

Geographic and ecological features of phlebotomine sand flies (Diptera: Psychodidae) as leishmaniasis in Central Iran

Hassan Vatandoost¹ · Jalil Nejati² · Abedin Saghaipour³ · Alireza Zahraei-Ramazani¹

Received: 13 May 2017 / Accepted: 6 October 2017 / Published online: 26 October 2017
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Abstract Phlebotomine sand flies occur throughout the tropics and subtropics, as well as in temperate regions of the world. They are vectors of human and canine leishmaniasis and sand fly fevers caused by phleboviruses. This study was aimed to determine the geographic and ecological characteristics of phlebotomine sand flies as vectors of leishmaniasis and to prepare a checklist of phlebotomine sand flies. The study was conducted in Qom province, central Iran, between April and November 2016. Qom is located in latitude 34.6399°N and longitude 50.8759°E with average annual minimum and maximum temperatures of 16.5 and 49 °C, annual rainfall of 150 mm and relative humidity of 84 and 28%, respectively. Sand flies were collected by sticky paper traps from Qom city and its six districts. The sand flies collected were separated from the sticky paper traps using an insulin syringe and kept in 70% ethanol for species identification using taxonomic keys. Also, a literature review was performed using all published reports on phlebotomine sand flies in this province during 1999–2015. A total of 28,410 sand flies from two genera

and 14 species were collected. *Phlebotomus papatasi*, the main vector of zoonotic cutaneous leishmaniasis and arboviruses, and *Phlebotomus sergenti*, the vector of anthroponotic cutaneous leishmaniasis, were the predominant species followed by *Phlebotomus kandelakii*, *P. major* and *P. alexandri*. Fourteen species from two genera mostly from wet and mountainous areas were identified in the study area. Kahak and Markazi districts were identified as high-risk foci with numerous leishmaniasis vectors species; we recommend intensifying and strengthening of vector control programme in the area of study.

Keywords Checklist · Phlebotomine sand flies · Ecology · Leishmaniasis · Iran

Introduction

Phlebotominae sand flies are haematophagous insects that belong to the family psychodidae, order Diptera and sub-order Nematocera (Lewis 1982). The subfamily phlebotominae contains six genera, of which *Phlebotomus* (13 subgenera), *Sergentomyia* (ten subgenera) and *Chinius* (four species) are found in the Old World (Akhoundi et al. 2016). These insects feed on a wide range of hosts, including humans and other vertebrates (Artemiev 1978). Presently, more than 800 species of sand flies have been described and less than 10% transmit *Leishmania* parasite to humans both in the old world and in the new world from more than 80 countries (Kato et al. 2010; Depaquit et al. 2010). Three species of *Leishmania* parasite (*L. infantum*, *L. major*, *L. tropica*) have been incriminated as causative agents of human leishmaniasis infection (Nadim and Seyedi-Rashti 1971; Parvizi et al. 2008). Visceral leishmaniasis (VL) is endemic in Khalazestan district of Qom,

✉ Abedin Saghaipour
abed.saghafi@yahoo.com

Hassan Vatandoost
hvatandoost1@yahoo.com

Alireza Zahraei-Ramazani
azahraei@tums.ac.ir

¹ Department of Medical Entomology and Vector Control, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

² Health Promotion Research Center, Zahedan University of Medical Sciences, Zahedan, Iran

