



Original Article

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.apjtb.org



doi: 10.4103/2221-1691.281463

Impact Factor: 1.59

Diversity of Phlebotomine sand flies (Diptera: Psychodidae) in mountainous and plain areas of an endemic focus of anthroponotic cutaneous leishmaniasis in Iran

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ABSTRACT

Objective: To determine the diversity of sand flies in different biotopes of mountainous and plain areas of Bam County as the most infected focus of anthroponotic cutaneous leishmaniasis in southeast Iran, and synanthropic index of *Phlebotomus sergenti* Parrot, and *Phlebotomus papatasi* Scopoli as the main vectors of cutaneous leishmaniasis in Iran.

Methods: Sand flies were captured once a month using sticky traps in domestic, peri-domestic, agricultural, and sylvatic biotopes in the plain and mountainous areas. Alpha diversity indices, including richness, evenness, Shannon-Wiener; beta diversity indices (Jaccard's and Sorensen's similarity indices) and synanthropic index were calculated.

Results: A total of 2664 specimens of 9 sand fly species were collected from mountainous (47%) and plain (53%) areas. Species richness, species evenness, and Shannon-Wiener indices were obtained as 9, 0.637, and 1.399, respectively in the mountainous area. *Phlebotomus sergenti* and *Phlebotomus papatasi* were constant species with the synanthropic index of -18.463 and -29.412, respectively. In addition, species richness, species evenness, and Shannon-Wiener indices were 4, 0.690, and 0.956, respectively in the plain area. *Phlebotomus sergenti* and *Phlebotomus papatasi* were dominant species with the synanthropic index of +9.695 and +36.207, respectively. Similarity indices were low among different biotopes of plain and mountainous areas.

Conclusions: A basic knowledge about the diversity of sand flies in various biotopes is essential to design sound control programs. Biodiversity and synanthropic indices of sand flies are different in plain and mountainous areas due to the difference in biotic and abiotic factors between the two areas.

KEYWORDS: Sand flies; Diversity; Richness; Evenness; Synanthropic index; Iran

1. Introduction

Phlebotomine sand flies, with about 900 species, are the most important vectors of pathogens such as bacteria (*Bartonella bacilliformis*), viruses (Phlebovirus and Vesiculovirus), and protozoa (*Leishmania*) all over the world[1,2].

Up till now, 53 species of sand flies have been identified in Iran, of which 34 species belonging to the *Phlebotomus* genus and 19 to the *Sergentomyia* genus[3]. Kerman Province locating in the southeast of Iran is one of the most important foci of anthroponotic cutaneous leishmaniasis (ACL), and Bam is the most infected County in Kerman[4].

Some ecological studies such as species richness, species evenness, degree of presence or occurrence, synanthropic index and Jaccard's and Sorensen's similarity indices are important to study the composition of sand flies species in different biotopes, spatial separation among the populations and the degree of avoidance or attraction of sand flies to human places[2,5].

The diversity of sand flies depends on the topographic conditions, climate, type of soil and vegetation in each area, therefore, in areas

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How to cite this article: Yousefi S, Zahraei-Ramazani AR, Rassi Y, Aflatoonian MR, Yaghoobi-Ershadi MR, Aghaei-Afshar A, et al. Diversity of Phlebotomine sand flies (Diptera: Psychodidae) in mountainous and plain areas of an endemic focus of anthroponotic cutaneous leishmaniasis in Iran. Asian Pac J Trop Biomed 2020; 10(5): 201-207.

Article history: Received 16 August 2019; Revision 30 September 2019; Accepted 18 February 2020; Available online 6 April 2020

with similar ecological conditions, despite geographical distance, similar species of sand flies are found and vice versa[6,7].

This study is considered as the first research on diversity of sand fly species in different biotopes of mountainous and plain areas of the Bam County and aims to compare the composition of sand flies species between these two areas, identify the main high-risk biotopes due to presence of vector species and suggest appropriate control strategies according to the diversity of sand flies and vectors behavior in these environments[5,8].

2. Materials and methods

2.1. Study area

The study was conducted from March 2017 to December 2018 in mountainous (Dehbakri) and plain (Khajeh-Asgar) areas in Bam County, Kerman Province, in the southeast of Iran (Figure 1).

