Diversity, ecology, and seasonality of sand flies (Diptera: Psychodidae) of the Jenin District (Palestinian Territories)

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ABSTRACT: The diversity, ecology, and seasonality for sand flies from two localities in Jenin District, the Palestinian Territories, were studied. A total of 12,579 sand flies (5,420 *Phlebotomus* and 7,159 *Sergentomyia*) were collected during the study period. The genera *Phlebotomus* and *Sergentomyia* are represented by 13 and nine species and subspecies, respectively. Species account was given for all collected species. CDC light traps yielded 7,649 (60.8%) of the total captured sand flies, while sticky traps and aspirators contributed to 36.4 and 2.8% of the total collected specimens, respectively. *Phlebotomus sergenti* and *P. syriacus* showed two peaks, one in July and one in October. *Phlebotomus tobbi* showed one peak towards the end of the summer in September and August, while *P. papatasi* showed a bimodal peaks pattern, one in June and one in October. *Phlebotomus canaaniticus* showed a peak in August. *P. perfiliewi transcaucasicus* and *P. neglectus* showed a peak in October. *Sergentomyia dentata* showed one peak in August and increasing numbers from June to August, declining afterwards. Other species, such as *S. theodori*, had one peak in June, *S. taizi* had steady numbers across the summer, and *S. christophersi* had a peak in August. *Journal of Vector Ecology* 42 (1): 120-129. 2017.

Keyword Index: Sand fly, Diptera, Psychodidae, Jenin, Palestinian Territories, diversity, ecology, seasonality.

INTRODUCTION

Leishmaniasis is an endemic disease in most countries of the Middle East (Ashford and Bettini 1987, Bettini and Gradoni 1986, Klaus and Frankenburg 1999). Both forms of leishmaniasis, visceral leishmaniasis (VL), and cutaneous leishmaniasis (CL) have become serious public health problems in Palestine. Several studies addressed the epidemiology of leishmaniasis in the Palestinian Territories (Alvarado et al. 1989, Klaus et al. 1994, Qubain et al. 1997, Abdeen et al. 2002, Al-Jawabreh et al. 2003, Amro et al. 2009).

The sand flies of Palestine were studied from the Jordan Valley (Müller et al. 2011a and 2011b), Jerusalem Mountains (Orshan et al. 2010, Moncaz et al. 2012), the north (Svobodova et al. 2006), and Jenin area (Sawalha et al. 2003, Depaquit and Le'ger, 2004). Regionally, the sand flies of neighboring countries were identified in Egypt (Lane 1986), Jordan (Lane et al. 1988, Kamhawi et al. 1995, Kanani et al. 2015), Lebanon (Haddad et al. 2003), and Saudi Arabia (Lewis and Buttiker 1982).

The present study discusses the diversity, ecology, and seasonal emergence of sand flies in Jenin District (Palestinian Territories), with comparison of different trapping methods.

MATERIALS AND METHODS

Study area

This study was carried out in Jenin district (32° 20' N, 35° 8' E), northern West Bank, Palestinian National Authority. Altitude ranges between 100 and 200 m asl, with annual rain fall of 450-

500 mm. Two study sites, Silet Al Harthiyyeh and Ta'anek, were selected.

Silet Al Harthiyyeh is located near agricultural fields mostly planted with almond and olive trees. Small caves and crevices in rocks are also abundant. Although raising animals has decreased in the last years, domestic animals like sheep, goats, and chickens are still found and are more abundant in Ta'anek.

Collection, dissection, and identification of sand flies

Fifty-six sampling sites, including houses, open fields, animal sheds, and caves were selected in the two study areas (34 in Silet Al Harthiyyeh and 22 in Ta'anek). A total of 16 CDC miniature light traps (John W. Hock Co., Gainesville, FL, U.S.A.) and 40 castor oil–soaked papers (21.3x29.5 cm) stapled vertically on wooden stakes at a height of 20 cm were used on a daily basis from May to November, 2011. All traps were set in fixed sites, operated just before sunset, and collected the following morning after sunrise. Additionally, sand flies were collected from houses by means of an aspirator (48 person-h).