³ Department of Public Health, Faculty of Health, Qom University of Medical Sciences, Qom, Iran

and recently some autochthonous cases were officially reported by CDC (Rakhshanpour et al. 2014; Fakhar et al. 2004). Similarly, zoonotic cutaneous leishmaniasis (ZCL) is hypoendemic in the rural areas of Markazi and Kahak districts of Qom with 20% of all new cases seen among indigenous residents of 15 years and above. (Nateghi Rostami et al. 2013). However, cases of anthroponotic cutaneous leishmaniasis (ACL) have never been reported from the study area according to epidemiological studies (Saghafipour et al. 2017). Sand flies are also vectors to some viruses, and the frequency of human infection with viral *Phlebotomus* fever is high in Iran (Tesh et al. 1976). Antibodies against three *Phlebotomus* virus serotypes (Nepalese, Sicilian and Karimabad) were observed in the serum and blood specimens of Esfahan residents which is an endemic province neighbouring the study areas. Adler, Theodor and Lourie, in 1930, were the first to study the faunistic status of phlebotominae sand flies in Iran. Presently, there are 56 reported phlebotominae species in Iran from the genera *Phlebotomus* (subgenera *Adlerius*, *Euphlebotomus*, *Larrousius*, *Paraphlebotomus*, *Phlebotomus* and *Synphlebotomus*) and *Sergentomyia* (subgenera *Grassomyia*, *Parrotomyia*, *Parvidens*, *Rondanomyia*, *Sergentomyia* and *Sintonius*) (Kassiri et al. 2011; Zahraei-Ramazani et al. 2015). The fauna and distribution of phlebotominae sand flies from different areas of Qom province have been studied and documented between 1999 and 2000 (Akhavan et al. 2003). Many studies on sand flies have been conducted from the year 2000 to date in Qom; therefore, this study describes the preparation of a checklist of phlebotominae sand fly species, their geographic distribution and important ecological characteristics. To accomplish these, all articles published in different areas of the province have been reviewed, in addition to the field works that were conducted.

Materials and methods

Study area

Qom province is bordered by Tehran province to the north, Isfahan province to the south, Semnan province to the east and Markazi province to the west, with an area of approximately 11,240 square kilometres (0.68% of the total area of Iran as shown in Fig. 1). The altitude is 928 m, latitude 34.6399°N and longitude 50.8759°E. This study was conducted from April to November 2016 in Qom city and 15 villages (Khor Abad, Sarm, Ghobadbezan, Mir Abad, Malek Ghaleh, Qomrood, Zavarian, Rahjerd, Khadijeh Khatoon, Tagharood, Pachian, Mahmood Abad, Chahak, Kohandan and Mehrzamin) of all the six districts in the province; of which Kahak, Markazi, Salafchegan, Jafarieh and

Khalajestan districts were with an elevation of almost 1500 m above sea level. The average annual minimum and maximum temperatures are 16.5 and 49 °C in January and July, respectively. The total annual rainfall is 150 mm with relative humidity of 84 and 28% in December and June, respectively (Iran Meteorological Organization 2016). Qom province has two topographic and climatic regions: (A) the arid areas along the edge of the central desert and (B) the semi-arid region with a moderate climate and four distinct seasons (Table 1, Fig. 1).

Collection methods and identification

Sand flies were collected using sticky paper traps (15 × 21 cm² papers coated with castor oil) during their monthly and seasonal activities. The paper traps were placed before sunset and collected the next day before sunrise. Captured sand flies were separated from the sticky paper traps using an insulin syringe and kept in 70% ethanol for morphological identification. The head and genitalia of each sand fly were disconnected within a drop of ethanol on a slide, and afterwards, they were placed on a drop of Puri's media between a slide and cover slip (Smart et al. 1965). Available taxonomic keys were used for the identification of specimens (Seyedi-Rashti and Nadim 1992; Artemiev 1978; Theodor and Mesghali 1964; Lewis 1982). Simultaneously, literature review was done on existing researches, including published articles, and detailed information on phlebotomine sand flies in Qom province from 1999 through 2015 was sought.

