

Original Article**Richness and Diversity of Phlebotomine Sand Flies (Diptera: Psychodidae) in North Khorasan Province, Northeast of Iran**

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Abstract

Background: We aimed to determine the species composition, richness and diversity indices of the Phlebotomine sand flies at different topographic condition in visceral (VL) and cutaneous leishmaniasis (CL) foci in the northeast of Iran.

Methods: This cross-sectional study was conducted during 2016 in North Khorasan Province. The sampling was focused on rural regions, where human cases of VL and/or CL were diagnosed and reported during last 5 years. Sand flies were collected three times each twenty days during peak periods of seasonal activity. Seven collection methods were used. Some Alpha and Beta diversity indices were calculated.

Results: Overall, 7253 sand flies were collected and identified. They were from 19 species of Phlebotominae sandflies. *Phlebotomus sergenti* and *Ph. papatasi* were the most prevalent (84.9%) species in the study area. Species richness (S) was very different in three areas and were 18, 8, and 4 respectively but Evenness (E) were 0.357, 0.345, and 0.380, so evenness was almost equal in the study areas. Shannon Index (H') and Margalef Richness Index were calculated 1.033, 0.718, 0.527 and 2.117, 0.8998, 0.4006 respectively.

Conclusion: The sand fly fauna in North Khorasan Province was very rich and often included some of the most important proven or suspected vectors of leishmaniasis. Species diversity indices (Shannon index, and Simpson's index) were not high due to decreasing in evenness. The Margalef richness index could accurately reflect the biodiversity of sand flies between three subtidal locations.

Keywords: Richness, Diversity, Sandflies, *Leishmania*, Iran

Introduction

Phlebotominae sandflies (Diptera: Psychodidae: Phlebotominae) are widespread in the tropical and subtropical regions. They may carry and transmit etiologic agents of arboviruses and bartonellosis but the most important agents that they transmit are *Leishma-*

nia, protozoan parasites (1). There are three main forms of leishmaniasis, visceral leishmaniasis (VL), various forms of cutaneous leishmaniasis (CL), and mucocutaneous leishmaniasis. Endemic leishmaniasis transmission has been reported from at least 98 countries

and three territories on five continents. Cutaneous leishmaniasis is more widely distributed and approximately 0.7 to 1.2 million new cases occur each year. Ten countries with highest estimated CL cases are Afghanistan, Algeria, Colombia, Brazil, Iran, Syria, Ethiopia, North Sudan, Costa Rica and Peru, together account for 70% to 75% of global estimated CL incidence (2).

Approximately 800 species of phlebotomine sand flies are distributed in the world. In Iran, 47 species of sand flies have been identified and the presences of some species are doubtful (3–5). CL is reported from all provinces and is endemic in more than half of the 31 provinces in Iran. VL is endemic in northwest, west, southwest, central and north-east of Iran and is reported sporadically from all provinces of the country (5–7).

The first entomological studies in North Khorasan Province were conducted during 1975 in Esfarayen County and *L. major* detected in *Phlebotomus papatasi* (8). In Shirvan County molecular infection of leishmanial due to *L. infantum* was observed in *P. kandelakii* (9). Cutaneous leishmaniasis is reported from all counties and VL is reported from five out of eight counties of North Khorasan Province (10). Some counties in Northern half of the province have been reported to be endemic for VL with more than 160 new cases in the last second decade (11).

Biodiversity is a primary interest of ecologists and is a contraction of biological diversity. Species, ecosystem, and genetic diversity are three components of biodiversity. Diversity can be represented in various types of Alpha (α), Beta (β) and Gamma (Σ). Alpha diversity is the variation of species within a community or habitat, Beta diversity is in taxonomic composition between communities, and Gamma diversity is the total variation of an area and combination of both alpha and beta diversity (12).

