



International Journal of Current Research and Academic Review

ISSN: 2347-3215 Volume 3 Number 9 (September-2015) pp. 62-69

www.ijcrar.com



Anuran Study and Identification of Arak County, Markazi, Iran

T. Moslehi, A. Shayestehfar* and A. Pesaeakloo

Department of Biology, Faculty of Science, Arak University, 38156-88138,
Arak, Markazi, Iran

*Corresponding author

KEYWORDS

Anura,
Arak
County

A B S T R A C T

Amphibians are commonly viewed as indicators of regional environmental health because of their dual life cycle, sensitivity to environmental changes, and dependence on water. In order to clarify species ranges and identify of factors influencing distribution, we surveyed 5 sites to known systematic and population structure and describes patterns of geographic variation in morphometric characters of anuran in Arak county. During field work in the sampling areas since Februarys to June 2013, 105 specimens were collected from different habitats of Arak county. Sampling carried out by hand and also with the help of collection net. The specimens were compared according to morphology and morphometry, studies to determine of the status of the intra-specific variation of the populations. Several metric and meristic characters were measured and all the specimens were identified using recent recognition keys for anuran. In total, 4 species belong to 4 Genera and 3 Families of Amphibians including: *Bufo viridis* from Bufonidae, *Rana ridibunda* from Ranidae, and *Hyla savignyi* from Hylidae, were collected, studied and identified that *Rana camerani* and *Hyla savignyi* were reported for the first time from this area.

Introduction

Although taxonomic and faunistic studies on the herpeto fauna of Iran began during the late 18th century (Anderson, 1999), the study of amphibians and reptiles of Iran has undergone rapid progress during the last decades as a result of several factors (Rastegar-Pouyani *et al.*, 2008). The Iranian Plateau Amphibian have been surveyed by some foreign (e.g., Blandford, 1876; Nesterov, 1916; Anderson, 1957; Anderson, 1963; Tuck, 1974; Leviton *et al.*, 1992) and

native (e.g., Balouch and Kami, 1995; Sharifi and Assadian, 2002; Hezaveh, 2007; Bahmani *et al.*, 2012; Rastegar-Pouyani *et al.*, 2013).

Arak has a continental climate that is, in general, relatively cold and dry. Its weather is warm and dry in summer, windy and cool in autumn, cold and snowy in winter, and mild in spring. The maximum temperature may rise up to 35 degrees Celsius in summer

and may fall to below -25 degrees Celsius in winter. The average rainfall is around 350 mm (mostly snow fall) and the annual relative humidity is 46%. The Amphibian of Iran consists of 13 species and five subspecies of frogs and toads belonging to five genera and four families, eight species of salamanders belonging to four genera and two families (Rastegar-Pouyani *et al.*, 2008). Prior to the present study, two of these species had been recorded from Arak province that is; *Bufo pseudepidalea varibiliss* and *Rana pelophylax ridibunda*. Thus, of the 4 species which were found during this survey, 2 are reported for the first time from this area. In this study describes patterns of geographic variation in

morphometric characters of Arak amphibians to determine whether or not morphological characters differ significantly from one locality to another, and to characterize these differences.

Materials and Methods

The study area is located in the central regions of the Iranian Plateau between 33° 30' and 35° 35' N and 48° 57' and 51° E (Fig. 1). The region is bordered on the north by Tehran and Ghazvin province, to the south by Isfahan and Lorestan province, to the west by the Hamadan province and to the east by Teheran, Ghom and Isfahan province.

