

Original Article

Species Composition of Phlebotominae Sand Flies (Diptera: Psychodidae) in Mashhad City, an Endemic Focus of Anthroponotic Cutaneous Leishmaniasis, Northeastern Iran

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Abstract

Background: Phlebotomine sand flies are the primary vectors of cutaneous leishmaniasis. We conducted a field survey and comprehensive literature review to investigate the species composition and bionomics of sand flies in Mashhad, a major pilgrimage and tourist city in northeastern Iran. This location is critical due to its high population mobility, which can influence disease dynamics.

Methods: Sand flies were collected monthly from spring to winter 2022 using sticky paper traps at 108 sites across Mashhad's diverse ecological zones. Species were identified, and population densities were calculated. Climatic data for 2022 were sourced from the Mashhad Meteorological Station. Using SPSS version 26, we employed ANOVA to compare seasonal species densities and Pearson correlation to analyses relationships between climatic parameters and sand fly abundance.

Result: A total of 3,270 sand fly specimens were collected, representing two genera. The majority (98%) belonged to the genus *Phlebotomus*, while the remaining 2% were classified under *Sergentomyia*. The identified species and their respective relative abundances were as follows: *Ph. sergenti* (76.29%), *Ph. papatasi* (18.53%), *Ph. alexandri* (1.10%), *Ph. ansari* (1.00%), *Se. sumbarica* (1.31%), *Ph. caucasicus* (0.73%), *Ph. major group* (0.61%), and *Se. sintoni* (0.42%). Statistical analysis revealed significant seasonal variations in the abundance and distribution of sand fly species, which were strongly associated with environmental parameters such as temperature, wind speed, and relative humidity.

Conclusion: The bionomics, diversity, and population density of sand flies vary across different locations and times, a factor that must be considered in advanced studies, prevention, and control programs.

Keywords: Sand flies; Leishmaniasis; Bionomics; Diversity; Iran

Introduction

Leishmaniasis is a common vector-borne parasitic disease affecting both humans and animals. This disease has been prevalent in tropical and subtropical regions worldwide, posing a significant health problem. Cutaneous leishmaniasis, caused by *Leishmania* (Family: Trypanosomatidae), is distributed all over the world (1). The main vectors of *Leishmania* are

Phlebotomine sand flies (Diptera: Psychodidae) (2). More than 90 species and subspecies of sand flies serve as vectors for various forms of leishmaniasis worldwide (3). In the Old World, sand fly vectors belong to the genus *Phlebotomus*, whereas in the New World, they belong to the genus *Lutzomyia* (4). More than one billion people live in high-risk areas of leishman-

iasis (5), with at least 12 million individuals globally being affected by the disease, and about two million cases being reported each year. The disease causes ten thousand deaths annually (6). This disease has been observed in different parts of Iran. In 2019, approximately 87% of new cases worldwide occurred in ten countries, including Iran, Afghanistan, Algeria, Brazil, Colombia, Iraq, Libya, Pakistan, Syria, and Tunisia (7). Currently, there are two clinical forms of cutaneous leishmaniasis in Iran, which are Anthroponotic Cutaneous Leishmaniasis (ACL) that caused by *Leishmania tropica* and Zoonotic Cutaneous Leishmaniasis (ZCL) that caused by *Leishmania major* (8). Khorasan, Esfahan, and Kerman Provinces are the important foci of leishmaniasis in Iran (9). The main vector of ACL in Iran is *Phlebotomus sergenti*, with humans and dogs serving as reservoir hosts (10, 11).

