Online ISSN: 2588-3526

Journal of Wildlife and Biodiversity

Volume 5 (3): 21-34 (2021) (http://www.wildlife-biodiversity.com/)

Research Article

Overview of taxonomy and prediction potential distribution of *Bufotes sitibundus* (Anura: Bufonidae) using environmental factors

Fatemeh Fakharzadeh[1]* Mahboubeh Sadat Hosseinzadeh[2,3]

[1]Department of Biology, Faculty of Sciences, Shahid Chamran University of Ahvaz, Ahvaz, Iran

[2]Department of Biology, Faculty of Sciences, University of Birjand, Birjand, Iran

[3]Research Group of Drought and Climate Change, University of Birjand, Birjand, Iran

*Email: ffakharzadeh2015@gmail.com

Received: 17 October 2020 / Revised: 5 January 2021 / Accepted: 9 January 2021 / Published online: 9 January 2021. Ministry of Sciences, Research, and Technology, Arak University, Iran.

How to cite: Fakharzadeh, F., Hosseinzadeh, M. (2021). Overview of taxonomy and prediction potential distribution of *Bufotes sitibundus* (Anura: Bufonidae) using environmental factors. Journal of Wildlife and Biodiversity,5(3), 21-34. **https://doi.org/ 10.22120/jwb.2021.138255.1191**

Abstract

Bufotes sitibundus spreads from Greece eastwards through Turkey, to Syria, Jordan, and Lebanon. It is also reported from Iraq and Iran and is distributed through the Caucasus and Russia to Kazakhstan. Formerly, this species was considered *Bufotes viridis* with three subspecies, but recent molecular studies have changed the name of the taxon. However, with respect to the patchy distribution range of the species, species distribution models (SDM) are useful methods to predict and identify the potential distribution and suitable habitat for the species. In this study, 62 coordinates of *B. sitibundus* with 30 variables including six informative bioclimatic environmental variables, namely Bio1 (annual mean temperature), Bio2 (mean diurnal range), Bio4 (temperature seasonality), Bio15 (precipitation seasonality), Bio16 (precipitation of wettest quarter), Bio19 (precipitation of coldest quarter and water vapor pressure (kPa), solar radiation (kJm-2 day-1) for each month of the year, were analyzed. Results show that the solar radiation of the seventh month, the water vapor pressure of the third month, and bio19 (Precipitation of Coldest Quarter) have the highest contribution in distribution patterns of the *B. sitibundus*. It seems that *B. sitibundus* is a flexible species and can adapt to different habitats, different altitudes, and different environmental conditions. Therefore, the distribution range of this species is likely to be larger than what has been reported so far.

Keywords: Bioclimatic variables; species distribution models; *Bufotes viridis* complex

Introduction

Bufonidae is nearly a cosmopolitan in distribution. Among Bufonidae, the genus *Bufo* (sensu lato) is the most problematic group. Since there are no synapomorphies to define *Bufo*, several investigators have questioned the genus' monophyly (e.g., Graybeal & Cannatella, 1995; Graybeal, 1997). At first, Frost et al. (2006) combined the former "*Bufo*" *viridis* group with a new genus described as *Pseudepidalea* and suggested that *Bufo* be divided into several genera. Dubois & Bour (2010) then showed that *Pseudepidalea* is a junior synonym of *Bufotes* (Rafinesque, 1815). A molecular study (Stöck et al., 2006) showed that green toads of Asia Minor, the Middle East, and northern Eurasia form a separate clade. Since the range of this clade includes the type locality (Stöck et al., 2001a), they referred to these populations as *B. variabilis* (Pallas, 1769). Recently, Dufresnes et al. (2019) stated that the Middle Eastern green toads might instead be considered *B. sitibundus* (Pallas, 1771), which is the oldest name for this species.

B. sitibundus spreads from Greece eastwards through Turkey to Syria, Jordan, and Lebanon. Besides, it is reported from Iraq, Iran and is distributed through the Caucasus and Russia to Kazakhstan. Moreover, there are some isolated *B. sitibundus* in Denmark, northern Germany, and southern Sweden. This species has been reported previously from Iran by *B. viridis* (Baloutch & Kami, 1995; Stöck et al., 2001a) with three subspecies. *B. v. viridis*, which was distributed in northwest Iran, *B. v. kermanensis* that was reported from Kerman and Hormozgan provinces, and *B. viridis* ssp., which was distributed in western and southwestern Iran.

B. sitibundus is now distributed in western and Central Iran (Fakharzadeh et al., 2014, 2018). It is a member of *B. viridis* complex, which is the only known anuran complex that comprises diploid, tetraploid, and triploid bisexually reproducing taxa and broadly spreads in the Palearctic range (Stöck et al., 2001b,2006). Green toads are widely distributed in Iran. Cytogenetic shreds of evidence reveal that all three ploidy levels (2n, 3n, 4n) exist in Iran. A Karyological study of several populations of *B. sitibundus* showed that they are diploid (Fakharzadeh et al., 2014, 2015, 2018).

Species distribution models (SDMs) are practical models, which correlate field observations to environmental variables based on statistically or theoretically derived response surfaces (Franklin, 1995; Guisan & Zimmermann, 2000). Species records can be simple presence only, presence-absence, or abundance observations based on field sampling or data from other sources such as natural history collections (Graham et al., 2004; Guisan & Thuiller, 2005). The predictive models of species distributions are essential for various ecology and conservation applications (Graham et al., 2004). SDMs can provide more information about all species, particularly the rarely known species, by mapping potential distribution ranges. One can identify the sites where searches are more promising and should be considered for conservation programs (Peterson et al., 2000).