Results

The checklist result

A checklist, taxonomy and notes on the geographic and some ecological characteristics of each species of the phlebotomine sand flies in Qom province are described in the present study. Combining the results of field study and reviewed articles, 28,410 adult sand flies were identified from a total of 14 species representing six subgenera (four subgenera of the *Phlebotomus* genus and two subgenera of the *Sergentomyia* genus) (Table 1). In the present field research, a total of 10,461 adult sand flies were collected, where 79.14% of the specimens belonged to the genus *Phlebotomus* and 20.86% belonged to the *Sergentomyia* genus. The result of our literature review on the phlebotomine sand flies as disease vectors in Qom province showed that Akhavan et al. (2003) were the first researchers who studied the fauna of sand flies in the Ghanavat area of the province from 1999 to 2001 (Akhavan et al. 2003). Other researchers studied the fauna of

Fig. 1 Map of Iran in which the position of Qom Province and its five districts; 1. Jafarabad, 2. Kahak, 3. Khalajestan, 4. Markazi, 5. Salafchegan (★ Qom City)

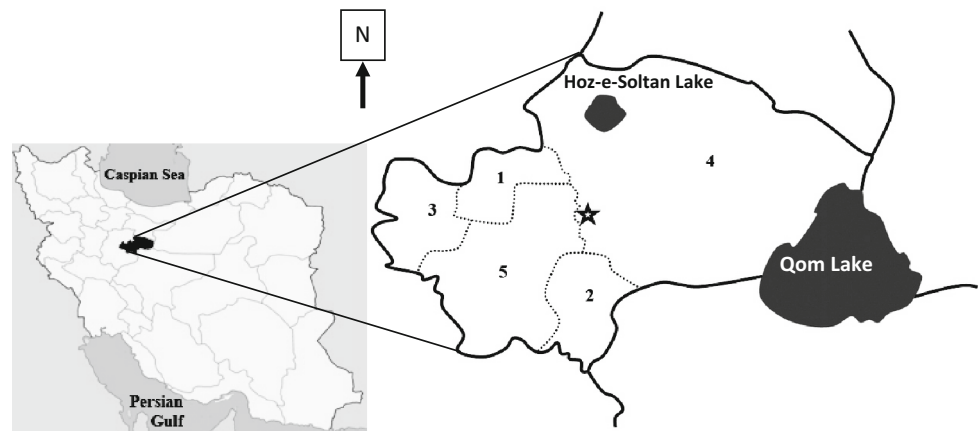


Table 1 Distribution of various species of sand flies in different districts and Qom city of Qom province

Sand fly species	Area					
	Dry zone and lowland plains				Wet zone and mountainous areas	
	Qom city	Markazi	Jafar Abad	Salafchegan	Kahak	Khalajestan
<i>P. papatasi</i>	+	+	+	+	+	–
<i>P. sergenti</i>	+	+	+	+	+	+
<i>P. alexandri</i>	+	+	+	+	+	+
<i>P. caucasicus</i>	+	+	+	+	+	+
<i>P. caucasicus</i> group	+	+	+	+	+	+
<i>P. kandelakii</i>	–	–	–	–	+	+
<i>P. tobbi</i>	–	–	–	–	+	+
<i>P. major</i> (probably <i>P. neglectus</i>)	–	–	–	–	–	+
<i>P. halepensis</i>	–	–	–	–	–	+
<i>P. brevis</i>	–	–	–	–	–	+
<i>P. (adlerius)</i> group	–	–	–	–	–	+
<i>S. sintoni</i>	+	+	+	+	+	+
<i>S. theodori</i>	+	+	+	+	+	+
<i>S. pawlowski</i>	–	+	–	–	–	–

sand flies from rural Ghahan district and Kahak area of Qom province, and published their work in Persian (Farzin-Nia and Hanafi-Bojd 2007; Saghaipour et al. 2014). Also Abedi-Astaneh et al. studied the ecological niche model of *Phlebotomus papatasi* in Qom province (Abedi-Astaneh et al. 2015). These species are listed below in order of genera and species. Data on their geographic distribution, their climatic distribution and their temperature distribution are summarised in Tables 1, 2, 3 and illustrated in Fig. 1.