Dehbakri (29° 3'13.89" N 57°54'37.75" E) is situated in the southwest of Bam at an altitude of 2052 m above sea level with 325 mm of mean annual rain fall, 14.1 °C of mean temperature, and a relative humidity of 45.5%. The main agricultural products are walnuts (*Juglans regia*) and apricots (*Prunus armeniaca*) and the most important domestic animals in this area include goat (*Capra aegagrus*), sheep (*Ovis aries*), cattle (*Bos taurus*), and chicken (*Gallus gallus*).

Khajeh-Asgar (29° 7'18.42" N 58°15'43.37" E) is situated in the west of Bam at an altitude of 1163 m above sea level with 61.3 mm of average annual rainfall, 24.2 °C of mean temperature, and a relative humidity of 31.6%. Date (*Phoenix dactylifera*) is the most

important agricultural product of this area and the major domestic animals include goats, cows and chickens[9].

2.2. Collection and identification of sand flies

Sand flies were collected once a month in four biotopes (domestic, peri-domestic, agricultural, and sylvatic) in plain and mountainous areas using 20 sticky traps in each biotope.

Captured sand flies were preserved in ethanol (96%), mounted in Puri's medium and identified using morphological identification key of Rassi *et al.*[10].

2.3. Data analysis

To assess the diversity of sand fly populations, alpha and beta diversity indices were used.

2.3.1. Alpha diversity indices

Species richness (S) referred to the number of various species represented in an ecological community or region[11].

Shannon-Wiener index indicates species biodiversity, $H = -\sum_{i=1}^s p_i \ln p_i$ where p_i is the number of specific species divided by the total number of collected sand flies in each biotope[12].

Evenness: $E = H/\ln S$ where H is the Shannon–Wiener index and S is species richness[12].

Degree of presence: $C = n/N \times 100$, referred to the number of places where a specific species is captured divided by the total number of study sites. According to this index, sand fly species were classified as constant species that were found in 50% or more of the study

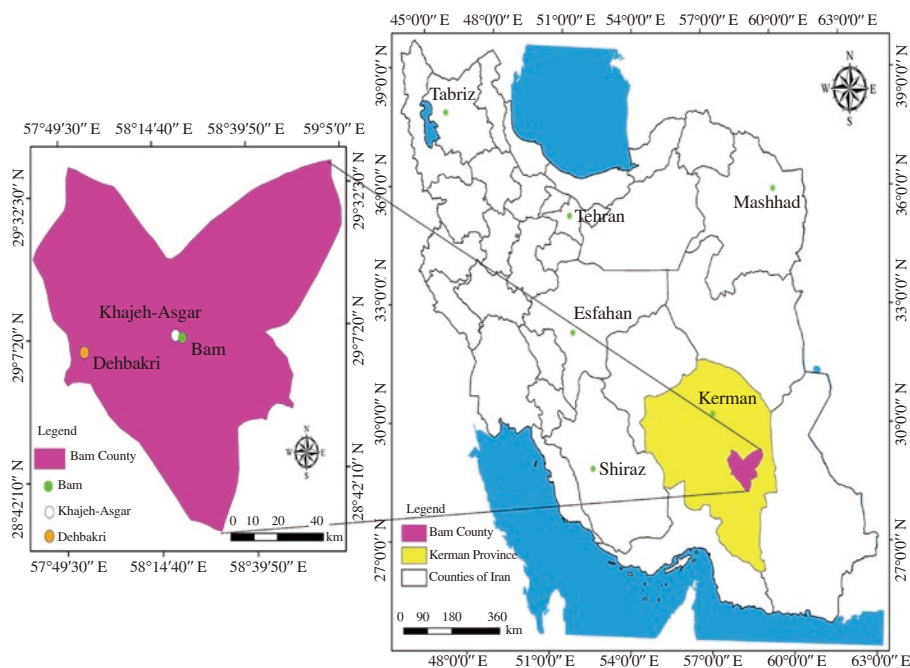


Figure 1. The study area in Bam County, Kerman Province, Iran.

places (biotopes); common species that were captured in 25%–49% of the study places; accidental species that were found in 12.5%–24% of the study places; very accidental species that were captured in less than 12% of the study places[13].

2.3.2. Beta diversity indices

Jaccard's similarity index: $S_j = a/(a+b+c)$

Sorensen's similarity index: $S_s = 2a/(2a+b+c)$

Where a represents the number of species (richness) found in both biotopes, b is the number of species found in the first biotope and c is the number of species in the second biotope[14].

