■ ORIGINAL ARTICLE

Cutaneous Leishmaniasis Situation and Predicting the Distribution of *Phlebotomus papatasi* and *P. sergenti* as Vectors of Leishmaniasis in Ardabil Province, Iran

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Abstract: Cutaneous leishmaniosis (CL) is the most common form of leishmaniasis.CL caused by *L. major* and *L. tropica* is endemic in 17 provinces of Iran. This study was carried out to elucidate situation of CL in Ardabil province and to predict distribution of *Phlebotomus papatasi* and *Phlebotomus sergenti* (Diptera: Psychodidae) as vectors of CL in the region. In this cross-sectional study, data on CL patients were collected from local health centers of Ardabil province, Iran during 2006-2018 to establish a geodatabase using ArcGIS10.3. A total of 20 CL cases were selected randomly and skin samples were collected and analyzed by PCR method. MaxEnt 3.3.3 model was used to determine ecologically suitable niches for the main vectors. A total, 309 CL human cases were reported and the highest incidence rate of disease was occurred in Bilasavar (37/100,000) and Germi (35/100,000). A total of 2,794 sand flies were collected during May to October 2018. The environmentally suitable habitats for *P. papatasi* and *P. sergenti* were predicted to be present in northern and central areas of Ardabil province. The most variable that contributed ratio in the modeling were Isothermality and slope factors. Ardabil province is possibly an endemic are for CL. The presence of *P. papatasi* and *P. sergenti* justifies local transmission while the vectors of CL are existing in the northern and central areas of the province.

Key words: *Phlebotomus papatasi*, *Phlebotomus sergenti*, cutaneous leishmaniasis, vector, Iran

INTRODUCTION

Cutaneous Leishmaniasis (CL) is one of the neglected tropical disease caused by different *Leishmania* species which is endemic to more than 100 countries, annually 700,000-1,200,000 new cases of CL and visceral leishmaniasis (VL) in the world [1]. Four common clinical types of leishmaniasis are usually presented; CL, mucocutaneous leishmaniasis (MCL), diffuse cutaneous leishmaniasis (DCL) and VL. CL is the most common form of leishmaniasis, 2 types of CL were reported [2]. In Iran, 2 types of CL is endemic; zoonotic cutaneous leishmaniasis (ZCL) and anthroponotic cutaneous leishmaniasis (ACL) [3]. Annually, more than 20,000 cases of CL were reported in Iran. ACL is caused by *Leishmania tropica* and transmitted by

Phlebotomus (*Para-phlebotomous*)*sergenti* [4,5]. ACL was reported from Tehran, Mashhad, Shiraz, Kerman, Yazd, Bam and etc. [6,7]. In Iran, ZCL is caused by *L. major* and transmitted by *Phlebotomus papatasi* and small rodents are the main reservoir. ZCL is endemic in 17 out of 31 provinces, Iran [3,8,9]. Over 800 species of sand flies are known but only over 30 transmit *Leishmania* [8]. 44 spices of sand flies are reported in Iran and 10 species is doubtful [9-11]. Ardabil province is located in northwest of Iran and is the main endemic areas of VL and annually 15-20 new cases were reported [12,13]. To our knowledge through search on internet, no study is conducted on CL in the northwest of Iran including Ardabil Province. Previously, *Leishmania* parasite has been reported from 2 spices of *Sergentomyia dentata* and *S. sintoni* as well as *Leishmania* was isolated from *P. papatasi* using molecular methods. The parasite species has not been identified [14,15]. This study aims to explain about CL situation in Ardabil province and to explore distribution of *P. papatasi* and *P. sergenti* (Diptera:Psychodidae) as possible vectors of leishmaniasis in the province.

MATERIALS AND METHODS

Study area

Ardabil Province is located in the north-west of Iran (37.45-39.42°N, 47.30-48.55°E). The province has an area of 17,953 km² and a population of 1,270,000, based on 2016 census. This province consists of 10 counties, 21 districts, 26 cities, 71 rural districts and 1,477 permanent villages [16].

