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Distribution and ecological aspects of sand flies (Diptera: Psychodidae) species in Northeastern Iran

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ABSTRACT

Objective: To determine both the distribution and the ecological characteristics of sand flies in Golestan Province, northeast of Iran in 2016. **Methods:** In this study, 34 villages were selected based on their geographical conditions. Sticky paper traps were used for collecting the sand flies. Sampling was carried out in each of villages from May to November. In each village, 60 traps for indoors and 60 for outdoors were monthly installed. The species of all collected sand flies were determined using approved morphological keys. Pearson coefficient correlation was used to find the relationship between the number of collected *Phlebotomus papatasi* from different villages and incidence rate of zoonotic cutaneous leishmaniasis as well as the number of positive cases of the disease. The altitude of the studied villages was extracted from digital elevation model of the area using GIS and vegetation cover density index of the province was extracted from Modis satellite imagery and distribution map of sand flies drawn up. **Results:** Overall, 5 428 sand flies were collected and identified, belonging to 18 species. *Phlebotomus wenyoni* was reported for the first time from the area in this study. The frequency of sand flies in the villages located in northeast of the Golestan province (the plateau area, lower altitude, arid and semi-arid climates, and lower vegetation cover density), were more than other villages in this province. There was a significant correlation between the number of collected *Phlebotomus papatasi* and incidence rate of the zoonotic cutaneous leishmaniasis cases in different villages ($r=0.837$, $P=0.019$) as well as the number of positive cases of the disease ($r=0.688$, $P<0.001$). **Conclusions:** In the northeastern areas of Golestan Province which is known as the endemic foci of zoonotic cutaneous leishmaniasis, the abundance of sand flies were more and the conditions for their growth and development were more appropriate.

1. Introduction

Zoonotic cutaneous leishmaniasis is one of the most important

vector-borne diseases in Golestan Province and is endemic in north and northeast areas of the province; it may be regarded as one of the most important health problems in these areas[1-3]. The disease

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is transmitted by infected female sand flies (Diptera: Psychodidae) to animals and humans. Up to date, approximately 1 000 species of sand flies have been identified worldwide[4] and 98 species are known as proven or suspected vectors of human leishmaniasis[5]. According to studies conducted in Iran since 1930 up to date, a total of 48 species of sand flies have been reported from two genera: *Phlebotomus* and *Sergentomyia*[6]. So far, approximately 18 species of sand flies have been reported in Golestan Province, northeast of Iran including: *Phlebotomus papatasi* (*Ph. papatasi*), *Phlebotomus caucasicus* (*Ph. caucasicus*), *Phlebotomus mongolensis*, *Phlebotomus sergenti* (*Ph. sergenti*), *Phlebotomus alexandri*, *Phlebotomus kazeruni*, *Phlebotomus brevis* (*Ph. brevis*), *Phlebotomus adleri* sp, *Sergentomyia sintoni* (*S. sintoni*), *Sergentomyia clydei* (*S. clydei*), *Sergentomyia sogdiana*, *Sergentomyia dentata*, *Sergentomyia hodegson*, *Sergentomyia antennata*; *Sergentomyia grekovi*, *Sergentomyia hodgsoni*, *Sergentomyia tiberiadis*. Among the collected sand flies in the earlier studies, two species of *Ph. papatasi* and *S. sintoni* have been known as the dominant species sand flies, where *Ph. papatasi* was the major vector of zoonotic cutaneous leishmaniasis with an infection rate of 1% in the province[3,7-14].

