

Original Article:

Morphological Identification of Sandflies and Their Species' Diversity

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ABSTRACT

Introduction: Leishmaniasis is a zoonosis disease, presented in three forms including cutaneous, mucocutaneous, and visceral (kala-azar). The parasite, *Leishmania* spp, is transferred by the causative agent of the disease, namely the sand-fly. Accurate identification of sand-flies helps to prevent and treat the disease. **Materials and Methods:** This study was conducted to identify sand-fly species distributed in Birjand, east of Iran. Sand-flies were collected from different parts of Birjand city, Iran through using sticky traps in summer 2015. Captured female specimens were mounted and identified by specific keys. **Result:** In this study, a total of 1480 samples of sand-flies were collected, out of which 945 sand-flies in areas were related to animal and 535 sand-flies in areas related to human. The most prevalent species, among 654 collected female specimens were *Phlebotomus sergenti* (22.17%) and *Sergentomyia dentata* (49.54%). **Conclusion:** According to the current results, the dominant species among disease vectors of cutaneous leishmaniasis is the urban type. Because of the presence of *Leishmania tropica*, the city's health system must take necessary care to control cutaneous leishmaniasis.

Keywords: Sand-flies, Cutaneous Leishmaniasis, *Phlebotomus sergenti*, *Leishmania*

INTRODUCTION

Sand-flies (*Diptera: Psychodidae, Phlebotominae*) are of significant public health importance in many parts of the world as vectors of the causative agents of leishmaniasis, bartonellosis and sand-fly fever [1]. Sand-flies are the only proven vectors of leishmaniasis with approximately 800 species being recorded in five major genera; *Phlebotomus* and *Sergentomyia* in the Old World, and *Lutzomyia*, *Brumptomyia* and *Warileya* in the New World [2]. However, the only assigned species in *Phlebotomus* and *Lutzomyia* are the putative vectors of *Leishmania* [2, 3]. Leishmaniasis remains one of the most neglected diseases across the world. It is endemic in 98 countries, putting 350 million people at risk, with about two million new cases every year [4]. The disease is considered as the most important vector-borne disease in Iran. It is endemic in 17

out of 31 provinces of the country [5]. Studies on the fauna of *phlebotominae (Diptera: Psychodidae)* sand-flies of Iran indicated that two genera, *Phlebotomus* and *Sergentomyia* comprising 44 species are present in the country. *Phlebotomus papatasi* and *Ph. sergenti* are introduced as vectors of Zoonotic cutaneous leishmaniasis (ZCL) and Anthroponotic cutaneous leishmaniasis (ACL) to the human in the city, respectively [5, 6]. Cutaneous leishmaniasis (CL) is one of the increasing infections in Iran as it is almost doubled (from 11,505 to 22,705 cases) over a nine-year period in 2001–2009 [7]. The annual cases of CL reported from South Khorasan province reflect that their origin is mainly from neighboring provinces. Traveling and immigration pattern in line with the geographical and climatic conditions in South Khorasan province creates the favorable location

for the vectors activity of leishmaniasis so that during 2008 and 2009, 1.14 and 9.24 percent of people were infected in South Khorasan province, respectively. Moreover, according to Shayeste, et al. in 2011 the rate of the disease detected in the province increased by 2.2% compared with the previous year [8]. Therefore, identification of sandflies in order to fight the leishmaniasis vectors and control the disease at the provincial level seems critical.

The aim of this study was to correct sand-fly species identification is very important for designing strategies for surveillance and control of leishmaniasis in given endemic areas.

MATERIALS AND METHODS

We started sandfly collection after analyzing the study area. The following formula is used to determine the prevalence of Leishmaniasis infected embryos and also to determine the species of sand-flies:

$$N = \frac{Z^2 p(1-P)}{d^2} = \frac{1.96 \times 1.96 \times 0.2 \times 0.8}{0.02 \times 0.02} = 600$$

N=Sample size Z=Coefficient of confidence
p=Specific precision d=Error rate.

