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Ecological Niche Modeling of West Nile Virus Vector in Northwest of Iran

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ABSTRACT

Objectives: West Nile virus (WNV) is a microorganism and the leading cause of the spread of fatal diseases in horses, birds, and humans. The etiologic agent of West Nile fever belongs to the genus *Flavivirus* that is transmitted by *Culex* mosquitoes in Iran and throughout the world. We aimed to evaluate the distribution of WNV vectors in northwest of Iran ecological niche modeling. ***Methods***: This cross-sectional study was conducted in Ardabil province in one year, from January to December with the samples captured from May to November 2017. Forty-five locations from all 10 counties of the province were selected randomly to determine the seasonal activity of mosquitoes. Larval collection was carried out twice a month. MaxEnt version 3.3, ArcMap 10.3 software, jackknife, and crew methods were used to determine the impact of climatic change and environmental factors on the distribution of mosquito species. ***Results:*** A total of 2000 larva were collected, 1789 (89.5%) of which were *Culex* larvae. Seven species of *Culex* genus were identified, including *Culex pipiens, Cx. modestus, Cx. theileri, Cx. hortensis, Cx. perexiguus, Cx. tritaeniorhynchus,* and *Cx. mimeticus*. Two important ecological niche areas were identified in the north and south of the province. The annual temperature and rainfall in the cold seasons were the most important factors affecting the distribution of *Culex* species larva. *Cx. pipiens* was identified as the main vector of WNV vectors with high frequency in Ardabil province. ***Conclusions:*** Two large areas were found as the ecological niches of larvae of these species. It is recommended that additional investigations be carried out on infection in adult female *Cx. pipiens* and its hosts in these areas.

Mosquito-borne diseases are one of the most important health problems in the world.[1] At least 3531 species of mosquitoes (Culicidae, Diptera) including 112 genera have been reported in the world.[2] *Culex* genus is classified into 26 subgenera and 769 species.[3] *Culex pipiens* is distributed in tropical and subtropical regions such as Africa, Asia, Australia, and the tropical islands. In addition to human biting, they transmit numerous important diseases to humans. They include agents of parasitic diseases such as *Wuchereria bancrofti*, *Brugia malayi*, *Brugia timori* and the important viral disease agents such as West Nile virus (WNV), Sindbis virus, Japanese encephalitis, St. Louis, Western Horse Encephalitis, Rift Valley Fever (RVF), and Oropouche fever.[4–6] WNV is the re-emerging pathogen which threatens human health.[7] The agent of West Nile fever belongs to the genus *Flavivirus* transmitted by *Culex* mosquitoes.[8] West Nile fever was reported for the first time in Uganda in 1937.[9] In Iran, WNV has been reported in several regions, especially central, southwestern, and northern Iran,[10] with the infection rate reported to be 1.3–5%.[10–12] Many studies have been carried out on the distribution of mosquitoes, especially larvae and distribution of *Culex* genus adults or larvae in different parts of Iran.[13–16] Various studies conducted on mosquitoes in the northwest of Iran, including Ardabil province have reported a high frequency of