In general, sand flies have different ecological and behavioral patterns. Some of them tend to rest in indoors places and others in outdoors. Some species of sand flies prefer to live within active burrows of rodents (reservoir of disease) across the plain areas, and the others in mountainous areas. Sand flies from Iran belong to three regions: the Afrotropical, the Oriental and the Palearctic[15]. However, due to influence of temperature and relative humidity on survival of these sand flies, they can be found in areas where conditions are appropriate in terms of temperature and relative humidity[15]. Other important factors have also contributed to distribution of the sand flies in different areas of the world. So many studies have been conducted around the world to identify these factors. The mean temperature of the wettest and driest quarters, the annual mean temperature and minimum temperature in the coldest month[16, 17], land cover type[18], the altitude from the sea level[19] and rainfall[19-21] have been reported as effective factors for distribution of different species of sand flies. Moreover, a study in Iran showed that among climate and geographic factors, the mean temperature of the wettest quarters played a more important role to distribution of *Ph. papatasi*[17]. A study in Golestan province[22] also indicated that some factors such as slope, annual mean temperature, the altitude from the free sea level and vegetation cover were known as the most important factors to distribution of sand fly species. Therefore, due to the fact that Golestan province has three different climatic areas, *i.e.* plateau, mountainous, forest and coastal zones, this study was carried out to determine distribution and ecological

aspects of sand flies in Golestan province of Iran.

2. Materials and methods

2.1. Study area

Golestan province with an area of 20 437.74 square kilometers covers about 1.3 percent of the total area of Iran. It is located in the northeast of the country and bordered from the north to the Republic of Turkmenistan, from the south to Alborz mountain range and Semnan province, from the east to the Khorassan-e Shomali province and from the west to the Caspian Sea and Mazandaran province. According to the latest divisions of the year 2011, this province consists of 14 counties, 25 cities, 60 districts and 1 764 villages. The province is widespread with diverse ecology and climate conditions. With regard to the sea, forest and mountainous areas climate conditions of province is classified to temperate mountain, cold mountain (3 000 meters high), a mild Mediterranean, arid and semi-arid conditions, so that as we move from southern to northern parts, the amount of rainfall and relative humidity decreases and degree of temperature increases. In regards to topography of the province, this region is divided into three distinct areas including mountainous, plain areas, and evenly posts. The mountainous areas are located in southern parts containing the highest peaks of the province. Mountain areas are located in the foothills of the southern and eastern parts of province with coarse sediments as alluvial fans make of this land. In mountain areas due to the high permeability of the soil, groundwater aquifers with water in wells and canals are exploited. Due to retreat of Caspian Sea post and plain regions were created with severe water erosion and compaction of alluvial rivers. The lowest parts of the province (Caspian Sea 32 meters below sea level) is located in this area where most of the province's population living[23].

2.2. Villages selection

In this study, 34 villages were selected based on the wide range of different geographical zones including: 18 villages from wet zone including: 15 villages from hillside and mountainous areas and 3 villages from coastal areas. Also, 16 villages from arid and semi-arid area including: 15 villages from plain areas and 1 village from hillside areas. Among the selected villages, seven were located in endemic areas of zoonotic cutaneous leishmaniasis and 27 villages in non-endemic areas.

2.3. Sand fly collection

This study was carried out during the activity period of sand flies from early May to November 2016. Sticky paper traps coated with castor oil were used to collect sand flies, so that for each month (May- November) 60 indoor and 60 outdoor sticky traps were installed before sunset and were collected the next morning before sunrise. All sand flies on traps were removed using an insulin needle and were placed for two minutes in acetone. Then, sand flies were preserved in 70% ethanol and delivered to the entomology laboratory. All specimens were mounted in Puris' medium and identified using approved morphological keys[24, 25].

According to the results of previous studies in Golestan Province[3, 7-14] that reported seasonal monthly activity of sand flies from May to November, we also sampled these months and for the other months, the number of collected specimens was considered zero.

2.4. Data analysis

Pearson coefficient correlation was used to find the relationship between the number of collected *Ph. papatasi* (primary and main vector of zoonotic cutaneous leishmaniasis) from different counties and disease incidence rate as well as the number of positive cases of the disease. To perform the test, first the average number of *Ph.*

Table 1
Sand flies collected in different zones of Golestan Province, 2016.