Checklist of phlebotomine sand flies recorded in Qom province

Genus Phlebotomus (Rondani and Berte 1840)

(A) Subgenus *Phlebotomus* (Rondani and Berte 1840).

1. *P. papatasi* (Scopoli 1786).

Distribution: *P. papatasi*, the main vector of ZCL and arboviruses, was collected from all areas (Qom city, Markazi, Kahak, Jafar Abad and Salafchegan districts of the province), except for Khalazestan district. Note: It is the main vector of *L. major* from animals to humans in central Asia and appears to be a vector of *L. major* in Iran and 21 other countries in Old World (WHO 2010). *Leishmania major* has been isolated from *P. papatasi* in Markazi and Kahak areas of this province (Saghaipour et al. 2017; Kassiri et al. 2011). This species is a troublesome biting pest. In view of the widespread distribution of *P. papatasi* and the high prevalence of leishmaniasis in many areas of Iran, one can conclude that human infections from sand fly-transmitted viruses are common (Tesh et al. 1976).

Table 2 Fauna and the number of collected sand flies from the endemic area leishmaniasis in Qom province, central Iran, 2015

Data	Area					
	Qom city	Markazi	Jafar Abad	Salafchegan	Kahak	Khalajestan
Latitude (N)	34°15'	34°67'	36°61'	34°35'	36°61'	34°67'
Longitude (E)	50°30'	50°28'	50°20'	50°86'	50°86'	50°28'
Elevation	830	850	840	900	930	950
Monthly total of precipitation (mm)	122	119	115	124	212	217
Average of minimum temperature (°C)	19	21	18	16	9	8
Average of maximum temperature (°C)	24	25	24	22	14	14

(B) Subgenus *Paraphlebotomus* (Theodor 1948).

2. *P. sergenti* (Parrot 1917).

Distribution: Qom city and all the five rural districts in this province. Note: It is the vector of *L. tropica* in Iran and 17 other countries in the Old World, and *P. sergenti* is also a vector of *L. aethiopica* in Ethiopia (WHO 2010). In an endemic focus of ACL in Iran, *P. sergenti* was found to be the predominant species throughout the entire period of its activity (Zahraei-Ramazani et al. 2007). Also, *P. sergenti* was studied for *Leishmania* infection using PCR techniques in Markazi and Kahak districts of Qom province, but *Leishmania* species could not be isolated from the sand fly species (Saghafipour et al. 2017; Rassi et al. 2011).

3. *P. alexandri* (Sintoni 1928).

Distribution: Qom city and all the five rural districts in the province. Note: This species readily bites man in Afghanistan (Artemiev 1978). It is the vector of *L. infantum* in China, Oman and Turkey and transmits *L. donovani* in Iraq (WHO 2010). Also, it has been found to be naturally infected with promastigotes and is a suspected vector of VL in Iran (Azizi et al. 2006).

4. *P. caucasicus* (Marzinovski 1917).

5. *P. caucasicus*

They are group of three female sand flies species belonging to the subgenera *Paraphlebotomus* (*P. caucasicus*, *P. mongolensis* and *P. andrejevi*) that are not identified by current identification keys (Rassi and Hanafi-Bojd 2006). Distribution: Sand flies belonging to *P. caucasicus* and *P. caucasicus* group were collected in Qom city and all five rural districts in this province. Note: This group was considered to transmit VL and Kazakhstan in central Asia, and it plays a major role in disseminating *L. major* in the former Soviet Union (Lewis 1982). This species was found to naturally contain *L. major* MON26 in Esfahan province (Yaghoobi-Ershadi et al. 1994). This species was also found naturally to vector promastigotes in a new focus of VL in north-west of Iran (Rassi et al. 2004).

(C) Subgenus *Adlerius* (Nitzulescu 1931).

6. *P. brevis* (Theodor and Mesghali 1964).

