

## **New data on distribution of amphibians and reptiles in the Aral Sea Basin and surrounding areas of Kazakhstan.**

### **Part I. The Green Toads of *Bufo viridis* complex (Amphibia: Anura)**

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**Abstract.** First the data on distribution of the toads of *Bufo viridis* complex are presented for Aral Sea Basin and surrounding areas of Southern and Western Kazakhstan. Karyological analysis revealed a presence of diploid toad populations in the valley of the middle and low flows of Syr-Darya River; in the northern coast of Aral Sea and the desert areas between the last and Irgyz-Turgay Basin; in the northern part of Karatau Range. The only tetraploid population was discovered in the central part of Karatau Range.

### **Introduction**

From a time of first record of tetraploid green toad population in the mountains of Kirgizyia (Masik et al., 1976) and a first description of a tetraploid species *Bufo danatensis* Pisanets, 1978 (Pisanets, 1978) the toads of *Bufo viridis* complex continue to be one of the most problematical and curious taxonomic groups of amphibians. For near of quarter of century a huge number of data on distribution of diploid, triploid and tetraploid populations were collected, a series of new taxa were described and some ideas on origin and evolution of tetraploid toads were proposed. Recently Stöck et al. (2001a) has undertaken a deep taxonomic revision of the Asian *Bufo viridis* group comprising 23 nominal taxa. He presented data on type localities, nomenclatural and systematic histories, karyotypes and distribution; proposed current taxonomic status and tentative identification key as well as indicate the regions lack available data on *B. viridis* complex (Stöck et al., 2001a: Fig. 16).

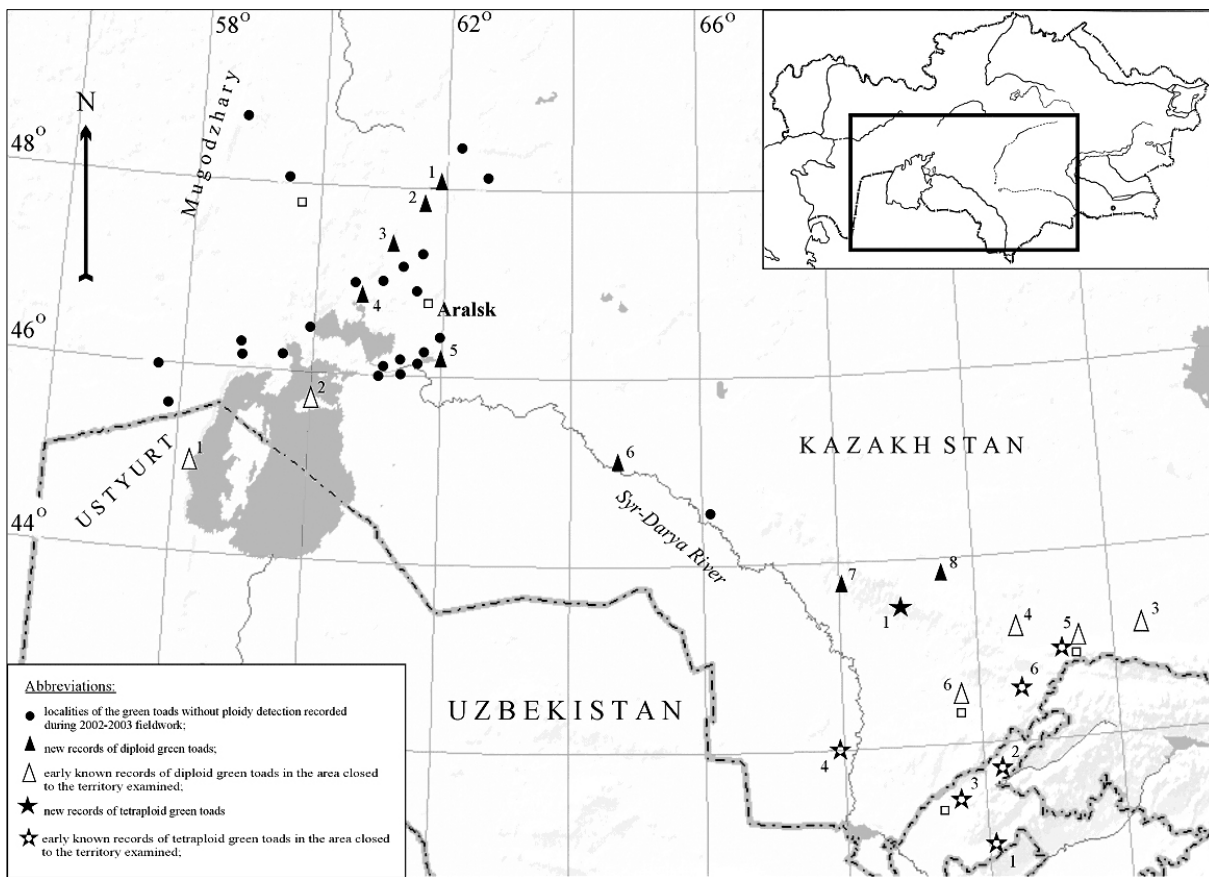
To present time the green toad populations with identified karyotypes are know from Iran, Tadjikistan, Turkmenistan, Uzbekistan, Kirgyzstan, Kazakhstan, Afganistan, Pakistan, Turkey, India, Mongolia and China (See literature references in Stöck et al. (2001b). In spite of a number of the green toad records with ploidy detection known for southern regions of Central Asia, a vast territory of northern deserts and steppes still stayed out of study. Except of few finds, a huge area between the Caspian and Aral Seas, the deserts between Syr-Darya and Amu-Darya Rivers including the rivers valleys and the territories of Central, Northern and Eastern Kazakhstan lack distributional data on the toads of *Bufo viridis* complex with identified karyotype.

