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Anuran Karyological Study of Khorasan Province

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Abstract: In addition to the morphological, skeletal and biometric characteristics, karyological study is the key to the animal identification and classification. Due to the insufficient studies on amphibians in Khorasan Province (Iran), this research studies five areas of this province, namely Mashhad, Sarakhs, Bojnourd, Shirvan and Tibaad. Totally, 100 samples were collected in these regions from April to November, which were transferred alive to the lab. In the laboratory, the viminoblastin was injected to the samples proportional to their weights and consequently, the bone marrows were extracted. The obtained solution was dropped from a height of 60 to 70 cm on the frozen slides placed on an inclined surface. Prepared slides were stained and photographed to study the chromosomes. Relative length and arms ratio of the chromosomes were used to recognize the centromere position and chromosomal type. Finally, based on the above studies the related idiograms were derived. This research illustrates that all frogs have a set of 26 ($2n = 26$) chromosomes and all toads have a set of 22 chromosomes. It is concluded that all collected frogs and toads from the different stations are *Rana ridibunda* and *Bufo viridis* species, respectively. This research shows no sex dimorphism in all samples.

Key words: Anura, *Bufo viridis*, karyotype, *Rana ridibunda*

INTRODUCTION

Morphology of karyotype, which includes number, shape and size of chromosomes, forms one class of the phenotypic characteristics, which is usually independent of the genotype (Chiraelli and Capana, 1973; Green and Sessions, 1991). Most of the amphibians do not have a large number of chromosomes. Nevertheless, their chromosomes are relatively large in size and easy to obtain, so the amphibians have an excellent position in chromosomal research.

So far, the karyological studies on Anura in Great Khorasan (Fig. 1), are limited to a few cases (Nehmati, 1998). Kami and Baluch (1994) has done an extensive investigation on Iran Amphibian and recognized various species of Amphibian in Iran. Some other studies have been conducted on Amphibians in the regions close to Iran. For example, Balletto *et al.* (1985) have studied the Amphibians of the Arabian Peninsula. In Turkey, a research was carried out on the cytogenetic study of the pelobates syriacus (Amphibia, Anura)

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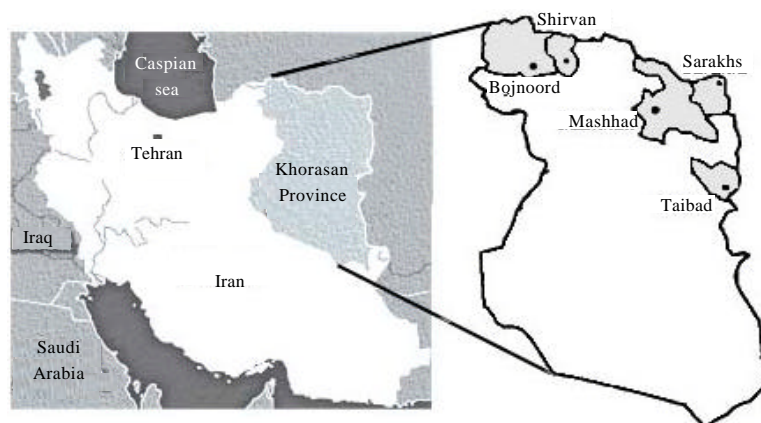


Fig. 1: Great Khorasan map and the studied areas

which examined cytological aspects of the chromosomes (Ugurtas *et al.*, 2001). Their method is similar to the method described in this study. Moreover, Al-Shehri and Al-saleh (2005) have presented a comprehensive research on karyotype of amphian specially Anura in Saudi Arabia.