Zone	T	EN/E	Altitude (m)	Village number	<i>Ph. papatasi</i>	<i>Ph. caucasicus</i>	<i>Ph. caucasicus group</i>	<i>Ph. mongolensis</i>	<i>Ph. sergenti</i>	<i>Ph. brevis</i>	<i>Ph. kazruni</i>	<i>Ph. adleri</i> (sp)	<i>Ph. alexandri</i>	<i>Ph. weenyoni</i>	<i>S. sinuoni</i>	<i>S. chydai</i>	<i>S. danata</i>	<i>S. hodgsoni</i>	<i>S. gorkoni</i>	<i>S. tiberiadis</i>	<i>S. sogdiana</i>	<i>S. antennata</i>	Total			
Dry	P	E	100	3	338	8	8	13	2	0	0	2	0	0	1 336	7	0	0	5	0	0	3	1 722			
			101-200	4	405	64	148	154	17	10	53	26	4	0	532	46	0	1	2	1	41	2	1 506			
	M	NE	100	6	297	0	0	0	5	0	0	2	0	0	388	2	6	0	0	0	0	0	700			
			101-200	2	113	18	16	15	11	0	7	0	0	0	156	5	5	0	9	0	0	0	355			
Wet	M	NE	100	10	99	0	0	0	6	1	0	0	0	4	92	0	0	0	0	0	0	0	202			
			101-300	2	117	0	0	0	2	1	0	0	0	0	46	0	43	6	0	0	0	0	215			
			301-600	1	119	0	3	6	27	0	0	0	0	0	9	0	0	0	0	0	0	0	164			
			600	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0			
	C	NE	0	3	65	0	0	0	0	0	0	0	0	0	86	0	0	0	0	0	0	0	151			
			Total				34	1 614	122	187	216	130	24	72	34	4	4	2 833	60	54	7	20	1	41	5	5 428
			Percent					29.73	2.25	3.45	3.98	2.39	0.44	1.33	0.63	0.07	0.07	52.19	1.11	0.99	0.13	0.37	0.02	0.76	0.09	100

NE: Cutaneous leishmaniasis non-endemic areas; E: Cutaneous leishmaniasis endemic areas; T: Topographical condition of areas; M: Mountain and forestall areas; P: Plate areas; C: Coastal areas.