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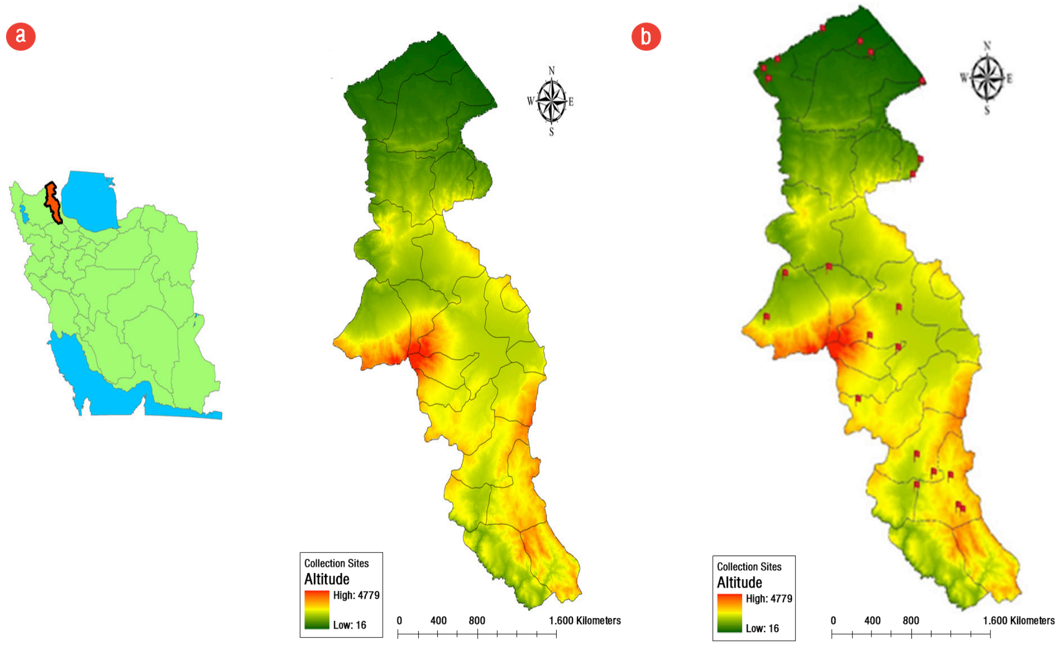

Figure 1: Sampling locations and detection sites for *Culex pipiens* in the study area, Ardabil province, northwest of Iran, 2017.

Culex species.[17–19] Infection with WNV has not been reported in Ardabil province. However, Dirofilaria infection with *Culex* has been reported in this province.[20] Determining the distribution of vectors based on the Ecological Niche Model (ENM) can serve as a guideline to understand the ecology and distribution of diseases. It also calls for accurate planning to control and prevent this disease, which has been implemented in high-risk areas.[21] MaxEnt is one of the most important tools for determining the ENM of one or more species by using environmental data while predicting the probability of the presence of species in different places.[22] Our study sought to predict the distribution of WNV vectors namely, *Cx. pipiens* larva, using ENM in Ardabil province in northwest of Iran.

METHODS

Ardabil province is located at 38.2514N and 48.2973E in the northwest of Iran, sharing a common border with the Republic of Azerbaijan to the north. The province has an area of 17 953 square kilometers with 10 cities and a population of 1.27 million. About two-thirds of the province consists of mountainous areas with high altitude while the rest are flat areas. The northern region of the province (Moghan plain) has a low altitude and a relatively warm climate, with the central and southern regions having cold climates [Figure 1].

We randomly selected 46 locations to collect larvae, which occurred twice monthly during seasonal activity of mosquitoes. Larval sampling was carried out by the dipping method (350 mL dipper) from January to December 2017 with the samples captured from May to November. Sampling was carried out from different parts in each habitat, with 10 dipping loads in each habitat. The larvae were stored in a lactophenol solution, and after about a week they became transparent so that microscopic slides were made using a Faure's medium. The third- and fourth-instar larvae were identified using the appropriate identification keys.[23–27]

For modeling and determining the ENM, WNV vectors were analyzed using MaxEnt 3.3 software. To predict the distribution of *Cx. pipiens* with a presence probability of more than 70%, the maps of 23 climatic and environmental factors were used, which were taken at a resolution of 30 m/s from the website (http://www.worldclim.org/bioclim.htm) and Modis satellite [Table 1]. In these maps, the areas with a high probability of this species were marked with red, and areas with low presence were identified with green. Jackknife and crew analysis were used in MaxEnt to investigate the effects of environmental and climatic variables on *Culex* species distribution

Table 1: Variables used for MaxEnt modeling of *Culex pipiens* distribution in Ardabil province, northwest of Iran.