Globally, 22.5% of the amphibian species are classified as data deficient, whereas the percentage in the other class of animals like birds and mammals is much less (Stuart et al., 2004). The mentioned problems make it necessary to concentrate on surveying their distribution pattern, suitable habitat, practical environmental factors, and population estimation. Identifying the relationship between amphibian species and habitats, associated with the environmental elements are the key factors that approximately dictate the density and abundance of the amphibian species (Allmon, 1991; Crump & Scott, 1994; Marsh & Pearman, 1997). However, the distribution of *B.*

sitibundus is not entirely known, and based on scientific sources (e.g., Avci et al., 2018; Frost, 2019), this species' status is still data deficient (D.D). So, the present study can better understand this species' geographical distribution and recognize the environmental factors that affect the distribution.

In this study, the geographic distribution of *Bufotes sitibundus* was digitized by integrating new data from Iran into the globally available data. To predict potential distribution, the authors use MaxEnt software to find the current habitat suitability of *B. sitibundus* in the world. Additionally, the analysis reveals the most suitable habitats associated with informative environmental variables. According to the global decline of the amphibian population, the technique and similar methods can provide more knowledge for scientists to identify and manage conservation activities and priorities.

Material and methods

A total of 62 coordinates of *B. sitibundus* (Table1) were collected and used in the modeling approach includes 22 occurrence records from our fieldwork, 24 from Global Biodiversity Information Facility (GBIF: www.gbif.org), and 16 from literature sources (Stöck et al., 2006). The fieldwork has been conducted between March 2015 to October 2018 in the breeding season. Toads were captured by hand at night. Specimens obtained from fieldwork have been checked for any morphological changes (Baloutch & Kami, 1995) for different altitudes.

Table 1. Information of point localities of *B. sitibundus* has been used in the study.

#	Longitude	Latitude	Locality	Reference
1	48.7450	31.3833	Kourosh neighborhood, Ahvaz, Khuzestan Province	This study
2	48.5333	32.5167	Lavi Spring near Khammat village, Choqa-zanbil, Khuzestan Province	
3	52.4333	29.0167	Firoozabad road, Mehkuh Olia village (80 km south of Shiraz), Fars Province	
4	52.4333	29.8167	Phase 4 of Sadra town, Shiraz, Fars Province	
5	49.5333	32.7500	Jahangirkhani village, 10 km north of Hosseiniye region, Andimeshk, Khuzestan Province	
6	53.4333	28.3500	Barous Plain 11km east of Mobarakabad, Qir-Jahrom road, Fars Province	
7	54.1358	31.6333	Baghestan neighborhood, Dehbala village, Shirkouh, Taft Yazd province	
8	48.0667	38.1333	Kodak Park, Danesh square, Sarein, Ardabil Province	
9	45.2667	37.3667	Dolama village, Uremia – Naqadeh road, 30 km southeast of Uremia, West Azerbaijan Province	
10	46.1667	38.0333	The residential thermal power plant of Tabriz, Tabriz, East Azerbaijan Province	
11	48.2833	38.3000	Tourist residential complex near the entrance of Ali Sadr cave, Ali Sadr village, Hamedan, Hamedan province	
12	55.6333	29.4667	Khoramabad region, Sirjan, Kerman Province	
13	57.1167	30.2833	Kerman, Kerman Province	
14	52.2167	27.6833	Akhtar village,15 and16 phases of Asaloyeh refinery, Residential complex of Oil and Gas Company, Asalouyeh,	

			Bushehr Province	

Continues (Table 1)

15	46.2833	37.4833	Eshan village to the Alavian Dam, Maragheh, East Azerbaijan Province	This study
16	46.0000	37.3167	Gharehchopogh, Bonab, East Azerbaijan Province	
17	46.3333	35.7167	Qamishlehvillage, Marivan, Kordestan Province	
18	50.1500	31.7500	Kalmat village, 10 km East of Dehdez, Khuzestan Province	
19	50.2333	30.5833	Pardis park ,Behbahan, Khuzestan Province	
20	49.2833	31.9333	Tombi region,15 km South of Masjed Soleyman, Khuzestan Province	
21	51.6685	32.6445	Esfahan, Esfahan Province	
22	51.4100	33.9850	Kashan, Esfahan Province	
23	35.7000	32.9000	Golan province, Israel	GBIF
24	35.0972	32.8901	Hazafon province, Israel	
25	12.9000	55.5830	Sweden, Malmö, Limhamn	Stöck et al.(2006)
26	35.9000	33.0000	Israel	GBIF
27	57.7000	30.4000	Shahdad, Kerman Province, Iran	
28	51.0000	35.8000	District 11, Karaj, Alborz Province,Iran	
29	51.5000	35.8000	Hesarak, District 1, Tehran, Tehran Province, Iran	
30	51.8000	35.7000	Phase 2, Pardis, Tehran Province, Iran	
31	46.1000	35.6000	Kurdistan Province, Iran	
32	52.0000	35.9000	Mazandaran Province, Iran	
33	47.8000	30.5000	Basrah, Iraq	
34	11.8000	54.7500	Denmark, Falster, a few km S Nykøbing Falster,	Stöck et al.(2006)
35	11.2500	54.9000	Denmark, NW Lolland	
36	53.2310	29.1950	Central Iran, Qasr-e-Sásán,Iran	
37	10.6330	53.5830	Germany, Schleswig-Holstein, Woltersdorf near Lübeck,	
38	20.2660	39.5000	Greece, Epirus, S Igoumenitsa	
39	36.0160	34.2500	Lebanon, Lebanon mountains, above Bcharre, Cedrus forest, 2300 m a.s.I., Bischo	
40	40.1500	35.3330	Syria, Dayr az Zawr, Hotel Al Waha, left Euphrat bank	
41	42.3000	38.6600	Turkey, Nemrut Dagh and E of Nemrut	
42	35.0799	36.6787	Adana, Turkey	GBIF
43	42.5270	43.2570	Russia, Caucasus, Terskol	Stöck et al.(2006)
44	43.7600	39.0000	Turkey, Karahan-Kars lli, Van Golu (N) Karahan Koyu	
45	50.3070	49.2130	Kazakhstan, Djangalinsky Rayon, village Djangala,	
46	53.3540	51.0000	Kazakhstan, W 80 km E of Uralsk city, Berezka river	
47	22.3670	37.5160	Greece, Peloponnese	
48	36.0000	33.4830	Doura Europos, Syria	
49	47.5000	42.9670	Russia, Dagestan Autonomous Republic Sary Kum Sand Dunes, at Kumtorkala Railroad Station	
50	44.6265	42.6175	Georgia, Mtskheta-Mtianeti	GBIF
51	48.2397	32.0676	Shush, Khuzestan, Iran	
52	44.6257	42.6178	Mtskheta-Mtianeti Georgia	
53	44.6548	42.5964	Mtskheta-Mtianeti Georgia	
54	41.6334	41.6466	Parnavaz Mepe Street, Batumi, Adjara, Georgia	
55	27.6373	40.4971	Balikesir, Turkey	
56	45.0601	40.9017	Tavush, Armenia	

