https://doi.org/10.18502/jad.v19i2.20191

Original Article

Predicting the Distribution of *Culex pipiens* (Diptera: Culicidae) in Golestan Province of Iran Using an Ecological Niche Model

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(Received 04 Jan 2022; accepted 15 Mar 2025)

Abstract

Background: *Culex pipiens* is one of the most important vectors of mosquito-borne pathogens in Iran. This study aimed to predict the distribution of this species in Golestan Province, Iran and to determine the factors affecting its distribution.
Methods: Mosquito larvae were collected by using the dipping method from 56 locations across all 14 counties of the province, between April and October 2016. Species were identified morphologically, and the spatial distribution of *Cx. pipiens* was modeled using the maximum entropy (MaxEnt software Ver. 3.3.3) model.
Results: The results of our study show that areas located in the central part of Golestan Province are the most environmentally suitable habitat for *Cx. pipiens*. Jackknife test of variable importance showed that the following factors had the greatest influence on the distribution of *Cx. pipiens*: proximity to human settlements, precipitation of coldest quarter (mm), precipitation of wettest quarter (mm), precipitation of wettest month (mm), and mean temperature of coldest quarter (ºC).
Conclusion: This study concludes that the MaxEnt model is a very suitable model for studying the ecology of *Cx. pipiens* and precipitation, and temperature play a major role in the distribution of this species.

Keywords: House mosquito; *Culex pipiens*; Ecological niche modeling; Maximum entropy

Introduction

Culex pipiens (Diptera: Culicidae) is one of the most medically important species of the family Culicidae. It is well known for its human nuisance biting, and plays a major role in the transmission of several pathogens, including *Wuchereria bancrofti*, *Brugia malayi* and some pathogens of Arboviral diseases such as West Nile fever, Sindbis fever, Rift Valley fever, St. Louis encephalitis, Japanese encephalitis and Western equine encephalitis (1–3).

Culex pipiens is widely distributed in temperate areas of Europe, Asia, Africa, America, and Australia (4). Studies conducted in Iran have indicated a wide spatial distribution of this species in most parts of the country (5–7). The distribution of this species in all counties of Golestan Province has also been reported by recent studies (8–9). The widespread occurrence of this species has been attributed to its high adaptability to various larval habitats, which leads to an increase in species abundance and spread (7). Because of the importance of *Cx. pipiens* in the transmission of diseases, its ecology has attracted the attention of many researchers. Studies conducted in Iran have shown a diverse larval habitat for this species, but mostly larval specimens of this species were collected from rice fields, agricultural streams,

http://jad.tums.ac.ir
Published Online: June 30. 2025

https://doi.org/10.18502/jad.v19i2.20191

swamps, Bog wetlands, discarded tires and holes and barrels filled with water (9–12). In recent years, Ecological Niche Models, including the Maximum Entropy (MaxEnt) Model, have been used to study the distribution of this species in different geographical areas worldwide. These studies have demonstrated that ecological factors such as proximity to human settlements, precipitation, distance to rivers, temperature, slope, elevation and seasonality of enhanced vegetation index are the most important factors affecting the distribution of this species (13–17). The Ecological Niche Model (MaxEnt) showed that the following factors had the greatest impact on the ecological niche of *Cx. pipiens* larvae in Northwest of Iran: precipitation of coldest quarter, maximum temperature of warmest month, slope of the area, precipitation of wettest quarter, and direction of slope (18).

The MaxEnt model is one of the best ecological niche models for modeling species distribution in order to interpolate and discover areas of undocumented habitats of species (13). Similar to other ecological niche models, the MaxEnt model uses species occurrence records to estimate the species' potential occurrence in the study area. The model provides a probability of a species' presence between 0–1 for the different study points (14, 19), such that the closer the obtained probability for a study point (location) to the number 1, the higher the suitability of that location for the species distribution (20).

Given the high adaptability of this species to different larval habitats and its high medical importance, as well as the suitability of the climatic conditions in Golestan Province for the growth and development of the Culicidae mosquitoes, especially *Cx. pipiens*, the present study was conducted to predict the distribution of this species in Golestan Province of Iran using the MaxEnt model. Predicting the distributions of species is useful in providing more understanding about the ecology and possible control measures for this species.

