Original Article

Bioecological Study on the Sand Flies (Diptera: Psychodidae, Phlebotominae) in Sari County, North of Iran

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Abstract

Background: Phlebotomine sand flies are vectors of *Leishmania* species, the causative agents of leishmaniasis in the world. Present study aimed to evaluate the bioecological aspects of sand flies in different ecotopes in Sari County, north of Iran.

Methods: Sand flies were collected from four villages in mountainous, forest, plain and peri-urban areas monthly using sticky traps in May–October 2016. Mounted specimens were identified using valid identification keys under optical microscope. The Arc GIS 10.5 software was applied for showing the distribution of sand flies. Shannon-Weiner, Simpson and Evenness species diversity indices were calculated.

Results: Generally, 334 specimens were captured and identified, namelly *Phlebotomus kandelakii*, *Ph. papatasi*, *Ph. major*, *Ph. sergenti*, *Ph. longiductus*, *Ph. halepensis*, *Ph. tobbi*, *Sergentomyia dentata*, *Se. theodori*, *Se. sintoni*, *Se. antennata* and *Se. sumbarica*. The most common species was *Ph. kandelakii* (n= 128, 38.32 %). The highest Simpson index (0.81) and abundance (N= 141) were recorded in the mountaineous area. Shannon diversity index was higher in the forest (H'= 1.53) and the highest evenness index was in the plain area (J'= 0.93). The highest richness (S= 9) and Shannon indices (H'= 1.57) were observed in June.

Conclusions: *Phlebotomus kandelakii*, *Ph. sergenti*, *Ph. tobbi*, *Ph. longiductus*, *Se. theodori*, *Se. antennata* and *Se. sumbarica* were recorded for the first time in the study area. Since some species are incriminated for leishmaniasis transmission, further studies are required in the northern regions of Iran to timely control measures planning.

Keywords: Sand fly; Ecology; Biodiversity; Northern Iran

Introduction

Sand flies belong to the order Diptera, suborder Nematocera, family Psycodidae and subfamily Phlebotominae (1, 2). They are vectors of various types of leishmaniasis. It is a neglected tropical disease caused by obligate protozoan parasites of the genus *Leishmania* (Trypanosomatida: Trypanosomatidae) transmitted by the bites of infected female sand flies that host animals such as canids, rodents, marsupials, hyraxes or humans (2, 3). Leishmaniasis is endemically extended to extensive areas of the tropics, subtropics and Mediterranean basins of about

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102 countries, with a total of 350 million people at risk and 12 million infected (2, 4, 5). Almost 53 species of *Leishmania* have been presented in diferrent parts of the world. Of these, 31 species are mammalian parasites, and 20 species are pathogenic to humans. *Leishmania* parasites cause four main clinical types of visceral, cutaneous, diffuse cutaneous and mucocutaneous leishmaniasis (6). Cutaneous leishmaniasis is the well-known form of the disease and is estimated to occur 87% of its global incidence in ten countries including Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Libya, Tunisia, Pakistan and Iraq (7).

Phlebotomine sand flies are belonging to two genera of *Phlebotomus* in the old world and *Lutzomyia* in the new world. According to the latest classification, 53 species of sand flies have been documented from different parts of Iran, including 34 *Phlebotomus* species of six subgenera and 19 *Sergentomyia* species of six subgenera (8). *Phlebotomus sergenti sergenti* and *Ph. sergenti similis* were considered as subspecies, and the presence of *Ph.* (*Adlerius*) *kabulensis*, *Ph.* (*Adl.*) *salangensis*, *Ph.* (*Adl.*) *turanicus*, *Ph.* (*Larroussius*) *langeroni*, *Ph.* (*Transphlebotomus*) *mascittii*, *Ph.* (*Lar.*) *smirnovi*, *Ph.* (*Euphlebotomus*) *caudatus* and *Se.* (*Grassomyia*) *indica* were doubtful in the country (9). *Phlebotomus* (*Adl.*) *comatus* is reported as the new species from northwest of the country by Zahraei-Ramazani et al. (10).

Sand flies are commonly observed and adapted to natural environments (human and animal places), especialy *Ph. papatasi* and *Ph. sergenti*. This may be advantageous due to the potential breeding and resting sites and easy food source, which can ultimately enhance the risk of transmitting pathogens to humans and domestic animals (11). Also, various environmental and physiological conditions of sand flies can influence their capacity to transmit the parasite (12). Therefore, understanding the biological and ecological aspects of sand flies is essential.

Geographic information systems (GIS) are software tools regarded to determine the probability of presence, biodiversity, distribution and abundance of vectors and to predict the risk map of vector-borne diseases (13, 14). Spatio-temporal epidemiological researches by GIS can be applied to predict disease distribution in the regions at risk (15). GIS technique is also applied for insect ecology by combining climatic and insect parameters to assess pest risk areas (16).