Captured sand flies were removed from the light traps or castor oil-soaked papers, washed in a 2% detergent solution, stored in 70% alcohol, mounted in Berlese's medium, sexed, and identified based on available taxonomic keys (Lewis 1982, Lane 1986, Léger et al. 1997, Kakarsulemankhel 2010). Both habitat types and methods of collecting were analyzed using the Simpson Diversity Index.

RESULTS

Species composition and sex ratio

A total of 12,579 sand flies (5,420 *Phlebotomus* and 7,159 *Sergentomyia*) was collected during the study period. The genus *Phlebotomus* is represented by 13 species and subspecies (Table 1, Figures 1 - 3). *Phlebotomus sergenti* and *Phlebotomus major* were the most and least common species, respectively. For the genus *Sergentomyia*, nine species were found, of which *Sergentomyia dentata* was the most common (Table 1).

The sex ratio among the 22 sand fly species varied greatly (Table 1). Among the rare species caught, *P. jacusieli, P. kazeruni, P. major*, and *P. saltiae*, only females were collected. On the contrary, only males of *P. halepensis* were trapped. Higher female to male ratios were observed among *P. tobbi, S. dentata*, and *S. fallax*, while high male to female ratios were recorded for *P. neglectus*, *P. syriacus*, *P. canaaniticus*, and *P. sergenti* (Table 1).

Sand fly yield of different trapping methods

Table 2 summarizes methods of sand fly collections and habitat types for the Silat El Harthiyya and Ta'ank sites. CDC light traps yielded 7,649 (60.8%) sand flies, while sticky traps and aspirators contributed to 36.4 and 2.8% of the total collected specimens, respectively.

Of the total catches, 6,865 flies (54.6%) were collected from Silat El Harthiyya using 234 light traps nights and 650 sticky paper traps for 26 nights, while 5,715 flies (45.4%) were collected from Ta'ank using 182 light traps nights and 390 sticky paper traps for 26 nights. Overall, CDC light traps yielded the highest diversity index (0.83782), followed by sticky traps (0.61507) and aspirators (0.53238).

Habitats of sand flies

From the inside of houses, there were 912 sand flies caught, representing 14 species. By far, *P. papatasi* was the most trapped fly from indoors, followed by *S. dentata*. A total of 7,770 flies were caught from outdoors using CDC light traps and sticky papers representing 20 species. *Sergentomyia fallax, P. tobbi, P. sergenti, P. perfiliewi transcaucasicus*, and *P. sergenti* were the most common species.

In animal sheds, 16 species were caught, whereas *P. tobbi* was the most common, followed by *P. syriacus* and *P. papatasi*. Fourteen species were caught from hyrax caves, with *S. dentata* as the most common species. On the other hand, 20 species were trapped from caves not used by the hyrax. *Phlebotomus sergenti*, *P. syriacus*, and *P. canaaniticus* were found in high numbers.

Sand flies collected from other caves yielded the highest diversity index (0.73552), followed by open fields (0.69237), whereas indoor and outdoor habitats yielded diversity indexes of 0.68 and 0.66, respectively, while hyrax caves and animal sheds gave the lowest indexes (0.568 and 0.559, respectively).

Seasonality of sand flies

Seasonal abundance of the genus *Phlebotomus* varied greatly (Table 3). *Phlebotomus sergenti* and *Phlebotomus syriacus* showed two peaks, one in July and one in October, corresponding to temperatures ranging from 23.3-27.6° C during this period, with relatively steady numbers from June through October (Figure

Species	Fen	nale	M	Total	
Species	No.	%	No.	%	
P. alexandri	3	50	3	50	6
P. halepensis	0	0	7	100	7
P. jacusieli	2	100	0	0	2
P. kazeruni	5	100	0	0	5
P. major	1	100	0	0	1
P. neglectus	11	21.6	40	78.4	51
P. syriacus	254	36.5	441	63.5	695
P. canaaniticus	81	18.3	362	81.7	443
P. papatasi	461	54.8	380	45.2	841
P. perfiliewi transcaucasicus	149	76	47	24	196
P. saltiae	4	100	0	0	4
P. sergenti	604	35.9	1,077	64.1	1,681
P. tobbi	1,148	77.2	340	22.8	1,488
S. adleri	1	33.3	2	66.7	3
S. africana	246	42.1	338	57.9	584
S. antennata	13	72.2	5	27.8	18
S. christophersi	38	66.7	19	33.3	57
S. dentata	3,472	74.1	1,211	25.9	4,683
S. fallax	1,007	71.4	403	28.6	1,410
S. taizi	101	72.7	38	27.3	139
S. theodori	59	56.2	46	43.8	105
S. tiberiadis	93	58.1	67	41.9	160
Total	7,753		4,826		12,579