The presence of disease vectors and the occurrence of outbreaks are determined by a range of environmental factors, including topography, climate, soil conditions, vegetation, and sanitation infrastructure (12). In addition, each of these factors has the potential to independently influence the attraction of Phlebotomine sand flies to human habitats (13, 14). To date, over 53 species of sand flies have been recorded in Iran (34 *Phlebotomus* and 19 *Sergentomyia* species) (15). Previous studies have recorded several species of sand flies in Mashhad city, including *Ph. sergenti*, *Phlebotomus ansari*, *Phlebotomus papatasi*, *Sergentomyia sintoni*, and *Sergentomyia sumbarica* (16). The incidence of urban leishmaniasis in Mashhad city has witnessed a substantial surge since 2002. This increase can be attributed to the high abundance of *Ph. sergenti*, particularly in the city's periphery and mountainous areas, with notable concentrations in older buildings and socioeconomically disadvantaged neighborhoods. Moreover, the burgeoning population in this region has resulted in escalated waste generation and suboptimal waste management practices in the proximity of Mashhad City, giving rise to apprehensions regarding the am-

plification of vector populations and the concomitant diffusion of the disease. The period of activity for Phlebotomine sand fly species has been documented to extend from April to November (17). Due to the special conditions of this city and the annual arrival of millions of Iranian and foreign travelers, Mashhad is an endemic of urban leishmaniasis (16). In recent years, a thorough investigation regarding the presence of potential Phlebotomine sand fly species in the endemic focus of Mashhad City has been lacking. The identification of sand flies is very important to distinguish behaviour and advanced studies of vectors, and ultimately for the prediction, prevention, and control of disease. Consequently, the current study endeavours to identify and assess the occurrence and abundance of prevalent cutaneous leishmaniasis vectors amidst varying climatic conditions and across different seasons within Mashhad City in the year 2022. The results of this research are expected to contribute to the development and implementation of targeted control strategies against the vectors responsible for transmitting this disease.

Materials and Methods

Study area

Khorasan-Razavi Province is situated in northeastern Iran. Mashhad city, which is the capital of Khorasan-Razavi, is a populated city with 3,062,242 inhabitants of which 1,527,439 are females and 1,534,803 males (18, 19) (Fig. 1). The number of religious pilgrims and tourists in this city is about 20 million every year (20). The local climate in this area is characterized by variability, ranging from moderate to cold and arid conditions (21, 22). During summer, maximum temperatures can reach approximately 43 °C, while winter temperatures plummet below freezing, reaching around -22 °C. Over the past 60 years, the average rainfall in Mashhad has been 250 mm, but in recent years, it has shown a significantly decreasing trend, reaching only 90 mm. The average

annual relative humidity is also 53.4%. The dominant wind in Mashhad is southeast wind, and the average wind speed per year is 2.9 km/h (23).

Sand fly Collection

Based on the geographical distribution of positive cutaneous leishmaniasis cases, 12 areas within Mashhad City were selected for sand fly collection (Fig. 2). The sticky paper traps (castor oil coated white papers 15×21 cm²) were used for collecting the specimens in outdoor biotopes including yard, rodent burrow, stone and wall crevices, wall cracks, bird nests, riverbank, courtyard, dog houses and rubble. Collections were conducted twice a month from the spring to the end of the winter in 2022 (24). Following collection, the sticky paper traps were carefully transported to the Medical Entomology Laboratory at Mashhad University of Medical Sciences for species identification. The sand fly specimens were carefully extracted from the sticky traps using a needle, washed in a 1% acetone solution, degreased, and preserved in 70% ethanol (25).

Morphological Study

For species identification, the head and two terminal segments of each sand fly specimen were cut and transferred to a drop of Puri's media placed between a slide and cover slip (26). Identification of the sand flies was carried out using the morphological keys (27, 28).

Data Analysis

To determine the relative abundance of sand flies within each season, the following index was computed:

$$\frac{\text{The number of specific species that captured in each season}}{\text{Total number of the same species collected throughout the entire year}}$$

Similarly, to assess the relative abundance of each sand fly species within a year, the following index was calculated (26):

$$\frac{\text{The number of specific species captured in the year}}{\text{Total number of species collected throughout that year}}$$

Data on ACL were input into Microsoft Excel. The Pearson correlation coefficient was

employed to investigate the association between climatic data and the presence of Phlebotomine sand fly species. Furthermore, the Pearson correlation test was performed to analyse correlations across different time periods (29). The relative abundance of species during various seasons was visually presented in a graph prepared using IBM SPSS version 26. The correlation coefficient ranges from -1.0 to 1.0, where a positive correlation signifies a direct relationship between two variables, indicating that an increase in one variable corresponds to an increase in the other. Conversely, a negative correlation signifies an inverse relationship between the variables. Conversely, a correlation coefficient of zero denotes independence between the variables. A 95% confidence interval was used; a p-value of less than 0.05 ($P < 0.05$) was considered statistically significant.