In current paper we try to improve a deficiency of our knowledge on distribution of the green toads of *Bufo viridis* complex and represent the results of our investigation in Aral Sea Basin and surrounding areas of Southern and Western Kazakhstan.

### **Material and methods**

Field work was carried out in May –June 2002-2003 mainly in Aral Sea Basin (Western, Northern and Northeastern Aral Sea regions including the low flow of Syr-Darya River), Mugodzhar Mountains, Irgyz-Turgay Basin and partially in the valley of the middle flow of Syr-Darya River and Karatau Range (Fig. 1). The toads were collected in the first hours of the night when the reproductive activity was maximal. Only adult specimens with well-developed secondary sexual characters were sampled for measurements. Next day after capture the toads were anaesthized in MS-222 (Sandoz), weighed and measured in 20 body-proportion characters with a digital caliper (Mitutoyo mod. 500-321) calibrated to 0.01 mm. The results of morphometrical analysis will be presented elsewhere. The adult specimens from 9 localities (totally 53 specimens) were taken alive to laboratory for a karyological analysis.

From each specimen 100-200 µl of venous blood were incubated for 4 days at 25°C in MEM (Minimal Eagle Medium, GIBCO) with 20% calf serum and 3% Phytohaemagglutinin M. Chromosome preparations were produced by conventional air-drying method, using KCl 0.075M as hypotonic solution. Standard staining method was performed using Giemsa 5% in phosphate buffer pH 7.



**Fig. 1.** New records of the green toads of *Bufo viridis* complex with ploidy detection (black triangles and black stars) and early known data on diploid and tetraploid green toad distribution (transparent triangles and black stars with transparent center) in the Aral Sea Basin and surrounding areas of Southern and Western Kazakhstan. Black circles indicate the records of 2002-2003 fieldwork without ploidy detection

**New 2n records (black triangles):** 1 - 120 km N Aralsk Town, 5 km W Ushki Urotchishchye (47°53'N; 61°46'E, 80-90 m), 20.05.2002; 2 - 100 km to N direction by the road Aralsk - Irgyz (47°35'N; 61°30'E, 90 m), 20.05.2002; 3 - Shokissu Pump-Station, ~15 km NNW Saksaulskaya Rail-way Station (47°14'N; 61°05'E, 120 m), 15.05.2002; 4 - 3 km E Akеспе Village, Kizilbulak Spring (46°48'N; 60°34'E, 60 m), 11-12.05.2002; 5 - Syr-Darya River delta, E coast of Kamishlibash Lake (46°10'N; 61°55'E, 80 m), 06.05.2002; 6 - valley of the middle flow of Syr-Darya River, 13 km SW Zhalagash Village (45°02'N; 64°38'E, 122 m), 07.05.2002; 7 - valley of the middle flow of Syr-Darya River, 45 km NW Turkestan Town, vicinity of Intaly (=Maidantal) Village (43°41'N; 67°57'E, 354 m), 05.05.2002; 8 - NE foothills of Karatau Range, S and W coasts of Kizilkol Lake (43°44'N; 69°31'E, 353 m), 04.05.2002 - for all samples ploidy level was detected by chromosome count.