2.3.3. Synanthropic index (SI)

SI measures the relation of sand fly species with human settlements. The equation is $SI = (2a+b-2c)/2$ where a is the percentage of vector species [*Phlebotomus sergenti* (*P. sergenti*) & *Phlebotomus papatasi* (*P. papatasi*)] in domestic biotope, b is the percentage of vector species in peri-domestic biotope and c is the percentage of vector species in agricultural and sylvatic biotopes. This index was calculated according to Nuorteva[15] with modifications.

3. Results

A total of 2664 specimens of 9 sand fly species were collected in different biotopes of mountainous (47%) and plain (53%) areas. *P. sergenti* was the dominant species in the region. The activity of sand flies in the mountainous area was found to start in May and end in November, but in the plain area, it started in April and continued until December (Table 1).

3.1. Biodiversity of sand flies in the mountainous area

A total of 1259 sand flies belonging to 9 species were collected in mountainous area including *P. sergenti* (44.956%), *P. papatasi* (2.701%), *Phlebotomus longiductus* (0.079%), *Sergentomyia baghdadis* (*S. baghdadis*) (30.421%), *Sergentomyia dentata* (*S. dentata*) (11.597%), *Sergentomyia sintoni* (6.672%), *Sergentomyia theodori* (2.462%), *Sergentomyia antennata* (1.033%), and *Sergentomyia paulowski* (0.079%).

Species richness, species evenness, and Shannon-Wiener indices were 9, 0.637, and 1.399, respectively (Table 2). The Shannon-Wiener index was 1.536, 0.964, 0.927, and 0.594 in sylvatic, peri-domestic, domestic, and agricultural biotopes, respectively with difference. Calculation of the degree of presence or occurrence showed that *P. sergenti*, *P. papatasi*, and *S. baghdadis* were constant species, whereas *S. dentata*, *Sergentomyia antennata*, *Sergentomyia sintoni*, and *Sergentomyia theodori* were common species and *Phlebotomus longiductus* and *Sergentomyia paulowski* were very accidental species. Synanthropic index of the two main vectors of cutaneous leishmaniasis namely *P. sergenti* and *P. papatasi* was -18.463 and -29.412, respectively (Table 3).

Table 1. Monthly activity of *Phlebotomus sergenti* in mountainous and plain areas of Bam County in Kerman Province between March 2017 and December 2018.

Months	Mountainous area		Plain area	
	frequency	Sand fly/sticky trap	frequency	Sand fly/Sticky trap
April	0	0.0	40	0.50
May	15	0.2	163	2.00
June	44	0.6	100	1.00
July	69	0.9	351	4.00
August	114	1.0	36	0.50
September	159	2.0	73	0.90
October	92	1.0	20	0.25
November	73	1.0	28	0.35
December	0	0.0	9	0.10
January	0	0.0	0	0.00
Total	566	-	820	-

3.2. Biodiversity of sand flies in the plain area

A total of 1405 sand flies belonging to 4 species were captured. The relative abundances of these species were as follows: *P. sergenti* (58.363%), *P. papatasi* (10.320%), *S. baghdadis* (30.320%), and *S. dentata* (0.996%). Species richness, evenness, and Shannon-Wiener indices were 4, 0.690 and 0.956, respectively (Table 2). Shannon-Wiener index was different in sylvatic (0.97), peri-domestic (0.63), domestic (1.026), and agricultural (0.806) biotopes. According to the degree of presence or occurrence, *P. sergenti*, *P. papatasi*, and *S. baghdadis* were constant species and *S. dentata* was a common species. The synanthropic index of *P. sergenti* and *P. papatasi* was +9.695 and +36.207, respectively (Table 3).