57	45.4935	42.4874	Georgia	

Continues (Table 1)

58	55.3745	30.2313	Meymand, Kerman Province, Iran	GBIF
59	26.2182	37.6350	Aegean, Greece	
60	51.4734	28.0655	Bushehr province, Iran	
61	50.4629	35.9493	Qazvin province, Iran	
62	52.9987	27.2530	Bastak, Hormozgan, Iran	

Maximum Entropy (MaxEnt) is a general-purpose algorithm for creating predictions or inferences from the presence-only modeling of species distributions, suitable for all existing applications involving presence-only datasets. The MaxEnt method is used to estimate a target probability distribution by finding the probability distribution of maximum entropy (i.e., most spread out or closest to uniform), subject to a set of constraints that represent our incomplete information about the target distribution. When MaxEnt is applied to presence-only species distribution modeling, the study area's pixels make up space on which the MaxEnt probability distribution is defined; pixels with known species occurrence records constitute the sample points (Philips et al., 2006). A wide variety of methods are used to develop such models and their predictions (Elith & Burgman, 2003; Rushton et al., 2004).

In most cases, the evaluations of predictive performance focus on comparing predictions against observations at a particular set of sites (Fielding & Bell, 1997). Although such evaluation data should ideally be independent or formed through resampling the modeling data, in many cases, models are evaluated using the training data itself. A broad range of statistics such as Kappa, an area under the receiver operating characteristic curve (ROC), and correlation coefficients can be used to assess whether predictions are suitably accurate for their intended use (Moisen & Frescino, 2002; Pearce & Ferrier, 2000; Rushton et al., 2004). The method we propose here addresses this by facilitating the visualization of species' predicted responses to environmental variables, even where these relationships are not apparent from the model itself (Elith et al., 2005). In total, 19 variables related to bioclimatic seasonality and annual trends of temperature and precipitation were downloaded from the WorldClim database version 2 (http://www.worldclim. org/) for the present period (1970–2000, Table 2).

According to Litvinchuk et al. (2018), six informative bioclimatic variables have been selected to analyze: Bio1 (annual mean temperature;°C × 10), Bio2 (mean diurnal range;°C × 10), Bio4 (temperature seasonality; standard deviation×100), Bio15 (precipitation seasonality; CV), Bio16 (precipitation of wettest quarter; mm), and Bio19 (precipitation of coldest quarter; mm). Additionally, water vapor pressure (kPa), solar radiation (kJ m-2 day-1) for each month of the year were used to analyze species modeling. The average map model in ASCII file was digitized using DIVA-GIS v7.1.7.2 (Hijmans, 2009). A tool algorithm, Maximum Entropy modeling (MaxEnt) has been used. MaxEnt is a mighty presence-pseudoabsence algorithm (Elith et al., 2006). Many authors have suggested that it is one of the most efficient approaches for predicting species' potential distributions (e.g., Elith et al., 2006, 2011; Phillips et al., 2006). The performance of MaxEnt for various parameter settings is described as follows. Performance is also measured in terms of 'area under the receiver-operating characteristic curve' (AUC) (Philips et al., 2006; Phillips & Dudík, 2008). Herein, MaxEnt software version 3.4.1 (Phillips et al., 2006) was used with default settings with ten replicates, which is a technique that has been proven to achieve high predictive accuracy (Phillips & Dudík, 2008). MaxEnt was used with default settings when separating records into training and test samples randomly (75 and 25%, respectively), convergence threshold, and the maximum number of iterations (0.00001 and 500, respectively).

The AUC was used for model evaluation, which surveys a model's ability to distinguish between sites where a species is 'present' versus 'absent' (Phillips et al., 2006; Elith et al., 2006). According to Manel et al. (2001), models with AUC = 0.5 mentions a performance equivalent to random; AUC > 0.7 mentions useful performance, AUC > 0.8 mentions good performance, and AUC ≥ 0.9 mentions excellent performance. Besides, MaxEnt performance was evaluated using the Kappa and true skill statistic (TSS) (Allouche et al., 2006).

Table 2. Description of 19 variables related to bioclimatic seasonality and annual trends of temperature and precipitation.

Abbreviation of variables	Description of variables
BIO1	Annual Mean Temperature
BIO2	Mean Diurnal Range (Mean of monthly (max temp - min temp))
BIO3	Isothermality (BIO2/BIO7) (100)
BIO4	Temperature Seasonality (standard deviation* 100)
BIO5	Max Temperature of Warmest Month
BIO6	Min Temperature of Coldest Month
BIO7	Temperature Annual Range (BIO5-BIO6)
BIO8	Mean Temperature of Wettest Quarter
BIO9	Mean Temperature of Driest Quarter
BIO10	Mean Temperature of Warmest Quarter
BIO11	Mean Temperature of Coldest Quarter
BIO12	Annual Precipitation
BIO13	Precipitation of Wettest Month
BIO14	Precipitation of Driest Month
BIO15	Precipitation Seasonality (Coefficient of Variation)
BIO16	Precipitation of Wettest Quarter
BIO17	Precipitation of Driest Quarter
BIO18	Precipitation of Warmest Quarter
BIO19	Precipitation of Coldest Quarter

Results

The AUC, AUC training, of our model was 0.987. As has been mentioned in material and methods, AUC ≥ 0.9 shows the excellent performance of the model. Also, the area under the receiver-operating characteristic (ROC) curve has been shown in figure 1. Additionally, other statistics scales have been evaluated for testing the accurate performance of *B. sitibundus*, including true skill statistic (TSS) = 0.677; Kappa = 0.484. The scales indicate the performance of the model accurately as well.