Study area

Khorasan-e-jonoobi province with 151,193km² covers 9.17% of the total area in Iran. It is located in the East of the country, and its provincial capital is the city of Birjand (coordinates:59°13"N, 32°53"E; 1491 m over sea level) (Figure1). The county had a population of approximately 259,506 out of which 88.63% resided in urban areas and 11.37% in rural vicinities in 2012. The county contains one city, six rural districts, and 380 villages. The climate of Birjand city is of a desert and semi-desert climate type and comprises mountainous areas, foothills, and plains. Due to its location (located near an arid region), it experiences a dry climate, with low humidity and rainfall. In 2014, the average of maximum and minimum mean temperatures in different stations across the province was reported as 24°C and -8°C in July and January, respectively. The average relative humidity is 33% [9].



Figure 1. Location of Birjand city in Khorasan-e-jonoobi province

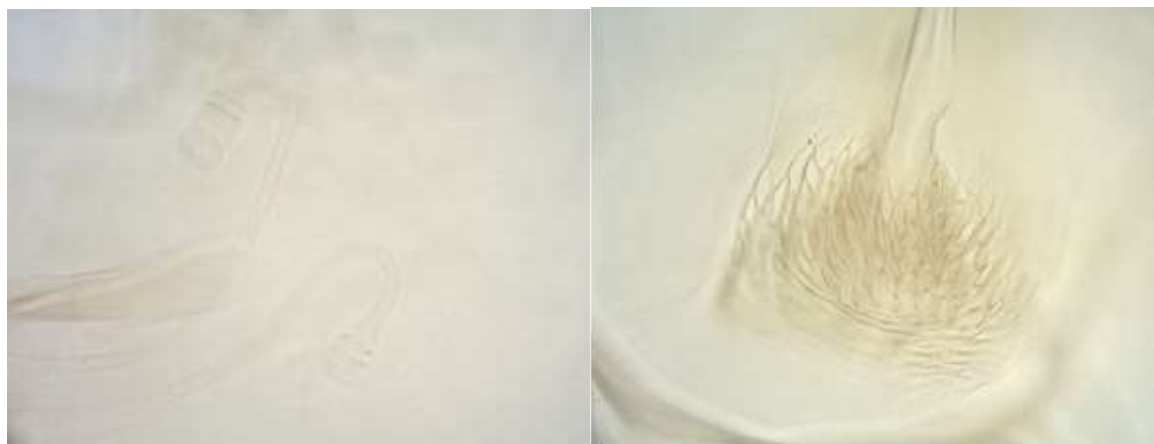
Sand-fly Collection

The collections were carried out in 2015, during the activity seasons of adult sandflies. All studied locations were at the edge of rural villages on the cultivated plains, mostly at the

edges of 3 regular sampling villages and 3 random ones, near Birjand. Three types of traps were used for collecting sandflies; first, sticky papers which were placed overnight in bedrooms, storerooms, toilets, and ruined outhouses and the

entrances to gerbil burrows, and second CDC miniature light traps which were set overnight to sample sandflies in domestic animal shelters; and third, a manual aspirator, sometimes used by a single collector to capture sandflies resting inside houses in mornings [10]. For statistical analysis, SPSS software was used. Specimens were then defatted by acetone and stored in the vials containing 70% ethanol. To identify sandflies, permanent assembly of Puri solution was used.

The only diagnostic morphological characters, namely Identification of species of sand-flies was conducted by examining the pharyngeal armature and spermathecae of females and examining cibarium teeth and other important morphological characteristics, based on valid diagnostic keys (Figure 2,3) [11-13]. This study was approved by the vice-chancellor for research of Birjand University of Medical Sciences (No: 1124).