Biodiversity is the set of differences between organisms at all levels of the life spectrum, from genes and species to higher levels of taxonomy, including habitat and ecosystem types, and is represented in different types of Alpha (α), Beta (β) and Gamma (𝛶). It is an important ecological issue because changes in the biodiversity of communities can propably lead to the emergence of some dominant species, create new ecological niches for proliferation of them, and enhance the prevalence of sand fly-borne diseases (17). No studies have been conducted on the biodiversity of sand flies in Mazandaran Province. Therefore, the study of biodiversity in the region is momentous and can clarify the transmission of disease to humans and reservoirs.

Mazandaran Province is considered as one of the sporadic foci of leishmaniasis. In previous studies, sporadic cases of cutaneous and visceral leishmaniasis have been documented in some parts of the Province (18-20), some of which have a history of travel to endemic areas of leishmaniasis. Since Mazandaran Province borders with the provinces that are endemic foci of the disease, disregarding the characteristics of the vectors can lead to an increase in the incidence of this disease. Few studies have been carried out in the area to identify vectors that complete the disease cycle. Therefore, this study focuses on collecting sand flies in Sari County of Mazandaran Province and identifying their ecological and biological characteristics. This data set will serve as the basis for other epidemiological studies and the design of disease control strategies.

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Materials and Methods

Study area

This descriptive cross-sectional study was conducted from May to October 2016 in Sari County, the capital of Mazandaran Province, which is located in the central part of the province, on the foothills of Alborz Mountains in north of Iran with a longitude of 53°5′ and a latitude of 36°4′. The county is divided into two major mountainous and plain areas. Sari has a moderate and humid climate in summer and a relatively cold climate in winter. The southern mountainous part of the county has rather long and very cold winters. The average daily temperature and annual rainfall were reported 17.7 °C and 824.4mm, respectively. The maximum and minimum temperatures were in August (36.6 °C) and December and February (2.7 °C), respectively. The maximum and minimum average daily relative humidity were 82% in November and 72% in September, respectively.

In this study, sampling areas were selected in such a way that appropriate coverage in the entire study area is ensured by considering the diversity in terms of geography and type of ecosystem. Accordingly, four villages were selected from each mountainous (Era), forest (Shekta), plain (Kordkheil) and, peri urban (Zoghal Chal) areas of Sari County (Fig. 1). In each area, altitude, longitude and latitude were recorded using a global positioning system (GPS) device. In addition, due to the lack of information about the distribution of leishmaniasis vectors in Sari, the areas in which the health center had reported the admission of native leishmaniasis patients in the past years, received more attention.

Sand fly collection (Sampling) and identification

In this study, sampling was conducted once a month to determine the seasonal and monthly activity of sand flies from the beginning to the end of the sand fly activity season. Specimens were collected using sticky paper traps coated with castor oil. During sampling in each selected area, three permanent stations were selected for trapping and 60 sets of sticky traps were installed per station: 15 traps in human indoor places (bathrooms, bedrooms, warehouses), 15 in animal indoor places (stables, poultry houses, animal habitats) and 30 in outdoors (yards) before sunset and picked up the next morning before sunrise. Sand fly specimens were taken from sticky paper using a fine needle, washed in acetone, and stored in small vials with 70% ethyl alcohol, then transferred to the Department of Entomology, Faculty of Health, Mazandaran University of Medical Sciences for determination of abdominal condition, mounting and identification. The main morphological characters used in this study were pharyngeal armature, cibarial teeth, female spermatheca and male genitalia. To determine the physiological condition of the abdomen, each sample was dissected separately in physiological serum using dissecting needles under a stereomicroscope. To prepare a permanent microscopic slide, the head and the last three segments of the abdomen were seperated. The isolated pieces were then mounted on a clean slide in Puri medium. In the case of the genus *Phlebotomus*, the head was mounted upwards and in the case of the genus *Sergentomyia*, the head was mounted downwards on the slide (21). The mounted slides were immediately transferred to an incubator at 37 °C. After drying the slides were identified according to valid morphological keys (22).

GPS points of the collected sand flies were used as a layer for analysis. Arc GIS 10.5 software was used to prepare the distribution map of sand flies in the study area. The GIS layer containing sand fly data was transferred to Arc Map which is the main component of Arc GIS software for geospatial processing.

Statistical analyzes were performed using SPSS v.20 (IBM, New York, USA). Nonparametric Chi-Square and Binomial statistical tests were used to compare the data. P values less than 0.05 were considered significant.

