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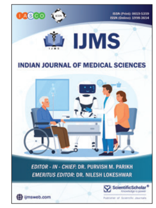

Original Article

Species diversity and vector competence of sand flies in an urban cycle of cutaneous leishmaniasis in Shiraz, Southwestern Iran

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ABSTRACT

Objectives: Detailed entomological profiling, including species composition and natural infection rates, is crucial for understanding transmission dynamics and designing effective control strategies in these unique ecosystems.

Materials and Methods: An integrated entomological survey was conducted in the historic urban district of Shiraz, southwestern Iran, during the 2024 active season. Sand flies were collected using a multi-method approach combining monthly deployment of adhesive traps, Centers for Disease Control miniature light traps, and manual aspiration from resting sites. Specimens were morphologically identified using taxonomic keys. A subset of female sand flies was analyzed for *Leishmania* infection via a nested polymerase chain reaction protocol targeting kinetoplast minicircle DNA.

Results: A total of 435 sand flies were collected. *Phlebotomus sergenti* was the dominant species, constituting 53.6% of the fauna, followed by *Phlebotomus papatasi* (34.0%), *Sergentomyia tiberiadis* (7.1%), and *Sergentomyia clydei* (5.3%). Spatial distribution was significantly biased toward outdoor environments (68.5% of captures). Activity followed a unimodal seasonal pattern, with a peak in the hot summer months (August). Molecular analysis of 112 females revealed a *Leishmania tropica* infection rate of 14.3% (7/52) in *P. sergenti*. No *Leishmania* infections were detected in *P. papatasi* or *Sergentomyia* species.

Conclusion: This study confirms *P. sergenti* as the predominant and competent vector of *L. tropica* in urban Shiraz. It's marked by exophily and a distinct seasonal peak, highlighting critical vulnerabilities in the transmission cycle. These findings underscore the necessity of moving beyond broad interventions to develop spatially and temporally targeted vector control strategies that address the specific ecology of *P. sergenti* in historic urban landscapes.

Keywords: Cutaneous leishmaniasis, Iran, *Leishmania tropica, Phlebotomus sergenti,* Species diversity, Urban transmission, Vector competence

INTRODUCTION

Cutaneous leishmaniasis (CL) remains a formidable global health challenge, with transmission dynamics intricately linked to the complex ecology of phlebotomine sand fly vectors.[1] The distribution and burden of this disease are largely dictated by the presence of competent vector species, their population dynamics, and their interaction with susceptible hosts within specific environmental niches.[2] Understanding these entomological parameters – species composition, seasonal abundance, and infection rates – is therefore fundamental to deciphering transmission cycles and designing effective, location-specific control programs.[3]

In urban and peri-urban landscapes, transmission ecology undergoes significant modification.[4] Urbanization alters microhabitats, creates novel breeding sites, and concentrates human populations, potentially shifting vector species dominance, prolonging seasonal activity, and facilitating new parasite-vector associations.[5] In the Middle East, including Iran, such environments have seen the stabilization of anthroponotic cycles, particularly those involving *Leishmania tropica*, yet the precise entomological drivers underpinning these urban foci are often inadequately characterized.[6] While Phlebotomus sergenti is recognized as a primary vector for *L. tropica*, and *P. papatasi* for *Leishmania* major, their relative abundance, seasonal phenology, and realized vector competence can vary dramatically between geographic locations and habitat types, influencing local disease epidemiology.[7,8]

A critical knowledge gap persists regarding the integrated entomological profile of CL vectors within historic urban centers, where unique architectural and socioecological

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Received: 10 August 2025 **Accepted:** 12 January 2026 **Published:** 17 March 2026 **DOI:** 10.25259/IJMS_248_2025

conditions prevail.[9] Comprehensive data on local sand fly species diversity is essential, as the presence of secondary or suspected vector species can complicate transmission networks.[10,11] Perhaps most important is the direct assessment of natural infection rates within vector populations, which provides definitive evidence of a species' role in transmission and can distinguish between active zoonotic, anthroponotic, or mixed cycles.[12,13]

In southern Iran, a region endemic for both anthroponotic cutaneous leishmaniasis (ACL) and zoonotic cutaneous leishmaniasis (ZCL), historic cities such as Shiraz represent significant and persistent foci of human disease.[14] Previous studies have indicated the involvement of *P. sergenti*, but a holistic analysis linking detailed species inventory, precise seasonal fluctuation, and molecular evidence of parasite infection within the context of an urban environment is lacking. Such an integrated entomological assessment is necessary to move beyond general assumptions and provide evidence-based insights for targeted intervention.

