

Bioecology of Sandflies (Diptera: Psychodidae: Phlebotominae) in Khorramshahr County, the Endemic Focus of Zoonotic Cutaneous Leishmaniasis in Khuzestan Province, Iran (2017–2018)

Hamid Kassiri^{1,2}, Samaneh Najafi^{1,2}

¹Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, ²Department of Medical Entomology, School of Health, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

ORCID:

Hamid Kassiri: 0000-0001-8447-5481

Abstract

Aims: The aim of this study was to determine the fauna and bioecology of sandflies in Khorramshahr County, southwest of Iran, where cutaneous leishmaniasis (CL) is endemic. **Materials and Methods:** Sandflies were caught indoors and outdoors by sticky paper traps in different areas. Sandflies were removed by needle and put in acetone and then transferred and preserved in 70% ethanol. In the laboratory, they were mounted in the Puri's medium and identified using identification key. The species richness, relative abundance, monthly prevalence, gender, and abdominal situation of sandflies in indoor and outdoor resting places were determined. **Results:** A total of 7172 sandflies representing 11 species were collected in 11 areas. Almost 29.9% of sandflies were collected from indoor places and 70.1% from outdoor places. About 60.8% of the specimens were *Phlebotomus* species while 39.2% were *Sergentomyia*. *Phlebotomus papatasi* and *Sergentomyia sintoni* were the most common species among *Phlebotomus* and *Sergentomyia* genera, respectively. The highest sex ratio was 1100 for *Sergentomyia theodori*. In total, 53.7% of sandflies were male and 46.3% were female. The majority of captured sandflies collected from indoor and outdoor places had an unfed stomach. The sandflies were more active in June and September. **Conclusion:** More detailed studies on leptomonaad infection of the suspected vectors and amastigote infection of the potential reservoirs of the CL are recommended to detect the epidemiological characteristics of the disease in this county.

Keywords: Ecology, Iran, sandfly, sex ratio, species composition

INTRODUCTION

Sandflies belong to the order Diptera, the suborder Nematocera, and the family Psychodidae. Although approximately 800 species of these insects have been identified, only 81 are medically significant and play a role in the transmission of some diseases to humans. Only female sandflies are blood feeders, and they feed on blood after sunset and at night.^[1]

The Phlebotominae subfamily of sandflies is responsible for the transmission of at least three diseases to humans: bartonellosis, papatasi fever, and leishmaniasis.^[1] Among these, the last

two diseases are common in Iran. Leishmaniasis, a common zoonotic disease, manifests itself as cutaneous, visceral, and mucocutaneous lesions in most parts of the world.^[2,3] Despite the fact that cutaneous leishmaniasis (CL) is not a fatal disease, it has always received special attention due to the patient's long-term struggle with wounds and the lengthy healing process.^[4,5]

Address for Correspondence: Dr. Hamid Kassiri, Infectious and Tropical Diseases Research Center, Health Research Institute, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. E-mail: hamid.kassiri@yahoo.com

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CL is present in Iran in two forms: zoonotic CL caused by *Leishmania major* and anthroponotic CL caused by *Leishmania tropica*, both of which are transmitted by female sandflies of *Phlebotomus* species. In the zoonotic form, wild rodents, particularly rats, are considered reservoirs of the disease, while in the anthroponotic form, sick humans and canines are considered reservoirs. The main vector in the first form is *Phlebotomus sergenti* and in the second form is *Phlebotomus papatasi*.^[4-7] CL is more common than other types, with approximately 1.5 million new cases reported annually. In Iran, the number of CL patients reduced from 23202 in 2008 (incidence rate 32/100,000) to 13,124 in 2019 (incidence rate 15.8/100,000).^[8-11]

