

Original Article

A Comparative Study on the Biodiversity and Species Richness of Phlebotomine Sand Flies (Diptera: Psychodidae) in Kermanshah and Khuzestan Provinces of Iran

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Abstract

Background: Leishmaniasis is one of the most important vector-borne and neglected tropical diseases in many parts of the world. The main objective of this study was to determine the biodiversity of phlebotomine sand flies (Diptera: Psychodidae, Phlebotominae) in Khuzestan and Kermanshah Provinces of Iran.

Methods: Sampling was conducted in Khuzestan and Kermanshah Provinces using sticky paper traps and CDC light traps. The samples were then stored in 96% alcohol-containing vials, mounted, and identified. Afterward, the alpha diversity (using Simpson's, Shannon-Weiner's diversity, evenness, Maghalef's, Menhinick's, Hill N_1 and N_2 indices) and beta diversity indices (using Sorensen's and Jaccard's coefficients) were calculated.

Results: A total of 4302 sand flies were caught and identified which comprised mostly of *Phlebotomus papatasi*, *Ph. alexandri* and *Ph. sergenti*. It was found that among the four counties of Ahvaz, Shush, Shushtar and Dezful in Khuzestan Province, Shush had the lowest, and Shushtar, the highest amount of species diversity and evenness. In the four studied counties of Kermanshah Province, species diversity in Kermanshah County had the lowest amount and Sarpol-e-Zahab, the highest. The species richness in Kermanshah County was the lowest, while Qasr-e-Shirin County had the highest amount.

Conclusion: An overview of the biodiversity of phlebotomine sand flies in Kermanshah County in Kermanshah Province, and Shush County in Khuzestan Province, showed less stability in community structure of these vectors, which can be counted as an alarm for emerging dominant vectors among the studied counties and have the potential to increase the prevalence of leishmaniasis.

Keywords: Leishmaniasis; Phlebotominae; Species Richness; Biodiversity; Iran

Introduction

Leishmaniasis is a vector-borne and neglected tropical disease and a tropical infection of poverty as it is associated with malnutrition, population displacement, poor housing, weak

immune system, and lack of financial resources. Among all parasitic diseases, mortality from leishmaniasis is second only to malaria (1). Leishmaniasis affects 102 countries, areas, or

territories worldwide (2). The disease causes considerable human morbidity and socio-economic burden to endemic societies. Two forms of the disease, visceral leishmaniasis (VL) and cutaneous leishmaniasis (CL), are endemic in Iran, and zoonotic cutaneous leishmaniasis (ZCL) is more common (about 80%) in the rural areas of 19 out of 31 provinces of the country (3). Phlebotomine sand flies (Diptera: Psychodidae, Phlebotominae) are the well-known vectors of leishmaniasis (4). Iran's most geographic and climatic conditions are suitable for the growth and proliferation of rodents and sand flies as reservoirs and vectors of this disease (5).

Up until now, 53 species of sand flies have been identified in Iran, of which 34 species belonging to the genus *Phlebotomus* and 19 to the genus *Sergentomyia* (6). Two provinces of Khuzestan and Kermanshah were chosen according to the purposes of previous research done in 2011–2012 (7). Khuzestan Province in the southwest of Iran is considered to be an important endemic focus of ZCL due to the environmental impact of the warfare (8). In 2012, the annual incidence of CL was 9.5 to 21.7 per 100000 inhabitants in the province (4). This variation may be due to migration of non-immune people to endemic areas, people living near rodent colonies, migration of rodents and increasing synanthropic index for the reservoir, and lack of knowledge and attitude toward ZCL (9). On the other hand, Kermanshah Province was chosen to study as a non-endemic focus for CL.

Alpha (or within habitats) diversity is the most common form of inventory diversity, which reflects species packing within a community. Species diversity is a central theme in ecology and has two separate components named species richness (S) and evenness (E). Two indices of Shannon–Wiener (Shannon index) and Simpson's index are commonly used for species biodiversity which differ in their emphasis on species richness (Shannon-Wiener) or abundance (Simpson's) (10).

Beta (or between habitat) diversity is the category of differentiation diversity that measures the variation in taxonomic composition between areas of alpha diversity. Some ecological studies such as species richness, species evenness, degree of presence or occurrence, synanthropic index and Jaccard's and Sorensen's similarity indices are important to study the composition of sand fly species in different biotopes, spatial separation among the different populations and the degree of attraction or avoidance of Phlebotominae sand flies to human places (11, 12).