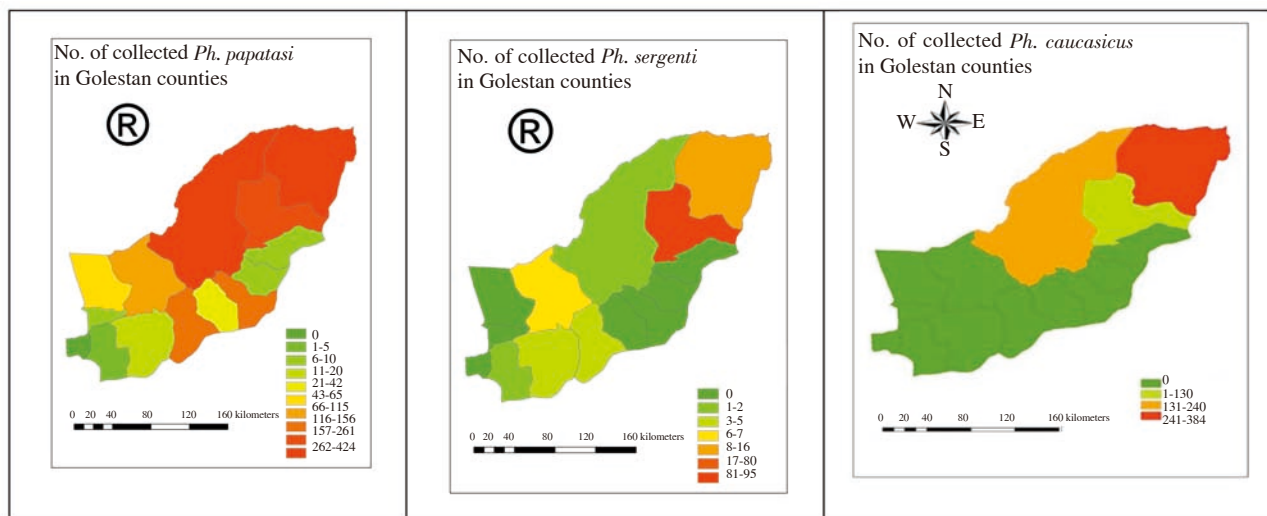


Figure 1. Geographical distribution of *Ph. papatasi*, *Ph. sergenti* and *Ph. caucasicus* group in Golestan Province, 2016.

papatasi in different villages was calculated according to below formula:

$$\frac{\text{Total number of collected } Ph. \textit{papatasi} \text{ from each county}}{\text{The number of installed traps}}$$

Moreover, the disease incidence it was calculated by dividing the number of positive cases of the disease by the population at risk of this disease (population without scar of cutaneous leishmaniasis). Then the correlation between the entered data by entering the data in SPSS version 18.

Map of elevation layers, were obtained from the World-Clim

database, version 1.4[26]. This database provides climate layers at a spatial resolution of 1 km² derived from the data of weather stations from 1950 to 2000[27]. Also, map of normalized differentiated vegetation index (NDVI) was extracted from Modis satellite imagery for August 2014 in spatial resolution of 1 000 m.

3. Results

In this study, a total of 5 428 specimens of sand flies were collected and 2 407 (44.34%) specimen belonging to the *Phlebotomus* genus and the other species belonging to the *Sergentomyia* genus. In this