Numerous molecular studies have been conducted in Iran to detect and isolate the parasite that causes zoonotic cutaneous leishmaniasis (ZCL) in sandflies. For example, in a cross-sectional study conducted by Oshaghi *et al.* in Arzuiyeh County in Kerman Province, using the semi-nested PCR technique, 92 (77%) of 172 female sandflies caught were reported to be *P. papatasi*, and *L. major* was reported to be at 6 (6.5%) *P. papatasi*.^[12,13] In another study conducted by Kassiri *et al.* in Chabahar County in Sistan-Baluchestan Province, out of 667 *P. papatasi* and 465 *Phlebotomus salehi*, 14 (2.1%) and 5 (1.07%) sandflies infected with *L. major* parasite were identified, respectively, using the PCR technique.^[14]

In a study in Sistan-Baluchistan Province in 1997 and based on a paper in 2020 about sandflies in Iran,^[15] Kassiri *et al.* collected and identified *Phlebotomus salengensis* (Artemiev, 1978), *Phlebotomus kabulensis* (Artemiev, 1978), *Phlebotomus similis* (Perfiliew, 1963), *Sergentomyia dreyfussi turkestanica* (Theodor and Mesghali, 1964), *Sergentomyia indica* (Theodor, 1931), *Sergentomyia squamipleuris* (Newstead, 1912), and *Sergentomyia (Rondanomyia) sp.* in Iran.

This study was carried out to investigate the fauna, monthly activity, and other ecological aspects of sandflies in Khorramshahr County, Southwestern Iran.

MATERIALS AND METHODS

The altitude of Khorramshahr (30°26'23"N 48°09'59"E) is 3 m above the sea level. It is a plain county and has a hot and desert climate. It is a highly humid county with hot summer and mild winter.

This descriptive research was guided to characterize the bioecology of phlebotomine sandflies. Since this county is a CL-endemic focus, eleven villages and cities with higher prevalence of CL were chosen for collecting sandflies: Arayez, Kofaishie, Honaishieh, Haffar, Manikh, Ghazbanieh, Chomeh, Maslavi, Shalheh, Sabeh as well as the Khorramshahr City.

Trapping was done every 15 days during the sandflies' activity season. Sampling was done three times from each region both outdoors and indoors. Sandflies were then removed from papers impregnated with castor oil and transferred to a container of acetone for a few seconds. Finally, the acetone was

evacuated and 70% ethanol was added to the container with the sandflies. To identify the species, sandflies were then placed on the Puri's medium drop placed on the slide. Data related to sandflies were inputted into SPSS (IBM SPSS software/Singapore, version 22.0), presented in descriptive tables, and its diagram was drawn. The sex ratio of all the species was calculated as: no. of males/no. of females × 100. The study was approved by the Committee of Ethics in Research, Ahvaz Jundishapur University of Medical Sciences, and registered as IR.AJUMS.REC.1396.806.

RESULTS

In total, 7172 sandflies were collected from indoor and outdoor places by sticky traps and their species were determined. Sandfly species were isolated and identified separately based on whether they were collected indoors or outdoors. Out of these sandflies, 2142 sandflies (29.9%) were collected from indoor places and 5030 sandflies (70.1%) from outdoor places. Totally, 4358 sandflies (60.8%) were *Phlebotomus*, Rondani and Bert 1980, and 2814 (39.2%) were *Sergentomyia*, Franca and Parrot 1920. This study identified 11 sandfly species: *P. papatasi*, Scopoli 1786 (39.7%); *Phlebotomus alexandri*, Sinton 1928 (21.1%); *Sergentomyia sintoni*, Pringle 1953 (32.3%); *Sergentomyia dentata*, Sinton 1933 (5.24%); *Sergentomyia tiberiadis*, Adler, Theodor, and Lourie 1930 (0.8%); *Sergentomyia baghdadis*, Adler and Theodor 1929 (0.3%); *Sergentomyia iranica*, Lewis and Mesghali 1961 (0.25%); *Sergentomyia theodori*, Parrot 1942 (0.17%); *Sergentomyia antennata*, Newstead 1912 (0.1%); *S. squamipleuris*, Grassomyia 1942 (0.03%); and *Sergentomyia palestinensis*, Bott 1967 (0.01%) [Table 1].