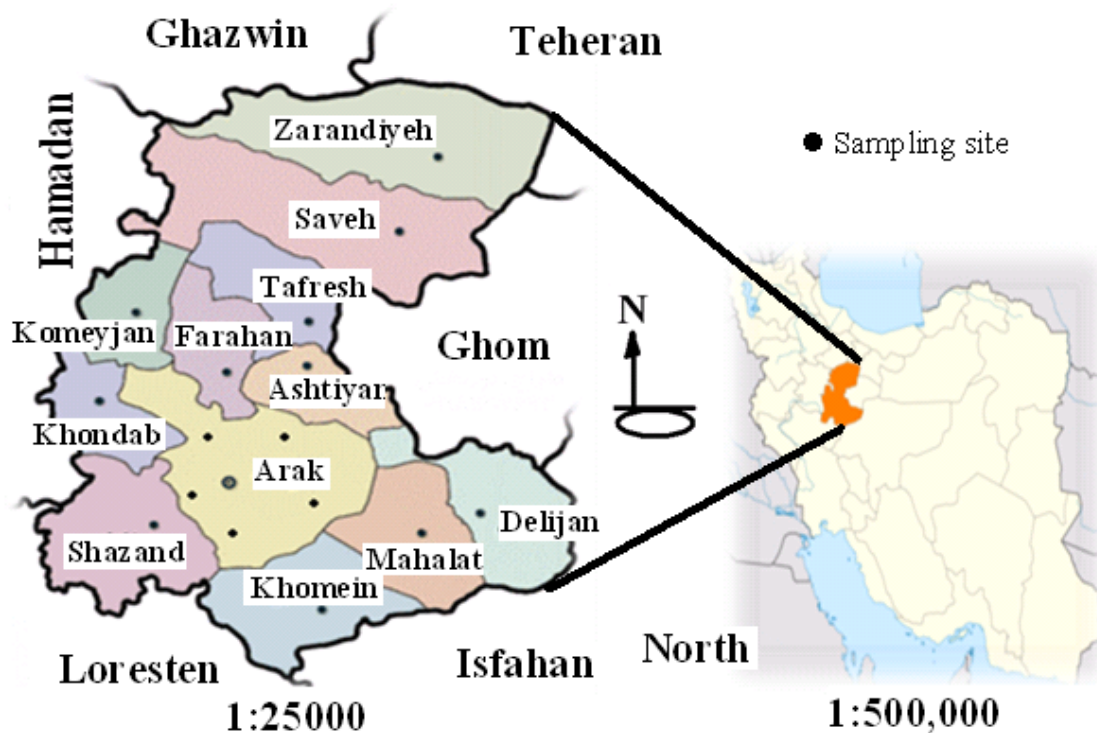


Fig.1 Topography of sampling sites in Arak province

The study was carried out between Februarys to June 2013. The senior author searched habitats in two-month periods, at night and day times. Night searching was

done with a flashlight. Most specimens were captured by hand and some with hunting net. Specimens were transferred alive to the zoology laboratory. Several metric and

meristic characters were measured and most of them were preserved in 75% ethanol specimens were stored in the Arak University Zoological Museum at Arak city. Specimens were identified according to Rastegar-Pouyani, *et al.* (2011) and Faivovich (2005).

Result and Discussion

During field survey a total of 105 specimens were collected in all of the study areas. Encompassing 4 species in 4 genera and 3 families including: *Bufo pseudepidalea varibiliss* from Bufonidae, *Rana pelophylax ridibunda* from Ranidae, *Rana macrocnemis camerani* from Ranidae and *Hylasavignyi* from Hylidae, were collected, studied and identified.

It is important to mention that *Rana camerani* and *Hylasavignyi* were reported for the first time in this area. Two *R. camerani* (males) were collected from the shahrak gerdoo located in Arak in cool water, and five *Hylasavignyi* (3 males and 2 females) were collected from the Aghilabad and Adeshteh Village that were on a bush or leaf (Table 1).

Morphometric analysis

The phonetic studies between population of two species, 13 characters in frog and 16 characters in toad were measured (Table 2).

Independent sample T-test and ANOVA test were carried out in male and female specimens of five populations of *Rana ridibunda ridibunda* and *Bufo pseudepidalea viridis* to investigate sexual dimorphism in this population. In these analyses none of the characters exhibited significant sexual differences among male and female populations of *Rana ridibunda ridibunda*, but in *Bufo pseudepidalea viridis* two characters (Pa.l and Pa.w) were significant (P<0.05). On the other hand, results from ANOVA variance analysis for *Rana ridibunda ridibunda* population suggested statistically significant differences in 5 characters including Sp.p, Sp.c.r, L.o, Lt.p, N.d, among 5 studied population that these characters are good for population separating (Table 3). In *Bufo pseudepidalea aviridis* population also suggested statistically significant differences in 3 characters Lt.p, Sp.p, Nd among 5 populations (Table 4).

Table.1 Showing the location, Height, number of male and female of collected Amphibians (Februarys to June 2013)

Station	Longitude	Latitude	Height	Number							
				Rana				Bufo		Hyla	
				ridibunda		camerani		Male	Female	Male	Female
				male	Female	Male	Female				
1	34° 24' 41"	49° 30' 09"	1870	8	5	-	-	5	3	1	1
2	34° 10' 50"	49° 32' 02"	1870	6	4	2	-	5	6	2	1
3	34° 03' 06"	49° 56' 57"	1799	3	9	-	-	5	4	-	-
4	33° 58' 37"	49° 02' 00"	1712	4	4	-	-	5	5	-	-
5	34° 00' 25"	49° 54' 40"	1724	2	4	-	-	3	5	-	-