Among environmental variables, solar radiation includes the most contributed variables. There are three influential environmental factors includes solar radiation of the seventh month (37.2%), bio19 (Precipitation of Coldest Quarter, 23.2%), and water vapor pressure of the third month (8.1%) (Table 3).

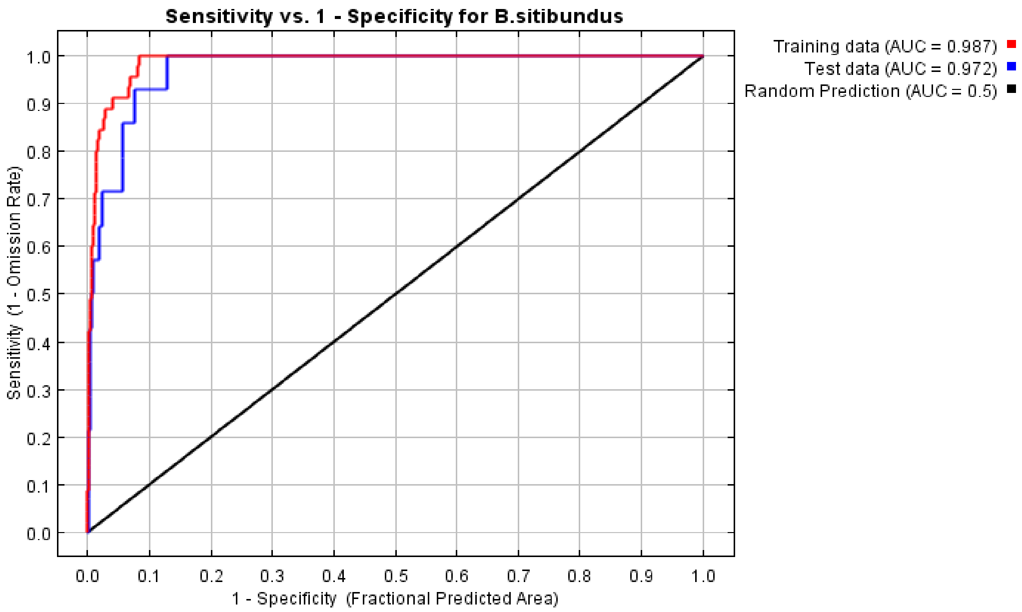

Figure 1. The results of the area under the receiver operating characteristic (ROC) curve developing *B. sitibundus* habitat suitability model. The red (training) line shows the "fit" of the model to the training data. The blue (testing) line indicates the fit of the model to the testing data and is the real test of the model's predictive power.

These variables have a significant amount of information, which is not represented by the other variables. In the jackknife test, The environmental variables with the highest gains for *B. sitibundus* are solar radiation of seventh, eighth, and sixth month (Fig. 3) as the model gain decreases significantly when omitted implies that these variables have a significant amount of information.

Besides, our data from the field shows that *B. sitibundus* can be found from low lands (e.g., Fig4.B) in Khuzestan plain (12 m a.s.l.), the vicinities of Persian Gulf (2m a.s.l.) to high lands in mountain Shirkouh in Central Iran (2190 m a.s.l.) and Alisadr cave (e.g., Fig 4.A) in Hamedan (2000 m a.s.l.).

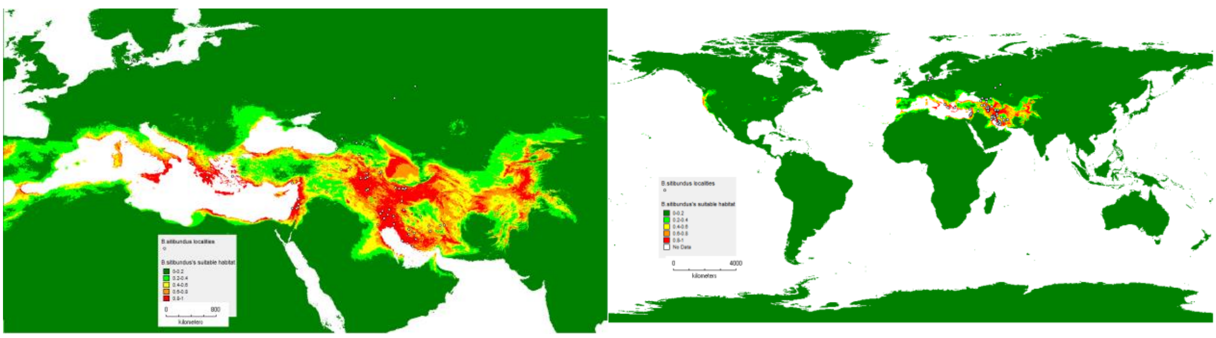

Figure 2. Potential distribution of *B. sitibundus* resulting from the average MaxEnt model. Empty circles showed occurrence records of *B. sitibundus*. Legend: red to green showed high habitat suitability to low habitat suitability, with more intense colors indicating more extreme values.

Table 3. Contribution percentages of important variables included in the best-fitting distribution model for *B. sitibundus*. Vapr and srad stand for water vapor pressure and solar radiation, respectively.