**New 4n records (black stars):** 1 - SW slopes of Karatau Range, 20 km ESE Kentau Town (43°27.153'N; 68°50.035'E, 527 m), 05.05.2002 - chromosome count.

**Early known 2n records (transparent triangles):** 1 - W Aral Sea Region, Ustyurt Plateu, 15-20 km N Kassarma Urotchishchye against Vozrozhdeniye Island (coll. V. A. Cherlin, ZISP 5571) - flow cytometry; 2 - Aral Sea, Barsakelmes Island (coll. N. B. Ananjeva, ZISP 5769) - flow cytometry; 3 - Akyr-Tyube, a village between Tien-Shan Mountains and the edge of the Muyun-Kum Desert (Borkin et al., 2001) - flow cytometry; 4 - Karatau Mountains, Berkara River, 75 km WNW Dzhambul City (Borkin et al., 2001) - chromosome count; 5 - Dzhambul City (Roth, Ráb, 1986) - chromosome count; 6 - Chimkent City environs (Mezhzherin, Pisanets, 1995) - external morphology, chromosome count.

**Early known *An* records (black stars with transparent center):** 1 - Kuraminsky Range, 3000 m (Borkin et al., 1986) – chromosome count; 2 – entry of Chatcal Nature Reserve, 5 km E Burchmulla, 80 km E Tashkent (41°35'N, 70°07'E, 900 m) (Stöck, 1998) - chromosome count and/or erythrocyte size, calls; 3 – Tashkent (41°16'N, 69°13'E, 450 m) (Borkin et al., 1986; Roth, Ráb, 1986; Kudryavcev et al., 1988; Stöck, 1998) – chromosome count, flow cytometry, erythrocyte size, calls; 4 - SE Kizilkum Desert, 50 km SW Bairkum Village (42°02'N; 67°25'E, 250 m) (Dujsebayaeva et al., 1997; Castellano et al., 1998) – chromosome count; 5 - Dzhambul City (Roth, Ráb, 1986) – chromosome count; 6 – Western Tien-Shan, Jabagly Village (42°25'N, 70°32'E, 1100 m) (Dujsebayaeva et al., 1997; Castellano et al., 1998) – chromosome count.

All the localities visited were also checked in respect of the eggs and larvae presence especially if the adult toads were not found. Species identification of the larvae was carried out according to Bannikov et al. (1977) and Kuzmin (1995) and developmental stages were determined according to Gosner (1960).

To analyze the known and new data on distribution of diploid and tetraploid green toads in the region examined we revised literature sources, checked museum collections (Zoological Institute of Russian Academy of Sciences, Saint-Petersburg – ZISP; Zoological Museum of Moscow State University, Moscow – ZMMSU; Institute of Zoology, Almaty – IZKAZ) and used a database "Amphibians of the USSR" (State Register of the Databases of Russian Federation no. 0229803415).

### Results

33 records of the green toads have been registered in the vast area placed between 43°-49°N and 58°-70°E. The toad samples from 9 localities were analyzed in respect of ploidy level. Rare finds of the toads in the valley of the middle flow of Syr-Darya River and in Karatau Range can be explained not rarity of the amphibians themselves but rather passing character of our research in these regions. However, we have registered three new localities of the diploid toads and one locality with tetraploid population. One diploid toad locality was found in the middle flow of Syr-Darya River in 13 km southwest of Zhalagash Village and two other - in the northern part of Karatau Range namely in the southwestern range slopes near Intaly Village and in the northeastern range foothills along the southern and western coasts of Kizilkol Lake (Fig. 1). The only tetraploid toad population was recorded in the low mountain zone of the central part of Karatau Range (20 km southeast of Kentau Town, Fig. 1). All the finds were fixed in lowland zone between 120 and 530 m a. s. l.

