

On the Distribution of Diploid and Tetraploid Green Toads of the *Bufo Viridis* Complex (Anura; Bufonidae) in Southern Kazakhstan

TATJAINA DUJSEBAYEVA¹, SERGIO CASTELLANO², CRISTINA GIACOMA², EMILIO BALLETO²,
AND GAETANO ODIERNA³

¹Department of Biology, Kazakh State University, Timirjaseva Street, 46, Almaty, 480121, Kazakhstan;

²Department of Animal Biology, University of Torino, Via Accademia Albertina 17, I-10123, Torino, Italy;

³Department of Comparative and Evolutionary Biology, University of Napoli, Via Mezzocannone 8, I-80134, Napoli, Italy

Abstract. -115 specimens of Green Toads belonging to the *Bufo viridis* complex were sampled from 8 populations in south Kazakhstan during the breeding seasons of 1994 and 1995. Subsequent karyological analysis revealed a single diploid population (Kopa) with a $2n=22$ karyotype and large snout-vent length. All other seven populations proved tetraploid with a $4n=44$ karyotype and smaller size. Information on the distribution of diploid and tetraploid toads in southern Kazakhstan is discussed in connection with previous data.

Key words: Amphibia, Bufonidae, diploid *Bufo viridis*, tetraploid *Bufo danatensis* Kazakhstan, geographic distribution.

Introduction

Following the discovery of some polyploid Asiatic Green Toad populations (Masik et al. 1976) and the description of a new species, *Bufo danatensis* Pisanetz (Pisanetz 1978), two distinct species, diploid *Bufo viridis* and tetraploid *Bufo danatensis*, have been known to occur in ex Soviet central Asia, where only *Bufo viridis* Laurenti, 1768 was formerly recognized.

Using literature data on a number of populations (Bachmann et al. 1978; Pisanetz 1978; Pisanetz and Szczerbak 1979; Pisanetz et al. 1985; Toktosunov 1984), as well as their own data, Borkin et al. (1986a) have drawn a preliminary map of the distribution of diploid and tetraploid Green Toads of the *Bufo viridis* complex in the territory of the ex Soviet Union. Only 3 records of tetraploid *Bufo danatensis* were known for Kazakhstan at the time, whereas diploid *Bufo viridis* had not yet been reported from this region (Borkin et al. 1986a, p. 141).

During the following nine years many more data became available on the distribution of diploid and tetraploid Green Toads in Middle Asia, including Kazakhstan and its bordering countries (China, Mongolia) (Borkin et al. 1986a, 1986b, 1986c, 1996; Fichtman 1989; Orlova and Terbish 1986; Orlova and Uteshev 1986; Schneider and Egiaryan 1995; Wu Min and Zhao Yajiang 1987; Fichtman 1989; Zhao Ermi 1995). Although some of these data relate to

Kazakhstan, however, the vast territory of this Republic is still very poor studied in this respect.

In 1994-1995 a combined expedition by the Department of Animal Biology of the University of Torino (Italy) and the Department of Biology of Kazakh State University at Almaty (Kazakhstan) carried out some field work on the *Bufo viridis* complex in south Kazakhstan. One of the aims of this expedition was to obtain new data on the geographic distribution of diploid and tetraploid toads in this territory.

Materials and Methods

115 Green Toads were sampled from 8 populations in South Kazakhstan (Fig. 1) during the breeding seasons (May-June) of 1994 and 1995. Preliminary species identification was based on morphometric comparisons and records of mating calls. In order to obtain the exact determination of the ploidy level of each specimen a karyological analysis was subsequently carried out at Naples University. From each specimens 100-200 μ l of venous blood were incubated for 4 days at 25°C in MEM (Minimal Eagle Medium, GIBCO) with 20% calf serum and 3% Phytoemagglutinin M. Chromosome preparations were produced by conventional air-drying method, using KCl 0.075 M as hypotonic solution. Standard staining method were performed using Giemsa 5% in phosphate buffer pH 7.