Table 1. The numbers of male and female of sand fly species collected during May to November, 2011, in Silet Al Harthiyyeh and Ta'anek, in Jenin District, northern West Bank.

		(Sticky trap		Aspirator			
Species	Indoor	outdoor	Animal sheds	Hyrax caves	Other caves	outdoor	Open fields	Indoor
P. alexandri	0	2	1	0	3	0	0	0
P. halepensis	0	4	2	0	1	0	0	0
P. jacusieli	0	0	0	0	2	0	0	0
P. kazeruni	0	1	0	0	4	0	0	0
P. major	0	1	0	0	0	0	0	0
P. neglectus	1	15	1	18	16	0	0	0
P. syriacus	14	69	150	5	359	59	37	2
P. canaaniticus	3	20	49	1	342	17	11	0
P. papatasi	246	62	120	1	16	160	8	228
P. perfiliewi transcaucasicus	21	110	54	1	7	2	0	1
P. saltiae	0	2	1	0	0	1	0	0
P. sergenti	19	323	75	16	1,104	41	52	51
P. tobbi	45	361	900	4	119	35	20	4
S. adleri	1	1	0	0	1	0	0	0
S. afrecana	15	170	6	0	18	345	27	3
S. antennata	0	1	0	11	3	1	2	0
S. christophersi	2	26	2	0	11	7	9	0
S. dentata	151	1,481	21	217	153	2,284	342	34
S. fallax	40	235	12	17	120	854	129	3
S. taizi	2	19	1	1	67	40	9	0
S. theodori	4	23	0	32	5	26	4	11
S. tiberiadis	2	31	1	14	50	29	24	9
Total	566	2,957	1,396	338	2401	3,901	674	346

Table 2. Sand fly species collected by different methods during May to November, 2011, from Silet Al Harthiyyeh and Ta'anek.

4). *Phlebotomus tobbi* showed one peak towards the end of the summer in September and August. *Phlebotomus papatasi* showed a bimodal peaks pattern, one in June and one in October, while *P. canaaniticus* showed a peak in August. *P. perfiliewi transcaucasicus* and *P. neglectus* showed a peak in October. The other species have no distinct seasonal pattern since they were found in low numbers.

August, with steady numbers until October, but then declined afterward. *Sergentomyia africana* showed two peaks, one in July and one October, while *S. tiberiadis* showed the highest numbers in October. Other species such as *S. theodori* had one peak in June, *S. taizi* had steady numbers across the summer, and *S. christophersi* had a peak in August.

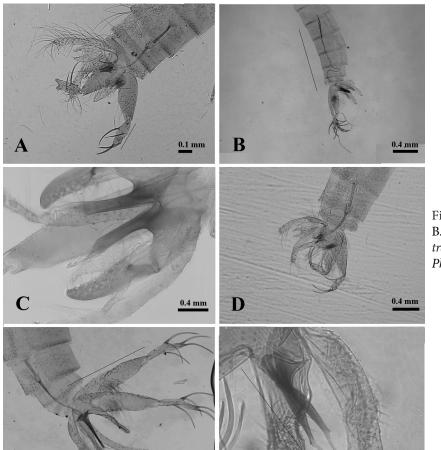
For sand flies of the genus *Sergentomyia*, *S. dentata* showed one peak in August and increasing numbers from June to August, declining afterwards (Table 4). *Sergentomyia fallax* peaked in