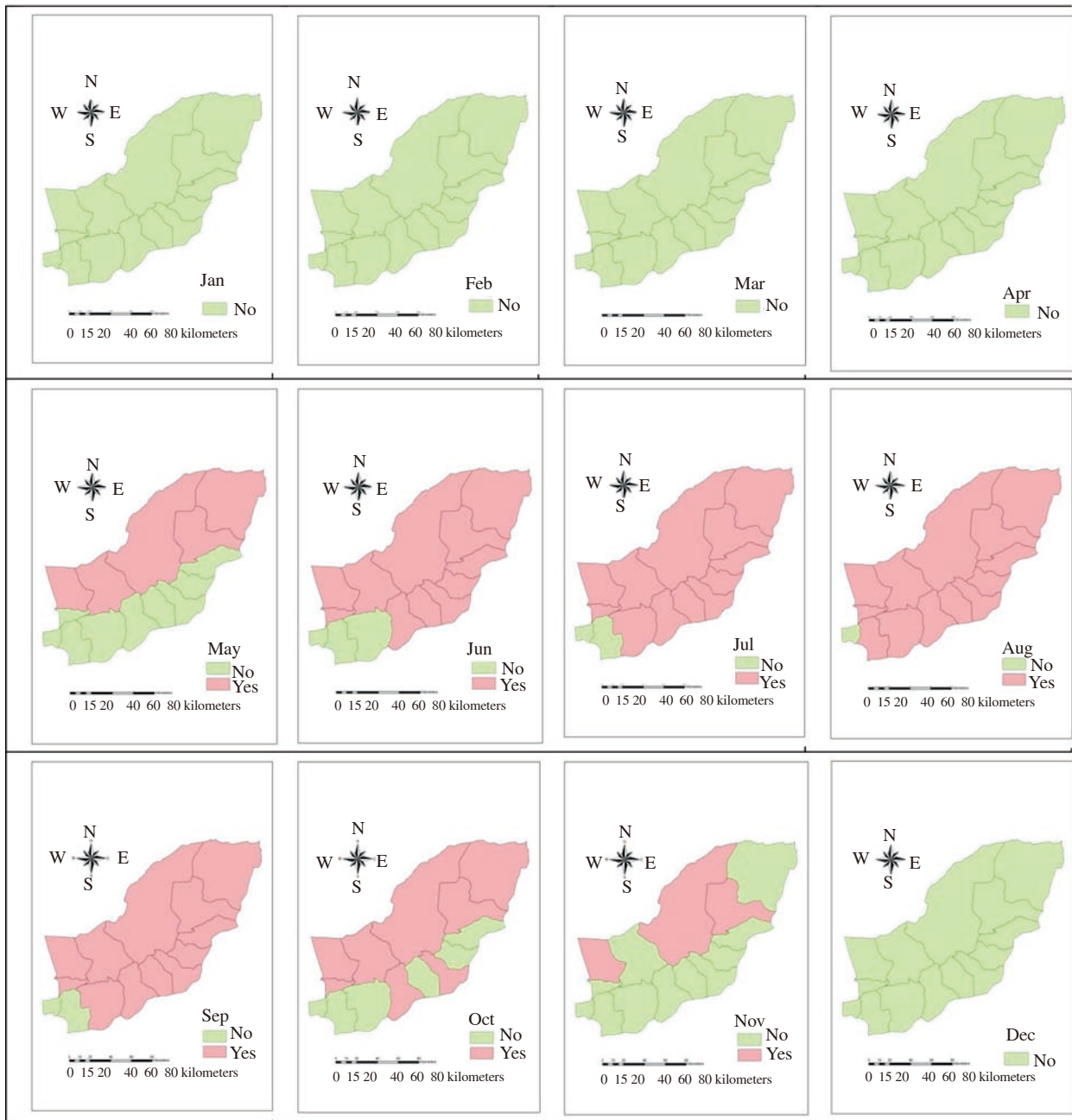


Figure 2. Monthly activity of *Ph. papatasi* in different counties of Golestan province, Iran, 2016.

study, a total of 18 species of sand flies were known in Golestan Province of which *S. sintoni* characterized as the dominant species in the region in relation to 52.19% of all species and *Ph. papatasi* (29.73%) was in lower ranking (Table 1). *Ph. wenyoni* was also reported for the first time from Golestan Province.

The most frequency of *Ph. papatasi* and *Ph. caucasicus* group were seen in northeast of Golestan Province and *Ph. sergenti* in Kalaleh County, east of this province (Figure 1). Sand flies monthly activity extended from May to November, and they were active for 7 month of year in north, northeast and east of counties (Figure 2).

Visual comparison of satellite images of vegetation cover, altitude and distribution of *Ph. papatasi* in different counties of Golestan Province indicated that frequency of this species in northeast of Golestan Province with lower vegetation cover and altitude from sea level was higher than other areas (Figure 3, 4).

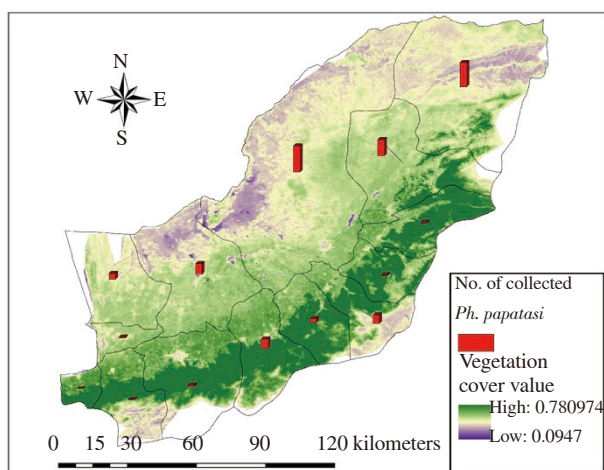


Figure 3. Geographical distribution of *Ph. papatasi* (No. per village) and NDVI in Golestan province, Iran, 2016.