Data collection

CL patients' data are collected from the source document of each patient from Health Center of Ardabil province from 2006 to 2018, the information in regard to demographic, travel history, site and number of lesion was recorded and analyzed. Sand flies were collected from 30 areas during May to November 2018. The study areas were selected based on the frequency of human CL in the past 10 years. Five urban areas and 25 villages were selected, and their geographic coordinates were recorded using Global Positioning System. Sand flies were collected using caster oiled papers (60 papers for indoors and outdoors of each area). Then, the collected sand flies were put in acetone and then stored in the 70% alcohol. The speci-

mens were mounted by addition of a single drop of Puris' medium and identified using relevant identification keys [17,18].

PCR method

A sample was prepared from the slide which was used for direct smear, the skin materials were isolated from the slide by scratching, DNA was extracted and used for PCR amplification according to the procedure previously described [19,20]. PCR will be carried out using the primers for leishmania-specific pair fragment of kinetoplast DNA, F: (5′TCGCAGAACGCCCCTACC, 3′) and R: (5′AGGGGTTGGTGTAAAATAGG 3′). Amplification; 35 cycle, denaturation at 94°C for 1 min, annealing at 60°C for 45 sec, extension at 72°C for 1 min and final extension at 72°C for 7 min.

Modeling

The MaxEnt version 3.3.3 was used to predict the presence of *P. papatasi* and *P. sergenti* sand flies in order to prepare the distribution map at 72 locations (30 locations from *P. sergenti* and 42 locations for *P. papatasi*) in Ardabil Province northwest of Iran (Table 1). Jackknife test was used to analyze the relationship between weather variables and distribution of sand

Table 1. Variables used for MaxEnt modeling of cutaneous leishmaniasis distribution in Ardabil Province, northwest of Iran

Variable	Description	Contribution (%)	
		Phlebotomus papatasi	*Phlebotomus sergenti*
Bio1	Annual mean temperature (°C)	0	0
Bio2	Mean diurnal range: mean of monthly (max temp-min temp) (°C)	0	0
Bio3	Isothermality: (Bio2/Bio7) × 100	0.2	24.1
Bio4	Temperature seasonality (SD × 100)	0.6	0.4
Bio5	Maximum temperature of warmest month (°C)	0	4.3
Bio6	Minimum temperature of coldest month (°C)	0	0
Bio7	Temperature annual range (Bio5 – Bio6) (°C)	0	3.2
Bio8	Mean temperature of wettest quarter (°C)	2.8	19.7
Bio9	Mean temperature of driest quarter (°C)	0	0
Bio10	Mean temperature of warmest quarter (°C)	0.6	10.2
Bio11	Mean temperature of coldest quarter (°C)	0.8	0
Bio12	Annual precipitation (mm)	0.1	0
Bio13	Precipitation of wettest month (mm)	7.6	0.5
Bio14	Precipitation of driest month (mm)	6.3	0
Bio15	Precipitation seasonality (coefficient of variation)	0.2	4.6
Bio16	Precipitation of wettest quarter (mm)	0.5	0
Bio17	Precipitation of driest quarter (mm)	1.2	0
Bio18	Precipitation of warmest quarter (mm)	1.1	0
Bio19	Precipitation of coldest quarter (mm)	0.5	0.5
Altitude	Elevation from the sea level (m)	0	0
Slope	Slope of the area (%)	56.4	12.1
Aspect	direction of slope (degree)	6.2	13.8
NDVI	-1 to +1	14.9	0

flies and the relevant variables were identified with percentages and non-relevant variables were assigned zero. The output of the MaxEnt model was included in the ArcMap 10.4.1 software and was analyzed by Jackknife test and descriptive statistical.