Particularly, a few researches on *Bufo* and *Rana* can be found in the study. Spasic-Boskovic *et al.* (1999) have studied population composition and genetic variation of water frogs (Anura Ranidae) from Yougslavi. Also, Spasic-Boskovic *et al.* (2000) have investigated on karyotype of two species of *Bufo* from Yougslavi. Another research is devoted to karyological comparison of water frog (*Rana cf. ridibunda*) population from Bahrain, Eastern Saudi Arabia and Egypt that has been studied by Saeed (1997). Dujsebaveva *et al.* (1997) have investigated on diploid and tetraploid of *Bufo viridis* complex (Anura: Bufonidae) in Southern Kazakhstan. Also Stock (2005) have studied on multiple origin of tetraploid taxa in Eurasian *Bufo viridis* subgroup. Finally, Mindrescu and Ghiorghita (2008) have done a cytogenetic investigation on *Rana idibunda*. and *Rana esculenta* fom the North of Moldavia.

In this study, the karyological and morphological research on 100 samples of Anura collected from five areas in Khorasan Province (Mashhad, Sarakhs, Bojnourd, Shirvan and Tibad) is studied. These areas are shown in Fig. 1.

MATERIALS AND METHODS

Morphological Studies

In this research, 16 morphological characters, such as absence or presence of paratoid glands, vomerian teeth, dorsal fold, temporal spot, locomotion organs and etc. (Kami and Baluch, 1994; Balletto *et al.*, 1985), were used to study all collected samples.

Karyological Studies

For this research, all samples were transferred alive to the Bio-systematic lab of Ferdowsi University of Mashhad, where this research was conducted from April 2003 to December 2006. The number of the collected samples from each area in Khorasan (Fig. 1), is given in Table 1. Totally, 100 samples were collected in these regions from April to November of 2004, 2005 and 2006.

Table 1: Number of samples of each area

Sampling areas	No. of frogs	No. of toads
Tibad	-	20
Mashhad	13	3
Shirvan	24	3
Sarakhs	-	16
Bojnoord	21	-
Total = 100		

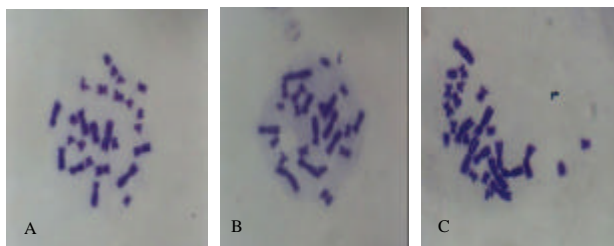


Fig. 2: Karyotype of *Rana ridibunda* (A) Bojnoord, (B) Mashhad and (C) Shirvan

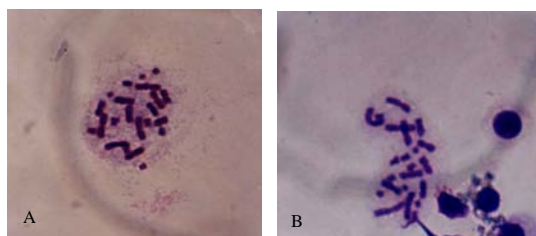


Fig. 3: Karyotype of *Bufo viridis* (A) Sarakhs and (B) Tibad

At first for each gram of the sample weight 0.01 mL vimnoblantine was injected under the stomach skin. About 1 h later, the bone marrow was extracted and put it in KCl. Then the solution was kept for 15 to 20 min in incubator (37°C) and centrifuged three times. Before each centrifuge a fixator containing methanol and acetic acid (3:1) was used (Schmid, 1978a, b).

This solution was dropped from a height of 60 to 70 cm distance on the frozen slides placed on an inclined surface. After staining the prepared slides, they were studied using microscope and then photographed (Fig. 2, 3). Each chromosome was separated and then the length of short and long arms and total length of chromosome were measured. Based on Levan (1964) classification, chromosomes types were determined and arranged (according to their length) to obtain a chromosomal set.

RESULTS

Morphology and Identification of Species

The mentioned morphological characters were divided into three categories. The first category included those characters that were more important in species identification and amphibian classification, namely absence or presence of dorsal fold, temporal spot, vocalization organs, hind locomotion organs position and etc. (Kami and Baluch, 1994). On the other hand, some of them such as vertebral line and color diversity, especially at body

dorsal, were different between populations of one area and probably had influenced by environmental factors. The third category included those characters used to recognize animal at supra species, namely absence or presence of vomerian teeth, head shape, tongue shape and disk plates at the end of fingers. Based on the morphological characters used in this study, it was concluded that all of toads are from *Bufo viridis* species and all of collected frogs are from *Rana ridibunda* species.