Materials and Methods

Study area

Golestan Province (53°57′-56°23′ E, 36°30′-38°08′ N), covering a land area of 20437.74 square kilometers, constitutes approximately 1.3 percent of the total land area of Iran (21). It is located in the northeastern region of the country and bordered by the Republic of Turkmenistan to the north, the Alborz mountain range and Semnan province to the south, Khorassan-e Shomali Province to the east and the Caspian Sea and Mazandaran Province to the west. According to the latest provincial delimitation of the country in the year 2016, this province consists of 14 counties (Fig. 1). The province has diverse ecological features and has different climatic conditions. Considering the presence of sea, forests, and mountains in the area, the climatic condition of Golestan Province is classified into cold mountain (3000 meters high), temperate mountain, mild Mediterranean arid and semi-arid regions, such that as we move from southern to northern areas, the amount of rainfall and relative humidity decreases and the degree of temperature increases. The mountainous areas are located in the southern parts, and the lowest parts of the province are formed by areas around the Caspian Sea at an altitude of 32 meters below sea level (21).

Sampling method and the sites with a presence record of *Culex pipiens*

This study was carried out during the activity period of mosquitoes in the study area from early April to October 2016. In each of the 14 counties of the province, three to five villages and cities (a total of 56) were selected based on the land area, geographical conditions and relative distances apart.

At least three suitable habitats for sampling were chosen in the selected villages and cities, and larval sampling was carried out using the standard dipper (350 ml). Ten dips were taken from each larval habitat at each sampling time. Sampling was done once in each of the months of spring, summer, and autumn. All captured larvae in each dipper were counted and kept in special containers. Larval containers were labelled with the identity of the related larval habitat and transferred to the medical entomology laboratory in the Health Center of Kalaleh County for species identification. In the laboratory, larvae were preserved in lactophenol medium and were then mounted on microscope slides in de Faure's formula. Using standard taxonomic keys (22), larvae were identified morphologically at the species level.

All villages and cities where *Cx. pipiens* was collected were considered as the species presence points and their coordinates were extracted in ArcMap 9.3 software. The data obtained were entered into Excel software Version 2003 for use in our model.

Bioclimatic and environmental variables

The bioclimatic variables used in this study (Table 1) were obtained from the WorldClim-Global climate database version 1.4 (http://www.worldclim.org/current) at a spatial resolution of ˷1 km or 30 arc-s. The variables were derived from the dataset of weather stations for the period 1950–2000 (23–24). Slope and aspect were derived from the elevation layer at the same resolution using the surface analyst tool in ArcMap 9.3 software, and they were saved as new layers. Normalized difference vegetation index (NDVI) was extracted from MODIS satellite imagery for August 2014, and soil type (shapefile format) was obtained from the National Cartographic Center, Iran. Also, population data from the census in 2016 were used to create a proximity to human settlements variable. For all the villages and cities of Golestan Province, where the population lived in this, the value for the variable was 1 and it was 0 otherwise. All layers were converted to ASCII format in ArcGIS.

Modeling the potential occurrence of *Culex pipiens*

The Maximum Entropy (MaxEnt software Ver. 3.3.3) Model was used in this study to predict the most appropriate ecological niches for *Cx. pipiens*. For running the model, 80% of the 56 species presence points were used for training, and the remaining 20% were employed as a test for the model.

The data on environmental variables were stored in ASCII format, required for running the MaxEnt model. This modeling can be performed successfully even with very small sample sizes (i.e., less than 100 observations) (25). The jackknife test was used to assess the contribution of each variable in the model. The area under the receiver operating characteristic (ROC) curve (AUC) was used to assess the model performance (19).

Results

In the present study, *Cx. pipiens* was collected from 56 villages and cities in Golestan Province (N= 1,657). The number of collected species in the different counties of the province is given in Table 2.

Larvae were collected from full and partial sunlight and shaded habitats, mud and rocky substrate habitats, sweet water, salty water and brackish water wetlands, as well as other natural and artificial larval habitats. In the present study, this species was mostly collected from sweet water wetlands (94.8%), artificial habitats (73.1%), temporary habitats (70.5%), stagnant water habitats (68.4%), clear water habitats (67.2%), mud substrate (60.5%), and vegetation sites (57%).

Overall, we identified 56 villages and cities in different areas of Golestan Province that qualified for the study and were entered into the model as presence points for predicting the occurrence of *Cx. pipiens*. Forty-five presence points were used for training and eleven presence points were used as a test for the model. The results obtained from the model show that areas located in the western and central parts of Golestan Province have a higher presence probability for the species compared to other areas of the province (Fig. 2). AUC values for the training points and test points were 0.898 and 0.776, respectively (Fig. 3).