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Biodiversity of species and statistical analyses

The number of taxa (S), number of individuals (n), Simpson's diversity index (D), Shannon's diversity index (H) and Pielou's evenness index (J) were calculated to estimate Alpha-biodiversity with the following equations:

Shannon index: $H' = -\sum P_i \times lnP_i$

Where, p_i is the relative abundance of the i^{th} species (n/N), N; the total number of collected individuals.

Evenness index (Pielou's index formula): $J' = H'/log(S)$

Where, H′ is the Shannon-Wiener function and *S* is the total number of species observed.

Simpson's index:

$$1 - D = 1 - \sum_{i=1}^{s} P_i^2$$

where, $P_i=n_i/N$; n_i, the relative abundance of a species calculated as the proportion of individuals of a given species to the total number of individuals in a community, N (23). Software past- v.3 (Paleontological Statistics Software Package) was applied to perform whole developed calculations (24).

Results

A total of 334 sand flies were collected using sticky traps from May to October 2016 in the study area. Two hundred and four (61. 07%) specimens belonged to genus *Phlebotomus* and 128 (38.32%) specimens to genus *Sergentomyia,* including six subgenera of which 146 (43.71%) subgenus *Larroussius*, 123 (36. 82%) *Sergentomyia*, 52 (15.56%) *Phlebotomus*, 2 (0.52%) *Adlerius*, 1 (0.29%) *Paraphlebotomus*, and 1 (0.29%) *Parrotomyia* (nine samples (2.69%) were undetectable) (Table 1).

In the present study, among 12 species of Phlebotominae, the most frequent *Phlebotomus* species was *Ph. kandelakii* (38.32%) followed by *Ph. papatasi* (15.56%) and *Ph. major* (5.08 %). *Sergentomyia dentate* with 22.45% (n= 75) was the most frequent *Sergentomyia* sand flies followed by *Se. theodori* (n= 27, 8.08%) and *Se. sintoni* (n= 15, 4.49%) (Table 1).

In terms of gender, out of 334 phlebotominae, 194 (58.08%) were identified male and 140 (41.91%) were female. The male to female ratio was 1:0.72. This difference was statistically significant (P= 0.004).

From the perspective of abdomen physiological condition, among the 140 female sand flies, 18 (12.85%) were fed, 120 (85.71%) were unfed, and 1 (0.71%) was semi-gravid and the abdomen physiological condition was unclear in one sample because of color change (Table 1). This difference was significant (P= 0.004). The results showed that 107 (46.3%) sand flies were collected from human hosts and 124 (53. 7%) from animal hosts. In total, 231 (69%) sand flies were collected from indoors and 103 (31%) were from outdoors (P= 0.004). The most prevalent species were *Ph. kandelakii* and *Se. dentata* with rate of 34.19% and 23.8% in indoors, and 47.57% and 19.41% in outdoors, respectively (Table 1). Genus *Phlebotomus* was mostly observed in indoors (141/204, 69.1%) (P= 0.004). *Phlebotomus kandelakii*, *Ph. papatasi*, and *Ph. major* were caught more in indoor places, and consequently, they have more access to human and animal hosts (Table 1).

The activity of sand flies started in early-May and ended in late-October. The highest abundance of sand flies was observed in June (n= 102, 30.53%) and July (n= 155, 46.40%) (P= 0.004). Among the collected species, *Ph. papatasi* was active in all months of the year. The highest prevalence of *Ph. kandelakii* was observed in July (n= 124, 80%) and *Ph. papatasi* and *Se. dentata* in June (Fig. 2).

Analysis of alpha-biodiversity indices showed that the highest richness (S) was observed in Shekta area (S= 8) and in June (S= 9), while the lowest was found in Kordkheil (S= 3) and in October (S= 2). The highest value of Shannon was in Shekta (H'= 1.53) and evenness was in Kordkheil (J'= 0. 93) and both in May (H'= 1.57, J'= 0.95). The maximun values of Shannon were presented in Shekta and

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May, whereas Simpson index indicated that the highest dominance was in Era (D= 0.81) and in July (D= 0.67), due to the presence of the dominant species *Ph. kandelakii*. Other data on the status of sand fly biodiversity by region and month are shown in Table 2 and 3.

Figure 3 shows that *Ph. kandelakii*, *Ph. papatasi* and *Se. dentata* are the most prevalent species in Era, Zoghal Chal, Shekta and Kordkheil areas, respectively. *Phlebotomus longiductus*, *Ph. halepensis*, and *Se. sumbarica* were collected only from the mountainous area (Era) and the foothills. While *Ph. kandelakii* and *Ph. major* were collected from both mountainous area (Era) and forest (Shekta). *Phlebotomus papatasi* and *Se. antennata* were collected from both forest and peri urban areas (Zoghal Chal). *Sergentomyia theodori*, *Se. dentata*, and *Se. sintoni* were collected from three areas: the plain (Kordkheil), forest and peri urban. *Phlebotomus sergenti* was collected from forest and *Ph. tobbi* from peri urban area. Temporal distribution of *Ph. kandelakii* in the study region is shown in Figure 4. This species was not collected from study area in May.