The main purpose of this study is to determine species richness and biodiversity of the sand flies, and the applied purpose would be determining the areas with high potential in leishmaniasis transmission to implement prevention and control programs.

Materials and Methods

Study area

Khuzestan Province (29° 57'–33° 00'N, 47° 40'–50° 33' E) is in the southwest of the country and divided into 27 counties. Topographic elevations in the province vary between 0 and 3740m (13). This province consists mostly of lowlands and is generally warm, however, northeastern parts are more elevated and have a more moderate climate.

Kermanshah Province (33° 41'–35° 17'N, 45° 24'–48° 06'E) is situated in the west part of the country and contains 14 counties. Mean topographic elevation in the province is 1342 m (14). The province is mostly mountainous, and overall, the weather condition is cool and to some extent rainy during colder seasons.

Sand fly collection

During the activity seasons of sand flies in 2011 and 2012, sand flies were collected using 4239 sticky paper traps and 124 CDC light traps from indoors (human dwelling and stables) and outdoors (mountainous, foothill, and plain areas) in Khuzestan and Kermanshah Provinces, shown in Fig. 1 and 2 (7). In this study, samples were

mounted using Puri's medium. They were then identified using the sand fly identification key of the Eastern Mediterranean Region (15).

Data process and analysis

To determine alpha and beta diversity, four counties from each province were selected and their data were processed in Microsoft office Excel 2016 software using related indices and coefficients. The average and standard deviation of all the results were calculated and, their P-value was indicated by performing the Analysis of Variance (ANOVA) test in IBM SPSS Statistics 26 software.

Alpha diversity indices

To determine the species diversity, Shannon–Weiner index (H'): $H' = -\sum(p_i \times \ln p_i)$ was used where ' p_i ' can be calculated by dividing n_i , the number of species, by N , the total number of collected individuals (16). Also, indices Hill N_1 ($N_1 = e^{H'}$) to determine the species diversity exponentially, and Hill N_2 ($\frac{1}{D} = \frac{1}{\sum p_i^2}$) was used where e is the Euler's number and $\frac{1}{D}$ is the symbol for Hill N_2 (17).

Species richness was estimated by calculating Margalef's index (D_{Mg}) which can be calculated through this equation: $(D_{Mg}) = (s-1)/\ln N$ (17) and Menhinick's index $D_{Mn} = \frac{s}{\sqrt{N}}$ which is more reliable (18).

Evenness index (E) was calculated by dividing H' index by natural logarithm (\ln) of the number of collected species (S) (17).

To determine the dominant vector's diversity, Simpson's index (D) was calculated using the formula: $D = \frac{\sum n(n-1)}{N(N-1)}$. The result is a number between 0 and 1, and 0 indicates unlimited diversity, and on the contrary, 1 represents no diversity at all (19).

Beta diversity indices

Beta diversity was also estimated intra-provincially by determining the similarity be-

tween different communities using Jaccard's (Sj) and Sorensen's Indices (Ss). Sj: $a/(a+b+c)$ and Ss: $2a/(2a+b+c)$ are the equations for calculating Jaccard's and Sorensen's Indices, respectively. In both equations, a represents the number of species (richness) found in both biotopes and b is the number of species found in the first biotope and c biotope is the number of species in the second (20).

Results

A total of 4302 sand flies were collected from both provinces which included 17 and 12 species belonging to the genus *Phlebotomus* and *Sergentomyia*, respectively. Species composition and relative abundance of collected sand flies from the studied areas are shown in Table 1. 39.3% of the collected sand flies were identified as male and 60.7% as female. In Khuzestan Province, *Ph. papatasi* and *Ph. alexandri* were the most prevalent species, which respectively consisted of 57.3% and 29.5% of the total sand flies. In Kermanshah Province, *Se. dentata* was the most prevalent species (29%), but it is neither a proven nor a probable vector, *Ph. papatasi*, *Ph. alexandri*, and *Ph. sergenti* were consecutively the dominant vector species, with 24.3%, 9.1% and 9.02% of the total samples, respectively.