This study was therefore designed to elucidate the entomological foundations of CL transmission in the historic urban landscape of Shiraz. Specifically, we aimed to characterize the species diversity and relative abundance of phlebotomine sand flies and evaluate the vector competence of the predominant species through molecular detection of *Leishmania* DNA. By synthesizing these data, this investigation seeks to clarify the vectorial capacity driving the urban cycle of CL in this important endemic focus.

This study comprehensively evaluates the CL transmission cycle in Shiraz. Using a multidisciplinary approach to characterize the faunistic distribution and infection rates of sand fly vectors across urban microhabitats.

MATERIALS AND METHODS

Study area

The study was conducted in Shiraz, the capital of Fars Province in southwestern Iran (29°36′N, 52°32′E). Shiraz has a semi-arid climate characterized by hot summers and mild winters. The survey focused on the historic urban district known for its dense residential buildings, narrow alleys, and aging masonry structures that provide favorable resting and breeding microhabitats for sand flies.

A multi-method entomological survey was conducted during 2024 to comprehensively assess the sand fly population within the study area. To ensure a robust representation of the sand fly community across different habitats and behaviors, complementary collection techniques were employed, including passive sticky traps and active light traps.

The primary method for monitoring seasonal abundance and distribution was the use of adhesive (sticky) traps. These were deployed monthly across nine fixed residential units. In each unit, 10 traps were placed indoors, and an additional 30 traps were positioned in adjacent outdoor microhabitats such as courtyards, storerooms, and alleyways. This standardized monthly effort of 120 traps (90 indoor, 30 outdoor) over the 9-month active season resulted in a total sampling effort of 1080 indoor and 360 outdoor trap-nights. Traps consisted of castor oil-coated white A4 paper sheets, which were collected after a 24-h exposure period.

To complement the passive trapping and to enhance the collection of phototactic species and potentially increase female catch rates, Centers for Disease Control (CDC) miniature light traps were utilized concurrently. On each sampling night, two CDC light traps were operated from dusk to dawn in peridomestic locations. This active collection method provides a valuable counterpoint to sticky trap data, as it can attract sand flies from a wider radius and is less selective toward highly mobile males.

In addition, manual aspiration using handheld battery-powered aspirators was performed for 30 min per site during each visit to collect resting sand flies from specific microhabitats such as wall cracks, crevices, and the bases of furniture, both indoors and outdoors. This method targets flies that may avoid other types of traps.

All collected specimens, regardless of the capture method, were processed uniformly. They were carefully removed from the traps or aspirator collection bags, cleared and preserved in Puri's medium, and subsequently mounted on microscope slides in Berlese fluid for detailed morphological examination.[15]

Morphological identification

Sand flies were identified to species level based on morphological characters of the head, pharynx, cibarium, and genitalia using established taxonomic keys for Iranian phlebotomine sand flies.[7,16] Species nomenclature followed the classification proposed by Killick-Kendrick.[3] Specimens were categorized by sex and genus (*Phlebotomus* or *Sergentomyia*), and their relative abundances were calculated as percentages of the total collection.

Molecular detection of *Leishmania* in sand flies

DNA extraction

Only female sand flies were processed for molecular analysis. Individual specimens were homogenized in 1.5 mL microtubes using sterile pestles. Genomic DNA was extracted using a modified protocol adapted from Aransay *et al.*[17] Briefly, 150 µL of lysis buffer (1% SDS, 25 mM NaCl, 25 mM EDTA) was added, and samples were incubated at 65°C for 30 min. After adding 100 µL of potassium acetate (3 M, pH 7.2) and incubation on ice, samples were centrifuged, and DNA was precipitated with absolute ethanol. The pellet was washed, dried, and resuspended in 50 µL of TE buffer.