(A) **(B)**
Figure 2. (A) Spermatheca of sand-flies *Ph. sergenti*, (B) pharyngeal armature of sand-flies *Ph. sergenti*



Figure 3. The middle teeth in cibarium, shorter than lateral ones at *S. dentate*

RESULTS

This study was an experiment conducted on the sandflies in Birjand, Iran. Out of 1480 sandflies, samples were collected and identified from different villages of Birjand. A total of 945 sandflies were captured from outdoors including gerbil borrowers, yards, ruins and cave crave; and

535 sandflies were captured from indoors including animal shelters, bedrooms, and bathrooms. The total 826 males and 654 female sandflies were identified through morphological characters. Males are not involved in the transmission cycle of the parasite since male sandflies are not blood feeder. Therefore, males

had no pathogenic importance and were not identified and were excluded from the study. The identified female species belonged to two genus *Phlebotomus* and *Sergentomyia*, with 42.36 percent belonging to the genus *Phlebotomus* and 57.64 percent to the genus *Sergentomyia*.

The genus *Phlebotomus* included *Ph. sergenti*, *Ph. papatasi*, *Ph. caucasicus* group, *Ph. major* group, *ph. salehi*, *ph. kazeruni* and *Ph. Alexandri*. *Ph. sergenti* with a frequency of 22.17 percent was the most frequent; in other words, it was the predominant species of the genus *Phlebotomus* in the studied area. *Ph. alexandri* with 0.31 percent had the least abundance. The other species had

the following abundance: *Ph. papatasi* with 7.95 percent, *Ph. caucasicus* group with 7.03 percent, *Ph. the major* group with 2.45 percent, *Ph. salehi* with 1.83 percent and *Ph. kazeruni* with 0.61 percent in the region. The two species *S. dentata* and *S. sintoni* belong to the genus *Sergentomyia* with the frequency of 49.54 percent and 8.1 percent in the region, respectively (Figure 4). Among examined females, 654 (74.16 percent) were found with an empty stomach, 11.93 percent pregnant, 2.45 percent semi-pregnant and 11.47 percent with collected blood. They are shown in table 1.

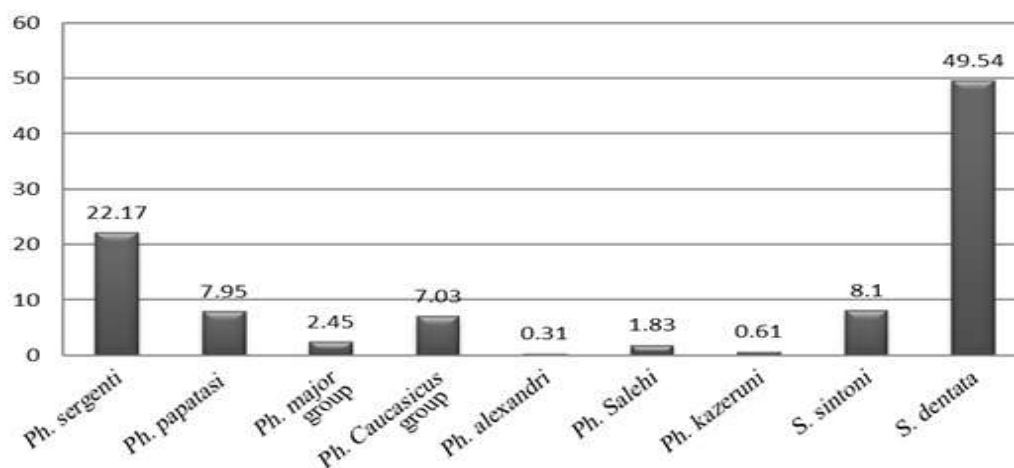


Figure 4. Distribution of sand-flies in the studied area, Birjand city during 2015

Table 1. Number and species of blood-fed phlebotominae sand-flies captured, using sticky traps in the locality of Birjand city during 2015

Percent	No.	Stomach situation				Sand-flies species
		empty	Blood-eaten	Semi Pregnant	Pregnant	
22/17	145	69(%47/58)	36(%24/83)	9(%6/21)	31(%21/38)	<i>Ph. sergenti</i>
7/95	52	29(%55/77)	11(%21/15)	2(%3/85)	10(%19/23)	<i>Ph. papatasi</i>
7/03	46	41(%89/13)	2(%4/35)	1(%2/17)	2(%4/35)	<i>Ph. caucasicus group</i>
2/45	16	9(%56/25)	2(%12.5)	1(%6/25)	4(%25)	<i>Ph. major group</i>
1/83	12	12(%100)	-	-	-	<i>Ph. Salehi</i>
0/61	4	4(%100)	-	-	-	<i>Ph. kazeruni</i>
0/31	2	-	1(%50)	-	1(%50)	<i>Ph. alexandri</i>
8/1	53	278(%85/8)	20(%6/17)	2(%0/62)	24(%7/41)	<i>S. dentata</i>
49/54	324	43(%81/13)	3(%5/66)	1(%1/89)	6(%11/32)	<i>S. sintoni</i>
100	654	485(%74/16)	75(%11/47)	16(%2/45)	78(%11/92)	Total