Results

Throughout the Spring Season

During the spring season, the highest relative abundance was recorded for *Ph. papatasi* (53.79%). During this season, the average temperature was 21°C. Furthermore, the abundance of *Ph. ansari* showed a significant positive correlation with average wind speed ($r = 0.73$, $p = 0.007$), which averaged 7.74 km/h for the period.

Throughout the Summer Season

Throughout the summer season, eight sand fly species were captured. Among them, *Ph. sergenti* demonstrated the highest relative abundance (45.37%), whereas *Se. sintoni* exhibited the lowest (5.78%). The abundance of *Ph. sergenti* was significantly correlated with both the average temperature ($r = 0.70$, $p = 0.01$) and average wind speed ($r = 0.83$, $p < 0.001$). Similarly, *Ph. papatasi* abundance was significantly correlated with average temperature ($r = 0.62$, $p = 0.027$) and wind speed ($r = 0.83$, $p < 0.001$). In contrast, the apparent fluctuation in *Ph. caucasicus* abundance (62.50%) in relation to wind speed showed a non-significant trend ($r = 0.57$, $p = 0.055$).

Throughout the Autumn Season

Throughout autumn, the abundance of *Ph. sergenti* was significantly correlated with both average relative humidity ($r = 0.68$, $p = 0.020$) and average temperature ($r = 0.71$, $p = 0.013$). In contrast, the abundance of *Ph. papatasi* was only correlated with average relative humidity ($r = 0.68$, $p = 0.020$).

Throughout the Winter Season

During the winter, only *Ph. sergenti* was identified, with a relative abundance of 1.40%. Its abundance was significantly correlated with the average temperature ($r = 0.83$, $p < 0.001$) and average wind speed ($r = 0.83$, $p < 0.001$) for that season, which were 2.5 °C and 5.96 km/h, respectively.

Throughout the Year

Throughout the year, a total of 2495 *Ph. sergenti* were collected, making it the most common species in outdoor resting sites with a relative abundance of 76.29%. In contrast, *Se. sintoni* was the least common, with a relative abundance of only 0.42%.

Checklist of Phlebotomine Sand Fly Species Recorded in Khorasan Province

A checklist of Phlebotomine sand fly species recorded in Khorasan Province was compiled based on previously collected and described species from the genera *Phlebotomus* and *Sergentomyia*, including *Ph. sergenti*, *Ph. ansari*, *Ph. papatasi*, *Se. sintoni* and *Se. sumbarica* (30). The paragraph below summarizes all published reports on sand fly species in Khorasan Province from 1957 to 2023.

In 1957, Lewis (31) documented the presence of *Ph. ansari*, *Sergentomyia clydei*, *Se. sintoni* and *Se. sumbarica* in Khorasan-Razavi (Sabzevar). In 1967, Mesghali et al. (32) reported the occurrence of *Ph. sergenti*, *Ph. papatasi* and *Se. clydei* in the same region. Nadim et al. (33) recorded the presence of *Se. sintoni* in 1968, and later in 1977, Nadim et al. (34) reported *Ph. sergenti* in Khorasan-Razavi (Neyshabur). In 1991, Nadim et al. (35) document-

ed the occurrence of *Ph. sergenti* and *L. tropica* in the province.