Distribution: This sand fly was only collected from Khalazestan district. However, infection of this sand fly with *Leishmania* was not detected. Note: Although this possibility has not yet been investigated, this sand fly has been suspected to be a potential vector of VL in Iran and a suspected vector of *L. infantum* in Kazakhstan (Sadlova et al. 2003).

7. *P. halepensis* (Theodor 1958).

Distribution: It was only collected from Khalazestan district. The *Leishmania* infection of this sand fly was not recorded. Note: *P. halepensis* appears to be the vector of *L. donovani* in Gruziya, and may possibly be a vector in Turkestan, and has been considered to be a main vector in Transcaucasia, Central Asia and Kazakhstan (Lewis 1982). It can potentially be the vector of *L. infantum* in Georgia and Syria (WHO 2010).

8. *P. adlerius*

They are all-female sand flies species belonging to the subgenera *Adlerius* that cannot be distinguished with current identification keys (Rassi and Hanafi-Bojd 2006). Distribution: These sand flies were captured only in the Khalazestan district.

(D) Subgenus *Larroussius* (Nitzulescu 1931).

9. *P. kandelakii* (Shurenkova 1929).

Distribution: *P. kandelakii* was collected from the villages of Kahak and Khalajestan districts. Note: In Afghanistan, this sand fly species bites man and large number of animals. It appears to be the vector of *L. infantum* in Iran, Armenia, Azerbaijan and Georgia (WHO 2010). It is naturally infected with *Leishmania* spp. promastigotes in north-western Iran and suspected to be a probable vector of VL in the region (Rassi et al. 2005).

10. *P. major* (Annandale 1910).

Distribution: In the present study, this species was captured and identified only in the village of Khalajestan

Table 3 Geographic, climatic and temperature variation in different areas of phlebotominae sand flies in Qom province

Site	Outdoor			Indoor			Total		
	Male No. (%)	Female No. (%)	Total No. (%)	Male No. (%)	Female No. (%)	Total No. (%)	Male No. (%)	Female No. (%)	Total No. (%)
<i>P. papatasi</i>	1675 (45.79)	1983 (54.21)	3658 (100)	816 (44.81)	1005 (55.19)	1821 (100)	2491 (23.82)	2988 (28.56)	5479 (52.38)
<i>P. sergenti</i>	121 (39.16)	188 (60.84)	309 (100)	382 (78.76)	103 (21.24)	485 (100)	503 (4.81)	291 (2.78)	794 (7.59)
<i>P. alexandri</i>	22 (62.86)	13 (37.14)	35 (100)	27 (28.13)	69 (71.87)	96 (100)	49 (0.47)	82 (0.78)	131 (1.25)
<i>P. caucasicus</i>	45 (100)	–	45 (100)	61 (100)	–	61 (100)	106 (1.01)	–	106 (1.01)
<i>P. caucasicus</i> group	–	629 (100)	629 (100)	–	156 (100)	156 (100)	–	785 (7.50)	785 (7.50)
<i>P. kandelakii</i>	173 (80.46)	42 (19.54)	215 (100)	235 (78.86)	63 (21.14)	298 (100)	408 (3.90)	105 (1)	513 (4.90)
<i>P. tobbi</i>	3 (100)	0 (0)	3 (100)	0 (0)	9 (100)	9 (100)	3 (0.03)	9 (0.09)	12 (0.12)
<i>P. major</i>	15 (28.30)	38 (71.70)	53 (100)	105 (29.66)	249 (70.34)	354 (100)	120 (1.15)	287 (2.74)	407 (3.89)
<i>P. halepensis</i>	0 (0)	–	0 (0)	34 (100)	–	34 (100)	34 (0.33)	–	34 (0.33)
<i>P. brevis</i>	1 (100)	–	1 (100)	3 (100)	–	3 (100)	4 (0.04)	–	4 (0.04)
<i>P. (adlerius) group</i>	–	5 (100)	5 (100)	–	9 (100)	9 (100)	–	14 (0.13)	14 (0.13)
<i>S. sintoni</i>	1007 (51.12)	963 (48.88)	1970 (100)	50 (39.06)	78 (60.94)	128 (100)	1057 (10.10)	1041 (9.96)	2098 (20.06)
<i>S. theodori</i>	30 (56.60)	23 (43.40)	53 (100)	19 (67.86)	9 (32.14)	28 (100)	49 (0.46)	32 (0.31)	81 (0.77)
<i>S. pавilowski</i>	0 (0)	3 (100)	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	3 (0.03)	3 (0.03)
Total	3092 (44.29)	3887 (55.71)	6979 (100)	1732 (49.74)	1750 (50.26)	3482 (100)	4824 (46.11)	5637 (53.89)	10,461 (100)

