

# Morphological Identification of Leishmaniasis Vectors and Their Species Diversity in Fars Province, Southern Iran

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#### Abstract

**Background:** Sandflies are the vectors of at least eight different diseases, the most important of which is cutaneous leishmaniasis (CL). CL is a major public health problem in Iran, with annual cases increasing to more than 20,000 in 2019. Fars Province has the second-highest number of cases with more than 3000 cases in 2019 in Iran. This study aimed to survey the fauna and different species of sandflies (Diptera: Psychodidae: Phlebotominae) in Fars Province, southwest Iran.

**Methods:** This is a cross-sectional study. Sandflies were collected in urban and rural regions using the sticky-traps method, and then they were cleared and fixed in puris medium and identified using the pictorial key.

**Results:** A total of 1071 of sandflies were collected. The dominant species consisted of 5 species of *Phlebotomus* (*Ph. papatasi* 55.4%, *Ph. alexandri* 17.4%, *Ph. sergenti* 5.6%, *Ph. caucasicus* 1.4%, *Ph. ansari* 0.46% and 6 species of *Sergentomyia* (*Ser. sintoni* 10.8%, *Ser. antennata* 5.4%, *Ser. tiberiadis* 1.4%, *Ser. tobbi* 1%, *Ser. baghdadis* 0.5%, and *Ser. halepensis* 0.09%).

**Conclusion:** This investigation showed that *Ph. papatasi* was the most prevalent species playing a crucial role in the transmission of cutaneous leishmaniasis in Fars Province. Species of *Ph. papatasi* and *Ph. sergenti* are the main vectors of CL in Iran. The healthcare system must take steps to control cutaneous leishmaniasis, raise awareness of the disease, and apply effective ways to prevent it.

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**Keywords:** Leishmaniasis, Psychodidae, Sandflies, Morphology

## Introduction

Leishmaniasis is transmitted by sandflies and is present in 88 countries, especially in the Middle East region and southwest Asia, of which 72 are developing and 13 are developed countries, with an annual prevalence and incidence of 12 and 2 million, respectively.<sup>1</sup> Approximately, 90% of cutaneous leishmaniasis have been reported in eight countries, and Iran is one of those countries.<sup>2</sup> Leishmaniasis, especially CL, in Iran has a long history and is transmitted to humans through sandfly infection bites in both urban and rural types.<sup>3-6</sup>

Sandflies are also capable of transmitting Bartonellosis and arbovirus diseases in addition to leishmaniasis.<sup>7</sup> The disease is caused by different species of *Leishmania* parasite, a protozoan parasite belonging to the family Trypanosomatidae that is capable of infecting humans and other mammals.<sup>8</sup> The parasite has two forms, namely the Amastigote form that is found in vertebrates (humans, rodents and dogs), and the Promastigote form that is found in sandflies.<sup>9</sup> Two types of leishmaniasis exist in Iran:<sup>10</sup> visceral and zoonotic. Cutaneous leishmaniasis is the most prevalent worldwide, with two forms of anthroponotic cutaneous leishmaniasis (ACL) caused by *L. tropica*

and zoonotic cutaneous leishmaniasis (ZCL) caused by *L. major*, respectively. ZCL is transmitted to humans by *Ph. sergenti* and *Ph. papatasi* in Turkey, Egypt, Israel, Iran, Saudi Arabia, and Northern India.<sup>11-16</sup> The prevalence of leishmaniasis is high in Khorasan, Fars, Isfahan, Khuzestan and Kerman provinces of Iran.<sup>16</sup> Fars Province in the south of Iran is one of the most important endemic areas of zoonotic cutaneous leishmaniasis, and most of its cities, including Jahrom, Fasa, Marvdasht, Kharameh, Arsanjan and Neyriz, have endemic diseases.<sup>17-19</sup> It is noteworthy that uncontrolled urban development and population growth on the outskirts of cities cause an increase in sandfly populations, leading to the outbreak of leishmaniasis. Furthermore, change of housing patterns to the apartment in cities does not decrease the incidence of the disease among the inhabitants of apartments on various floors, especially the lower floors.<sup>20</sup> The present study aimed to investigate the faunae and monthly activities of sandflies as the vectors of leishmaniasis. The findings of this study are suggested to be used for future programs to control leishmaniasis.

## Methods

### Study Area

Fars province is located in the south of Iran, (29°37'N 52°32'E) (Figure 1) and its neighboring provinces are Boushehr, Isfahan, Yazd, Kohgiluyeh and Boyer-Ahmad, and Kerman. It has a total of 4,851 million inhabitants (est. 2016), living over 122,608 km<sup>2</sup> of the territory; slightly more than half (67.6%) of the population is urban. People at risk of leishmaniasis approach 80% of the population since most of the territory is infested with the mosquito vectors, *Ph. papatasi* and *Ph. sergenti*.

