* (1993) of cattle sheds and residential areas showed high rainfall increased the sand fly density. However, this study indicated limestone and cave habitats displayed a different pattern of sand fly density with rainfall. This may be attributable to weather changes and general moisture of the cave related to rainfall (McClure *et al*, 1967). The species profile would be more accurate if the collection of sand flies was done monthly. In Kenya, *P. guggisbergi* was the predominant species living in caves (90.1%) (Johnson *et al*, 1999). Cave dwelling probably protects it from rainfall. The cooler temperature may inhibit reproduction or delay development, keeping the population low during the months with the lowest temperatures at night. The combination of warm weather and short rains may provide favorable conditions for an increase in population levels (Johnson *et al*, 1999). These explanations may also be applicable to the survival of *P. stantoni* in Batu Caves. Favorable conditions in the Batu Caves may be a factor in generating good breeding conditions.

In Batu Caves, *P. stantoni* had high population numbers throughout the year, unlike in Gunung Senyum. This species was more tolerant to cave conditions and became dominant, whereas the same conditions suppressed other species in this cave. Batu Caves had a high density of sand flies, but a smaller number than Gunung Senyum and other main caves. Annual religious activities, renovation and modification of the interior of Batu Caves may have contributed to this situation. The fluctuating rainfall in 2001 did not appear to affect the *P. stantoni* population, although it did affect other species of sand flies.

At Gunung Senyum *Sergentomyia perturbans* outnumbered six other *Sergentomyia* spp. *Sergentomyia perturbans* was the most dominant species, although no members of this species was caught between February and April, probably due to the cool period with high rainfall. In contrast, *S. perturbans* in Batu Caves did not appear to be affected by rainfall except during August. This may be due to low rainfall during the previous month of July. In Malaysia, this species was first recorded in Lubok Paku, Rantau Panjang (Lewis, 1957) and later in Gua Che Yatim (Quate and Fairchild, 1961), Tanjong Rabok, Ulu Langat Forest Reserve (Quate, 1962a) and Gunong Besout Forest Reserve (Lewis, 1978). This species is also found world-wide in Bangladesh, Myanmar, Cambodia, India, Java Island, Lao PDR and Vietnam (Lewis, 1978). In India, *S. perturbans* is associated with foothill jungle (Sinton, 1924).

Biting activity studies at Gua Senyum and Gua Kota Gelanggi showed that most of *Phlebotomus* spp bit human but none of *Sergentomyia* spp did. The biting activity of sand flies in limestone areas was correlated with the nocturnal activity of sand flies (Guernaoui *et al*, 2006). Both *Phlebotomus* and *Sergentomyia* species were caught in the village using a light trap and a sticky trap, but no biting was reported. In limestone areas, blood meal and autogenicity studies of cave sand flies should be carried out in order to understand their life cycle. Besides humans, animals such as bats, swallows, pigeons and reptiles observed in the caves may serve as blood sources for the sand flies. Surprisingly, at Batu Caves biting activity was not over 3 days even though light trap collection was high. The blood preference of sand flies in Batu Caves may be different from other caves. No other studies in Malaysia have show human biting by sand flies.

Trapping sand flies using light traps and bare leg landing catch is influenced by environmental conditions, including wind, temperature, rain and the presence of moonlight (WHO, 1990). These factors can alter the yield of traps placed in open areas. Their proportion may not truly reflect the relative abundance of species since their reaction to traps may differ. For example, *P. ariasi* and *P. perfiliewi* are attracted to light and are readily caught in light traps, whereas *P. argentipes* are light shy (WHO, 1990). In this study, *P. stantoni* and *S. perturbans* were easily caught in light traps. However, a comparative study using various sampling techniques in West Bengal, India, showed that hand capture was the most effective method for sampling adult *P. argentipes* (Hati, 1987). Thus, the study of this vector population in this country should be a continuous effort to determine natural fluctuations in density in relation to meteorological data and other factors.

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