district. Note: This species is one of the principal vectors of VL in the Eastern Mediterranean (WHO 2010). In Iran, it has been found in all areas where human cases of VL have been reported, and natural promastigote infection of this species has been reported in endemic focus of VL in Ghir, Fars province, south of Iran (Sahabi et al. 1992).

11. *P. tobbi* (Addler, Theodor and Lourie 1930).

Distribution: *P. tobbi* was collected from the villages of Kahak and Khalajestan districts. Note: This species is the vector of VL (Lewis 1982) and is distributed in the Eastern Mediterranean area (Theodor and Mesghali 1964), Transcaucasia and is the vector of leishmaniasis in Albania, Croatia, Cyprus, Greece, Israel, Palestine, Syria and Turkey (WHO 2010).

Genus Sergentomyia (Franca and Parrot 1920)

(A) Subgenus *Rondanomyia* (Theodor 1958.)

12. *S. povlovskyi* (Perfiliev 1933).

Distribution: This sand fly species was collected from villages within the Markazi district. Note: The biting habit of this species is similar to *S. hodgsoni*, which prefers to take blood meal from birds and reptiles, and their possibility to suck human blood has not been investigated (Artemiev 1978; Theodor and Mesghali 1964).

(B) Subgenus *Sergentomyia* s. str.

13. *S. theodori* (Parrot 1942).

Distribution: *S. theodori* was found in Qom city and all the five rural districts in this province. Note: The biting habit of this species is very similar to other species of the genus *Sergentomyia* (Artemiev 1978).

14. *S. sintoni* (Pringle 1953).

Distribution: this sand fly species was captured from Qom city and all of its five rural districts. Note: Infection from *S. sintoni* has been observed in Mashhad, Khuzistan, Bakran, Shahrood, Varamin and Turkeman-Sahara (Seyedi-Rashti et al. 1994).

Ecological study results

In the dry zones and lowland plains (Markazi, Jafar Abad, Salafchegan districts and Qom city), some species of sand flies belonging to *Phlebotomus* and *Paraphlebotomus* such as *P. papatasi*, *P. sergenti*, *P. alexandri*, *P. caucasicus* and *P. caucasicus* spp. were collected. Within the *Sergentomyia* genus, *S. sintoni* and *S. theodori* were observed in dry zones and lowland plains (Table 1). In the wet zone and mountainous areas (Kahak and Khalajestan districts), most of the sand flies collected were found these areas. For

example, sand flies belonging to the subgenera *Larroussius* as probable vectors of VL in Iran, such as *P. kandelakii*, *P. tobbi* and *P. major*, were collected and identified, but the results showed that *P. papatasi* was not collected from Khalajestan district as a mountainous area (Table 1). In this study, some species of sand flies such as *P. sergenti* and *P. kandelakii* were collected more in indoors than in outdoors (Table 2). In the wet and mountainous district of Khalajestan, with a high monthly precipitation (217 mm), an average maximum temperature of 14 °C plays an important role in the diversity and distribution of the sand flies in this area (Tables 2, 3).