Table.2 Showing the phonetic characters studied from collected Amphibians (Februarys to June 2013)

Number	Abbreviation	Definition
1	L	Body Length
2	L.C.	Head length (from tip of snout to the posterior edge of tympanum)
3	D.R.O	Rostrum length (from tip to anterior corner of eye)
4	Spcr	Rostrum wide
5	L.O.	Length of eye (distance from anterior corner to posterior corner to its posterior)
6	Lt.p.	Eyelash wide
7	Sp.p	Distance between two eyelashes
8	L.tym.	Tympanum diameter (largest size)
9	F	Length of femur (from hip joint to top of knee)
10	T	Length of tibia (from top of knee to beneath wrist)
11	D.P.	First Digit
12	C.int	Metatarsal Tubercles
13	L.F.	Length of Leg
14	N.D.	Distance between two Nostril
15	Pa.1	Length of Parotoid Gland
16	Pa.w	Wide of Parotoid Gland

Table.3 Showing the statistically significant differences in 5 studied characters of *Rana pelophylax ridibunda*

Component Matrix^{a,b}

	Component	
	1	2
Sp.c.r	.889	-.092
L.o	.687	-.396
Lt.p	.958	.010
Sp.p	.111	.943
N.d	.914	.262

The two multivariate techniques, that is: Principal Component Analyses (PCA) and Discriminate Function Analysis (CVA) were employed to explain the patterns of morphological variation among five populations of *Rana ridibunda ridibunda* and also five populations of *Bufo pseudopidalea viridis* in the principal component analysis, as shown in table 4. For *Rana pelophylax ridibunda* five principal

components have captured, the first principle component show 60.5% variance which Lt.p, N.d are mainly responsible for this variation and the second principle component show 22.46% variance of this total, which Sp.p, Sp.c.r and N.d are mainly responsible for this variation; species distribution based on two principle component show that second principle component separated W population from S,

SW population mainly attributed to Sp.p, Sp.c.r and N.d that were explained by PC2. These results show that Sp.p, Sp.c.r and N.d are most important characters in separating populations respectively in *Rana pelophylax ridibunda* population (Figure 2). But in *Bufo pseudupidalea viridis* none of characters

show important role in separating population (Table 4) and (Figure 3).

The power of PCA comes with plotting the values of these newly constructed variables (principal components). Scatter gram plots of the PC1 against PC2, is given in figure 2.

Table.4 Showing the statistically significant differences in 5 studied characters of *Bufo pseudupidalea viriabilis*

Component Matrix^{a,b}

	Component	
	1	2
Lt.p	-.054	.997
Sp.p	.958	-.073
N.d	.953	.130

Fig.2 Showing the statistically significant differences in 5 studied populations of *Rana pelophylax ridibunda*

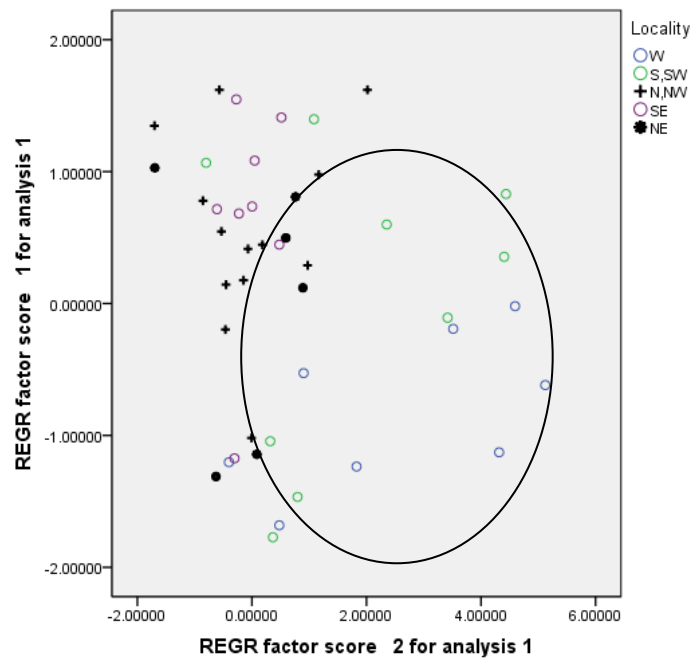
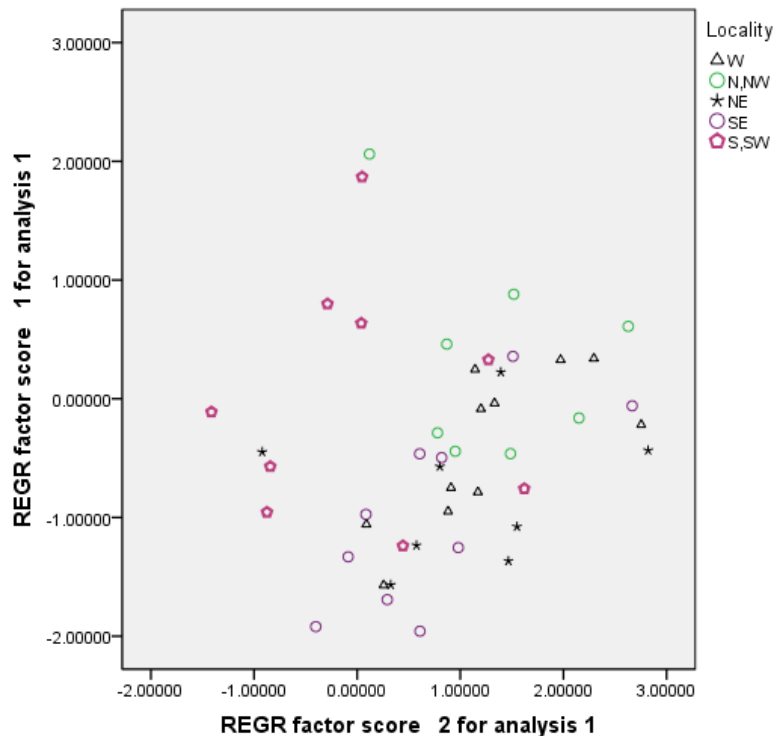