Nested polymerase chain reaction (PCR) for *Leishmania* detection

A nested PCR targeting the conserved region of the kinetoplast minicircle DNA was performed to detect *Leishmania* infection.[18] The first amplification used external primers CSB2XF (5′-CGA GTA GCA GAA ACT CCC GTT CA-3′) and CSB1XF (5′-CTA TTT TAC ACC AAC CCC CAG TT-3′). Each 20 µL reaction contained 5 µL of DNA template, 10 µL of PCR master mix (AccuPower PCR PreMix, Bioneer), and 10 pmoL of each primer. Cycling conditions were: initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30 s, 55°C for 60 s, and 72°C for 90 s; final extension at 72°C for 10 min. For the second round, 2 µL of the primary product served as template with internal primers 13Z (5′-ACT GGG GGT TGG TGT AAA ATA G-3′) and LiR (5′-TCG CAG AAC GCC CCT ACC CC-3′) under identical cycling conditions.[19] PCR products were separated on 1.5% agarose gel, stained with Safe Stain, and visualized under UV light. A 650-bp band indicated *L. major,* and a 760-bp band indicated *L. tropica*. Positive controls (DNA from reference strains) and negative controls (no-template reactions) were included in each run.

Statistical analysis

Species diversity was expressed as relative abundance. Seasonal activity was analyzed by plotting monthly capture data. Vector competence was assessed as the proportion of infected females among the tested specimens of each species. Descriptive statistics were performed using Statistical Package for the Social Sciences v.26.

RESULTS

Sand fly collection and species composition

A total of 435 sand fly specimens were collected during the 2024 survey period using the integrated multi-method approach. Standardized sticky trapping accounted for the majority of captures (n = 390, 89.7%), providing the core dataset for seasonal and spatial analysis. The complementary active methods – CDC light traps and manual aspiration – contributed an additional 45 specimens (10.3%), enhancing the collection of phototactic species and flies from cryptic resting sites.

Morphological identification of all specimens revealed the presence of four species belonging to two genera [Table 1].

The genus *Phlebotomus* was dominant, comprising 88.5% (n = 385) of the total fauna. Within this genus, *P. sergenti* was the most abundant species, representing 53.6% of all sand flies collected. *Phlebotomus papatasi* was the second most frequent species (34.0%). Specimens from the genus *Sergentomyia* were less common, with *Sergentomyia tiberiadis* and *S. clydei* accounting for 7.1% and 5.3% of the total, respectively [Figures 1 and 2].

Spatial and temporal distribution

Spatial analysis revealed a significant difference in sand fly density between environments (χ^2 = 42.1, P < 0.001). The majority of the specimens (68.5%, n = 298) were collected from outdoor sites, including courtyards and alleyways, compared to 31.5% (n = 137) captured indoors.

Adult sand fly activity exhibited a distinct unimodal seasonal pattern. No specimens were collected during the winter months (December to February). Activity commenced in April, increased sharply to a peak in August, and declined through September and October, ceasing entirely by November. *P. sergenti* and *P. papatasi* followed this general pattern, with their highest abundances recorded in the summer months.

Table 1: Species composition and relative abundance of sand flies collected in the historic urban district of Shiraz, 2024.

Species	Number	Percentage
Phlebotomus sergenti	233	53.6
Phlebotomus papatasi	148	34.0
Sergentomyia tiberiadis	31	7.1
Sergentomyia clydei	23	5.3
Total	435	100.0

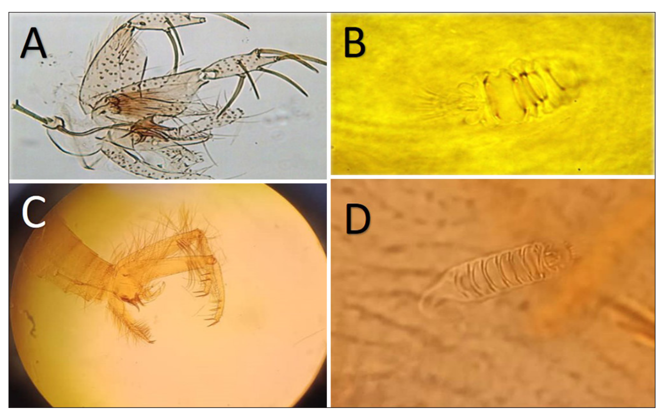

Figure 1: Sand flies caught in Shiraz from southwestern Iran during 2024; (A) Male genitalia of *Phlebotomus sergenti*, (B) Female spermatheca of *P. sergenti*, (C) Male genitalia of *Phlebotomus papatasi*, (D) Female spermatheca of *P. papatasi.*