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The results obtained from the Jackknife test show that the following bioclimatic factors have the highest effect on the distribution of *Cx. pipiens*: proximity to human settlements, precipitation of coldest quarter (mm), precipitation of wettest quarter (mm), precipitation of wettest month (mm), and mean temperature of coldest quarter (°C) (Fig. 4).

Figure 5 shows the response of *Cx. pipiens* to the four bioclimatic factors with a major influence on its distribution, a direct relationship between the occurrence of *Cx. pipiens* and the four most important bioclimatic factors (proximity to human settlements, precipitation of coldest quarter (mm), precipitation of wettest quarter (mm) and min temperature of coldest month) can be observed. In other words, an increase in these variables increases the probability of the occurrence of the species.

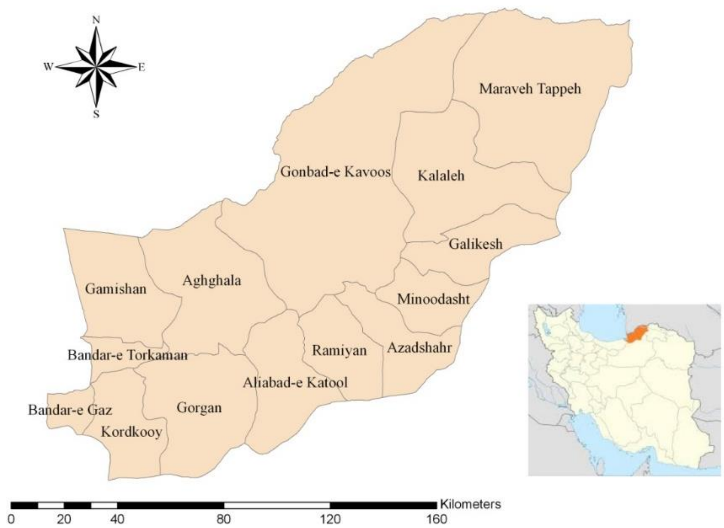

Fig. 1. Golestan Province and its counties, northeast of Iran

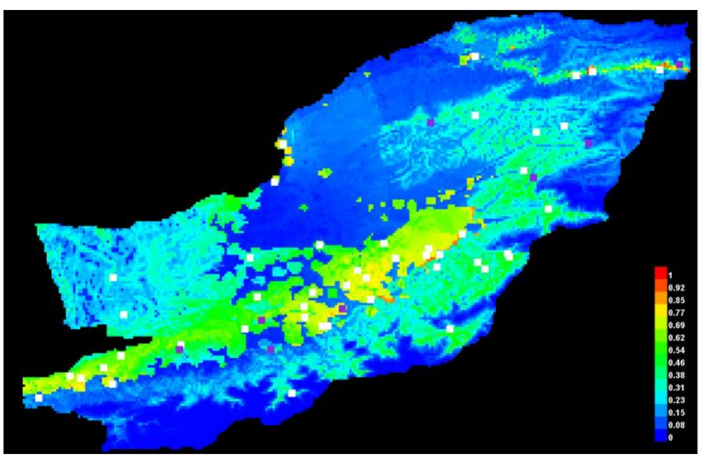

Fig. 2. Prediction of *Culex pipiens* presence at different parts of Golestan Province using the MaxEnt model. The blue areas show a lower presence probability of *Cx. pipiens,* whereas the red areas indicate areas with a higher presence probability of the species. The white points denote the presence points of the species which were used for training the model, whereas the purple points denote the presence points which were used for testing the model