Species	May	Jun	Jul	Aug	Sep	Oct	Nov
P. sergenti	9	245	482	318	243	354	30
P. tobbi	67	49	148	240	450	478	56
P. papatasi	70	263	159	48	98	191	12
P. syriacus	27	47	147	109	59	230	76
P. canaaniticus	13	31	80	159	51	97	12
P. perfiliewi transcaucasicus	5	6	35	27	64	59	0
P. neglectus	1	5	6	6	14	19	0
P. halepensis	0	0	2	0	1	4	0
P. alexandri	1	0	1	3	1	0	0
P. kazeruni	0	2	2	1	0	0	0
P. (synphlebotomous) saltiae	1	2	1	0	0	0	0
P. jacusieli	0			1	1	0	0
P. major major	0	1	0	0	0	0	0

Table 3. Number of sand fly species of the genus *Phlebotomus* collected during May to November, 2011, in Silet Al Harthiyyeh and Ta'anek.

Table 4. Number of sand fly species of the genus *Sergentomyia* collected during May to November, 2011, in Silet Al Harthiyyeh and Ta'anek.

Species	May	Jun	Jul	Aug	Sep	Oct	Nov
S. dentata	59	340	1058	1648	876	697	5
S. fallax	12	50	162	384	362	366	74
S. africana	6	85	143	74	111	134	31
S. tiberiadis	0	23	16	39	48	33	1
S. taizi	2	36	24	36	16	21	4
S. theodori	15	44	11	14	17	4	0
S. christophersi	6	4	4	23	15	5	0
S. antennata	0	7	11	0	0	0	0
S. adleri	0	2	0	1	0	0	0



0.1mm

R

Figure 1. Male genitalia. A. *Phlebotomus alexandri*. B. *Phlebotomus halepensis*. C. *Phlebotomus perfiliewi transcaucasicus*. D. *Phlebotomus papatasi*. E. *Phlebotomus syriacus*. F. *Phlebotomus tobbi*.

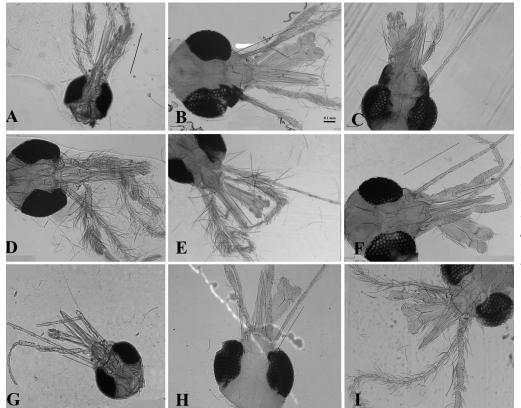


Figure 2. Head. A. Phlebotomus alexandri. B. Phlebotomus halepensis. C. Phlebotomus jacusieli. D. Phlebotomus kazeruni. E. Phlebotomus major. F. Phlebotomus papatasi. G. Phlebotomus perfiliewi transcaucasicus. H. Phlebotomus syriacus. I. Phlebotomus tobbi.

E

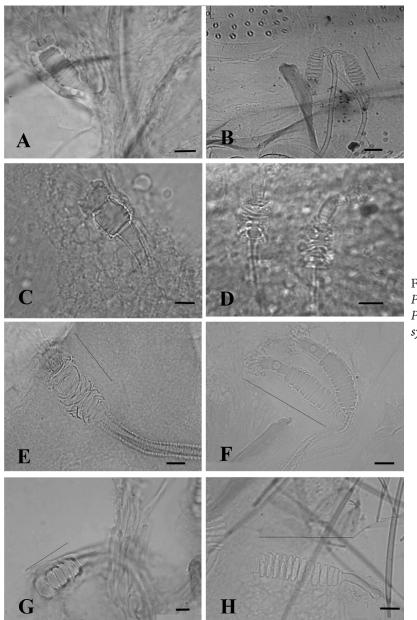


Figure 3. Spermatheca. A. Phlebotomus alexandri. B. Phlebotomus jacusieli. C. Phlebotomus kazeruni. D. Phlebotomus major. E. Phlebotomus papatasi. F. Phlebotomus syriacus. G. Phlebotomus sergenti. H. Phlebotomus tobbi.