Variables	Description	Contribution, %
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)	6.8
Bio4	Temperature seasonality (SD × 100)	0.0
Bio5	Maximum temperature of the warmest month (°C)	21.2
Bio6	Minimum temperature of the coldest month (°C)	0.0
Bio7	Temperature annual range (Bio5−Bio6) (°C)	3.2
Bio8	Mean temperature of wettest quarter (°C)	2.1
Bio9	Mean temperature of driest quarter (°C)	0.0
Bio10	Mean temperature of warmest quarter (°C)	0.0
Bio11	Mean temperature of coldest quarter (°C)	0.0
Bio12	Annual precipitation (mm)	0.1
Bio13	Precipitation of wettest month (mm)	1.7
Bio14	Precipitation of driest month (mm)	0.0
Bio15	Precipitation seasonality (coefficient of variation)	6.2
Bio16	Precipitation of wettest quarter (mm)	7.0
Bio17	Precipitation of driest quarter (mm)	0.0
Bio18	Precipitation of warmest quarter (mm)	0.0
Bio19	Precipitation of coldest quarter (mm)	21.9
Altitude	Elevation from the sea level (Beiranvand R, #1)	3.6
Slope	Slope of the area (%)	17.7
Aspect	Direction of slope (degree)	6.9
NDVI	-1 to +1	2.1

NDVI: normalized difference vegetation index.

and consideration of its ecological niche. This model can be used to evaluate the location of the samples' breeding places and their frequency in one place. The best breeding place and habitat for the species can be determined by this model.[28]

RESULTS

Out of 2000 collected larvae from 46 collection sites in Ardabil province [Figure 1a], 1789 (89.5%) were identified as *Culex* larvae. Seven species of *Culex* genus were collected from 22 location sites [Figure 1b] and identified as follows: *Cx. pipiens* (71.5%), *Cx. modestus* (2.8%), *Cx. theileri* (16.8%), *Cx. hortensis* (6.6%), *Cx. perexiguus* (1.0%), *Cx. tritaeniorhynchus* (0.8%), and *Cx. mimeticus* (0.4%). *Cx. pipiens* was collected with a high frequency in all the counties, especially in northern (21.3%) and southern parts (12.9%) of the province while *Cx. mimeticus* was collected with a low frequency

Table 2: Composition and localities of the larvae of *Culex* mosquitoes collected in Ardabil province, northwest of Iran, 2017.

Species	Kowsar	Bilehsavar	Sareyn	Khalkhal	Pars-abad	Ardabil	Namin	Nir	Meshkin-shahr	Germi	Total
Cx. modestus	8	0	0	12	14	6	0	2	8	0	50
Cx. hortensis	19	7	4	14	26	12	0	16	12	8	118
Cx. pipiens	79	72	0	231	381	236	36	9	217	18	1279
Cx.theileri	49	5	41	37	57	16	0	18	68	10	301
Cx. perexiguus	2	10	0	0	6	0	0	0	0	0	18
Cx. tritaeniorhynchus	1	6	0	0	6	0	0	0	0	2	15
Cx. mimeticus	0	0	0	0	6	2	0	0	0	0	8
Total	158	100	45	294	496	272	36	45	305	38	1789

Table 3: The larval habitat characteristics and occurrence percentages of different *Culex* mosquitoes larvae in Ardabil province, northwest of Iran, 2017.

Larval breeding site	*Cx. hortensis*	*Cx. modestus*	*Cx. pipiens*	*Cx. theileri*	*Cx. perexiguus*	*Cx. ritaeniorhynchus*	*Cx. mimeticus*
Habitat status, %							
Permanent	95.0	90.0	75.0	71.5	68.0	71.0	95.0
Temporary	5.0	10.0	25.0	28.5	32.0	29.0	5.0
Water, %							
Stagnant	18.0	27.0	33.0	30.0	14.0	15.5	15.0
Slow running	82.0	73.0	67.0	70.0	86.0	84.5	85.0
Vegetation, %							
Without vegetation	3.0	15.0	23.0	22.5	20.0	19.0	21.5
With vegetation	97.0	85.0	77.0	76.5	80.0	81.0	78.5
Type of bed, %							
Clay	15.0	30.0	45.0	47.0	43.0	44.0	70.0
Sand	25.0	15.0	35.0	30.0	20.0	25.0	20.0
Water status							
Stone or cement, %	60.0	55.0	15.0	23.0	37.0	31.0	10.0
Opaque	12.0	2.0	10.0	5.0	25.0	30.0	80.0
Transparent	88.0	98.0	90.0	95.0	75.0	70.0	20.0
Ligh status,%							
Sunny	0.0	4.0	12.0	26.0	15.0	38.0	40.0
Shaded	5.0	36.0	17.0	20.0	19.0	17.0	20.0
Semi-shade	95.0	60.0	61.0	54.0	66.0	45.0	40.0
Habitat type, %							
Natural	92.0	85.0	78.0	86.0	95.5	87.5	75.0
Artificial	8.0	15.0	22.0	14.0	4.5	12.5	25.0