3.3. Similarity between the mountainous and plain areas

To determine the similarity index between various biotopes of the mountainous and plain areas, Jaccard's and Sorensen's coefficients were calculated. In the mountainous area, the lowest rate of similarity ($S_j=0.154$, $S_s=0.267$) was observed between agricultural and sylvatic biotopes and the highest rate of similarity ($S_j=0.286$, $S_s=0.444$) was found between peri-domestic and domestic biotopes. In contrast, in the plain area, the lowest rate of similarity ($S_j=0.300$, $S_s=0.462$) was observed between domestic and peri-domestic, sylvatic and agricultural environments while the highest rate of similarity ($S_j=0.333$, $S_s=0.500$) between sylvatic and peri-domestic, agricultural and peri-domestic, agricultural and sylvatic biotopes. Calculation of similarity index among the different biotopes of mountainous and plain areas showed that the lowest similarity rate ($S_j=0.200$, $S_s=0.333$) was between the sylvatic biotope of the mountainous area and the domestic biotope of plain area. The highest level of similarity ($S_j=0.308$, $S_s=0.471$) was found between peri-domestic and domestic biotopes of mountainous with peri-domestic, sylvatic, and agricultural biotopes of plain area (Tables 4 and 5).

Table 2. Alpha diversity indices of sand flies in mountainous and plain areas of Bam County in Kerman Province between March 2017 and December 2018.

Sand fly species	Mountainous area				Plain area			
	Frequency	Proportion (Pi)	Log _e pi	Pi log _e pi	Frequency	Proportion (Pi)	Log _e pi	Pi log _e pi
<i>Phlebotomus sergenti</i>	566	0.450	-0.799	-0.359	820	0.584	-0.538	-0.314
<i>Phlebotomus papatasi</i>	34	0.027	-3.612	-0.098	145	0.103	-2.271	-0.234
<i>Phlebotomus longiductus</i>	1	0.001	-7.138	-0.006	-	-	-	-
<i>Sergentomyia baghdadis</i>	383	0.304	-1.190	-0.362	426	0.303	-1.193	-0.362
<i>Sergentomyia dentata</i>	146	0.116	-2.154	-0.250	14	0.010	-4.609	-0.046
<i>Sergentomyia antennata</i>	13	0.010	-4.573	-0.047	-	-	-	-
<i>Sergentomyia sintoni</i>	84	0.067	-2.707	-0.181	-	-	-	-
<i>Sergentomyia pawlowski</i>	1	0.001	-7.138	-0.006	-	-	-	-
<i>Sergentomyia theodori</i>	31	0.025	-3.704	-0.091	-	-	-	-
Total	1 259	-	-	-	1 405	-	-	-

Table 3. Synanthropic index of *Phlebotomus sergenti* and *Phlebotomus papatasi* in mountainous and plain areas of Bam County in Kerman Province between March 2017 and December 2018.

Biotope/Sand fly species	Domestic		Peri-domestic		Natural		Total	Synanthropic index
	Frequency	Percentage	Frequency	Percentage	Frequency	Percentage		
Mountainous area <i>Phlebotomus sergenti</i>	80	14.134	201	35.512	285	50.353	566	-18.463
<i>Phlebotomus papatasi</i>	6	17.647	8	23.529	20	58.824	34	-29.412
Plain area <i>Phlebotomus sergenti</i>	47	5.732	537	65.488	236	28.780	820	+9.695
<i>Phlebotomus papatasi</i>	23	15.862	101	69.655	21	14.483	145	+36.207

*The percentage of *Phlebotomus sergenti* in natural biotope (50.353) = Sylvatic (47.173) +Agricultural (3.180) biotopes of the mountainous area. *The percentage of *Phlebotomus papatasi* in natural biotope (58.824) = Sylvatic (58.824) +Agricultural (0) biotopes of the mountainous area. *The percentage of *Phlebotomus sergenti* in natural biotope (28.780) = Sylvatic (6.829) +Agricultural (21.951) biotopes of the plain area. *The percentage of *Phlebotomus papatasi* in natural biotope (14.483) = Sylvatic (11.035) +Agricultural (3.448) biotopes of the plain area.

Table 4. Jaccard's similarity index between different biotopes of mountainous and plain areas of Bam County in Kerman Province between March 2017 and December 2018.