S. theodori, *S. antennata*, *S. squamipleuris*, and *S. palestinensis* were captured only in outdoor places. *P. papatasi*, *P. alexandri*, *S. sintoni*, *S. dentata*, *S. baghdadis*, and *S. tiberiadis* were collected from both indoor and outdoor places [Tables 2 and 3]. The abundance of phlebotomine sandfly species of human dwellings was as follows: *P. papatasi* ($n = 268$, 50.4%), *P. alexandri* ($n = 89$, 16.7%), *S. sintoni* ($n = 166$, 31.2%), *S. dentata* ($n = 7$, 1.32%), and *S. tiberiadis* ($n = 2$, 0.38%). Meanwhile, the abundance of phlebotomine sandfly species of stables was as follows: *P. papatasi* ($n = 746$, 46.3%), *P. alexandri* ($n = 373$, 23.2%), *S. sintoni* ($n = 423$, 26.3%), *S. antennata* ($n = 53$, 3.3%), *S. tiberiadis* ($n = 10$, 0.6%), *S. baghdadis* ($n = 3$, 0.18%), and *S. iranica* ($n = 2$, 0.12). *P. papatasi* ($n = 1014$, 47.3%), *S. sintoni* ($n = 589$, 27.5%), and *P. alexandri* ($n = 462$, 21.6%) accounted for the majority of sandflies in human habitats and stables.

With respect to gonotrophic cycle stages of female sandflies based on abdominal appearance, the majority of captured sandflies collected from indoor and outdoor places had an unfed stomach. Abdominal status of phlebotomine sandflies of indoor places was as follows: empty ($n = 1402$, 65.5%),

Table 1: Total abundance of phlebotomine sandfly species of Khorramshahr County, Southwestern Iran (2017-2018)

Species	Males, n (%)	Females, n (%)	Frequency of <i>Phlebotomus</i> or <i>Sergentomyia</i> population, n (%)	Frequency of total sandfly population (%)
<i>Phlebotomus papatasi</i>	1964 (69)	882 (31)	2846 (65.3)	39.7
<i>Phlebotomus alexandri</i>	1218 (80.6)	294 (19.4)	1512 (34.7)	21.1
<i>Sergentomyia sintoni</i>	499 (21.6)	1813 (78.4)	2312 (82.2)	32.3
<i>Sergentomyia dentata</i>	89 (23.5)	289 (76.5)	378 (13.4)	5.24
<i>Sergentomyia tiberiadis</i>	43 (72.9)	16 (27.1)	59 (2.1)	0.8
<i>Sergentomyia baghdadis</i>	5 (20.8)	19 (79.2)	24 (0.85)	0.3
<i>Sergentomyia iranica</i>	15 (83.3)	3 (16.7)	18 (0.64)	0.25
<i>Sergentomyia theodori</i>	11 (91.7)	1 (8.3)	12 (0.4)	0.17
<i>Sergentomyia antennata</i>	7 (87.5)	1 (12.5)	8 (0.29)	0.1
<i>Sergentomyia squamipleuris</i>	0	2 (100)	2 (0.08)	0.03
<i>Sergentomyia palestinensis</i>	1 (100)	0	1 (0.04)	0.01
Total	3852 (53.7)	3320 (46.3)	7172	100

Table 2: Abundance of phlebotomine sandfly species of outdoor places in Khorramshahr County, Southwestern Iran (2017-2018)

Species	Males, n (%)	Females, n (%)	Frequency of <i>Phlebotomus</i> or <i>Sergentomyia</i> population, n (%)	Frequency of total sandfly population (%)
<i>Phlebotomus papatasi</i>	1201 (65.6)	631 (34.4)	1832 (63.6)	36.4
<i>Phlebotomus alexandri</i>	793 (75.5)	257 (24.5)	1050 (36.4)	20.9
<i>Sergentomyia sintoni</i>	706 (41)	1017 (59)	1723 (80.2)	34.3
<i>Sergentomyia dentata</i>	263 (82.7)	55 (17.3)	318 (14.8)	6.4
<i>Sergentomyia tiberiadis</i>	36 (76.6)	11 (23.4)	47 (2.2)	0.9
<i>Sergentomyia baghdadis</i>	15 (71.4)	6 (28.6)	21 (1.1)	0.4
<i>Sergentomyia iranica</i>	13 (81.2)	3 (18.8)	16 (0.7)	0.3
<i>Sergentomyia theodori</i>	11 (91.7)	1 (8.3)	12 (0.5)	0.2
<i>Sergentomyia antennata</i>	8 (100)	0	8 (0.37)	0.14
<i>Sergentomyia squamipleuris</i>	0	2 (100)	2 (0.09)	0.04
<i>Sergentomyia palestinensis</i>	0	1 (100)	1 (0.04)	0.02
Total	3046 (60.6)	1984 (39.4)	5030	100