Variable	Percent contribution	Permutation importance
srad_7	37.2	1.5
bio19	23.2	12.5
vapr_3	8.1	0.4
srad_8	7.1	0.1
bio2	5.4	1.5
bio15	5.3	0.9
bio4	3.5	0
srad_9	2.7	33.9
srad_6	2.3	39.4
srad_5	1.1	0.5
srad_4	1	0.5
vapr_4	0.6	0.7
srad_3	0.5	2.6
bio1	0.4	0
srad_12	0.3	0
vapr_10	0.3	3.9
vapr_2	0.3	0
vapr_6	0.3	0.3
vapr_11	0.2	0.3
varp_8	0.2	0
vapr_5	0.1	0.5
bio16	0.1	0.1

Discussion

Our modeling findings are partially compatible with the known distribution of *B. sitibundus* (Frost, 2019) except for Russia, Denmark, Germany, and Sweden. On the contrary, the results show that Russia, Germany, Denmark, Sweden, and central Iran are not suitable habitats for *B. sitibundus*. Surprisingly, the species has been recorded from these areas. It seems the regions, Russia, Germany, Denmark, Sweden, and central Iran, are located in the marginal zone of species distribution. It is probably there are no optimum conditions for *B. sitibundus*. The figure of modeling distribution showed the area as low suitable habitat in green (Fig. 2). According to our results, the species' local populations have been recorded from Russia, Denmark, Germany, and Sweden.

Moreover, as mentioned above, the northernmost distribution of the species belongs to these regions. On the other hand, the map shows that central Iran is not a highly suitable habitat for the species, but *B. sitibundus* has been found in this region (e.g., Shirkouh, Taft, Yazd province and Esfahan; see Table1). All distribution patterns indicate that microhabitat for the species is necessary, providing the environmental condition for surviving the local populations. Therefore, the AUC values of all the mentioned models were excellent. The standard deviation (SD) of the models was also very low, which implies the models' sound performance (Manel et al., 2001).

Besides, our data from the field shows that *B. sitibundus* was found from low lands to high lands. Therefore, *B. sitibundus* is a flexible species adapted to different environmental conditions and diverse habitats.

Based on Stöck et al. (2001a) and Fakharzadeh et al. (2014), although this species tends to colonize different habitats, central Iran's deserts are barriers that prevent it from spreading through the East of Iran. In Iran, partially resulted from suitable habitats in northern Iran are occupied by another species *B. perrini*.

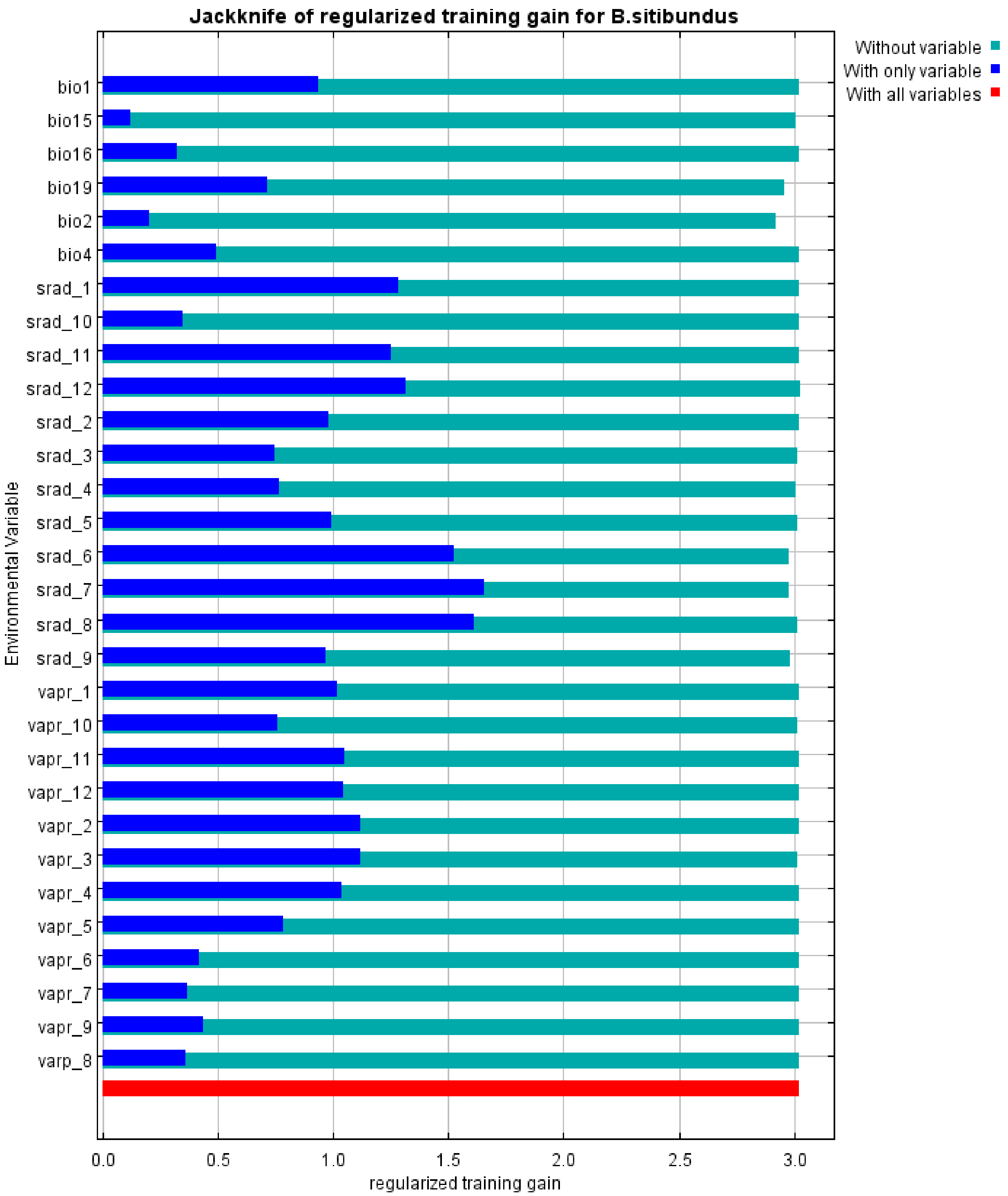

Figure 3. Results of Jackknife evaluations of the importance of the variables used for our *B. sitibundus* MaxEnt model.