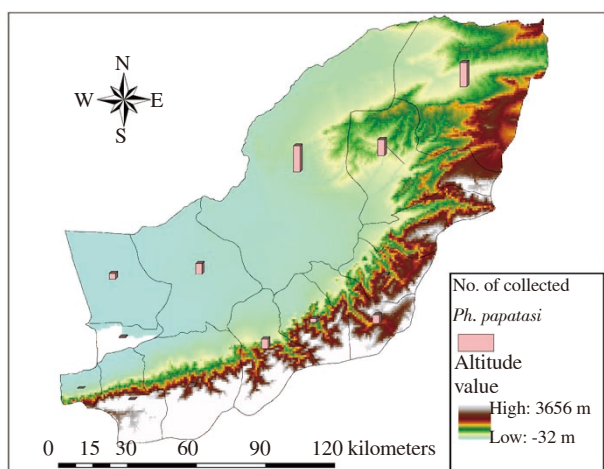


Figure 4. Geographical distribution of *Ph. papatasi* (No. per village) and altitude of sea level in Golestan province, Iran, 2016.

From 15 villages sampled in the plateau areas of the Golestan

Province, a total of 4 283 specimens of sand flies (285.5 sand flies per village) were collected, while 994 sand flies (62.1 sand flies per village) were collected from 16 villages of mountainous areas. In coastal areas, this figure was 50.3 per village (Table 1).

Sand flies were collected from an altitude of -23 m from the sea level to an altitude of 598 m. The highest number of sand flies were collected in a village with an altitude of 14 m from the sea level. No sand flies were collected in villages with an altitude above 900 m.

In this study, there was a significant relationship between the number of collected *Ph. papatasi* and the incidence rate of cutaneous leishmaniasis in different endemic and non-endemic counties of Golestan Province ($r=0.837$, $P=0.019$), as there was a significant relationship between the number of collected *Ph. papatasi* and number of positive cases of this disease ($r=0.688$, $P<0.001$) (Table 2).

Table 2

Number of positive cases and incidence of zoonotic cutaneous leishmaniasis and collected of sand flies in counties of Golestan Province, 2016.

Counties	No. positive cases	Incidence (per 100 000)	No. <i>Ph. papatasi</i> (per village)
Maraveh Tapeh	69	121.5	132
Gonbad-e kavus	334	99.4	141
Aqqala	37	28.7	57
Ramiyan	24	27.3	21
Gomishan	9	14.0	32
Aliabad-e katul	18	12.8	52
Azadshahr	10	10.1	46
Gorgan	39	8.7	7
Kalaleh	10	8.5	87
Kordkuy	5	6.9	1
Bandar-e Gaz	2	3.8	0
Bandar-e torkman	0	0.0	5
Galikesh	0	0.0	3
Minudasht	0	0.0	3
Total	557	31.7	-

4. Discussion

Comparing the species collected in this study with the previous studies in Golestan Province[3,7-14], *Ph. wenyoni* was also reported for the first time from Golestan Province. The abundance of sand flies in the villages located in the northeast of Golestan Province including plateau area, arid and semi-arid climates as well as lower vegetation cover density were more than other regions in this province.

The distribution of cutaneous leishmaniasis was very similar to distribution of sand flies, and the number of the positive cases of the disease and their incidence in the northeast villages of Golestan Province were significantly higher than other areas of the province. Similar to the results of this study, two studies conducted in Golestan Province[28, 29] showed distribution of cutaneous leishmaniasis in northeast of the province were higher than other areas. It was also reported that the number of collected sand flies in the Kalaleh County located in east of the province[10] was more than the number

of collected sand flies in Aliabad-e Katool County located in central part of the province[14]. The presence probability of *Ph. papatasi* was reported in the northeast of the Golestan province more than other areas[22]. The association between the incidence of cutaneous leishmaniasis and vegetation cover density was investigated[29,30] and results showed an inverse relationship between the incidence rate of cutaneous leishmaniasis and vegetation cover density which was consistent with our study. Similar to our study[31], others reported abundance of sand flies in plain area was more than mountainous and coastal areas; the abundance of sand flies in coastal area was more than mountainous areas. In another study[32], 56.8% sand flies had been collected from plain areas of Iranshahr County and mountainous areas, which was similar to the results of this study (81.2%). So we can conclude that situation in plain areas was more appropriate for growth and development of sand flies and distribution of sand flies in this areas are more than coastal and mountainous.