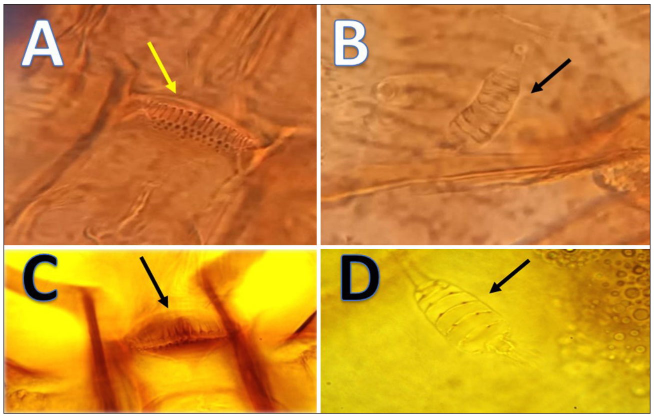

Figure 2: Sand flies caught in Shiraz from southwestern Iran during 2024; (A) Ciborium teeth of *Sergentomyia tiberiadis* (yellow arrow), (b) Female spermatheca of *S. tiberiadis* (black arrow), (C) Ciborium teeth of *Sergentomyia clydei* (black arrow), (D) Female spermatheca of *S. clydei* (black arrow).

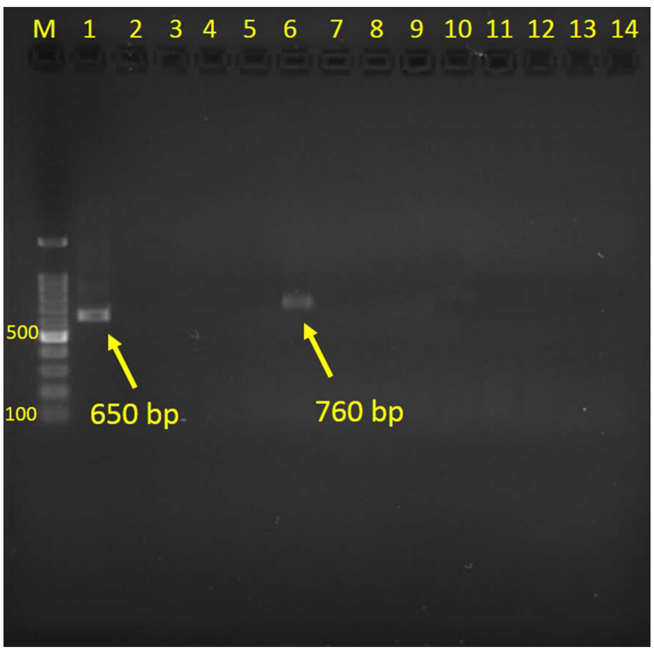

Figure 3: Representative agarose gel electrophoresis of nested polymerase chain reaction products for *Leishmania* detection in sand flies. Lanes: M, 100-bp DNA ladder; 1, *Leishmania major* positive control (650 bp); negative control; 6, *Leishmania tropica* positive *Phlebotomus sergenti* field samples (760 bp); Lanes 2–5, negative *Phlebotomus papatasi* field samples; Lanes 7–14, negative *P. sergenti* field samples.

Molecular detection of *Leishmania* and vector infection rates

A subset of 112 female sand flies, encompassing all collected species, was analyzed for natural *Leishmania* infection through nested PCR. Infection was detected exclusively in the predominant vector species, *P. sergenti*. Out of 52 female *P. sergenti* tested, 14.3% (*n* = 7) were found positive for *Leishmania tropica*, as indicated by the 760 bp amplification product [Figure 3]. No infections with *Leishmania major* (650 bp band) were detected in any *P. sergenti* specimens.

All tested specimens of *P. papatasi* (*n* = 41), *S. tiberiadis* (*n* = 12), and *S. clydei* (*n* = 7) were negative for both *Leishmania* species. The overall infection rate within the total tested female sand fly population was 6.25% (7/112).

DISCUSSION

This integrated entomological investigation provides a detailed characterization of the sand fly fauna implicated in the urban transmission of CL in Shiraz. The application of complementary collection methods offers a more comprehensive profile of the local vector community than single-method surveys typically afford, while molecular screening delivers direct evidence of vector–parasite associations.