https://doi.org/10.18502/jad.v19i2.20191

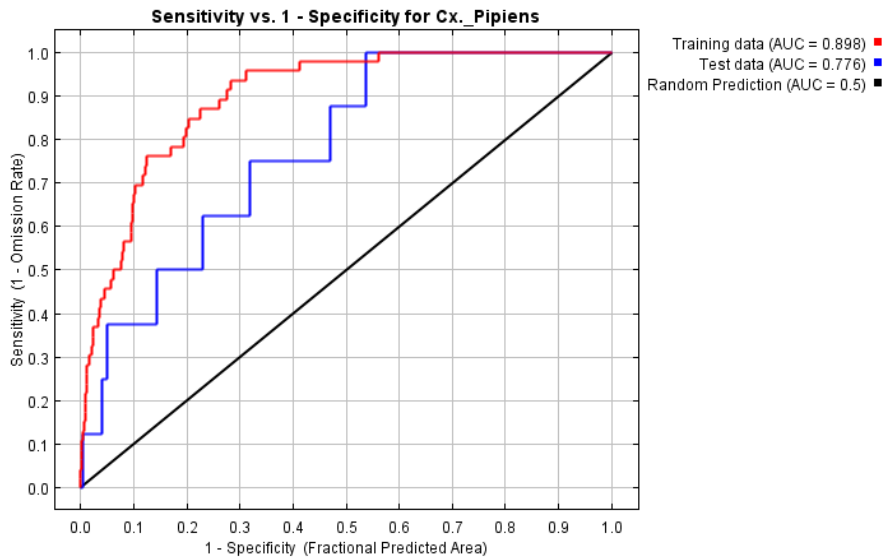

Fig. 3. The estimated area under the ROC curve (AUC) for training and testing the MaxEnt model for *Culex pipiens*

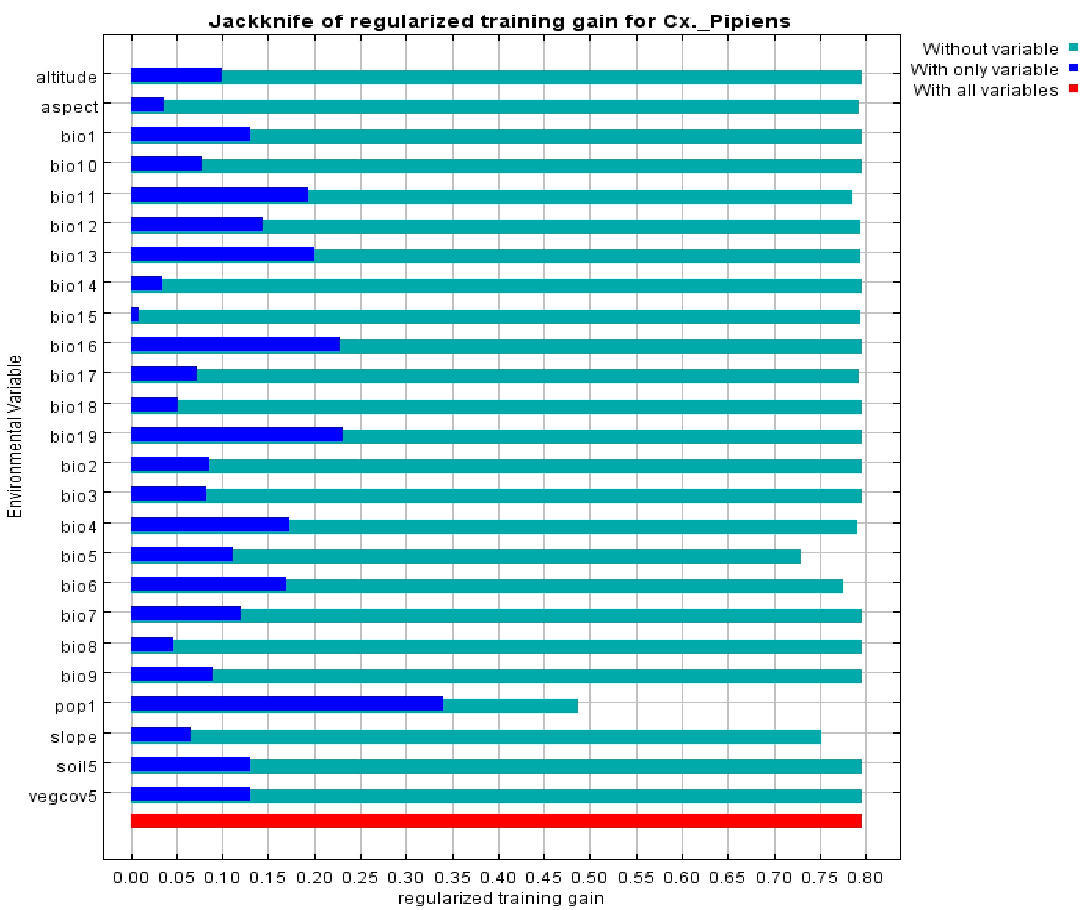

Fig. 4. Results of the Jackknife test for determining factors that effectively contribute to the distribution of *Culex pipiens*. The red bar shows model performance with all variables, the blue bar shows model performance with each variable alone, and the light sky blue color shows model performance without each variable

Table 1. Variables used for modeling the potential distribution of *Culex pipiens* and variable contribution in Golestan Province, Iran