RESULTS

Aspects of cutaneous leishmaniasis

Totally, 309 cases of CL were reported during 2005-2018 in Ardabil Province. The highest number (31cases) of CL was reported in 2009 and the lowest number (13 cases) in 2011 year (Fig. 1), CL were reported in 9 of 10 counties in Ardabil Province and the highest number was reported in Bilasavar region for 37/100,000 and Germi 35/100,000, about 50% of CL patients were reported from the north part, 39% from the central parts and 11% from south parts of Ardabil Province (Fig. 2), 71% of CL were males and 29% were females, The age range of the patients was 86% over 10 years and 14% under 10 years,
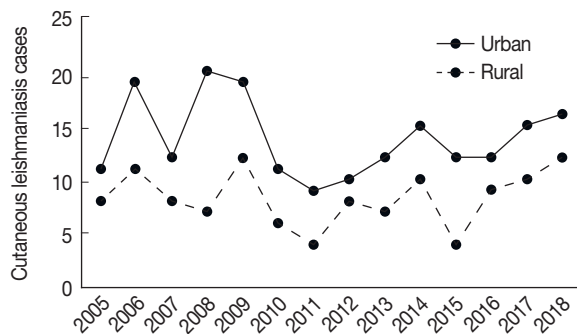
53% of the patients mentioned a history of travel outside the province. Annually, 20-30 CL cases were reported from Ardabil Province which 74.5% of CL reported from 13 urban areas and 24.5% from 46 rural areas, The clinical picture of the lesions showed that 65% of the lesions were dry with no secretion and 35% of the lesions were wet with secretion, most of the lesions (35.5%) was seen in hands and 30% in the face (Fig. 3), 47% of the patients present only a single lesion and 53% more than one lesion. The PCR results showed that the causative agents were *Leishmania tropica* in line 7 and *L. major* in lanes 1, 4, and 6 (Fig. 4).

Sand flies collected

A total of 2,794 sand flies were collected from 30 locations of Ardabil province, 40% of the total sand flies were belong to

Fig. 1. Trend of CL cases reported in urban and rural areas in Ardabil Province, northwest of Iran (2005-2018).

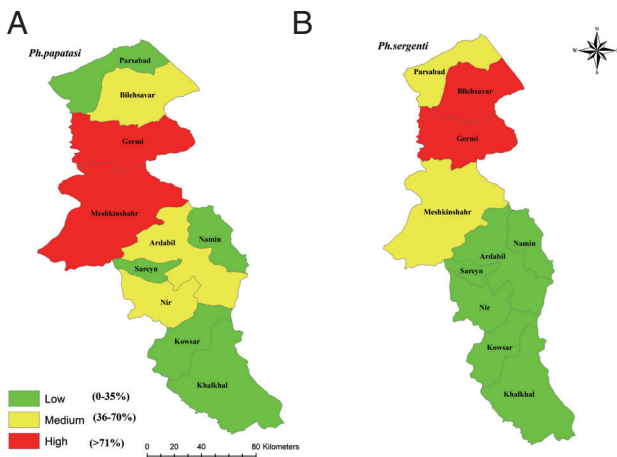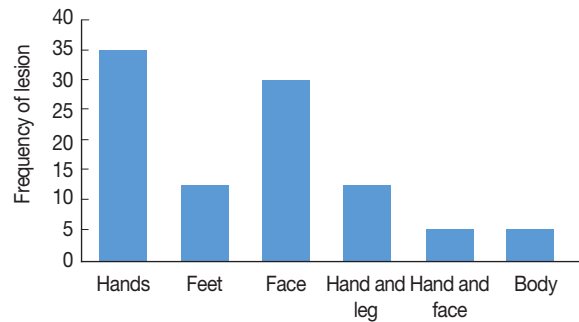**Fig. 2.** Frequency of *Phlebotomus papatasi* (A) and *Phlebotomus sergenti* (B) in northwest provinces, Iran, 2018.

Fig. 3. The frequency and location of lesion of CL in different areas, Ardabil Province, northwest Iran (2005-2018).
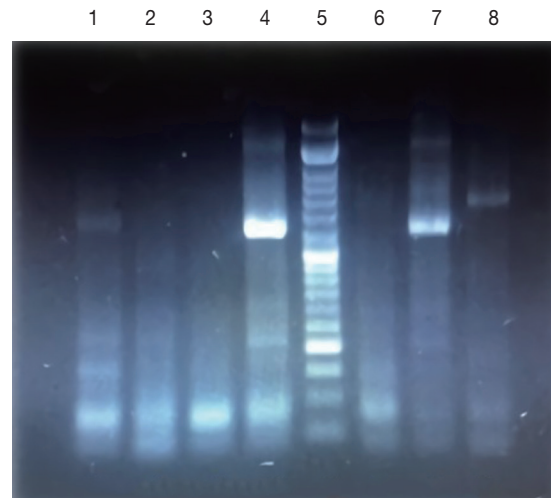