Species diversity is a central theme in ecology and has two separate components

named species richness (S) and evenness (E). Richness and evenness have been combined mathematically in various ways to calculate diversity indices based on proportional abundances of species (12). Two indices of Shannon–Wiener (Shannon index) and Simpson's index commonly used for species biodiversity. Simpson's index measures the probability of two individuals randomly selected from a sample belongs to the same species. Shannon index is one of the most well-known diversity indices and measures species richness and proportion of each species within a community (12, 13).

The two indices differ in their emphasis on species richness (Shannon–Wiener) or abundance (Simpson's). Typical values of Shannon–Wiener index are between 1.5 and 3.5 and rarely go beyond to 4.5 (12).

Diversity also can be measured as the variation in species composition among communities (Beta diversity). Several techniques have been developed to compare communities based on their species compositions. The Jaccard's similarity coefficient (CJ) is the simplest of these similarity measures. This index will always give a value between 0 and 1 and then multiply by 100 to be expressed as percentage of similarity (12).

Knowledge about the ecological aspects of sand fly species and biodiversity indices help in understanding the transmission dynamics of the disease to human and reservoirs.

The purpose of this study was to determine the species composition, richness and diversity indices of the Phlebotomine sand flies at different topographic condition e.g. mountain/plain in North Khorasan Province located in the northeast of Iran.

Materials and Methods

Study area

This cross-sectional study was conducted during 2016 in some rural areas of North Khorasan Province, between 36°37'–38°17'

N latitudes and 55°53'–58°20' E longitudes. The province has a desert, mountainous and temperate climate with cold winters and is bordered by Turkmenistan in the north. The total area is approximately 28434km² and situated in the northeast of Iran. Bojnurd is the capital city of the province. The Koppeh Dagh Mountain, mountain range on the border of Turkmenistan and Iran and The Eastern Alborz range located in this province.

Selection of the villages of study

The sampling was focused on rural regions. Three villages including A) Bacheh-Dareh located in Raz and Jargalan County, B) Kohne Jolgeh located in Maneh and Samalqan County and C) Arg located in Jajarm County were selected based on diagnosed human VL and/or CL cases during last 5 years. Collection site in Bacheh-Dareh was a valley 3km far from the village and sand fly captures have been restricted to outdoor and wild environments. Collection sites in Kohne Jolgeh were indoors and domestic environments and in Arg were indoors and outdoors and domestic environments. Several human VL and CL had been reported from sites of A and B but only human CL cases had been reported from site of C (Fig. 1).

Sand fly collection

Sand flies were collected three times each twenty days during the peak activity periods of sand flies. Sample collection started from early July and continued until late Aug 2016. Seven collection methods were used as follows: 1) Sticky paper Traps (SPT). We installed 10 paper traps for each rotation and totally 60 papers per night. 2) Light Trap (LTP), and 3) CDC light trap baited with carbon dioxide (CO₂) gas that obtained by a CO₂ gas tank (CLT). The light traps were suspended at 1.5m above the ground. 4) White Shannon Trap (WST), 5) Black Shannon Trap (BST) were made of white or black cloth and consisted of a large central compartment and

two smaller lateral ones. The measurements, in meters (width, length, and height), of the central and the lateral compartments were 1.3× 1.3× 2m respectively and suspended by cords from supports and base of the traps touched the ground. A portable stove was used as CO₂ and light source inside the traps. 6) Animal Baited Trap (ABT) and finally 7) Disney Trap (DST). All of the traps were placed randomly with a distance of 20m from each other. The traps were set before sunset, were changed every two hours and remained in operation during the night (20:00 to 08:00). As the traps were changed the new ones were replaced in the same location. Traps were rotated clockwise between the trap locations in site “A” and “C” but were fix in site “B”. Collected sand flies were stored in 96% ethanol alcohol. The specimens were mounted on glass slides in Puri's medium. Species identification was carried out according to morphological characters using pictorial keys of sand flies (14).

Determination of the species diversity

Species diversity based on Simpson index and Shannon-Wiener index, species richness based on Margalef index and Menhinick index and also Evenness based on Shannon-Wiener evenness index and Buzas and Gibson's evenness index have been calculated to estimate species biodiversity of Phlebotomine sand flies in the study area. Computation of these indices is shown in (Table 1).