Variables	Description	Percent contribution	Permutation importance
Alt	Altitude from the sea level (m)	0.1	1.3
Slope	Slope in degrees obtained from altitude (%)	18.5	8.9
Aspect	Aspect in degrees obtained from altitude (Direction)	0.5	0
BIO1	Annual mean temperature (ºC)	0	0
BIO2	Mean diurnal range (mean of monthly (max temp - min temp)) (ºC)	0	1.6
BIO3	Isothermality (BIO2/BIO7) (×100)	6.1	0.7
BIO4	Temperature seasonality (standard deviation ×100)	0.4	4.8
BIO5	Max temperature of warmest month (ºC)	9.9	8.2
BIO6	Minimum temperature of coldest month (ºC)	1.6	9.2
BIO7	Temperature annual range (BIO5-BIO6) (ºC)	2.6	0
BIO8	Mean temperature of wettest quarter (ºC)	0	0
BIO9	Mean temperature of driest quarter (ºC)	0	0
BIO10	Mean temperature of warmest quarter (ºC)	0.4	0
BIO11	Mean temperature of coldest quarter (ºC)	2.3	2.4
BIO12	Annual precipitation (mm)	0.4	5.8
BIO13	Precipitation of the wettest month (mm)	2	0.8
BIO14	Precipitation of driest month (mm)	0.2	0
BIO15	Precipitation seasonality (coefficient of variation)	1	5.7
BIO16	Precipitation of the wettest quarter (mm)	0	0
BIO17	Precipitation of driest quarter (mm)	1.1	2.4
BIO18	Precipitation of the warmest quarter (mm)	0	0
BIO19	Precipitation of coldest quarter (mm)	0.3	0
Pop1	Proximity to human settlements	52.5	48.3
Soil5	Type of soil	0	0
Vegcov5	Normalized difference vegetation index (NDVI)	0	0

Table 2. Abundance and larval habitat characteristics of *Culex pipiens* collected in Golestan Province of Iran, 2016

County / Habitats characteristics	Maraveh Tapeh	Kalaleh	Gonbad-e Kavus	Galikesh	Minoodasht	Azadshahr	Ramian	Aliabad-e Katul	Aghghala	Gorgan	Kordkouy	Bandar-e Gaz	Bandar-e Turkman	Gomishan	Total
Habitat situation															
Permanent	33	0	0	0	2	0	8	12	0	80	281	21	14	37	488
Transient	50	62	45	12	1	33	153	375	5	0	146	210	60	17	1169
Slow-running water	22	24	4	0	2	0	0	29	0	18	309	91	14	10	523
Stagnant water	61	38	41	12	1	33	161	358	5	62	118	140	60	44	1134
Vegetation situation															
Out of water	33	8	45	4	3	33	0	17	0	0	279	81	14	10	527
In the water level	0	0	0	8	0	0	0	183	0	0	25	115	39	0	370
Underwater	0	0	0	0	0	0	1	0	0	48	0	0	0	0	49
Without vegetation	50	54	0	0	0	0	160	187	5	32	123	35	21	44	711
Sunlight situation															
Full sunlight	0	62	41	0	0	0	111	12	5	32	212	60	0	2	537
Shaded	0	0	0	0	0	0	0	12	0	0	23	3	0	26	64
Partial sunlight	33	0	0	12	2	33	0	35	0	48	154	68	14	0	399

https://doi.org/10.18502/jad.v19i2.20191

Table 2. Continued ...

Sunlight shaded	50	0	4	0	1	0	50	328	0	0	38	100	60	26	657
Substrate type															
Mud	83	62	45	12	3	33	160	24	5	32	311	179	53	2	1004
Sand	0	0	0	0	0	0	0	209	0	18	0	52		0	279
Rock	0	0	0	0	0	0	1	0	0	30	48	0	0	0	79
Others	0	0	0	0	0	0	0	154	0	0	68	0	0	52	295
Water Situation (turbidity)															
Muddy	72	1	0	0	0	33	31	0	0	62	107	52	14	0	372
Clear	11	61	6	12	3	0	129	382	5	0	256	159	36	54	1114
Turbid	0	0	39	0	0	0	1	5	0	18	64	20	24	0	171
Water Situation (salinity)															
Sweet	78	61	45	12	3	33	129	387	0	80	427	228	34	54	1571
Salty	5	0	0	0	0	0	5	0	0	0	0	3	21	0	34
Brackish	0	1	0	0	0	0	27	0	5	0	0	0	19	0	52
Habitat Kind															
Natural	78	22	1	12	3	33	5	12	5	32	107	80	53	2	445
Artificial	5	40	44	0	0	0	156	375	0	48	320	151	21	52	1212