Table 3: Abundance of phlebotomine sandfly species of indoor places in Khorramshahr County, Southwestern Iran (2017-2018)

Species	Males, n (%)	Females, n (%)	Frequency of <i>Phlebotomus</i> or <i>Sergentomyia</i> population, n (%)	Frequency of total sandfly population (%)
<i>Phlebotomus papatasi</i>	783 (77.2)	231 (22.8)	1014 (68.7)	47.3
<i>Phlebotomus alexandri</i>	363 (78.6)	99 (21.4)	462 (31.3)	21.6
<i>Sergentomyia sintoni</i>	448 (76.1)	141 (21.2)	589 (88.4)	27.5
<i>Sergentomyia dentata</i>	49 (81.7)	11 (18.3)	60 (9)	2.8
<i>Sergentomyia tiberiadis</i>	10 (83.3)	2 (16.7)	12 (1.85)	0.56
<i>Sergentomyia baghdadis</i>	0	3 (100)	3 (0.45)	0.15
<i>Sergentomyia iranica</i>	2 (100)	0	2 (0.3)	0.09
Total	1655 (77.3)	487 (22.7)	2142	100

semi-gravid ($n = 342$, 16%), gravid ($n = 175$, 8.2%), and blood-fed ($n = 223$, 10.4%). Meantime, the abdominal status of phlebotomine sandflies of outdoor places was as follows: empty ($n = 3458$, 68.7%), semi-gravid ($n = 965$, 19.2%), gravid ($n = 451$, 9%), and blood-fed ($n = 156$, 3.1%).

In total, 3852 sandflies (53.7%) were male and 3320 sandflies (46.3%) were female. Moreover, 73% ($n = 3182$)

of *Phlebotomus* species were male and 27% ($n = 1176$) were female. On the other hand, 23.8% ($n = 670$) and 76.2% ($n = 2144$) of *Sergentomyia* species were male and female, respectively. In general, the majority of sandflies were male [Table 1]. Studies on the determination of sex percent and sex ratio (number of males per 100 females) showed that the highest of *P. papatasi*, *P. alexandri*, *S. tiberiadis*, *S. iranica*,

S. theodori, *S. antennata*, and *S. palestinensis* were male, whereas, the majority of *S. sintoni*, *S. dentata*, *S. baghdadis*, and *S. squamipleuris* species were female. The sex ratio of the sandflies of genus *Phlebotomus* and *Sergentomyia* was 270.6 and 31.25 males per 100 females, respectively. Furthermore, the sex ratio of phlebotomine species was 116. The sex ratio for *P. papatasi*, *P. alexandri*, *S. sintoni*, *S. dentata*, *S. tiberiadis*, *S. baghdadis*, *S. iranica*, *S. theodori*, *S. antennata*, *S. squamipleuris*, and *S. palestinensis* was 222.7, 414.3, 27.5, 30.8, 268.7, 26.3, 500, 1100, 700, 0, and 100, respectively.

Sandfly activity started in April and ended in January. The sandflies were more active in June and September in Khorramshahr County [Figure 1].