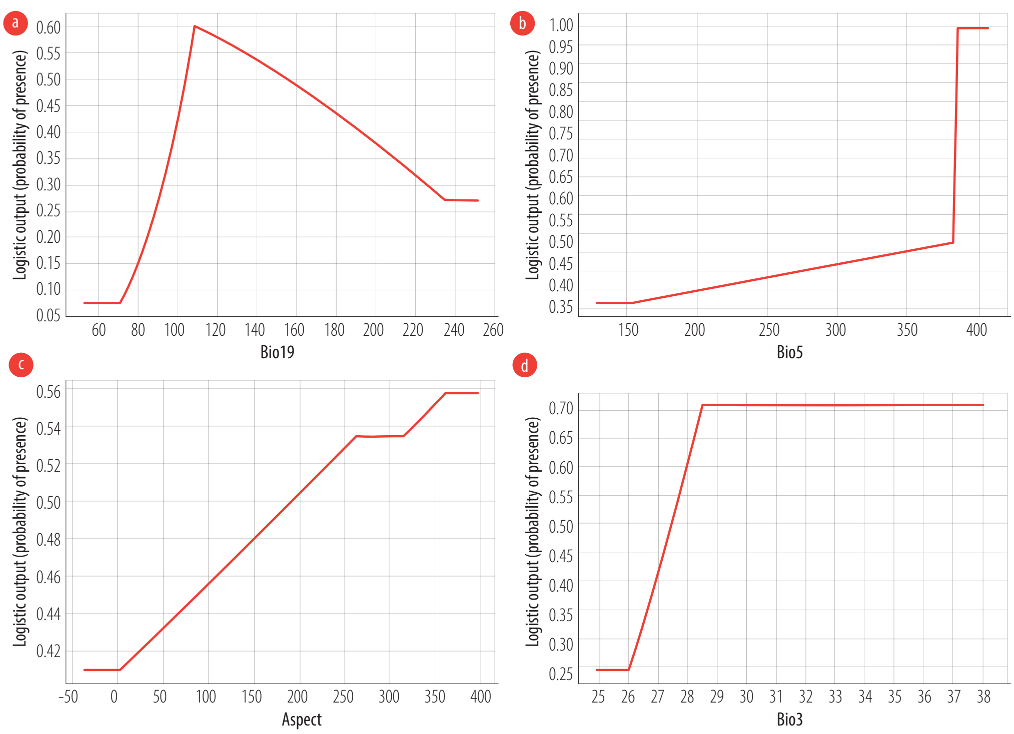

Figure 2: Result of important variables impact on ecological niche of *Culex pipiens* larvae in Ardabil province, northwest of Iran, 2017.