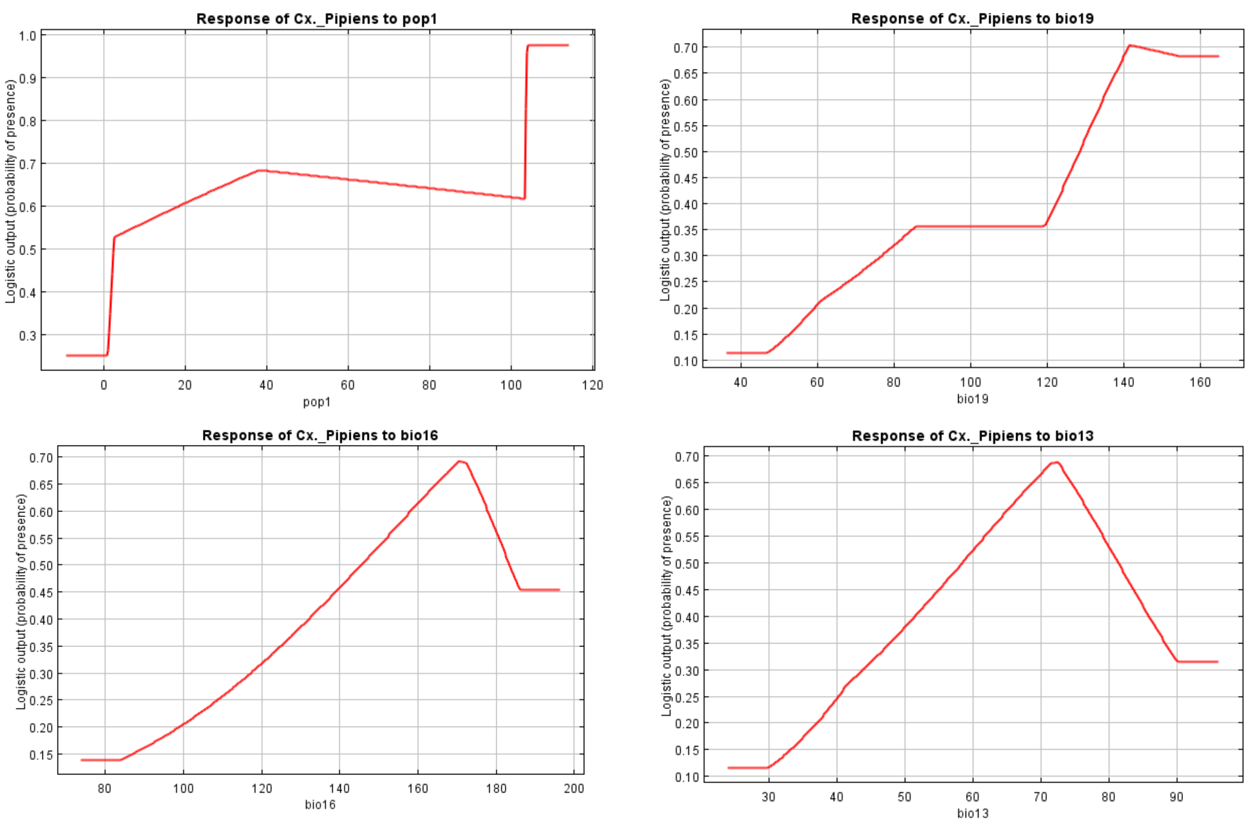

Fig. 5. Response curves of *Culex pipiens* to the four most influential environmental variables (pop1: Proximity to human settlements, bio19: Precipitation of coldest quarter (mm), bio16: Precipitation of wettest quarter (mm), bio6: Min temperature of coldest month (^{0}C))

Discussion

The presence of *Cx. pipiens* has been reported in almost all the provinces in Iran (5, 7–12, 26). In the present study, this species was collected in all the study sites in Golestan Province. The