Our findings confirm *P. sergenti* as the predominant and competent vector of *L. tropica* in this historic urban landscape. Its numerical dominance (53.6% of total captures) aligns with its recognized role as the primary vector of anthroponotic CL in other Iranian cities such as Isfahan and Kerman.[20,21] The significant outdoor abundance of sand flies, particularly *P. sergenti*, underscores a critical ecological trait. This exophilic tendency, documented in other urban foci,[3] suggests that peridomestic environments – courtyards, alleyways, and external wall surfaces – serve as major resting and possibly breeding sites. This has direct implications for control, indicating that interventions focused solely on indoor residual spraying may be insufficient. Outdoor space spraying or targeted environmental management, though logistically challenging in dense urban settings, should be considered during peak transmission months.[15]

The multi-method collection strategy proved valuable in capturing different facets of sand fly behavior. While sticky traps provided a standardized measure of seasonal abundance, the supplementary use of CDC light traps and aspiration helped sample species and physiological states (e.g., host-seeking females) that may be underrepresented by passive traps alone. This methodological triangulation strengthens the reliability of the species inventory and abundance estimates, a point emphasized in recent guidelines for vector surveillance.[22]

The distinct unimodal seasonal activity, peaking in the hot, dry summer months (April–October), is consistent with the known temperature-dependent biology of *Phlebotomus* species in Iran's semi-arid climate.[8] The correlation between rising temperatures and increased sand fly activity creates a predictable, narrow window of high transmission risk. This seasonality provides a clear temporal target for intensifying vector control measures, such as public awareness campaigns and the strategic application of insecticides, ideally just before and during the peak activity period.[11]

The molecular detection of *L. tropica* in 14.3% of female *P. sergenti* is the most critical finding, confirming its vector competence and active participation in the parasite's transmission cycle within the study area. This infection rate falls within the range reported for *P. sergenti* in other *L. tropica* foci in the Middle East.[8] The absence of *L. major* in the tested *P. papatasi* population, despite its substantial presence (34% of the fauna), is noteworthy. This suggests that while *P. papatasi* – the classic vector of rural zoonotic *L. major* cycles – is well-established in urban Shiraz, but it may not be actively transmitting *L. major* within this specific historic district. This could indicate a spillover of the vector from surrounding rural areas without the concomitant establishment of the zoonotic parasite cycle, or a very low, undetected level of enzootic transmission. The complete lack of *Leishmania* detection in *Sergentomyia* species, consistent with their generally accepted role as non-vectors of mammalian leishmaniasis in the Old World, further focuses attention on *Phlebotomus* species.[23,10]

Some limitations of this entomological study warrant consideration. The overall sand fly density, though thoroughly sampled, was moderate. This may reflect the true urban ecology of the site or the efficiency of the chosen collection methods. Future studies could benefit from incorporating additional techniques, like animal-baited traps, to better assess host-seeking rates. Furthermore, while the sample size for molecular analysis was sufficient to demonstrate infection, larger-scale screening across multiple seasons would provide more robust estimates of temporal variation in infection rates.

Data availability: The data supporting the findings of this study are available within the article.

CONCLUSION

This study delineates the entomological backbone of urban CL in Shiraz. It establishes *P. sergenti* as the key vector species, highlights its exophilic behavior and summer peak activity, and provides molecular confirmation of its role in transmitting *L. tropica*. These findings shift the intervention paradigm from a general, broad-based approach to a more precise strategy. Effective control should prioritize *P. sergenti* through integrated methods that target both indoor and outdoor habitats, with efforts concentrated during the predictable summer peak. This vector-centric evidence forms an essential foundation for public health authorities aiming to disrupt transmission in this and similar historic urban endemic foci.

Acknowledgment: Our appreciation goes to the staff at Shiraz School of Health. This research was undertaken under the auspices of Shiraz University of Medical Sciences (Approval No. IR. SUMS. SCHEANUT.REC.1402.153).

Ethical approval: The research/study was approved by the Institutional Review Board at Shiraz University of Medical Sciences, number IR. SUMS.SCHEANUT.REC.1402.153, dated 2023.

Declaration of patient consent: Patient's consent not require as there are no patients in the study.

Financial support and sponsorship: This research was funded by Linerase®, grant number Rep. n. 49/2023 Prot. N. 0121007 del June 05, 2023.

Conflicts of interest: There are no conflicts of interest.

Use of artificial intelligence (AI)-assisted technology for manuscript preparation: The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for assisting in the writing or editing of the manuscript, and no images were manipulated using AI.

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How to cite this article: Jokar A, Kalantari M, Soltani A, Asgari Q, Rezaee E, Nasiri Z, *et al*. Species diversity and vector competence of sand flies in an urban cycle of cutaneous leishmaniasis in Shiraz, Southwestern Iran. Indian J Med Sci. 2026;78:6-11. doi: 10.25259/IJMS_248_2025