Our investigation in the Aral Sea Basin have confirmed the early known data on a wide distribution of the green toads in the low flow of Syr-Darya River, northern and northwestern coasts of Aral Sea and Irgyz-Turgay Rivers Basin (Elpatjevsky, 1903; Zarudny, 1915; Sidoroff, 1925; Dinessman, 1953; Iskakova, 1959). The green toads still stay as quite abundant within lake system of Syr-Darya River delta (Kamishlibash, Karashalan, Tishchye-Bass Lakes), in artificial ponds and wells in the human settlements (Rayim, Amanutkel, Zhanakurilis Villages) and in the temporary spring pools in both continental and former Aral Sea bottom areas. Karashalan Lake (46°05'N; 61°05'E) and a bottom of former Bayan Lake (46°01'N; 61°00'E) represented the localities of most deep penetration of the green toads into the dried bottom of the Aral Sea. The altitude range of the records varied within 50-80 m a. s. l. Karyological analysis of the toad sample from Kamishlibash Lake (46°10'N; 61°55'E) showed the diploid chromosome set in all 12 specimens examined (Fig. 1).

A number of new finds was noted for Northern Aral Sea region – a territory between the northern coast of Aral Sea and Irgyz-Turgay Rivers junction. In the second half of May in 2002-2003 we recorded the adult toads and the larvae at 25-33 stages of development along the northern coast of Butakov Gulf, in Maliye Barsuki, Barshakum and Zhamankum Sands, in the southern foothills of Altynshokissu Hills (Shokissu Pump-Station - 47°14'N; 61°05'E) and in the upper sector of Termenbess Hill (47°07'N; 61°11'E), where one hundred years ago the green toads were first found by Berg (Elpatjevsky, 1903). All localities were registered in lowland range (mainly between 60 and 120 m with highest point in Termenbess Hill – 194 m). The toads inhabited the floods of fresh-water or slightly salty natural artesian water-holes and springs, in the wells and arik systems in the villages.

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Some finds were fixed in the temporary ponds along the roads. In the low flow of Turgay River quite numerous adult specimens were found in the stagnant reservoirs formed after spring flooding of the river. The toad eggs and larvae sometimes of a quite abundant masses were constantly fixed in the accumulations of rain and melted waters (ravines, ditches, pools along the roads), in well floods and irrigation systems in the villages. All specimens of four toad samples taken for karyological analysis from Northern Aral Sea region were diploid (Fig. 1).

New for Kazakhstan localities of the green toads (ploidy detection in progress) were recorded in the southern part of Bolshiye Barsuki Sands (Juzhnoye Village environs - 46°19'N; 58°41'E, 130 m; Zhaksikoyankuduk Hill - 46°13'N; 58°44'E, 159 m) and in the eastern part of Northern Ustyurt Plateau (Ozektyk Urotchishchye between Dongelek and Kosbulak Sors - 45°59'N; 57°28'E; Shiyoba Well 30 km east of Asmatay-Matay Sor - 45°36'N; 57°46'E; both localities at 93 m a. s. l.). One record was registered in the central part of Mugodzhary Mountains (foothills of the Two Brothers Mountain-48°39'N, 58°37'E, 395 m) and the other one to east direction from Mugodzhary Mountains – in the vicinity of Karakol' Winter House (30 km northwest of Shalkar Town - 47°59'N; 59°20'E, 204 m).

### Discussion

For Kazakhstan the distribution of the green toads of *Bufo viridis* complex until now was studied best of all in the southeastern and in the east section of the southern regions of the country (Northern and Western Tien-Shan and Dzhungsky Alatau with their foothills, Southern Balkhash Region, Ily River Valley, Alakol Depression and partially Southeastern Kizilkum Desert) where a maximal number of the toad records with identified karyotype was known (Borkin et al., 1986, 1995; Dujsebayaeva et al., 1997; Bassalayeva et al., 1998a; Castellano et al., 1998; Stöck, 1998; Stöck et al., 2001a, 2001b). Such data were rather rare for eastern regions – Ayaguz Town environs and Aksiir Farm in the east of Zaissan Depression (Borkin et al., 1986; Bassalayeva et al., 1998b). In Kazakhstan the tetraploid toads inhabited mainly mountainous and deserted biotopes in a wide altitude range (from 200 to near 3000 m a. s. l.) while the diploids preferred the lowlands or hilled areas in visibly narrow diapason of altitudes (100-1000 m a. s. l.) (Dujsebayaeva, Bassalayeva, 1999).