Karyology

Results of karyotype revealed that all samples of *Rana ridibunda* had a set of $2n = 26$ chromosomes clearly shown in Fig. 2. Table 2 shows arm ratio and chromosomal type of *Rana ridibunda* from Shirvan area according to Levan (1964) classification. Related idiogram was drawn which is shown in Fig. 4. It was observed that karyotype of *Rana ridibunda* from Shirvan consisted of five long pairs and eight short pairs of chromosome. Five long pairs were metacentric or submetacentric and eight short pairs of chromosome were metacentric submetacentric and acrocentric. Only one chromosome was acrocentric.

Table 3 also demonstrates the arm ratio and chromosomal type (based on Levan classification (1964)) of *Rana ridibunda* from Bojnoord area. The related idiogram is shown in Fig. 5. The karyotype of *Rana ridibunda* from Bojnoord consisted of five long pairs and eight short pairs of chromosome. Five long pairs were metacentric or submetacentric and eight short pairs of chromosome were metacentric submetacentric and acrocentric. Only one chromosome was acrocentric.

Table 4 shows the arm ratio and chromosomal type of *Rana ridibunda* from Mashhad. Accordingly, the related idiogram was drawn which is shown in Fig. 6. It was

Table 2: Arm ratios and type of centromeres of *Rana ridibunda* from Shirvan area

Chor. No.	Total length	Long arm	Short arm	Arm ratio	Type of centromere
1	1.37	0.73	0.64	1.1	M
2	1.25	0.76	0.49	1.6	M
3	1.14	0.76	0.38	2.0	Sm
4	1.07	0.63	0.44	1.4	M
5	0.93	0.52	0.41	1.3	M
6	0.58	0.31	0.27	1.1	M
7	0.54	0.30	0.24	1.3	M
8	0.50	0.43	0.07	6.1	Ac
9	0.48	0.33	0.15	2.2	Sm
10	0.46	0.30	0.16	1.9	Sm
11	0.45	0.26	0.19	1.4	M
12	0.43	0.28	0.15	1.9	Sm
13	0.40	0.24	0.16	1.5	M

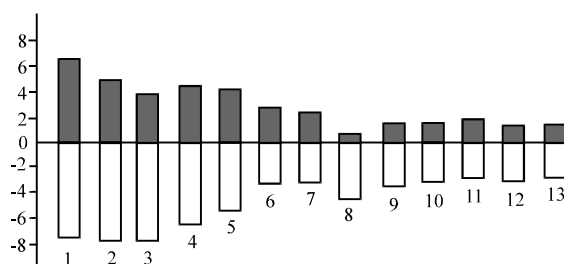


Fig. 4: Idiogram of *Rana ridibunda* samples collected from Shirvan area in Khorasan

Table 3: Arm ratios and type of centromeres of *Rana ridibunda* from Bojnoord area

Chor. No.	Total length	Long arm	Short arm	Arm ratio	Type of centromere
1	1.14	0.64	0.5	1.3	M
2	0.92	0.59	0.34	1.7	Sm
3	0.91	0.54	0.37	1.5	M
4	0.83	0.47	0.36	1.3	M
5	0.82	0.51	0.31	1.6	M
6	0.51	0.27	0.24	1.1	M
7	0.46	0.24	0.22	1.1	M
8	0.42	0.29	0.13	2.2	Sm
9	0.41	0.3	0.09	3.3	Ac
10	0.41	0.25	0.16	1.6	M
11	0.4	0.21	0.19	1.1	M
12	0.38	0.22	0.16	1.4	M
13	0.34	0.22	0.12	1.8	Sm

Table 4: Arm ratios and type of centromeres of *Rana ridibunda* from Mashhad area