DISCUSSION

The most abundant species among the 7172 sandflies caught were *P. papatasi* (39.6%), *S. sintoni* (32.2%), and *P. alexandri* (21.1%). The significance of *P. papatasi* is due to the reality that it is noticed as the definitive and main vector of ZCL in all regions investigated across the country and the globe.^[14] Moreover, *P. papatasi* is known as a vector of sandfly fever.^[1] *P. alexandri* was reported as potential vector and known as a proven leishmaniasis vector in the globe and Iran.^[4] Results of PCR showed that *P. alexandri* females from East Azerbaijan Province, Kermanshah Province, Fars Province, and Khuzestan Province were infected with *Leishmania infantum*; *Leishmania major*; *L. major*, *L. infantum*, *Leishmania donovani*, and *L. tropica*; and *L. major* and *L. infantum*, respectively.^[16] Meanwhile, *S. dentata* and *S. sintoni* were introduced as the vectors of lizard leishmaniasis.^[4,17,18] The current study's findings were consistent with those of a previous study conducted in 2003 on leishmaniasis vectors in the Bastak region of Hormozgan Province,^[19] as well as those of a previous study conducted in 2005–2006 on the fauna, abundance, and distribution of sandflies in Nourabad Mamasani, Fars Province, Iran.^[20] In Kassiri *et al.*'s research in Sistan-Baluchistan Province, ten *Phlebotomus* and eight *Sergentomyia* species were found outdoors, and nine *Phlebotomus* and 10 *Sergentomyia* species were found indoors. *P. papatasi* (58.4%)

was the dominant species captured indoors in the plain area. *Sergentomyia clydei*, Sinton 1928 (64.7%), was captured to be the greatest species outdoors in the plain area. *S. clydei* (19.8%) and *S. tiberiadis*, Alder, Theodor, and Lourie, 1930 (35%), were the dominant indoor and outdoor species from the mountainous region, respectively.^[21] In the study of sandfly fauna in Khash County, Southeast Iran, of the 21 species of sandflies identified, 9 and 12 belonged to *Phlebotomus* and *Sergentomyia* genus, respectively. The greatest species were *Phlebotomus kazeruni* Theodor and Mesghali, 1964, and *S. tiberiadis*, representing 39.3% of *Phlebotomus* spp. and 62.5% of *Sergentomyia* spp., respectively.^[22] The dominant species in the above study was *P. kazeruni*. Due to differences in ecological, geographical, climatic, and geological conditions between the two regions, this result was different from that of the present study in which *P. papatasi* was the dominant species. According to a comparison of these two studies, the difference is most likely due to geographical and ecological conditions, as well as climatic differences, because Khash is a dry mountainous region, whereas Khorramshahr is located in the plain and has a humid climate. In the study of phlebotomine sandflies in Nikshahr County, Southeastern Iran, 27% of the specimens were *Phlebotomus* species while 73% were *Sergentomyia*. *P. alexandri* and *S. tiberiadis* were the most abundant species among *Phlebotomus* spp. and *Sergentomyia* spp., respectively.^[23] In the study of Rudbar County in Northern Iran, ten species were identified. About 0.42% of the specimens related to *Sergentomyia* genus. The species *Phlebotomus perfiliewi*, *Phlebotomus neglectus*, *Phlebotomus halepensis*, *S. tiberiadis*, *S. baghdadis*, and *S. clydei* were reported for the first time in Guilan Province. The numbers were 79.8% and 57.7% for the most common species of *P. perfiliewi* and *Phlebotomus tobbi*, respectively.^[24] In the study of Kassiri and Farhadi-Nezhad in Abadan County, Southwestern Iran, 2 species of *Phlebotomus* and 11 species of *Sergentomyia* were captured. *P. papatasi* (45.64%), *P. alexandri* (31.31%), and *S. sintoni* (14.3%) accounted for the majority of collected species.^[4] In Chabahar County, Southeastern Iran, *P. papatasi* (34.83%), *S. clydei* (33.57%), *S. sintoni* (16.23%), and *P. salehi* (13.02%) were the four common species in the area.^[25] Geographical, topographic, ecologic, and climatic differences are the reason for the difference in the results in the study of sandfly fauna.