The regions mentioned put together no more than 1/5 or even 1/6 of Kazakhstan. So, the vast territories between the Caspian and Aral Seas, the deserts between Syr-Darya and Amu-Darya Rivers including the rivers valleys, Central and Northern Kazakhstan (north from Karatau Range and Balkhash Lake) as well as most part of Eastern Kazakhstan lacked distributional data on the toads of *Bufo viridis* complex with ploidy detected except of few sparse data. The last included the diploid toads from Guryev (present Atyrau) Town environs (Northern Caspian Sea Region: Schneider, Egiasaryan, 1995; Castellano et al., unpubl.) and from Tengiz Lake (Central Kazakhstan: Stöck et al., 2001b) and two records of tetraploid toads from Eastern Kazakhstan that were mentioned above. Analysis of museum collections undertaken by authors of present work additionally revealed some localities of the toads with ploidy detection. According to catalogue data of Zoological Institute of Russian Academy of Sciences (unpublished data of cytometry analysis), the toads caught by Vasilkovskaya near the villages Oitan and Miyali (Guryev District, Western Kazakhstan – ZISP 5678, 6456 accordingly) in 1991 and by Ananjeva in Barsakelmes Island (Aral Sea - ZISP, 5769) in the same year were diploid according to flow cytometrical analysis.

Our data significantly extended an image on the distribution of the green toads of *B. viridis* complex in Aral Sea Basin and surrounding areas of Western and Southern Kazakhstan. As it was turn out the diploid toads inhabited the valley of Syr-Darya River in its middle and low flows including the delta region, the northern coast of Aral Sea and area between that coast in the south and Irgyz-Turgay Rivers junction in the north (Fig. 1). While early diploid toad populations were detected only in the foothill zones of the southern part of Karatau Range we found the diploids also in the northern part of Karatau, namely in its southwestern slopes and in the northeastern foothill zone (Fig. 1). Such data confirmed an early concept on a wide distribution of the diploid toads in Kazakhstan from the western boundaries of the country to Ily River Valley in the east (Kuzmin, 1995). However, a necessity of diploid nominal green toad taxa identification noted by Stöck et al. (2001a) again arisen. This is especially important for the western part of Kazakhstan where according to preliminary results of

genetic analysis the northern and northeastern Aral Sea regions can represent a secondary contact zone of Central and Middle Asiatic green toad clades (Delpero et al., unpubl.).

The only tetraploid locality recorded during our 2002-2003 voyages was pointed in 20 km southeast of Kentau Town (the southwestern slopes of the central part of Karatau Range). This locality can be referred to one of the most western finds of tetraploid green toads in Kazakhstan together with 4n record from Southeastern Kizil-Kum Desert noted by Dujsebayaeva et al. (1997) (Fig. 1). Most close tetraploid populations are known from the northeastern foothills of Karatau Range (Dzhambul Town) and Western Tien-Shan (Talass Alatau Range, Chatkalsky and Kuraminsky Ranges) including its western foothills (Tashkent Town) (Borkin et al., 1986; Roth, Ráb, 1986; Dujsebayaeva et al., 1997; Stöck, 1998).

Although our data again confirmed the distribution of diploid toads only in lowland in Kazakhstan the low boundary of altitude range noted as 100 m a. s. l. (Dujsebayaeva, Bassalayeva, 1999) can be changed twice less and accepted now as 50 m a. s. l. (low delta of Syr-Darya River).

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## Резюме

*Дүйсебаева Т., Кастеллано С., Магни П., Одиерна Г.* Новые данные по распространению земноводных и пресмыкающихся в Приаралье и сопредельных районах Казахстана. Часть I. Зеленые жабы комплекса *Bufo viridis* (Amphibia: Anura).

Впервые представлены данные по распространению диплоидных и тетраплоидных жаб комплекса *Bufo viridis* в Приаралье и сопредельных районах Южного и Западного Казахстана. По результатам кариологического анализа диплоидные популяции обитают в долине среднего и нижнего течения р. Сырдарья, на северном побережье Аральского моря, в пустынных равнинах между северным берегом Арала и Иргиз-Тургайским бассейном, а также в северной части хребта Каратау. Единственная находка тетраплоидной популяции была сделана в центральной части хр. Каратау.