DISCUSSION

A total of 13 species of sand flies have been reported from the Jenin district based on a total collection of 4,082 flies made during June and December, 1998 (Sawalha et al. 2003). In the present study, we recorded an additional eight species from Jenin area (P. neglectus, P. alexandri, P. kazeruni, S. dentata, S. afrecana, S. antennata, and S. adleri). Additionally, P. perfiliewi transcaucasicus and P. papatasi were the most common Phlebotomus species, while S. theodori was the most abundant species, constituting 49.6% of all sand flies (Sawalha et al. 2003). Sawalha et al. (2003) did not consider caves and animal sheds, instead basing their study on altitudinal stratification indoors and outdoors. Our findings are different, whereas P. sergenti (13.4%) and P. tobbi (11.8%) were the most common Phlebotomus species, and S. dentata and S. fallax were the most common Sergentomyia species accounting for 37.2% and 11.2%, respectively. Sergentomyia dentata and P. syriacus were the most common species in Lebanon (Haddad et al. 2003). In southern Anatolia, Turkey, *P. tobbi* was the most abundant species, while *P. sergenti* accounted for 0.1% of collected sand flies (Bahrami et al. 2014).

Phlebotomus (Larroussius) neglectus is known in southern Europe, the Balkans, the states of the former Yugoslavia (Lewis 1982), Turkey, Lebanon, Syria, and Palestine (Haddad et. al. 2003, Sawalha et al. 2003, Kasap et al. 2013). This species is the vector of visceral leishmaniasis in Cyprus (Leger and Depaquit 2008). *Phlebotomus (Adierius) halepensis* is distributed in Jordan, Palestine, Syria, Turkey to Iran, and the southern states of the former Soviet Union (Lewis 1982). *Phlebotomus tobbi* has a wide range of distribution across southern Europe, and in the Mediterranean basin it is spread from the mid-northern side to the eastern side reaching as far as Iran (Lewis 1982). This species was found in high numbers in animal sheds and resting indoors during the daytime (Sawalha et al. 2003). Killick-Kendrick (1990) stated that *P. tobbi* is a suspected vector of visceral leishmaniasis in the Mediterranean region. It is also a confirmed vector of *L*.

infantum in Turkey and Cyprus and *L. donovani* in Syria (Rioux et al. 1998, Leger et al. 2000, Svobodova et al. 2009). *Phlebotomus (Larroussius) syriacus* is known from Jordan, Lebanon, Palestine, Syria, Turkey, the Caucasus, Crimea, and Saudi Arabia (Lewis 1982, Lewis and Buttiker 1982).

Phlebotomus (Larroussius) major is an eastern Mediterranean species, with records from Palestine, Syria, Sinai, the Caucasus, and Turkistan (Lewis 1982, Lane 1986). This species is a proven vector of visceral leishmaniasis. Previously, this species was considered to have three subspecies (P. major major, P. major syriacus, and P. major neglectus). Phlebotomus (Larroussius) perfiliewi transcaucasicus is a zoophilic sand fly that was not found to feed on humans and was present in many different habitats in the Jenin District (Sawalha et al. 2003). Its distribution extends from Palestine, Iraq, Iran, and the southern states of the former Soviet Union (Lewis 1982). This species is a vector of visceral leishmaniasis caused by L. infantum (Rassi et al. 2009). Phlebotomus (Paraphlebotomus) jacusieli was recorded from Palestine, Turkey, Lebanon, and Syria (Haddad et al. 2003, 2015) and the former southern states of the former Soviet Union (Lewis 1982). Phlebotomus (Paraphlebotomus) sergenti has a wide range of distribution extending from the Mediterranean Basin, through Palestine reaching India to the east, Saudi Arabia, Yemen, and the Ethiopian highlands southwards (Lewis 1982). Phlebotomus sergenti flies were found to harbor Leishmania tropica (Sawalha et al. 2003). Phlebotomus sergenti is considered the potential vector of Leishmania tropica that is highly endemic in the Middle East, such as in Syria and Iran. The distribution of Phlebotomus (Paraphlebotomus) alexandri extends from North Africa, the Arabian Peninsula, the Levant, and the southern states of the former Soviet Union, reaching as far as Pakistan eastwards (Kakarsulemankhel 2010, Lewis 1982). Phlebotomus (Paraphlebotomus) kazeruni is known from Egypt, central Saudi Arabia, Jordan, Palestine, southern Iran, and Afghanistan (Artemiev 1978, Lewis 1982). Within its range of distribution, it was found in low rocky deserts, remote wadis, and hills with sparse vegetation (Lewis 1982). Phlebotomus (Phlebotomus) papatasi has a wide range of distribution extending from Portugal and North Africa, reaching as far as Bangladesh to the east and Sudan to the south (Lewis 1982). This species is considered the main vector of Leishmania major in the Middle East (Janini et al. 1995). Phlebotomus (Synphlebotomus) saltiae was originally described from Lebanon from humid areas (Léger et al. 1997). Phlebotomus (Transphlebotomus) canaaniticus was collected in low numbers from Jenin and from the upper northern Jordan Valley and the Ajlun area in Jordan (Sawalha et al. 2003, Kanani et al. 2015).