Alpha diversity results in Table 2 show that among the four studied counties in Khuzestan province, Shushtar County had the highest probability of dominant vector presence, species diversity, and species evenness, albeit not significantly ($P=0.361$), also Ahvaz County showed the highest amount of species richness ($P=0.759$). Shush County on the other hand, had the lowest amount of all the mentioned indices. In Kermanshah Province, Kermanshah County also showed the lowest amount of these indices, whereas Javanroud County significantly had the highest amount of dominant vector presence ($P=0.029$), Sar-e-Pol Zahab County had the highest amount of species diversity ($P=0.007$) (significantly), and evenness ($P=0.132$),

and Ghasr-e- Shirin County was significantly considered to be the richest county, species-wise (P= 0.05).

Beta diversity results proved that among the four studied counties in Khuzestan Province, Ahvaz and Shush County had the least amount

of similarity in sand fly species, and equally, Dezful with Shushtar and Ahvaz showed the most (Table 3 and 4). As shown in Table 5 and 6, in Kermanshah Province, Javanroud County had the least similarity with Kermanshah County and the most with Ghasr-e- Shirin County.

Table 1. Species composition, abundance, and relative abundance of phlebotomine sand flies in Khuzestan and Kermanshah Provinces, Iran, 2011–2012

Species	Collection sites and Percentages			
	Khuzestan Province	%	Kermanshah Province	%
<i>Ph. papatasi</i>	1425	57.344	443	24.380
<i>Ph. alexandri</i>	735	29.577	166	9.135
<i>Ph. jacusieli</i>	3	0.120	3	0.165
<i>Ph. sergenti</i> s.l.	13	0.523	164	9.025
<i>Ph. mongolensis</i>	14	0.563	1	0.055
<i>Ph. kazeruni</i>	1	0.0402	0	0
<i>Ph. caucasicus</i> group	4	0.160	1	0.055
<i>Ph. mesghali</i>	1	0.040	1	0.055
<i>Ph. major</i>	0	0	72	3.962
<i>Ph. brevis</i>	0	0	2	0.110
<i>Ph. perfiliewi</i>	0	0	13	0.715
<i>Ph. chinensis</i> group	0	0	53	2.916
<i>Ph. halepensis</i>	0	0	2	0.110
<i>Ph. tobbi</i>	0	0	1	0.055
<i>Ph. keshishiani</i>	0	0	8	0.440
<i>Ph. wenyoni</i>	0	0	1	0.055
<i>Ph. longiductus</i>	0	0	1	0.055
<i>Se. clydei</i>	40	1.609	16	0.880
<i>Se. tiberiadis</i>	56	2.253	19	1.045
<i>Se. iranica</i>	17	0.684	1	0.055
<i>Se. squamipleuris</i>	7	0.281	2	0.110
<i>Se. dentata</i>	75	3.018	521	28.673
<i>Se. sintoni</i>	76	3.058	160	8.805
<i>Se. theodori</i>	12	0.482	63	3.467
<i>Se. antennata</i>	5	0.201	88	4.843
<i>Se. palestinensis</i>	1	0.040	1	0.055
<i>Se. mervynae</i>	0	0	2	0.110
<i>Se. africana</i>	0	0	1	0.055
<i>Se. pawlowskyi</i>	0	0	8	0.440
Total	2485	100.0	1817	100.0

Table 2. The alpha diversity indices for sand fly species in the studied counties of Khuzestan and Kermanshah Provinces, Iran, 2011–2012

Province	County	Shannon-Weiner diversity	Shannon-Weiner evenness	Simpson	Marghalef	Menhinick	Hill N ₁	Hill N ₂
		$H' = \sum_{i=1}^S (P_i) \frac{E_{H'} - \frac{H'}{\ln S}}{\ln P_i} \quad D = \frac{\sum n(n-1)}{N(N-1)} \frac{(S-1)}{\ln N} \quad D_{Mn} = \frac{S}{\sqrt{N}} \quad N_1 = e^{H'} \quad \frac{1}{D} = \frac{1}{\sum P_i^2}$						
Khuzestan	Shush	0.5933	0.2966	0.6599	0.8079	0.7776	2.0136	1.6661
	Shushtar	1.2820	0.5980	0.1581	1.5383	1.1180	3.9679	3.3570
	Ahvaz	0.9725	0.4682	0.3775	1.5083	1.1431	2.7669	2.4675
	Dezful	1.2423	0.4813	0.3739	1.3159	0.8977	3.7509	3.1337
Kermanshah	Javanroud	1.5706	0.5028	0.2527	1.9201	1.0817	4.8853	3.8484
	Kermanshah	0.5703	0.3111	0.6748	0.7056	0.4768	1.8051	1.5617
	Sar-e-Pol Zahab	1.5768	0.5435	0.2697	1.5537	0.8979	4.9137	3.8275
	Zahab							
	Ghasr-e-Shirin	1.5021	0.5018	0.3150	1.7301	1.7499	4.8173	3.6834