Chor. No.	Total length	Long arm	Short arm	Arm ratio	Type of centromere
1	1.44	0.78	0.66	1.2	M
2	1.28	0.81	0.47	1.7	Sm
3	1.14	0.63	0.51	1.2	M
4	1.14	0.78	0.36	2.2	Sm
5	0.89	0.5	0.39	1.3	M
6	0.62	0.36	0.26	1.4	M
7	0.56	0.43	0.13	3.3	Ac
8	0.51	0.27	0.24	1.1	M
9	0.5	0.32	0.18	1.8	Sm
10	0.48	0.28	0.2	1.4	M
11	0.47	0.33	0.14	2.4	Sm
12	0.42	0.27	0.15	1.8	M
13	0.41	0.26	0.15	1.7	Sm

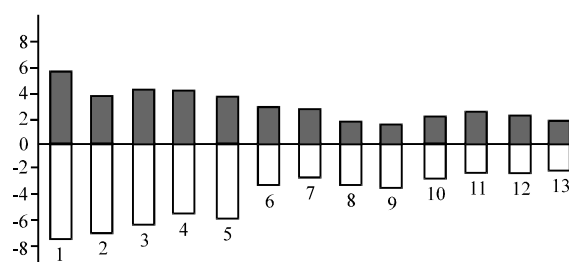


Fig. 5: Idiogram of *Rana ridibunda* samples collected from Bojnoord area in Khorasan

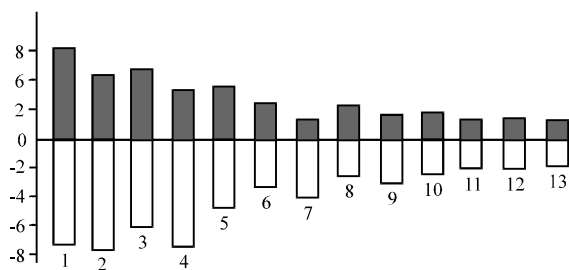


Fig. 6: Idiogram of *Rana ridibunda* samples collected from Mashhad area in Khorasan

observed that karyotype of *Rana ridibunda* from Mashhad consisted of five long pairs and eight short pairs of chromosome. Five long pairs were metacentric or submetacentric and eight short pairs of chromosome were metacentric submetacentric and acrocentric. Only one chromosome was acrocentric.

Karyotype of all samples of *Bufo viridis* showed a set of $2n = 22$ chromosomes clearly recognized in Fig. 3. Table 5 shows the arm ratio and chromosomal type (based on Levan classification (1964)) of *Bufo viridis* from Sarakhs area. Figure 8 shows the related idiogram. It was concluded that karyotype of *Bufo viridis* from Sarakhs comprised of six long pairs and five short pairs of chromosome. The six long pairs were metacentric and the pairs were five short metacentric and submetacentric. No acrocentric chromosome was observed.

Table 6 also shows the arm ratio and chromosomal type of *Bufo viridis* from Tibad area. The related idiogram is shown in Fig. 7. It was seen that karyotype of *Bufo viridis* from

Table 5: Arm ratios and type of centromeres of *Bufo viridis* from Sarakhs area

Chor. No.	Total length	Long arm	Short arm	Arm ratio	Type of centromere
1	0.95	0.48	0.47	1.0	M
2	0.93	0.50	0.43	1.2	M
3	0.83	0.47	0.36	1.3	M
4	0.74	0.45	0.29	1.6	M
5	0.63	0.39	0.24	1.6	M
6	0.62	0.32	0.30	1.1	M
7	0.35	0.22	0.13	1.7	Sm
8	0.33	0.20	0.13	1.5	M
9	0.33	0.18	0.15	1.2	M
10	0.28	0.17	0.11	1.5	M
11	0.24	0.14	0.10	1.4	M

Table 6: Arm ratios and type of centromeres of *Bufo viridis* from Tibad area

Chor. No.	Total length	Long arm	Short arm	Arm ratio	Type of centromere
1	1.25	0.67	0.58	1.2	M
2	1.15	0.65	0.50	1.3	M
3	1.00	0.57	0.43	1.3	M
4	0.92	0.58	0.34	1.7	M
5	0.75	0.40	0.35	1.1	M
6	0.72	0.40	0.32	1.3	M
7	0.41	0.28	0.13	2.2	Sm
8	0.41	0.24	0.17	1.4	M
9	0.36	0.20	0.16	1.3	M
10	0.30	0.16	0.14	1.1	M
11	0.23	0.13	0.10	1.3	M