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Published Online: June 30, 2025

larval habitats of this species in our study sites were diverse, ranging from permanent and temporary habitats to running and stagnant water habitats. Larvae were collected from full and partial sunlight habitats, shaded habitats, mud and rocky substrate habitats. In the present study, habitats with the highest number of collected species included the following: sweetwater wetlands, artificial habitats, temporary habitats, stagnant water habitats, clear water habitats, mud substrate and vegetation sites. In previous studies conducted by Azari-Hamidian et al. (7) in Gilan and Mousakazemi et al. (11) in Isfahan, this species was mostly collected from rice farms and natural habitats. Hasan (10) reported that the habitats formed of rain-filled pools and a leaking underground pump were positive for *Cx. pipiens* in Saudi Arabia. Significantly, shallow water, mud bottom, and absence of grasses, algae and predators were the common characteristics of habitats positive for *Cx. pipiens* larvae. *Culex pipens* larvae were also found to thrive better in stagnant habitats that are devoid of solid wastes and fully exposed to sunlight. Researchers in Tunisia (27) reported that the most favorable breeding sites for *Cx. pipiens* are man-made artificial habitats such as canals, storage lakes, swimming pools, gardens and storm water drainage systems. Findings from the above studies clearly indicate that the larvae of *Cx. pipiens* have higher adaptability to different larval habitats with variable ecological conditions. Thus, the higher distribution and abundance of this species in Iran and other countries (East Africa and the USA) can be attributed to the high adaptability of *Cx. pipiens* larvae to different habitats (1, 7–15, 26). In our study, the characteristics of the larval habitats of *Cx. pipiens* larvae in Golestan Province were similar in all counties.

The MaxEnt model was used to estimate the area under the receiver operating characteristic [ROC] curve (AUC). The AUC was calculated for each model using the proportion of the study area in which the species was predicted to be present. This is automatically generated by the MaxEnt, which constructs ROC curves using randomly selected pseudo-absences. For acceptable models, the AUC threshold was set to an extremely conservative value of 0.5 for both training and test data (28). The value of AUC in the MaxEnt model in our study was 0.898, which indicates excellent model prediction performance. Similar to this result, other studies conducted on *Cx. pipiens* in countries using the same model yielded the AUC values 0.946, 0.938, 0.908, and 0.838, which are also indicative of excellent model prediction performance (13–16). In the MaxEnt model, the closer the AUC to 1, the higher the accuracy of the species distribution prediction. A higher prediction accuracy can be achieved by selecting a wider study area with a high number of presence points for the species. The AUC values of the studies above were higher and closer to 1, probably due to a wider study area and a high number of presence points for the species.

Jackknife test revealed that proximity to human settlements, precipitation of coldest quarter (mm), precipitation of wettest quarter (mm), temperature seasonality and minimum temperature of coldest month (^{o}c) have the highest effect on the distribution of *Cx. pipiens* in our study area (Fig. 4). By omitting the proximity to human settlements variable from the model in the Jackknife test, the gain of the model decreased to the lowest point. Among the different variables used for modeling the distribution of *Cx. pipiens*, proximity to human settlements had the highest contribution (52.5%) and permutation importance (48.3%). This variable, therefore, appears to have the most information that is not present in the other variables. Also, there was a direct association between proximity to human settlements and the distribution of *Cx. pipiens*, such that increasing proximity to human settlements increases the presence probability of the species. The observed association may be since *Cx. pipiens* is an urban species that prefers urban habitats for its growth, including man-made artificial containers such as old tires and cans, as well as urban sewage, animal waste, contaminated ponds, and agricultural spells. This species is found more often in the vicinity of human living places (13, 29–30). The majority of the population in Golestan Province resides in the central and western areas of the province. Villages and towns in these areas are often overcrowded and located closer to one another compared to the other areas of the province and this provides more suitable conditions for the breeding of this species. In contrast, the distance between villages is longer in the northern areas of the province due to the presence of desert and arid climatic conditions. Suitable larval habitats for this species are thus limited and accordingly, the presence probability of this species is also low in these areas. The southern areas of the province are basically mountainous and forest areas with no residents, and the probability of the presence of this species is much lower. Similar to the findings of our study, in a study conducted in arid and semiarid regions of the Middle East, North Africa, and Iowa (13, 16), the most important predictor of habitat preference for *Cx. pipiens* was the human proximity to human settlements, which had the highest percent contribution to the occurrence of this species compared to other variables. In similar studies on the analysis of variable importance (31–32), a positive association between human proximity to human settlements and the mean number of female *Cx. pipiens* species was reported. In another study conducted in the Iowa region (13), approximately 89% of the occurrence records of this species fell inside or within four km of an incorporated city boundary. In another study, the following four factors were reported as the most important predictors of *Cx. pipiens* distributions; slope, mean temperature of coldest quarter (^{o}C), mean temperature of warmest quarter (^{o}C) and precipitation of driest month (mm). However, Mweya et al. (14) did not incorporate proximity to human settlements into their model for *Cx. pipiens* distribution studies.