Many studies showed that Perrin's green toad had been reported from the north and northeastern Iran; therefore, the existence of hybrid zones of the two species in the northern part of Iran is predictable (e.g., Beçak,2014; Malone & Fontenot, 2008).

In this study, solar radiation of the seventh month as the variable associated with solar energy and bio19 (Precipitation of Coldest Quarter), the water vapor pressure of the third month as water energy variable includes the most contribution distribution patterns of the *B. sitibundus*. According to Rodríguez et al. (2005), the distribution of amphibians depends strongly on solar energy. Both heat and moisture are required for reproduction. Among climate variables, temperature and precipitation are two fundamental drivers of amphibian distribution (Otto et al., 2007). Generally, amphibians require a combination of water energy balance and productivity to distribute (Rodríguez et al., 2005; Whittaker et al., 2007). Another study showed that reducing water availability and temperature increase are critical factors, which might cause decline and local extinction of amphibian species (Araújo et al., 2006). Water availability is a crucial factor in semi-arid and arid regions that dictate amphibians' distribution (Carey & Alexander, 2003). Amphibians are the most sensitive and vulnerable terrestrial vertebrates that need water for reproduction, metamorphism, and survival, and hence precipitation oscillations influence their lives (Araújo et al., 2006).

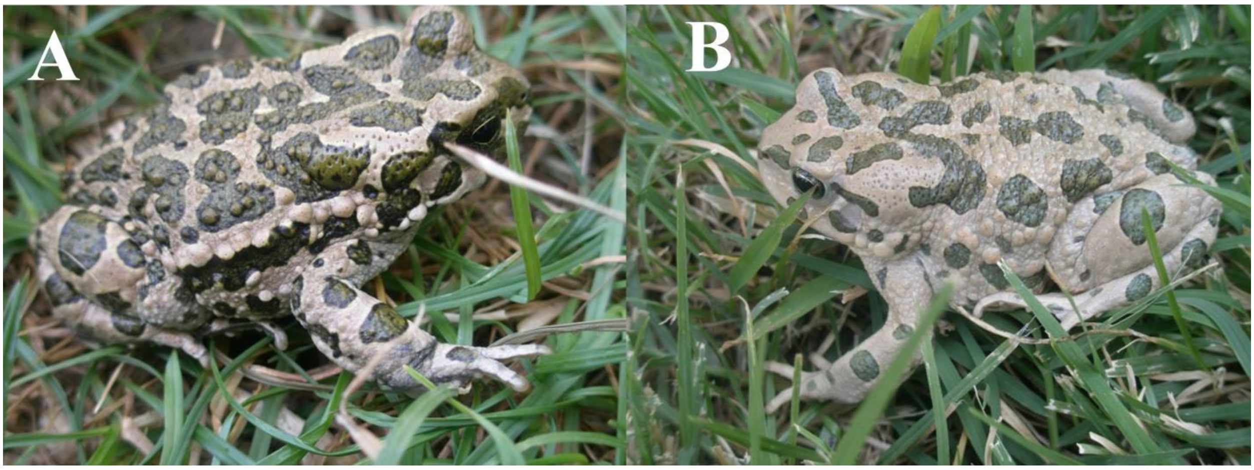

Figure 4. *B. sitibundus* from Hamedan, 2000 m a.s.l. (A) and Ahvaz, 12 m a.s.l. (B).

Our observations showed that green toads from northwestern Iran have more prominent tubercles than Western and southwestern specimens (Fig. 4.A and B), so it seems that there is a cline for this morphological character. Probably different subspecies may exist in the western half of Iran. More morphological and molecular studies in the future can confirm this issue.

Conclusion

We successfully mapped the target species' suitable habitats and extracted the most influential variables on the species distribution. Observation of the specimens in different altitudes from the lowest to highest has been recorded. Concerning the occurrence of *B. sitibundus* in diverse climates, the authors believe the species occur in microhabitat. The distribution range is probably more expansive than what has been already reported. Then, more field investigations can provide higher data on species distribution and habitat usage. Besides, all of our findings can help scientists to manage and solve the global decline of amphibian issue.,

Acknowledgment

The authors are grateful to Mr. Mahmood Abbaszadeh for his assistance during the fieldwork.

References

Allmon, W.D. (1991). A plot study of forest floor litter frogs Central Amazon Brazil. Journal of Tropical Ecology, 7(4), 503–522. https://doi.org/ 10.1017/S0266467400005885

Allouche, O., Tsoar, A., & Kadmon, R. (2006). Assessing species distribution models' accuracy: prevalence, Kappa, and the true skill statistic (TSS). Journal of Applied Ecology, 43(6), 1223-1232. https://doi.org/10.1111/j.1365-2664.2006.01214.x

Araújo, M.B., Thuiller, W., & Pearson, R.G. (2006). Climate warming and the decline of amphibians and reptiles in Europe. Journal of Biogeography, 33(10), 1712–1728. https://doi.org/ 10.1111/j.1365-2699.2006.01482.x

Avci, A., Tuniyev, B., Crnobrnja Isailovic, J., Lymberakis, P., Andrén, C., Cogalniceanu, D., Wilkinson, J., Ananjeva, N., Üzüm. N., Orlov, N., Podloucky, R., Tuniyev, S., Uğur, K., Nilson, G., Sharifi, M., Papenfuss, T., & Shafiei Bafti, S. (2018). Bufotes variabilis. The IUCN Red List of Threatened Species. Version 2018-2. http://www.iucnredlist.org. Downloaded on 14 November 2018.

Baloutch, M., & Kami, H.G.(1995). Amphibians of Iran. Tehran, Iran.Tehran University Press (in Persian).