Fig. 4. 1.5% agarose gel electrophoresis of PCR amplification for identification of *Leishmania* species on Giemsa-Stained slides. Lanes 1,4,7: Standard *Leishmania major*; Lanes 2,3,6: negative control; Lane 5: DNA Ladder (50 bp); Lane 8: *Leishmania tropica*.

Table 2. Fauna and number of collected sand flies in Ardabil Province, northwest Iran, 2018

Subgenus	Spices	Total collected	Male (%)	Female (%)	Outdoors (%)	Indoors (%)	P-value
Phlebotomus	*Phlebotomus papatasi*	662	71	29	55	45	< 0.05
Paraphlebotomus	*Phlebotomus sergenti*	340	74.7	25.3	75.6	24.4	< 0.05
	Phlebotomus caucasicus	30	73.3	26.7	63.3	36.7	< 0.05
	Phlebotomus mongolensis	49	100	0	89.8	10.2	< 0.05
	Phlebotomus jacusieli	6	100	0	100	0	< 0.05
	Phlebotomus andrejevi	31	100	0	93.6	6.5	< 0.05
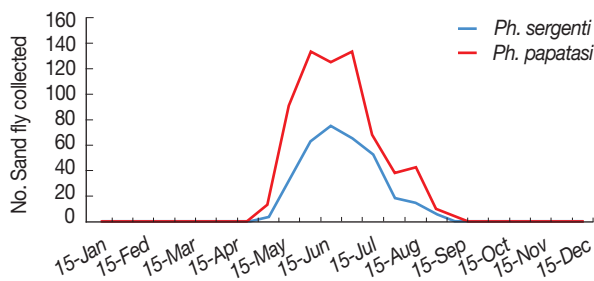
Fig. 5. Monthly activity of *Phlebotomus papatasi* and *Phlebotomus sergenti* in Ardabil province, Iran, 2018.

the 2 *phlebotomus* and *paraphlebotomus* subgenus and 60% to 6 other spices (Fig. 2). *P. papatasi* were collected from all of the study sites but *P. sergenti* was collected in 22 of the 30 sites.

P. papatasi

Only 1 spice of *Phlebotomus* subgenus was collected and 23.7% of the total collected sand flies were belong to this spices (M = 71%, F = 29%), 55% of *P. papatasi* were collected from outdoors and 45% from indoors (Table 2). The frequency of *P. papatasi* was the highest in all the study areas of Ardabil Province and the maximum number of samples were collected from in Meshkinshahr and Germy counties (Fig. 2). Monthly activity of *P. papatasi* in Ardabil province was started in 30 April and ended in 15 October (when density of sand flies reaches to zero), with one peak between in 15 June to 15 July (Fig. 5).

P. sergenti

Five spices of this *Paraphlebotomus* were collected in the study areas and 16.32% of total sand flies were belong to these spices (M = 74.7%, F = 25.3%), 75.5% of the species were collected from outdoors and 24.5% of indoors. The highest and lowest spices in paraphlebotomus subgenus was *P. sergenti* (74.6%) and *P. jacusieli* (1.3%), respectively (Table 2). The frequency of *P. sergenti* was the highest in north and central parts

and the highest number of the spices were collected from Germy and Bilasavar counties (Fig. 2). Monthly activity of *P. sergenti* in Ardabil Province was started in 15 May and ended in 30 September (when density of sand flies reaches to zero) with one peak in June (Fig. 5).

Ecological niche modeling
P. sergenti

The environmental suitability for *P. sergenti* was predicted to be present in most parts of northern Ardabil Province (Fig. 6A), although there are some areas of high environmental suitability in northeastern, northwestern regions as well. Warmer colors show areas with higher environmental suitability for *P. sergenti*.The areas under receiver operating characteristic (ROC) curve (AUC) for training and testing data was calculated as 0.904 and 0.933, respectively. The most variable seems to contribute the ratio in the modeling of *P. sergenti* was Bio3 (Isothermality) factor and Jackknife test results showed that the environmental variable with the highest gain in the predicting power of the model if used in isolation was Slope variable (Fig. 7A).