Fig.3 Showing the statistically significant differences in 5 studied populations of *Bufo pseudepidalea viriabilis*



The next stage of analysis aimed to provide more accurate discrimination keys, thus permitting the unambiguous determination of all the specimens. Initially, step wise Discriminate Function analysis was performed on populations. All discriminant functions had Eigen values greater than one and were found to be statistically significant. On the other hand, discriminate analysis distinctly separates populations of W and S, SW of *Rana pelophylax ridibunda*. The discriminate function gives complete classification for all the specimens of five studied populations of the genus *Rana pelophylax ridibunda* in Arak county.

Using of this analyze for *Bufo pseudepidalea viridis* population show that 3 characters including Sp.p, N.d, Lt.p, separated population of S,SW and N,NW from the other population of genus.

Cluster analysis based on centroids grouped populations also showed that in *Rana pelophylax ridibunda* two populations W, SW have a good distance and in *Bufo pseudepidalea viridis* also two populations N,NW and S,SW are separated from others population in the study area.

The Amphibian occurs in a wide range of different habitats including arid, semi-arid, tropical and subtropical regions (Anderson, 1957). Twenty species occur in Iran including six salamanders in three genera in two families and fourteen frogs and toads in four genera in four families. The amphibian fauna in Iran is most diverse in the northwestern of county, which have the greatest rainfall and running water throughout the year. Almost every geographic region has one or more species (Anderson, 1999). In this research four species found including *Rana pelophylax*

ridibunda, *Rana macrocnemis camerani*, *Hyla savignyi* and *Bufo pseudepidalea viridis*. This is the first report for *Rana camerani* and *Hyla savignyi* from Arak county. The analysis of morphometric variation in *Rana pelophylax* and *Bufo pseudepidalea viridis* shown that body length of females are larger than males in both of two species that seems reproduction excellence in female that carry a lot of eggs, which in agreement with Shine, 1979.

On the other hand, *Rana pelophylax ridibunda* shows sexual dimorphism in some morphological characters. But in *Bufo pseudepidalea viridis*, none of the characters shows significant differences between male and female.

As shown before, both monovariate and multivariate statistical analyzes represent some differences between the two species (*Rana pelophylax ridibunda* and *Bufo pseudepidalea viridis*).

Results of monovariate analysis of SVL seem to suggest a population separation between west to east of Arak counth. In addition to differences in meristic and metric characters, the differentiation of the above-mentioned species is also based on differences in dorsal color pattern in correlation with different geographic regions of occurrence. In relation to coloration and color pattern, the several color pattern features of *Rana pelophylax ridibunda* and *Bufo pseudepidalea viridis* were quantified, and also some of samples with teratology recognized that may be could say as a symbol of environmental pollution. It is important to mention that we could not find any source of chemical pollutants (fertilizers, insecticides, herbicides etc.) in these habitats. We guess most of these kinds of pollutants are produced by human activities as cultivated grounds.

Although morphometric variations are caused by a combination of genetic and environmental factors which in agreement with Castellano and Giacoma (1998) and Stöck *et al.* (2008a).

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