Beta diversity was also estimated by similarity between different communities using Jaccard's similarity coefficient. Berger-Parker dominance index and Lorenz graph of the collected sand flies within different collection sites were estimated. The statistical analyses were performed in SPSS version 18 (Chicago, IL, USA).

Results

An overall 7253 sand flies (19 species)

from three mentioned villages were collected and identified. Ten species belonged to the phlebotomus genus and nine species belong to the genus Sargentomia. The overall numbers of collected females were 3543 and consisted of 48.8% of specimens. *Phlebotomus sergenti* and *Ph. papatasi* were the most prevalent (84.9%) species in the study area. *Phlebotomus sergenti* was the most predominant species being recorded in all collected areas. Species composition and relative abundance of collected phlebotomine sand flies is shown in (Table 2).

Some of the most important Alpha diversity indices including, Species richness, Shan-

non Index, Simpson index, Buzas and Gibson's evenness index, Berger-Parker dominance index, Menhinick richness index, and Margalef richness index were calculated and are shown in Table 2. The Lorenzen curve is shown the cumulative percentage in relation to species rank or log species rank (Fig. 2).

The sand fly community similarity measured by Jacard's similarity coefficient. The similarity of the sand fly communities was the highest (42.1%) between community A and B and was the lowest (15.8%) between community A and C. Jacard's coefficient was 20% between community B and C.

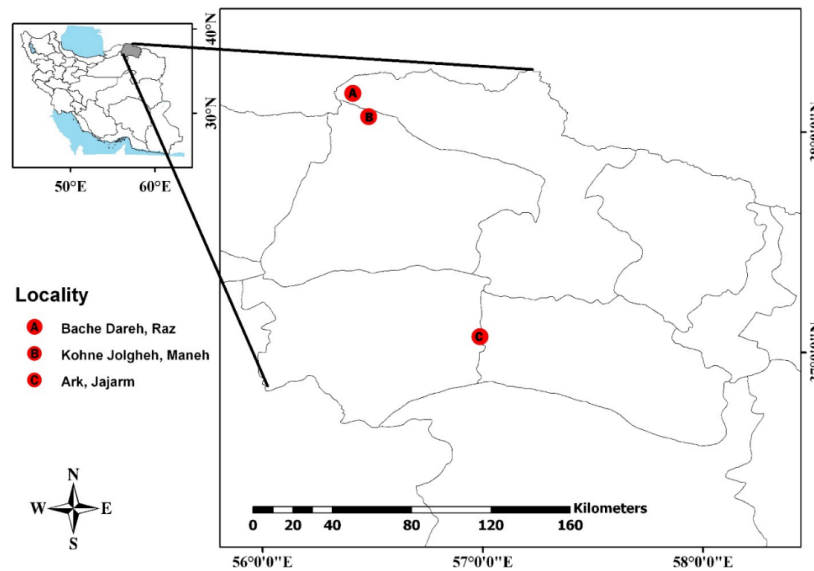


Fig. 1. Study area and geographical locations of phlebotomine sand flies, North Khorasan Province, Iran, 2016

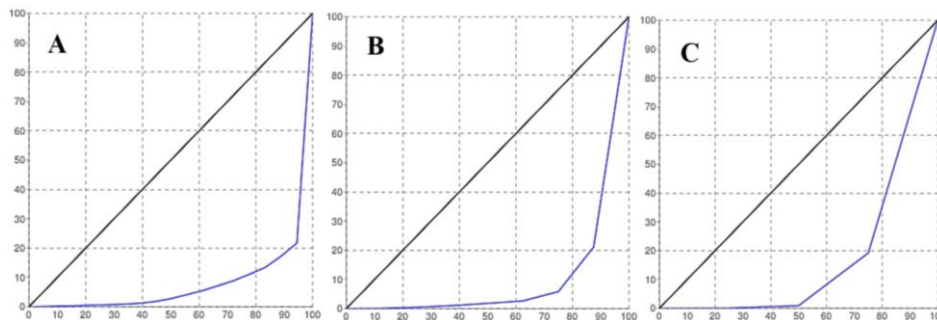


Fig. 2. The Lorenz graph for phlebotomine sand flies collected in different collection sites, North Khorasan Province, Iran, 2016. (A: Bacheh-Dareh village, B: Kohne Jolgeh village, C: Arg village)