Subsequent studies confirmed the continued presence of key vector species. In 2003, Yaghoobi-Ershadi et al. (36) reported *Ph. papatasi* and *Ph. sergenti* in Khorasan-Razavi and North Khorasan provinces. Moosa-Kazemi et al. (17) highlighted *Ph. sergenti* as a common sand fly vector in Khorasan and north-eastern Iran in 2007. Moghadam et al. (37) in 2015 emphasized that species from both *Phlebotomus* and *Sergentomyia* genera specifically *Ph. sergenti*, *Ph. papatasi*, *Se. sintoni*, and *Se. dentata* serve as prominent vectors of leishmaniasis in central and southern Khorasan. Yaghoobi-Ershadi (38) in 2016 confirmed *Ph. papatasi* and *Ph. sergenti* as vectors of urban and rural cutaneous leishmaniasis. Most recently, in 2021, Shorka et al. (39) reported the presence of *Ph. papatasi*, *Ph. ansari*, and *Ph. caucasicus* in Khorasan-Razavi (Sabzevar). Overall, these historical records demonstrate the consistent presence of medically important sand fly species, particularly *Ph. sergenti* and *Ph. papatasi*, in Khorasan Province over the past six decades, providing essential baseline information for vector surveillance and leishmaniasis control strategies.

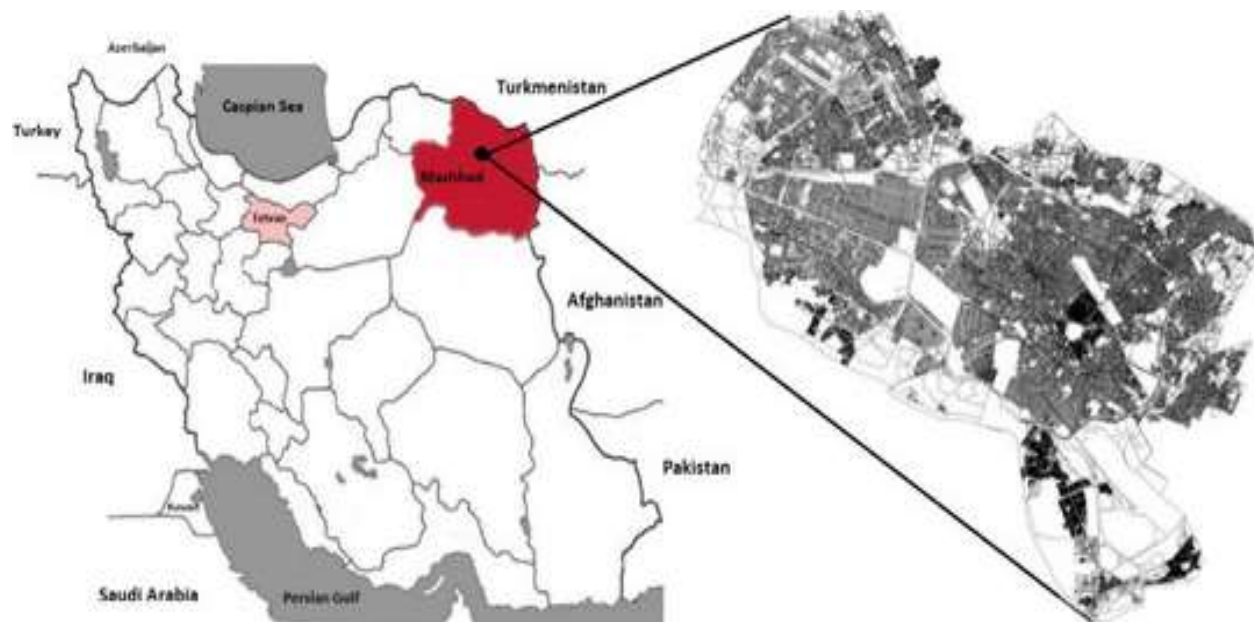
Table 1. Climate variables in Mashhad City, Iran, 2022