Biotope	Domestic ₁	Peri-domestic ₁	Sylvatic ₁	Agriculture ₁	Domestic ₂	Peri-domestic ₂	Sylvatic ₂	Agriculture ₂
Domestic ₁	-	4	5	2	3	4	4	4
Peri-domestic ₁	0.286	-	5	2	3	4	4	4
Sylvatic ₁	0.263	0.263	-	2	3	4	4	4
Agriculture ₁	0.222	0.222	0.154	-	2	2	2	2
Domestic ₂	0.273	0.273	0.200	0.286	-	3	3	3
Peri-domestic ₂	0.308	0.308	0.235	0.250	0.300	-	4	4
Sylvatic ₂	0.308	0.308	0.235	0.250	0.300	0.333	-	4
Agriculture ₂	0.308	0.308	0.235	0.250	0.300	0.333	0.333	-

Mountainous area biotopes: Domestic₁, Peri-domestic₁, Sylvatic₁, Agriculture₁; Plain area biotopes: Domestic₂, Peri-domestic₂, Sylvatic₂, Agriculture₂. Species richness in domestic, peri-domestic, sylvatic and agricultural biotopes of mountainous area is 5, 5, 9 and 2, respectively, and of plain area is 3, 4, 4 and 4, respectively.

Table 5. Sorensen's similarity index between different biotopes of mountainous and plain areas of Bam County in Kerman Province between March 2017 and December 2018.

Biotope	Domestic ₁	Peri-domestic ₁	Sylvatic ₁	Agriculture ₁	Domestic ₂	Peri-domestic ₂	Sylvatic ₂	Agriculture ₂
Domestic ₁	-	4	5	2	3	4	4	4
Peri-domestic ₁	0.444	-	5	2	3	4	4	4
Sylvatic ₁	0.417	0.416	-	2	3	4	4	4
Agriculture ₁	0.364	0.364	0.267	-	2	2	2	2
Domestic ₂	0.429	0.429	0.333	0.444	-	3	3	3
Peri-domestic ₂	0.471	0.471	0.381	0.400	0.462	-	4	4
Sylvatic ₂	0.471	0.471	0.381	0.400	0.462	0.500	-	4
Agriculture ₂	0.471	0.471	0.381	0.400	0.462	0.500	0.500	-

Mountainous area biotopes: Domestic₁, Peri-domestic₁, Sylvatic₁, Agriculture₁; Plain area biotopes: Domestic₂, Peri-domestic₂, Sylvatic₂, Agriculture₂. Species richness in domestic, peri-domestic, sylvatic and agricultural biotopes of mountainous area is 5, 5, 9 and 2, respectively, and of plain area is 3, 4, 4 and 4, respectively.

4. Discussion

This study is considered as the first research on biodiversity of sand flies in different biotopes of plain and mountainous areas of Bam County as the main focus of ACL in the southeast of Iran.

Based on our results, the activity period of the sand flies was longer in plain area than in mountainous areas due to the differences in the topographical condition between the two areas. The highest frequency of *P. sergenti*, as the main vector of ACL, in mountainous and plain areas was observed in September and July 2018,

respectively. A previous study[16] indicated that the highest activity of sand flies species in the plain area was during the 7th month of the year.

Abedi–Astaneh *et al.*[17] observed an increase in *P. sergenti* abundance in May and August in the plain area. A high abundance of this species was observed in June and September in the mountainous area of Qom Province in the center of Iran, but it was active from May to October in the west of Iran[18]. Orshan *et al.*[19] indicated that the seasonal activity of *P. sergenti* started in April and ended in December in Israel. The results of our study are similar to the above-mentioned studies[16,17,19] conducted in Bam, some parts of Qom Province, as well as Israel. There are some differences between our results with the study[18] conducted in the west of Iran, perhaps due to the differences in geographical conditions between the two areas. The richness of the sand fly species was reported as 11, 9, 14, 14, 10, and 19 respectively, in Khuzestan, Qom, Azarbaijan-e-shari, Ardabil, Azarbaijan-e-qarbi and north Khorasan Provinces[17,20,21]. The Shannon–Wiener index was higher in highland than lowland areas in north Khorasan, Khuzestan and Qom provinces[5,17,21]. The biodiversity index was higher at altitudes between 800–1500 m above sea level in Turkey, Saudi Arabia and Morocco[22–24].

Previous studies have shown that differences in biodiversity, richness, and evenness are attributed to the difference in altitude that affects abiotic (temperature, relative humidity, annual precipitation, breeding places, and resting places) factors and biotic (plant and animal resources) factors[25–27]. Our results showed that the biodiversity index and richness were higher in mountainous areas (2052 m above sea level) than in the plain area (1163 m above sea level). Due to climatic and geographical conditions, the number of plant and animal species, as well as proper breeding and resting places is higher in Dehbakri than in Khajeh-Asgar. Such results are in agreement with those obtained by Jahanifard *et al.*[5], Abedi–Astaneh *et al.*[17], and Arzamani *et al.*[21] in highland and lowland areas in Khuzestan, Qom, and North Khorasan Provinces, while Guernaoui *et al.*[28] revealed the biodiversity index was higher in plain area than in mountainous area.