Table 1. Different diversity indices of sand flies of the collection sites, North Khorasan Province, Iran, 2016

Index	Computation	Collection sites		
		A	B	C
specimens	Total number of specimens	3073	2392	1788
Species richness (S)	The number of species	18	8	4
Shannon Index (H')	$H' = \sum_{i=1}^s p_i \ln(p_i)$	1.033	0.718	0.527
Simpson Index	$\frac{\sum_{i=1}^s n_i(n_i - 1)}{N(N - 1)}$	0.6185	0.6468	0.6865
Shannon Evenness Index (E)	SHEI= H/ln (S)	0.357	0.345	0.380
Buzas and Gibson's Evenness Index	$e^{-\sum_{i=1}^s p_i \ln(p_i)}$	0.1561	0.2562	0.4235
Berger-Parker Dominance Index	$\frac{n_{max}}{N}$	0.7829	0.7889	0.8082
Menhinick Richness Index	$\frac{S}{\sqrt{\sum_{i=1}^s n_i}}$	0.3247	0.1636	0.0946
Margalef Richness Index	$\frac{S - 1}{\ln N}$	2.117	0.8998	0.4006

(A: Bacheh-Dareh village, B: Kohne Jolgeh village, C: Arg village)

Table 2. Species composition and relative abundance of phlebotomine sand flies in North Khorasan Province, Iran, 2016

Species	Collection sites			Total	%
	A	B	C		
<i>Ph. sergenti</i>	2406	1887	15	4308	59.4
<i>Ph. papatasi</i>	42	366	1445	1853	25.5
<i>Ph. alexandri</i>	137	76	0	213	2.9
<i>Ph. major</i>	52	9	0	61	0.8
<i>Ph. caucasicus</i>	6	2	0	8	0.1
<i>Ph. mongolensis</i>	9	0	0	9	0.1
<i>Ph. halepensis</i>	30	0	0	30	0.4
<i>Ph. longiductus</i>	5	0	0	5	0.1
<i>Ph. turanicus</i>	3	0	0	3	0.0
<i>Ph. ansarii</i>	0	0	1	1	0.0
<i>Se. sintoni</i>	4	0	327	331	4.6
<i>Se. sumbarica</i>	117	18	0	135	1.9
<i>Se. grekovi</i>	4	0	0	4	0.1
<i>Se. dentata</i>	19	14	0	33	0.5
<i>Se. theodori</i>	67	0	0	67	0.9
<i>Se. hodgsoni</i>	41	0	0	41	0.6
<i>Se. pawlowskyi</i>	72	0	0	72	1.0
<i>Se. dreyfussi turkestanica</i>	54	20	0	74	1.0
<i>Se. clydei</i>	5	0	0	5	0.1
Total	3073	2392	1788	7253	100.0

(A: Bacheh-Dareh village, B: Kohne Jolgeh village, C: Arg village)

Discussion

This study reports some proven or suspected vectors of CL and VL in Iran including *Ph. papatasi*, *Ph. sergenti*, *Ph. caucasicus*, *Ph. alexandri* and *Ph. major*. Among the collected species presence of *Sergentomyia dreyfussi turkestanica* in Iran was doubtful and we collected and confirmed the presence of this species in the country. *Phlebotomus turanicus* was reported from this region (3) and we collected and confirmed the presence of this species in Iran. *Phlebotomus kandelakii* had been reported as the probable vector of VL in North Khorasan Province (9) but we were not able to capture in this investigation.