Beçak, M.L. (2014). Polyploidy and epigenetic events in the evolution of Anura. Genetics and Molecular Research, 13 (3), 5995-6014. https://dx.doi.org/10.4238/2014.August.7.15

Carey, C., & Alexander, M.A. (2003). Climate change and amphibian declines: is there a link? Diversity and Distributions, 9(2), 111–121. https://doi.org/10.1046/j.1472-4642.2003.00011.x

Crump, M.L., & Scott, N.J, J.r. (1994). In: Heyer W.R., Donnelly M.A., M.c Diarmid R.W, Donnelly Heyek L.C., Foster M.S. (Eds.), Measuring and Monitoring Biological Diversity. Standard Methods for Amphibians. Washington DC, Smithsonian Institution Press.

Dubois, A., & Bour, R. 2010. The nomenclatural status of the nomina of amphibians and reptiles created by Garsault (1764), with a parsimonious solution to an old nomenclatural problem regarding the genus *Bufo* (Amphibia, Anura), comments on the taxonomy of this genus, and comments on some nomina created by Laurenti (1768). Zootaxa, 2447, 1–52. https://doi.org/10.11646/zootaxa.2447.1.1

Dufresnes, C., Mazepa, G., Jablonski, D., Caliari Oliveira, R., Wenseleers, T., Shabanov, D.A., Auer, M., Ernst, R., Koch, C., Ramírez-Chaves, H.E., Patrick Mulder, K., Simonov, E., Tiutenko A., Kryvokhyzha, D., Louis Wennekes, P., Zinenko, O.I., Korshunov, O.V., Al-Johany, A.M., Peregontsev, E.A., Masroor, R., … Litvinchuk, S. (2019). Fifteen shades of green: The evolution of Bufotes toads revisited. Molecular Phylogenetics and Evolution, 141, 1–25. https://doi.org/ 10.1016/j.ympev.2019.106615

Elith, J., & Burgman, M.A. (2003). Habitat models for PVA. In : C.A. Brigham& M.W. Schwartz, (Eds.), Population Viability in Plants. (Chapter 8).Springer-Verlag, New York press.

Elith, J., Ferrier, S., Huettmann, F., & Leathwick, J. (2005). The evaluation strip: A new and robust method for plotting predicted responses from species distribution models. Ecological Modelling, 186(3), 280–289. https://doi.org/10.1016/j.ecolmodel.2004.12.007

Elith, J., Graham, C.H., Anderson, R.P., Dudik, M., Ferrier, S., Guisan, A., Hijmans, R.J., Huettmann, F., Leathwick, J.R., Lehmann, A., Li, J, Lohmann, L.G., Loiselle, B.A., Manion, G., Moritz, C., Nakamura, M., Nakazawa, Y., Overton, J.M., Peterson, A.T., Phillips, S.J., Richardson, K., Zimmermann, N.E. (2006). Novel methods improve predictions of species distribution from occurrence data. Ecography, 29(2), 129–151. https://doi.org/10.1111/j.2006.0906-7590.04596.x

Elith, J., Phillips, S.J., Hastie, T., DudÍk, M., Chee, Y., &Yates, C.J. (2011). A statistical explanation of Maxent for ecologists. Diversity and Distributions, 17(1), 43–57. https://doi.org/ 10.1111/j.1472-4642.

Fakharzadeh, F., Darvish, J., Kami, H.G., Ghasemzadeh, F., Rastegar-pouyani, E. (2014). New karyological and morphometric data on poorly known *B. surdus* and *B. luristanicus* in comparison with data of diploid green toads of the Bufo viridis complex from South of Iran. Asian Herpetological Research, 5(3), 168 – 178. https://doi.org/ 10.3724/SP.J.1245.2014.00168

Fakharzadeh, F., Darvish, J., Kami, H.G., Ghassemzadeh, F., Rastegar-Pouyani, E., & Stöck, M. (2015). Discovery of triploidy in Palearctic green toads (Anura: Bufonidae) from Iran with indications for a reproductive system involving diploids and triploids. Zoologischer Anzeiger, 255, 25 – 31.http://dx.doi.org/10.1016/j.jcz.2015.01.001

Fakharzadeh, F., Darvish, J., Kami, H.G., Ghassemzadeh, F., & Rastegar-Pouyani, E. (2018). Karyological investigation of *Bufo variabilis* (Anura: Bufonidae) populations from northwest and southeast of Iran. Russian Journal of Herpetology, 25(1), 56–60. https://doi.org/0.30906/1026-2296.

Fielding, A.H., & Bell, J.F. (1997). A review of methods for the assessment of prediction errors in conservation presence/absence models. Environmental Conservation, 24, 38–49. https://doi.org/10.1017/S0376892997000088.

Franklin, J. (1995). Predictive vegetation mapping: geographic modeling of biospatial patterns in relation to environmental gradients. Progress in Physical Geography, 19(4), 474–499. https://doi.org/10.1177/030913339501900403

Frost, D.R., Grant, T., Faivovich, J., Bain, R.H., Haas, A., Haddad, C.F.B., de Sá, R.O., Channing, A., Wilkinson, M., Donnellan, S.C., Raxworthy, C.J., Campbell, J.A., Blotto, B.L., Moler, P., Drewes, R.C., Nussbaum, R.A., Lynch, J.D., Green, D.M. and Wheeler, W.C. (2006).The amphibian tree of life. Bulletin of the American Museum of Natural History, 297, 1-291. https://doi.org/ 10.1206/0003-0090(2006)297[0001:TATOL]2.0.CO;2

Frost, D.R. (2019). Amphibian Species of the World: An Online Reference. Version 6.0. American Museum of Natural History, New York, USA. http:// research.amnh.org/ herpetology/amphibia/index.html.

GBIF. (2020). Occurrence download https://www.gbif.org/species/5216588 accessed via GBIF.org on 2020.