**Procedure:** Samples were collected from Kherameh, Marvdasht, Kazeroon, Shiraz, Larestan, Mamasani, Zarrin Dasht, and Pasargad districts twice a year, using indoor and outdoor sticky-traps, spring season from mid-May to late June and summer from mid-August to late September in indoor and outdoor



**Figure 1:** Location of the study areas in Fars province, Southern Iran, 2019. ☆ Indicated the study area

locations. Since we aimed to identify mosquito species during the peak of mosquito activity in Fars province, we selected these two times based on studies conducted by other researchers.<sup>21</sup> A total of 30 sticky-traps were installed either indoor or outdoor (gardens and rodent burrows). To catch adult sandflies, we used sticky traps (20 cm \* 20 cm) impregnated with castor oil. The sticky-traps were placed in selected areas during the night and were collected the next morning before sunrise. The sandflies were separated from the adherent traps by brush, transferred to 70% alcohol-containing microtubes, and then identified in a puri's medium on mounted slides under a stereomicroscope. Diagnosis of sandflies was made by examining the morphology of male genitalia, female spermatheca, pharyngeal reinforcement and Cibarium teeth using the pictorial key of Seyedi-Rashti (1990).

**Data analysis:** In the current study, the SPSS V.19 software was used for data analysis. Additionally, to compare the median quality variable, we applied chi-square test.

## Results

A total of 1071 sandflies, including 514 (48%) males and 557(52%) females were collected from 8 regions during the study (Table 1). Furthermore, 206 (19.2 %) and 865 (80.8%) sandflies were caught indoors and outdoors, respectively (Table 1).

**Table 1:** Frequency of sandflies species based on sampling places and gender, Fars province, Southern Iran, 2019

	Species	Gender		Catching places	
		Male	Female	Indoor	Outdoor
1	<i>P. alexsandri</i> *	101	86	28	159
2	<i>S. antennata</i>	30	28	0	58
3	<i>P. ansari</i> *	0	5	0	5
4	<i>S. baghdadis</i>	1	5	0	6
5	<i>P. caucasicus</i>	15	1	0	16
6	<i>S. halepensis</i>	1	0	0	1
7	<i>P. papatasi</i> *	305	289	131	463
8	<i>P. sergenti</i> *	44	17	31	30
9	<i>S. sintoni</i>	39	77	17	99
10	<i>S. tiberadis</i>	10	6	0	16
11	<i>S. tobbi</i>	11	0	0	11
Total	-	557	514	207	864

\*The vector species in Iran

After morphological identification, 5 species of *Phlebotomus* [*Ph. papatasi*, *Ph. alexandri*, *Ph. sergenti*, *Ph. caucasicus*, *Ph. ansari*] and 6 species of *Sergentomyia* [*Ser. sintoni*, *Ser. antennata*, *Ser. tiberiadis*, *Ser. tobbi*, *Ser. baghdadis*, *Ser. halepensis*] were detected in the current study (Tables 1, 2). Most of the sandflies caught in Shiraz were *Ph. papatasi* (55.5%) and *Ph. alexandri* (17.46%) (Table 1). In this study, a significant difference was found between the regions and species of sandflies, including both genders. ( $P < 0.05$ ).

The most collected species of sandflies in indoor places was *Ph. papatasi*. It was also the most dominant species in all regions with 594 (55.4%) frequency, and 131 (22%) cases were captured in indoor places. The adult sex ratio was 52% males versus 48% females. In this study, since more mosquitoes were caught in outdoor places, the number of males was higher than that of females (Table 1). *Ph. alexandri* consists of 187 (17.5%) sandflies collected from Fars province and is considered to be the second abundant species of sandflies collected from

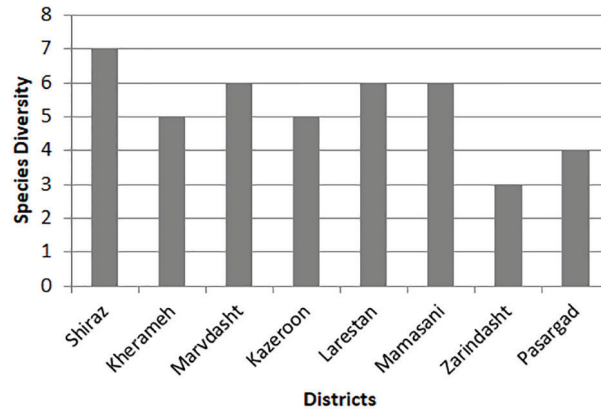


Figure 2: Species diversity based on districts in Fars Province, Southern Iran, 2019.