Table 1. Phlebotomine sand flies according to species, sex, and collection places in Sari County, north of Iran, 2016

Species	Human places		Animal places		Outside places		Total	
	Male	Female	Male	Female	Male	Female	N	%
Phlebotomus* (*Larroussius*) *kandelakii	15	19	21	24	36	13	128	38.3
Sergentomyia* (*Sergentomyia*) *dentata	7	22	13	13	7	13	75	22.5
Phlebotomus* (*Phlebotomus*) *papatasi	8	3	30	3	5	3	52	15.6
Sergentomyia* (*Sergentomyia*) *theodori	6	4	4	1	10	22	27	8.1
Phlebotomus* (*Larroussius*) *major	5	4	2	3	3	0	17	5.1
Sergentomyia* (*Sergentomyia*) *sintoni	5	3	2	0	4	1	15	4.5
Sergentomyia* (*Sergentomyia*) *antennata	2	1	2	0	1	0	6	1.7
Phlebotomus* (*Adlerius*) *longiductus	0	0	0	0	1	0	1	0.3
Phlebotomus* (*Adlerius*) *halepensis	0	0	1	0	0	0	1	0.3
Phlebotomus* (*Larroussius*) *tobii	0	0	1	0	0	0	1	0.3
Phlebotomus* (*Paraphlebotomus*) *sergenti	0	0	0	0	0	1	1	0.3
Sergentomyia* (*Parrotomyia*) *sumbarica	0	0	0	0	0	1	1	0.3
Unknown	0	3	2	2	1	1	9	2.7
Total	**48**	59	78	46	68	35	334	100.0