In this study, there was a significant relationship between the number of collected *Ph. papatasi* and the incidence rate of cutaneous leishmaniasis as well as the number of positive cases of the disease in different counties of the province. In the northeastern villages of the province with high frequency of *Ph. papatasi*, the incidence and number of positive cases of the disease were also high. A study conducted in Egypt[33] also showed there was an overlap between the geographical distribution of *Ph. papatasi* and distribution of zoonotic cutaneous leishmaniasis.

In the present study, the altitude of the sampling villages of sand flies from the sea level was also assessed. Sand flies were collected from an altitude of -23 m from the sea level to an altitude of 598 m. The highest number of sand flies were collected in a village with an altitude of 14 m from the sea level. No sand flies were collected in villages with an altitude above 900 m. In a conducted study in Turkey[34], which was similar to the results of our study, frequency of sand flies showed a negative correlation with the altitude and 56.1% of them were collected from areas with 0-199 m from sea level. According to our study, 68.7% of the sand flies in arid and semi-arid areas were collected from areas of 0-199 m. However, results of a study on the vectors of cutaneous leishmaniasis in Iran[17] showed that in areas with high potential for transmission of the disease, the presence probability of vectors (*Ph. papatasi* and *Ph. sergenti*) were higher than 60%, these areas had arid and semi-arid climates and an average altitude in these areas was estimated 990 m and 1 235 m, respectively. In a study conducted in Golestan Province[28] approximately 97.8% of patients were reported from arid and semi-arid regions with an altitude below 725 m from the sea level. *Ph. papatasi* known as the vector of zoonotic cutaneous leishmaniasis in Golestan Province[9], it was previously reported by all studies in Iran's different provinces[15]. This species was also collected from all counties of our studied areas, except the Bandar-e Gaz County, located in the west of the Golestan Province as well as villages which located in the mountainous and forest areas of the province. The abundance of this species in the northeast villages of Golestan Province, where had a geographic features including the plateau area, arid and semi-arid climate, was more than other areas of the

province and 71.5% of all collected specimens of this species were collected from this area. In a study conducted in Libya[19] areas with an altitude of less than 600 m were identified as suitable areas to distribution of this species, as in our study the abundance of this species was also higher in areas with lower altitudes than those with higher altitudes. Monthly activity of *Ph. papatasi* in plateau areas of the province continued from May to November for 7 months, but in the counties located in the mountainous and forest areas, the activity duration of this species lasted from June to October. Similar results were reported in the previous study of the Golestan Province[10,14]. In Iran[6], the activity of sand flies were estimated 10 months in the arid and semi-arid regions located in the southern part of the country and 7 to 8 months in the plateau areas located in center of Iran and 4 to 5 months in the cold regions located in the northwest areas of the country that was similar to the results of our study.

Another important species of sand flies in Golestan Province was *Ph. caucasicus* group known as the wild cycle vectors of *Leishmania major*[6,35,36] and also as the secondary vector of *Leishmania major* to human[36].