P. papatasi

The results of this study showed that the environmental suitability habitat for *P. papatasi* was in northern and central parts of Ardabil province (Fig. 6B). There are some areas of high environmental suitability in northern regions in borderline of Iran and Azerbaijan Republic country. The area under receiver operating characteristic (ROC) curve (AUC) for training and testing data was calculated as 0.866 and 0.568, respectively. The important variable which contributed ratio in the modeling of *P. papatasi* was slope and NDVI factors. The results of jackknife test showed that the Bio8 (Mean Temperature of Wettest Quarter) factors with highest gain in the predicting power of the model if used in isolation (Fig. 7B).
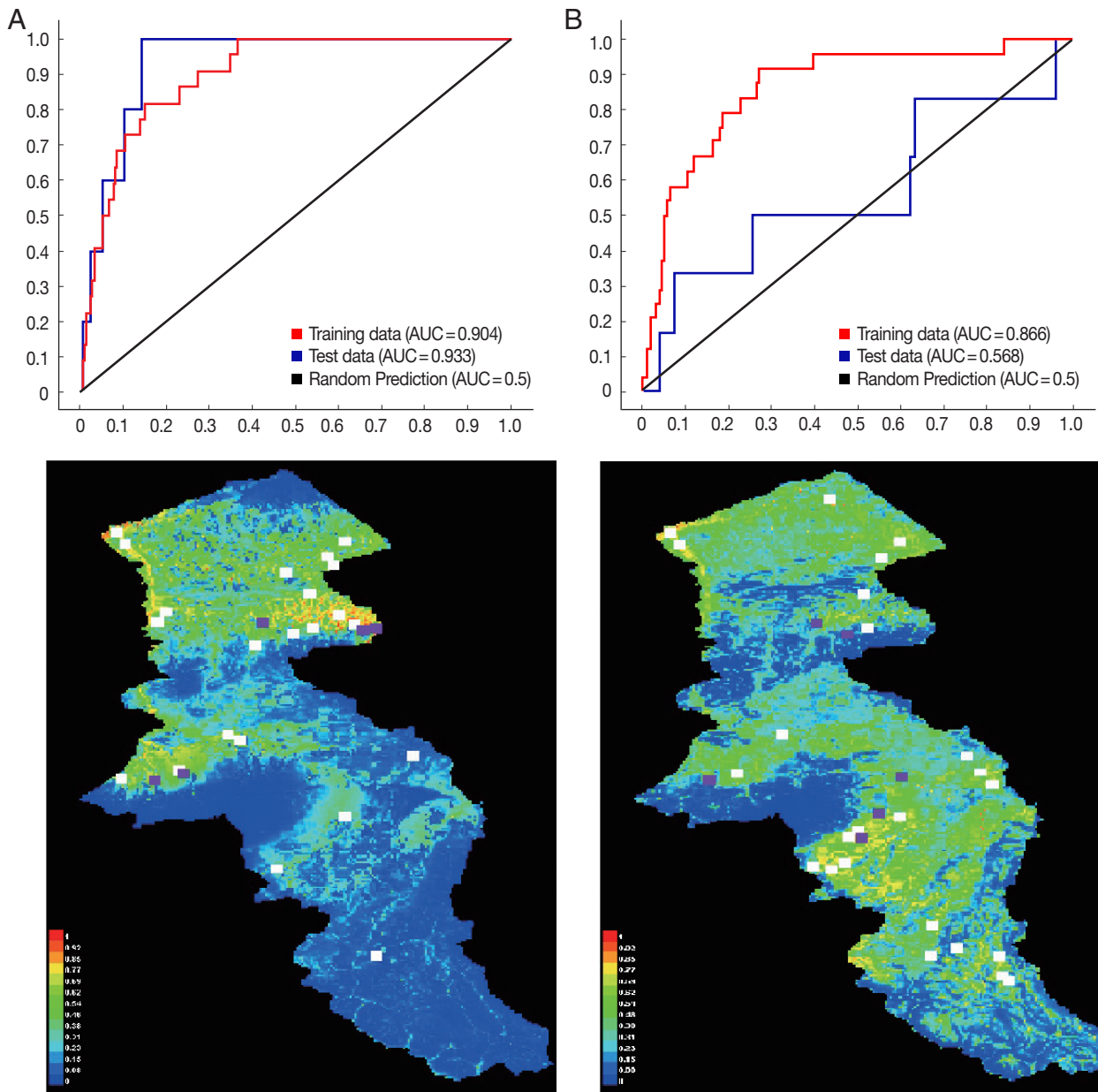
Fig. 6. Presentation of MaxEnt model (Upper) and receiver operating characteristic (Down) of two vectors of cutaneous leishmaniasis in northwest Iran. (A) *Phlebotomus sergenti*. (B) *Phlebotomus papatasi*.