Season	Temperature minimum (°C) ±2	Temperature maximum (°C) ±2	Temperature average (°C) ±2	Average relative humidity (%) ±5	Average rainfall (mm) ±1	Average Wind speed (km/h) ±2
Spring	8.00	38.10	21.40	63.12	2.20	7.74
Summer	17.10	43.60	30.74	37.30	0.00	7.59
Autumn	0.00	31.40	13.61	62.52	0.12	5.54
Winter	-16.00	18.10	2.57	60.49	0.80	5.96

Table 2. Frequency of Phlebotomine sand flies in various seasons relative to the same species, Mashhad city, Iran, 2022

Species	Spring		Summer		Autumn		Winter	
	Male No. (%)	Female No. (%)	Male No. (%)	Female No. (%)	Male No. (%)	Female No. (%)	Male No. (%)	Female No. (%)
<i>Ph. ansari</i>	8 (80.0)	2 (20.0)	4 (33.3)	8 (66.7)	5 (50.0)	5 (50.0)	0 (0.0)	0 (0.0)
* <i>Ph. caucasicus</i>	3 (60.0)	2 (40.0)	12 (80.0)	3 (20.0)	3 (75.0)	1 (25.0)	0 (0.0)	0 (0.0)
<i>Ph. papatasi</i>	287 (88.0)	39 (12.0)	103 (48.1)	111 (51.9)	28 (42.4)	38 (57.8)	0 (0.0)	0 (0.0)
<i>Ph. sergenti</i>	265 (49.1)	275 (50.9)	556 (48.9)	576 (51.1)	421 (54.7)	362 (45.9)	14 (40.0)	21 (60.0)
<i>Se. sumbarica</i>	6 (71.8)	1 (28.1)	13 (48.1)	14 (51.9)	4 (44.0)	5 (55.5)	0 (0.0)	0 (0.0)
<i>Se. sintoni</i>	0 (0.0)	0 (0.0)	5 (45.5)	6 (54.5)	1 (33.3)	2 (66.7)	1 (0.0)	0 (0.0)
<i>Ph. major</i>	1 (50.0)	1 (50.0)	5 (46.4)	6 (54.5)	4 (57.1)	3 (42.8)	0 (0.0)	0 (0.0)
<i>Ph. alexandri</i>	6 (42.9)	8 (57.1)	9 (60.0)	6 (40.0)	4 (57.1)	3 (42.8)	0 (0.0)	0 (0.0)

*: *Phlebotomus* (Par.) *caucasicus* group.