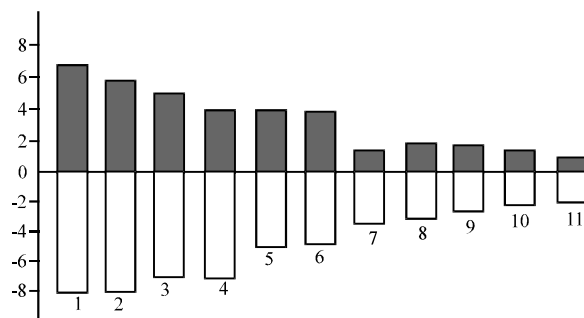


Fig. 7: Idiogram of *Bufo viridis* samples collected from Tibad area in Khorasan

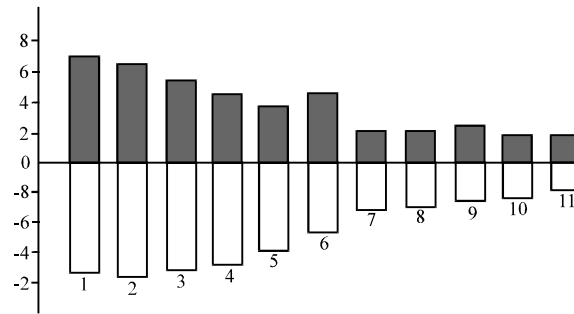


Fig. 8: Idiogram of *Bufo viridis* samples collected from Sarakhs area in Khorasan

Tibad consisted of six long pairs and five short pairs of chromosome, where the six long pairs were metacentric and the five short pairs were metacentric and submetacentric. There was no acrocentric chromosome.

DISCUSSION

As it was described earlier, all samples of *Rana ridibunda* had a set of $2n = 26$ chromosomes (that was according to the general plan of karyotype for this speciation) including five long pairs and eight short pairs of chromosome. Karyotype of all samples of *Bufo viridis* showed a set of $2n = 22$ chromosomes. In some papers such as Stock (2005) and Dujsebaveva (1997), the tetraploid form of *Bufo viridis* has been reported. However, in this investigation only diploid toads were found in the examined areas. Karyotype of all samples of *Bufo viridis* consisted of six long pairs and five short pairs of chromosomes, which was in total agreement with the results in Spasic-Boskovic *et al.* (1999, 2000). Collected frogs from different areas had Metacentric (M), Submetacentric (Sm) and Acrocentric (Ac) chromosomes. In this study among all samples of *Rana ridibunda* one chromosome was Acrocentric, but Mindrescu and Ghiorghita (2008) have pointed that in their study, water frogs (*Rana ridibunda*) have only Metacentric and Submetacentric chromosomes. They also mentioned that the chromosomes belonging to the 7th, 9th and 13th pairs of *Rana ridibunda* are more likely Acrocentric than meta- and submetacentric.

Collected toads had only Metacentric and Submetacentric chromosomes. Despite the results of Al-Shehri and Al-Saleh (2005), this study showed no sex dimorphism in all 100 samples, which was in total agreement with Saeed (1997).

This study demonstrated a thorough analysis of karyology of Anura in Khorasan province. In conclusion, all samples of *Rana ridibunda* had a set of $2n = 26$ chromosomes and Karyotype of all samples of *Bufo viridis* showed a set of $2n = 22$ chromosomes. Frogs had Metacentric (M), Submetacentric (Sm) and Acrocentric (Ac) chromosomes but toads had only Metacentric and Submetacentric chromosomes. For further investigation it is suggested that other areas of Khorasan Province (specially South of Khorasan that has never been studied) be investigated and compared with this study. Also, it is suggested to compare the species of Anura discussed here with those from Turkmenistan and Afghanistan, two countries nearby Khorasan province, to obtain precise results.

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