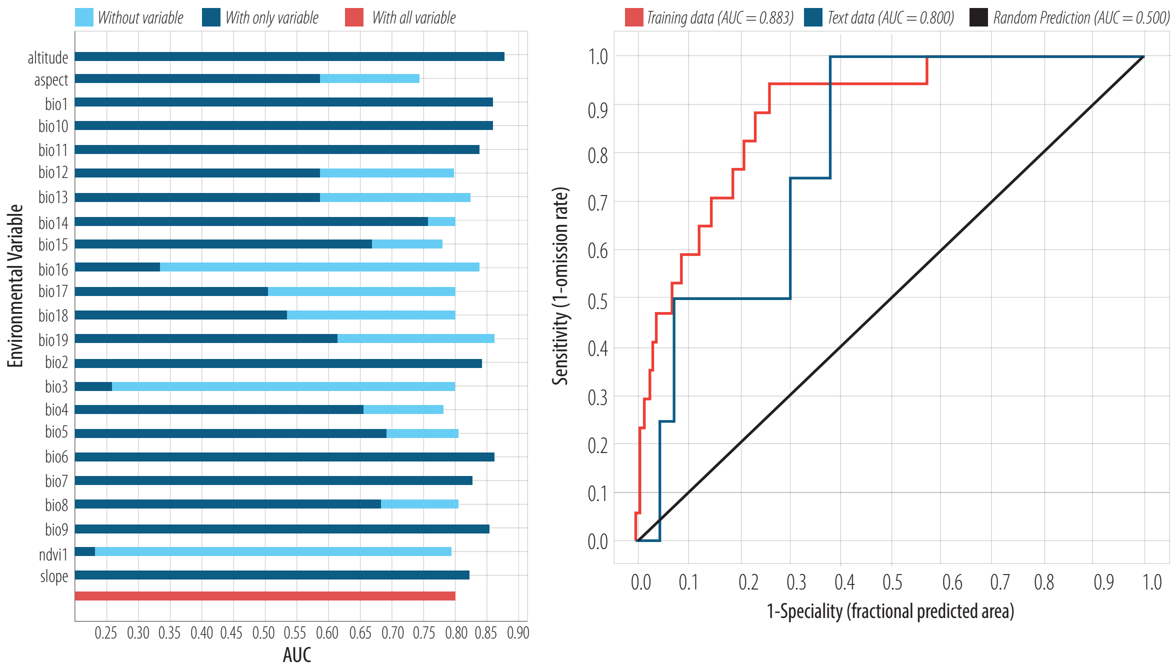

Figure 3: Result of jackknife test on variables importance for *Cluex. pipiens* larvae in Ardabil province, northwest of Iran, 2017.

in Ardabil and Parsabad counties [Table 2]. The maximum number of larvae of *Culex* mosquitoes was collected from Parsabad (27.2%), and the minimum was collected from Namin (2.0%) counties. More than 80% of the collected samples were from permanent habitats. The other physical characteristics of its breeding places, such as habitats with vegetation, stone or cement bed type, transparent water status, semi-shade, and natural habitat are listed in Table 3.

The ENM showed that the following factors had the greatest impact on the ecological niche of *Cx. pipiens* larvae: precipitation of coldest quarter (Bio19; 21.9%), maximum temperature of warmest month (Bio5; 21.2%), slope of the area (17.7%), precipitation of wettest quarter (7.0%), direction of slope (Aspect; 6.9%), and isothermality (Bio3; 6.8%) [Table 1 and Figure 2]. Whereas the jackknife test showed that the annual temperature range (Bio7) was the highest predictor of *Cx. pipiens* larvae in the north and south of Ardabil province. With the increase of rainfall (from 80 to 110 mm) and annual temperature ranges (increasing from 1.5 to 3.5 °C), the density of larvae in their habitats had also increased [Figure 3]. The results of the MaxEnt model indicated that the most ecologically suitable areas of *Cx. pipiens* larvae occurrence were identified in two hotspots in northern and southern Ardabil.