**Fig. 1.** Location of the city of Mashhad in Razavi Khorasan Province, Iran (19)

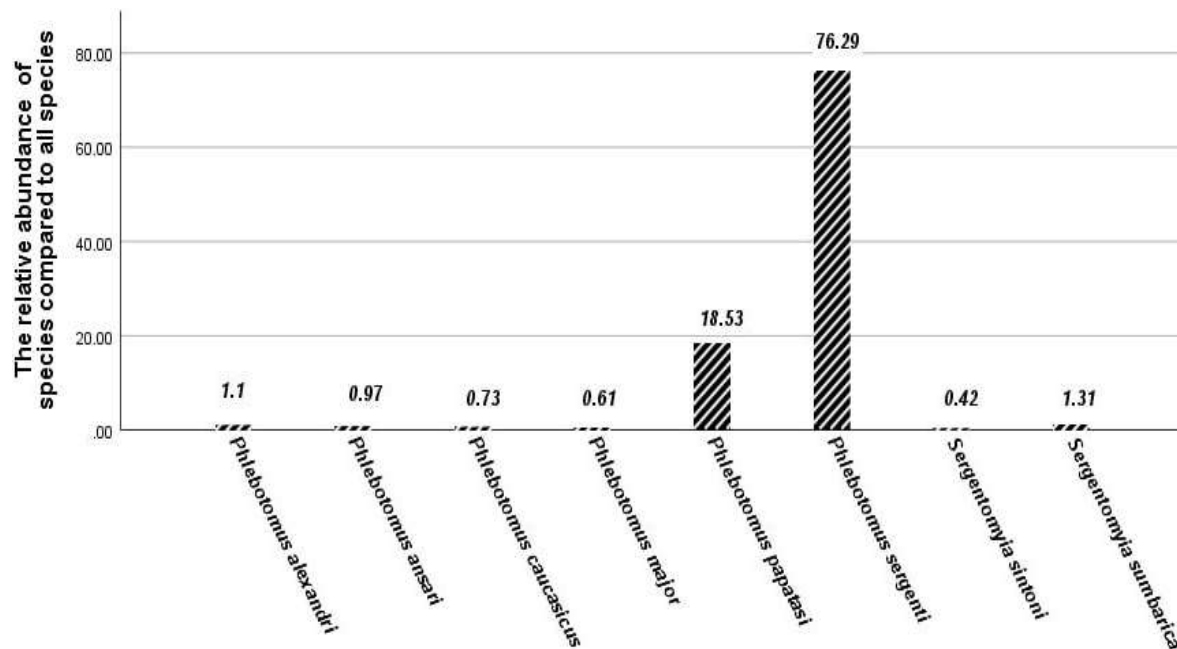


Fig. 2. The relative abundance chart of Phlebotomine sand fly species compared to all species in Mashhad City, Iran, 2022

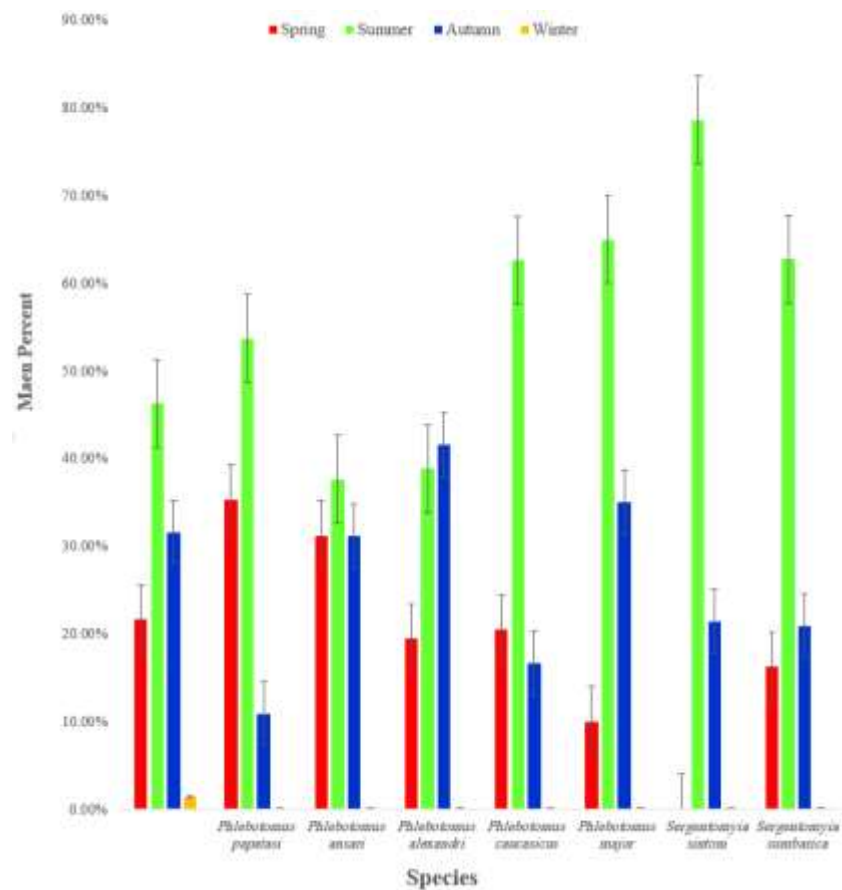


Fig. 3. Seasonal variation in the relative abundance of *Phlebotomus* and *Sergentomyia* species in Mashhad City, Iran, during 2022. Data are shown as mean $\pm$ standard error (SE)