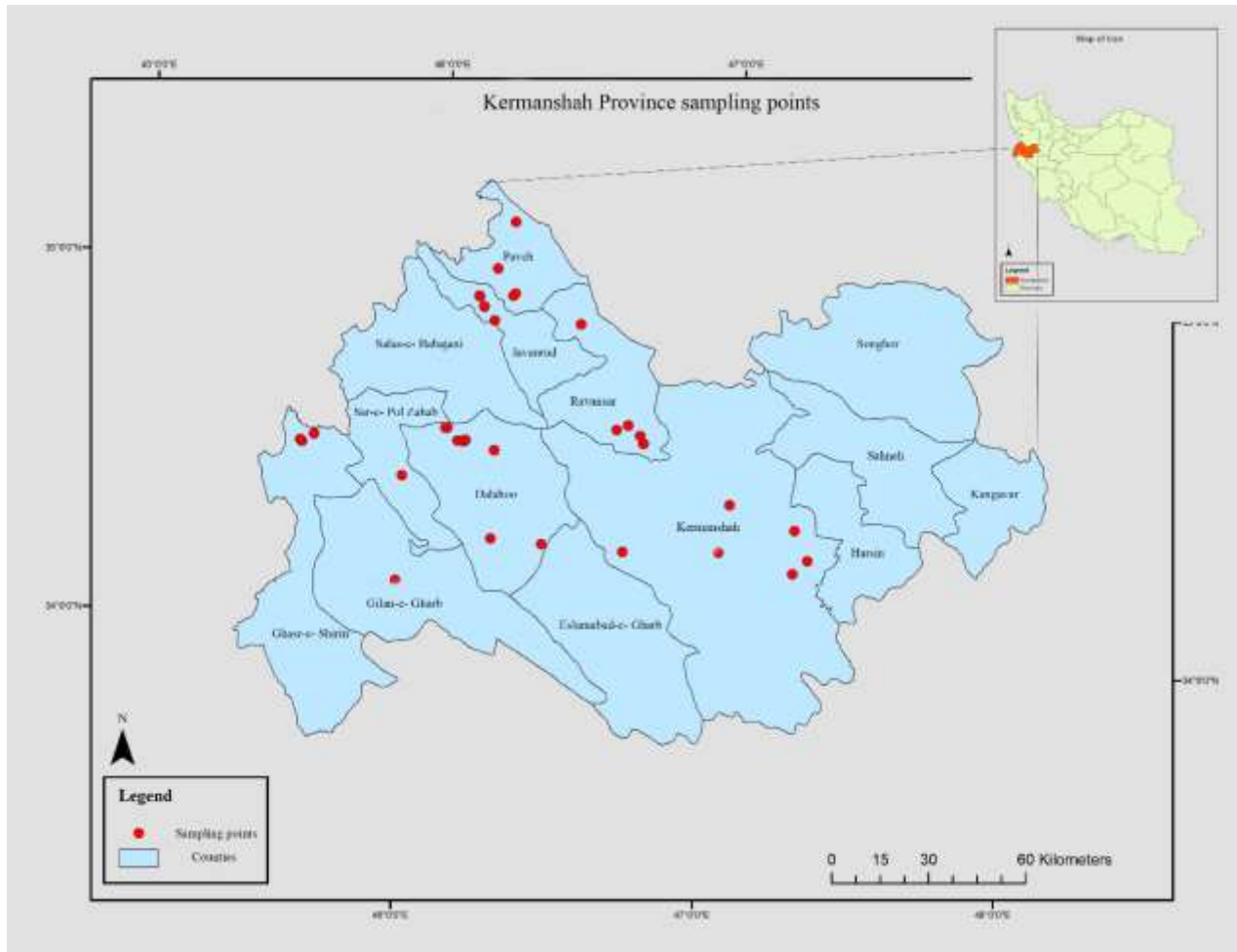


Fig. 1. Study areas in Kermanshah Province, Iran, 2011–2012

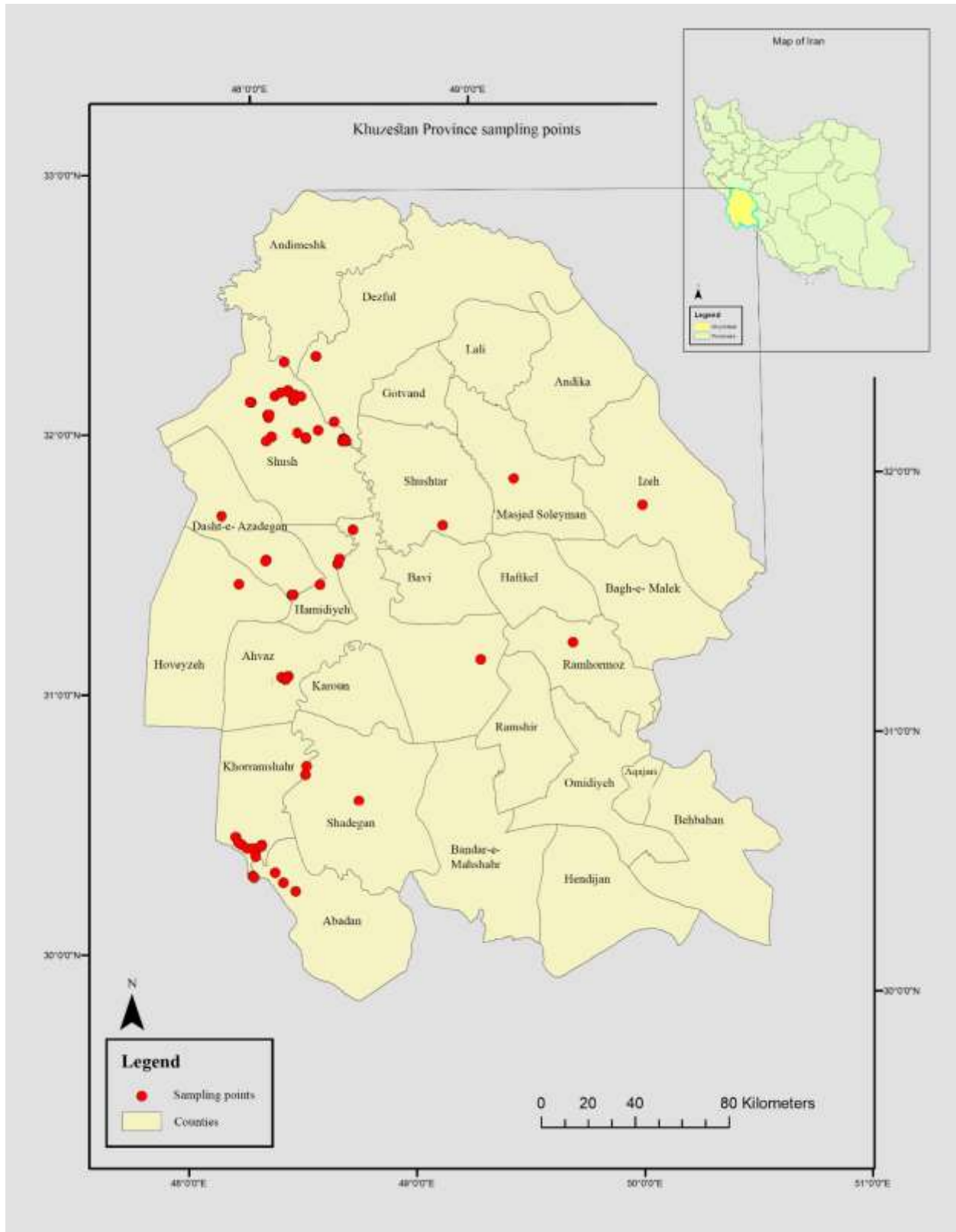


Fig. 2. Study areas in Khuzestan Province, Iran, 2011–2012

Table 3. The Jaccard’s and Sorensen’s coefficient (beta diversity) indices for four counties of Khuzestan Province, Iran, 2011–2012