Phlebotomus sergenti was the most predominant species throughout the study area and recorded in all localities. Our results are similar to another study that reported *Ph. sergenti* has a wide distribution in the country and includes and extends beyond the distribution of *L. tropica* (5).

Phlebotomus papatasi normally prefers to live in plain areas rather than in mountains. This species often abundant in areas of steppe and semi-arid zones where temperatures are high and such circumstance appears in community C. *Phlebotomus papatasi* showed a widespread distribution in this region and in community C about 81% of all specimens belong to this species. Low frequency of this species in mountains area such as community A could be explained by its preference to semi-arid areas (15, 16).

Phlebotomus alexandri and *Ph. major* were found in A and B collection sites recognized as the areas of the richest biodiversity of sandflies. *Phlebotomus major* has been reported from 17 out of 31 provinces and in all areas which human cases of ZVL have been reported mostly in mountainous areas (5). *Phlebotomus alexandri* has been reported as a probable vector of zoonotic visceral leishmaniasis (ZVL) in Iran (17) and is generally distributed in mountainous regions although

reported from almost all parts of Iran (5). These species were reported from different areas of Iran including plain and highlands. *Phlebotomus alexandri* prefers regions with a high percentage of relative humidity and warmer niches (16). Caspian Sea near of these villages provides relative humidity suitable for the presence of these species.

In the study area, nineteen species of sand flies collected and identified. This revealed sand flies species richness in this area was higher than some other regions in the country (15, 18, 19).

There were differences in species richness and diversity indices in three communities. In community A, the Shannon diversity index and richness were maximum ($H' = 1.033$, $S = 18$) and more than community B and C. There are only limited comprehensive studies on biodiversity of sand flies in Iran in which the authors calculated the relevant indices (15, 18-20). In a study in Qom Province, Shannon diversity index in mountainous areas ($H' = 1.36$, $S = 9$) was higher than in lowlands ($H = 0.66$, $S = 9$). The highest diversity in community A is similar to this investigation that showed more diversity in the mountainous area (15, 19), but in contrast to evenness. The evenness in Qom Province in the mountainous areas was 0.62, and in lowlands was 0.30, while in all communities in North Khorasan Province were similar (from $E = 0.345$ up to 0.380) and less than mountainous areas in Qom Province.

Species diversity, as indicated by the values of Shannon-Wiener index (H) consists of two components: species richness and evenness. A community is said to have high species diversity if many species are present and all species are nearly equally abundant but in all communities in the study area one or two species (*Ph. sergenti* and *Ph. papatasi*) consist of more than 80 percent of specimens as seen in table of species composition (Table.

1), Berger-Parker Dominance Index (Table 2) and Lorenz graph (Fig. 2) and therefore, evenness is less than other studies and species diversity is not much.

The similarity of the sand fly communities was different based on Jacard's similarity coefficient. The similarity of the sand fly communities was highest (from 40% up to 100%) between communities (18). The results were in contrast to our finding and showed more diversity of the sand fly communities in the North Khorasan Province. Alborz Mountain range and Aladagh Mountain in the middle parts of the province appear to be important geographical barriers for sand fly distribution. Future investigations are needed to clarify the role of natural barriers such as mountains for sand fly distribution.

Conclusion

The sand fly fauna in North Khorasan Province was very rich and often included some of the most important proven or suspected vectors of leishmaniasis. Species diversity indices (Shannon index, and Simpson's index) were not high due to decreasing in evenness. Some well-known species from mountainous areas such as *Ph. alexandri*, *Ph. major*, *Ph. halepensis*, *Ph. longiductus* and *Ph. turanicus* and plain area species such as *Ph. papatasi* and *Ph. caucasicus* were collected that shows the potential of transmission of a different kind of leishmaniasis in this province.

The species richness of sand flies was varied between three selected leishmaniasis foci in the North Khorasan Province and the Margalef richness index could accurately reflect the biodiversity of sand flies between three collection areas.

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