DISCUSSION

Annually, 20-30 cases of CL were reported from Ardabil province and in 47% of the cases showed no history of travel outside the province. Out of human cases, 17% were under 10 years old and 74.5% of CL reported from 13 urban areas as well as 65% of lesions were dry and without secretion that these symptoms are probably related to ACL. The important clinical symptoms for ACL including dry ulcer that most occur in face in urban areas [17].

In The results of this study show that dominant species of Ardabil province is a *P. papatasi*. In a study which was completed by Absavaran et al. [19], Dehkordi et al. [22], Ghorbani et al. [23], and; Moradi-Asl et al. [24] confirmed the current results. *P. papatasi* and *P. sergenti* are the main vectors of ZCL and ACL in Iran respectively[3]. In this study *P. papatasi* was collected from all regions of Ardabil Province with a high frequency in north and central parts and *P. sergenti* from north part of Ar-
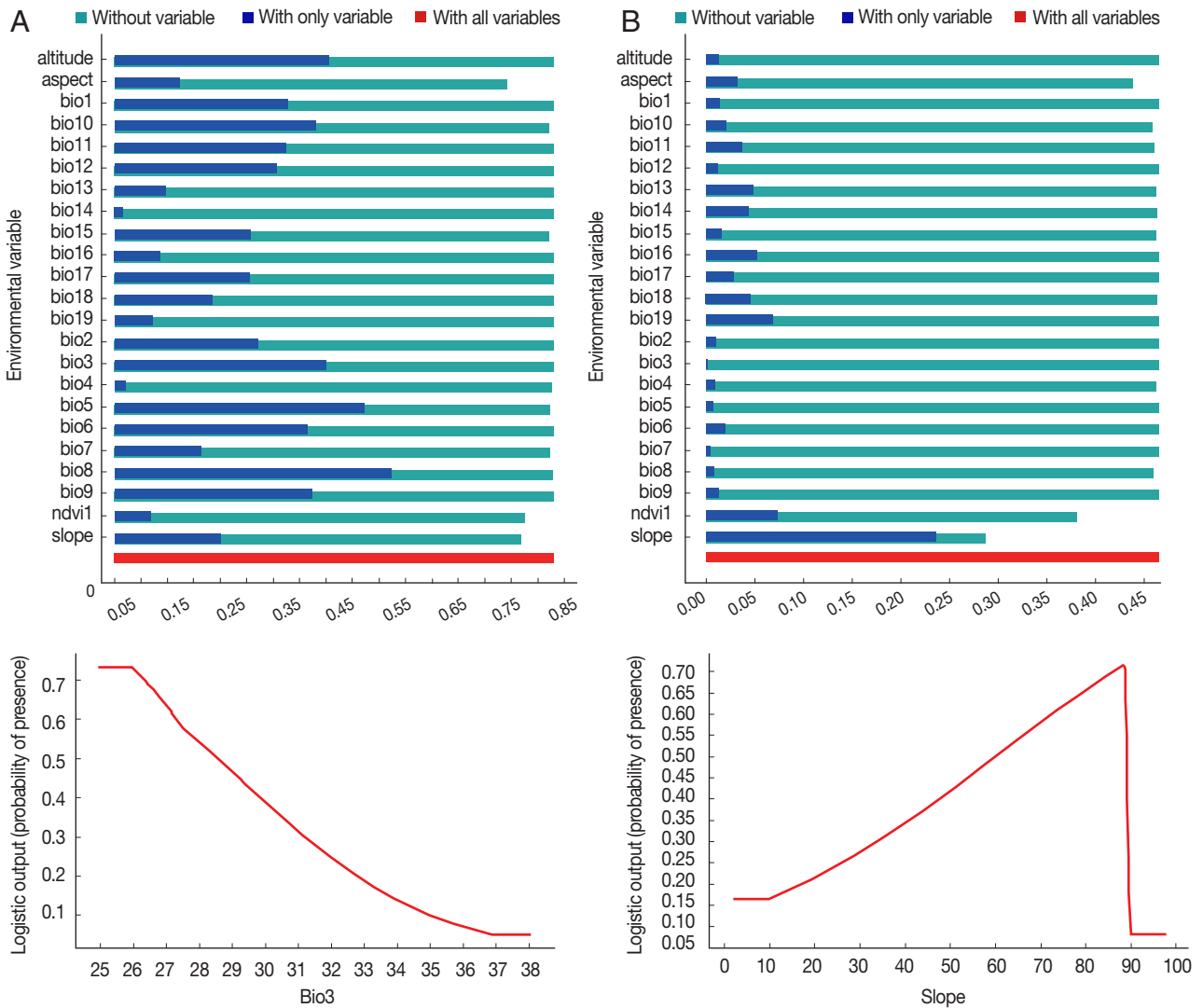
Fig. 7. Variable importance (upper) and the most important variable by jackknife test (down) for two vectors of cutaneous leishmaniasis in Ardabil Province. (A) *Phlebotomus sergenti*. (B) *Phlebotomus papatasi*.