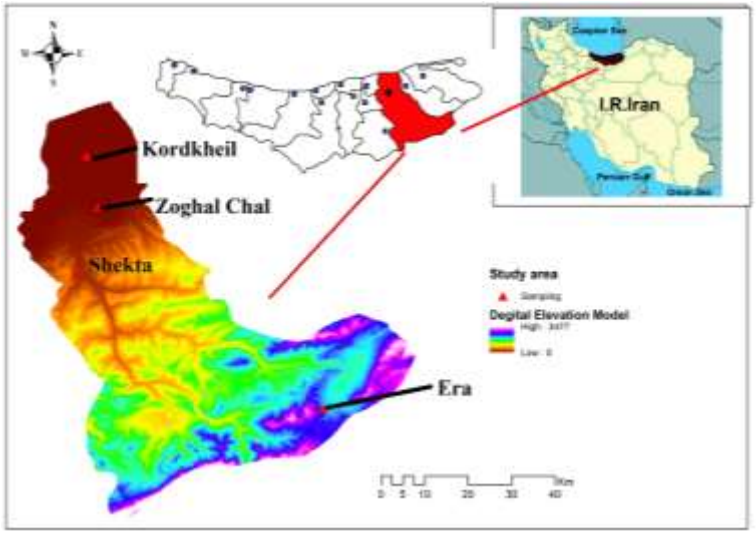

Fig. 1. Sampling sites in Sari County, north of Iran

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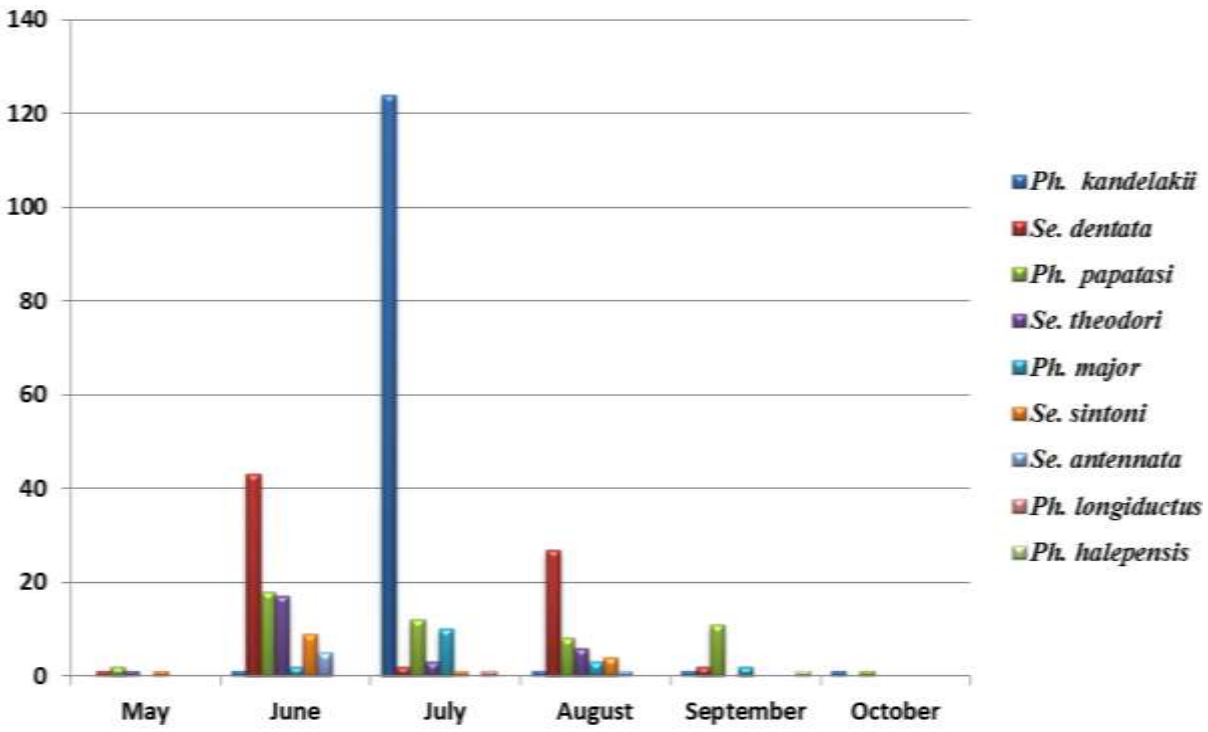

Fig. 2. Monthly frequency and percentage of sand flies, collected in Sari County, north of Iran, 2016

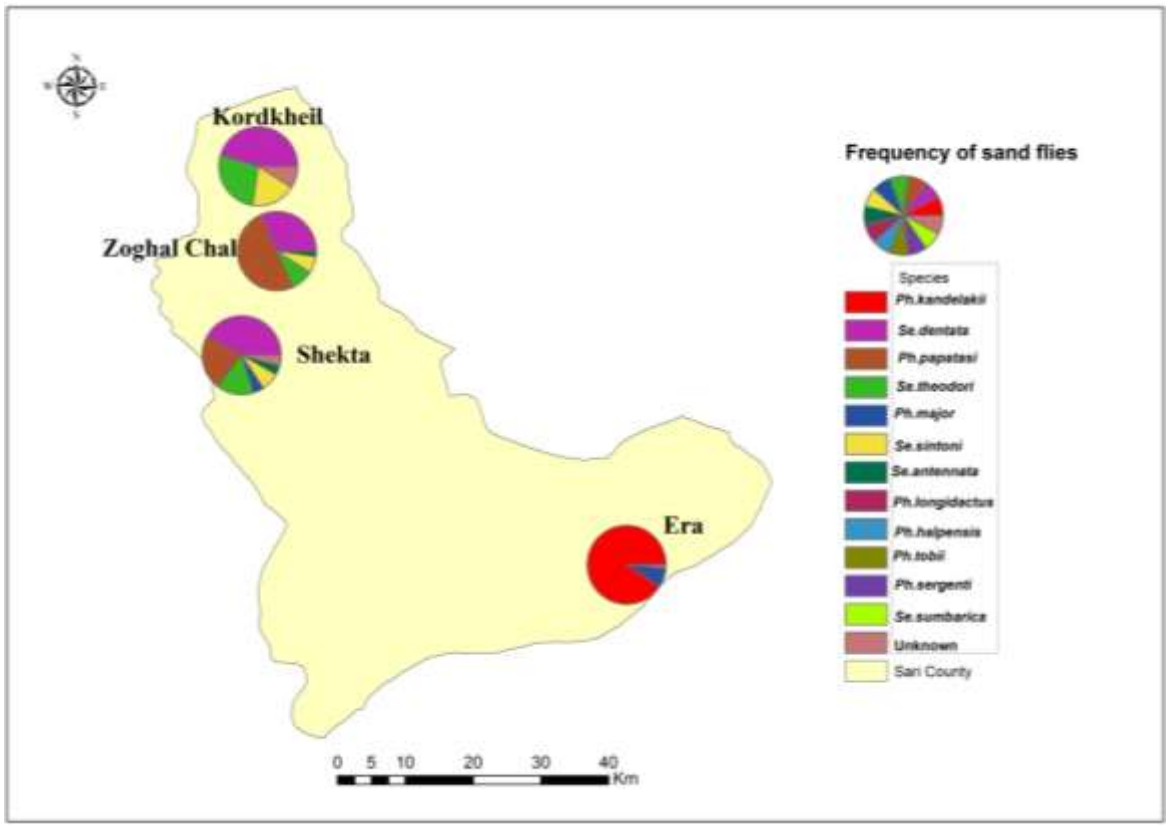

Fig. 3. Geographical distribution and frequency of phlebotomine sand flies in Sari County, north of Iran, 2016