Our results also indicated that, among the different biotopes in mountainous area, sylvatic environment showed the highest rate of Shannon–Wiener index or biodiversity (1.536), and species richness (9) while agricultural biotope had the lowest biodiversity (0.594) and richness (2), but with the highest evenness (0.857), indicating that in the agricultural environment, the low number of species was distributed more uniformly than in the other places. In the plain area, domestic biotope showed the highest rate of Shannon–Wiener index (1.026) and evenness (0.934), with the lowest richness (3).

The biodiversity index is influenced by two factors including species richness (number of species in a specific area) and species evenness (distribution of species in a population). The low or high rates of these factors can influence the biodiversity index[29]. As it was previously mentioned, biotic and abiotic factors determine the diversity of sand flies in each biotope[27,30–32]. Therefore, in places with similar ecological conditions, species composition is almost the same and vice versa.

Calculating Jaccard's, and Sorensen's similarity indices between the different biotopes of the mountainous area showed that, the lowest rate of similarity was between agricultural and sylvatic biotopes, because in this area, the highest and lowest species richness were in sylvatic and agricultural environments, respectively. In sylvatic

environments, conditions are more favorable for the life of different species of sand flies compared to the other biotopes, perhaps because of less human intervention and the presence of different plants and animal species as a suitable food source for these insects. Calculation of Jaccard's and Sorensen's coefficients in the plain area showed that the highest similarity was observed between every two biotopes except the domestic place that showed the lowest similarity with the other biotopes. Apparently, *P. sergenti*, *P. papatasi*, and *S. baghdadis* have been adapted with human dwellings and in other biotopes. *P. sergenti*, *P. papatasi*, *S. baghdadis*, and *S. dentata* are adapted to live in and can tolerate the diverse environmental conditions. Calculating similarity indices between different biotopes of mountainous and plain areas demonstrated that the lowest similarity was between domestic biotopes of the plain area and sylvatic biotope of the mountainous area. Generally, the similarity was low among different biotopes, but the highest similarity index was observed between domestic and peri-domestic biotopes of mountainous areas with peri-domestic, sylvatic, and agricultural biotopes of plain area. As it was mentioned above, biotic and abiotic factors determine the existence and survival of different sand fly species in these various environments[27,30–32].

Akhoundi *et al.*[20] stated that there was the highest rate of similarity ($S_j=1$) between Meshkin Shahr and Sarab locating in the northwest of Iran while the lowest rate of similarity was between Bileh Savar and Germi ($S_j=0.4$). Jahanifard *et al.*[5] reported that the highest rate of similarity was between agricultural and natural areas whereas the lowest rate of similarity between urban and natural biotopes of Shush County. Comparing similarity between Shush (71 m above sea level) and Khorramshahr (3 m above sea level) Counties (the southwest of Iran) indicated that the highest rate of similarity was between urban area of Shush and semi-urban area of Khorramshahr while the lowest rate of similarity was between urban biotope of Khorramshahr and natural area of Shush. Like Khuzestan Province, the similarity between sylvatic and agricultural biotopes was high in the plain area of Bam County, but it was low between agricultural and sylvatic biotopes of mountainous area, perhaps due to the high use of pesticide in agricultural biotope of the mountainous area, which can affect the biodiversity of sand flies[5].

Moreover, results showed that *P. sergenti* and *P. papatasi*, the main vectors of ACL and Zoonotic Cutaneous Leishmaniasis, are constant species in both mountainous and plain areas of Bam County. The synanthropic index of these species was found to be higher in plain area than in the mountainous areas. The synanthropic index of the sand flies was between -91.18 and -69.84 in Khuzestan Province[5]. Barata *et al.*[33] demonstrated that the synanthropic index of sand flies was between 0.4 and 100 in Brazil. The synanthropic index of *P. papatasi* was higher (-29.412 and $+36.207$) in mountainous and plain areas of Bam County, respectively, compared to Khuzestan Province (-83.34 and -69.84)[5]. Generally, synanthropic index is between -100 and $+100$ [34,35]. In mountainous area, this index was negative for both species of sand flies, but in plain area, synanthropic index was positive for *P. sergenti* and *P. papatasi*; nevertheless, it was low for *P. sergenti*, so that in Bam County, *P. sergenti* did not show a high degree of domiciliation in human environments which is extremely important in control programs. According to our results, in this region, *P. sergenti* is not an endophilic species and a large population of this vector rest and feed outdoors. Since indoor residual spraying, as an effective method, was used to control

endophilic vectors[36], it seems that this method is not an effective method to reduce the vector population in this area. However, this method has been used to control sand fly populations in Bam County[37].