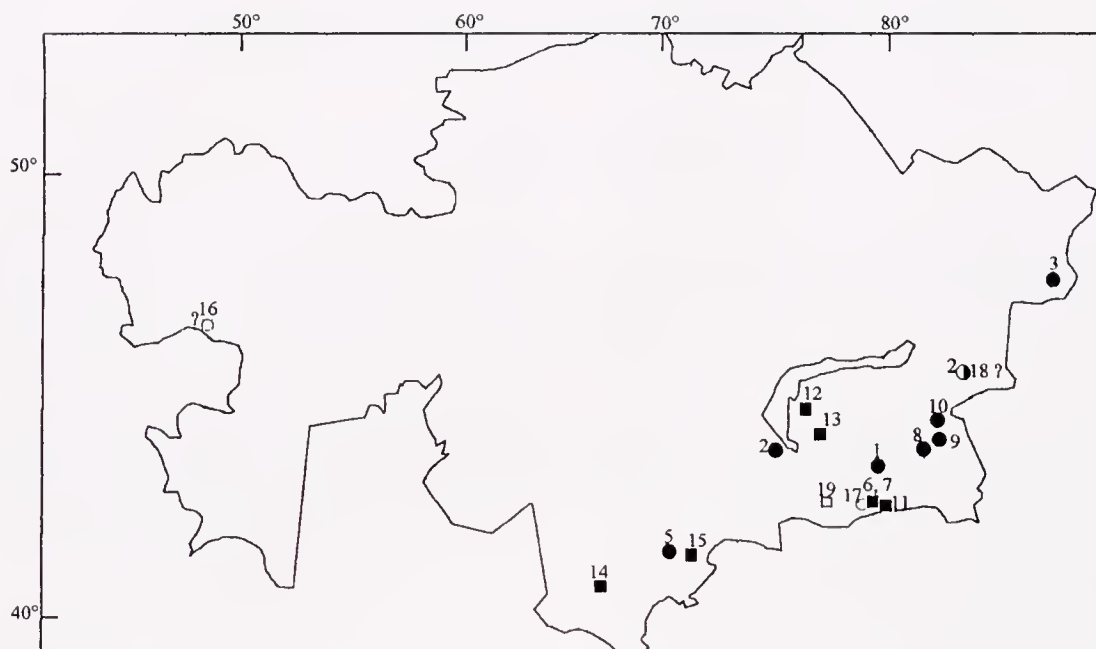


Figure 1. Known localities of diploid and tetraploid green toads, *Bufo viridis* complex in Kazakhstan. [Circle = data from literature; Square = our own findings. Dark = *Bufo danatensis* (4n); light = *Bufo viridis* (2n); hemidark = sympatric locality of *Bufo danatensis* and *Bufo viridis*. ? = attribution not supported by karyological analysis]

Bufo danatensis: 1 - Kapchagai, Ili River (Borkin et al., 1986a); 2 - desert near Burubaital Village, to the southwest of the Balkhash Lake (Egemberdieva, 1983); 3 - Aksir Farm, 12 km to the northwest of the Maikapchagay Village, Zaissan Depression (Szczerbak, Golubev, 1981); 4 - Chindzhaly River near Andreyevka Village, Taldy-Kurgan Region (Golubev, 1990); 5 - Chimkent Town, Chimkent Region (Mezherin, Pisanetz, 1991); 6, 7 - Almaty (Borkin et al., 1996; our data); 8 - Baschii Village, 1 km to the south of the Altyn-Emel Mountain Range (44° 10'N; 78° 45'E), Taldy-Kurgan Region (Borkin et al., 1996); 9 - Koyandytau Mountain Range, Taldy-Kurgan Region (Borkin et al., 1996); 10 - Ayan-Saz Point, the Borokhudzir River Valley, Taldy-Kurgan Region (Borkin et al., 1996); 11 - Big Almaty Lake, Zailiysky Alatau Range; 12 - Karaoj Village, to the south of the Balkhash Lake; 13 - Zhidely Channel, Ili River delta; 14 - Kizilkum Desert, 50 km to the southwest of the Bairkum Village, Chimkent Region; 15 - Jabagly Village, Chimkent Region (11-15, our data).

Bufo viridis viridis: 16 - Chornaya Rechka near Guryev Town, Guryev Region (Schneider, Egiasaryan, 1995).

Bufo viridis turanensis: 17 - Almaty (Mezherin, Pisanetz, 1991); 18 - Chindzhaly River near Andreyevka Village, Taldy-Kurgan Region (Golubev, 1990); 19 - a point in 20 km to the south of the Kopa Station, Almaty Region (our data).

Table 1. Some preliminary results of the study of green toad of *Bufo viridis* complex in South Kazakhstan. In 1994 and 1995, specimens have been collected in two different regions of Almaty town and are, therefore, considered as distinct samples

Locality	Year	Longitude	Latitude	Altitude (m)	Sample size males/females	Karyotype	SVL min-max mm
Almaty*	1994	76° 55'	43° 15'	900	13/1 (14)	4n	68.49-79.87
Almaty*	1995	76° 55'	43° 15'	900	15/14(29)	4n	68.48-90.88
Big Almaty Lake	1994	77° 00'	43° 04'	2300	12/1 (13)	4n	62.14-83.62
Kopa	1994	75° 47'	43° 25'	750	15/3 (18)	2n	85.15-106.33
Karaoj Village	1995	74° 47'	45° 54'	350	11/5 (15)	4n	59.06-65.24
Zhidely Channel	1995	75° 12'	45° 18'	370	3/1 (4)	4n	49.35-71.8
Kizil-kum Desert	1995	67° 25'	42° 02'	250	7/0 (7)	4n	67.87-78.79
Jabagly Village	1995	70° 32'	42° 25'	1100	11/2 (13)	4n	68.02-77.80

A map of the distribution of diploid and tetraploid Green Toads of the *Bufo viridis* complex was made by program Automap 1.1 (programmers V. Dubjansky and A. Kazimirov). Since the main purpose of this paper is to present some new information on the distribution of diploid and tetraploid toads in Kazakhstan, only a brief review of other results obtained has been summarized in Table 1.