dabil province, the same areas of CL reports in Bilasavar and Germi counties. This result showed that the vectors of CL is present in the Ardebil province with activity in June to July. Since 45% of *P. papatasi* and 24.5% of *P. sergenti* were collected from indoors, it seems that the sand flies have a feeding and resting in indoors places but invers, *P. sergenti* is more likely to outdoors places. In study of central Iran, 52% of collected sand flies were *P. papatasi* that 33% of them were captured in indoor places and the seasonal activity was reported May to September [25]. The average temperature and altitude level in the central and northwest of Iran has a significant difference and have a greatest impacts on seasonal activity and distribution of sand flies [17-26]. The results of this study showed that

the suitable habitat for *P. sergenti* in Ardabil Province were located in north part that this area have a lower than 1,500 m altitude. The results of MaxEnt model and jacknife test showed that isothermality and slope factors had the greatest effect on the distribution of *P. sergenti* in north part of Ardabil Province, respectively. With the slope increasing from 10 to 88 degrees, also frequency of *P. papatasi* has increased and after the 89 degrees the frequency of this spices has decreased. In one study, *Lutzomyia whitmani* the vector for CL in Southern Brazil was affected the east slope of the Andes [27] and the results of ecological niche model of *P. alexandri* and *P. papatasi* in the Middle East showed that elevation, minimum temperature in the coldest month, mean temperature in the wettest quarter, and

precipitation in the driest month were the most important role in modeling of this spices [28]. For *P. papatasi* in Ardabil Province the suitable habitat was identified in north and central parts. The important factors that impacted on ecological niche model of *P. papatasi* were slope and NDVI. In central of Iran, lowland areas provides good ecological niches for *P. papatasi* and max-min temperature were impacted on ecological niche model [16]. According to the findings, cutaneous leishmaniasis might be endemic in Ardabil province with low incidence. Moreover, *P. papatasi* and *P. sergenti* as vectors of CL are present in northern and central parts of the province, and there are the potential of transmission of CL in the areas. It is recommended to more studies be done in the area in respect of CL, reservoir and vectors.

ACKNOWLEDGMENTS

CONFLICT OF INTEREST

The authors declare no conflict of interest related to this study.

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