Discussion

This study, conducted from spring to winter 2022, resulted in the collection and identification of eight sand fly species in Mashhad, including six species of the genus *Phlebotomus* and two of the genus *Sergentomyia*. Some of these species are recognized as primary or secondary vectors of leishmaniasis worldwide. Our findings indicate that *Ph. sergenti* is the dominant species in the study area and is known as the main vector of ACL, consistent with previous studies conducted in this region (30, 32–36, 40). *Phlebotomus sergenti* was collected in all seasons and across all 108 sampling sites, highlighting its wide distribution in urban environments and its close association with human dwellings. This species is particularly prevalent in mountainous and foothill areas, often inhabiting older mud-constructed houses (26). Environmental factors were found to have a significant influence on sand fly abundance. In spring, increases in wind speed were associated with a reduction in the presence of sand flies, particularly *Ph. ansari*. During the summer season, higher average temperatures and wind speeds corresponded with a decline in the relative abundance of *Ph. sergenti* and *Ph. papatasi*. Similarly, *Ph. caucasicus* exhibited an inverse relationship with wind speed, where higher wind led to reduced abundance and lower wind speed was associated with increased numbers. These findings are important because these species are potential vectors of *L. major* and other leishmaniasis parasites, indicating that Mashhad may represent a potential focus for both ZCL and VZL transmission (41).

In autumn, decreasing relative humidity and rising temperatures promoted an increase in *Ph. sergenti*, whereas *Ph. papatasi* abundance decreased with higher relative humidity. These results suggest that these *Phlebotomus* species are sensitive to seasonal changes in humidity and temperature, with *Ph. sergenti* thriving under drier and warmer conditions. Despite winter being generally less favorable for sand fly survival, *Ph. sergenti* remained active. Tem-

perature increases led to higher abundance, while higher wind speeds reduced its presence. This demonstrates the species' ability to persist under adverse conditions and maintain its anthropophagic behavior, feeding on humans even when the climate is less favorable. Although *Se. sintoni* is not considered medically significant, its abundance increased during summer with higher temperatures and relative humidity. This species often co-occurred with *Ph. sergenti* in urban areas, suggesting potential ecological interactions that merit further investigation. Understanding these ecological associations is important for refining predictive models and developing targeted control strategies (42).

The continuous presence and abundance of *Ph. sergenti* across all seasons highlight its epidemiological importance. Given the high number of domestic and international visitors to Mashhad, the city represents a potential focus for leishmaniasis transmission. Therefore, implementing effective vector control measures is crucial. Optimal control programs should target periods when sand fly abundance is lowest to maximize efficacy. Increasing the number of sticky traps, both indoors and outdoors, especially in older buildings and hotels near the Imam Reza shrine, is recommended. Additionally, specialized studies on taxonomy, behavior, ecology, and periodic susceptibility testing are essential for effective management of sand fly populations.

In total, Mashhad hosts eight sand fly species out of the ten reported in Khorasan Razavi Province. The consistent presence and high abundance of *Ph. sergenti* indicate that the city is a potential focus for ACL. These findings provide valuable baseline information for monitoring sand fly populations, understanding environmental drivers of their abundance, and designing effective leishmaniasis control strategies in a highly visited urban and religious area.

Conclusion

Our study documents the diversity and seasonal dynamics of sand fly species in Mashhad, Iran, identifying eight species, with *Ph. sergenti* as the predominant and most epidemiologically significant vector. Environmental factors, including temperature, relative humidity and wind speed, shaped species abundance, yet *Ph. sergenti* persisted across all seasons, demonstrating its resilience. These findings underscore Mashhad as a potential focus for ACL and highlight the critical need for targeted vector control strategies in urban and densely populated areas. The data provide a valuable baseline for informing effective leishmaniasis surveillance and prevention programs.

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Ethical consideration

The study was approved by the Ethics Committee of Tehran University of Medical Sciences, Code number: IR.TUMS. SPH.REC. 1400.235.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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