Graham, C. H., Ferrier, S., Huettman, F., Moritz, C., & Peterson, A. T. (2004). New developments in museum-based informatics and application in biodiversity analysis. Trends in Ecology and Evolution, 19(9), 497–503. https://doi.org/10.1016/j.tree.2004.07.006

Graybeal, A., & Cannatella, D. (1995). A new taxon of Bufonidae from Peru, with descriptions of two new species and review of the phylogenetic status of supraspecific bufonid taxa. Herpetologica, 51(2), 105–131.

Graybeal, A. (1997). Phylogenetic relationships of bufonid frogs and tests of alternate macroevolutionary hypotheses characterizing their radiation. Zoological Journal of the Linnean Society, 119 (3), 297–338. https://doi.org/10.1111/j.1096-3642.1997.tb00139.x

Guisan, A., & Zimmermann, N.E. (2000). Predictive habitat distribution models in ecology. Ecological Modelling, 135,147–186. https://doi.org/10.1016/S0304-3800(00)00354-9

Guisan, A., & Thuiller, W. (2005). Predicting species distribution: offering more than simple habitat models. Ecology Letters, 8, 993- 1009. https://doi.org/10.1111/j.1461-0248.2005.00792.x

Hijmans, R., Guarino, L., Mathur, P.,& Jarvis, A. (2009). Diva-Gis version 7.1. 7.2: Geographic information system for biodiversity research. Manual available at: https://www. diva-gis. org.

Litvinchuk, S.N., Skorinov, D.V., Mazepa, G. O., &Borkin, L. J. (2018). Distribution of *Bufotes latastii* (Boulenger, 1882), endemic to the Western Himalaya. Alytes, 36 (1–4), 314–327.

Manel, S., William, H.C., Ormerod, S.J. (2001). Evaluating presence-absence models in ecology: the need to account for prevalence. Journal of Applied Ecology, 38(5), 291–931. https://doi.org/10.1046/j.1365-2664.2001.00647.x

Malone, J.H., & Fontenot, B.E. (2008). Patterns of reproductive isolation in toads. Plos ONE, 3 (12), e3900. https://doi.org/ 10.1371/journal.pone.0003900 Marsh, D.M.

Pearman, P.B. (1997). Effects of habitat fragmentation on the abundance of two species of leptodactylid frogs in an Andean montane forest. Conservation Biology, 11(6), 1323–1328. https://doi.org/10.1046/j.1523-1739.1997.95519.x

Moisen, G.G., & Frescino, T.S. (2002). Comparing five modeling techniques for predicting forest characteristics. Ecological Modelling, 157(2-3), 209–225. DOI: 10.1016/S0304-3800(02)00197-7

Otto, C.R., Snodgrass, J.W., Forester, D.C., Mitchell, J.C., & Miller, R. (2007). Climatic variation and the distribution of an amphibian polyploid complex. Journal of Animal Ecology, 76(6),1053–1061. https://doi.org/10.1111/j.1365-2656.2007.01300.x

Pearce, J., &Ferrier, S. (2000). Evaluating the predictive performance of habitat models developed using logistic regression. Ecological Modelling, 133(3), 225–245. https://doi.org/10.1016/S0304-3800(00)00322-7

Peterson, A.T., Egbert, S.L., Sanchez-Cordero, V., & Price, K.P. (2000). Geographic analysis of conservation priorities using distributional modeling and complementarity: endemic birds and mammals in Veracruz, Mexico. Biological Conservation, 93(1), 85-94. DOI: 10.1016/S0006-3207(99)00074-9

Phillips, S.J., Anderson, R.P., & Schapire, R.E. (2006).Maximum entropy modeling of species geographic distributions. Ecological Modeling, 190(3-4), 231–259. https://doi.org/10.1016/j.ecolmodel.2005.03.026

Phillips, S.J., Dudík, M. (2008). Modeling of species distributions with Maxent: new extensions and a comprehensive evaluation. Ecography, 31(2), 161–175. DOI: 10.1111/j.0906-7590.2008.5203.x

Rodríguez, M.Á., Belmontes, J.A., & Hawkins, B.A. (2005). Energy, water, and large-scale patterns of reptile and amphibian species richness in Europe. Acta Oecologica, 28(1), 65-70. https://doi.org/10.1016/j.actao.2005.02.006

Rushton, S.P., Ormerod, S.J., & Kerby, G. (2004). New paradigms for modeling species distributions? Journal of Applied Ecology, 41(2), 193–200. DOI: 10.1111/j.0021-8901.2004.00903.x

Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S., FIschman, D.L., & Waller, R.W. (2004). Status and trends of amphibian declines and extinctions worldwide. Science, 306, 1783–1786. DOI: 10.1126/science.1103538

Stöck, M., Günther, R., & Böhme, W. (2001a). *Bufo viridis* group: current status of nominal taxa and unsolved problems (Amphibia: Anura: Bufonidae). Zoological Abhandlungen Staatliches Museum fur Tierkunde Dresden, 51(18), 253 – 319.

Stöck, M., Frynta, D., Grosse, W-R., Steinlein, C., &Schmid, M. (2001b). A review of the distribution of diploid, triploid, and tetraploid green toads (*Bufo viridis* complex) in Asia, including new data from Iran and Pakistan. Asian Herpetological Research, 9, 77 – 100. https://doi.org/10.5962/bhl.part.15562

Stöck, M., Steinlein, C., Lamatsch, K., Schartl, M., &Schmid, M. (2005). Multiple origins of tetraploid taxa in the Eurasian *Bufo viridis* subgroup. Genetica, 124(2), 255 –272. doi: 10.1007/s10709-005-3085-9.

Stöck, M., Moritz, C., Hickerson, M., Frynta, D., Dujsebayeva, T., Eremchenko, V., Macey, J. R., Papenfuss, T. J., & Wake, D. B. (2006). Evolution of mitochondrial relationships and biogeography of Palearctic green toads (*Bufo viridis* subgroup) with insights in their genomic plasticity. Molecular Phylogenetics and Evolution, 41(3), 663 – 689. https://doi.org/10.1016/j.ympev.2006.05.026