Results and Discussion

Of 8 populations, only one, sampled near Kopa, in south eastern Kazakhstan, proved to have a diploid karyotype. As it is normally the case, this population also shows the largest snout-vent length (see Table 1).

Until now, diploid Green Toads were reported for the territory of Kazakhstan from 3 localities only, but only Mezherin and Pisanetz (1991) and Pisanetz (1991) supported the occurrence of *Bufo viridis turanensis* at Almaty with a karyological study. The record by Golubev (1990) of the presence of *Bufo viridis* in cohabitation with *Bufo danatensis* on the Chindzhily River, near the village of Andreyevka (eastern Kazakhstan) (Fig. 1) was based on differences in the breeding behaviour of two groups of specimens and lacks any karyological confirmation. Finally, Schneider and

Egiasaryan (1995) cited *Bufo viridis viridis* from Chornaya Rechka, near the town of Guryev (north western Kazakhstan, Fig. 1) on the basis of some advertisement-call characters. Although the mating calls and behaviour of some populations of *Bufo viridis* have been studied in full detail, a karyological analysis remains necessary for exact determination.

Records of tetraploid toads in Kazakhstan are more numerous (Fig. 1). Two of them, however, respectively from the Zaissan region (Szczerbak and Golubev 1979) and the Chindzhily River, near Andreyevka Village (Golubev 1990) need karyological confirmation.

Our karyological data show that, apart from a single locality (Kopa), all other populations examined consist of tetraploid specimens. The finding of tetraploid toad populations at the Zhidely Canal of the Ili River delta and at Karaoj Village concurs with data by Borkin et al. (1986a; 1996) in that the territory south of Lake Balkhash and the area along the Ili River are inhabited by tetraploid toads only (Fig. 1). Specimens from Zhidely Canal and Karaoj also show the smallest snout-vent lengths in comparison with toads from all other populations sampled in 1994 and 1995 (Table 1).

Our record of tetraploid toads within the town of Almaty (Table 1) is in an agreement with data by Borkin et al. (1996), who identified a tetraploid population in the same area by flow cytometry analysis, but may contradict data of Mezherin and Pisanetz (Mezherin and Pisanetz 1991; Pisanetz 1991, see above) who reported the presence of diploid *Bufo viridis turanensis* from Almaty. A high mountain population from the Big Almaty Lake (Transvalley Alatau Range, 2300 m) also has a tetraploid $4n=44$ karyotype (Table 1). For the time being, however, we cannot exclude the possibility of the presence in the Almaty region of diploid and tetraploid syntopic populations and further investigations are necessary in order to settle this point.

Mezherin and Pisanetz (1991) were first to report on the presence of a tetraploid population in the southeast region of Kazakhstan, near the town of Chimkent. All specimens from the south east examined by us (Kizilkum Desert and Jabagly Village, Fig. 1) are indeed tetraploid. They share a similar snout-vent length (Table 1) but inhabit ecologically different biotopes. At Kizilkum, toads were found at a pond located in a sand-dunes area and formed by water overflowing from a water hole (altitude 250 m). At Jabagly, toads were caught in small ponds and in a stream, by a mountain village, at an altitude of 1100 m.

Acknowledgments

We are very grateful to Vladimir Dubyansky for providing technical assistance in preparing this manuscript and for using program Automap 1.1.

Literature Cited

Bachmann, K., A. Konrad, E. Oeldorf, and H. Hemmer. 1978. Genome size in the green toad (*Bufo viridis*) group. *Experientia* 34:331-332.

Borkin, L. J., I. A. Caune, E. M. Pisanetz, and Y. M. Rozanov. 1986a. Karyotype and genome size in the *Bufo viridis* group. Pp. 137-142. In Z. Rocek (Ed.), *Studies in Herpetology*. Prague.

Borkin, L. J., Y. M. Rozanov, H. Terbish, and I. M. Caune. 1986b. [The distribution, karyology, taxonomy and variability of the toad of *Bufo viridis* group in Mongolia]. Pp. 120-143. In *Herpetological researches in MNR*. (In Russian).