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Table 2. Biodiversity indices of sand flies, based on spatial distribution in Sari County, north of Iran, 2016

Biodiversity indices	Era	Shekta	Kordkheil	Zoghal Chal
Number of species (*S*)	5	8	3	6
Abundance (N*)	141	129	10	45
Shannon (H')	0.39	1.53	1.03	1.27
Simpson (1-D)	0.81	0.28	0.38	0.35
Evenness (J')	0.29	0.59	0.93	0.59

*: Nine unknown specimens were not included in the biodiversity index analysis

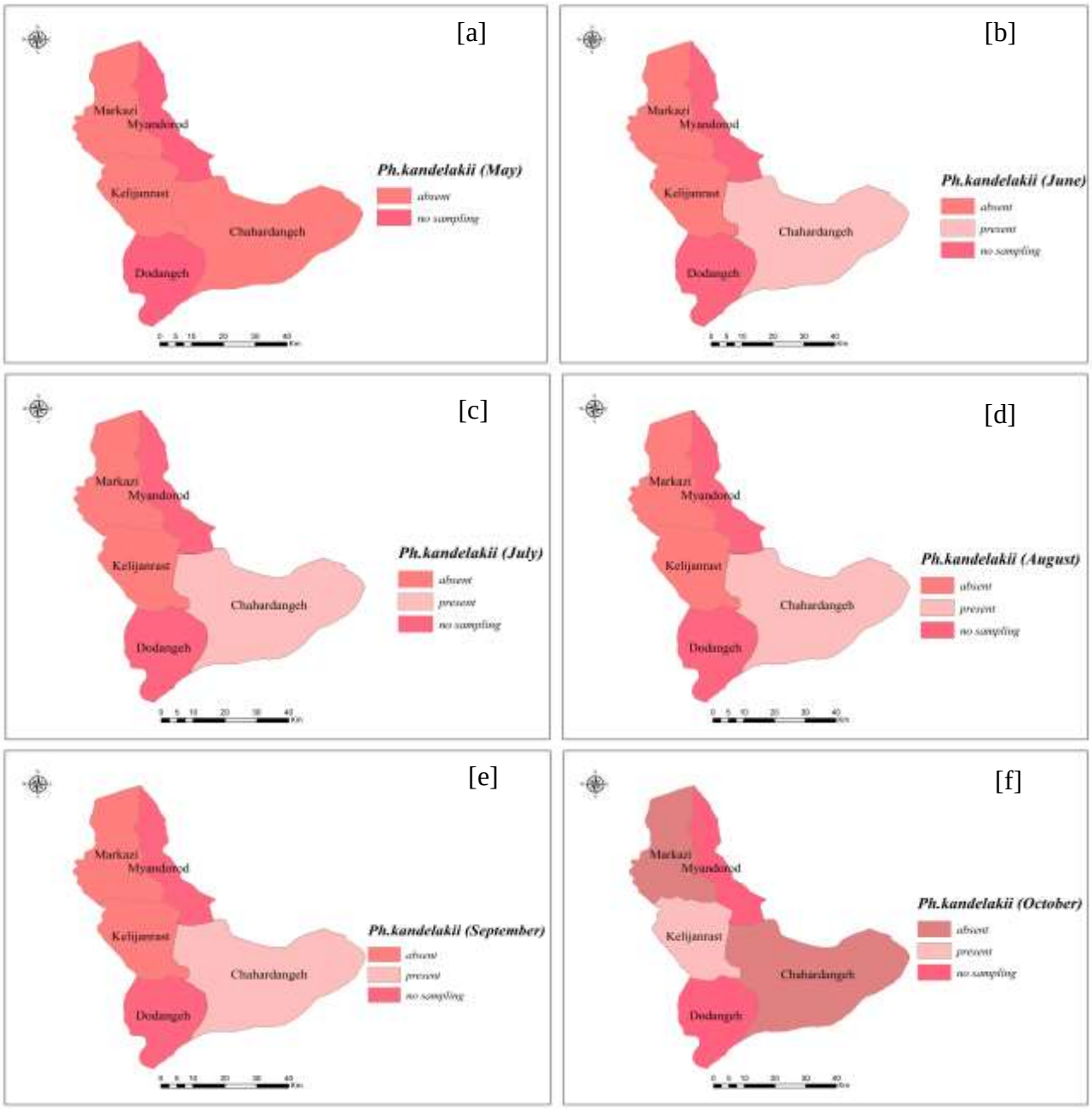

Fig. 4. Temporal distribution of *Phlebotomus kandelakii* in Sari County, north of Iran, a-f: May-October 2016