Sergentomyia (Sergentomyia) dentata is a common species with a wide range of distribution (Lewis 1982). Sergentomyia (Sergentomyia) fallax is known from West Africa to eastern Afghanistan, North Africa and Egypt (Lewis 1982), and Jordan, Lebanon, and Palestine (Haddad et al. 2003, Lane 1986). Sergentomyia (Sergentomyia) taizi was recorded from Yemen, Saudi Arabia, Egypt (Lewis 1982, Lane 1986), Jordan, Lebanon and Palestine (Lane et al. 1988, Sawalha et al. 2003). Sergentomyia(Sergentomyia) theodori was recorded from

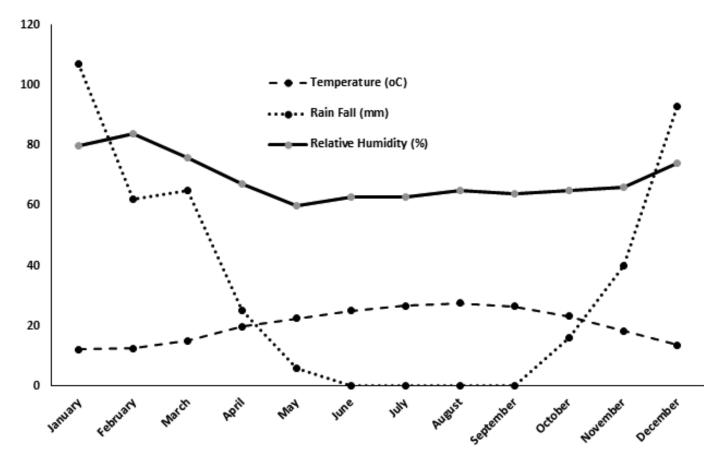


Figure 4. Annual rain fall, relative humidity and temperature at the Jenin District.

Yugoslavia, Turkey, Lebanon, Syria, Palestine, Cyprus, Egypt, Iraq, Iran, Pakistan, India, and Afghanistan (Lewis 1982). Sergentomyia (Sergentomyia) antennata is known from North Africa, the Sahara Desert from Ghana through Central African Republic, East Africa, the Arabian Peninsula, and Jordan (Lewis 1982, Lane 1986). Sergentomyia (Sintonius) tiberiadis is a northeastern African species with distribution extending from Djibouti, Ethiopia, Egypt, Sudan, the Arabian Peninsula and into Palestine and Jordan (Lane 1986). Sergentomyia (Sintonius) christophersi has a distribution range extending from Guinea to Ethiopia, Sudan, Egypt, Yemen, Saudi Arabia, reaching Pakistan (Lane 1986) Jordan, Lebanon, and Palestine (Kamhawi et al. 1995, Haddad et al. 2003). Sergentomyia (Sintonius) adleri is a widespread species with distribution extending from West Africa, across the Sahara Desert, through Central African Republic, East Africa, to the Arabian Peninsula and the Middle East (Lane 1986). Sergentomyia (Parrotomyia) africana is known in Africa, reaching Yemen. It is known from Jordan (Lane et al. 1988) and the Jordan Valley (Schlein et al. 1982).