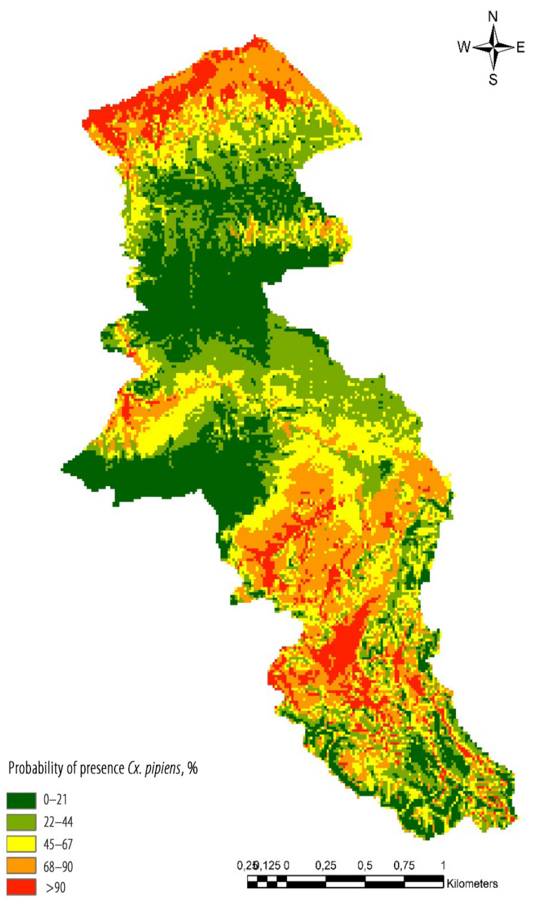

Figure 4: Ecologically suitable areas for *Cx. pipiens* larvae occurrence in Ardabil province, northwest of Iran, 2017.

The northern hotspot is the largest area comprising three counties of Parsabad, Garmi, and Bilehsavar. This area is located on the borderline of Iran and the Republic of Azerbaijan [Figure 4]. Moreover, the receiver operating characteristic/area under the curve (ROC/AUC) index for *Cx. pipiens* larvae in the ENM was 0.88.

DISCUSSION

Two large areas in the northwest Iran constitute a suitable habitat for *Cx. pipiens* larvae. Therefore, studies can be carried out to delve into the possible infection of WNV in mosquitoes and humans in this region. For the first time in the northwest of Iran, this study determined ENM for seven mosquitoes' larvae, which can be extended to other vectors. Previous studies have determined the ENM for *leishmania infantum* in humans (the agent of visceral leishmaniasis in Iran) and its vectors.[29,30] However, there is a dearth of studies on ENM for mosquito larvae. In one research in Chile, *Cx. pipiens* modeling predicted that shallow and transparent water comprised suitable habitats for larvae and temperature was reported to be among the most effective factors.[31] Likewise, most of the larvae were collected from transparent water in this study. Studies of modeling niche ecology have been carried out mostly on adult mosquitoes. Annual temperature range, precipitation of coldest quarter, maximum temperature of warmest month, slope and precipitation of driest quarter were the most important factors influencing the distribution and density of *Cx. pipiens* larvae. In modeling for *Cx. pipiens* for adults in America, it was found that non-forest regions with low vegetation coverage are appropriate for this species.[32] In Kenya, the results of modeling RVF vectors (*Cx. quinquefasciatus*, *Cx. univitattus, Mansonia africana,* and *M. uniformis*) using MaxEnt indicated that soil type, rainfall in dry seasons, seasonal rainfall, and isothermal process had the greatest impact on the vectors.[33] There is a plethora of studies and modeling carried out on West Nile vectors in the world. In one study about the effect of climate change on WNV vectors in USA in 2013, it was found that the increase in seasonal temperature and rainfall affected the frequency of *Cx. pipiens* and *Cx. Quinquefasciatus*[34] or in Florida, rain in warm and cold months of the year was reported to be the most important factor impacting *Cx. nigripalpus* and *Cx. Quinquefasciatus*.[35] Of the numerous studies on fauna and ecological characteristics of the larvae habitat done in Iran, some have been carried out in western Iran and West Azarbaijan province. In these studies,[14,36–38] the number of collected *Cx. pipiens* larvae was higher than other that of mosquito larvae like our findings, with the higher prevalence of *Cx. pipiens* larvae. However, in another study in the southeast of Iran,[39] *Cx. perexiguus* was found in the greatest abundance. The results of this research and their comparison with other studies show that *Culex* mosquitoes are also distributed in different parts of Ardabil province, which are like other regions of Iran. These vectors can transmit diseases to humans if there is a WNV cycle in this area.

CONCLUSION

Culex mosquitoes as the WNV vectors have a diversity of species in the Southwest of Iran. In addition, two large areas were found as ecological niches of larvae of *Cx. pipiens*, which call for more studies on infection in adult *Cx. pipiens* and their hosts in these regions.

Disclosure

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