In the present study, precipitation of the coldest quarter (mm) and precipitation of the wettest quarter (mm) were among the most important factors that affect the distribution of *Cx. pipiens*. These variables also showed a direct association with the presence probability of *Cx pipiens.* Similar to the results of our study, in another study conducted in Iran (18), precipitation of the coldest quarter (mm) was the most important factor on the distribution of *Cx pipiens.* The role of precipitation, especially in wet and cold seasons, is more prominent in the distribution of this species. Potential breeding sites for mosquitoes, like shallow temporary ponds, can be developed due to rainfall. An increased number of these sites, as well as their stability due to subsequent rainfalls, has a direct and positive impact on the abundance of mosquitoes, because it increases the survival and aquatic development of immature mosquitoes (33). Similar to the results of the present study, a direct association between precipitation and the population of *Cx. pipiens* was reported in other studies. In those studies, the abundance of this species markedly increased several weeks after precipitation (34–35). Mweya et al. (14) and Adham et al. (18) used MaxEnt modeling to study the distribution of *Cx. pipiens*, and they reported that precipitation has a moderate effect on the distribution of this species. It is worth mentioning that precipitation and temperature, during the early period of the year (spring and early summer), might remarkably influence *Cx. pipiens* population dynamics (36), but heavy precipitation can harm the population of this species. It has been reported that larvae of *Cx. pipiens* may be flushed out by heavy precipitation and longer exposure to rain (33, 37–38).

Temperature is one of the most important factors that affect the life cycle of *Cx. pipiens*, and plays an essential role in the abundance and distribution of this species. Growth and development of mosquitoes can be accelerated by relatively high temperatures (39–40). It seems that the activity of mosquitoes, especially female ones, is affected by temperature variations such that a decrease in temperature leads to a delay in their activities, including search for a host, until optimal temperature conditions are reached. Temperature change not only influences their activities but also affects their survival rate (33–35). In the present study, temperature seasonality and minimum temperature of the coldest month (°C) had a high gain in the model when used in isolation.

In the study of Mweya et al. (14) in 2013, after slope, the mean temperature of the coldest quarter and warmest quarter (°C) had the highest influence on the distribution of *Cx. pipiens*. In another study conducted by Ragab et al. (15) in 2025, after altitude, temperature seasonality had the highest impact on the distribution of *Cx. pipiens.* In the same study, this variable was found to have the highest permutation importance after elevation. Temperature and rainfall not only influence the growth, development and distribution of this species, but may also indirectly affect the diseases transmitted by mosquitoes (40). It has been reported that rainfall and temperature patterns are associated with disease transmission by mosquitoes in many parts of the world (41).

Elevation, slope, and normalized difference vegetation index (NDVI) are also important factors in the distribution of *Cx. pipiens*. In the present study, after proximity to human settlements, slope had the highest impact on the distribution of this species (Table 1). Similar to the results of our study, in another study (14), slope was the most important predictor of the distribution of this species. Slope plays a major role in the stability of larval habitats. In areas with low slope, larval habitats are more stable. In the present study, the abundance of *Cx. pipiens* was negatively associated with elevation and NDVI. Similar to the results of the present study, Yoo et al. (42) reported that *Cx. pipiens* abundance was negatively associated with elevation and NDVI. In another study (14), elevation had the highest permutation importance, but it was the third most important factor in terms of variable contribution in the modeling of the distribution of this species. However, it has been reported (16) in the Middle East and North Africa that there is a strong and positive relationship between the occurrence of *Cx. pipiens* and the maximum value of the enhanced vegetation index (EVI), but the association was negative between the mean and standard deviation of EVI and the occurrence of this species.

The limitations of this study are: The binary nature of the population density variable, and the potential for spatial autocorrelation for bioclimatic variables.

Conclusions

Precipitation and temperature play a major role in the distribution of *Cx. pipiens.* This species is known for its human nuance biting and is found more often in the vicinity of human living places. This study concludes that the MaxEnt model is a very suitable model for studying the ecology of different species of mosquitoes.

Acknowledgements

This research was approved by the Infectious Diseases Research Center, Golestan University of Medical Sciences (No. 970211). The authors would like to appreciate all staff of this center and the Health centers of Golestan Province for their support and cooperation.

Ethical considerations

The ethical code IR.GUMS.REC.1397 has been registered for this study.

Conflict of interest statement

The authors declare there is no conflict of interest.

https://doi.org/10.18502/jad.v19i2.20191

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