Male and female sandflies accounted for 53.7% and 46.3% of the specimens, respectively. Male sandflies were more common in this study. The sex ratios indicated that the sticky traps were more attractive for males. The sex ratio of the sandflies of genus *Phlebotomus* and *Sergentomyia* was 270.6 and 31.25, respectively. Furthermore, the overall sex ratio of phlebotomine species was 116. In the Chabahar, Khash, and Abadan sandfly studies, 59.1%, 74.7%, and 71.4% of the specimens were male. In Chabahar, the sex ratio was 241.3 for *Phlebotomus* genus, whereas it was 92.3 for *Sergentomyia* genus.^[25] In Rudbar County, 64.4% and 67.2% of phlebotomine sandflies were females when light traps and hand catches were

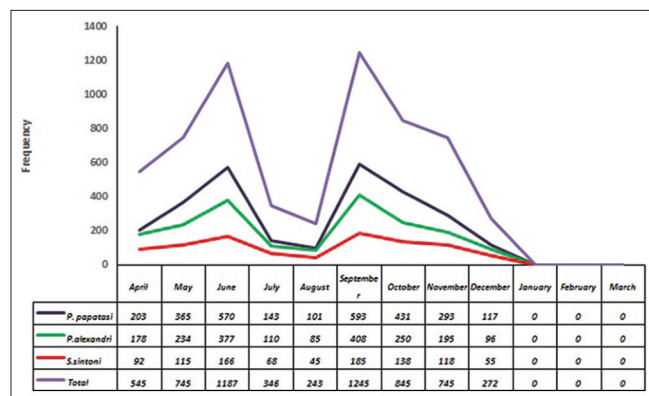


Figure 1: Monthly activity of phlebotomine sandflies in Khorramshahr County, Southwestern Iran (2017–2018)

applied, respectively. Furthermore, 91.2% of captured sandflies by sticky paper traps were males. The overall sex ratio (M: F) of phlebotomines was 0.69.^[24] In a study in Northwestern Iran, the maximum part of sandflies captured by light traps was female and by sticky paper traps was male. This greater frequency of males to females may be described by the greater hunting of sandflies by sticky paper traps than light traps in that study. They indicated a sex ratio of 1.1 for *Sergentomyia* and 1.3 for *Phlebotomus*.^[26] Findings of a survey in Bam city show 81.3% of captured sandflies were male and the sex ratio was 618.^[27] Sex ratio is not only a constant rate for all species but also in association with the collection method.

Abdominal appearance of female sandflies is applied for determining ovary development situation. A classification for abdominal appearances is as follows: gravid, semi-gravid, freshly fed, and empty (unfed). In the present study, the majority of sandflies collected from indoor and outdoor places had an empty abdomen (67.8%). In Norouzi *et al.*'s study, 71.6% of female phlebotomines were unfed.^[24] In Kassiri *et al.*'s study in Abadan, the analysis of physiological status of sandflies in outdoors and indoors revealed that most of them were unfed (66.15%). A study in Sistan-Baluchistan Province revealed that 38.4% and 50.9% dissected *P. papatasi* and *P. salehi* were unfed, respectively.^[28]

In the present study, sandfly activity began in April and ended in January. Furthermore, the sandflies were more active in June and September in Khorramshahr County. In the study of Abadan County, the sandflies were more active in June and September.^[4] Yaghoobi-Ershadi *et al.* in Rafsanjan County, Southeastern Iran, reported *P. papatasi* with its two peaks of activity happening at the beginning of both June and August.^[29] In Damghan County, Semnan Province, *P. papatasi* had one activity peak at the end of May and one at the beginning of September.^[30] Since the climatic conditions differ in various geographical parts of Iran, the peak activity of sandflies is expected to differ in various areas. Different surveys have proved this conclusion.

The limitations of this study were as follows: collection of sandflies by only one method and the impossibility of sampling from all areas of the county. The strengths of the research included sampling in all months and seasons and the relatively large number of specimens collected. The highlight of this study was the rich fauna of sandflies in the area.

CONCLUSION

The findings of the present research show that different species of sandflies are active in Khorramshahr County. Among them, *P. papatasi* and *P. alexandri* are introduced as the main and secondary vectors of ZCL. Leptomonad infection of *P. papatasi* and *P. alexandri* is recommended for this area.

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Conflicts of interest

There are no conflicts of interest.

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