In the present study, *Ph. sergenti* represented 3% of all collected sand flies, which is known as the vector of anthroponotic cutaneous leishmaniasis in Iran[36,37]. This species was reported in Iran from 26 provinces with three morphotypes[38] and is known as a mountainous species[6]. In our study, the frequency of this species in plain areas were more than other regions. In southeast of Iran[31,32,39], 96%, 97% and 98.5% of this species were collected from mountainous areas. In our study, this species was collected from villages with -3 to 598 m, but most of the specimens of this species were collected from Kalaleh County in the east of Golestan Province, so that 73.2% of the specimens of this species were collected from three villages in Kalaleh County, that located in hillside and plateau areas with 598 m, 424 m and 273 m from sea level. The abundance of this species has been very low at lower altitudes. In the studies conducted in Iran, this species has been collected from the height of 40-2 232 m, but the average altitude from the sea level for this species was estimated at 1 235 m[17]. In other countries, this species has also collected from the height of 1 600-2 000 m above sea level[34,40].

Ph. alexandri, which is known as a probable vector of visceral leishmaniasis in the south part of the country, was almost 0.07% of all collected sand flies. In the various studies conducted in Iran[6], this species was reported mainly in the central regions of the country. However, in studies in Sistan-Baluchistan Province, 91.6%, 96.1% and 98% of this species were reported from mountainous areas[31,32,39]. Therefore, it is necessary to was done more studies in mountainous areas of Golestan Province.

In the present study, collected san flies of *Adlerius* subgenus was in a low abundance. However, the abundance of this subgenus in areas with an altitude above 100 m was greater than those with a lower altitude. In other studies in Golestan Province, this species was also collected in a low abundance[10,14]. Species of this subgenus are mostly found in wild form in mountainous areas above 1 200 m from sea level in northwest of Iran[41], as in our study, *Ph. brevis* is mostly collected in mountainous areas. There are about 20 species in this

subgenus, known as primary or probably vectors of zoonotic visceral leishmaniasis in many parts of the world, however, some species of this subgenus are reported as the vectors of *Leishmania tropica*, *Leishmania major* and *Leishmania donovani*[42]. Six species of this subgenus has been reported in Iran[42].

In Iran, the sand flies of the subgenus *Larrousius* are considered as vectors of zoonotic visceral leishmaniasis[35]. From this subgenus, only the species *Phlebotomus major* has been reported before from this province[6].

Another species that was collected in the high abundance in Golestan Province was *S. sintoni*, known as a vector of lizard leishmaniasis in Afghanistan and Iran[6,7,15]. In most studies, this species has been collected[6] from Iran as well as Golestan province[6,7,8,10,14]. The distribution of this species was similar to *Ph. papatasi* in different areas of Golestan province, and were often collected in plain regions with low altitude and low vegetation[32,39]. But contrary of our result, in another study conducted in southeast of Iran[31], the abundance of this species in coastal areas was more than plain and mountainous areas.

Sergentomyia clydei and *S. dentata*, also considered as vectors of lizard leishmaniasis[6,43,44], were collected in a low abundance in this study. Both of these species were reported in previous studies of Golestan Province[10,14]. Similar to the results of these studies, *S. clydei* was also reported from Aqqala, Gonad-e Kavus and Marāveh Tapeh Counties, located in the north and northeastern parts of Golestan Province.

Sergentomyia hodgsoni, previously reported only from the provinces such as West Azarbaijan, Sistan and Baluchestan and Aliabad-e Katul County in Golestan Province[14,15], were collected in a low abundance in this study. It is a species that has been collected from mountainous areas (caves and gap of the rocks in mountains) but it was in a low abundance in plateau areas[15]. In the current study, this species was again collected from a village in Aliabad-e Katul County with an altitude of 221 m from the sea level and hillside areas and also from a village in Gonbad-e-Kavus County with 189 m altitude, hillside and arid and semi-arid areas.

Other species such as *Sergentomyia sogdiana*, *S. grekovi*, *S. antennata* and *S.tiberiadis* were also collected from the northeastern parts of Golestan Province in a low abundance which the villages of these areas were located in arid and semi-arid conditions with an altitude of less than 200 m. Because these species have been collected with low abundance, more studies are necessary for review their distribution and ecological characteristics.

Conflict of interest statement

We declare that we have no conflict of interest.

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