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Table 3. Biodiversity indices of sand flies based on temporal distribution in Sari County, north of Iran, 2016

Biodiversity indices	May	June	July	August	September	October
Number of species (S)	5	9	7	7	5	2
Abundance (N*)	6	97	153	50	17	2
Shannon (H')	1.57	1.57	0.75	1.4	1.1	0.69
Simpson (1-D)	0.22	0.27	0.67	0.34	0.45	0.5
Evenness (J')	0.95	0.54	0.30	0.45	0.61	1

*: Nine unknown specimens were not included in the biodiversity index analysis

Discussion

The study of leishmaniasis vectors is necessary as a prerequisit to many epidemiological studies. The results of this study can inform authorities to make evidence-based decisions regarding appropriate interventions to control vectors and the diseases they transmit.

In the present study, seven *Phlebotomus* species and five *Sergentomyia* species were collected. This is the first report after 1998 (25) on different biological and ecological aspects of sand flies in Sari County, Mazandaran Province, north of Iran.

In the study of Rahbarian et al. in Mazandaran Province, a total number of 194 sand flies were collected which included six species of *Ph. major*, *Se. sintoni*, *Ph. halepensis*, *Ph. andrejevi*, *Ph. papatasi*, and *Se. dentata* (25). Compared to previous studies, in the present investigation *Ph. kandelakii*, *Ph. sergenti*, *Ph. tobbi*, *Ph. longiductus*, *Se. antennata*, *Se. theodori*, and *Se. sumbarica* were reported as new records in Mazandaran Province. Other related studies in northern Iran were recently conducted in Gilan Province, neighboring Mazandaran Province, in which eleven species of sand flies were reported (26, 27). In the present study, twelve species of sand flies were identified, so the species richness is higher than the previous studies, it probably indicates a thorough sampling effort in the present study.

Regarding the sex of collected sand flies in this study, the sex ratio (male: female) was 1:0.7, which indicates that male sand flies were collected 1.4 times more than female sand flies. In accordance with this study, previous studies reported that the male to female ratio was 1:0.74 in Thiland (28), 1:0.92 in western Saudi Arabia (29) and 1:0.5 in central Iran (30). Another study in norhtwest of Iran reported a higeher sex ratio than the current study, in which male sand flies were captured 2.5 times more than female sand flies (31). The sticky paper trap method used in our study showed that males were more attracted to traps than females. Another study in Thiland using the method of CDC light trap, suggested that the frequency of collected male sand flies was higher than that offemales, which could be explained by the natural behavior of males that follow females to mate (28). In terms of the abdomen physiological condition, in our study most of the captured female sand flies were unfed, showing that they were more active and flew more than fed ones, probably for sugar or blood feeding.

One of the main findings of this study was the abundance of species such as *Ph. kandelakii*, *Ph. papatasi*, and *Ph. major* that are either proven or possible vectors of leishmaniasis in the world (32, 33). Additionally, *Se. sintoni* and *Se. dentata* were reported as lizard Leishmaniasis (34).

According to the current study, *Ph. kandelakii* was the most abundant species collected in indoors and outdoors in the study area. The value of Simpson dominance Index was high in some areas of study because of the abundance of this species. The higher dominance value in a region may indicate the higher potential of vector for disease transmission. The first report of visceral leishmaniasis in

Iran was reported from Mazandaran Province in northern Iran in 1949 (35). Until recently, visceral leishmaniasis was reported only based on epidemiological evidence and the high prevalence of sand fly vectors of Kala Azar. In previous studies, *Leishmania* promastigote infection was identified in *Ph. kandelakii* in the endemic focus of Meshkin-Shahr in northwestern Iran (36). In latter studies, *L. infantum* was detected in *Ph. kandelakii* using molecular methods at the same focus in northwestern (37) and in northeastern Iran in North Khorasan Province (38). Thus, according to previous studies, *Ph. kandelakii*, the most abundant species collected in this study area, is of paramount importance considering the fact that it is a vector of ZVL in Iran.

Phlebotomus papatasi was another species collected in this study. Various studies have shown that *Ph. papatasi* is the main and proven vector of ZCL which is distributed all over the country (9, 32). In the past studies, the parasitic infection of *Ph. papatasi* by *L. major*, *L. turanica* and *L. gerbilli* were identified (39). Presence of non-pathogenic *L. turanica* in *Ph. papatasi* and animal reservoirs can imply the role of this parasite in sustaining the disease cycle (40-44).