DISCUSSION

Our study showed that species diversity of sandflies in Birjand is more than the amount

listed in previous studies. Identification of the sand-fly vectors is crucial for any leishmaniasis control programs. This study also showed that the

primary vector of CL in Birjand is *Ph. sergenti*. This species was the most prevalent and the most infected *Phlebotomus* sand-fly species with the parasite *L. tropica* in this region and neighboring countries [14]. On the other hand, *S. dentata* was the most abundant sand-fly in Birjand city; however, it is not a competent vector for CL. It is already shown that this species is the principal vector for CL in the Old World, and has a relatively high preference for feeding on human [15]. Studies also showed a direct relationship between the abundance of sandflies and CL disease [16, 17]. It can be argued that sandflies species (*Ph. sergenti*) leishmaniasis is the main vector in the city of Birjand introduced. In this study, 9 species of sandflies (seven *Phlebotomus* and two *Sergentomyia* species) were collected and identified, among which *Ph. Sergenti*, *Ph. papatasi*, *Ph. alexandri*, *ph. salehi* and *ph. Kazeruni* are defined as proven vectors of leishmaniasis all across the world [18]. It is also proved that some species such as *S. dentata* and *S. sintoni* play as vectors of Lizard Leishmaniasis [19-21]. A study conducted by Moghaddam et al. on 174 collected sandflies from Khorasan-e-jonoobi province reported the occurrence of species *Ph. sergenti*, *Ph. papatasi*, *S. dentata* and *S. sintoni* and due to the specificity of the current study, more species diversity was observed. In a study conducted in the north of Mashhad, Berenji et al detected species *Ph. sergenti*, *Ph. papatasi*, *S. sintoni* and *S. sumbarica* and introduced *Ph. sergenti* as dominant species [22]. Additionally, Mehrabi Tavani et al in their study conducted in Taybad city in Khorasan Razavi province showed that the species *Ph. sergenti* is dominant species in this region [23]. This result is very similar to the results of the present study; perhaps the most important reason for this similarity is the almost-same climatic conditions of different regions in South and Razavi Khorasan province. However, in a similar study conducted by Behravan et al in Varamin city, *Ph. papatasi* was introduced as dominant species [24, 25]. These results are not in line with our findings, since different weather condition exists in our studied area. In this study, sand-flies vectors of lizard leishmaniasis, belonging to the

genus *Sergentomyia*, were also trapped from the studied areas. As these areas were rural areas and residential houses, located in the vicinity of farms and colonies of rodents, it has found a way to human areas [26].

In some countries, some species belonging to this genus are isolated as *L. major* parasites, which in terms of pathogenicity is considered as important [27]. Although the pathogenic pattern of this species (*S. dentata*) is not clear yet, higher species diversity in a studied area demands specific concern in epidemiological studies. Most captured female sand-flies had an empty stomach (16.74%). The empty stomach of sand-flies can be due to the lack of blood feeding and digestion. Empty stomach conditions show that parasite has passed blood digestion stages behind, and more importantly, it has been able to get rid of Peritrophic membrane (PM) and remain in the digestive tract [28]. Findings of the current study can take further steps in planning and stratification of potential risk for CL transmission in Birjand city.

CONCLUSION

These results will be a part of Cutaneous Leishmaniasis database of the Khorasan-e-jonoobi province and need to be completed by data on spatial and temporal distribution of human cases as well as fauna and of reservoir hosts.

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"The authors declare no conflict of interest"

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