Vector control is one of the most important methods for controlling metaxenic diseases. Vector control programs must be administered with regard to the ecology and biology of the vector in each place[38]. *P. sergenti* represented 47%, 36%, 14%, and 3%, of collected species respectively, in sylvatic, peri-domestic, domestic, and agricultural biotopes of the mountainous area. Therefore, the sylvatic biotope is a potential environment for the transmission of the disease due to the high frequency of *P. sergenti* as the main vector and stray dogs as a secondary reservoir host of ACL[39,40]. Peri-domestic biotope also had a high frequency of *P. sergenti*, this environment may play an important role in the maintenance and transmission of the disease because infected (as the main reservoir) and uninfected people (as sensitive hosts) have to go to this place to take care of their animals. Health education about sand fly behavior and use of repellents are considered as suitable approaches to prevent transmission of the disease for shepherds and climbers who have to go to sylvatic biotope for job reasons. Moreover, during summer in the mountainous area of Bam County, health education for the patients about covering their lesions[4], and using repellents are considered to be good preventive methods for disease transmission in peri-domestic biotope. The frequency of *P. sergenti* is low in the domestic environment and people tend to rest in yards in summer, so indoor residual spraying is not reasonable. Other methods like the use of insecticide-treated nets, early diagnosis, and treatment of patients can be effective to control the disease in this region.

P. sergenti represented 65%, 22%, 7%, and 6% of collected species, respectively in peri-domestic, agricultural, sylvatic, and domestic biotopes of the plain area. Therefore, the peri-domestic biotope is one of the potential environments for infection and transmission of ACL, due to the high frequency of *P. sergenti* and the presence of both infected and uninfected peoples as the owner of the livestock. Like the mountainous areas, indoor residual spraying is not cost-effective under normal conditions of the plain area. Accordingly, using repellents and insecticide-treated nets will be effective in controlling the diseases in the agricultural biotope, especially at harvest time. In the domestic environment, although the frequency of *P. sergenti* was low, most of the people spend the night in yards. Unfortunately, in plain area, people do not tend to use bed nets and insecticide-treated nets, because the interior space of the bed net is warm in those with small mesh (mesh size of 156 holes/inch², 25 holes/cm², 75 deniers) although these are suitable for protection against sand flies[41,42]. Therefore, health education about the necessity of using insecticide-treated nets or long-lasting insecticide-treated nets, early diagnosis, and treatment of patients and covering the lesion during the night can be effective to control the disease.

In conclusion, in the present study, diversity indices of sand flies and the synanthropic index of *P. sergenti* were found to be different in mountainous and plain areas of Bam County. *P. sergenti* as the main vector of ACL is constant species, meaning that it exists in all biotopes of mountainous and plain areas with relatively low synanthropic index. Therefore, humans are likely to be bitten by this vector in all biotopes especially in peri-domestic and natural environments (agricultural and sylvatic). Indoor residual spraying is expensive and ineffective for controlling the disease in this region

with side effects on the environment and non-target organisms. Therefore, some methods such as health education about ecology and biology of the sand fly, covering the lesion by sterile cloths, using repellents, and long-lasting insecticide-treated nets with regard to nocturnal activity of *P. sergenti* and early diagnosis and treatment of patients will be effective for controlling ACL in the Bam County (the southeast of Iran).

Conflict of interest statement

We declare that there is no conflict of interest.

Funding

This research has been funded by Tehran University of Medical Sciences (Project No. 36142).

Authors' contributions

ARZR, YR, MRA, MRYE, AAA and SY designed the work and edited manuscript. SY collected the sand fly specimens. SY, MA and AP identified the sand flies. SY and AA Afshar performed the data analysis. SY wrote the manuscript. SY and ARZR performed critical revisions of the manuscript.

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