Another species collected in the current study was *Ph. major*, which was reported from 17 out of 31 provinces of Iran with human cases of ZVL and according to the current study, this species was mostly collected from mountainous areas (32, 45). Previous morphological and morphometric study in the northwest of the country, showed other morphotypes including *Ph. major neglectus* and *Ph. major krimensis* for this species (46). It is not usually difficult to identify the male Laroussius sand flies, but females of some species are not easily distinguishable. Further studies using morphological and molecular data are suggested.

In the present study, *Ph. kandelakii*, *Ph. papatasi* and *Ph. major* were mainly collected from indoor places, thus they had more access to human and animal hosts. In accordance with the current study, in an investigation on Phlebotominae ecology in an endemic cutaneous leishmaniasis focus in Isfahan conducted by Yaghoobi Ershadi in 2001, the most prevalent specie in indoors was *Ph. papatasi* (47). In another study in south of Iran, *Ph. papatasi* was collected mostly from indoors (48). In the studies conducted in Qom Province (49), central Iran and in Ardabil Province (31), northwest of the country, *Ph. kandelakii* was collected more from indoors.This finding could indicate the habituation of these species to human habitats.

In this study, *Ph. kandelakii* was collected from the mountains and peaked in June, while *Ph. papatasi* was collected from areas other than mountains. According to previous studies, *Ph. papatasi* usually prefers the plains over the mountains (50, 51). In the present study, the highest temporal distribution was associated to *Ph. papatasi*, which was collected in all months of sand fly activity in the area.

In the current study, sand fly activity began in May and continued until October, while the peak of seasonal activity was in July. According to our previous study in the same study area, in November, more disease transmission and higher incidence rate of leishmaniasis occured after the incubation period, from sand fly bites to nodule development (19). During a study conducted in an endemic cutaneous leishmaniasis focus in Golestan province, north east of Iran, sand flies were collected in 7 months of the year and the activity of sand flies was identified from May to November (52).

It has been admitted that phlebotomine sand flies have a more or less definite seasonal distribution pattern in Mediterranean countries, become visible in late spring or early summer, and are active until early autumn (53, 54). However, various studies have verified the bimodal pattern of temporal dispersion of phlebotomine sand flies with one peak in spring and another in summer or autumn (11, 55, 56).

One of the main findings of this study was the collection of eight species of Phlebotom-

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inae from the forest, five species from the mountains, three species from the plains, and six species from the peri-urban areas. The forest region had a richer collection of species composition compared to other regions due to its moderate and humid climate, which created a proper environment for sand fly development. In current study, twelve species were captured. Species diversity and richness in this study was high compared to some other species composition studies of sand flies in Iran. In the present study, species diversity, richness and evenness in the forest (H'= 1.53, S= 8, J'= 0.59) was higher than the mountainous area. Less diversity in mountainous area was because of the presence of the dominant species *Ph. kandelakii*, and the greater value of Simpson dominance index (D= 0.81). Contrary to our study, Shannon and evenness indices in the study of Qom (50) Province (H'= 1.36, J= 0.62) was higher in mountaineous area, and in North Khorasan (51) Province, Shannon index (H'= 1.033) was higher and evenness was similar (J'= 0.357) in mountainous area. In accordance with this study, in Khouzesten Province, south of Iran, species diversity was higher in plain areas (48). It should be mentined that the values of Shannon biodiversity index, affected by richness, eveness and Simpson, were high in the studied areas, jointly in the months of May and June. The high diversity indices could be because of humid climate due to low distance to the Caspian Sea and suitable condition for sand fly breeding.

Conclusion

This study determined some bioecological aspects of sand flies in Sari County, Mazandaran Province, northern Iran. The diversity and richness of the species was high, which could be because of the moderate climate due to its geographical proximity to the Caspian Sea.

According to the results of this study, leishmaniasis vectors *Ph. kandelakii*, *Ph. papatasi* and *Ph. major* were present in the study area. Considering Mazandaran Province as one of the sporadic foci of leishmaniasis and geographical proximity of Mazandaran Province with ZCL and ZVL foci of Golestan Province in the northeast and Azerbaijan Province in the northwest, respectively, and the lack of study of disease vectors in this region, health authorities are recommended to highten the surveillance system and conduct more studies on the ecology of sand flies and leishmanial infections, to prevent the emergence of a new focus of leishmaniasis.

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Ethical considerations

This project has been approved by Mazandaran University of Medical Sciences (MUMS) ethics committee and has been registered with the code IR.MAZUMS..REC.140011513.

Conflicts of Interest

Authors declare that there is no conflict of interest.

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