Discussion

In this study, the results show high species diversity of sand flies in Qom province, central Iran. The sand flies belonged to two genera, *Phlebotomus* and *Sergentomyia*, six subgenera and fourteen species. Faunistic studies of insects as vectors of arthropod-borne diseases are necessary prior to carrying out an ecological, biological or epidemiological studies (Saghafipour et al. 2017). Generally, sand fly species' diversity can be related to ecological and climatic factors like humidity, wind, temperature and precipitation (Jahanifard et al. 2014). Some areas of Qom province, such as Kahak and Khalajestan, have wet zones and mountainous cold climates, while other regions like Markazi and Salafchegan districts, as well as the city of Qom, have hot, dry weather and lowland plains. In the dry zones and lowland plains of Markazi, Jafar Abad and Salafchegan districts, and of Qom city, some species of sand flies belonging to *Phlebotomus* and *Paraphlebotomus*, like *P. papatasi*, *P. sergenti*, *P. alexandri*, *P. caucasicus* and *P. caucasicus* group, were collected. Species of *Sergentomyia* genus, namely *S. sintoni* and *S. theodori*, were observed in the dry zones and lowland plains. In the wet zones and mountainous areas of Kahak and Khalajestan districts, most of the samples were collected from these areas and identified. Although sand flies belonging to the subgenera *Larroussius* as probable vectors of VL in Iran, including *P. kandelakii*, *P. tobbi* and *P. major* (probably *P. neglectus*), were trapped and identified, the results showed that *P. papatasi* was not collected from Khalajestan district, as a mountainous area.

The probable and logical idea of having a greater diversity of sand flies in the wet and mountainous areas includes the presence of various species of plants, the availability of abundant rivers and the existence of rodents' active burrows near human dwellings. These factors cause the formation of various and diverse ecosystem that provide suitable breeding places for the growth and reproduction of various species. This study revealed that in this area, enough precipitation and an appropriate temperature in the Khalajestan district had an important impact on the sand flies' diversity and distribution.

Previous studies have demonstrated that the distribution of sand flies is dependent on local environmental factors such as precipitation and temperature (Ozbel et al. 2011). In Iran, previous studies on the ecology of sand flies showed that the most effective factors for predicting *P. papatasi* distribution are moderate temperatures in the wettest areas and seasonal rainfall (Hanafi-Bojd et al. 2015). In the current study, *P. papatasi* was not collected from Khalajestan district due to its mountainous nature. Usually, there are no rodents' burrows in the mountainous areas of Iran. For sand flies that were caught by other researchers in the mountainous areas of Iran (Rassi et al., 2004), it could be due to changes in host preference and a shift in breeding places by sand flies. It is more likely that the breeding places of sand flies have changed from rodent burrows to livestock ranches and barns, like those containing cattle, while barn expansion has changed the nutritional preference of sand flies from rodents to other animals. Yaghoobi-Ershadi et al. (2005) showed that sheep and cows were preferred hosts for *P. papatasi*. As it was observed in their study, the host preference pattern of *P. papatasi* in humans, sheep, cows, goats, rodents and birds was 31.2, 69.6, 63, 38.8 and 24.7%, respectively (Yaghoobi-Ershadi et al. 2005). In the present study, sex ratios show more males than females of *P. sergenti* and *P. kandelakii* were collected from indoor areas compared to outdoors. Perhaps the high frequency of males in these locations may be due to the formation of larval habitats indoors.

Acknowledgements The authors appreciate the efforts of the director and staff of the Qom Health Centre. Our appreciation also goes to Fatemeh Abedi-Astaneh, MSc student in Medical Science at Tehran University for completing the sand fly collection. This work was supported by Qom University of Medical Sciences.

Authors' contributions This study was planned by the corresponding author, who also participated in the collection of sand flies from the study area. Other authors helped in collecting the samples, identification and confirmation of the sand fly species, and the article was written under their scientific guidance.

Compliance with ethical standards

Conflict of interest The authors declare there is no conflict of interest.

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