Borkin, L. J., H. Terbish, and I. A. Caune. 1986c. [Tetraploid and diploid populations of the toad of *Bufo viridis* group from Mongolia]. *Doklades of Academy of Sciences USSR* 287(3):760-764. (In Russian).

Borkin, L.J., and S. L. Kuzmin. 1988. [The Amphibians of Mongolia]. Pp. 30-197. In *Amphibians and reptiles of MNR*. Moscow, Nauka. (In Russian).

Borkin, L. J., A. E. Vinogradov, Z. K. Brushko., and S. L. Kuzmin. 1996. New records of tetraploid toads (*Bufo viridis* group) from Alma-Ata and Taldy-Kurgan region, Kazakhstan. *Russian Journal of Herpetology* 2 (2):174-175.

Egemberdieva, G. Ch. 1983. [Differenciuyushchaya rol izolyacii v izmenchivosti pozvonochnykh zhivotnykh]. Pp. 81-114. In *Ekologo-geneticheskie osnovy izmenchivosti pozvonochnykh zhivotnykh*. Frunze. (In Russian).

Fichtman, B.S. 1989. [On the diagnosis of the green toad of *Bufo viridis* complex in Kirgizia]. Pp. 263-264. In *Problems of Herpetology*. Kiev, Naukova dumka. (In Russian).

Golubev, M. L. 1990. [New records of amphibians and reptiles in the territory of Kazakhstan]. *Vestnik Zoologii*, Kiev 5:76-78. (In Russian with English summary).

Masik, E. J., B. K. Kadyrova, A. T. Toktosunov. 1976. [Karyotype pattern in the green toad *Bufo viridis* in Kirgizia]. *Zoologichesky Zhurnal* 55:1740-1742. (In Russian with English summary).

Mezherin, S. V., and E. M. Pisanetz. 1990. [Genetic structure and origin of asiatic tetraploid toad, *Bufo danatensis* Pisanetz, 1978]. *Doklades of Ukrainian Academy of Sciences* 3B(8):71-73. (In Russian with English summary).

Mezherin, E. J., and E. M. Pisanetz. 1991. [Genetic divergence of green toads of *Bufo viridis* complex of USSR fauna]. *Doklades of Academy of Sciences of USSR* 317(10):222-226. (In Russian).

Orlova, V. F., and H. Terbish. 1986. [Materials on Dzhungarian Gobi Herpetofauna]. Pp. 95-110. In *Herpetological Researches in MNR*. Moscow. (In Russian).

Orlova, V. F., and V. Uteshev. 1986. The tetraploid toad of *Bufo viridis* group from Dzhungarian Gobi, Mongolia. Pp. 143-146. In Z. Rocek (ed.), *Studies in Herpetology*. Prague.

Pisanetz, E. M. 1978. [On new polyploid species of the toad, *Bufo danatensis* Pisanetz sp. n. from Turkmenia]. *Doklades of Ukrainian Academy of Sciences* 3B(3):280-284. (In Russian or Ukrainian).

Pisanetz, E. M. 1991. [The new dates of karyology *Bufo viridis*-complex (Amphibia, Bufonidae) and the questions of origin of asiatic tetraploid toads]. *Herpetological Researches*, Leningrad 1991(1):41-50. (In Russian with English summary).

- Pisanetz, E. M. 1992. Evidence of hybridization between di- and tetraploid toads (*Bufo viridis* complex) from Eurasia (Anura: Bufonidae). Pp. 357-360. In Z. Korsos and I. Kiss. (eds.), Proc. Sixth Ord. Gen. Meet. S.E. Budapest.
- Pisanetz, E. M., and N. N. Szczerbak. 1979. [Taxonomy of the green toads (Amphibia, Anura) from the USSR fauna]. Vestnik Zoologii 4:11-16. (In Russian with English summary).
- Schneider, H., and E. M. Egiaryan. 1995. The mating call of the green toad, *Bufo viridis viridis* (Anura, Amphibia) in Kazakhstan. Russian Journal of Herpetology 2(1):1-4.
- Toktosunov, A. T. 1984. [Ecological basis of altitude adaptation of the vertebrates of Tien-Shan]. Lenin-grad, Nauka. (In Russian).
- Wu Min, and Zhao Yajiang. 1987. [Preliminary study of the karyotype of green toad from Sinzsjan]. Zoological Researches 4:339-342. (In Chinese).
- Zhao Er-mi. 1995. [A checklist of Chinese amphibians and their distribution]. Pp. 1-15. In Er-mi Zhao (Ed.). Amphibian Zoogeographic Division of China. 1995 Supplement: Sichuan Journal of Zoology. Chengdu, Sichuan, China. (In Chinese).