Specimens of P. saltiae were collected in the Jenin district in 1998, but the specimens identified as P. synphleobotmus spp. were later identified as P. saltiae (Depaquit and Leger 2004). We found high numbers of P. sergenti in caves (16 in hyrax caves and 1,104 in other caves) accounting for 66.6% of collected specimens. It seems that this sand fly is a rock shelter-dwelling species. Phlebotomus sergenti rests and develops inside caves used by rock hyrax and rodents east of Jerusalem (Moncaz et al. 2012). Seven sand fly species were reported from the Galilee, north Palestine (Svobodova et al. 2006), whereas P. tobbi and P. sergenti were the most abundant species. Phlebotomus tobbi was the most common species in animal sheds. In Lebanon, it was mostly associated with stables, engorged with nucleated blood, suggesting that it feeds on poultry (Haddad et al. 2003). Phlebotomus syriacus was recovered from all types of studied habitats with high numbers indoors and in animal sheds. Our findings are consistent with Haddad et al. (2003) where this species was captured inside houses and barns. It was demonstrated that P. papatasi resting sites depend on vegetation cover, type of vegetation, and the presence of a mulch layer, and were absent in bare soil and little shade (Müller et al. 2011b). Also, this fly prefers disturbed habitats as resting sites. In the present study, most of the trapped P. papatasi were from indoors, suggesting that they prefer houses for resting (Müller et al. 2011b). In our study, P. papatasi was the most abundant Phlebotomus trapped indoors, with much fewer P. tobbi and P. sergenti. In contrast, Orshan et al. (2010) found that Phlebotomus papatasi was found to be the most abundant indoor sand fly, followed by P. sergenti and P. syriacus, while P. tobbi was the leastcollected species. In the present study, indoor habitats showed a medium diversity index, whereas open fields yielded the highest index.

Alexander (2000) gave a comprehensive account on different trapping methods used to collect sand flies. He showed that traps with CO_2 collect more flies than those without CO_2 , and traps with black color, heat, and moisture significantly caught more sand flies than the control traps (Kline et al. 2011). It was shown that CDC traps were more effective in trapping sand flies, accounting for about 89% of the total sand flies collected (Wheeler et al. 1996). The present study confirms this finding, where CDC traps showed

the highest diversity index among the other trapping methods. In the present study, CDC traps yielded 60.8% of the total captured flies. Our results showed that CDC traps yielded more *P. tobbi* trapped by sticky traps. Similar results were obtained in Lebanon (Haddad et al. 2003).

Phlebotomus tobbi was not collected during the cold months of the year in the Carmel Mountains but observed from July to September (Müller et al. 2011a). In this study, *P. tobbi* was observed from March to November, one peak towards the end of the summer in September and August. *Phlebotomus sergenti* flies were collected from May to November. Our results are similar to previous findings (Müller et al. 2011a), with highest numbers in July to September. Similar results were obtained for *P. syriacus*, *P. papatasi*, and *P. perfiliewi* similar (Müller et al. 2011b). All these fluctuations are related to temperature, rainfall, and relative humidity changes over the breeding season for the sand flies. A further assessment of related sand fly activities and seasonal dynamics with host animals (e.g., hyrax) and human exposure patterns is necessary.

A number of the trapped sand flies are involved in the transmission of the different *Leishmania* parasites, including *P. papatasi*, *P. perfiliewi*, *P. sergenti*, *P. syriacus*, and *P. tobbi* (Killick-Kendrick 1990). *L. infatum* DNA was detected in *P. tobbi*, suggesting that this sand fly may be considered as a vector (Bahrami et al. 2014). *Leishmania major* is known to be transmitted by *P. papatasi*, while *Leishmania tropica* is vectored by *P. sergenti* (Schlein et al. 1982, Janini et al. 1995, Svobodova et al. 2006). High numbers of both cutaneous and visceral leishmaniasis cases were reported from the Silet Al Harthiyyeh site, while fewer cases were reported from the Ta'anek site (Unpublished data, Ministry of Health).

Further monitoring and habitat preference studies of the vector should be conducted in order to relate population dynamics of sand flies with leishmaniasis outbreaks. The present data can help to plan control measures of sand flies in the study area.

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