	Shush -Shushtar	Shush -Ahvaz	Shush -Dezful	Shushtar - Ahvaz	Shushtar -Dezful	Ahvaz -Dezful
Cj	0.54545	0.41667	0.54545	0.46667	0.69231	0.69231
Cs	0.70588	0.58824	0.70588	0.63636	0.81818	0.81818

Table 4. The Jaccard's and Sorensen's coefficient (beta diversity) indices for four counties of Kermanshah Province, Iran, 2011–2012

	Javanrud - Kermanshah	Javanrud - Ghasr-e-Shirin	Javanrud -Sar-e-Pol Zahab	Kermanshah - Ghasr-e- Shirin	Kermanshah - Sar-e-Pol Zahab	Sar-e-Pol Zahab - Ghasr-e-Shirin
Cj	0.444444	0.578947	0.526316	0.533333	0.466667	0.5625
Cs	0.615385	0.733333	0.689655	0.695652	0.636364	0.72

Discussion

This study is considered as one of the first researches on biodiversity of sand flies in Kermanshah Province, and a complementary one in Khuzestan Province, as a main focus of CL in the southwest of Iran.

Our results indicated that after more than four decades (21), *Ph. papatasi* and *Ph. alexandri* are still active in all the studied counties, with *Ph. papatasi* being the dominant species. *Phlebotomus papatasi* normally prefers to live in plain areas rather than in mountains. Lower abundance of this species in mountainous areas such as the studied counties in Kermanshah Province could be explained by its preference to semi-arid and plain areas such as the studied counties in Khuzestan Province (12, 22).

Phlebotomus alexandri has been reported as a probable vector of zoonotic visceral leishmaniasis (ZVL) in Iran (7, 23). This species prefers regions with a high percentage of relative humidity and warmer niches (22), which may be the reason behind its higher abundance in the studied areas of Khuzestan Province, rather than Kermanshah Province which is less humid and warm.

From all the studied area in this study, 27 species of sand flies were collected and identified. This showed that species richness of sand flies in these areas was higher than some other

regions in the country (12, 24, 25).

Jahanifard et al. (12) found that the urban area of Shush County has the highest biodiversity and evenness, and the lowest species richness, which shows that the lowest species number in this area became more adapted to environmental conditions. The highest species richness was in the semi-urban area of this county which accounts for the high species number in peripheral areas of the county. It can be concluded that the natural habitat is under the stress of continuous drought in Shush County. However, in the present study, this county itself showed the lowest species richness and diversity among the four studied counties, which could lead to a rise of activities of dominant vectors and a probable increase in leishmaniasis case reports.

A study based in Kuhpayeh district in Isfahan Province (26) revealed the importance of elevation in sand fly diversity, where the species diversity in mountainous areas was higher than in plain areas and lowlands. The same conclusions were derived from more research throughout the recent years (25, 27), yet some indicated otherwise such as the study done by Sofizadeh et al. (28). Therefore, considering the result of the present study that shows higher diversity in plain areas rather than elevated ones,

the degree of species diversity cannot be simply based on the factor of elevation and, we must consider different aspects. Due to the restrictions of COVID-19, we weren't able to collect more samples from other counties as well for much more comprehensive research. We recommend the authorities take preventive measures in Shush County in Khuzestan Province and Kermanshah County in Kermanshah Province since they may contain a more suitable habitat for vectors. Further research on the biodiversity of the remaining counties in both provinces and other areas of the country is highly recommended. Moreover, determining the reasons behind species similarity between different counties in each province needs further research on various ecological, geographical, anthropological variables.

Conclusions

Based on the results, it can be concluded that Kermanshah County in Kermanshah Province, and Shush County in Khuzestan Province are ecologically unstable and more likely to welcome vector species, which is critical in planning control programs.

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Ethical considerations

This study has been reviewed and approved by the School of Public Health (SPH), Tehran University of Medical Sciences (TUMS) ethics committee and has been registered with the code IR.TUMS.SPH.REC.1399.206.

Conflict of interest

Authors declare that they have no competing interest.

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