Table 2: The number and percentage of species caught in the study areas, Fars province, Southern Iran, 2019

Row	Species	Shiraz	Kherameh	Marvdasht	Kazeroon	Larestan	Mamasani	Zarindasht	Pasargad	Frequency	Percentage%
1	<i>P. alexandri</i> *	34	0	77	2	51	17	4	2	187	17.5%
2	<i>S. antennata</i>	23	1	11	6	12	4	0	1	58	5.49%
3	<i>P. ansari</i> *	0	5	0	0	0	0	0	0	5	0.4%
4	<i>S. baghdadis</i>	1	0	0	3	1	1	0	0	6	0.5%
5	<i>P. caucasicus</i>	0	12	0	0	0	4	0	0	16	1.5%
6	<i>S. halepensis</i>	0	0	1	0	0	0	0	0	1	0.09%
7	<i>P. papatasi</i> *	190	45	140	14	101	9	88	7	594	55.5%
8	<i>P. sergenti</i> *	61	0	0	0	0	0	0	0	61	5.6%
9	<i>S. sintoni</i>	10	86	0	0	4	14	2	0	116	10.9%
10	<i>S. tiberiadis</i>	2	0	1	3	10	0	0	0	16	1.5%
11	<i>S. tobbi</i>	0	0	1	0	0	0	0	10	11	1.02%
Total		321	149	231	28	179	49	94	20	1071	100

\*The vector species in Iran

Table 3: Sandflies distribution in the regions, Fars province, Southern Iran, 2019

Row	Species	Shiraz	Kherameh	Marvdasht	Kazeroon	Larestan	Mamasani	Zarindasht	Pasargad
1	<i>P. alexandri</i> *	*		*	*	*	*	*	*
2	<i>S. antennata</i>	*	*	*	*	*	*		*
3	<i>P. ansari</i> *		*						
4	<i>S. baghdadis</i>	*			*	*	*		
5	<i>P. caucasicus</i>		*				*		
6	<i>S. halepensis</i>			*					
7	<i>P. papatasi</i> *	*	*	*	*	*	*	*	*
8	<i>P. sergenti</i> *	*							
9	<i>S. sintoni</i>	*	*			*	*	*	
10	<i>S. tiberiadis</i>	*		*	*	*			
11	<i>S. tobbi</i>			*					*

\*The vector species in Iran

the region (Table 2), which has the potential to transmit visceral leishmaniasis. Among the 11 species captured and identified in this study, 4 species in Fars province were the vector of *Leishmaniasis*. Vector species have been marked in the tables as bold. In this study, Shiraz with 7 species had the highest species diversity and Zarindasht with 3 species had the least species diversity (Table 3) (Figure 2).

## Discussion

In the current research, eleven important species of *Phlebotomus* and *Sergentomyia* subgenus were captured. Among the eleven identified species, two species of *Ph. papatasi* and *Ph. alexandri* were abundant, respectively. The studied species were collected in the spring and summer seasons. Rural cutaneous leishmaniasis is endemic in all the studied districts. *Ph. papatasi* is the most abundant species and is the only vector confirmed by different researchers as the main vector of cutaneous leishmaniasis in recent years in Iran using the molecular method.<sup>22-25</sup> Our studies indicate that *Phlebotomus papatasi* is the most abundant and dominant species in Fars Province.<sup>26</sup> The results of studies in Noorabad Mamasani on the fauna and abundance of sandflies in 2004-2005 are different from the results of this study, which may be owing to the place of catching and climate change in recent years.<sup>27</sup> Rasoolian et al. (2007) in their study identified one sub-genus from *Sergentomyia* genus (*Grassomyia* spp) and 10 other species consisting of 3 species from *Phlebotomus* genus (*Ph. papatasi*, *Ph. tobbi*, *Ph. sergenti*) and 7 from *Sergentomyia* genus.<sup>28</sup> This species diversity appears to be due to various factors such as the type of traps, environment, and climatic changes of the study area. Due to the drought in Fars province during the last 15 years, the conditions for the presence of some species of sandflies have been provided, and studies have demonstrated that climate change plays a role in the diversity of species.<sup>29</sup> Similar studies in Fars province and other parts of Iran have reported that *Phlebotomus papatasi* is the dominant species.<sup>6, 7, 18, 30-31</sup> In recent years, climate change has increased the incidence of leishmaniasis in Fars province for two reasons. First, the growth period of sandflies has increased in the endemic areas, so that the peak activity of mosquitoes was previously from June to mid-September, but now due to hot weather, it is from mid-May to mid-October. Second, new areas that previously did not have optimal weather conditions now have suitable conditions for sandfly activity. In addition, many studies have investigated the reservoir of leishmaniasis in Fars province, and it has been shown that four species *Meriones libycus*, *Meriones persicus*, *Tatera indica*, and *Gerbillus* spp. are the reservoirs of the disease.<sup>32-34</sup> Therefore, using rodenticide and raising the residents' awareness about mosquito bites are highly effective in controlling the disease.

## Conclusion

This article provides only useful information on the fauna of leishmaniasis vectors (sandflies) in one of the major endemic foci in the southwest of Iran. Fars province, especially Shiraz city, is one of the most important tourist resorts in Iran; therefore, the results of this study can be used to design an effective control program for leishmaniasis vectors.

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**Conflict of Interest:** None declared.

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